Characterisation of a-Adrenoceptors in the Carotid Vasculature:

Possible Implications for Migraine Therapy

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Characterisation of α-Adrenoceptors in the Carotid Vasculature:

Possible Implications for Migraine Therapy

Karakterisering van α-Adrenoceptoren in het Halsslagader Vaatbed:

Mogelijke Implicaties voor Migraine Therapie

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Dedicated to Jeanne Hazenberg
(✞ 10-06-1983)

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Part I

Introduction

Tension

The moment you are in tension,
you'll loose your attention
Then you're in total confusion,
and you'll feel irritation
Then you'll spoil personal relation,
ultimately you'll not get cooperation
Then you'll make things complication
Then your blood pressure may also rise caution,
and you'll have to take medication
In stead, understand the situation,
and try to think about the solution
Many problems will be solved with discussion,
which will work out better in your profession
Don't think it is my free suggestion,
it's only for your prevention
If you understand my intention,
you'll never come again to tension...

(Author unknown)

Chapter 1

Current pharmacological aspects of a -adrenoceptors

Historical perspective

The endogenous catecholamines noradrenaline (norepinephrine) and adrenaline (epinephrine), which are released upon activation of the sympathetic nervous system, play essential roles in the regulation of a host of physiological responses. Cardiovascular function is tightly regulated by the autonomic nervous system, i.e. by the sympathetic and parasympathetic nervous system. Sensory nerves monitor the volume and pressure status of the heart and blood vessels, as well as the metabolic state of cardiac and systemic tissues. This information is processed by the nervous system, and impulses sent *via* the autonomic motor nerves modulate cardiac rate and contractility, as well as coronary and systemic vascular resistance. In addition to these direct controls over the cardiovascular system, the sympathetic nervous system innervation of the kidney influences fluid and electrolyte balance[1].

Noradrenaline is the major neurotransmitter in the sympathetic nervous system, whereas adrenaline is the primary hormone secreted by the adrenal medulla. Several decades ago, adrenoceptors (adrenergic receptors) were introduced to explain the difference in actions of noradrenaline and adrenaline[1] The introduction of the concept *receptor*, however, has its origin at the beginning of the 20th century with the pioneering work of Langley[2], Dale[3] and Ehrlich[4]. Studying the effects of denervation of sympathetic nerves, it was Langley[2] who first introduced the term *receptive substance*, which was later[4] constituted into the well-used appellation receptor. In today's discipline of pharmacology, it is considered that receptors play key functions in the generation of many biological responses. Strikingly, even before the term receptor was introduced, Dale[3] showed the first evidence for multiple types of receptors, demonstrating that ergot alkaloids not only blocked the pressor actions of adrenaline, but also revealed a vasodilator component, a phenomenon now known as *epinephrine reversal*. It was the revolutionary work of Ahlquist[5] that convincingly established the coexistence of adrenoceptors mediating different physiological responses. He showed that the order of potencies of a series of adrenergic agonists in inducing vasoconstriction was opposite to their rank order for producing

vasodilatation and alterations in myocardial contractility. It was stated that the adrenoceptor mediating vasoconstriction should be termed the ***a**-adrenotropic* and the receptor mediating vasodilatation and myocardial contraction should be termed the ***b**-adrenotropic* receptor[5]. Several important advances in the field of receptor research were achieved based on the work of Gaddum[6], Clark[7] and Michaelis & Menten[8], leading for example to the *occupancy theory*; stating that *the response of a drug is linearly related to the number of receptors occupied*. In the following decades, the existing theories were improved and together with others (such as *spare receptors*[9] and *relative efficacy*[10]), the concepts of receptor, receptor theory and drug action were refined to its present state[see 11, 12-14]. These theories have subsequently been supported and challenged by the introduction of specific agonists (*sympathomimetics*, which stimulate adrenoceptors) and antagonists (*sympatholytics*, which prevent agonist-induced effects by adrenoceptor-blockade) at α-adrenoceptors (e.g. phenylephrine and phentolamine, respectively) and β-adrenoceptors (e.g. isoproterenol and propranolol, respectively). Despite the fact that the range of physiological functions mediated by the sympathetic nervous system is so diverse as mentioned above, many of the actions produced by compounds that act at α- and/or β-adrenoceptors are understandable in terms of known physiological effects of the catecholamines.

After the initial division of adrenoceptors into α- and β-adrenoceptors, proposed by Ahlquist[5], it took several decades to demonstrate that these adrenoceptors could be subdivided into several subtypes (α_1, α_2 and β). In the following sections of this chapter, an attempt has been made to summarise several pharmacological aspects of α_1- and α_2-adrenoceptors, whereas those of β-adrenoceptors (subdivided into β_1-, β_2-, β_3- and, probably, β_4-subtypes; see Figure 1.1) have been described extensively elsewhere[see 15, 16-20].

As reviewed by Hoyer & Humphrey[21], it is important to note that the nomenclature for the classification of transmitter receptors is based on three criteria: *structural* (gene structure/amino acid sequence), *operational* (pharmacological/ ligand-defined/recognitory) and *transductional information* (receptor-effector coupling). Thus, none of these criteria has priority and that as much information as

feasible on these three aspects should be collected before the name of a receptor can be regarded upon.

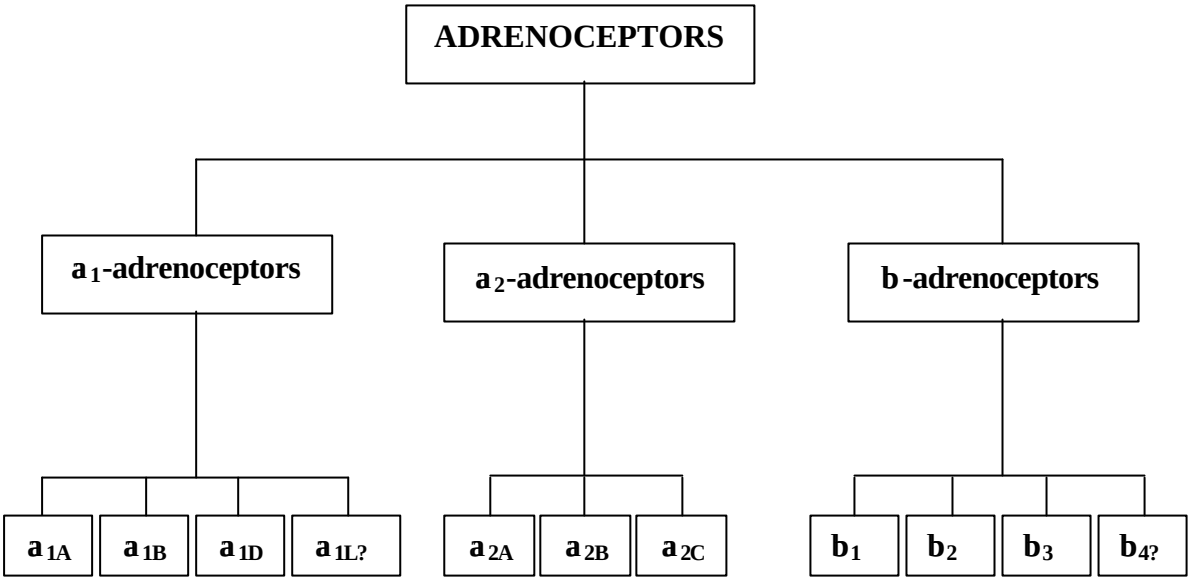

Figure 1.1. Current classification of adrenoceptors (modified from[17]). Please note that the pharmacological status of the α_{1L}- and β_4-adrenoceptor are unclear.

Subdivision of a-adrenoceptors

Besides the knowledge that α-adrenoceptors can produce vasoconstriction[5], already back in the 1970's it was shown that blockade of α-adrenoceptors (by phenoxybenzamine or phentolamine) could produce an increase in noradrenaline release induced by electrical stimulation, due to a direct action distinct from uptake blockade[22, 23]. Since several α-adrenoceptor agonists (such as clonidine) caused a decrease in noradrenaline release induced by electrical stimulation, it became clear that these effects were mediated by α-adrenoceptors on sympathetic nerve terminals, by so-called *presynaptic* or *prejunctional* α-adrenoceptors[24]. Over the years, it became evident that prejunctional α-adrenoceptors on sympathetic nerve terminals (i.e. *autoreceptors*) could mediate noradrenaline release *via* a negative feedback regulation, but that α-adrenoceptors could also mediate the release of other neurotransmitters (e.g. histamine or acethylcholine), acting as *heteroreceptors*[see 25, 26]. Thus, the first subclassification of α-adrenoceptors into α_1- and α_2-adrenoceptors was based on an anatomical location, with the α_2- being prejunctional (acting as a release modulation receptor), and the α_1- being postsynaptic

(mediating vascular smooth muscle contraction[27]). Already at the end of the 1970's, it became clear that two types of vascular α-adrenoceptors existed, the prazosin-sensitive α_1- and the prazosin-insensitive α_2-adrenoceptors[28]. Timmermans & Van Zwieten[29] demonstrated that yohimbine acted as an effective antagonist of the pressor actions produced by BHT933, clonidine and phenylephrine; in contrast, prazosin did not affect the pressor actions of BHT933, but it strongly diminished those to phenylephrine in pithed rats. Based on the above, it seems evident that α-adrenoceptor classification schemes featuring anatomical[27] or physiological function[30] were not sufficient to account for all these data. Instead, the currently accepted pharmacological classification of α-adrenoceptors into α_1 and α_2 subtypes has been based on the affinity and relative potency of selective agonists and antagonists[26, 31]. As discussed by Ruffolo *et al.*[32], an α_1-adrenoceptor is one that is activated by methoxamine, cirazoline or phenylephrine and is blocked by low concentrations of prazosin or WB 4101. A response elicited by BHT933, UK 14304 or BHT 920 that is antagonised by selective concentrations of yohimbine, rauwolscine or idazoxan should be defined as being mediated by α_2-adrenoceptors[26, 31].

Thus, as described above, α-adrenoceptors can be classified into two families, namely α_1- and α_2-adrenoceptors. In turn, these receptors can be further subdivided into different subtypes (see Figure 1.1). Eventually, detailed information on these receptors may help to understand the effects produced by the endogenous catecholamines and, hopefully, will lead to a better application of sympathetic ligands in many areas of therapeutics.

Table 1.1. Summary of α_1-adrenoceptor subtype characteristics (modified from Docherty[33], Bylund[34] and Alexander & Peters[19]).

	a_{1A}	a_{1B}	a_{1D}
Previous names	α_{1a}, α_{1c}	α_{1B}	α_{1A}, $\alpha_{1a/d}$
Functional response(s)	rat *vas deferens* contraction, rat renal artery contraction, rat caudal artery contraction, rat isolated perfused kidney vasoconstriction control of blood pressure	rat spleen contraction, role in rat tail contraction, control of blood pressure?	rat aorta contraction, control of blood pressure (hypertension)?
Ligand binding assay	rabbit liver, rat submandibular gland	rat liver and spleen, transfected CHO and HEK 239 cells	rat aorta, transfected CHO and HEK 239 cells
Non-selective agonists	phenylephrine, cirazoline, methoxamine	phenylephrine, cirazoline, methoxamine	phenylephrine, cirazoline, methoxamine
Selective agonists	A61603, oxymetazoline	none	none
Non-selective antagonists	prazosin	prazosin	prazosin
Selective antagonists	e.g. 5-methylurapidil, RS 17053	L-765,314	BMY7378
Potency order	noradrenaline ≥ adrenaline	noradrenaline = adrenaline	noradrenaline > adrenaline
Receptor distribution	brain, prostate, *vas* deferens, heart, blood vessels	spleen, kidney, brain, heart, blood vessels	brain, rat aorta, blood vessels
Tissue function(s)	smooth muscle and myocardial contraction	smooth muscle contraction	smooth muscle contraction
Sensitivity to CEC	+ / -	+++	++
Second messenger system(s)	activation of $G_{q/11}$, increase in PI turnover with elevation of $[Ca^{2+}]_i$, activation of voltage-gated Ca^{2+} channels		
Notes	A61603 also displays high affinity at α_2-adrenoceptor subtypes; There are four known isoforms	CEC also affects other receptors	the rat aorta appears to contain other α_1-adrenoceptor subtypes

Abbreviation: CEC, Chloroethylclonidine; PI, Phosphoinositol; CHO, Chinese Hamster Ovary; HEK 293, Human Embryonic Kidney Cells.

Heterogeneity of α_1-adrenoceptors

Shortly after the division of α_1- and α_2-adrenoceptors, evidence began to emerge that was inconsistent with a single vascular α_1-adrenoceptor[26, 31]. The initial subclassification of α_1-adrenoceptors into α_{1A}- and α_{1B}-subtypes was determined by receptor binding experiments, using the competitive antagonist WB 4101 and the alkylating agent chloroethylclonidine (CEC). Whereas the α_{1A}-subtype displayed a moderate affinity for WB 4101 and was CEC-insensitive, α_{1B}-adrenoceptors exhibited a low affinity for WB 4101 but were sensitive to CEC. Following the initial cloning of the hamster α_{1B}-adrenoceptor[35], two additional cDNAs were cloned[36-38]. The relationship between the pharmacologically defined (native) subtypes and the cDNA (recombinant) clones was controversial for several years[31]. However, after the cloning and analysis of the different human α_1-adrenoceptor subtypes, together with the development of more subtype-selective drugs, it is now generally agreed that the three native expressed subtypes (α_{1A}, α_{1B} and α_{1D}) can be distinguished pharmacologically and exhibit equivalency to the cloned and expressed α_1-adrenoceptor subtypes in various tissues[31]. Interestingly, four splice variants of the human α_{1A}-adrenoceptor have recently been cloned, which differ in length and sequence of their C-terminal domains[39, 40]. Because these splice variants of the human α_{1A}-adrenoceptor are awaiting further pharmacological characterisation[21], they are not included in Figure 1.1. However, it has been shown that human cloned α_{1A}-adrenoceptor isoforms display the α_{1L}-adrenoceptor pharmacology in functional studies[41]. The physiological significance of these α_{1A}-adrenoceptor splice variants is presently unknown, but may manifest interesting pharmacological features. Table 1.1 shows a summary of the current α_1-adrenoceptor pharmacological characteristics.

Signal transduction mechanisms of α_1-adrenoceptors

For some years it has been known that all three α_1-adrenoceptor subtypes mediate their responses *via* G-protein coupled receptors through a $G_{p/11}$ mechanism and involves activation of phospholipase C-dependent hydrolysis of phosphatidylinositol 4,5-diphosphate[31, 42, 43] (see Table 1.1). Activation of this enzyme results in the

generation of inositol (1,4,5)-triphosphate, which acts on the inositol triphosphate receptor in the endoplasmatic reticulum to release stored Ca^{2+} and diacylglycerol that (together with Ca^{2+}) can activate protein kinase C. The production of these second messengers results in an activation of both voltage dependent and independent Ca^{2+}-channels, which can cause smooth muscle contraction in particular tissues. Additional effectors that can couple to α_1-adrenoceptors include phospholipase D, mitogen-activated protein kinase pathway or the release of arachidonic acid and other substances, including *reactive oxygen species*[33, 43]. The mitogen-activated protein kinase pathway involves a longer term response and causes increased cell growth and may be important in vascular smooth muscle, for example the prostate[43]. However, a given α_1-adrenoceptor subtype may be coupled to a variety of different systems or different effector mechanisms may be linked to different subtypes. An understanding of the mechanisms involved, the receptor subtypes (α_{1A}, α_{1B} or α_{1D}) and the changes to these under different physiological and pathological states, may provide additional targets for therapeutic interventions.

Location and function of α_1-adrenoceptor subtypes

Radioligand binding assays have shown that α_1-adrenoceptors are expressed in a large number of tissues from several species[44] (see Table 1.1). Since highly subtype-selective antagonists and specific antibodies to these subtypes are not yet available[42], it has been very difficult to define the precise location of α_1-adrenoceptors in both the central and peripheral nervous systems. Thus, mapping the distribution of these subtypes (α_{1A}, α_{1B} and α_{1D}) has been performed largely by combining the analysis of *m*RNA expression patterns with results from radioligand and functional studies, which are mainly performed in rats, mice and humans[42].

In the last decade, increasing information has become available on the expression, distribution and functions of *central* α_1-adrenoceptors[45]. It is relatively straightforward to allocate physiological responses to adrenoceptor activation in the peripheral nervous system (see below). However, it has proved more difficult to define which of the effects of noradrenaline is mediated by a specific receptor class (i.e. α_1, α_2 or β) or subclass (e.g. α_1- or α_2-adrenoceptor subtypes) in the central nervous system[45]. Based on ligand autoradiography and *in situ* hybridisation

studies, it has been revealed that α_1-adrenoceptor subtypes are widely distributed in the brain of several species, although there is substantial variation in subtype expression between brain regions[46, 47]. In the central nervous system, it has been shown that α_1-adrenoceptors are located predominantly postsynaptically where they mediate an excitatory effect[48]. In this context, drugs activating α_1-adrenoceptors have been shown to induce electrophysiological excitatory changes and to facilitate transmission in the brain areas related to locomotion (causing arousal). As reviewed by Sirviö & MacDonald[45], there is a substantial amount of evidence suggesting the role of α_1-adrenoceptors in the modulation of attention and memory (see later). Nevertheless, the series of events following activation of central sympathetic neurones remains poorly understood.

In contrast to the central nervous system, the distribution of α_1-adrenoceptor subtypes in the *periphery* has been investigated more extensively[44]. Peripheral α_1-adrenoceptors are located on both vascular and non-vascular smooth muscle (e.g. prostate, *vas* deferens, heart or liver) where activation of the receptor results in contraction[49]. Investigations employing functional, radioligand binding and molecular methods have demonstrated the existence of multiple α_1-adrenoceptor subtypes in vascular smooth muscle from several species, including rat, rabbit, dog and human[44, 50]. It has been shown that all α_1-adrenoceptor subtypes are present throughout the arterial and venous system, a finding that substantiates the important role of α_1-adrenoceptors in blood flow regulation and the maintenance of vascular resistance. In general, establishing the α_1-adrenoceptor subtype-specificity mediating vasoconstriction in a particular vascular bed has been hampered, amongst others, by: (*i*) the lack of highly selective agonists and/or antagonists; (*ii*) the co-existence of multiple α_1-adrenoceptor subtypes; and (*iii*) regional differences in the distribution and density[44]. All three α_1-adrenoceptor subtypes are expressed in heart, although the α_{1A} may be the dominant cardiac subtype in humans[17, 51]. It must be noted that around 90% of the total α_1-adrenoceptor *m*RNA message pool expressed at very high levels in peripheral arteries is for α_{1A}-adrenoceptors[52]. In agreement with the latter, there is good evidence obtained from both *in vitro* and *in vivo* studies that the α_{1A} is the main α_1-adrenoceptor subtype in the sympathetic regulation of vascular tone and

blood pressure[33, 44, 53]. Human *vas deferens* and other smooth muscles appear to express predominantly α_{1A}- and α_{1D}-adrenoceptors[42]. Some α_{1B}-adrenoceptor message and protein can also be found in vascular smooth muscle[54]; however, limited information is available on the exact role of α_{1B}-adrenoceptors in peripheral blood vessels of humans[54]. Interestingly, recent findings obtained from experiments using knockout mice (lacking the α_{1B}-subtype), provide evidence that α_{1B}-adrenoceptors may mediate vasopressor and aorta contractile responses induced by α_1-adrenoceptor agonists[46]; whether this is species dependent needs further investigations. In this context, it has been reported that rat liver contains predominantly α_{1B}-adrenoceptors[55-57], but α_{1A}-adrenoceptors in humans[51]. By comparing the protein expression patterns and the results from radioligand binding and functional experiments, it is reasonable to conclude that most tissues express mixtures of α_1-adrenoceptor subtypes[42], which complicates pharmacological characterisation in functional studies. Therefore, tissues that have been used as model systems to characterise the pharmacological and signalling properties of particular subtypes (α_{1A}: rat *vas* deferens, rat submaxillary gland, rat kidney; α_{1B}: rat spleen, rat liver; α_{1D}: rat aorta) most likely express multiple receptor subtypes. This may contribute to the confusion surrounding the properties of the native subtypes[42]. The exception appears to be the rat liver (see above), which seems to express only the α_{1B}-subtype[55-57].

Selective agonists and antagonists at α_1-adrenoceptors

It must be emphasised that the current knowledge concerning the α_1-adrenoceptor subtype-selectivity mediating functional responses has been facilitated by the identification of selective antagonists at these receptor subtypes. Both the affinity and selectivity of these compounds for α_1-adrenoceptor subtypes have been determined primarily by competition of radioligand binding to expressed recombinant subtypes in different cell lines. The affinities of some of the commonly used antagonists for the different human α_1-adrenoceptor subtypes are shown in Table 1.2.

Table 1.2. Binding affinity constants (pK_i values) for cloned human α_1- and α_2-adrenoceptor subtypes.

Antagonist	**a_{1a}**	**a_{1b}**	**a_{1d}**	**a_{2a}**	**a_{2b}**	**a_{2c}**
Prazosin	9.8[a]	9.6[a]	10.2[a]	5.3[f]	6.4[f]	6.7[f]
	9.9[n]	9.5[n]	10.4[n]	5.7[g]	6.5[g]	7.2[g]
5-Methylurapidil	9.0[o]	7.5[o]	7.9[o]	6.2[k]	6.4[k]	6.9[k]
	9.1[a]	7.4[a]	8.0[a]			
L-765,314	6.4[b]	8.7[b]	7.5[b]	n.d.	n.d.	n.d.
BMY7378	6.6[c]	7.2[c]	9.4[c]	5.1[c*]	5.1[c*]	5.1[c*]
	6.8[p]	7.0[p]	9.0[p]			
Rauwolscine	6.3[j]	6.3[j]	6.3[j]	9.5[d]	9.4[d]	9.9[d]
	5.1[m]	5.3[m]	5.3[m]	8.9[h]	8.9[h]	9.3[h]
BRL44408	n.d.	n.d.	n.d.	8.2[e]	6.2[e]	6.8[e]
				7.6[f]	6.0[f]	6.4[f]
Imiloxan	<4[i]	<4[i]	<4[i]	5.8[f]	6.9[f]	6.0[f]
				6.5[h]	7.2[h]	6.8[h]
MK912	n.d.	n.d.	n.d.	8.9[e]	8.9[e]	10.2[e]
				9.1[h]	9.1[h]	10.2[h]

Data taken from: [a], Shibata *et al.*[58]; [b], Patane *et al.*[59]; [c], Goetz *et al.*[60]; [d], Bylund *et al.*[48]; [e], Uhlen *et al.*[61]; [f], Devedjan et *al.*[62]; [g], Lomasney *et al.*[63]; [h], Jasper *et al.*[64]; [i], Michel & Whiting[65] (pA_2 value); [j], Michel & Whiting[66] (pA_2 value); [k], Hieble *et al.*[67]; [l], Kenny *et al.*[68]; [m], Forray *et al.*[69]; [n], Schwinn *et al.*[70]; [o], Saussy *et al.*[71]; [p], Craig *et al.*[72]; *, Value for rat receptor; n.d., not determined. For full names of the antagonists, see list of abbreviations.

Until a decade ago, most antagonists (including prazosin), showed little or no selectivity between the three known α_1-adrenoceptor subtypes[26]. In recent years, however, a variety of ligands with varying degrees of selectivity have been developed (see Table 1.1). For example, 5-methylurapidil[73] and (+)-niguldipine[74] were introduced as potent and selective α_{1A}-adrenoceptor antagonists, showing 80 to 500-fold higher affinity for α_{1A}- over the α_{1B}-subtype and a low affinity for the α_{1D}-subtype[see 31]. Recently, several more potent and selective α_{1A}-adrenoceptor antagonists have been developed, including SNAP 5089[75], Rec 15/2739[76], RS-17053[77], A131701[78] and RWJ-38063[79]. Similarly, several moderate to high potent and selective α_{1A}-adrenoceptor agonists have become available, such as oxymetazoline (partial agonist), cirazoline[80, 81], SDZ NVI 085[82], A61603[83] and NS-49[84]. Since the initial subclassification of α_1-adrenoceptors, the alkylating agent chloroethylclonidine has been used to characterise the α_{1B}-adrenoceptor[26, 33]. Because chloroethylclonidine alkylates several other receptors as well[26, 85, 86], other antagonists have been developed displaying moderate selectivity at α_{1B}-adrenoceptors, including spiperone, cyclazosin[87] and L-765,314[88]. The partial 5-HT_{1A} receptor agonist BMY7378 is considered to be a selective α_{1D}-adrenoceptor antagonist, with about 100-fold higher affinity at α_{1D}-adrenoceptors over the α_{1A}- or α_{1B}-subtype[60]. To date, selective α_{1B}- or α_{1D}-adrenoceptor agonists are, unfortunately, not available[18]. In agreement with the latter, there is a clear need for more selective agonists and antagonists at α_{1B}- and α_{1D}-adrenoceptors in order to make a more precise evaluation of the distribution and functional roles mediated by the α_1-adrenoceptor subtypes. However, the limited number of potential therapeutic applications seems to reduce this possibility.

Existence of other α_1-adrenoceptor subtypes?

Besides the existence of α_{1A}- α_{1B}- and α_{1D}-adrenoceptor subtypes (see above), several lines of evidence suggest that the current classification of α_1-adrenoceptors cannot explain all the α_1-adrenoceptor-mediated actions in a satisfactory manner. Firstly, as reviewed by Docherty[33], confusing evidence exists suggesting the existence of peripheral prejunctional α_1-adrenoceptors, mediating both inhibition (prostaglandines

and adenyl purines) and facilitation of the release of several substances (acethylcholine and noradrenaline). In agreement with the latter, Széll *et al.* have recently shown that activation of prejunctional α_{1A}-adrenoceptors facilitates acetylcholine release and enhances neurogenic contraction in the rat urinary bladder[89] However, these results need to be validated in other species[21]. Secondly, Muramatsu *et al.*[90] have shown that some blood vessels and other tissues with smooth muscle (e.g. prostate) from different species may posses additional α_1-adrenoceptor subtype(s), namely, the so-called ***a_{1L}-adrenoceptors***. This apparent different subclassification of α_1-adrenoceptors is based on the affinity of prazosin, showing that α_1-adrenoceptors can be subdivided into three subtypes: (*i*) the α_{1H}-adrenoceptor having high affinity ($K_B < 1$ nM) for prazosin (i.e. α_{1A}, α_{1B} and α_{1D}); (*ii*) the α_{1L}-adrenoceptor having lower affinity prazosin ($K_B > 2$ nM) and yohimbine ($K_B > 300$ nM); and (*iii*) the α_{1N}-adrenoceptor that shows a comparable affinity for prazosin as displayed at α_{1L}-adrenoceptors, but shows higher affinity for yohimbine ($K_B < 100$ nM). Furthermore, the α_{1L}- and α_{1N}-adrenoceptors can also be distinguished by their affinity for HV 723, which shows higher affinity for α_{1N}-($K_B = 0.4$-1 nM) than for α_{1L}-adrenoceptors ($K_B = 2$-7 nM). However, to date, any putative additional subtype that shows low affinity for prazosin has resisted identification by biochemical or molecular techniques[42]. Notwithstanding, the lack of identification does not automatically imply the absence of these receptor subtype(s) in certain tissues, keeping in mind that they could represent a therapeutic target for selective drug action[91].

Table 1.3. Summary of α_2-adrenoceptor subtypes characteristics (modified from Docherty[33], Bylund[34] and Alexander & Peters[19]).

	a_{2A} / a_{2D}	a_{2B}	a_{2C}
Previous name(s)	α_2-C10, RG20	α_2-C2, RNG	α_2-C4
Functional response(s)	prejunctional inhibition in most adrenergic nerves, central hypotensive action in several species	pressor responses in mutated mouse, pressor responses in pithed rat	contraction of human saphenous vein, inhibition of neurotransmitter release, inhibition of adenylate cyclase in different cell lines
Ligand binding assay	human platelet (α_{2A}), rat submandibular gland (α_{2D})	neonatal rat lung, rat kidney	different cell lines
Non-selective agonists	clonidine, BHT933, UK 14304, BHT920	clonidine, BHT933, BHT920	clonidine, BHT933, BHT920
Selective agonists	oxymetazoline (partial agonist), moxonidine?	none	none
Non-selective antagonists	rauwolscine	rauwolscine	rauwolscine
Selective antagonists	BRL44408, BRL48962	ARC239, imiloxan	MK912
Potency order	adrenaline > noradrenaline	adrenaline > noradrenaline	adrenaline > noradrenaline
Receptor distribution	platelet, brain, spinal cord, adipocyte cells, aorta, kidney, brain, spleen (human and rat)	liver, spleen, heart (human), kidney, neonatal rat lung (rat), thalamus, olfactory tubercle, septum, cerebellum (rat)	brain human and rat), heart, lung, aorta, kidney, brain (human)
Tissue function(s)	involved in hypotension, sedation, analgesia, anaesthesia	vasoconstriction	not established
Second messenger system(s)	activation of $G_{i/o}$, inhibition of adenylate cyclase, decrease in cAMP levels, inhibition of voltage-gated Ca^{2+} channels, activation of Ca^{2+} dependent K^+ channels (except for α_{2B}-adrenoceptors)		
Notes	α_{2A}- (human, dog, pig and rabbit) and α_{2D}-adrenoceptors (rat, mouse and cow) are species orthologues	ARC239 also displays moderate affinity for α_{2C}-adrenoceptors, prazosin display moderate affinity	

Abbreviation: cAMP, cyclic Adenosine Mono Phosphate.

Heterogeneity of α_2-adrenoceptors

The first suggestion that α_2-adrenoceptors could be subdivided was based on the findings that prazosin, previously considered as a selective α_1-adrenoceptor antagonist, produced potent inhibition of[^{3}H]rauwolscine binding in certain tissues and cell lines[48]. Initially, the prazosin-insensitive α_2-adrenoceptor (in human platelets) was designated as the α_{2A}-adrenoceptor subtype, while the prazosin-sensitive α_2-adrenoceptor (in neonatal rat lungs) was referred to as the α_{2B}-adrenoceptor subtype. Correlating the affinity constants of several α_2-adrenoceptor antagonists with their inhibitory potency against α_2-adrenoceptor binding, two additional α_2-adrenoceptor subtypes (α_{2C} and α_{2D}) were identified[26]. The α_{2C}-adrenoceptor was initially identified in OK cells, an opposum kidney cell line[92] and, subsequently, in native opossum kidney[93]. Currently, it is known that combining ligand binding and molecular cloning studies four distinct subtypes of α_2-adrenoceptors have been identified, namely the α_{2A}-, α_{2B}-, α_{2C}- and α_{2D}-subtype[17, 94]. Nevertheless, the α_{2D}-adrenoceptor subtype (found in the rat, mouse and cow) is currently believed to be a species orthologue of the human α_{2A}-adrenoceptor subtype (found in human, dog, rabbit and pig) and, therefore, cannot be recognised as an additional receptor subtype[31, 95, 96]. Table 1.3 shows a summary of the current α_2-adrenoceptor pharmacological characteristics.

Signal transduction mechanisms of α_2-adrenoceptors

α_2-Adrenoceptors are a part of the large family of G-protein coupled receptors and mediate their functions though a variety of G-proteins, including $G_{i/o}$[85]. In agreement with this, several studies have shown that α_2-adrenoceptor activation of these pathways was pertussis toxin sensitive. These findings suggest that GTP-binding proteins of the G_i/G_o subfamily are responsible for eliciting these effects[85]. In contrast, other studies have shown that pertussis toxin did not block α_2-adrenoceptor-mediated inhibition of noradrenaline release from cardiac sympathetic neurones in pithed rats[97] or isolated mouse atria[98]. After activation of the receptor and G-protein coupling, it has long been known that (pre- and postsynaptically) α_2-adrenoceptors are negatively coupled to adenylate cyclase,

attenuating cAMP production in target cells[33, 85]. Regarding *presynaptic* α_2-adrenoceptors, several lines of evidence suggest that opening of K^+-channels and inhibition of Ca^{2+}-channels are the two modes of action to produce presynaptic inhibition[95]. Additional signalling pathways have also been identified for presynaptic α_2-adrenoceptors, including accelerating Na^+/H^+-exchange, activation of phospholipase A_2, phospholipase D and phospholipase C[85]; however, their exact involvement in the signal transduction pathways upon presynaptic α_2-adrenoceptor (subtype) stimulation needs to be validated. In vascular smooth muscle, vasoconstrictor α_2-adrenoceptors seem to be positively linked to Ca^{2+}-channels, stimulating intracellular calcium influx upon stimulation[33]. This suggestion has been based on several *in vitro* studies showing that the α_2-adrenoceptor-mediated contractions are reduced by `Ca^{2+}-channel antagonists' and are nearly abolished in Ca^{2+}-free medium[85]. Other studies, however, show that α_2-adrenoceptor agonists enhance contractions to α_1-adrenoceptor agonists, an effect that is not mediated by enhancement of inositol triphosphate accumulation, but it may be mediated by inhibition of forskolin-stimulated cAMP production[see 33, 99].

The above mentioned studies emphasise the uncertainty regarding the signalling mechanisms that are involved in the physiological responses to stimulation of specific α_2-adrenoceptor subtypes in a given tissue. With the development of selective ligands, particularly antagonists, at these α_2-adrenoceptor subtypes (e.g. see Table 1.2), this matter will hopefully be elucidated in the near future[33].

Location and function of α_2-adrenoceptors

As mentioned before, α_2-adrenoceptors are located both pre- and postsynaptically where they mediate an inhibitory (e.g. for adenylate cyclase) and an excitatory (e.g. an increase in Ca^{2+} concentration) role in both the central and peripheral nervous systems[95]. As reviewed by Docherty[33] and Hieble[20], prejunctional α_2-adrenoceptors (most likely the α_{2A}- and/or α_{2C}-subtypes, depending on species) are located on most adrenergic nerves and primarily mediate prejunctional inhibition. On the other hand, postjunctional α_2-adrenoceptors are located on vascular smooth muscle and activation results in vasoconstriction[100].

Functional presynaptic α_2-adrenoceptors in rat submandibular gland, rat *vas deferens*, rat cerebral cortex, pithed rat heart and mouse atria resemble the α_{2D}-adrenoceptor subtype, whereas those in rabbit and human cerebral cortex, dog mesenteric artery and human saphenous vein resemble the α_{2A}-adrenoceptor subtype[see 33]. In contrast, other studies have shown that (postsynaptic) α_{2C}-adrenoceptors mediate contraction of the human isolated saphenous vein[101, 102]. Since both presynaptic α_{2A}- and α_{2C}-adrenoceptors are targets for the neural release of noradrenaline (at least when the noradrenaline-transporter is blocked[103]), it would be very interesting to elucidate their individual pharmacological profiles (e.g. binding properties to known compounds), signal transduction pathways, second messengers and their potential differences in function[103]. A valuable tool in dissecting the role of these receptors in the regulation of a variety of physiological responses, for example blood pressure[100], is by means of using genetically engineered mice deficient in each of the α_2-adrenoceptor subtypes[104-106]. The use of these knock-out mice confirmed the earlier findings that α_{2A}-adrenoceptors, which appear to be the major subtype in brain areas involved in cardiovascular regulation[107], play a critical role in regulating sympathetic outflow[106]. In agreement with this hypothesis, it has been shown that α_{2A}-adrenoceptor mutants or knockout mice, which display higher blood pressure, heart rate and plasma noradrenaline levels compared with their wild type counterparts, were unable to exhibit hypotension in response to α_2-adrenoceptor agonists[106]. The distribution of α_{2B}-adrenoceptors is restricted only to limited areas of the central nervous system, namely the thalamus and the *nucleus tractus solitarii* area of the brain stem[107], but is abundant in the vascular smooth muscle cells of the arterial wall and mostly responsible for a peripheral vasoconstrictor action[108-110]. In agreement with the latter, recent findings demonstrated that α_{2B}-adrenoceptor-deficient anephric mice were unable to raise their blood pressure in response to an acute hypertonic saline stimulus[100, 106]. These data are in agreement with studies using pithed rat preparations, where the α_{2B} is the main subtype mediating hypertension[20, 28, 111, 112]. On the contrary, Duka[113] reported that vasoconstriction mediated by direct activation of vascular α_2-adrenoceptors in mice is attributable to the postsynaptic

α_{2A}-adrenoceptor subtype, which is consistent with other findings that *m*RNA for the α_{2A}-, but not α_{2B}-, adrenoceptor could be detected on the arterial wall of rabbits[114]. In fact, the pressor responses attributed to α_{2B}-adrenoceptor stimulation, as reported by different investigators[108-110], could in fact be attributable to the involvement of central, rather than peripheral, α_{2B}-adrenoceptors[113]. As mentioned before, the human isolated saphenous vein is a preparation in which predominantly postsynaptic α_{2C}-adrenoceptors mediate contraction, whereas the involvement of α_1-adrenoceptors is limited (if any)[101]. No haemodynamic responses mediated by α_{2C}-adrenoceptors are known so far[20, 33, 106, 109]. As for other isolated tissues, α_2-adrenoceptors contribute to a predominantly α_1-adrenoceptor-mediated response[100, 115, 116], such as the rat cremaster arterioles and venules, rat tail vasculature, rabbit *corpus cavernosum*, guinea-pig *cauda epididymis* and mouse *vas deferens*[33]. In any case, since the involvement of specific α_2-adrenoceptor subtypes in any given functional response could be species dependent (as mentioned above), further investigation will be required to validate this matter in humans.

The development of potent and more selective α_2-adrenoceptor subtype-selective antagonists (see next section) will be very useful in the pharmacological characterisation of specific subtypes in certain vascular beds, especially considering potential clinical implications.

Selective agonists and antagonists at α_2-adrenoceptor subtypes

Although selective compounds are now being developed to differentiate between α_2-adrenoceptor subtypes in radioligand binding assays, there is currently no ligand available that is highly selective in functional studies (for affinity constants, see Table 1.2). Therefore, pharmacological characterisation is based on the affinity of a range of compounds that exhibit different affinities for the subtypes compared to their activity in functional studies. Attempts have been made to develop selective postsynaptic α_2-adrenoceptor antagonists (e.g. SKF 104078)[117]. However, these attempts failed because these antagonists also blocked presynaptic α_2-adrenoceptors in rat *vas deferens*, human saphenous vein, pithed heart and guinea-pig atrium, in the same dose-range as it blocked the postsynaptic α_2-adrenoceptors[see 33]. In contrast to the α_1-adrenoceptor, a few α_2-adrenoceptor subtype-selective antagonists have

been identified to date (see Table 1.2.). Initially, prazosin had been used to distinguish α_{2B}- and α_{2A}-adrenoceptors; however, other antagonists have recently been developed showing higher selectivity for α_{2B}- over the α_{2A}-subtype (e.g. ARC 239 or BRL 41992). BRL44408 is a potent and selective $\alpha_{2A/D}$-adrenoceptor antagonist, both in radioligand binding and functional studies[101, 118, 119], whereas moxonidine[120] and oxymetazoline (which also shows intrinsic activity at α_{1A}-adrenoceptors) display agonist properties at these receptors[121]. Most of the currently available α_{2B}-adrenoceptor antagonists (e.g. prazosin, ARC 239, SKF 104856 or spiroxatrine) also block α_1-adrenoceptors, the only current known exception being imiloxan[122]. The pharmacological differences between α_{2B}- and α_{2C}-adrenoceptors are very subtle, with currently no available antagonist showing clear selectivity. Examples of moderate selective $\alpha_{2B/C}$-adrenoceptor antagonists are MK912, ARC 239 and rauwolscine[26, 33]. Although these ligands can be used in binding studies to determine the presence of a certain α_2-adrenoceptor subtype in a particular tissue, one should be cautious when using these ligands for the characterisation of the above receptors in *in vivo* studies.

Therapeutic applications of drugs interacting with a adrenoceptors

Whereas both noradrenaline and adrenaline are sometimes used for therapeutic purposes (e.g. anaphylactic shock or cardiac arrest), most of the clinical used sympathetic ligands (agonists and antagonists) are structural analogues, have a variety of advantages (e.g. higher oral bioavailability, prolonged duration of action and more subtype-selectivity) and are used in the treatment of a variety of ailments, such as hypertension, asthma, benign prostate hypertrophy and hyperplasia and anaphylactic reactions[see 26, 85]. Detailed information about the α_1- and α_2-adrenoceptor subtypes by which these drugs are clinically efficacious could be very beneficial, especially when the potential side effects are taken into account.

α_1-Adrenoceptors have been one of the most widely studied families of receptors because of its major physiological importance in the control of blood pressure, blood flow, digestion, airways, reproduction, pupil diameter, endocrine and metabolic processes and in behaviour[33]. For several decades, it is known that non-selective α_1-adrenoceptor antagonists, such as prazosin, terazosin, doxazosin or trimazosin, are

efficacious in the treatment of hypertension[44, 85, 123, 124] and benign prostatic hyperplasia[125-128]. However, due to the frequent occurrence of side effects using these drugs (e.g. hypotension), there is a clear need for more α_1-adrenoceptor subtype-selective agents to treat these and other disorders[128-130]. As for the treatment of benign prostatic hyperplasia (BPH), the relatively selective α_{1A}-adrenoceptor antagonist, tamsulosin, which causes only moderate blood pressure changes, proved to be a better option than the non-selective α_1-adrenoceptor antagonists[131]. Recently, Pulito *et al.* demonstrated the uroselectivity of a novel α_{1A}-adrenoceptor subtype-selective antagonist, RWJ-38063; this compound was a more effective agent in the treatment of BPH symptoms with minimal effects on the vasculature and presents a more positive side effect profile than other α_1-adrenoceptor antagonists currently available[79]. In this context, further studies are required to ascertain the mechanisms (involvement of α_{1L}-adrenoceptors?) involved in the functional uroselectivity (over e.g. blood pressure) of α_1-adrenoceptor antagonists[128-130]. In other words, when it is generally agreed that the α_{1A}- is the main subtype of α_1-adrenoceptors in the sympathetic control of e.g. blood pressure[44], which receptors mediate the reported uroselectivity of the aforementioned selective α_{1A}-adrenoceptor antagonists? Similarly, several reports from both *in vitro* and *in vivo* studies suggest that activation of α_1-adrenoceptors, particularly α_{1A}-adrenoceptors by NS-49, increases urethral tone without causing an associated vasopressor response[84, 132]. Interestingly, investigating potential differences in pharmacological profiles of the four recently cloned splice variants of the human α_{1A}-adrenoceptor[39, 40] or the cloning and further characteristation of the putative α_{1L}-adrenoceptors[90], could be very helpful to clarify this matter[41]. Other therapeutic applications of drugs which interact with peripheral α_1-adrenoceptors are urinary incontinence (agonists[72, 133]), anal fissures (antagonists[134]), erectile dysfunction (antagonists[see 135, 136, 137]), nasal congestion (agonists), as adjunct to local anaesthesia (agonists)[see 127, 138]. Interestingly, α_1-adrenoceptors have recently been implicated in cardiac hypertrophy (antagonists[see 20]). Potential clinical applications of central α_1-adrenoceptors are with regard to sexual activity and

erectile dysfunction, as reviewed by Giuliano & Rampin in 1999[139]. In this context, the general idea is that the brain noradrenergic transmission facilitates sexual activity. In different animal models, it has been shown that activation of central α_1-adrenoceptors or blockade of central α_2-adrenoceptors (see below) increases sexual motivation and facilitates copulation[see 139]. However, direct evidence for central control of penile erection by the noradrenergic transmission is not yet available. Besides the link with penile erection, central α_1-adrenoceptors may also play a role in the modulation of attention and memory formation[see 45, 140].

For several decades, it has been known that α_2-adrenoceptor agonists, such as moxonidine, clonidine, guanabenz, guanfacine and α-methyldopa, are effective in the treatment of hypertension[see 141]. The underlying mechanisms of these drugs is assumed by inducing peripheral sympathoinhibition and a reduction in elevated blood pressure as a result of the stimulation of central α_{2A}-adrenoceptors[141, 142]. Besides acting at α_{2A}-adrenoceptors, several of these compounds have been shown to display affinity at imidazoline I_1-binding sites, located in the rostroventrolateral part of the brainstem[see 143]. The argument supporting that hypotension is induced *via* a selective action at imidazoline I_1-binding sites, presumes that compounds such as rilmenidine and moxonidine act preferentially through this site[64, 144, 145]. However, based on several lines of evidence, the involvement of imidazoline I_1-sites in controlling systemic blood pressure is questionable and remains unclear[see 20, 120, 146, 147, 148]. As reviewed by Piascik *et al.*[85], other therapeutic applications of drugs interacting with α_2-adrenoceptors are for example as adjunct to general anaesthesia (agonists[149]), hypertension (antagonists[20]), depression (antagonists[150]), obesity (antagonists[see 20]), schizophrenia (antagonists[151]), attention deficit disorder (agonists[152]), sedation/analgesia (antagonists[153]), peripheral nociceptive responses (agonists[154, 155] or in sexual disorders such as impotence (antagonists[138, 139, 156]). In addition, whereas prostatic tone is mainly mediated by α_{1A}-adrenoceptors, as described above, *in vitro* studies have shown that α_2-adrenoceptors are also present in this tissue[157]. This latter finding, which implies that presynaptic α_2-adrenoceptor blockade elicits prostatic obstruction *via*

α_1-adrenoceptors[126], could open new possibilities for the therapeutic use of α_2-adrenoceptor agonists in the treatment of urinary dysfunction.

Possible role of α-adrenoceptors in the treatment of migraine

Migraine is a syndrome that affects a substantial fraction of the world's population, with a higher prevalence in women (15-18%) than in men (6%[158]). According to the diagnostic criteria proposed by the Headache Classification of the International Headache Society[159], a migraine attack can be divided into distinct phases (predromitory phase, aura phase, headache phase, resolution and recovery phase) and is characterised by an intense, pulsating and throbbing headache, nausea, vomiting and photo- and/or phonopobia. For obvious reasons, the pathophysiology of migraine has been a matter of great interest for many researchers during several decades. Whereas limited information is available concerning the underlying mechanisms for the initiation of a migraine attack, the subsequent events leading to the aura or headache phase can be explained by a neurovascular hypothesis[see 160, 161, 162]. In this context, several experimental animal models have been developed to explain the efficacy of acutely acting antimigraine drugs, as reviewed by De Vries *et al.*[162]. The predictive antimigraine value of these models are based on: (*i*) the involvement of *the trigeminovascular system* (i.e. inhibition of plasma protein extravasation[163, 164] or central trigeminal inhibition[see 165]); (*ii*) *vasoconstriction of the cranial extracerebral vascular beds* (e.g. carotid vasculature or isolated blood vessels[161, 166, 167, 168]); or (*iii*) a combination of both hypotheses. It may be pointed out that the present project (described in this thesis) is exclusively based on vasoconstriction in the carotid vasculature of anaesthetised dogs and pigs.

Among others, Wolff[169] proposed an important role of vasodilatation of extracranial (extracerebral) arteries that are supplied by the (external) carotid artery (e.g. temporal artery) in migraine headache. Recognising several clinical signs accompanying a migraine attack (facial pallor, increased pulsations in the temporal artery and a clearly swollen frontal vein on the side of the headache), Heyck[170] investigated the underlying mechanisms in healthy volunteers and migraineurs. Subsequently, measuring the oxygen saturation difference between arterial (femoral artery) and venous (external jugular vein) blood ($AVSO_2$ difference) before, during

and after a migraine attack, Heyck (1969) reported a decreased $AVSO_2$ difference during the headache attack. Interestingly, this decrease in $AVSO_2$ difference was normalised after the attack had abated (either spontaneously or after successful treatment). Based on these rather preliminary studies, Heyck (1969) proposed that vasodilatation during the migraine headache phase primarily involves carotid arteriovenous anastomoses (see Figure 1.2.), but not arterioles[see also 171].

Arteriovenous anastomoses are large precapillary communications between arteries and veins and are present in most structures, including the cheeks, lips, forehead, nose, ears, nasal mucosa, eyes and dura mater of several species, including humans and pigs[161]. In conscious pigs, it has been shown that carotid arteriovenous anastomoses are under a sympathetic constrictor tone[172, 173], only shunting a small fraction (< 3%) of the total carotid blood flow[174]. Whether this is also the case in humans is unknown, but opening of carotid arteriovenous anastomoses, leading to shunting of a large amount of arterial blood directly into the venous circulation, may explain the facial pallor, lowering of facial temperature and increased vascular pulsations. Interestingly, it was already shown by Wolff[175] that the reported increased pulsations (an apparent *index* of quantitatively increased blood flow) in the temporal artery in migraineurs were absent after treatment with ergotamine. The relatively high usage of oxygen from the blood circulating in the fronto-temporal region suggests that the increased blood flow may serve other purposes, possibly protective, thermal control[170]. And indeed, arteriovenous anastomoses play an important role in the thermoregulation as established peripherally in the hind limbs of sheep[176]. Admittedly, the subsequent events leading to headache (and associated symptoms) are not completely understood. Nevertheless, it could be argued that increased vascular pulsations activate *stretch receptors*, increasing the activity of neuropeptide-containing (e.g. CGRP) perivascular sensory nerve endings; this may, ultimately, cause pain and other associated symptoms[see 161, 162, 167].

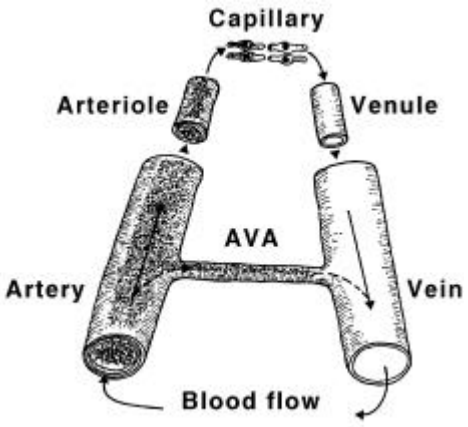

Figure 1.2.
Schematic representation of an arteriovenous anastomosis[161].

In line with the proposal that carotid arteriovenous anastomoses are dilated and play a role in the pathogenesis of migraine (see above), it is reasonable to believe that α-adrenoceptors could be involved in the vascular tone of the carotid circulation, which may provide a potential avenue for the development of new antimigraine drugs. It has been well established that several acutely acting antimigraine agents, including the ergots (ergotamine and dihydroergotamine) and the triptans (sumatriptan and the *second generation antimigraine drugs*), produce potent vasoconstriction in the canine and porcine carotid vasculature, a response mediated by 5-$HT_{1B/1D}$ receptors[177-179]. Interestingly, the canine carotid vasoconstrictor responses of the ergot alkaloids are mediated by 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors[177, 180]. On the other hand, the vasoconstriction of porcine carotid arteriovenous anastomoses by sumatriptan is mediated exclusively by 5-HT_{1B} receptors[178]; notwithstanding, that produced by the ergots involves, in addition to 5-$HT_{1B/1D}$ receptors, unidentified receptors/mechanisms[181]. The above lines of evidence, combined with the high affinity of ergotamine and dihydroergotamine at α-adrenoceptors[182], lead us to suggest that their therapeutic efficacy[see 183] may be partly explained by an action mediated *via* α-adrenoceptors. However, the possible involvement of α-adrenoceptors in the porcine carotid vasculature has been hampered, mainly on the basis that porcine carotid arteriovenous anastomoses were described to be insensitive to sympathetic nerve stimulation or intracarotid infusions of noradrenaline[184]. This discrepancy is striking since in conscious pigs arteriovenous anastomoses are under a vasoconstrictor sympathetic tone, which involves α_1-adrenoceptors[174]. In fact, sympathetic nerve stimulation as well as exogenously administered noradrenaline causes α-adrenoceptor-mediated vasoconstriction of arteriovenous anastomoses in the hind limb of several species[172, 185-188]. Finally, several *in vitro* experiments have shown that stimulation of α-adrenoceptors results in contraction of the isolated carotid artery of several species, including the dog[189, 190], rabbit[191] and pig[192].

Aims of this thesis

1. To investigate whether α_1- and α_2-adrenoceptors are operative *in vivo* mediating porcine (arteriovenous anastomotic) and canine (external) carotid vasoconstriction.

2. Based on the previous point, to identify, by pharmacological means, the specific subtypes of α_1- (α_{1A}, α_{1B} or α_{1D}) and α_2-(α_{2A}, α_{2B} or α_{2C}) adrenoceptors mediating (selective) carotid vasoconstriction in these *in vivo* experimental models.

3. To analyse the porcine and/or canine carotid vascular effects of some known antimigraine drugs (e.g. isometheptene) and to investigate whether specific α_1- and/or α_2-adrenoceptor subtypes mediate these potential carotid vasoconstrictor responses.

Chapter 2

Pharmacological evidence that a_1- and a_2-adrenoceptors mediate porcine vasoconstriction of carotid arteriovenous anastomoses

Summary Vasoconstriction of carotid arteriovenous anastomoses may be involved in the therapeutic action of acutely acting antimigraine agents, including the triptans and ergot alkaloids. While 5-$HT_{1B/1D}$ receptors mediate the effect of triptans, ergotamine and dihydroergotamine also interact with α-adrenoceptors. In the present study, we investigated the potential role of α_1- and α_2-adrenoceptors in mediating vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs. Ten minute intracarotid infusions of phenylephrine (1, 3 and 10 $\mu g\,kg^{-1}\,min^{-1}$) or BHT933 (3, 10 and 30 $\mu g\,kg^{-1}\,min^{-1}$) produced dose-dependent decreases in total carotid and arteriovenous anastomotic conductances; no changes were observed in the capillary fraction. The carotid vascular effects of phenylephrine and BHT933 were selectively abolished by prazosin (100 $\mu g\,kg^{-1}$, i.v.) and rauwolscine (300 $\mu g\,kg^{-1}$, i.v.), respectively. The responses to phenylephrine and BHT933 were not affected by the selective 5-$HT_{1B/1D}$ receptor antagonist GR127935 (500 $\mu g\,kg^{-1}$, i.v.). These results show that both α_1- and α_2-adrenoceptors can mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs. Since vasoconstrictor activity in this *in vivo* model is predictive of antimigraine activity, an agonist activity at particularly the α_2-adrenoceptor subtypes, in view of their less ubiquitous nature, could provide migraine abortive potential. Thus, the present results may aid further understanding of the mode of action of some current antimigraine agents and may eventually be helpful in the development of future treatment in migraine.

Based on: Willems *et al., Br. J. Pharmacol. (1999)* ***127****, 1263-1271.*

Introduction

Vasodilatation of carotid arteriovenous anastomoses may be involved in the pathophysiology of migraine headache[161, 170]. In line with this proposal, it has previously been shown that several acutely acting antimigraine agents, including the ergots (ergotamine and dihydroergotamine) and triptans, potently vasoconstrict porcine carotid arteriovenous anastomoses predominantly *via* 5-$HT_{1B/1D}$ receptors[166, 181]. Interestingly, the carotid vasoconstrictor effect of ergot alkaloids involves, in addition to 5-$HT_{1B/1D}$ receptors, unidentified receptor mechanisms[166, 181]. Since the ergot derivatives display high affinity at α-adrenoceptors[182], their therapeutic efficacy may be partly explained by an action mediated *via* these receptors. Indeed, we recently reported that canine external carotid vasoconstriction by ergot alkaloids also involves α-adrenoceptors[177]. In this context, the possible involvement of α-adrenoceptors in the porcine carotid vasculature has been hampered, mainly on the basis that porcine carotid arteriovenous anastomoses were described to be insensitive to sympathetic nerve stimulation or intracarotid infusions of noradrenaline[184]. This discrepancy is striking since in conscious pigs arteriovenous anastomoses are under a vasoconstrictor sympathetic tone[172-174]. In fact, sympathetic nerve stimulation as well as exogenously administered noradrenaline causes α-adrenoceptor-mediated vasoconstriction of arteriovenous anastomoses in the hind limb of several species[185-188].

In the light of the above, the present study was designed to investigate the potential role of α_1- and α_2-adrenoceptors in the vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs. For this purpose, we made use of the selective agonists, phenylephrine (α_1) and BHT933 (6-ethyl-5,6,7,8-tetrahydro-4H-oxazolo[4,5-d]azepin-2-amine dihydrochloride; α_2), and the selective antagonists, prazosin (α_1), rauwolscine (α_2)[85, 193], in a well-defined *in vivo* model predictive of antimigraine activity[85, 161, 162, 193]. In addition, GR127935 (N-[methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide was used to exclude the possible role of 5-$HT_{1B/1D}$ receptors[194-196]. The results obtained may provide useful information concerning the mode of action of some currently used antimigraine agents and, possibly, new avenues for the development of antimigraine agents.

Methods

General

After an overnight fast, 40 domestic pigs (Yorkshire x Landrace; 10-14 kg) were anaesthetised with azaperone (120 mg, i.m.), midazolam hydrochloride (5 mg, i.m.) and sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (20 $mg\,kg^{-1}\,h^{-1}$). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy (see below), leads to an increase in heart rate and vasodilatation of arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbital-anaesthetised pigs (70-80% of carotid blood flow) than in those under fentanyl/thiopental anaesthesia (~19% of carotid blood flow[174]. A high basal carotid arteriovenous anastomotic flow is particularly useful for investigating the effects of drugs that vasoconstrict arteriovenous anastomoses.

A catheter was placed in the inferior vena cava *via* the left femoral vein for infusion of antagonists and sodium pentobarbital. Another catheter was placed in the aortic arch *via* the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, the right common carotid artery and the external jugular vein were dissected free and the accompanying vagosympathetic trunks were cut between two ligatures in order to prevent a possible influence *via* baroreceptor reflexes on agonist-induced carotid vascular responses. The right external jugular vein was catheterised for withdrawal of venous blood samples for determining blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Two hub-less needles, each connected to a polyethylene tube, were inserted into the right common carotid artery and used for agonist (phenylephrine or BHT933) infusion and

radioactive microspheres injection, respectively. The microspheres were injected against the direction of blood flow for uniform mixing.

Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Arterial blood pressure, heart rate and right common carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept around 37 °C and the animal was continuously infused with saline to compensate for fluid loss.

Distribution of carotid blood flow

The distribution of common carotid blood flow was determined with 15.5±0.1 μm (s.d.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbital and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 10 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120-167, KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed programs[197]. The fraction of carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the total common carotid blood flow at the time of the injection of the microspheres labelled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping *via* carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the common carotid blood flow[198]. Vascular conductance

(10^{-2} ml min^{-1} $mmHg^{-1}$) was calculated by dividing blood flow (ml min^{-1}) by blood pressure (mmHg), multiplied by hundred.

Experimental protocol

After a stabilisation period of about 60 min, baseline values of heart rate, mean arterial blood pressure, common carotid blood flow and its distribution, as well as arterial and jugular venous blood gases were measured. Thereafter, the animals were divided into four groups receiving i.v. infusions (0.5 ml min^{-1} for 10 min) of either distilled water (vehicle; n=14), prazosin (100 µg kg^{-1}; n=10), rauwolscine (300 µg kg^{-1}; n=10) or GR127935 (500 µg kg^{-1}; n=6). After a waiting period of 15 min, all variables were reassessed. Subsequently, each group was divided into two subgroups receiving 10-min intracarotid infusions (0.1 ml min^{-1}) of phenylephrine (cumulative doses: 1, 3, and 10 µg kg^{-1} min^{-1}) or BHT933 (cumulative doses: 3, 10 and 30 µg kg^{-1} min^{-1}). The number of animals in the different groups receiving phenylephrine and BHT933 infusions were: vehicle (n=7 each), prazosin (n=7 and 3, respectively), rauwolscine (n=3 and 7, respectively) and GR127935 (n=3 each). All variables were collated again 10 min after the start of each agonist infusion.

It may be pointed out here that GR127935, which is a potent and selective 5-$HT_{1B/1D}$ receptor antagonist[194-196], was used to rule out the remote possibility that the effects of phenylephrine and/or BHT933 were mediated *via* 5-$HT_{1B/1D}$ receptors. The effectiveness of the blockade of 5-$HT_{1B/1D}$ receptors by GR127935 was confirmed by evaluating decreases in the total carotid blood flow and conductance by sumatriptan[196]. Sumatriptan (30, 100 and 300 µg kg^{-1}, i.v.; every 10 min over a period of 5 min each) was administered in animals treated with GR127935 after a near complete recovery from the effects of the last dose of phenylephrine or BHT933 had been achieved (~90 min).

Data presentation and statistical analysis

All data have been expressed as the mean±s.e.mean. The percent changes from baseline (i.e. after treatment with vehicle, prazosin, rauwolscine and GR127935) values caused by the different doses of phenylephrine or BHT933 within each group of animals were calculated. A two-way repeated measures ANOVA with Bonferroni's correction (SigmaStat 1.0, Jandel Corporation, Chicago, IL, USA) was

used to establish whether these changes were statistically significant ($P<0.05$, two-tailed) when compared with the baseline in each group as well as with the corresponding agonist dose in the vehicle-treated group.

Drugs

Apart from the anaesthetics azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and sodium pentobarbital (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: prazosin hydrochloride (Bufa Chemie b.v., Castricum; The Netherlands), GR127935, sumatriptan succinate (both from GlaxoWellcome, Ware, Herts, UK; courtesy: Dr. H.E. Connor), phenylephrine hydrochloride, BHT933 and rauwolscine dihydrochloride (all from Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands). Finally, heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of blood in the catheters. All drugs were dissolved in distilled water. For prazosin, rauwolscine and GR127935 a short period of heating was needed. The doses of the drugs refer to their respective salts.

Ethical approval

The local ethics committee dealing with the use of animals in scientific experiments approved the protocol.

Results

Systemic and carotid haemodynamic effects of vehicle, prazosin, rauwolscine and GR127935

As shown in Table 2.1, the administration of vehicle (5 ml of distilled water) did not produce any change in systemic or carotid haemodynamic variables. On the other hand, prazosin ($100\ \mu g\ kg^{-1}$, i.v.), rauwolscine ($300\ \mu g\ kg^{-1}$, i.v.) and GR127935 ($500\ \mu g\ kg^{-1}$, i.v.) decreased mean arterial blood pressure by 7±2%, 9±3% and 13±2%, respectively, which only in the case of rauwolscine was accompanied by a significant decrease in arteriovenous anastomotic blood flow by 18±5%. Additionally, rauwolscine increased capillary blood flow and conductance by 24±7% and 38±9%, respectively. These effects of rauwolscine were noticed in several tissues

(ear, skin, muscle, bones, salivary gland, fat and tongue; data not shown). No other significant changes were noticed with prazosin, rauwolscine or GR127935.

Table 2.1. Effects of vehicle, prazosin, rauwolscine or GR127935 on heart rate (HR; beats min^{-1}), mean arterial blood pressure (MABP; mmHg), $AVSO_2$ (%) and total carotid (Total), AVA and capillary (Cap) blood flow (BF; ml min^{-1}) and conductance (Con; 10^{-2} ml min^{-1} $mmHg^{-1}$) in anaesthetised pigs (n=40).

Variable	*Vehicle* (n=14)		*Prazosin* (100 µg kg^{-1}, n=10)		*Rauwolscine* (*300 mg kg^{-1}, n=10*)		*GR127935* (*500 mg kg^{-1}, n=6*)	
	Before	***After***	***Before***	***After***	***Before***	***After***	***Before***	***After***
HR	102±3	100±2	103±4	100±4	101±4	100±4	97±2	94±3
MABP	96±2	93±2	98±2	90±2[a]	91±2	82±3[a]	102±2	88±2[a]
$AVSO_2$	11±3	11±3	5±2	6±2	7±3	9±2	7±3	7±2
Total BF	117±8	116±7	135±6	125±6	124±12	109±10	125±20	108±14
Total Con	121±7	124±6	139±6	139±7	137±14	133±12	124±22	123±15
AVA BF	87±8	87±7	106±8	98±7	97±12	78±10[a]	97±17	77±13
AVA Con	90±7	93±7	109±8	109±8	107±12	94±11	96±17	87±14
Cap BF	30±2	29±2	28±4	27±3	26±3	32±2[a]	28±7	31±5
Cap Con	31±2	32±4	29±4	30±3	29±3	39±3[a]	28±8	35±5

All values have been presented as the mean±s.e.mean. a, $P<0.05$ vs. values before vehicle, prazosin, rauwolscine or GR127935.

Systemic haemodynamic changes by phenylephrine and BHT933

Systemic haemodynamic variables measured before and after the two agonists in animals treated with vehicle, prazosin, rauwolscine or GR127935 are shown in Table 2.1. In animals treated with vehicle, phenylephrine elicited an immediate and dose-dependent increase in heart rate by up to 29±6%. This increase was not affected by treatment with prazosin, rauwolscine or GR127935. BHT933 did not cause any change in heart rate. Moreover, no changes in mean arterial blood pressure were observed after infusions of phenylephrine or BHT933.

In vehicle-treated animals, both phenylephrine and BHT933 showed a trend to increase the difference between arterial and jugular venous oxygen saturation ($AVSO_2$) by up to 1036±325% and 254±140%, respectively (Table 2.2). However, statistical significance was achieved only with the highest dose of phenylephrine. The phenylephrine-induced response was selectively antagonised by treatment with prazosin.

Table 2.2. Changes in heart rate (beats min^{-1}), mean arterial blood pressure (mmHg) and $AVSO_2$ (%) induced by intracarotid phenylephrine or BHT933 in anaesthetised pigs treated i.v. with vehicle (5 ml), prazosin (100 μg kg^{-1}), rauwolscine (300 μg kg^{-1}) or GR127935 (500 μg kg^{-1}).

Treatment group	**Phenylephrine (μg kg^{-1} min^{-1})**				**BHT933 (μg kg^{-1} min^{-1})**			
	Baseline*	**1**	**3**	**10**	**Baseline***	**3**	**10**	**30**
Heart rate								
Vehicle	95±2	99±3	106±3	123±5[a]	105±4	104±4	105±4	104±4
Prazosin	98±5	99±5	103±5	115±6[a]	96±5	96±5	95±5	95±4
Rauwolscine	91±2	91±3	93±2	111±3[a]	102±6	101±6	101±6	99±5
GR127935	94±5	96±6	101±8	118±15[a]	96±3	95±3	92±4	93±3
Mean arterial blood pressure								
Vehicle	96±2	97±3	96±4	99±5	89±2	93±3	92±3	94±3
Prazosin	86±3	84±3	83±3	79±3	92±2	94±3	92±4	93±4
Rauwolscine	83±1	82±1	82±2	81±3	79±3	78±3	78±3	79±3
GR127935	86±3	83±1	82±1	84±1	90±1	89±1	85±3	83±5
$AVSO_2$								
Vehicle	6±2	7±2	13±3	39±6[a]	17±4	21±4	24±4	32±2
Prazosin	4±1	4±1	4±1	7±1[b]	11±4	20±8	25±6	29±3
Rauwolscine	5±1	6±3	8±2	22±8	12±3	12±2	14±2	14±3

All values have been presented as the mean±s.e.mean. *****, Values after administration of vehicle, prazosin, rauwolscine or GR127935. a, $P<0.05$ vs. baseline (ANOVA); b, $P<0.05$ vs. vehicle group (ANOVA). The number of animals receiving phenylephrine and BHT933 in the vehicle, prazosin, rauwolscine and GR127935 group were: phenylephrine (7, 7, 3 and 3, respectively); BHT933 (7, 3, 7 and 3, respectively).

Carotid haemodynamic responses to phenylephrine and BHT933

Absolute values of total carotid vascular conductance and its arteriovenous anastomotic and capillary fractions before and after infusions of phenylephrine and BHT933 in the four groups of animals treated with vehicle, prazosin, rauwolscine or GR127935 are shown in Figures 2.1 and 2.2, respectively.

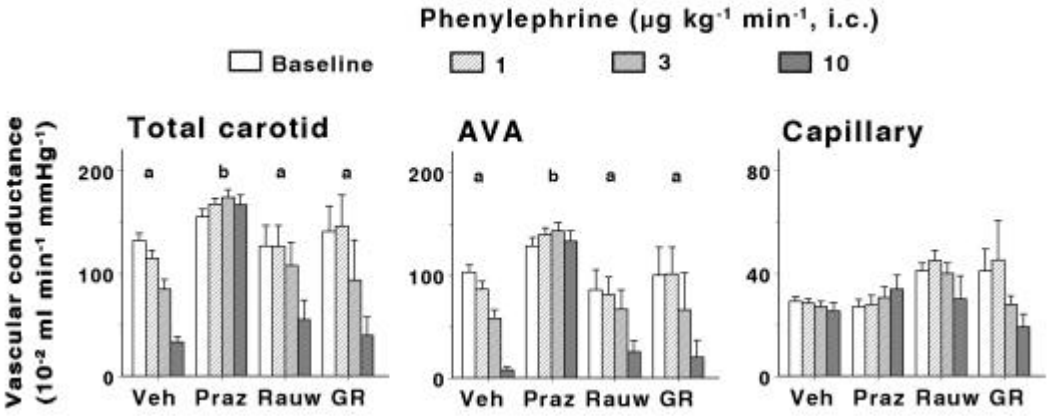

Figure 2.1. Effects of 10-min intracarotid infusions of phenylephrine on total carotid, arteriovenous anastomotic (AVA) and capillary conductances in anaesthetised pigs treated i.v. with vehicle (Veh; n=7), prazosin (Praz; 100 µg kg^{-1}; n=7), rauwolscine (Rauw; 300 µg kg^{-1}; n=3) or GR127935 (GR; 500 µg kg^{-1} n=3). All data are presented as mean±s.e.mean. a, $P<0.05$ vs. baseline (ANOVA); b, $P<0.05$ vs. vehicle group (ANOVA).

In vehicle-treated animals, both phenylephrine and BHT933 caused dose-dependent decreases in total carotid conductance (maximum change: 74±4% and 59±4%, respectively). These decreases were solely due to changes in arteriovenous anastomotic conductances, since capillary (nutrient) vascular conductance remained unchanged. Phenylephrine-induced changes were absent in animals treated with prazosin, but not in those treated with rauwolscine or GR127935 (Figure 2.1).

On the contrary, BHT933-induced effects were absent in animals treated with rauwolscine, but not in those treated with prazosin or GR127935 (Figure 2.2).

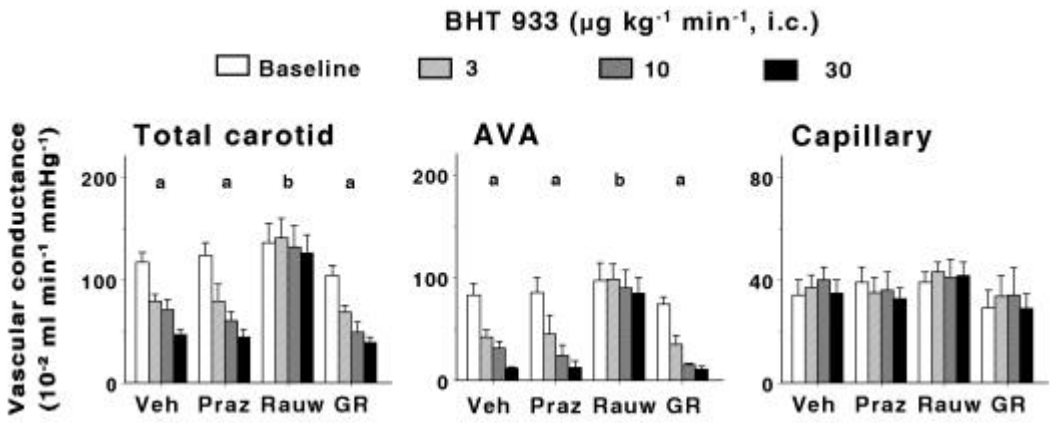

Figure 2.2. Effects of 10-min intracarotid infusions of BHT933 on total carotid, arteriovenous anastomotic (AVA) and capillary conductances in anaesthetised pigs treated i.v. with either vehicle (Veh), prazosin (Praz; 100 µg kg^{-1}), rauwolscine (Rauw; 300 µg kg^{-1}) or GR127935 (GR; 500 µg kg^{-1}). All data are presented as mean±s.e.mean. a, P<0.05 vs. baseline (ANOVA); b, P<0.05 vs. vehicle group (ANOVA).

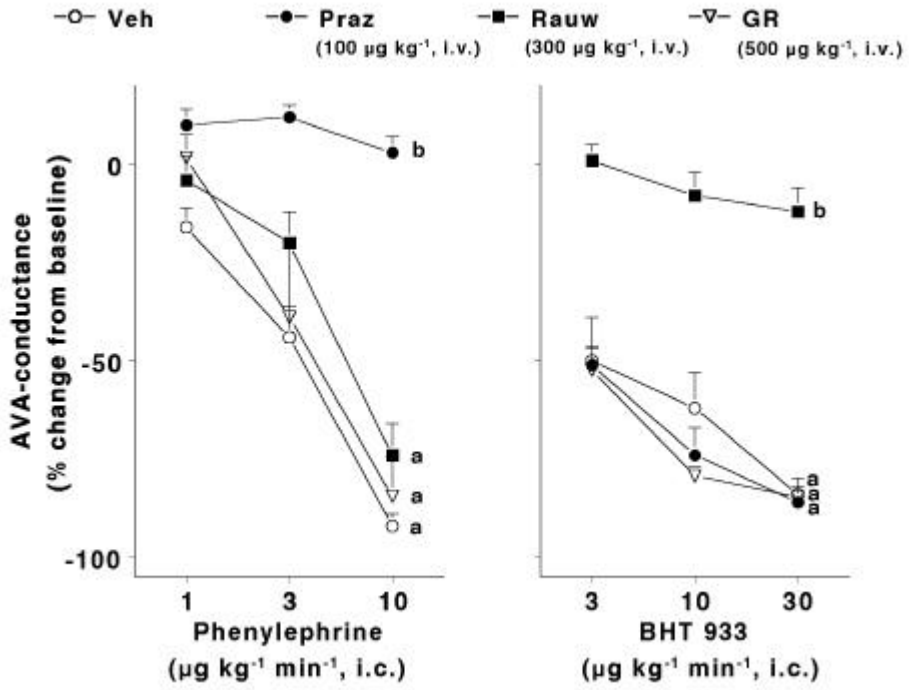

Figure 2.3. Percent changes in arteriovenous anastomotic (AVA) conductance induced by 10-min intracarotid infusions of phenylephrine or BHT933 in anaesthetised pigs treated i.v. with vehicle (Veh), prazosin (Praz; 100 µg kg^{-1}), rauwolscine (Rauw; 300 µg kg^{-1}) or GR127935 (GR; 500 µg kg^{-1}). All data are presented as mean±s.e.mean. a, P<0.05 vs. baseline (ANOVA); b, P<0.05 vs. vehicle group (ANOVA).

In Figure 2.3 percent changes (from baseline) observed with phenylephrine and BHT933 in carotid arteriovenous anastomotic conductance have been compared in the four groups of animals. It is clearly shown that prazosin and rauwolscine selectively

blocked the decreases in carotid arteriovenous anastomotic conductance induced by, respectively, phenylephrine and BHT933. The responses to the two agonists were not affected in animals treated with the potent and selective 5-$HT_{1B/1D}$ receptor antagonist GR127935.

Figure 2.4 shows the effects of phenylephrine and BHT933 on tissue vascular conductances in animals treated with vehicle. Neither phenylephrine nor BHT933 induced any change in vascular conductance in the ear, skin, salivary gland, fat and tongue. On the other hand, phenylephrine decreased vascular conductance in bone, eye and dura mater. These phenylephrine-induced conductance decreases were absent in animals treated with prazosin (data not shown). In vehicle-treated animals (Figure 2.4), but not in rauwolscine-treated animals (data not shown), BHT933 increased muscle and brain vascular conductances. The responses to the two agonists were similar in GR127935-treated animals as in animals treated with vehicle (data not shown).

Since in the present experiments mean arterial blood pressure was not affected by intracarotid infusion phenylephrine or BHT933, the above described agonist-induced changes in vascular conductances were qualitatively and quantitatively similar to those observed in blood flow (data not shown).

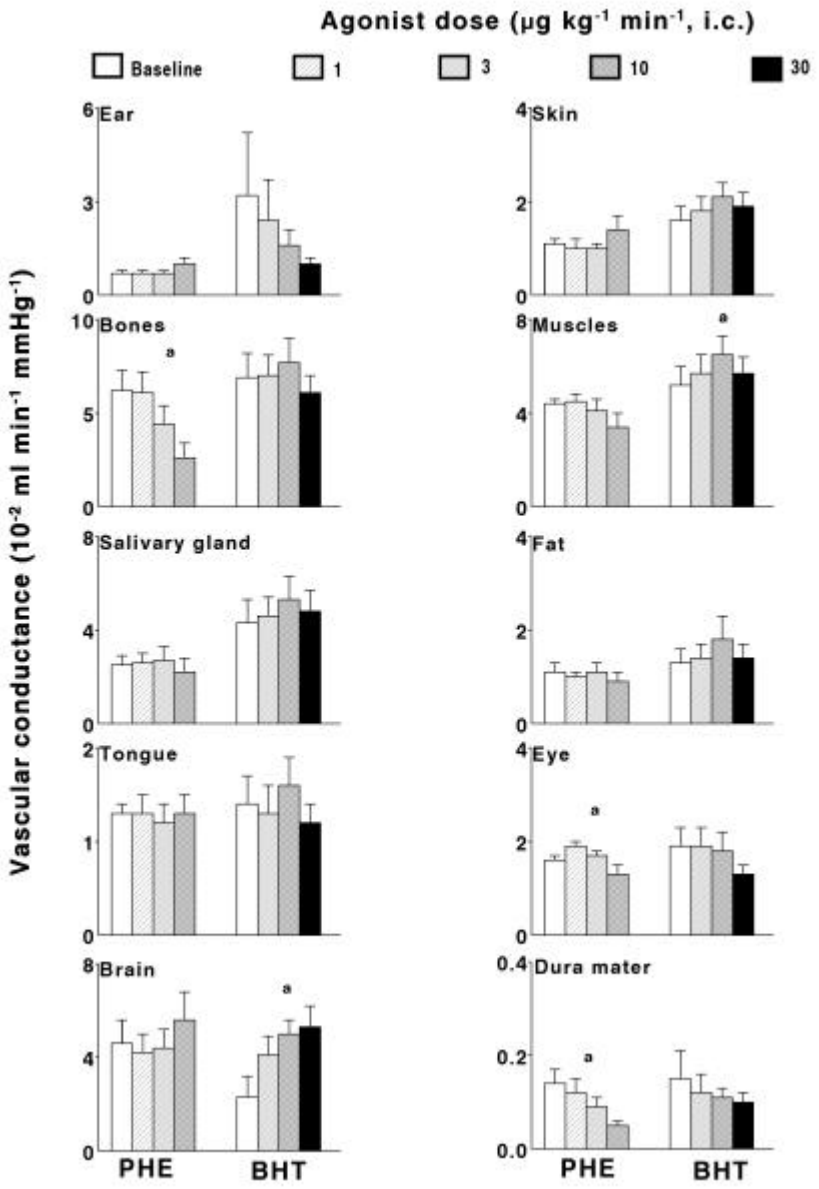

Figure 2.4. Effect of 10-min i.c. infusions of phenylephrine (PHE) or BHT933 (BHT) on the distribution of total carotid conductance in different ipsilateral cranial tissues in anaesthetised pigs treated with vehicle (n=7 each).
All data are presented as mean±s.e.mean. a, P<0.05 vs. baseline (ANOVA); b, P<0.05 vs. vehicle group (ANOVA).

Carotid vascular responses to sumatriptan in animals treated with GR127935

As reported earlier[196], sumatriptan (30, 100 and 300 µg kg^{-1}, i.v.) did not decrease either total carotid blood flow (% changes: -4±3, -5±3 and -6±3, respectively) or conductance (% changes: 8±4, 5±4 and 3±3, respectively) in animals treated with GR127935. In several previous publications from our laboratory[166], we have shown that these doses of sumatriptan consistently cause dose-dependent decreases in the total carotid conductance. For example, the changes in the carotid vascular conductance reported by De Vries *et al.*[196] with 30, 100 and 300 µg kg^{-1}, i.v. of sumatriptan in vehicle-treated pigs (n=5) were -22±4, -42±5 and -49±7, respectively.

Discussion

General

Several *in vitro* studies show that both α_1- and α_2-adrenoceptors mediate vascular effects in carotid vessels, including those of the dog[189] and pig[192]. Nevertheless, very few studies have investigated whether these receptors are operative in the carotid circulation *in vivo.* Verdouw *et al.*[184] reported that intracarotid *bolus injections* of noradrenaline elicited short-lasting and phentolamine-sensitive decreases in carotid and arteriovenous anastomotic conductance in pigs, whereas intracarotid *infusions* of noradrenaline were devoid of carotid vasoconstriction. Notwithstanding, the mechanisms involved in this response to noradrenaline were not further analysed, particularly in terms of different subtypes of α-adrenoceptors. Apart from the implications discussed below, the present study clearly shows that both α_1- and α_2-adrenoceptors can mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs.

Systemic and carotid haemodynamic effects of vehicle, prazosin and rauwolscine

Prazosin and rauwolscine produced moderate decreases in mean arterial blood pressure, most likely due to their blocking properties at, respectively, α_1- and α_2-adrenoceptors[199-201]. Importantly, no changes in carotid or arteriovenous anastomotic conductance were observed after treatment with prazosin or rauwolscine. This observation seems to contradict the results of Den Boer *et al.*[174], who reported that prazosin causes a potent vasodilatation of carotid arteriovenous anastomoses. However, in contrast to their study where fentanyl/thiopentane was employed as anaesthetic, we used sodium pentobarbital, which attenuates sympathetic tone and potently vasodilates carotid arteriovenous anastomoses (see also the Method section). Interestingly, rauwolscine, but not prazosin, increased capillary conductance, which was noticed in the ear, skin, muscle, bones, salivary gland, fat and tongue. Although we do not have a clear explanation, it may be that α_2-adrenoceptors mainly maintain vascular tone in these cranial tissues. Alternatively, rauwolscine may have activated 5-$HT_{1B/1D}$ receptors, at which the compound displays partial agonist property[202]. 5-$HT_{1B/1D}$ receptors can indeed mediate vasodilatation in porcine coronary arteries[203] and cranial tissues[196].

Systemic haemodynamic effects of phenylephrine and BHT 933

No changes in blood pressure or heart rate were observed after BHT 933. In contrast, phenylephrine produced a moderate increase in heart rate, which remained essentially unchanged in animals treated with prazosin, rauwolscine or GR127935. Since this tachycardia was absent after propranolol (500 $\mu g\,kg^{-1}$, i.v., n=3; unpublished observations), it would appear that phenylephrine has a direct action at cardiac β-adrenoceptors, as shown earlier[204].

Carotid haemodynamic changes: role of α_1- and α_2-adrenoceptors

Intracarotid infusions of phenylephrine and BHT 933 dose-dependently decreased total carotid conductance, which in both cases was exclusively caused by vasoconstriction of carotid arteriovenous anastomoses. Consistent with the closure of arteriovenous anastomoses[171], both phenylephrine and BHT 933 showed a trend towards increasing A-VSO_2; this was however only significant after phenylephrine. The activity of the above agonists implies, but does not categorically prove, that α_1- and α_2-adrenoceptors are involved.

The involvement of α_1-adrenoceptors in the vasoconstriction of carotid arteriovenous anastomoses is strengthened by the finding that prazosin completely blocked the effects of phenylephrine. It should be noted that in the dose employed prazosin acted selectively at α_1-adrenoceptors, since the drug did not modify BHT 933-induced carotid vasoconstriction. α_1-Adrenoceptors also mediate vasoconstriction within the canine external carotid vascular bed[200] and seem to maintain vascular tone in porcine carotid arteriovenous anastomoses[174]. On similar grounds, the fact that rauwolscine completely antagonised BHT 933-induced responses indicates that α_2-adrenoceptors also mediate carotid arteriovenous anastomotic vasoconstriction. This conclusion is substantiated by previous results demonstrating that clonidine vasoconstricts porcine carotid arteriovenous anastomoses[184] and that α_2-adrenoceptors partly mediate canine external carotid vasoconstriction by ergotamine and dihydroergotamine[177]. Furthermore, prazosin selectively attenuated the phenylephrine-induced increases in A-VSO_2.

Taken together, the results of the present study clearly show that activation of both α_1- and α_2-adrenoceptors results in a vasoconstriction of carotid arteriovenous anastomoses. Interestingly, our results seem in contradiction with earlier experiments in pigs, where intracarotid infusions of noradrenaline did not induce carotid vasoconstriction, but rather increased total carotid and arteriovenous anastomotic blood flow[184]. The suggestion that the absence of noradrenaline-induced vasoconstrictor effect was due to the putative α_1-blocking properties of the anaesthetic azaperone[174] seems not to be the case, as we used similar anaesthetic regimen as Verdouw and colleagues[184]. A more likely explanation could be that noradrenaline, when continuously and slowly infused, also induces a β-adrenoceptor-mediated vasodilator effect in the carotid circulation, which negates carotid vasoconstriction. Interestingly, Cohen & Coffman[205] have provided evidence for the presence of β-adrenergic vasodilator mechanism in human digital arteriovenous shunts.

Possible interactions of phenylephrine and BHT 933 with 5-HT$_{1B/1D}$ receptors

As pointed out earlier, 5-HT$_{1B/1D}$ receptors mediate the potent and selective vasoconstriction of carotid arteriovenous anastomoses caused by acutely acting antimigraine drugs in the pig[161, 162]. One could therefore argue that a part of the phenylephrine- and/or BHT 933-induced carotid vasoconstriction might also involve 5-HT$_{1B/1D}$ receptors. This possibility can however be discounted as treatment of the animals with the potent and selective 5-HT$_{1B/1D}$ receptor antagonist GR127935[194, 195, 206] did not modify carotid vascular haemodynamic changes induced by either phenylephrine or BHT 933. Importantly, the dose of GR127935 used in this study (500 μg kg^{-1}, i.v.) completely blocks sumatriptan-induced decreases in total carotid (and arteriovenous anastomotic) blood flow and conductance[196]. This was apparently also the case in our present experiments where no changes in total carotid blood flow and conductance were observed with sumatriptan in animals treated with GR127935. From these data we can exclude the possibility that 5-HT$_{1B/1D}$ receptors play a role in the phenylephrine- or BHT 933-induced vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs.

Effects of phenylephrine and BHT 933 on cranial tissue conductances

Although the effects of α-adrenoceptor stimulation have been extensively studied on isolated blood vessels, the use of intracarotid injection of radiolabelled microspheres allowed us to study these effects in the different cranial tissues *in vivo.* Stimulation of prazosin-sensitive α_1-adrenoceptors resulted in vasoconstriction in bones, dura mater and, to a lesser extent, in the eye, whereas BHT 933 was devoid of tissue vasoconstrictor effects. In fact, BHT 933 produced vasodilatation in brain vasculature, as also reported after clonidine [184]. This may be due to an interaction with endothelial vasodilator α_2-adrenoceptors as previously reported in other blood vessels [192, 207-209]. Indeed, the increase in brain vascular conductance was absent in the rauwolscine-treated group.

Possible clinical implications

Lastly, we would like to consider the possible clinical relevance of the vasoconstriction of the carotid arteriovenous anastomoses induced by phenylephrine and BHT 933. It has been suggested that vasodilatation of these 'shunt' vessels may play an important role in the pathophysiology of migraine [161, 170]. Indeed, to date all migraine abortive agents vasoconstrict carotid arteriovenous anastomoses in different animal species [161, 210]. While carotid arteriovenous anastomoses cannot be directly investigated in humans, sumatriptan has been shown to vasoconstrict arteriovenous anastomoses in the human forearm [211]. In addition, ergotamine, dihydroergotamine, clonidine and isometheptene (only dogs), all of which produce vasoconstriction in the carotid vascular bed in dogs and pigs [177, 181, 184, 212], interact with α-adrenoceptors. At least, a part of the therapeutic effect of these drugs may be related to their agonist action at these receptors. Thus, our results demonstrating the role of α_1- and α_2-adrenoceptor in mediating vasoconstriction of carotid arteriovenous anastomoses imply that selective agonists at, particularly, α_2-adrenoceptors (in view of the less ubiquitous nature of α_2-adrenoceptors compared to α_1-adrenoceptors) should have potential antimigraine properties. In this connection, further studies, which fall beyond the scope of the present investigation, will be required to ascertain the specific subtypes of α_1- (α_{1A}, α_{1B}, α_{1D}) and α_2- (α_{2A}, α_{2B}, α_{2C}) adrenoceptors mediating the vasoconstrictor response mentioned above.

Eventually, these results may be helpful in the development of antimigraine agents in the future.

Chapter 3

a_1-Adrenoceptor subtypes mediating vasoconstriction in the carotid circulation of anaesthetised pigs: possible avenues for antimigraine drug development

Summary It has recently been shown that the α-adrenoceptors mediating vasoconstriction of porcine carotid arteriovenous anastomoses resemble both α_1- and α_2-adrenoceptors, but no attempt was made to identify the specific subtypes involved. Therefore, the present study was designed to elucidate the specific subtype(s) of α_1-adrenoceptors involved in the above response, using the α_1-adrenoceptor agonist phenylephrine and α_1-adrenoceptor antagonists 5-methylurapidil (α_{1A}), L-765,314 (α_{1B}) and BMY 7378 (α_{1D}). Ten-min intracarotid infusions of phenylephrine (1, 3 and 10 $\mu g\,kg^{-1}.min^{-1}$) induced a dose-dependent decrease in total carotid and arteriovenous anastomotic conductance. These carotid vascular effects were potently attenuated by 5-methylurapidil ($1000\,\mu g\,kg^{-1}$; i.v.), abolished by L-765,314 ($1000\,\mu g\,kg^{-1}$; i.v.) and only slightly attenuated by BMY 7378 ($1000\,\mu g\,kg^{-1}$; i.v.). These results, coupled to the binding affinities of the above antagonists at the different α_1-adrenoceptors, suggest that both α_{1A}- and α_{1B}-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs. In view of less ubiquitous nature of α_{1B}- compared to α_{1A}-adrenoceptors, the development of potent and selective α_{1B}-adrenoceptor agonists may prove to be important for the treatment of migraine.

Based on: Willems *et al., Cephalalgia (2000)* ***submitted***.

Introduction

Although the pathophysiology of migraine has not yet been completely unravelled, there is little doubt that vasodilatation of large cephalic arteries and, possibly, arteriovenous anastomoses is involved in the headache phase of migraine[170, 213-215]. Indeed, over the years we have shown that two important groups of drugs that are highly effective in the acute treatment of migraine, i.e. the triptans and ergot alkaloids, potently constrict carotid arteriovenous anastomoses[161, 215, 216]. While the carotid vasoconstrictor effect of sumatriptan as well as some other triptans is mediated by the 5-HT_{1B} receptor[167, 178, 215], the ergot-induced vasoconstriction involves, in addition to 5-HT_{1B} receptors[217], also α-adrenoceptors[177].

There are several reasons to believe that α-adrenoceptors may regulate vascular tone of carotid arteriovenous anastomoses, providing a potential avenue for the development of new antimigraine drugs. It is well known that stimulation of α-adrenoceptors results in vasoconstriction of the isolated carotid artery[189-192, 218]. Administration of α-adrenoceptor antagonists to conscious pigs as well as pigs under thiopentone anaesthesia results in an increase in arteriovenous anastomotic blood flow[173, 174]. More recently, we have shown that both α_1- and α_2-adrenoceptors mediate the vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs[219].

The objective of the present study was to elucidate the subtype(s) of α_1-adrenoceptors –α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes[26, 33, 42, 95] – involved in the vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs. For this purpose, we investigated the effects of 5-methylurapidil, L-765,314 and BMY 7378, which are preferential antagonists, respectively, at α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (see Table 1.2 for affinity constants[60, 88, 220]), on the carotid vasoconstriction induced by the α_1-adrenoceptor agonist phenylephrine in a well-defined *in vivo* animal model predictive for antimigraine activity[161, 221].

Materials and methods

General

After an overnight fast, 41 domestic pigs (Yorkshire x Landrace; female; 10-14 kg) were anaesthetised with azaperone (120 mg, i.m.), midazolam hydrochloride (5 mg, i.m.) and sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (20 $mg\,kg^{-1}.h^{-1}$). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy (see below), leads to an increase in heart rate and vasodilatation of arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbital-anaesthetised pigs (70-80% of carotid blood flow; present results) than in conscious (<5% of carotid blood flow[173]) or fentanyl/thiopental anaesthetised (~19% of carotid blood flow[174]) pigs. A high basal carotid arteriovenous anastomotic blood flow is particularly useful for investigating the effects of drugs that constrict these 'shunt' vessels.

A catheter was placed in the inferior vena cava *via* the left femoral vein for the administration of sodium pentobarbital, vehicle (distilled water) or the antagonists. Another catheter was placed in the aortic arch *via* the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, bilateral vagosympathectomy was performed in order to prevent the possible influence *via* baroreceptor reflexes on phenylephrine-induced carotid vascular responses. Two hub-less needles, each connected to a polyethylene tube, used for the administration of radioactive microspheres and phenylephrine, respectively, were inserted into the right common carotid artery against the direction of blood flow for uniform mixing.

Total common carotid blood flow was measured with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow

601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Arterial blood pressure, heart rate and total carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept about 37 °C and the animal was continuously infused with physiological saline to compensate fluid losses.

The Ethical Committee of the Erasmus University Rotterdam, dealing with the use of animals in scientific experiments, approved the protocols followed in this investigation.

Distribution of carotid blood flow

The distribution of carotid blood flow was determined with 15.5±0.1 µm (s.d.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbital and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 10 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120-167 KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed computer programs[197]. The fraction of carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the carotid blood flow at the time of the injection of the microspheres, labelled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping *via* carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the total carotid blood flow[161]. Vascular conductance (ml min^{-1} mmHg^{-1}) was calculated by dividing blood flow (ml min^{-1}) by mean arterial blood pressure (mmHg).

Experimental protocol

After a stabilisation period of at least 60 min, baseline values of heart rate, mean arterial blood pressure, total carotid blood flow and its distribution into arteriovenous anastomotic and capillary fractions, as well as arterial blood gases were measured. Thereafter, the animals were divided into seven groups, receiving i.v. infusions (0.5 ml min^{-1} for 10 min) of either vehicle (distilled water; n=8), 5-methylurapidil (300 or 1000 µg kg^{-1}; n=6 each dose), L-765,314 (300 or 1000 µg kg^{-1}; n=6 and 3, respectively) or BMY 7378 (300 or 1000 µg kg^{-1}; n=6 each dose). After a waiting period of 15 min, all variables were reassessed. Subsequently, the animals received cumulative doses of phenylephrine (1, 3 and 10 µg $kg^{-1}.min^{-1}$) infused into the right common carotid artery (0.1 ml min^{-1} for 10 min). All variables were collated again 10 min after the start of each agonist infusion.

At least 90 min after the last microsphere injection, the animals received i.v. bolus injections of phenylephrine (3 and 10 µg kg^{-1}) and peak changes in mean arterial blood pressure were noted.

Data presentation and statistical analysis

All data have been expressed as means±s.e.m. In order to correct for potential baseline differences caused by the antagonists or vehicle, the percent changes induced by phenylephrine from the values after administration of the different antagonists or vehicle were calculated in each group. The significance of the percent changes induced by the different doses of phenylephrine within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations[222]. Percent changes caused by phenylephrine in the different treatment groups were compared to the percent changes caused by the corresponding phenylephrine dose in the vehicle-treated group using Student's unpaired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

Apart from the anaesthetics azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v.

Mijdrecht, The Netherlands) and sodium pentobarbital (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: L-phenylephrine hydrochloride, 5-methylurapidil, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro [4,5]decane-7,9-dione dihydrochloride) dihydrochloride (all from Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands) and L-765,314 (4-amino-2-[4-[1-(benzyloxycarbonyl)-2(S)-[[(1,1-dimethylethyl)amino] carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline hydrochloride; Merck & Co., Inc., West Point, PA 19486, USA). Finally, heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of blood in the catheters.

All drugs were dissolved in distilled water. A short period of heating was needed to dissolve 5-methylurapidil (acidified to pH=6.8-7.0 with 0.1 M HCl) and L-765,314. The doses of the drugs refer to their respective salts.

Results

Baseline values and effect of antagonists per se

Baseline values of haemodynamic variables in anaesthetised pigs (n=41) were: heart rate (100±2 beats.min^{-1}), mean arterial blood pressure (93±2 mmHg), total carotid blood flow (123±6 ml min^{-1}) and conductance (132±6 10^{-2} ml min^{-1} mmHg^{-1}), arteriovenous anastomotic blood flow (95±6 ml min^{-1}) and conductance (102±6 10^{-2} ml min^{-1} mmHg^{-1}) and capillary blood flow (28±2 ml min^{-1}) and conductance (30±2 10^{-2} ml min^{-1} mmHg^{-1}). There were no major differences between the baseline values in the different groups of animals.

No haemodynamic changes were observed with vehicle or BMY 7378 (data not shown). 5-Methylurapidil (1000 µg kg^{-1}) slightly decreased mean arterial blood pressure (-8±4%) and increased heart rate (9±1%) as well as capillary blood flow (35±4%) and conductance (49±9%). L-765,314 (1000 µg kg^{-1}) decreased mean arterial blood pressure (-9±4%) and increased capillary conductance (27±11%).

Systemic haemodynamic responses to intracarotid infusions of phenylephrine

As shown in Table 3.1, after treatment with vehicle intracarotid infusions of phenylephrine (1, 3 and 10 µg kg^{-1}.min^{-1}) caused a dose-dependent increase in heart rate by up to 28±5%, without affecting mean arterial blood pressure. This phenylephrine-induced tachycardia was slightly less (maximal increase: 11±3%) after

300 $\mu g\,kg^{-1}$ of 5-methylurapidil (probably due to higher initial value), but was not different after the highest dose of 5-methylurapidil (1000 $\mu g\,kg^{-1}$). In animals treated with either 1000 $\mu g\,kg^{-1}$ of L-765,314 or BMY7378, a small decrease in mean arterial blood pressure (9±1 or 8±4%, respectively) was observed with phenylephrine; however, when compared to the corresponding blood pressure change in vehicle-treated animals, statistical significance was not reached.

Carotid haemodynamic responses to intracarotid infusions of phenylephrine

Absolute values of total carotid, arteriovenous anastomotic and capillary vascular conductances in the different groups of animals before and after intracarotid infusions of phenylephrine are shown in Figure 3.1. In animals treated with vehicle, phenylephrine produced a dose-dependent decrease in total carotid conductance by up to 75±4%. Since phenylephrine did not change the vascular conductance in the capillary fraction, the decrease in total carotid conductance was exclusively caused by a decrease in the arteriovenous anastomotic fraction (maximal response: 92±3%).

Carotid haemodynamic responses to intracarotid infusions of phenylephrine

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Table 3.1. Changes in heart rate and mean arterial blood pressure induced by 10-min intracarotid infusions of phenylephrine in anaesthetised pigs treated i.v. with either vehicle, 5-methylurapidil, L-765,314 or BMY 7378.

Treatment group	Dose (µg kg^{-1})	Baseline*	Phenylephrine (mg kg^{-1} min^{-1})		
			1	3	10
Heart rate (beats.min^{-1})					
Vehicle	5 ml	95±2	98±2	105±3[a]	121±5[a]
5-Methylurapidil	300	112±5	111±6[b]	112±5[b]	123±4[ab]
	1000	101±3	103±4	106±4	117±3[a]
L-765,314	300	98±5	101±5	107±5[a]	129±7[a]
	1000	93±2	93±3	97±4	112±6[a]
BMY 7378	300	99±4	101±3	104±3	119±5[a]
	1000	109±6	112±6	117±6[a]	127±6[a]
Mean arterial blood pressure (mmHg)					
Vehicle	5 ml	97±2	97±3	96±3	99±5
5-Methylurapidil	300	80±5	82±5	79±6	83±6
	1000	95±5	89±5[b]	87±5	89±6
L-765,314	300	84±6	83±7	84±8	87±8
	1000	84±3	80±4[a]	78±2[a]	79±4[a]
BMY 7378	300	85±3	85±4	84±3	90±3
	1000	91±5	88±5	86±4	84±4[a]

*****, Values after treatment with respective antagonist or vehicle; a, $P<0.05$ vs. baseline; b, $P<0.05$ vs. response (% response from respective baseline) to the corresponding phenylephrine dose in animals treated with vehicle.

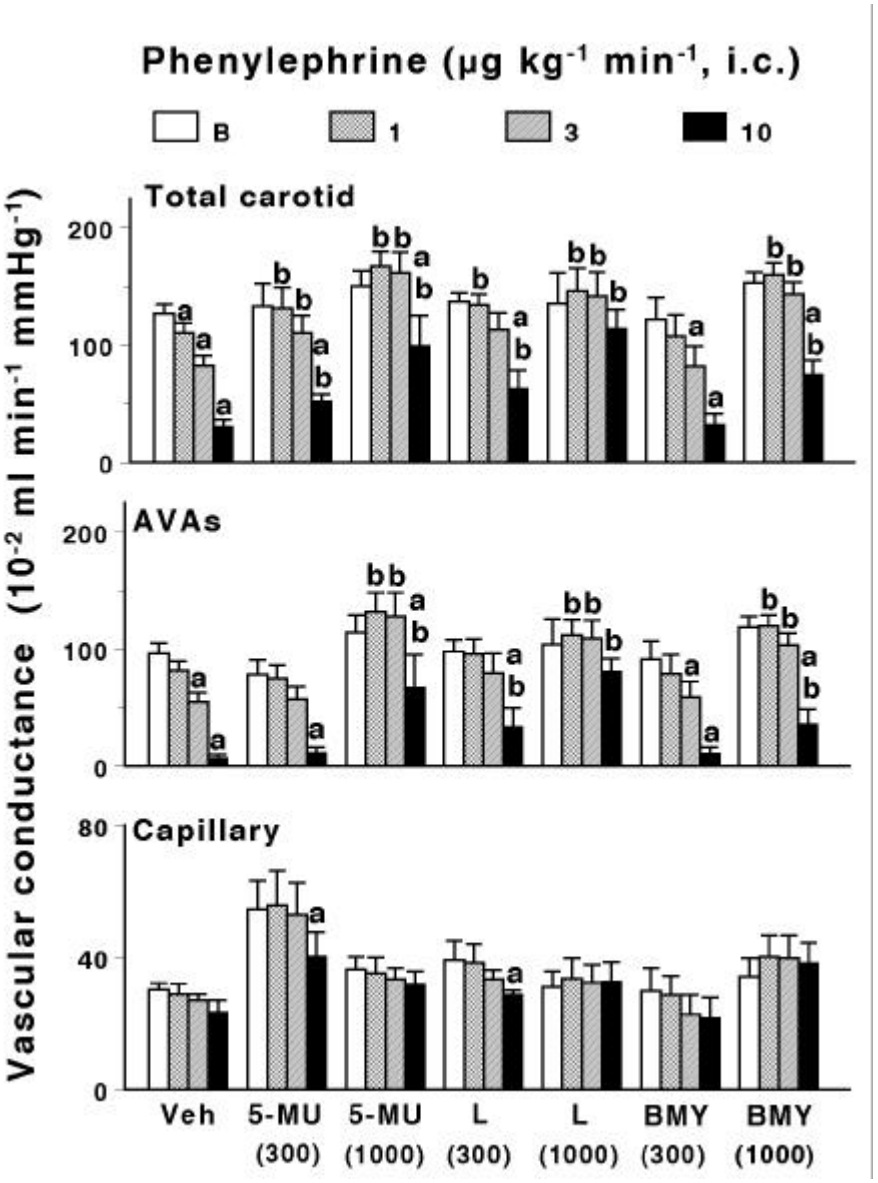


Figure 3.1. Effects of 10-min intracarotid infusions of phenylephrine on total carotid, arteriovenous anastomotic (AVA) and capillary vascular conductances in anaesthetised pigs treated i.v. with either vehicle (Veh), 5-methylurapidil (5-MU; 300 or 1000 µg kg^{-1}), L-765,314 (L; 300 or 1000 µg kg^{-1}) or BMY 7378 (BMY; 300 or 1000 µg kg^{-1}). a, $P<0.05$ vs. baseline (B; values after treatment); b, $P<0.05$ vs. response (% response from respective baseline) of the corresponding phenylephrine dose in vehicle-treated animals.

As shown in Figures 3.1 and 3.2, the constrictor effect of phenylephrine on carotid arteriovenous anastomoses was not affected by 300 µg kg^{-1} of either 5-methylurapidil or BMY 7378, but was significantly attenuated by 300 µg kg^{-1} of L-765,314 and 1000 µg kg^{-1} of 5-methylurapidil and BMY 7378. Furthermore, after the highest dose of L-765,314, the responses to phenylephrine were clearly abolished and the values did not significantly differ from those before phenylephrine infusion.

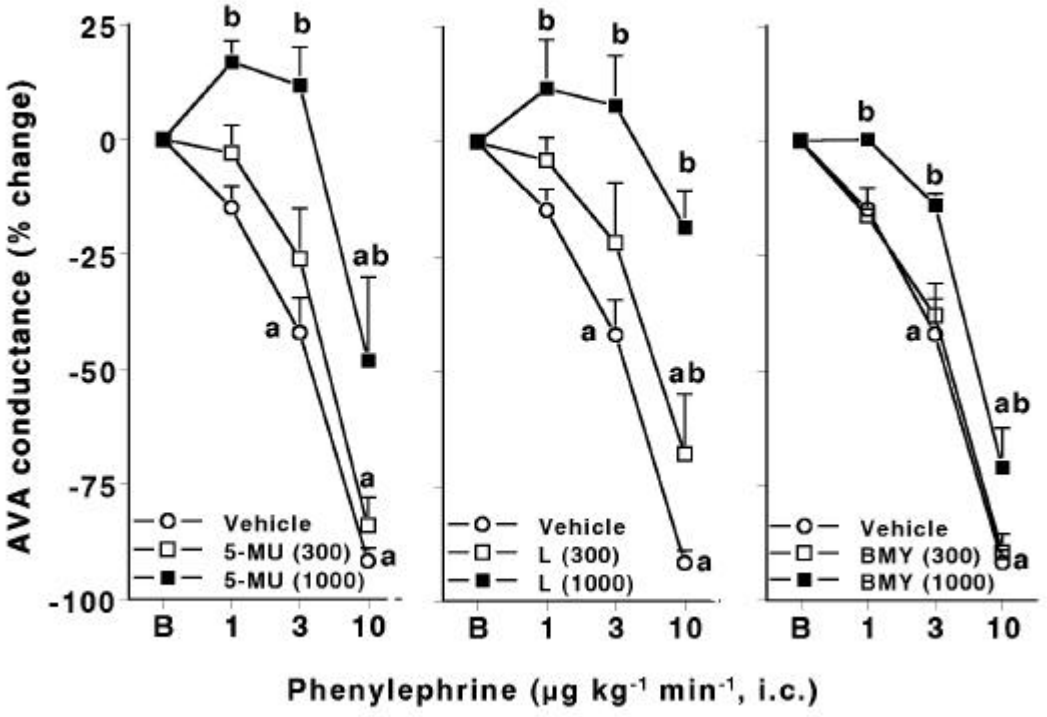


Figure 3.2. Percent changes in arteriovenous anastomotic (AVA) conductance induced by 10-min intracarotid infusions of phenylephrine in anaesthetised pigs treated i.v. with either vehicle (Veh), 5-methylurapidil (5-MU; 300 or 1000 µg kg^{-1}; *left graph*), L-765,314 (L; 300 or 1000 µg kg^{-1}; *middle graph*) or BMY 7378 (BMY; 300 or 1000 µg kg^{-1}; *right graph*). a, $P<0.05$ vs. baseline (B; values after treatments); b, $P<0.05$ vs. response (% response from respective baseline) of the corresponding phenylephrine dose in vehicle-treated animals. Note that the control curves are identical in each graph.

Since in the present experiments mean arterial blood pressure was not affected by the intracarotid infusions of phenylephrine, the responses in vascular conductances were qualitatively and quantitatively similar to those in blood flow (data not shown).

Changes in mean arterial blood pressure by i.v. bolus administration of phenylephrine

In vehicle-treated animals, bolus injections of phenylephrine (3 and 10 µg kg^{-1}, i.v) produced a dose-dependent increase in mean arterial blood pressure, yielding peak responses of 28±3 and 51±3%, respectively (Figure 3.3). These phenylephrine-induced vasopressor responses were significantly attenuated by 1000 µg kg^{-1} of 5-methylurapidil (peak responses: 17±3 and 31±4%, respectively), but were not affected by L-765,314 (300 or 1000 µg kg^{-1}), BMY 7378 (300 or 1000 µg kg^{-1}) or 300 µg kg^{-1} of 5-methylurapidil.

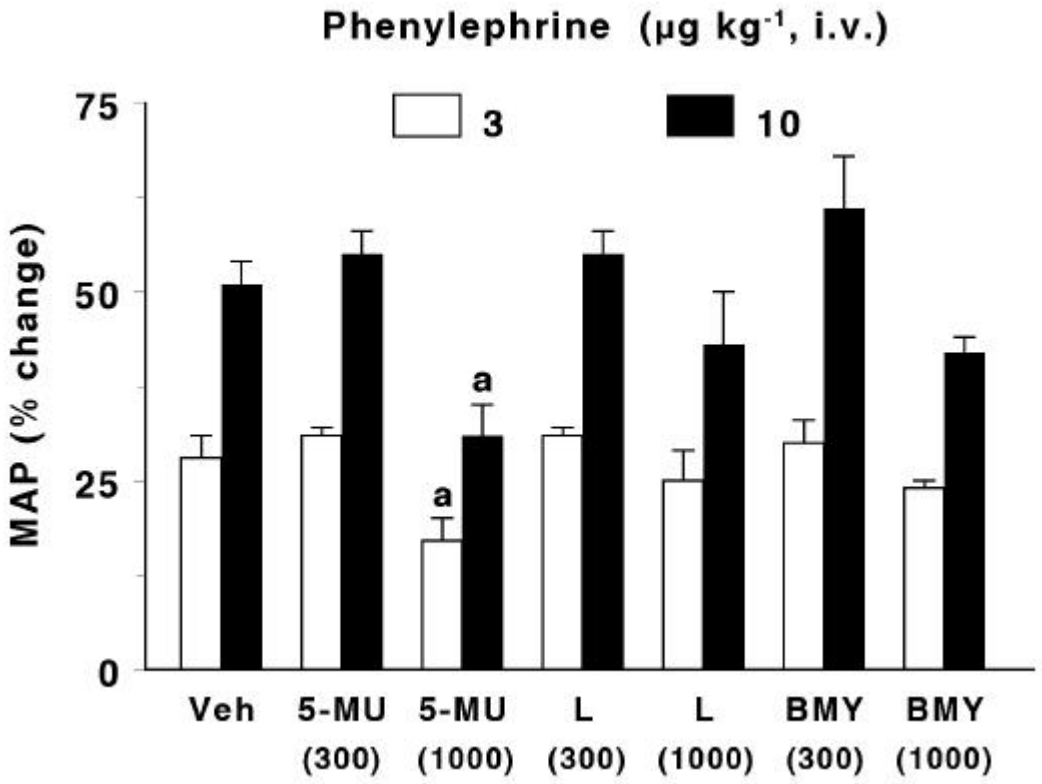


Figure 3.3. Peak changes in mean arterial blood pressure (MAP) induced by bolus injection of phenylephrine (3 or 10 µg kg^{-1}, i.v.) in animals treated i.v. with either vehicle (Veh), 5-methylurapidil (5-MU; 300 or 1000 µg kg^{-1}), L-765,314 (L; 300 or 1000 µg kg^{-1}) or BMY 7378 (BMY; 300 or 1000 µg kg^{-1}). a, $P<0.05$ vs. percent response of the corresponding phenylephrine dose in vehicle-treated animals.

Discussion

General

We have recently shown that both α_1- and α_2-adrenoceptors mediate vasoconstriction of arteriovenous anastomoses within the carotid vascular bed in anaesthetised pigs[219]. This conclusion was based on the findings that (*i*) intracarotid administration of phenylephrine (α_1-adrenoceptor agonist) and BHT933 (α_2-adrenoceptor agonist) decreased total carotid blood flow exclusively confined to the arteriovenous anastomotic fraction, without affecting mean arterial blood pressure; and (*ii*) these effects of phenylephrine and BHT933 were selectively antagonised by the α_1- and α_2-adrenoceptor antagonists, prazosin and rauwolscine, respectively[219]. This is also in agreement with findings previously reported in the external carotid vascular bed of anaesthetised dogs[223].

Based on radioligand binding, molecular biology and isolated tissue experiments, it is known that α_1-adrenoceptors are a heterogeneous group of receptors, currently subdivided into α_{1A}, α_{1B} and α_{1D} subtypes[26, 31]. As reviewed by Vargas and Gorman[44], the α_{1A}-adrenoceptor subtype, which is widely distributed throughout the body and is the major subtype regulating systemic vascular resistance and blood pressure; α_{1D}-adrenoceptors seem to play only a minor role in blood pressure regulation[224]. Whereas there is limited information concerning the vascular effects mediated by α_{1B}-adrenoceptors, it has been shown that vasoconstriction of the isolated carotid artery of the dog and rabbit mainly resembles the cloned α_{1B}-adrenoceptor[44]. However, no information is available on the subtype mediating vasoconstrictor effects within the carotid arterial bed *in vivo*. Therefore, the objective of the present study was to identify the α_1-adrenoceptor subtype(s) that mediate vasoconstriction in the carotid vasculature in anaesthetised pigs, with particular emphasis on the arteriovenous anastomotic fraction, which may be of relevance to migraine therapy[161, 162, 170, 216].

In recent years, some selective antagonists at α_1-adrenoceptor subtypes have been developed (Table 1.2). In the present study, we made use of 5-methylurapidil and BMY 7378, which have frequently been used to characterise α_{1A}- and α_{1D}-adrenoceptors, respectively (Table 1.2[26, 42, 60, 68-70, 225]). To block

α_{1B}-adrenoceptors, we employed L-765,314, which shows a moderate to high selectivity for cloned α_{1b}-adrenoceptors over cloned α_{1a}- or α_{1d}-adrenoceptors[88]. Many studies have used the clonidine derivative, chloroethylclonidine, for this purpose[26, 33]. However, it is now evident that chloroethylclonidine alkylates several other receptors as well[26, 85, 86].

Systemic and carotid haemodynamic effects of different antagonists

Whereas the vehicle was devoid of any systemic and carotid haemodynamic effects, administration of the α_{1A}-adrenoceptor antagonist 5-methylurapidil produced a small hypotension and tachycardia. Similar hypotensive effect was observed earlier with 5-methylurapidil[226] and may be related to either blockade of vascular smooth muscle α_{1A}-adrenoceptors, which play an important role in the maintenance of vascular tone[44] or to its agonist properties at central 5-HT_{1A} receptors[227]. Admittedly, we do not have a clear-cut explanation for the slight hypotension and increase in capillary conductance produced by L-765,314, since it has been shown by Piascik *et al.*[54] that α_{1B}-adrenoceptors play only a minor role in the contraction of peripheral blood vessels *in vitro*.

Changes in heart rate and mean arterial blood pressure by phenylephrine

Intracarotid infusions of phenylephrine produced only minor systemic haemodynamic responses in vehicle-treated animals. The tachycardia (Table 3.1), which was also observed with i.v. phenylephrine (data not shown), most likely involves an interaction with β-adrenoceptors[219]. Furthermore, i.v. administration of phenylephrine (3 and 10 $\mu g\,kg^{-1}$) induced a dose-dependent increase in blood pressure (Figure 3.3), which was antagonised by 100 $\mu g\,kg^{-1}$ of prazosin (100 $g\,kg^{-1}$), but not by 300 $\mu g\,kg^{-1}$ of rauwolscine (Willems *et al.*, unpublished observations). In view of the antagonism of this response by 5-methylurapidil, but not by L-765,314 (α_{1B}-adrenoceptor antagonist) or BMY 7378 (α_{1D}-adrenoceptor antagonist) (Figure 3.3), the pressor response to phenylephrine is likely to be mediated by α_{1A}-adrenoceptors. In keeping with the approximately 10-fold higher affinity at the cloned α_{1a}-adrenoceptor displayed by prazosin compared to 5-methylurapidil (Table 1.2), a 10-fold higher dose of 5-methylurapidil (1000 $\mu g\,kg^{-1}$) was needed to produce antagonism of the

phenylephrine-induced vasopressor response. Thus, as previously discussed[44], these results support the role of α_{1A}-adrenoceptors in the increase in peripheral vascular resistance and concomitant hypertensive effect upon activation.

Carotid haemodynamic responses to intracarotid infusions of phenylephrine

As previously reported[219], phenylephrine caused a pronounced and dose-dependent decrease in total carotid conductance, which was exclusively caused by vasoconstriction of carotid arteriovenous anastomoses; nutrient vascular conductance was not modified (Figure 3.1). These carotid vasoconstrictor responses were not affected by treatment with 300 $\mu g\,kg^{-1}$ of the α_{1D}-adrenoceptor antagonist BMY 7378. This dose of BMY 7378 should be sufficient to block α_{1D}-adrenoceptors in view of comparable affinities of prazosin and BMY 7378 at the cloned human α_{1d}-adrenoceptor (Table 1.2) and the fact that 100 $\mu g\,kg^{-1}$ of prazosin abolished this response[219]. On this basis, the involvement of α_{1D}-adrenoceptors in the cranial vasoconstriction induced by phenylephrine in anaesthetised pigs seems highly unlikely. Nevertheless, as shown in Figures 3.1 and 3.2, the higher dose of BMY 7378 (1000 $\mu g\,kg^{-1}$) produced a slight, but significant, attenuation in the phenylephrine-induced total carotid and arteriovenous anastomotic vasoconstriction. Since BMY7378 displays a moderate affinity at α_{1A}- and α_{1B}-adrenoceptors (pK_i: 6.6 and 7.2, respectively; Table 1.2), a non-selective blockade of these receptors (which can mediate carotid vasoconstriction; see below) is a likely explanation. Indeed, in 2 experiments a combination of 5-methylurapidil (1000 $\mu g\,kg^{-1}$) and BMY 7378 (1000 $\mu g\,kg^{-1}$) did not cause any more attenuation of the phenylephrine-induced arteriovenous vasoconstriction than 5-methylurapidil (1000 $\mu g\,kg^{-1}$) alone (see below).

The α_{1B}-adrenoceptor antagonist L-765,314 abolished the vasoconstriction of carotid arteriovenous anastomoses by phenylephrine, a finding that supports the role of α_{1B}-adrenoceptors in this effect. Interestingly, the α_{1A}-adrenoceptor antagonist, 5-methylurapidil was also able to antagonise the cranial vasoconstrictor effects of phenylephrine, but in contrast to L-765,314, the highest dose of phenylephrine still elicited a 50% decrease in arteriovenous anastomotic conductance (Figures 3.2 and 3.3). Thus, at similar doses, L-765,314 acted as a more potent antagonist when compared to 5-methylurapidil. The above findings lead us to conclude that both α_{1A}-

and α_{1B}-adrenoceptors mediate the phenylephrine-induced vasoconstriction of carotid arteriovenous anastomoses, whereas the α_{1D}-adrenoceptor plays a minor role, if any.

Admittedly, a critique of the above conclusion may be that, unlike *in vitro* studies, the exact concentration of α_1-adrenoceptor antagonists at the receptor site can be influenced by pharmacokinetic differences in such an *in vivo* investigation. However, current techniques do not allow us to study carotid arteriovenous anastomoses *in vitro* and, to some extent, we have tried to ensure effective antagonist concentration at the receptor site by using as high dose of antagonists as possible.

Possible clinical implications

Lastly, we would like to consider possible clinical implications of the present results showing that α_{1A}- and α_{1B}-adrenoceptors mediate the vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs. To date all acutely acting antimigraine agents, such as the triptans and ergot alkaloids, potently constrict carotid arteriovenous anastomoses[210, 216]. Moreover, vasodilatation of these 'shunt' vessels may be involved in the pathophysiology of the headache phase of migraine[161, 170, 216]. Since a vasoconstrictor effect in this experimental model seems to be highly predictive for antimigraine efficacy, an α_{1A}- (such as A61603[83]) or α_{1B}-adrenoceptor agonist (which is yet to be developed) should be able to abort migraine headaches. Of the two α_1-adrenoceptor subtypes, the α_{1B}-adrenoceptor is an interesting target for future antimigraine drugs, especially when considering that this receptor, unlike the α_{1A}-adrenoceptor, does not seem to be much involved in the vasoconstriction of the peripheral blood vessels[44, 54]. Indeed, our results suggest that the hypertensive effect produced by intravenous administration of phenylephrine is predominantly mediated *via* the α_{1A}-, but not α_{1B}-adrenoceptor (Figure 3.3). An α_{1B}-adrenoceptor agonist may have a major advantage over the currently available acute antimigraine drugs, which all constrict human isolated coronary artery[228], where, importantly, the α_{1B}-adrenoceptor is not present[50].

In conclusion, the present study shows that both α_{1A}- and α_{1B}-adrenoceptors mediate vasoconstriction of porcine carotid arteriovenous anastomoses produced by phenylephrine. Since the α_{1B}-adrenoceptor subtype is not much involved in vasoconstriction of the systemic vasculature, a cranioselective vasoconstriction may be achieved using selective α_{1B}-adrenoceptor agonists, which may prove effective in migraine.

Acknowledgements

We would like to thank Dr. M.A. Patane (Merck & Co. Inc., USA) for generously providing L-765,314.

Chapter 4

A61603-induced vasoconstriction in porcine carotid vasculature: possible involvement of novel receptor(s)

Abstract It has recently been shown that the pharmacological profile of α_1-adrenoceptors mediating vasoconstriction of porcine carotid arteriovenous anastomoses resembles that of α_{1A}- and α_{1B}-adrenoceptor subtypes. In an attempt to verify the involvement of α_{1A}-adrenoceptors, we used the potent α_{1A}-adrenoceptor agonist A61603 (N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydro-naphthalen-1-yl]methane sulphonamide) and found that intracarotid (i.c.) administration of A61603 (0.3-10 $\mu g\,kg^{-1}$) dose-dependently decreased porcine carotid blood flow and vascular conductance. This decrease was exclusively due to a vasoconstriction of carotid arteriovenous anastomoses; the capillary blood flow and conductance remained unchanged. Surprisingly, the responses to A61603 were little modified by prior i.v. treatment with 5-methylurapidil (1000 $\mu g\,kg^{-1}$), prazosin (100 $\mu g\,kg^{-1}$) or a combination of prazosin and rauwolscine (100 and 300 $\mu g\,kg^{-1}$, respectively). The 5-$HT_{1B/1D}$ receptor antagonist GR127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4' (5-methyl-1, 2,4-oxadiazol-3-yl) [1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate; 500 $\mu g\,kg^{-1}$) and ketanserin (500 $\mu g\,kg^{-1}$) also failed to modify carotid vascular responses to A61603, but, interestingly, methiothepin (3000 $\mu g\,kg^{-1}$) proved to be a potent antagonist. Taken together, the present results show that A61603 is a relatively poor agonist at the α_{1A}-adrenoceptor in anaesthetised pigs and that the carotid vasoconstriction produced by A61603 is mediated by novel, methiothepin-sensitive receptor(s).

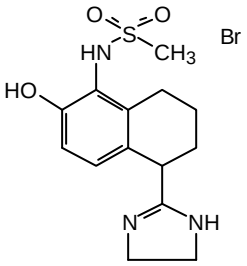
Based on: Willems *et al., Eur. J. Pharmacol. (2000)* ***Submitted***.

Introduction

There seems to be little doubt that the headache phase of migraine is associated with vasodilatation of cranial blood vessels. Indeed, sumatriptan as well as all 'second-generation' triptans potently constrict human isolated cranial arteries as well as carotid arteriovenous anastomoses in anaesthetised animals, mainly *via* the 5-HT_{1B} receptor[196, 215]. In an attempt to explore new avenues for the development of antimigraine agents, we recently reported that phenylephrine and BHT933 (6-ethyl-5,6,7,8-tetrahydro-4H-oxazolo [4,5-d] azepin-2-amine dihydrochloride) constrict carotid arteriovenous anastomoses in anaesthetised pigs *via* α_1- and α_2-adrenoceptors, respectively[219]. Subsequent studies suggest that the phenylephrine-induced response is mediated by the α_{1A}- and α_{1B}- adrenoceptor subtypes, but not by the α_{1D} subtype[229].

To confirm the involvement of α_{1A}-adrenoceptors, in the present study we studied the effects of a potent and selective α_{1A}-adrenoceptor agonist, A61603 (N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methane sulphonamide) (see Table 4.1[33, 83], on regional carotid blood flow in anaesthetised pigs.

Table 4.1. Chemical structure and pharmacological profile, i.e. binding affinity (pK_i), potency (pEC_{50}) and intrinsic activity (i.a.), of A61603 at several cloned human receptor subtypes*.

Chemical structure		**a_{1A}**	**a_{1B}**	**a_{1D}**	**a_{2A}**	**a_{2B}**	**a_{2C}**	**5-HT_{1B}**	**5-HT_{1D}**
	***pK_i***	7.1	4.8	4.9	7.3	6.5	6.2	5.2	5.6
	pEC_{50}	8.9	4.4	4.6	7.5	7.1	7.7	ND	ND
	i.a.	1.2	0.1	0.1	0.8	0.8	0.9	ND	ND

Data were taken from[72]; *, Affinity (pK_i value) of A61603 at other receptor subtypes ($H_{1/2}$, $D_{1/2/3}$, 5-$HT_{1/2/7}$ or β) was < 5.5; ND; not determined.

The response to A61603 was characterised by using selective α-adrenoceptor antagonists, 5-methylurapidil (α_{1A}), prazosin (α_1) and a combination of prazosin (α_1) and rauwolscine (α_2). Similarly, the effects of GR127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4' (5-methyl-1, 2,4-oxadiazol-3-yl) [1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate; 5-$HT_{1B/1D}$), ketanserin (5-HT_2, α_1) and methiothepin (5-$HT_{1/2}$), in doses sufficient to block their respective receptors[219, 230, 231], were also investigated. Surprisingly, the results suggest that A61603 constricts porcine arteriovenous anastomoses by a non-adrenergic mechanism.

Materials and Methods

General

After an overnight fast, 33 domestic pigs (Yorkshire x Landrace; female; 10-14 kg) were anaesthetised with azaperone (120 mg, i.m.), midazolam hydrochloride (5 mg, i.m.) and sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (20 mg $kg^{-1}.h^{-1}$). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy (see below), leads to an increase in heart rate and vasodilatation of carotid arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal carotid arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbital-anaesthetised pigs (70-80% of carotid blood flow; present results) than in conscious (<5% of carotid blood flow[173] or fentanyl/thiopental anaesthetised pigs(~19% of carotid blood flow[174]. A high basal carotid arteriovenous anastomotic blood flow is particularly useful for investigating the effects of drugs that constrict these 'shunt' vessels.

A catheter was placed in the inferior vena cava *via* the left femoral vein for infusion of vehicle (distilled water), the antagonists and sodium pentobarbital. Another catheter was placed in the aortic arch *via* the left femoral artery for the

measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, the right common carotid artery was dissected free and bilateral vagosympathectomy was performed in order to prevent a possible influence *via* baroreceptor reflexes on A61603-induced carotid vascular responses. Two hub-less needles, each connected to a polyethylene tube, used for the administration of radioactive microspheres and A61603, respectively, were inserted into the right common carotid artery against the direction of blood flow for uniform mixing.

Total common carotid blood flow was measured with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Arterial blood pressure, heart rate and carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept at about 37 °C and the animal was continuously infused with physiological saline to compensate fluid losses.

The Ethical Committee of the Erasmus University Medical Centre Rotterdam, dealing with the use of animals in scientific experiments, approved the protocols followed in this investigation.

Distribution of total common carotid blood flow

The distribution of total common carotid blood flow was determined with 15.5±0.1 µm (s.d.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbital and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in *via*ls. The radioactivity in these *via*ls was counted for 10 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120-167 KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and

^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed programs[197]. The fraction of right common carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the common carotid blood flow at the time of the injection of microspheres, labelled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping *via* carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the total common carotid blood flow[161]. Vascular conductance (10^{-2} ml min^{-1} mmHg^{-1}) was calculated by dividing blood flow (ml min^{-1}) by mean arterial blood pressure (mmHg) multiplied by hundred.

Experimental protocol

After a stabilisation period of at least 60 min, values of heart rate, mean arterial blood pressure, total common carotid blood flow, as well as arterial blood gases were measured. Thereafter, the animals (n=33) were divided into seven groups, receiving i.v. infusions (0.5 ml min^{-1} for 10 min) of either vehicle (distilled water; 5 ml, n=6), 5-methylurapidil (1000 µg kg^{-1}, n=6), prazosin (100 µg kg^{-1}, n=3), a combination of prazosin and rauwolscine (100 and 300 µg kg^{-1}, respectively, n=6), GR127935 (500 µg kg^{-1}, n=3), ketanserin (500 µg kg^{-1}, n=3) or methiothepin (3000 µg kg^{-1}, n=6). After 15 min, baseline values of heart rate, mean arterial blood pressure, arterial blood gases, total common carotid blood flow and its distribution into arteriovenous anastomotic and capillary fractions (injection of the first batch of microspheres) were measured. Subsequently, all animals received of A61603 (cumulative total doses: 0.3, 1, 3 and 10 µg kg^{-1} at the rate of 0.1 ml min^{-1} over 10 min infused into the right common carotid artery). Ten min after the start of each A61603 infusion, the animals received a different batch of microspheres and all variables were collated again.

After the carotid and systemic haemodynamic variables had been returned to baseline values, we analysed the systemic haemodynamic effects of i.v. bolus injections of A61603 (1, 3, 10 and 30 µg kg^{-1}) in the different groups of animals.

Data presentation and statistical analysis

All data are presented as the mean±s.e.mean. Percent changes from baseline values (i.e. after vehicle or the antagonists) caused by the different doses of A61603 within each group of animals were calculated. Duncan new multiple-range test, together with two-way ANOVA (SigmaStat 1.0, Jandel Corporation, Chicago, IL, USA), was used to establish whether these changes were statistically significant ($P<0.05$, two-tailed) when compared to the baseline in each group as well as with the corresponding dose of A61603 in the vehicle-treated group.

Drugs

Apart from the anaesthetics azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and sodium pentobarbital (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: A61603 hydrobromide (Tocris Cookson Ltd., Bristol, UK), rauwolscine hydrochloride (Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands), 5-methylurapidil (Byk Gulden, Konstanz, Germany), prazosin hydrochloride (Bufa Chemie b.v., Castricum, The Netherlands), GR127935, sumatriptan succinate (both from GlaxoWellcome, Herts, UK; courtesy: Dr. H.E. Connor), ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium) and methiothepin maleate (Hoffman La Roche b.v., Mijndrecht, The Netherlands). Finally, heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of blood in the catheters.

All drugs were dissolved in distilled water (vehicle). A short period of heating was needed to dissolve prazosin, rauwolscine, GR127935, 5-methylurapidil (acidified to pH=6.8-7.0 with 0.1 M HCl) and methiothepin (1 % of ascorbic acid was added). The doses of the drugs refer to their respective salts.

Results

Systemic and carotid haemodynamic variables after different antagonists

Baseline values of these variables in the 33 pigs used in the present investigation were: mean arterial blood pressure, 94±3 mmHg; heart rate, 101±3 beats min^{-1}; total common carotid blood flow, 133±7 ml min^{-1} and total common carotid conductance, 144±8 10^{-2} ml min^{-1} $mmHg^{-1}$. No significant differences were observed between the

values of haemodynamic variables collated before and after the administration of the vehicle (distilled water) or different antagonists used in this study (Table 4.2).

Table 4.2. Absolute values in mean arterial blood pressure, heart rate and total carotid blood flow before and after i.v. administration of vehicle and various antagonists used in anaesthetised pigs.

Treatment	**Dose (mg kg^{-1})**	**Mean arterial blood pressure (mmHg)**		**Heart rate (beats min^{-1})**		**Total carotid blood flow (ml min^{-1})$^{\#}$**	
		Before	*After*	*Before*	*After*	*Before*	*After*
Vehicle	5 ml	96±4	94±5	106±3	105±3	145±13	139±12
5-Methylurapidil	1000	102±4	95±7	103±5	116±6	149±12	152±15
Prazosin	100	92±5	77±7	112±6	108±5	147±11	128±13
Prazosin and rauwolscine	100 and 300	105±4	93±5	104±6	100±6	125±12	104±14
GR127935	500	102±3	91±7	96±3	92±3	183±31	178±26
Ketanserin	500	105±5	98±5	94±4	89±5	134±10	129±7
Methiothepin	3000	103±4	108±4	105±6	105±6	134±10	120±10

a, $P<0.05$ vs. the vehicle-treated group. #, The corresponding values of total carotid conductance 'Before' and 'After' were not significantly different ($P>0.05$) for any of the treatments and are not shown in the Table.

Systemic haemodynamic responses to A61603

A61603 (0.3, 1, 3 and 10 µg kg^{-1}, i.c.) produced a dose-dependent increase in mean arterial blood pressure (Figure 4.1; upper panel), without affecting heart rate (data not shown). This vasopressor response was antagonised (even reverted to hypotension) after treatment with methiothepin (3000 µg kg^{-1}), but remained by 5-methylurapidil (1000 µg kg^{-1}), prazosin (100 µg kg^{-1}), a combination of prazosin and rauwolscine (100 and 300 µg kg^{-1}, respectively), GR127935 (500 µg kg^{-1}) or ketanserin (500 µg kg^{-1}).

The pressor responses following i.v. bolus injection of A61603 (1, 3, 10 and 30 µg kg^{-1}) are shown in Figure 4.1 (lower panel); heart rate remained unaffected (data not shown). Treatment with 5-methylurapidil slightly attenuated these responses, which were markedly reduced by methiothepin; the other antagonists were ineffective.

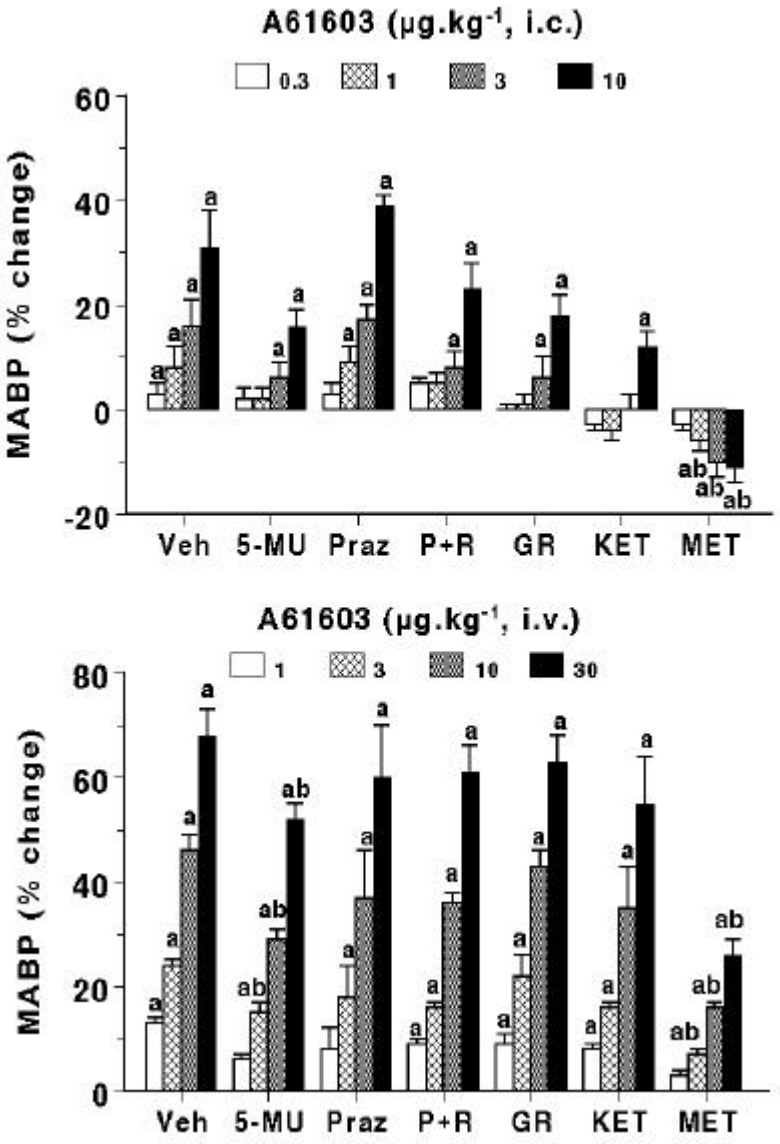


Figure 4.1. Changes in mean arterial blood pressure (MABP) produced by i.c. (*upper panel*) or i.v. (*lower panel*) administration of A61603 in anaesthetised pigs, treated i.v. with either vehicle (Veh; distilled water), 5-methylurapidil (5-MU; 1000 µg kg^{-1}), prazosin (Praz; 100 µg kg^{-1}), a combination of prazosin and rauwolscine (P+R; 100 and 300 µg kg^{-1}, respectively), GR127935 (GR; 500 µg kg^{-1}), ketanserin (KET; 500 µg kg^{-1}) or methiothepin (MET; 3000 µg kg^{-1}). Data is presented as mean±s.e.mean. a, P<0.05 vs. baseline; b, P<0.05 vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

Carotid haemodynamic responses to A61603

Absolute values of total carotid, arteriovenous anastomotic and capillary conductances before and after i.c. infusions of A61603 (0.3-10 µg kg^{-1}) in the seven groups of animals are shown in Figure 4.2. In animals treated with vehicle, A61603 produced a dose-dependent decrease in total carotid conductance. This effect was restricted to the carotid arteriovenous anastomotic fraction, since the capillary fraction

remained unmodified. The A61603-induced changes were clearly attenuated in animals treated with methiothepin, but not in those treated with prazosin, GR127935 or ketanserin. 5-Methylurapidil only attenuated the decrease in the total carotid blood flow by the highest dose of A61603. The treatment with prazosin and rauwolscine combination affected the A61603-induced decreases in the total carotid (highest two doses) and its carotid arteriovenous anastomotic fraction (highest dose).

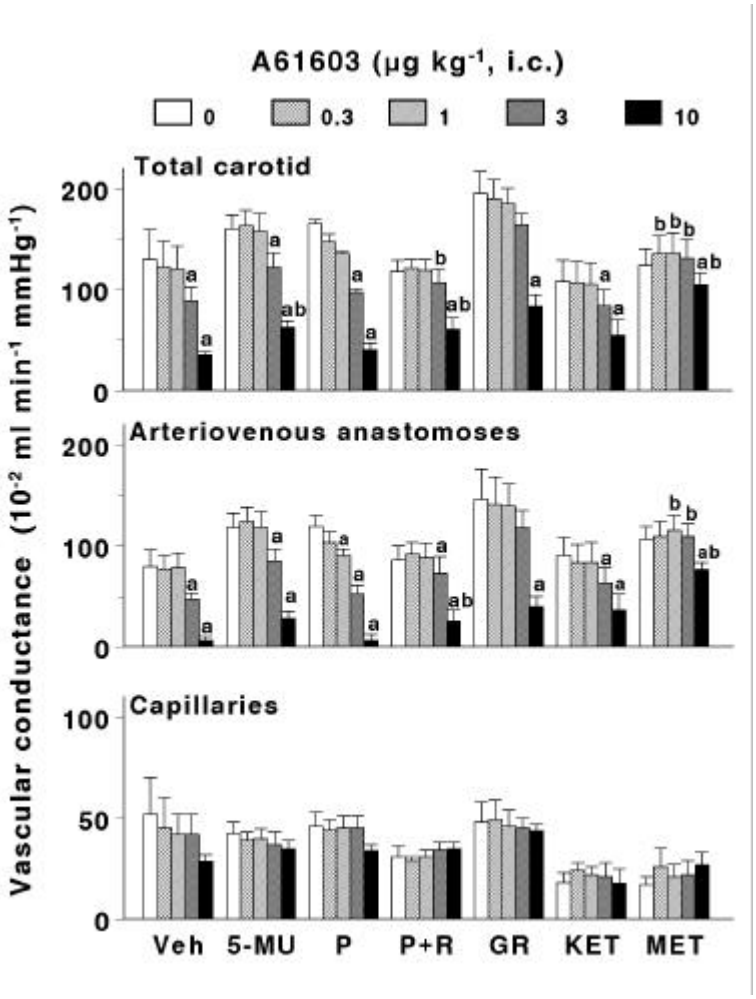


Figure 4.2. Porcine cotal carotid, arteriovenous anastomotic and capillary vascular conductances before and after i.c. administration of A61603. Treatments: vehicle (Veh; 5 ml distilled water), 5-methylurapidil (5-MU; 1000 µg kg^{-1}), prazosin (Praz; 100 µg kg^{-1}), a combination of prazosin and rauwolscine (P+R; 100 and 300 µg kg^{-1}, respectively), GR127935 (GR; 500 µg kg^{-1}), ketanserin (KET; 500 µg kg^{-1}) or methiothepin (MET; 3000 µg kg^{-1}). Data is presented as mean±s.e.mean. a, $P<0.05$ vs. baseline; b, $P<0.05$ vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

Figure 4.3 compares decreases in carotid arteriovenous anastomotic blood flow by A61603 as percent changes from baseline values in control pigs (vehicle-treatment) and in pigs treated with the different antagonists. It can be observed that the

responses to A61603 were clearly antagonised by methiothepin and only slightly by the combination of prazosin and rauwolscine; 5-methylurapidil, prazosin, GR127935 and ketanserin were ineffective.

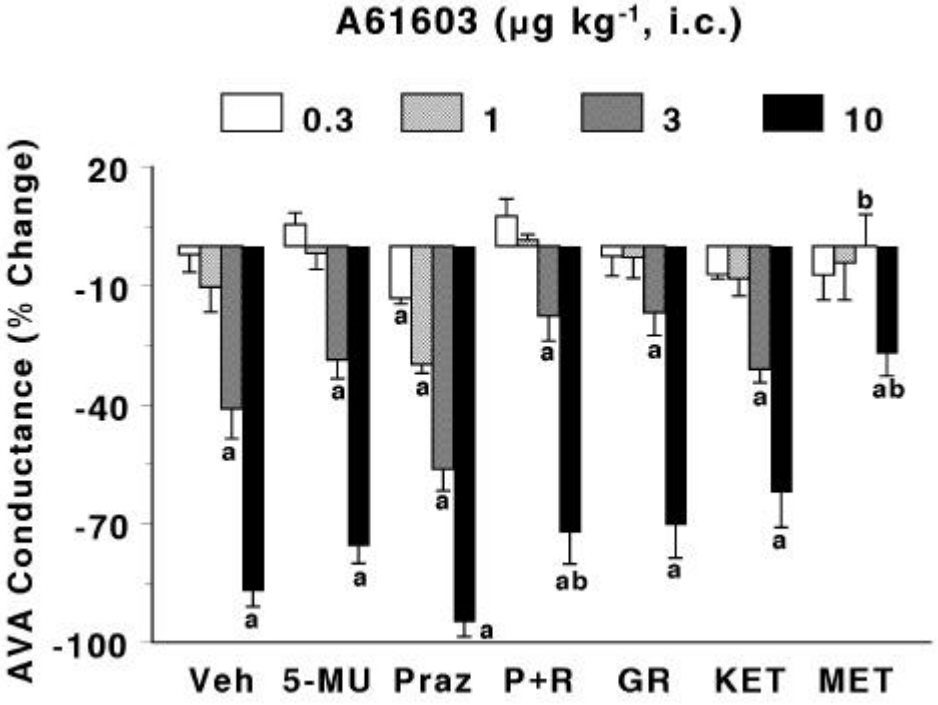


Figure 4.3. Changes in carotid arteriovenous anastomotic (AVA) conductance following i.c. administration of A61603 in anaesthetised pigs treated i.v. with either vehicle (Veh; 5 ml distilled water), 5-methylurapidil (5-MU; 1000 µg kg^{-1}), prazosin (Praz; 100 µg kg^{-1}), a combination of prazosin and rauwolscine (P+R; 100 and 300 µg kg^{-1}, respectively), GR127935 (GR; 500 µg kg^{-1}), ketanserin (KET; 500 µg kg^{-1}) or methiothepin (MET; 3000 µg kg^{-1}). Data is presented as mean±s.e.mean. a, $P<0.05$ vs. baseline; b, $P<0.05$ vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

Discussion

Consideration of known receptors that mediate carotid vasoconstriction

A number of studies have shown that sumatriptan produces vasoconstriction in the carotid vasculature of several species *via* GR127935-sensitive 5-$HT_{1B/1D}$ receptors; these species include the dog[232], pig[196]and rabbit[233, 234]. It is now known that these receptors mediating vasoconstriction are of the 5-HT_{1B} subtype[177, 178, 217]. In line with this proposal, the therapeutic efficacy of sumatriptan in migraine may be explained by carotid vasoconstriction mediated by the 5-HT_{1B} receptor. Furthermore, the canine external carotid vasoconstriction by ergotamine and dihydroergotamine involves 5-$HT_{1B/1D}$ receptors as well as α-adrenoceptors[177].

Since the ergots display reasonable affinity at α-adrenoceptors[182], their carotid vasoconstrictor effects in the pig may also be explained by these receptors. In this respect, we recently showed that: (*i*) both α_1- and α_2-adrenoceptors mediate vasoconstriction of porcine carotid arteriovenous anastomoses[219]; and (*ii*) these α_1-adrenoceptors belong to α_{1A} and α_{1B} subtypes, but not the α_{1D} subtype[229].

Except for A61603 (α_{1A}-adrenoceptor agonist), potent and selective agonists at α_1-adrenoceptor subtypes are unfortunately not available in order to verify this hypothesis. Therefore, in the present study we used A61603 to confirm the possible involvement of α_{1A}-adrenoceptors in the vasoconstriction of porcine carotid arteriovenous anastomoses.

Pharmacological profile of A61603

A61603 (Table 4.1) is a tetrahydro-1-napthyl imidazoline derivative that has been reported to show potent α_{1A}-adrenoceptor-agonist properties[33, 83]. As described previously[83, 235], A61603 is 35-fold more potent at human cloned α_{1a}- than at α_{1b}- or α_{1d}-adrenoceptors in radioligand binding studies and 100 to 300-fold more potent than noradrenaline and phenylephrine in isolated canine prostate strips and rat *vas deferens* (α_{1A}-adrenoceptors). In contrast, A61603 is only 40-fold more potent than phenylephrine at α_{1B}-adrenoceptors (rat spleen) and 35-fold less potent at α_{1D}-adrenoceptors (rat aorta)[83, 235]. Although the compound displays low affinity (pK_i<6) for other receptors (see Table 4.1), it has been shown to display a reasonable affinity and agonist property at α_2-adrenoceptor subtypes[72]. In anaesthetised dogs, A61603 increases intra-urethral as well as diastolic arterial blood pressure[83]. In agreement with the latter, A61603 produces pressor responses in conscious rats at 50- to 100-fold lower doses than those of phenylephrine, and tamsulosin (α_{1A}-adrenoceptor antagonist) causes a marked shift of the A61603-induced response curve[83].

Systemic and carotid haemodynamic effects of A61603; involvement of a new receptor

A61603 produced a dose-dependent increase in blood pressure when administered by either i.c. (0.3-10 μg kg^{-1}) or i.v. (1-30 μg kg^{-1}) routes (Figure 4.1). In view of the

high affinity of A61603 at the α_{1A}-adrenoceptor (Table 4.1) and the important role of α-adrenoceptors in the regulation of vascular tone[44], it was surprising that the hypertensive response to A61603 was little affected by 5-methylurapidil, prazosin or a combination of prazosin and rauwolscine. On the other hand, A61603-induced pressor response was markedly attenuated (i.v.) or even converted to hypotension (i.c.) by methiothepin.

Similar results were obtained with respect to the carotid haemodynamics. As shown in Figure 4.2, A61603 (0.3-10 $\mu g\,kg^{-1}$, i.c.) produced a dose-dependent decrease in the porcine carotid blood flow, exclusively due to a vasoconstriction of carotid arteriovenous anastomoses. This selective carotid vasoconstriction was apparently maximal because a higher dose of A61603 (30 $\mu g\,kg^{-1}$) did not produce an additional decrease in total carotid conductance (maximal change: 80±4%; n=6).

The A61603-induced vasoconstriction of carotid arteriovenous anastomoses was, unexpectedly, resistant to blockade by the potent and selective α_{1A}-adrenoceptor antagonist 5-methylurapidil[26, 42]. Since prazosin was also ineffective in attenuating this response, it seems plausible to conclude that α_1-adrenoceptors do not play an important role. As mentioned before, both α_1- and α_2-adrenoceptors can mediate vasoconstriction in the porcine carotid arterial bed[219]. For this reason and for the fact that A61603 also has affinity for α_2-adrenoceptors (see Table 4.1[72]), we applied a combination of prazosin and rauwolscine to investigate the possible involvement of α_2-adrenoceptors. The combination of prazosin and rauwolscine produced only a slight attenuation in the A61603-induced vasoconstriction of carotid arteriovenous anastomoses, which implies, at most, a limited involvement of α_2-adrenoceptors. Similarly, the fact that GR127935 as well as ketanserin did not significantly modify this response excludes the possible involvement of 5-$HT_{1B/1D}$ and 5-HT_2 receptors, respectively. Although A61603 shows only a low affinity at these receptors (see Table 4.1[72]), the exclusion of 5-$HT_{1B/1D}$ receptors is of interest, considering the affinity of benzylimidazoline derivatives related in structure to A61603 at 5-$HT_{1B/1D}$ receptors[236]. As reported elsewhere, the involvement of 5-HT_{1F} receptors in the carotid vasoconstriction of pigs and dogs (external) carotid vascular bed has been categorically excluded[162, 166, 180, 231]. Moreover, an endothelium-dependent vasoconstriction *via* the release of pro-constrictor

cyclo-oxygenase products[237, 238] seems also unlikely, based on the lack of effect of indomethacin (3000 $\mu g\,kg^{-1}$, i.v.; data not shown) on A61603-induced decrease in total carotid conductance. Similarly, a combination of indomethacin, prazosin, rauwolscine, GR127935 and ketanserin, at the doses previously mentioned, also failed to attenuate the decreases in total carotid conductance produced by i.c. infusions of A61603 (data not shown).

To strengthen the hypothesis that α-adrenoceptors and 5-HT receptors do not play a role in this response, we decided to test methiothepin in this porcine model. This drug displays high affinity at 5-$HT_{1/2}$ receptors[227] as well as $\alpha_{1/2}$ adrenoceptors[182]. It may be noted that a relatively high dose of methiothepin (3000 $\mu g\,kg^{-1}$) was required to abolish sumatriptan-induced carotid vasoconstriction in anaesthetised dogs and pigs[180, 182, 231, 239]; while a lower dose (1000 $\mu g\,kg^{-1}$) was ineffective. As shown in Figs. 4.2 and 4.3, treatment of the animals with methiothepin markedly attenuated the A61603-induced vasoconstriction in the porcine carotid vascular bed. Since all currently known vasoconstrictor receptors/mechanisms were blocked by their respective antagonist/inhibitor, this latter finding may in turn imply the involvement of another, possibly novel, receptor and/or mechanism in the vasoconstriction of carotid arteriovenous anastomoses by A61603. Since this *in vivo* animal model is predictive for antimigraine activity[161], this possible novel receptor could be a potential new target for the development of antimigraine agents in the future. Admittedly, as an antimigraine drug, such an agonist must be devoid of systemic vasoconstrictor properties.

In conclusion, the present results show that A61603 does not behave as a potent and selective α_{1A}-adrenoceptor agonist in the pig and that the vasoconstriction of porcine carotid arteriovenous anastomoses by A61603 is primarily mediated by a novel methiothepin-sensitive receptor/mechanism.

Acknowledgements

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Chapter 5

Porcine carotid and systemic haemodynamic effects of S19014: an experimental study to assess its antimigraine potential

Abstract Taking into account the drawbacks associated with the use of triptans (5-$HT_{1B/1D}$ receptor agonists), particularly the cardiovascular liability, attempts are being made to explore other avenues for the treatment of migraine. Recently, it has been shown that both α_1- and α_2-adrenoceptors can mediate vasoconstriction of porcine carotid arteriovenous anastomoses, which has effectively served as an experimental model predictive of antimigraine activity. The present study was set out to investigate the carotid vascular effects of a newly synthesised α-adrenoceptor agonist S19014 (spiro[(1,3-diazacyclopent-1-ene)-5:2'-(4',5'-dimethylindane)]) in this model. Intravenous administration of S19014 (1-30 $\mu g\,kg^{-1}$) produced a dose-dependent, initial short lasting vasopressor response and a decrease of total carotid blood flow and conductance. The decrease of total carotid blood flow by S19014 was exclusively due to vasoconstriction of carotid arteriovenous anastomoses; the total capillary blood flow (especially in muscles, fat, bone, salivary gland and dura mater) was increased. Whereas prazosin was ineffective, rauwolscine attenuated the carotid haemodynamic and initial vasopressor responses poduced by S19014. The above results suggest that the systemic and carotid vascular effects produced by S19014 in anaesthetised pigs are mainly mediated by α_2-adrenoceptors and that S19014 could be effective in the treatment of migraine.

Based on: Kapoor *et al., Communicated to Servier (Paris, France).*

Introduction

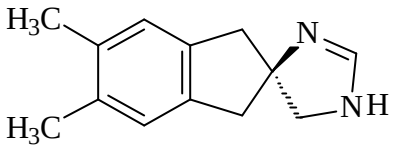
Vasodilatation of cranial large arteries and arteriovenous anastomoses has been proposed to play an important role in the pathophysiology of migraine headache[161, 170]. Indeed, to date all acutely acting antimigraine agents, i.e. the triptans and ergots, constrict isolated cranial vessels as well as arteriovenous anastomoses within the carotid vasculature[167, 177, 216, 231]. While the effect of triptans seems to be mediated exclusively by the 5-HT_{1B} receptor[178, 217], that of ergot alkaloids also involves other receptors[181, 240], including the α-adrenoceptors, which mediate the carotid vasoconstriction in anaesthetised dogs[177].

Stimulation of α-adrenoceptors produces contraction of isolated carotid artery of several species, including the dog[189, 190], rabbit[241, 242] and pig[192]. Also, *in vivo* studies have shown that exogenously administered α-adrenoceptors agonists (e.g. phenylephrine and BHT933) potently constrict carotid arteriovenous anastomoses[219] and there is evidence that α-adrenoceptors may regulate the vascular tone of carotid arteriovenous anastomoses[174]. Thus, it may be possible that α-adrenoceptors may provide a potential target for the development of novel antimigraine drugs.

Cordi *et al.*[243] have described a series of compounds with α-adrenoceptor agonist affinity/activity. One such compound, S19014 (spiro[(1,3-diazacyclopent-1-ene)-5:2'-(4',5'-dimethylindane)]), displays high affinities at the different α_1- and α_2-adrenoceptor subtypes (for structure and affinity values, see Table 5.1). Interestingly, S19014 shows a wide variation in its efficacy (maximum effect, E_{max}) and potency (EC_{50}, concentration needed to cause 50% of E_{max}) in contracting rabbit, dog and human isolated saphenous vein (EC_{50}: 18, 79 and 8500 nM, respectively; E_{max}: 92, 49 and 36% of K^+-induced contraction, respectively), rabbit aorta (EC_{50}: 816 nM; E_{max}: 36% of K^+-induced contraction) and dog femoral artery (practically inactive)[244].

The present study was designed to investigate the systemic and carotid haemodynamic effects of S19014 in anaesthetised pigs in order to assess its potential as an antimigraine agent.

Table 5.1. Chemical structure of S19014 and its binding affinities (pK_i) at human cloned α_1- and α_2-adrenoceptor subtypes.

Chemical structure	***pK$_i$ values****	
	**a$_{1a}$:** 7.66	**a$_{2a}$:** 8.98
	a$_{1b}$: 7.80	**a$_{2b}$:** 8.33
	a$_{1d}$: 7.65	**a$_{2c}$:** 8.75

*, Unpublished data from Institut de Recherches Servier (Paris, France).

Methods

General

After an overnight fast, 27 domestic pigs (Yorkshire x Landrace; 10-14 kg) were anaesthetised with azaperone (120 mg, i.m.), midazolam hydrochloride (10 mg, i.m.) and sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (20 mg kg^{-1} h^{-1}). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy, leads to an increase in heart rate and vasodilatation of arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbital-anaesthetised pigs (70-80% of carotid blood flow) than in those under fentanyl/thiopental anaesthesia (~19% of carotid blood flow[174]). A high basal carotid arteriovenous anastomotic flow is particularly useful for investigating the effects of drugs that vasoconstrict these shunt vessels.

A catheter was placed in the inferior vena cava *via* the left femoral vein for the infusions of the different treatments (Table 5.2) and S19014. Another catheter was placed in the aortic arch *via* the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen,

Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, both the common carotid artery and the external jugular vein were dissected free and bilateral vagosympathgectomy was performed in order to prevent a possible influence *via* baroreceptor reflexes on S19014-induced carotid vascular responses. The right external jugular vein was catheterised for withdrawal of venous blood samples for determining blood gases in order to determine arterio-jugular oxygen saturation difference (A-VSO_2). A hub-less needle, was connected to a polyethylene tube and inserted into the right common carotid artery and was used for radioactive microspheres injection. The microspheres were injected against the direction of blood flow for uniform mixing.

Right common carotid blood flow was determined with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Arterial blood pressure, heart rate and right common carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept around 37 °C and the animal was continuously infused with saline to compensate for fluid loss.

Distribution of carotid blood flow

The distribution of common carotid blood flow was determined with 15.5±0.1 µm (s.d.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbital and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120-167, KeV, ^{113}Sn: 355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed programs [197]. The fraction of

carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the total common carotid blood flow at the time of the injection of the microspheres labelled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping *via* carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the common carotid blood flow[198]. Vascular conductance (ml min^{-1} $mmHg^{-1}$) was calculated by dividing blood flow (ml min^{-1}) by mean arterial blood pressure (mmHg).

Experimental protocol

After a stabilisation period of at least 60 min, values of heart rate, blood pressure and total carotid blood flow and conductance were measured. Thereafter, the animals (n=27) were divided into four groups. Whereas the first (n=6) and second groups (n=6) remained untreated, the animals in the third (n=8) and fourth group (n=7) were treated intravenously (0.5 ml min^{-1} for 10 min) with prazosin (100 µg kg^{-1}) and rauwolscine (300 µg kg^{-1}), respectively. After a waiting period of fifteen minutes, baseline values of blood pressure, heart rate and total carotid blood flow were collated and the distribution of carotid blood flow into arteriovenous anastomotic and capillary fractions as well as A-VSO_2 difference were determined. Subsequently, the animals in the first group received four consecutive intravenous infusions (1 ml min^{-1} for 5 min) of distilled water (vehicle), whereas those in the second, third and fourth group received intravenous infusions (1 ml min^{-1} for 5 min) of S19014 (1, 3, 10 and 30 µg kg^{-1}) in a cumulative manner. Systemic and carotid haemodynamic variables were reassessed 10 min after every administration of vehicle (first group) or S19014 dose (other three groups).

Data presentation and statistical analysis

All data have been expressed as the mean ± s.e.mean. The significance of the difference between the variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations[222]. Percent changes

(from baseline values) caused by S19014 (1, 3, 10 or 30 $\mu g\,kg^{-1}$) in the animals treated with either prazosin or rauwolscine were compared with the corresponding doses in the control group using Student's unpaired t-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

Apart from the anaesthetics azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and sodium pentobarbital (Sanofi Sante b.v., Maasluis, The Netherlands,), the compounds used in this study were: prazosin hydrochloride (Bufa Chemie b.v., Castricum; The Netherlands), rauwolscine dihydrochloride (RBI, Natick, USA) and S19014 (spiro[(1,3-diazacyclopent-1-ene)-5:2'-(4',5'-dimethylindane)]; Gift from Dr. C. Rochat, Institut de Recherches Servier, Paris, France). Finally, heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of blood in the catheters.

All drugs were dissolved in distilled water (vehicle), however a short period of heating was needed to dissolve prazosin. The doses of the drugs refer to their respective salts.

Ethical approval

The local ethics committee dealing with the use of animals in scientific experiments approved the protocol.

Results

Baseline values

Baseline values in anaesthetised pigs (n=27) before any treatment were: heart rate (105±2 beats min^{-1}), mean arterial blood pressure (101±2 mmHg), total carotid blood flow (148±6 $ml\,min^{-1}$) and total carotid vascular conductance (140±6 $ml\,min^{-1}\,mmHg^{-1}$).

Systemic and carotid haemodynamic effects of the treatments per se

Table 5.2 shows the systemic and carotid haemodynamic effects before and after intravenous administration of vehicle (distilled water), prazosin ($100\,\mu g\,kg^{-1}$) or rauwolscine ($300\,\mu g\,kg^{-1}$). Whereas treatment of the animals with either vehicle or

prazosin did not produce any changes, rauwolscine elicited a small, but significant, decrease in mean arterial blood pressure (9±2%).

Table 5.2. Absolute values of heart rate, mean arterial blood pressure and total carotid blood flow in anaesthetised pigs, before and after intravenous infusions of vehicle (distilled water), prazosin or rauwolscine.

Treatment group	**Vehicle**		**Prazosin (100 µg kg^{-1})**		**Rauwolscine (300 µg kg^{-1})**	
	Before	*After*	*Before*	*After*	*Before*	*After*
Heart rate (*beats min^{-1}*)	102±2	101±3	114±3	112±3	98±4	99±5
MABP (*mmHg*)	97±3	95±3	98±3	91±4	108±2	99±2[a]
Total CBF (*ml min^{-1}*)	140±13	142±13	140±14	126±12	154±7	136±6
A-VSO_2 *(%)*	9±2	8±2	8±2	10±2	6±3	6±2

MABP, mean arterial blood pressure; Total CBF, total carotid artery blood flow; A-VSO_2, arterio-jugular venous oxygen saturation difference. a, $P<0.05$ 'Before' vs. 'After' administration of treatment (5 ml of each).

Systemic haemodynamic and A-VSO_2 effects of S19014

As shown in Table 5.3, intravenous administration of vehicle produced only moderate decreases in heart rate (maximum change: 4±1%) and mean arterial blood pressure (maximum change: 47±1%). On the other hand, S19014 (1, 3, 10 and 30 µg kg^{-1}) produced a small, but significant, decrease in heart rate (maximal response: 3±1%) and an initial, dose-dependent increase in mean arterial blood pressure (maximal responses were: 4±0, 8±1, 14±1, 21±2%, respectively). However, blood pressure was constant and back to baseline values at the time of microsphere injection (see Table 5.3); we even observed a small, but significant, hypotension (maximal response: -5±2%). The time for S19014 to produce this initial vasopressor response was 2.9±0.3 min. In addition, S19014 produced an increase in arterial jugular venous oxygen saturation difference (A-VSO_2; maximal response: 10±2%).

The effects of S19014 on heart rate and mean arterial blood pressure were not affected by prazosin, however, we observed (at the time of microsphere injection) a

small hypotension after rauwolscine, produced by the highest of S19014 (-7±4%). Interestingly, treatment of the animals with rauwolscine produced a right-ward shift of the above mentioned initial vasopressor response (maximal responses were: -9±1, -5±3, 1±3 and 14±1%, respectively), while prazosin was ineffective (data not shown). It may be noted that whereas prazosin was ineffective, rauwolscine showed a tendency to attenuate the S19104-induced increase in $AVSO_2$, however, this latter effect was not significant (see Table 5.3).

Table 5.3. Systemic haemodynamic effects of vehicle *per se* and S19014 in the absence (control) or presence of either prazosin (100 µg kg^{-1}) or rauwolscine (300 µg kg^{-1}).

Treatment	**S19014 (µg kg^{-1}, i.v.)**				
	Baseline	**1**	**3**	**10**	**30**
Heart rate (beats min^{-1})					
Vehicle†	102±2	101±3	100±3[a]	98±3[a]	98±4[a]
Control	102±4	102±5	101±4[a]	100±4[a]	100±4[a]
Prazosin	112±3	110±3	110±3	110±3	108±2
Rauwolscine	99±5	98±5[b]	98±6	98±6	98±6
Mean arterial blood pressure (mmHg)					
Vehicle†	97±3	95±3	96±3[a]	96±3[a]	90±3[a]
Control	101±3	99±3	96±4[a]	96±4[a]	96±3[a]
Prazosin	91±4	88±4	86±4[a]	84±4[a]	83±3[a]
Rauwolscine	98±2	94±2	93±3	93±3	91±3[b]
Arterio-jugular venous oxygen saturation difference (%)					
Vehicle†	9±2	8±2	8±2	8±2	10±3
Control	7±2	8±2	8±2	9±3*	10±2[a]
Prazosin	10±2	11±3	13±2[a]	12±2[a]	15±2[a]
Rauwolscine	6±2	6±2	6±1	7±2	8±2[a]

a, P<0.05 vs. baseline; b, P<0.05 vs. control; †, four consecutive infusions of 5 ml of distilled water were given after baseline.

Carotid haemodynamics of S19014

Absolute values of total carotid, arteriovenous anastomotic and capillary blood flow and conductance in the different groups of animals are shown in Figure 5.1. Whereas

vehicle was devoid of any carotid haemodynamic effects, S19014 (1, 3, 10 and 30 $\mu g\,kg^{-1}$) produced dose-dependent decreases in total carotid and arteriovenous anastomotic blood flow (maximum change: 18±2 and 34±5%, respectively) and conductance (maximum change: 14±2 and 30±4%, respectively. In contrast, S19014 increased capillary blood flow and conductance (maximum change: 40±23 % and 49±27%, respectively). These changes in vascular conductance were particularly noted in muscle (maximum change: 18±9%), bone (maximum change: 55±26%), fat (maximum change: 168±142%), salivary gland (maximum change: 91±57%) and dura mater (maximum change: 636±593%), while those in the others (skin, eye, brain, ear or tongue) remained unchanged. These S19014-induced haemodynamic responses were attenuated in animals treated with rauwolscine, while treatment with prazosin was without effect.

Figure 5.2 depicts percent changes (from baseline values) in carotid arteriovenous anastomotic conductance by S19014 (1, 3, 10 and 30 $\mu g\,kg^{-1}$) in control and prazosin- or rauwolscine-treated animals. While prazosin (100 $\mu g\,kg^{-1}$) did not modify the vasoconstrictor effect of S19014 on carotid arteriovenous anastomoses, treatment of the animals with rauwolscine (300 $\mu g\,kg^{-1}$) clearly did.

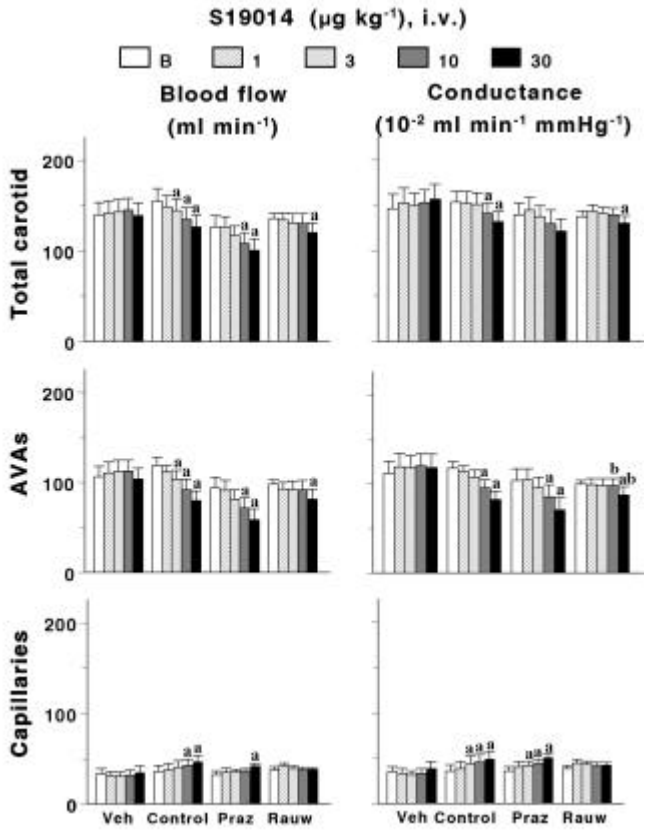


Figure 5.1. Effects of four consecutive i.v. infusions of vehicle (distilled water) *per se* and S19014 (1, 3, 10 and 30 µg kg^{-1}) in the absence (control) or presence of prazosin (Praz; 100 µg kg^{-1}) or rauwolscine (Rauw; 300 µg kg^{-1}) on porcine total carotid, arteriovenous anastomotic (AVA) and capillary fraction blood flow and conductance. All values are expressed as mean±s.e.mean. * P<0.05 vs. baseline; # vs. response produced by corresponding dose in control animals.

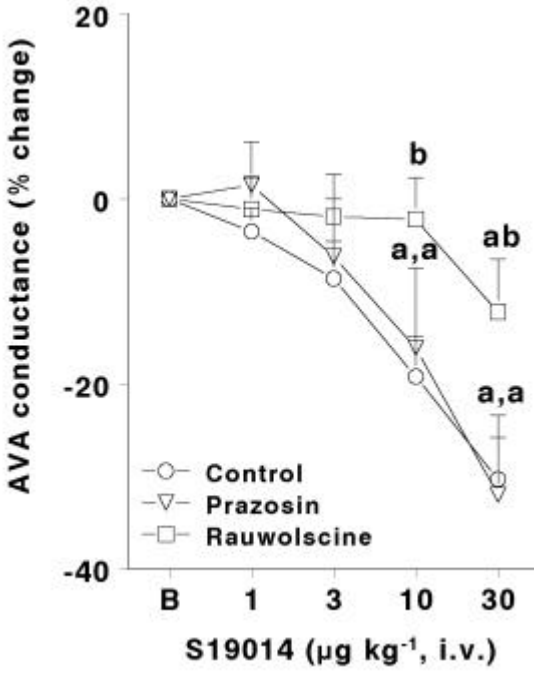


Figure 5.2. Effect (% change compared to aseline) of S19014 (1, 3, 10 and 30 µg kg^{-1}) n porcine arteriovenous anastomotic (AVA) onductance in the absence (control) or resence of prazosin (Praz; 100 µg kg^{-1}) or uwolscine (Rauw; 300 µg $^{-1}$kg). All values ave been expressed as mean±s.e.mean. , P<0.05 vs. baseline, # P<0.05 vs. response roduced by corresponding dose in control nimals.

Discussion

General

It is generally agreed that α-adrenoceptors are divided into α_1- and α_2-adrenoceptors and play an important role in the regulation of the vascular resistance and blood pressure[17, 20, 26, 31, 33, 245]. Recently, we have shown that both α_1- and α_2-adrenoceptors can mediate canine (external) and porcine (arteriovenous anastomotic) carotid vasoconstriction[219, 223]. In this context, several lines of evidence have demonstrated that vasoconstriction in this vascular bed is predictive for antimigraine activity[167]. The present study was designed to investigate the carotid

and systemic haemodynamic effects of the newly developed α-adrenoceptor agonist S19104 in anaesthetised pigs and whether the effects of S19104 involve α_1- and/or α_2-adrenoceptors.

The major findings of the present study were: (*i*) S19014 caused relatively little systemic haemodynamic changes; (*ii*) S19014 caused vasoconstriction of porcine carotid arteriovenous anastomoses in a model predictive for antimigraine activity; and (*iii*) this vasoconstrictor effect was markedly attenuated by rauwolscine, but not by prazosin. The doses of prazosin (100 µg kg^{-1}) and rauwolscine (300 µg kg^{-1}) are sufficient to selectively block α_1- and α_2-adrenoceptors, respectively[246].

Systemic haemodynamic effects of S19014

As described earlier[246], the treatments used in the present study (vehicle, prazosin and rauwolscine) were devoid of major systemic haemodynamic effects. It may be noted that the S19014-induced bradycardia and hypotension are probably due to its vehicle (see Table 3). In any case, the comparable bradycardic effect produced by sumatriptan (5±1%) in the same experimental set-up[240, 247] is of little clinical relevance in migraine therapy[215].

On the other hand, intravenous administration of S19014 produced a moderate short-lasting dose-dependent vasopressor response, which, being amenable to blockade by rauwolscine but not prazosin, was mediated by α_2-adrenoceptors. Indeed, pressor responses can be elicited *via* both α_1- and α_2-adrenoceptor subtypes[33]. It may be pointed out that in our previous experiments[246] no pressor changes were observed with either phenylephrine (α_1-adrenoceptor agonist) or BHT933 (α_2-adrenoceptor agonist) in anaesthetised pigs. This apparent discrepancy is due to the fact in these experiments phenylephrine and BHT933 were slowly infused into the carotid artery[Willems, 1999 #7], while we injected S19014 intravenously.

Carotid haemodynamic effects of S19014

As reported previously[246], intravenous administration of vehicle, prazosin or rauwolscine did not produce major carotid haemodynamic changes (Table 5.2). On the other hand, S19014 produced a dose-dependent vasoconstriction in the carotid

vasculature of anaesthetised pigs, an effect exclusively caused by vasoconstriction of carotid arteriovenous anastomoses; the vascular conductance in the capillary fraction was increased. In accordance with the vasoconstriction of carotid arteriovenous anastomoses[161, 170], S19014 produced an increase in A-VSO_2 difference. Although, as shown in Table 5.1, S19014 displays only a little less affinity at the three α_1-adrenoceptor subtypes (pK_i: 7.65-7.80) than at the three α_2-adrenoceptor subtypes (pK_i: 8.33-8.98), the vasoconstriction of carotid arteriovenous anastomoses by S19014 was antagonised by rauwolscine and not at all by prazosin. Interestingly, the vasoconstrictor responses to S19014 are variable in potency and efficacy[244], and this may suggest that α-adrenoceptor subtypes at which S19014 is efficacious may be unevenly distributed throughout the body.

Possible clinical implications

Both *in vitro*[248, 249] and *in vivo*[161, 167, 177, 250] experimental models demonstrating vasoconstrictor properties have consistently shown their value in predicting therapeutic potential of drugs in the acute treatment of migraine. Therefore, the results obtained with S19014 in the present experiments suggest that this compound may well have antimigraine properties. In this connection, it is interesting to compare the efficacy of S19014 with that of triptans and ergot alkaloids in the present porcine model. As can be observed in Figure 5.3, S19014 (30 $\mu g\,kg^{-1}$) and sumatriptan (30 $\mu g\,kg^{-1}$) were equi-effective in constricting porcine carotid arteriovenous anastomoses, but we do not know if higher doses of S19014 will exhibit higher efficacy with relatively little systemic haemodynamic effects (e.g. hypertension). Our experience with this porcine model is largely limited to 5-$HT_{1B/1D}$ receptor agonists and, in view of the fact that porcine α-adrenoceptors have not yet been cloned and compared with the human receptors, we do not know how the porcine carotid vascular responses mediated *via* α-adrenoceptors would be predictive of antimigraine efficacy in humans. Nevertheless, it will be worthwhile to explore this aspect with S19014, which mainly acts *via* α_2-adrenoceptors, which are less ubiquitous than α_1-adrenoceptors.

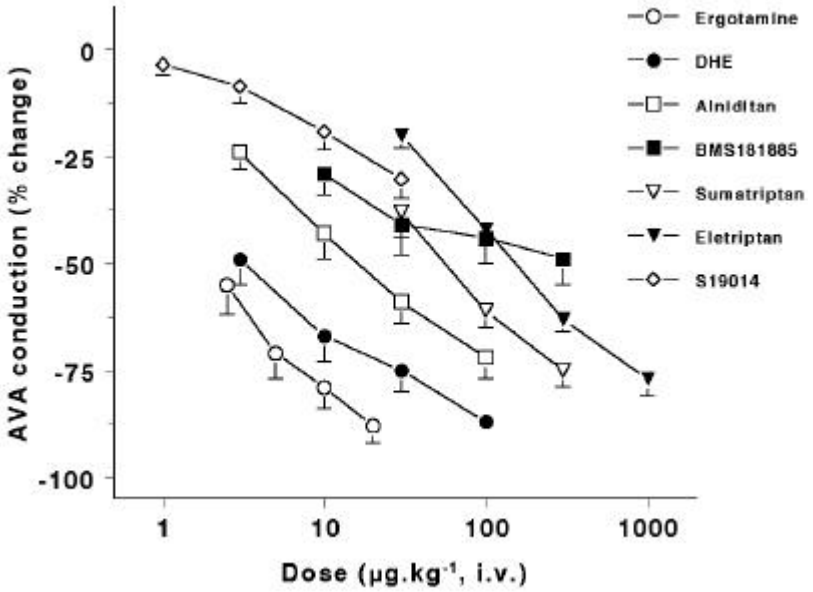


Figure 5.3 Comparison of the contractile effect of S19014 (present experiments) and some antimigraine drugs[167] on porcine carotid arteriovenous anastomoses.

As with the currently available antimigraine agents[215, 228], we are aware of the potential liability of α-adrenoceptor agonists in constricting peripheral blood vessels, for example the coronary artery[251, 252]. However, in contrast to sumatriptan, S19014 (in concentration less than 1 µM) did not contract human isolated coronary arteries (MaassenVanDenBrink *et al.*, unpublished data). In agreement with the latter, except for a dose-dependent decrease in the blood flow to the lungs, S19104 did not affect the distribution of cardiac output to the different tissues of anaesthetised pigs (Kapoor *et al.*, unpublished data).

Acknowledgements

We would like to thank Dr. C. Rochat (Institut de Recherches Servier, Paris, France) for providing S19014.

Chapter 6

Pharmacological identification of the major subtypes of adrenoceptors involved in the canine external carotid vascular effects of adrenaline and noradrenaline

Summary This study investigated the potential effects of adrenaline and noradrenaline on the external carotid blood flow of vagosympathectomised dogs and the receptor mechanisms involved. One minute (1 min) intracarotid infusions of adrenaline and noradrenaline produced dose-dependent decreases in external carotid blood flow without changes in blood pressure or heart rate. These responses, which remained unaffected after saline, were: (*i*) mimicked by the adrenoceptor agonists, phenylephrine (α_1) and BHT933 (6-Ethyl- 5,6,7,8- tetrahydro- 4H- oxazolo [4,5-d] azepin-2-amine dihydrochloride; α_2); (*ii*) abolished after phentolamine (2000 $\mu g\,kg^{-1}$) unmasking a vasodilator component (subsequently blocked by propranolol; 1000 $\mu g\,kg^{-1}$); and (*iii*) partly blocked by rauwolscine (30 and 100 $\mu g\,kg^{-1}$), and subsequently abolished by prazosin (100 $\mu g\,kg^{-1}$). Accordingly, rauwolscine (100 and 300 $\mu g\,kg^{-1}$) markedly blocked the responses to BHT933 without affecting those to phenylephrine; likewise, prazosin (100 $\mu g\,kg^{-1}$) markedly blocked the responses to phenylephrine without affecting those to BHT933. These results show that both α_1- and α_2-adrenoceptors mediate vasoconstriction within the canine external carotid circulation. Moreover, after blockade of α_1/α_2-adrenoceptors, both adrenaline and noradrenaline exhibit a β-adrenoceptor-mediated vasodilator component.

Based on: Willems *et al., Life Sci. (2000)* **In Press**.

Introduction

In contrast to the established role of serotonin (5-hydroxytryptamine; 5-HT) receptors in the carotid vascular beds of dogs and pigs[167, 231, 253], little is known about the receptors and/or mechanism(s) by which adrenaline and noradrenaline produce their effects in these vascular beds. In this respect, it has been recently shown that both α_1- and α_2-adrenoceptors mediate the vasoconstriction to, respectively, phenylephrine and BHT933 in the carotid circulation of anaesthetised pigs[219].

To the best of our knowledge, however, no study has reported whether the above catecholamines produce external carotid vascular effects in anaesthetised dogs *via* adrenoceptors. In view of the relevance that the canine external carotid model has had in the development of anti-migraine drugs[161, 162, 177, 216], the present study in the canine external carotid circulation investigates: (*i*) the effects produced by intracarotid infusions of adrenaline and noradrenaline as well as by the adrenoceptor agonists phenylephrine (α_1) and BHT933 (α_2); and (*ii*) the mechanisms involved in the effects (if any) produced by the above compounds. For the latter purpose, we made use of the classical adrenoceptor antagonists, phentolamine (α) and propranolol (β) and of the more specific antagonists, prazosin (α_1) and rauwolscine (α_2).

Methods

General methods

Experiments were carried out in 33 dogs (15-31 kg) not selected for breed or sex. The animals were anaesthetised with an intravenous (i.v.) bolus injection of sodium pentobarbitone (30 mg kg^{-1}) and additional amounts (1 mg kg^{-1}) were provided when required. All dogs were intubated with an endotracheal tube and artificially respired with room air using a Palmer ventilation pump at a rate of 20 strokes min^{-1} and a stroke volume of 13-16 ml kg^{-1}, as previously established by Kleinman & Radford[254]. Catheters were placed in the inferior vena cava *via* a femoral vein for the administration of the antagonists and in the aortic arch *via* a femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of blood pressure. After each drug administration, the venous catheter was flushed with 3 ml of saline. Mean blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures: MAP = DAP + (SAP-DAP)/3. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co., Quincy, MA, U.S.A.) triggered from the blood pressure

signal. The right common carotid artery was dissected free and the corresponding internal carotid and occipital arteries were ligated. Thereafter, an ultrasonic flow probe (4 mm, R-Series) connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, N.Y., U.S.A.) was placed around the right common carotid artery and the flow through this artery was considered as the external carotid blood flow[255]. Bilateral cervical vagosympathectomy was systematically performed in order to prevent possible baroreceptor reflexes induced by the intracarotid administration of the different sympathomimetic agents; these were administered into the carotid artery by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.) with a catheter inserted into the right cranial thyroid artery. Blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). Body temperature of the animals was maintained between 37-38°C.

Experimental protocol

After the animals had been in a stable haemodynamic condition for at least 60 min, baseline values of heart rate, mean blood pressure and external carotid blood flow were determined. Then, the animals (n=33 in total) were divided into two groups.

The first group (n=15) received consecutive 1 min intracarotid infusions of adrenaline (0.1, 0.3, 1 and 3 $\mu g\,min^{-1}$) and noradrenaline (0.1, 0.3, 1 and 3 $\mu g\,min^{-1}$) and the changes produced in external carotid blood flow, mean blood pressure and heart rate were noted. At this point, the dogs were subdivided into three subgroups which received i.v. bolus injections of, respectively: (*i*) physiological saline (0.03, 0.1 and 0.3 $ml\,kg^{-1}$; n=3); (*ii*) phentolamine (2000 $\mu g\,kg^{-1}$; followed by a continuous infusion of 1000 $\mu g\,kg^{-1}$.h in order to maintain a continuous blockade of α-adrenoceptors,) and, subsequently, propranolol (1000 $\mu g\,kg^{-1}$) (n=6); and (*iii*) rauwolscine (30 and 100 $\mu g\,kg^{-1}$) and, subsequently, prazosin (100 $\mu g\,kg^{-1}$) (n=6). Then, the responses to adrenaline and noradrenaline, at the doses and sequence listed above, were elicited again 15 minutes after each dose of saline or the above mentioned antagonists.

The second group (n=18) received consecutive 1 min intracarotid infusions of phenylephrine (0.3, 1, 3 and 10 μg min) and BHT933 (3, 10, 30 and 100 $\mu g\,min^{-1}$) and the changes produced in the above haemodynamical parameters were noted. Then

the dogs we subdivided into three subgroups which received i.v. bolus injections of, respectively: (*i*) physiological saline (0.03 and 0.1 ml kg^{-1}; n=6); (*ii*) prazosin (100 µg kg^{-1}; n=6); and (*iii*) rauwolscine (100 and 300 µg kg^{-1}; n=6). 15 min later, the responses to phenylephrine and BHT933 were elicited again.

The dose-intervals between the different doses of agonists ranged from 5 and 15 min, as in each case we waited until the changes in external carotid blood flow has returned to baseline values. The dosing with the agonists was sequential, whereas that with physiological saline and the antagonists was cumulative. The Ethical Committee of the Department of Pharmacology (CINVESTAV-IPN), dealing with the use of animals in scientific experiments, approved the protocol of this investigation..

Data presentation and statistical evaluation

All data in the text, figures and tables are presented as the mean±s.e.mean. The peak changes in external carotid vascular conductance (external carotid blood flow divided by mean blood pressure; calculated as percent change from baseline) produced by the different doses of agonists before and after a particular dose of saline or antagonist within one group of animals were compared using an analysis of variance followed by the Student-Newman-Keuls test[222]. Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

Apart from the anaesthetic (sodium pentobarbitone), the compounds used in this study were: (-)-adrenaline, (-)-noradrenaline tartrate, L-phenylephrine hydrochloride, BHT933 dihydrochloride, rauwolscine hydrochloride and propranolol hydrochloride (all from RBI/Sigma Chemical Company, St. Louis, MO, USA); phentolamine mesylate (Research Biochemicals International, Natick, MA, USA); and prazosin hydrochloride (Bufa Chemie b.v., Castricum; The Netherlands).

All drugs were dissolved in physiological saline. The doses of the antagonists refer to their respective salts, while those of the agonists refer to their free base.

Results

Systemic and carotid haemodynamic responses produced by the different treatments

Baseline values of heart rate, blood pressure and external carotid blood flow in the thirty three anaesthetised dogs were: 161±7 beats min^{-1}, 131±9 mmHg, 139±18 ml min^{-1}, respectively (Table 1). The specific systemic and external carotid haemodynamic values observed after different treatments (saline, phentolamine, propranolol, prazosin and rauwolscine) are shown in Table 1. Phentolamine and prazosin produced a decrease in blood pressure (20±8 and 13±5%, respectively), while propranolol caused a decrease in heart rate (29±5%). It must be pointed out, however, that none of the treatments affected external carotid blood flow (see Table 1) or the corresponding vascular conductance (data not shown).

Table 6.1. Absolute values of heart rate, mean blood pressure and external carotid blood flow in vagosympathectomised dogs before (baseline) and after treatment with phentolamine (2000 µg kg^{-1}), propranolol (1000 µg kg^{-1}), prazosin (100 µg kg^{-1}) or rauwolscine (300 µg kg^{-1}).

Treatment group	Heart rate (beats min^{-1})		Mean arterial blood pressure (mmHg)		External carotid blood flow (ml min^{-1})*	
	Before	***After***	***Before***	***After***	***Before***	***After***
Saline[c]	119±9	119±9	109±7	109±7	131±12	131±12
Phentolamine	170±8	187±7	152±7	122±10[a]	128±9	106±8
Propranolol[b]	160±8	113±5[a]	119±6	117±8	128±10	116±12
Prazosin	178±5	182±4	129±10	115±15[a]	185±40	175±41
Rauwolscine[c]	180±3	185±7	145±17	145±17	125±20	131±19

[a], The corresponding carotid conductances were not significantly different (P>0.05) for any of the treatments, but are not shown for the sake of clarity; [b], effect of the first dose, but the subsequent doses were similarly without significant effect; [c], given after phentolamine; [d], 30 or 100 µg kg^{-1} were similarly without significant effect; ***,** P<0.05 before *versus* after.

Systemic and haemodynamic changes to the agonists in the different groups of animals

As shown in Figure 6.1, 1 min intracarotid infusions of adrenaline, noradrenaline, phenylephrine and BHT933 produced dose-dependent decreases in external carotid conductance with a rank order of agonist potency of: adrenaline = noradrenaline >

phenylephrine ≥ BHT933. These effects were not accompanied by significant changes in heart rate or mean arterial blood pressure (data not shown).

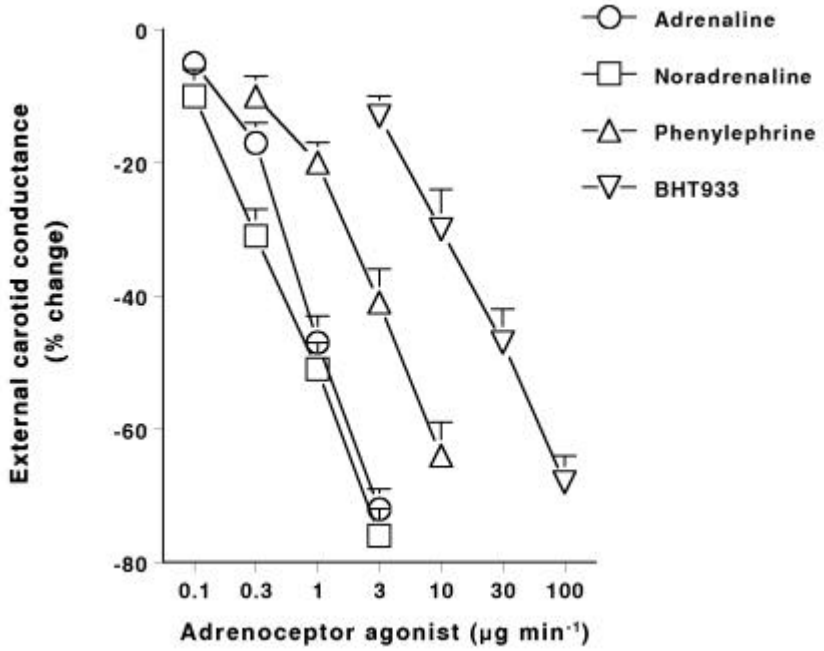


Figure 6.1. Percent changes in external carotid conductance (compared to baseline) produced by consecutive 1-min intracarotid infusions of adrenaline (n=15), noradrenaline (n=15), phenylephrine (n=18) and BHT933 (n=18) in anaesthetised, vagosympatectomised dogs.

Figure 6.2 shows that adrenaline and noradrenaline produced dose-dependent decreases in external carotid conductance which remained without significant changes after saline (maximal percent changes were: -72±3 and -76±4%, respectively). After phentolamine (2000 µg kg^{-1}), the vasoconstrictor responses to adrenaline and noradrenaline were abolished and, in both cases, a dose-dependent vasodilator effect was unmasked (maximal responses were: 76±12 and 22±5%, respectively). This vasodilator component was abolished by the subsequent administration of propranolol (1000 µg kg^{-1}; see Figure 6.2). Moreover, after rauwolscine (30 and 100 µg kg^{-1}) a partial blockade of the responses to adrenaline and noradrenaline was produced, and the subsequent administration of prazosin (100 µg kg^{-1}) abolished these responses. It may be noted that in the case of adrenaline a vasodilator component was unmasked, as observed with phentolamine (see above).

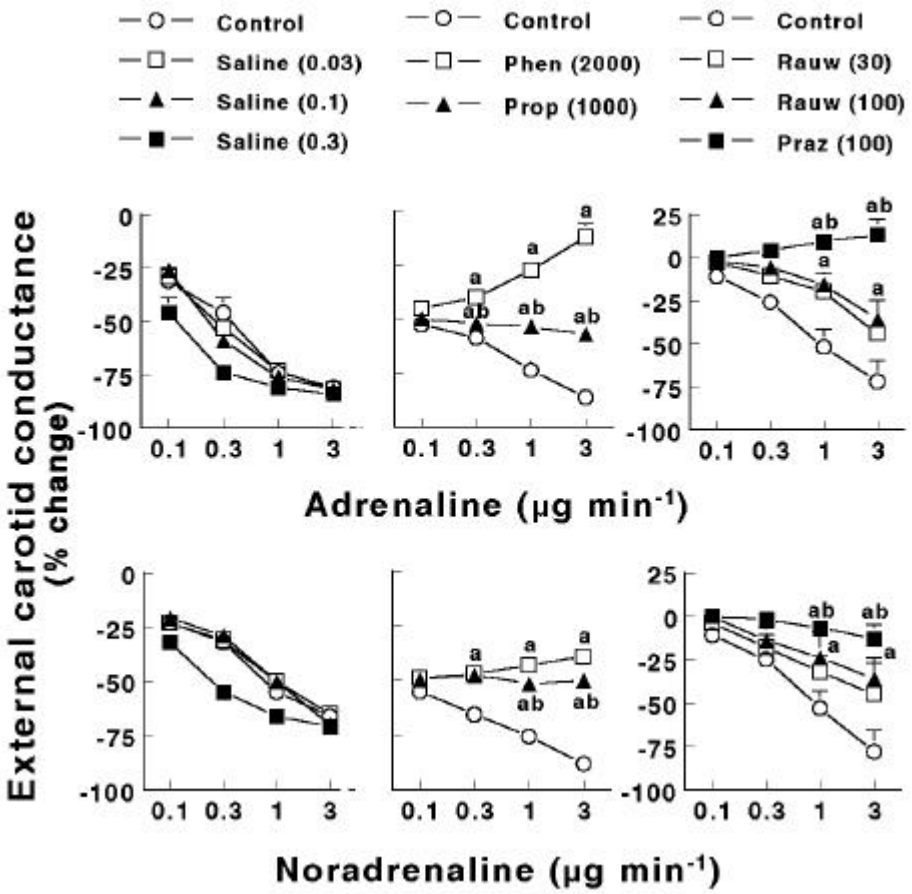


Figure 6.2. Effect of i.v. administration of saline (0.03, 0.1 and 0.3 ml kg^{-1}, n=3; *left panel*); phentolamine (Phen; 2000 µg kg^{-1}) followed by propranolol (Prop; 1000 µg kg^{-1}) (n=6; *middle panel*); or rauwolscine (Rauw; 30 and 100 µg kg^{-1}) followed by prazosin (Praz; 100 µg kg^{-1}) (n=6; *right panel)* on the responses to 1 min intracarotid infusions of adrenaline (*upper graphs*) and noradrenaline (*lower graphs*) in anaesthetised vagosympathectomised dogs. [a], Significantly different from the corresponding control response; [b], significantly different from the corresponding response after phentolamine (2000 µg kg^{-1}; *middle panel*) or rauwolscine (100 µg kg^{-1}; *right panel*).

Similarly, Figure 6.3 shows that intracarotid infusions of the adrenoceptor agonists, phenylephrine (α_1; 0.3-10 µg min^{-1}) and BHT933 (α_2; 3-100 µg min^{-1}) produced dose-dependent decreases in external carotid conductance (maximal percent changes were: -64±5 and -68±4%, respectively). After saline, the external carotid vasoconstrictor responses to these agonists remained without significant changes (see Figure 6.3, *left panel*). Moreover, after prazosin (100 µg kg^{-1}), the vasoconstrictor responses to phenylephrine were markedly blocked whilst those to BHT933 remained essentially unaltered (see Figure 6.3, *middle panel*). Similarly, after rauwolscine (100 and 300 µg kg^{-1}) the responses to BHT933 were markedly blocked whilst those to phenylephrine remained unaltered (see Figure 6.3, *right panel*). It is noteworthy

that after 100 µg kg^{-1} of rauwolscine, 300 µg kg^{-1} did not produce a further blockade on the responses to BHT933 (see Figure 6.3., *right panel*).

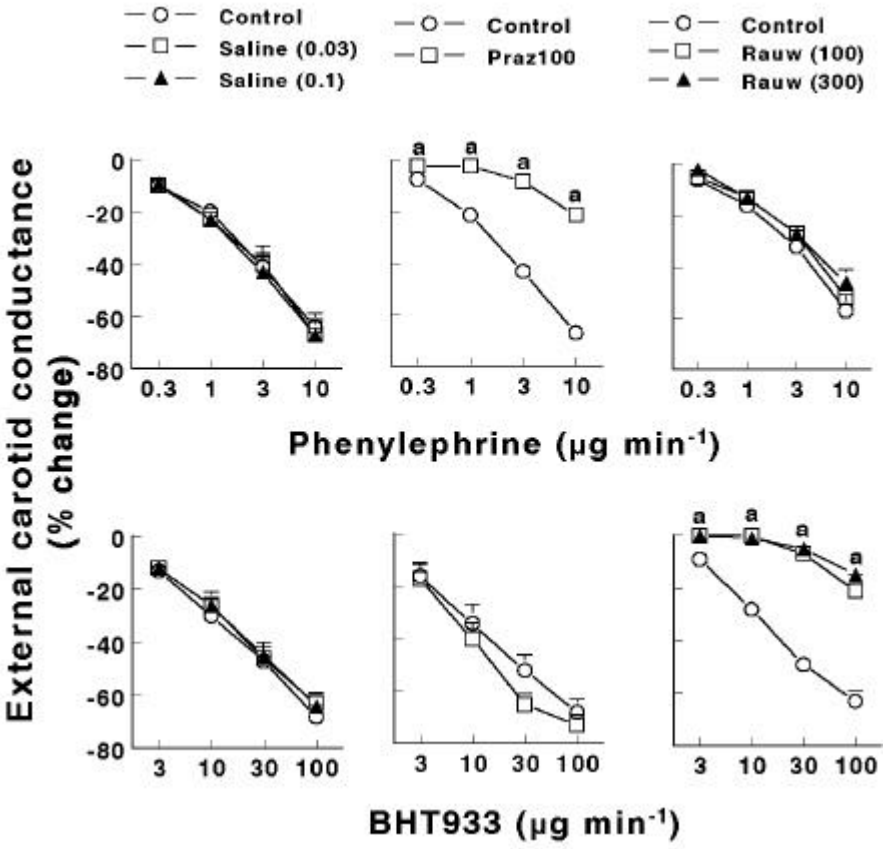


Figure 6.3. Effect of i.v. administration of saline (0.03 and 0.1 ml kg^{-1}, n=6; *left panel*), prazosin (Praz; 100 µg kg^{-1}, n=6; *middle panel*) or rauwolscine (Rauw; 100 and 300 µg kg^{-1}, n=6; *right panel*) on the external carotid vasoconstrictor responses elicited by consecutive 1 min intracarotid infusions of phenylephrine (*upper graphs*) and BHT933 (*lower graphs*) in anaesthetised vagosympathectomised dogs. [a], Significantly different from the corresponding control response.

Discussion

General

Several studies have demonstrated that stimulation of α-adrenoceptors produces contraction of isolated carotid artery rings, including those of the dog[189, 190], rabbit[241] and pig[192]. Others have shown *in vivo* that α-adrenoceptors mediate vasoconstriction in the carotid circulation of anaesthetised cats[184] and pigs[219]. However, these *in vivo* studies, instead of using adrenaline and/or noradrenaline (the endogenous ligands), employed synthetic agonists (e.g. clonidine) in their

pharmacological approach. Curiously enough, this has also been the case for the canine external carotid circulation. Thus, it has been shown that buspirone and ipsapirone (both anxiolytic agents) as well as ergotamine and dihydroergotamine (both antimigraine agents) produce external carotid vasoconstriction mediated by, respectively, α_1-[256] and α_2-[223] adrenoceptors. To the best of our knowledge, no study has reported the effects produced by adrenaline and noradrenaline on the canine external carotid bed. Thus, the significance of this experimental model in the development of antimigraine drugs[161, 162, 177, 216], coupled to the established heterogeneity of α_1 and α_2 adrenoceptors (see below), led us to pose a future problem, namely: the formal pharmacological characterisation of the subtypes of α_1- and α_2-adrenoceptors mediating canine external carotid vasoconstriction. Within this framework it should be firstly demonstrated whether this vascular bed is responsive to adrenaline and noradrenaline. If so, then the effects of agonists/antagonists at α_1 (e.g. phenylephrine/prazosin) and α_2 (e.g. BHT933/rauwolscine) adrenoceptors should be investigated. Indeed, the present study undertook this approach including the analysis of the effects by the classical adrenoceptor antagonists, phentolamine (α) and propranolol (β). The doses used of the above antagonists were sufficient to block their respective receptors[180, 219, 231]. Apart from the implications discussed below, this study shows that both α_1- and α_2-adrenoceptors mediate the vasoconstriction caused by adrenaline and noradrenaline within the external carotid circulation of anaesthetised vagosympathectomised dogs.

Systemic and carotid haemodynamic changes produced by different treatments

Treatment with saline or rauwolscine did not produce significant changes on systemic or carotid haemodynamics (see Table 6.1). In contrast, the significant hypotension produced after prazosin or phentolamine can be explained by blockade of α_1-adrenoceptors which regulate vascular tone and blood pressure[33, 44]. Similarly, the significant bradycardia produced after propranolol is most likely explained by blockade of cardiac β_1-adrenoceptors[17].

External carotid responses produced by adrenaline and noradrenaline

The vasoconstrictor responses to adrenaline and noradrenaline were reproducible as they were not significantly modified after three i.v. bolus injections of saline (Figure 6.2, *left panel*). Thus, the fact that the $\alpha_{1/2}$-adrenoceptor antagonist, phentolamine, abolished these responses unmasking a vasodilator component suggests the involvement of vasoconstrictor $\alpha_{1/2}$-adrenoceptors (Figure 6.2, *middle panel*). Since the subsequent administration of propranolol completely blocked this vasodilator component (Figure 6.2, *middle panel*), the involvement of vasodilator β-adrenoceptors is established. Although several lines of evidence have shown that β_1-adrenoceptors may mediate vasodilatation *in vivo* in the dog coronary artery[257] and rat choroidal vasculature[258], the β_1-adrenoceptor antagonist, metoprolol, did not block the above adrenaline-induced external carotidvasodilatator response (unpublished observations). Thus, the possible involvement of β_2- (rather than β_1) adrenoceptors is supported by the rank order of agonist vasodilator potency of adrenaline>>noradrenaline[259] (Figure 6.2). Obviously, this hypothesis requires further experiments. In any case, our results are in agreement with those reported in several human vascular beds (e.g. forearm or digital vessels), where α- and β-adrenoceptors have been shown to mediate vasoconstriction and vasodilatation, respectively [205, 260, 261].

Which α-adrenoceptor subtype(s) mediate(s) external carotid vasoconstriction?

The fact that rauwolscine partly blocked the responses to adrenaline and noradrenaline (Figure 6.2, *right panel*) may be explained by the capability of these catecholamines to stimulate both α_1- and α_2-adrenoceptors. Thus, after rauwolscine, the remaining stimulation of α_1-adrenoceptors may have overshadowed the blockade of α_2-adrenoceptors. Hence, the subsequent administration of prazosin abolished the remaining α_1-adrenoceptor-mediated responses. The vasodilator responses unmasked after prazosin (Figure 6.2, *right panel*) were smaller than those obtained after phentolamine (Figure 6.2, *middle panel*) perhaps because the latter is a much less selective antagonist. Thus, phentolamine may have blocked additional vasoconstrictor mechanisms resistant to rauwolscine and/or prazosin. In any case, the above results clearly suggest that both α_1- and α_2-adrenoceptors mediate external carotid

vasoconstriction. This suggestion is indeed reinforced when considering that the same doses of prazosin and rauwolscine selectively antagonised the responses to phenylephrine and BHT933 (see Figure 6.3), as previously shown in anaesthetised pigs[219]. A higher dose of prazosin (300 $\mu g\,kg^{-1}$; data not shown) or rauwolscine (300 $\mu g\,kg^{-1}$; see Figure 6.3) did not produce a further blockade of the responses to phenylephrine and BHT933, respectively.

Possible clinical implications

Finally, we would like to discuss the possible clinical implications of the present results in terms of the (neuro)vascular theory of migraine[160, 162, 215]. In this context, several experimental models have been developed to explain the efficacy of acutely acting antimigraine drugs[161, 162, 177, 216]. The predictive antimigraine value of these models seems to be higher in those considering the vasoconstriction of cranial extracerebral vascular beds such as the carotid vasculature [161, 166, 168]. Accordingly, some acute antimigraine agents, including the triptans (sumatriptan and the *second generation* triptans), ergot alkaloids (ergotamine and dihydroergotamine) and others (clonidine and isometheptene) produce carotid vasoconstriction in the dog and pig [166, 168, 177, 215, 262]. Interestingly, the canine external carotid vasoconstriction to ergotamine and dihydroergotamine involves 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors[177]. Thus, since both α_1- and α_2-adrenoceptors mediate vasoconstriction in the carotid circulation of dogs (present results) and pigs[219], it is tempting to hypothesise that selective agonists at these receptors (or their respective subtypes; see conclusion below) may have antimigraine potential. Evidently, this hypothesis will be validated with the advent of subtype-selective agonists at α_1- or α_2-adrenoceptors, which are not yet available.

In conclusion, the canine external carotid vasoconstrictor responses to adrenaline and noradrenaline are mediated by both α_1- and α_2-adrenoceptors. Moreover, both catecholamines produced external carotid vasodilatation after blockade of α_1- and α_2-adrenoceptors with phentolamine, which most likely involves β- (probably β_2) adrenoceptors. Further studies, which fall beyond the scope of the present investigation, will be required to ascertain the specific subtypes of α_1- (α_{1A}, α_{1B}, α_{1D}), α_2- (α_{2A}, α_{2B}, α_{2C}), and β- (β_1, β_2, β_3, β_4)-adrenoceptors mediating the above mentioned vascular responses.

Acknowledgements

The skillful technical assistance of Mr. Arturo Contreras is gratefully acknowledged. A part of this project has been supported by the Foundation 'Vereniging Trustfonds Erasmus Univestiteit Rotterdam' and the 'Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)', both from The Netherlands.

Chapter 7

The role of several a_1- and a_2-adrenoceptor subtypes mediating vasoconstriction in the canine external carotid circulation

Summary It has recently been shown that both α_1- and α_2-adrenoceptors mediate vasoconstriction in the canine external carotid circulation. The present study set out to identify the specific subtypes (α_{1A}, α_{1B} and α_{1D} as well as α_{2A}, α_{2B} and α_{2C}) mediating the above response. Consecutive 1 min intracarotid infusions of phenylephrine (α_1-adrenoceptor agonist) and BHT933 (α_2-adrenoceptor agonist) produced dose-dependent decreases in external carotid blood flow, without affecting mean arterial blood pressure or heart rate. The responses to phenylephrine were selectively antagonised by the antagonists, 5-methylurapidil (α_{1A}) or BMY7378 (α_{1D}), but not by L-765,314 (α_{1B}), BRL44408 (α_{2A}), imiloxan (α_{2B}) or MK912 (α_{2C}). In contrast, only BRL44408 or MK912 affected the responses to BHT933. The above results support our contention that mainly the α_{1A}, α_{1D}, α_{2A} and α_{2C}-adrenoceptor subtypes mediate vasoconstriction in the canine external carotid circulation.

Based on: Willems *et al., Br. J. Pharmacol. (2000)* ***In Press***.

Introduction

Although the underlying mechanisms for the initiation of a migraine attack are poorly understood, the clinical efficacy of several acute antimigraine agents can be explained by their ability to produce a cranio-selective vasoconstriction[162, 168, 215]. This has been shown for the triptans (e.g. sumatriptan) and the ergots (ergotamine and dihydroergotamine) in dogs as well as pigs[167, 177, 215, 240]. In this respect, the carotid vasoconstrictor responses to sumatriptan are exclusively mediated by serotonin 5-HT_{1B} receptors[178, 217], whilst those to the ergots seem to involve both 5-HT_{1B} and α_2-adrenoceptors[177].

Since both α_1- and α_2-adrenoceptors mediate the vasoconstriction to adrenaline and noradrenaline in the canine external carotid circulation[246], the present study was designed to identify the specific subtypes (α_{1A}, α_{1B} and α_{1D} as well as α_{2A}, α_{2B} and α_{2C}) mediating the above response. For this purpose, the external carotid vascular bed vasoconstrictor responses to the α-adrenoceptor agonists, phenylephrine (α_1) and BHT933 (α_2), were analysed before and after administration of selective antagonists at α_1- and α_2-adrenoceptor subtypes (see Table 1.2). The results obtained may open new avenues for the development of future antimigraine agents.

Materials and methods

General

Experiments were carried out in a total of 42 dogs (16-30 kg) not selected for breed or sex. The animals were anaesthetised with an intravenous (i.v.) bolus injection of sodium pentobarbitone (30 mg kg^{-1}) and additional amounts (1 mg kg^{-1}, i.v.) were provided when required throughout the experiment. All dogs were intubated with an endotracheal tube and artificially respired with room air; for this purpose, a Palmer ventilation pump was used at a rate of 20 strokes min^{-1} and a stroke volume of 13-16 ml kg^{-1}, as previously established by Kleinman & Radford[254]. Catheters were placed in the inferior vena cava *via* a femoral vein for the administration of antagonists and in the aortic arch *via* a femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of blood pressure.

After administration of each antagonist dose, the venous catheter was flushed with 3 ml of saline. Mean blood pressure (MAP) was calculated from the systolic (SAP)

and diastolic (DAP) arterial pressures: MAP = DAP + (SAP-DAP)/3. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co., Quincy, MA, U.S.A.) triggered from the blood pressure signal. The common carotid artery was dissected free and the corresponding internal carotid and occipital arteries were ligated. Bilateral cervical vagosympathectomy was systematically performed in order to prevent possible baroreceptor reflexes produced by the intracarotid infusions of phenylephrine or BHT933. These compounds were administered into the carotid artery by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.) with a catheter inserted into the right cranial thyroid artery. Thereafter, an ultrasonic flow probe (4 mm R-Series) connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, N.Y., U.S.A.) was placed around the common carotid artery and the flow through this artery was considered as the external carotid blood flow[255]. Blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The body temperature of the animals was maintained between 37-38°C.

Experimental protocol

After the animals (n=42) had been in a stable haemodynamic condition for at least 60 min, baseline values of mean blood pressure, heart rate and external carotid blood flow were determined. After collecting these data, the animals were divided into two groups. The first group (n=36) received consecutive intracarotid infusions (1 ml min^{-1}; during 1 min) of phenylephrine (0.3, 1, 3 and 10 µg min^{-1}) and BHT933 (3, 10, 30 and 100 µg min^{-1}). At this point, the dogs were subdivided into six subgroups (n=6 each) and the effects produced by the above infusions of phenylephrine and BHT933 were elicited again after i.v. treatment with each dose of either: (*i*) 5-methylurapidil (100 and 300 µg kg^{-1}); (*ii*) L-765,314 (100 and 300 µg kg^{-1}); (*iii*) BMY7378 (100 and 300 µg kg^{-1}); (*iv*) BRL44408 (300 and 1000 µg kg^{-1}); (*v*) imiloxan (300 and 1000 µg kg^{-1}); or (*vi*) MK912 (100 and 300 µg kg^{-1}).

The second group (n=6) was subdivided into two subgroups (n=3 each). The first subgroup received consecutive intracarotid infusions of phenylephrine (0.3, 1, 3 and 10 µg min^{-1}) as previously described. Then, the responses elicited by these infusions of phenylephrine were elicited again after i.v. administration of 5-methylurapidil (100 µg kg^{-1}) and, subsequently, BMY7378 (100 µg kg^{-1}). Likewise, the second subgroup

received consecutive intracarotid infusions of BHT933 (3, 10, 30 and 100 $\mu g\ min^{-1}$) and the responses produced were elicited again after i.v. administration of BRL44408 (1000 $\mu g\ kg^{-1}$) and, subsequently, MK912 (100 $\mu g\ kg^{-1}$).

The dose-intervals between the different doses of agonists ranged between 5 and 15 min, as in each case we waited until the external carotid blood flow had returned completely to baseline values. Moreover, after administration of a specific dose of an antagonist, a period of 15-25 min was allowed to elapse before the responses to the respective agonists were elicited again. The dosing with the agonists was sequential, whereas that with the antagonists was cumulative. The Ethical Committee of the CINVESTAV-IPN dealing with the use of animals in scientific experiments approved the protocols of the present investigation.

Data presentation and statistical analysis

All data in the text, figures and tables are presented as mean±s.e.mean. The peak changes in external carotid blood flow were expressed as percent change from baseline. The difference between the variables within one group of animals was compared using an analysis of variance (randomised block design) followed by the Student-Newman-Keuls test[222]. Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

Apart from the anaesthetic (sodium pentobarbitone), the compounds used in this study were: L-phenylephrine hydrochloride, BHT933, 5-methylurapidil and BMY7378 dihydrochloride (all purchased from RBI, Zwijndrecht, The Netherlands); L-765,314 (gift: Merck & Co., Inc., West Point, PA, U.S.A.); BRL44408 (gift: Dr. T.J. Verbeuren; Servier, Suresnes, France); imiloxan hydrochloride (gift: Dr. R. Eglen; Roche Bioscience, Palo Alto, CA, U.S.A.) and MK912 (gift: Dr. W.L. Henckler; Merck & Co.; New Jersey, NJ, U.S.A.). All drugs were dissolved in physiological saline; a short period of heating was needed to dissolve 5-methylurapidil or L-765,314 (acidified to pH=6.8-7.0 with 0.1 M HCl). The doses of the antagonists refer to their respective salts, whilst those of the agonists refer to their free base.

Results

Systemic and carotid haemodynamic effects of the different treatments

Baseline values of heart rate, mean arterial blood pressure and external carotid blood flow in the 42 dogs were, respectively, 131±4 beats min^{-1}, 132±3 mmHg and 134±9 ml min^{-1}. The systemic and carotid haemodynamic values before and 15-25 min after i.v. administration of the different compounds are shown in Table 7.1. None of the compounds produced significant changes under these conditions (P>0.05). However, *immediately* after its administration, BRL44408 (1000 µg kg^{-1}) produced a transient, though significant, increase (35±10%) in mean blood pressure (from 128±13 mmHg to 170±14 mmHg; n=6). This vasopressor effect, which was not accompanied by significant changes in heart rate and external carotid blood flow or conductance (data not shown), returned to baseline values after 25 min. In contrast, no immediate haemodynamic changes were observed in the other subgroups.

Table 7.1. Absolute values of heart rate, mean arterial blood pressure and external carotid blood flow and conductance in anaesthetised dogs before and after i.v. treatment with 5-methylurapidil (300 µg kg^{-1}), L-765,314 (300 µg kg^{-1}), BMY7378 (300 µg kg^{-1}), BRL44408 (1000 µg kg^{-1}), imiloxan (300 µg kg^{-1}) or MK912 (300 µg kg^{-1}).

Treatment (n=6 each)	**Heart rate (beat min^{-1})**		**Mean arterial blood pressure (mmHg)**		**External carotid blood flow (ml min^{-1})**		**External carotid conductance (ml min^{-1} $mmHg^{-1}$)**	
	Before	***After***	***Before***	***After***	***Before***	***After***	***Before***	***After***
5-Methylurapidil	131±5	117±7	136±9	122±8	146±17	103±10	111±18	86±9
L-765,314	130±5	131±5	138±8	138±7	144±28	144±30	112±30	111±30
BMY7378	142±10	134±10	129±8	122±8	124±16	126±20	99±16	106±21
BRL44408	117±13	112±11	128±13	148±10	141±40	102±27	127±48	51±21
Imiloxan	109±5	118±6	131±7	128±10	106±14	131±16	84±15	108±20
MK912	158±12	163±12	133±5	122±11	149±11	135±7	113±9	115±10

Note that none of the above treatments produced significant changes ($P>0.05$). The lower doses of the above compounds were similarly without significant effect. Non-significant effects were also produced by the administration of 5-methylurapidil followed by BMY7378 or of BRL44408 followed by MK912 (not shown).

Systemic and haemodynamic changes to the agonists in the different groups of animals

Intracarotid infusions of phenylephrine (α_1-adrenoceptor agonist; 0.3-10 µg min^{-1}; Figure 7.1) and BHT933 (α_2-adrenoceptor agonist; 3-100 µg min^{-1}; Figure 7.2) produced dose-dependent decreases in external carotid blood flow (maximal percent changes were: -64±5 and -68±4%, respectively). These responses to phenylephrine and BHT933, which were not accompanied by significant changes in heart rate or blood pressure (not shown), have been previously demonstrated to remain unaffected after two i.v. bolus injections of physiological saline[246].

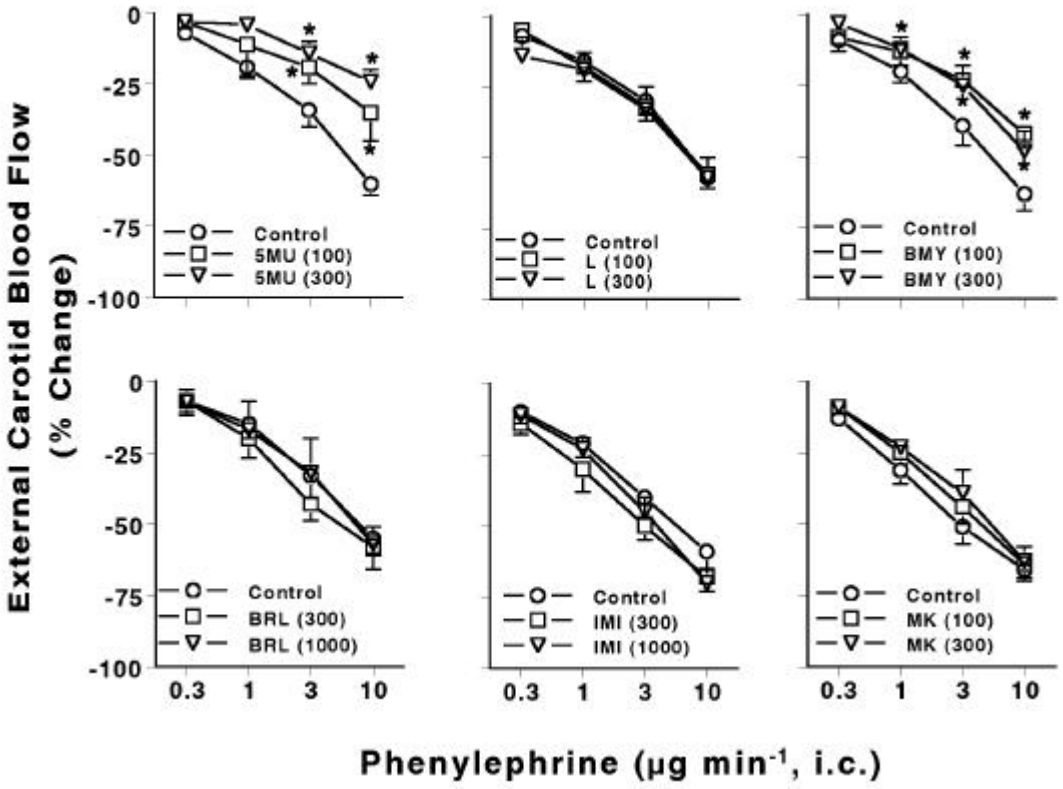


Figure 7.1. The effect of subsequent i.v. administration of 5-methylurapidil (5MU; 100 and 300 µg kg^{-1}), L-765,314 (L; 100 and 300 µg kg^{-1}), BMY7378 (BMY; 100 and 300 µg kg^{-1}), BRL44408 (BRL; 300 and 1000 µg kg^{-1}), imiloxan (IMI; 300 and 1000 µg kg^{-1}) or MK912 (MK; 100 and 300 µg kg^{-1}) on the external carotid vasoconstrictor responses produced by consecutive 1 min intracarotid (i.c.) infusions of phenylephrine in anaesthetised dogs. *, P<0.05 vs. corresponding dose in control curve.

Figures 7.1 and 7.2 also show the effects of subsequent i.v. administration of the antagonists, 5-methylurapidil (α_{1A}), L-765,314 (α_{1B}), BMY7378 (α_{1D}), BRL44408 (α_{2A}), imiloxan (α_{2B}) or MK912 (α_{2C}) on the external carotid vasoconstrictor

responses produced by the above agonists. The vasoconstrictor responses to phenylephrine remained unaffected after L-765,314 (up to a dose of 300 µg kg^{-1}), but were significantly attenuated after 5-methylurapidil or BMY7378 (both 100 µg kg^{-1}) (maximal responses: -35±10 and -42±5%, respectively; Figure 7.1, *upper panel*). The blockade produced by 100 µg kg^{-1} of these antagonists was maximal, since a higher dose (300 µg kg^{-1}) of either antagonist did not produce a further blockade (maximal responses: -24±4 and -48±7%, respectively; Figure 7.1, *upper panel*). Similarly, a higher dose of L-765,314 (1000 µg kg^{-1}) did not affect the phenylephrine-induced vascular responses (data not shown). It is worth noting that the blockade produced by the above antagonists was specific, as their corresponding doses did not significantly modify the external carotid vasoconstrictor responses to BHT933 (Figure 7.2, *upper panel*).

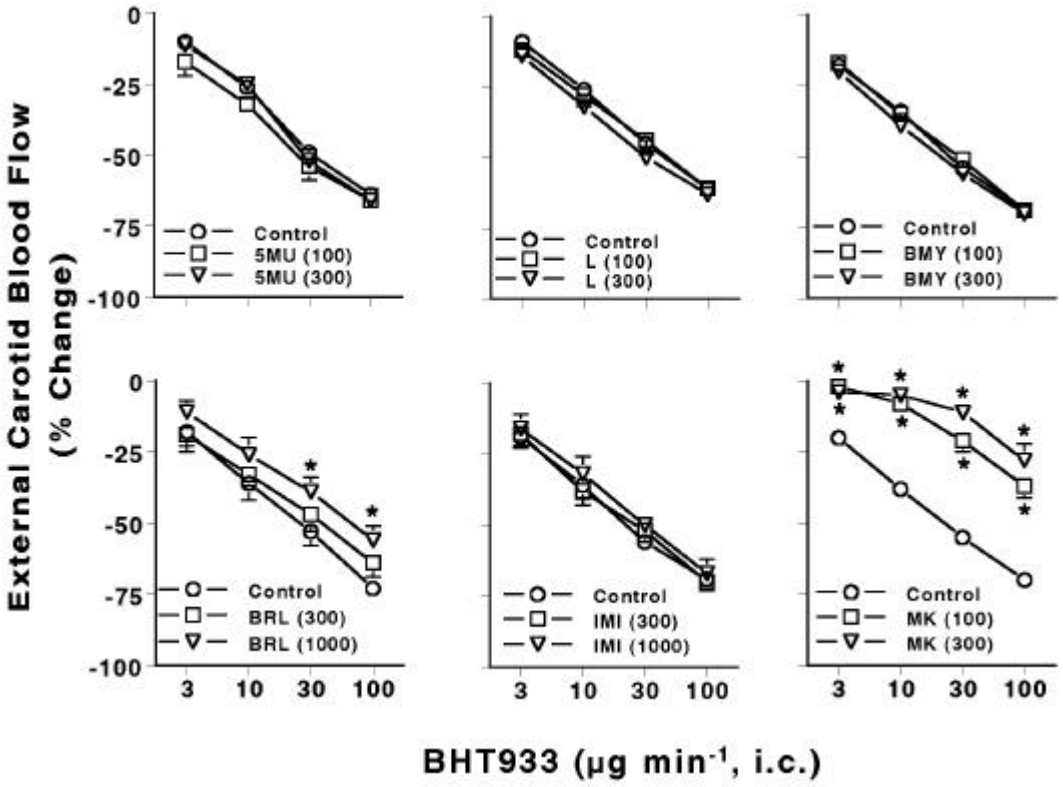


Figure 7.2. The effect of subsequent i.v. administration of 5-methylurapidil (5MU; 100 and 300 µg kg^{-1}), L-765,314 (L; 100 and 300 µg kg^{-1}), BMY7378 (BMY; 100 and 300 µg kg^{-1}), BRL44408 (BRL; 300 and 1000 µg kg^{-1}), imiloxan (IMI; 300 and 1000 µg kg^{-1}) or MK912 (MK; 100 and 300 µg kg^{-1}) on the external carotid vasoconstrictor responses produced by consecutive 1 min intracarotid (i.c.) infusions of BHT933 in anaesthetised dogs. *, P<0.05 vs. corresponding dose in control curve.

Moreover, the vasoconstrictor responses to BHT933 remained unaffected after imiloxan (up to 1000 $\mu g\,kg^{-1}$), but were significantly attenuated after BRL44408 (1000 $\mu g\,kg^{-1}$) or MK912 (100 $\mu g\,kg^{-1}$) (maximal responses: -56±5 and -37±4%, respectively; Figure 7.2, *lower panel*). The blockade produced by 100 $\mu g\,kg^{-1}$ of MK912 was maximal, since a higher dose (300 $\mu g\,kg^{-1}$) did not produce a further blockade (maximal response: -28±6%). It should be highlighted that the antagonism produced by BRL44408 or MK912 (at the doses mentioned above) was specific, as they did not significantly modify the external carotid vasoconstrictor responses to phenylephrine (Figure 7.1, *lower panel*). Based on its affinity profile at the different α_2-adrenoceptor subtypes (see Table 1.2), we did not test a higher dose of BRL44408.

It should be pointed out that the decreases in external carotid blood flow produced by phenylephrine or BHT933 were similar to those observed in the corresponding vascular conductance (data not shown).

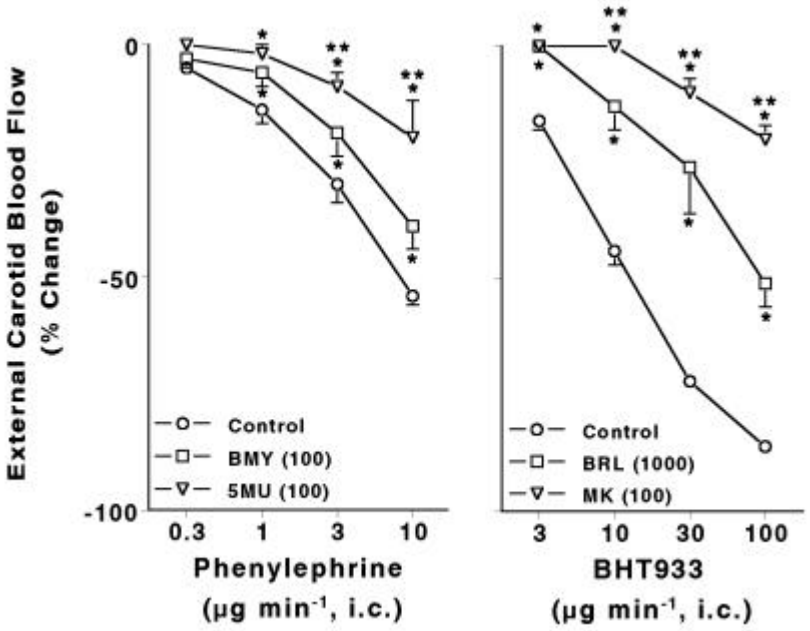


Figure 7.3. The effect of subsequent i.v. administration of: (i) BMY7378 (BMY; 100 $\mu g\,kg^{-1}$) and 5-methylurapidil (5MU; 100 $\mu g\,kg^{-1}$) on the external carotid vasoconstrictor responses to intracarotid (i.c.) infusions of phenylephrine (*left panel*); or (ii) BRL44408 (BRL; 1000 $\mu g\,kg^{-1}$) and MK912 (MK; 100 $\mu g\,kg^{-1}$) on the vasoconstrictor responses to i.c. infusions of BHT933 (*right panel*) in anaesthetised dogs. *, $P<0.05$ vs. corresponding dose of agonist in control curve. **, $P<0.05$ vs. corresponding dose of agonist after administration of the first antagonist (*i.e.* BMY7378 in the left panel or BRL44408 in the right panel).

Figure 7.3 (*left panel*) shows that the vasoconstrictor responses to phenylephrine were significantly attenuated by BMY7378 (100 $\mu g\,kg^{-1}$), as previously observed

(Figure 7.1, *upper panel*). It is noteworthy that the subsequent administration of 5-methylurapidil (100 $\mu g\,kg^{-1}$) produced a further blockade of the phenylephrine-induced responses over the one previously produced by BMY7378 (Figure 7.3, *left panel*). By analogy, Figure 7.3 (*right panel*) shows that the responses to BHT933 were significantly attenuated by BRL44408 (1000 $\mu g\,kg^{-1}$), as previously shown (Figure 7.2, *lower panel*). The subsequent administration of MK912 (100 $\mu g\,kg^{-1}$) produced a further blockade of the responses to BHT933 over the one previously produced by BRL44408 (Figure 7.3, *right panel*).

Discussion

General

According to the International Union of Pharmacology Subcommittee on Nomenclature for Adrenoceptors, α-adrenoceptors have been divided into α_1- and α_2-adrenoceptors with subdivisions into α_{1A}, α_{1B} and α_{1D} and α_{2A}, α_{2B} and α_{2C} subtypes, respectively[17, 26, 31, 33, 245]. We recently demonstrated that both α_1- and α_2-adrenoceptors mediate vasoconstrictor responses in the porcine[219] as well as canine[246] carotid arterial bed. The present study set out to identify which α_1- and α_2-adrenoceptor subtypes mediate this latter vasoconstrictor response, employing antagonists with a moderate to high subtype selectivity: 5-methylurapidil (α_{1A}), L-765,314 (α_{1B}), BMY7378 (α_{1D}), BRL44408 (α_{2A}), imiloxan (α_{2B}) and MK912 (α_{2C}); Table 1.2. Our results show that mainly α_{1A}- and α_{1D}-adrenoceptors mediate the canine external carotid vasoconstrictor responses to phenylephrine, whereas those to BHT933 are mainly mediated by α_{2A}- and α_{2C}-adrenoceptors. Admittedly, the differences in antagonist potency observed against phenylephrine- and BHT933-induced vascular responses may be partly due to differences in the metabolism of antagonists or by affinity differences between canine and human α_1- and α_2-adrenoceptor subtypes; however, these data are not available at present.

Systemic and carotid haemodynamic effects of the treatments

Apart from the α_{2A}-adrenoceptor antagonist, BRL44408, none of the antagonists used in this investigation produced any significant haemodynamic response. Even with BRL44408, the immediate increase in blood pressure was short lasting. This effect

may have been due to a direct activation of vascular α_1-adrenoceptors, as shown with other α_2-adrenoceptor antagonists in anaesthetised rats[263]. Alternatively, such a vasopressor effect could also be explained by a blockade of prejunctional α_2-adrenoceptors leading to an enhancement of neuronal release of noradrenaline[201].

Role of α_1- and α_2-adrenoceptor subtypes in canine external carotid vasoconstriction

The blockade produced by 5-methylurapidil and BMY7378 (both 100 $\mu g\,kg^{-1}$) of the responses to phenylephrine, being maximal as a higher dose (300 $\mu g\,kg^{-1}$) did not produce a further blockade, was selective as these compounds failed to antagonise the responses to BHT933. These results favour a predominant involvement of α_{1A}- and α_{1D}-adrenoceptors. Admittedly, since 5-methylurapidil shows a reasonable affinity at α_{1A}- and α_{1D}-adrenoceptors (pK_i: 9.0 and 7.9, respectively), we cannot categorically exclude a possible blockade of phenylephrine-induced responses resulting from antagonism of both receptors. However, the fact that 5-methylurapidil produced a further blockade of the responses to phenylephrine after a high dose of BMY7378 suggests the additional role of α_{1A}-adrenoceptors. Thus, after BMY7378 and 5-methylurapidil (both 100 $\mu g\,kg^{-1}$) the maximal response to phenylephrine (-20±8%; Figure 7.3) did not significantly differ from that after prazosin (maximal response: -11±3%[246]). In the light of these findings, the role of α_{1B}-adrenoceptors seems questionable, a view consistent with the fact that L-765,314 (up to 1000 $\mu g\,kg^{-1}$; data not shown) did not affect the responses to phenylephrine.

The blockade produced by BRL44408 (1000 $\mu g\,kg^{-1}$) and MK912 (100 and 300 $\mu g\,kg^{-1}$) of the responses to BHT933 was selective as these compounds failed to antagonise the responses to phenylephrine. It should be pointed out that the blockade after 100 $\mu g\,kg^{-1}$ of MK912 was maximal, since a higher dose (300 $\mu g\,kg^{-1}$) did not produce a further blockade. We decided not to test a higher dose of BRL44408, since it displays a reasonable affinity at α_{2A}-adrenoceptors (pK_i: 8.2) and the doses used (300 and 1000 $\mu g\,kg^{-1}$) should be sufficient to block α_{2A}-adrenoceptors. These results imply the coexistence of α_{2A}- and α_{2C}-adrenoceptor subtypes. This suggestion gains weight when considering that MK912 produced a further blockade of the responses to BHT933 (particularly at its highest dose) after the blockade produced by a high dose

of BRL44408. Accordingly, after BRL44408 (1000 µg kg^{-1}) and MK912 (100 µg kg^{-1}) the maximal response to BHT933 (-20±3%; Figure 7.3) did not significantly differ from that after rauwolscine (maximal response: -15±3;[246]). Considering the above, the role of α_{2B}-adrenoceptors seems unlikely, a view reinforced by the fact that imiloxan (up to 1000 µg kg^{-1}) failed to block the responses to BHT933.

Resemblance of the canine external carotid α_{1A}, α_{1D}, α_{2A} and α_{2C}-adrenoceptors to other contractile α_1- and α_2-adrenoceptor subtypes

As reviewed by Vargas & Gorman[44] as well as Docherty[33], a number of studies shows that both α_1- and α_2-adrenoceptor subtypes can mediate smooth muscle contraction. Some of the pharmacological preparations employed include rat *vas deferens* (α_{1A}[264]), rat aorta (α_{1D}[152]), porcine common digital artery (α_{2A}[265]) and dog saphenous vein (α_{2C}[266]). Other blood vessels suggest a functional coexistence α_{1A}- and α_{1D}-adrenoceptor subtypes, e.g. rat renal artery[267]. The α_{1B} and α_{2B} subtypes, although not involved in canine external carotid vasoconstriction, seem to mediate vasoconstriction of the rabbit cutaneous resistance arteries (α_{1B}[268]) and the rat kidney (α_{2B}[33]).

In a number of tissues, admittedly, α_2-adrenoceptors contribute to a predominantly α_1-adrenoceptor mediated response[33]. However, to the best of our knowledge, the present study seems to be one of the first to show *in vivo* the functional role of specific α_1 (α_{1A} and α_{1D})- and α_2 (α_{2A} and α_{2C})-adrenoceptor subtypes mediating vasoconstriction in a vascular preparation (the canine external carotid bed).

Possible clinical implications

To date, all acutely-acting antimigraine agents, including the triptans and the ergots alkaloids, produce a potent vasoconstriction in the carotid circulation of dogs [166, 177, 269] and pigs[161, 167]. In contrast to the well-established role of serotonin 5-HT$_{1B}$ receptors in the carotid vasoconstrictor effects of the triptans[178, 217], the ergots seem to involve both 5-HT$_{1B}$ and α_2-adrenoceptors[177]. However, irrespective to the mechanisms involved, a selective vasoconstriction within the

carotid circulation is an important property of antimigraine drugs. Therefore, we submit that the development of selective agonists at α_{1A}, α_{1D}, α_{2A} and α_{2C}-adrenoceptor subtypes may have potential therapeutic usefulness in the treatment of migraine.

Taken together, the present results suggest that both α_{1A}- and α_{1D}-adrenoceptors mediate the canine external carotid vasoconstrictor responses to phenylephrine, while those to BHT933 are mainly mediated by α_{2A}- and α_{2C}-adrenoceptors.

Acknowledgements

The skilful technical assistance of Mr. Arturo Contreras Bustos is gratefully acknowledged. The authors also thank the pharmaceutical companies (see Drugs section), CONACyT (Mexico), the 'Vereniging Trustfonds Erasmus Univestiteit Rotterdam' and the 'Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)' for their support.

Chapter 8

Pharmacological profile of the mechanisms involved in the external carotid vascular effects of the antimigraine agent isometheptene in anaesthetised dogs

Abstract The present study set out to investigate the external carotid vascular effects of isometheptene in vagosympathectomised dogs, anaesthetised with pentobarbital. 1 min intracarotid (intra-arterial; i.a.) infusions of isometheptene (10, 30, 100 and 300 $\mu g\ min^{-1}$) produced dose-dependent decreases in external carotid blood flow, without affecting blood pressure or heart rate. The vasoconstrictor responses to 100 and 300 $\mu g\ min^{-1}$ of isometheptene were clearly attenuated in animals pretreated with reserpine (5000 $\mu g\ kg^{-1}$). Moreover, after prazosin (an α_1-adrenoceptor antagonist; 100 $\mu g\ kg^{-1}$), the responses to isometheptene remained unaltered in either untreated or reserpine-treated dogs. In contrast, the responses to isometheptene were attenuated by rauwolscine (an α_2-adrenoceptor antagonist; 300 $\mu g\ kg^{-1}$) in untreated animals, and were practically abolished in reserpine-treated dogs. Further investigation into the specific α_2-adrenoceptor subtypes, using selective antagonists, showed that BRL44408 (α_{2A}) and MK912 (α_{2C}) markedly attenuated this response, while imiloxan (α_{2B}) was ineffective. The involvement of 5-HT_{1B} and 5-HT_{1D} receptors seems highly unlikely since antagonists at 5-HT_{1B} (SB224289) and 5-HT_{1D} (BRL15572) receptors (both at 300 $\mu g\ kg^{-1}$) were ineffective. On this basis, it is concluded that isometheptene-induced canine external carotid vasoconstriction is mediated by both indirect (a tyramine-like action) and direct (acting at receptors) mechanisms, which mainly involve α_{2A}- and α_{2C}-adrenoceptors. The involvement of α_1- and α_{2B}-adrenoceptors as well as 5-$HT_{1B/1D}$ receptors seems limited, if any.

Based on: Willems *et al., Naunyn-Schmied. Arch. Pharmacol. (2000)* **Submitted**

Introduction

Based on several animal studies, it has been suggested that vasoconstriction in the carotid circulation is predictive for antimigraine activity[166, 216]. Indeed, the triptans (e.g. sumatriptan) and the ergots (ergotamine and dihydroergotamine) produce potent vasoconstriction in porcine (arteriovenous anastomoses), rabbit and canine (external) carotid vasculature[177, 215]. Whereas the sumatriptan-induced carotid vasoconstriction in anaesthetised dogs and pigs is mainly mediated by 5-HT_{1B} receptors[178, 215, 217], the responses to the ergots in anaesthetised dogs also involve α_2-adrenoceptors[177]. We have shown that both α_1- and α_2-adrenoceptors mediate vasoconstriction in the carotid vasculature of anaesthetised pigs[219] and dogs[246]. The pharmacological profile of α_1- and α_2-adrenoceptors mediating canine external carotid vasoconstriction resembles that of the $\alpha_{1A/D}$- and $\alpha_{2A/C}$-adrenoceptor subtypes, respectively[270].

In the light of the above developments, it is important to examine the pharmacological properties isometheptene (for chemical formulae, see Figure 8.1), which is employed, either alone or in combination with an analgesic (acetaminophen, paracetamol) and a sedative (dichloralphenazone), in the treatment of migraine[271-275].

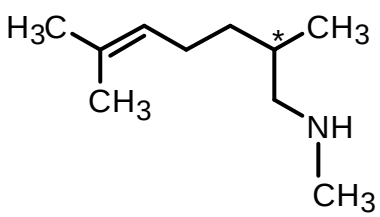


Figure 8.1. Chemical formulae of isometheptene (methyl-iso-octenylamine); a racemic mixture of two stereoisomers (chiral centre denoted by asterisks).

It is believed that isometheptene owes its therapeutic effect to a sympathomimetic action leading to cranial blood vessel vasoconstriction, which is observed at dose levels below those affecting arterial blood pressure[272]. Indeed, we have also reported that isometheptene can constrict carotid arteriovenous anastomoses in anaesthetised cats[212]. Therefore, the present study was designed to investigate whether isometheptene can produce canine external carotid vasoconstriction in a model predictive for antimigraine activity and, if so, to elucidate its mechanism of

action using different antagonists at α_1-, α_2-adrenergic and 5-$HT_{1B/1D}$ receptor subtypes (Table 1.2) and the monoamine depletor reserpine.

Materials and methods

General

Experiments were carried out in a total of 59 dogs (15-31 kg^{-1}) not selected for breed or sex. The animals were anaesthetised with an intravenous (i.v.) bolus injection of sodium pentobarbitone (30 mg kg^{-1}) and additional amounts (1 mg kg^{-1}, i.v.) were provided when required throughout the experiment. All dogs were intubated with an endotracheal tube and artificially respired with room air; for this purpose, a Palmer ventilation pump was used at a rate of 20 strokes min and a stroke volume of 13-16 ml kg^{-1}, as previously established by Kleinman & Radford[254]. Catheters were placed in the inferior *vena cava via* a femoral vein for the administration of antagonist doses and in the aortic arch *via* a femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of blood pressure. After the administration of each treatment (vehicle or antagonists), the venous catheter was flushed with 3 ml of saline. Mean blood pressure (MAP) was calculated from the systolic (SAP) and diastolic (DAP) arterial pressures: MAP = DAP + (SAP-DAP)/3. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co., Quincy, MA, U.S.A.) triggered from the arterial blood pressure signal. The common carotid artery was dissected free and the corresponding internal carotid and occipital arteries were ligated. Cervical vagosympathectic trunks were cut in order to prevent possible baroreceptor reflexes produced by isometheptene. Thereafter, an ultrasonic flow probe (4 mm R-Series), connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, N.Y., U.S.A.), was placed around the common carotid artery and the blood flow through this artery was considered as the external carotid blood flow[255]. A catheter was inserted into the cranial thyroid branch of the common carotid artery for infusions (1 ml min, for 1 min) of isometheptene using a Harvard model 901 pump (Harvard Apparatus Co. Inc., Millis, MA, U.S.A.). Blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The body temperature of the animals was maintained between 37-38°C.

Experimental protocol

After the animals had been in a stable haemodynamic condition for at least 60 min, baseline values of mean blood pressure, heart rate and external carotid blood flow were determined. The animals were then divided into two groups: the first remained untreated (n=48) and the second was pretreated with reserpine (5 mg kg^{-1}, i.p.) 24 h prior to the start of experiments (n=11). The animals in first group were further divided into eight subgroups (n=6 each), receiving i.v. infusions (1 ml min^{-1}, during 5 min) of vehicle (physiological saline; 0.03 ml kg^{-1}), prazosin (100 µg kg^{-1}), rauwolscine (300 µg kg^{-1}), BRL44408 (1000 µg kg^{-1}), imiloxan (1000 µg kg^{-1}), MK912 (300 µg kg^{-1}), SB224289 (300 µg kg^{-1}) or BRL15572 (300 µg kg^{-1}). The reserpinised animals (second group) received either vehicle (n=5), prazosin (n=3) or rauwolscine (n=3). Subsequently, after a waiting period of 15 min, each animal received consecutive intracarotid infusions (1 ml min^{-1}; during 1 min) of isometheptene (10, 30, 100 and 300 µg min^{-1}) and the changes in systemic and carotid haemodynamic variables were determined. The dose-intervals between the different doses of isometheptene ranged between 5 and 15 min, as in each case we waited until the external carotid blood flow had returned to baseline values. Moreover, after administration of a specific dose of an antagonist, a period of 15-25 min was allowed to elapse before the responses to isometheptene were elicited again.

The Ethical Committee of CINVESTAV-IPN dealing with the use of animals in scientific experiments approved the protocols of the present investigation.

Data presentation and statistical analysis

All data in the text and figures are presented as mean±s.e.mean. The peak changes in external carotid blood flow were expressed as percent changes from baseline. The significance of the percent changes induced by the different doses of isometheptene within one (sub)group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations[222]. Percent changes caused by isometheptene in the different treatment groups were compared to the percent changes caused by the corresponding isometheptene dose in the vehicle-treated group using Student's unpaired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

Apart from the anaesthetic sodium pentobarbitone, the compounds used in this study were: isometheptene (Carnick Laboratories, Cedar Knolls, NJ, USA), prazosin hydrochloride (Bufa Chemie b.v., Castricum; The Netherlands), rauwolscine hydrochloride, reserpine (RBI, Zwijndrecht, The Netherlands), BRL44408 (2-[2H-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazole; gift: Dr. T.J. Verbeuren; Servier, Suresnes, France), imiloxan hydrochloride (gift: Dr. R. Eglen; Roche Biosience, Palo Alta, CA, USA), MK912 ((2S,12bS)- 1'3'-dimethylspiro (1,3,4,5',6,6',7,12b-octahydro- 2H-benzo[b] furo[2,3-a] quinazoline)-2,4-pyrimidin-2'-one (L-657743; gift: Dr. W.L. Henckler; Merck & Co.; NJ, USA), SB224289 (2,3,6,7-tetrahydro-1'-methyl-5-[2'-methyl-4'(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl]furo[2,3-f]indole-3-spiro-4`-piperidine hydrochloride) and BRL15572 (1-(3-chlorophenyl)-4-[3,3-diphenyl (2-(S,R) hydroxypropanyl) piperazine] hydrochloride (both gifts: Dr. A.A. Parsons, SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK). Reserpine was dissolved in 5% v v^{-1} acetic acid, while 20% vv^{-1} propylene glycol was used for SB224289 and BRL15572. All other drugs were dissolved in physiological saline (vehicle). A short period of heating was needed to dissolve prazosin. The doses of the antagonists refer to their respective salts, while those of isometheptene and reserpine refer to free base.

Results

Systemic and carotid haemodynamic changes following administration of antagonists

Baseline values of heart rate, mean arterial blood pressure and external carotid blood flow in anaesthetised dogs (group 1, n=48) were: 149±5 beats min^{-1}, 132±3 mmHg and 158±7 ml min^{-1}, respectively. In animals treated with reserpine (n=11), heart rate (89±6 beats min^{-1}), mean arterial blood pressure (87±7 mmHg) and external carotid blood flow (89±7 $mlmin^{-1}$) were significantly ($P<0.05$) lower.

The systemic and carotid haemodynamic values in anaesthetised dogs before and 15-25 min after i.v. administration of vehicle (saline) or the different treatments (prazosin, rauwolscine, BRL44408, imiloxan, MK912, SB224289 and BRL15572) remained unchanged (data not shown), except for a moderate hypotensive response after prazosin (13±5%). However, immediately after its administration, BRL44408 (1000 $\mu g\,kg^{-1}$) produced an increase (35±10%) in mean arterial blood pressure. This vasopressor effect, which was not accompanied by significant changes in heart rate or

external carotid blood flow (data not shown), returned to baseline values within 25 min.

Systemic and carotid haemodynamics of isometheptene

As shown in Figure 8.2, intracarotid infusions of isometheptene (10, 30, 100 and 300 µg min^{-1}) produced a dose-dependent decrease in external carotid blood flow (maximal response: 60±3%); no changes were observed in blood pressure and heart rate (data not shown). At doses that selectively block α_1- and α_2-adrenoceptors, respectively[246], prazosin (100 µg kg^{-1}) did not modify, while rauwolscine (300 µg kg^{-1}) clearly attenuated the responses to isometheptene. Furthermore, pretreatment of the animals with the monoamine depletor reserpine produced a marked attenuation of the isometheptene-induced responses. Whereas subsequent treatment of the reserpinised animals with prazosin was without any effect, the external carotid vasoconstrictor effect of isometheptene was absent in animals treated with rauwolscine.

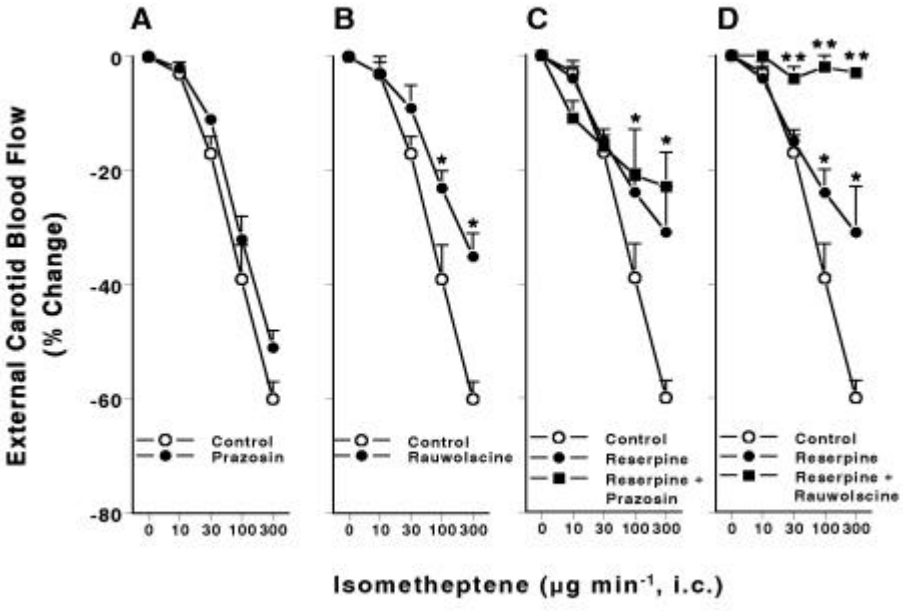


Figure 8.2. Effects of prazosin (100 µg kg^{-1}) and rauwolscine (300 µg kg^{-1}) on the external carotid vasoconstrictor effects produced by consecutive 1-min intracarotid (i.c.) infusions of isometheptene in control (A and B, respectively) and in animals treated with reserpine (5000 µg kg^{-1}, i.p. 24 h prior to the experiments) (C and D, respectively). *, $P<0.05$ vs. corresponding dose in control curve; **, $P<0.05$ vs. corresponding dose in reserpine curve.

We did not investigate the involvement of the α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1D}), since prazosin was ineffective against isometheptene. On the other hand, because rauwolscine produced a marked attenuation, the involvement of specific α_2-adrenoceptor subtypes (α_{2A}, α_{2B}, α_{2C}) in isometheptene-induced carotid vasoconstriction in anaesthetised dogs was elucidated using α_2-adrenoceptor subtype-selective antagonists, BRL44408 (α_{2A}), imiloxan (α_{2B}) and MK912 (α_{2C}); see Table 1.2. As presented in Figure 8.3, imiloxan (1000 µg kg^{-1}) was ineffective, but BRL44408 (1000 µg kg^{-1}) as well as MK912 (300 µg kg^{-1}) markedly attenuated isometheptene-induced decreases in external carotid blood flow (maximal response: -29±4 or -37±3%, respectively).

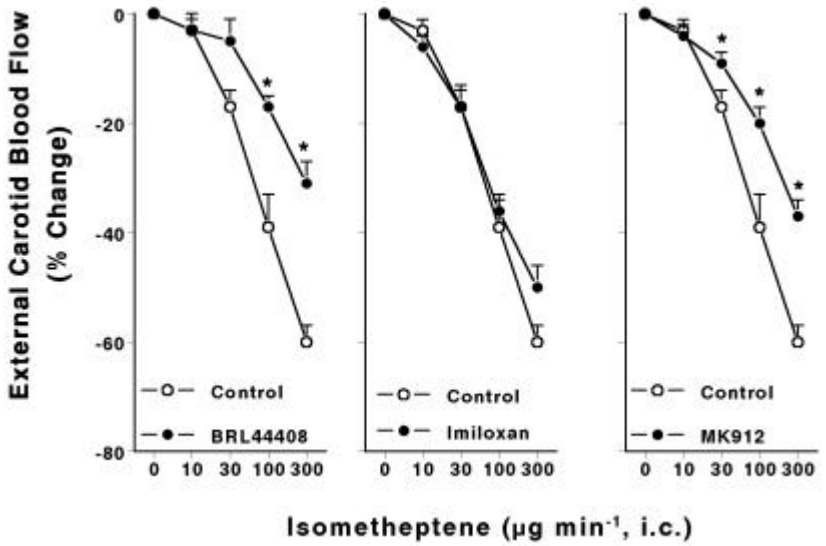


Figure 8.3. Effects of BRL44408 (1000 µg kg^{-1}; *left panel*), imiloxan (1000 µg kg^{-1}; *middle panel*) and MK912 (300 µg kg^{-1}; *right panel*) on the external carotid vasoconstrictor effects produced by consecutive 1-min intracarotid (i.c.) infusions of isometheptene in anaesthetised dogs. *, P<0.05 vs. corresponding dose in control curve.

The selective 5-HT$_{1B}$ (SB224289) and 5-HT$_{1D}$ (BRL15572) receptor antagonists, both at a dose of 300 µg kg^{-1}, did not attenuate the isometheptene-induced external carotid vasoconstriction (Figure 8.4).

As mentioned before, mean arterial blood pressure (or heart rate) was not affected by intracarotid infusions of isometheptene in the present experiments. Therefore, the changes in external carotid blood flow described above were qualitatively and

quantitatively similar to those observed in external carotid vascular conductance (data not shown).

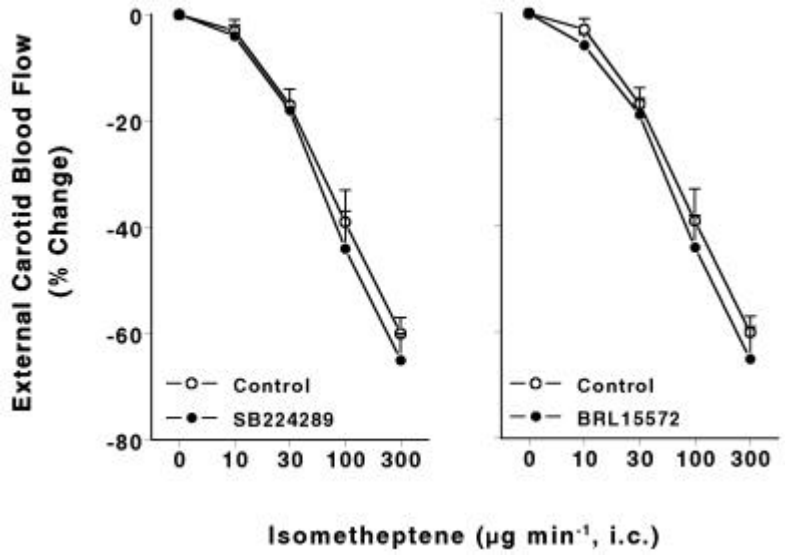


Figure 8.4. Effects of i.v. administration of SB224289 (300 µg kg^{-1}; *left panel*) or BRL15572 (300 µg kg^{-1}; *right panel*) on the external carotid vasoconstrictor effects produced by consecutive 1-min intracarotid (i.c.) infusions of isometheptene in anaesthetised dogs. There was no significant difference (P>0.05) between the effects of isometheptene in control and antagonist-treated animals.

Discussion

Systemic and carotid haemodynamic changes after antagonists

As demonstrated previously[246], treatment of the animals with vehicle (saline) did not cause any change in blood pressure, heart rate or external carotid blood flow in anaesthetised dogs. The lower baseline in heart rate and blood pressure observed in animals pretreated with reserpine is apparently due to depletion of sympathetic neurotransmitter noradrenaline[201].

The slight hypotension in control (reserpine-untreated) animals after prazosin can be explained by blockade of α_1-adrenoceptors that regulate vascular tone and blood pressure[33, 44]. On the other hand, the observed short-lasting hypertension after BRL44408 (35±10%), which was not accompanied by any change in heart rate or external carotid blood flow, was returned to baseline value (P>0.05) after 25 min. This response can most likely be explained by activation of vascular α_1-adrenoceptors, as reported in anaesthetised rats[263]. The other antagonists used in the present study were devoid of any systemic or carotid haemodynamic changes.

External carotid vascular effects of isometheptene

Intracarotid infusions of isometheptene produced a dose-dependent vasoconstriction in the external carotid circulation in anaesthetised dogs. Since this vasoconstrictor response was not accompanied by changes in heart rate or mean arterial blood pressure, it may be concluded that isometheptene produced a cranio-selective vasoconstriction. In view of the relevance that this animal model has had in the development and pharmacological characterisation of antimigraine drugs[161, 162, 167, 177, 269], the present results may suggest that the clinical efficacy of isometheptene in migraine may be due to its vasoconstrictor properties, as previously suggested[212, 272-274, 276]. The fact that reserpine markedly attenuated isometheptene-induced vasoconstrictor responses strongly suggests that a part of the carotid vasoconstrictor effect is due to an indirect sympathomimetic action[201]. The dose of reserpine (5 mg kg^{-1}, i.p.) is sufficient to abolish the external carotid vasoconstriction produced by tyramine, in the same experimental set-up (Villalón, unpublished observations).

Involvement of $\alpha_{1/2}$-adrenoceptors

Recently, we have shown that both α_1- and α_2-adrenoceptors are operative *in vivo* and can mediate carotid vasoconstriction in anaesthetised pigs and dogs[219, 246]. Subsequent investigations revealed that the pharmacological profile of α_1- and α_2-adrenoceptor subtypes mediating canine external carotid vasoconstriction resembles that of $\alpha_{1A/D}$- and $\alpha_{2A/C}$-adrenoceptors, respectively[270]. In the present experiments, we observed that isometheptene-induced carotid vasoconstrictor responses remained unchanged after prazosin (100 µg kg^{-1}), but were clearly diminished by rauwolscine (300 µg kg^{-1}) in both untreated and reserpine-treated dogs. The doses of prazosin and rauwolscine used by us are sufficient to selectively abolish carotid vasoconstrictor effects of phenylephrine[219] and BHT933 (6-ethyl-5,6,7,8- tetrahydro- 4H- oxazolo [4,5-d] azepin-2-amine dihydrochloride[219]), respectively. It may also be noted that rauwolscine, which only partly affected isometheptene responses in untreated dogs, was able to completely eliminate its reserpine-resistant part (see Figure 8.2). These results demonstrate that, like amphetamine and ephedrine[201], isometheptene behaves as a dual-acting

sympathomimetic agent. Unlike these two compounds that seem to act on both α_1- and α_2-adrenoceptors[201], isometheptene only stimulated α_2-adrenoceptors in this vascular bed. Furthermore, it is interesting to recall that amphetamine, ephedrine and isometheptene all have chiral centres and, therefore, it could well be that one of the stereoisomers releases noradrenaline, while the other interacts with the postsynaptic receptors.

Having established the role of α_2-adrenoceptors, we subsequently investigated the subtype-specificity of isometheptene using relatively selective antagonists at α_{2A}- (BRL44408), α_{2B}- (imiloxan) and α_{2C}- (MK912) adrenoceptors (for affinity constants, see Table 1). While imiloxan was ineffective, both BRL44408 and MK912 markedly attenuated the vascular effects of isometheptene (see Figure 8.3). These observations suggest that carotid vasoconstriction by isometheptene, as is the case with BHT933[270], is mediated mainly by α_{2A}- and α_{2C}-adrenoceptors.

Involvement of 5-HT$_{1B/1D}$ receptors

Several studies have shown that the vasoconstrictor effect of triptans is mediated predominantly by the 5-HT$_{1B}$ receptor[168, 215, 216, 250, 270]. Recently, SB224289[277] and BRL15572[278] have been shown to possess high degree of selectivity for the human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors, respectively. Since SB224289 (300 $\mu g\,kg^{-1}$), which effectively blocks the carotid vascular effect of sumatriptan in the dog[217], was ineffective against isometheptene, the role of 5-HT$_{1B}$ receptors can be ruled out. In view of the ineffectiveness of BRL15572, the same can probably be said about the involvement of the 5-HT$_{1D}$ receptor. However, we must point out to a caveat that we do not know the affinity constant of BRL15572 for the canine 5-HT$_{1D}$ receptor; our recent data show that this compound, unexpectedly, has little affinity at the cloned porcine 5-HT$_{1D}$ receptor[279].

Based on the present results, we conclude that intracarotid infusion of isometheptene produces a cranio-selective vasoconstriction in the canine external carotid vascular bed, *via* both direct and indirect sympathomimetic mechanisms. The direct mechanism seems to involve α_{2A}- and α_{2C}-adrenoceptors.

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Chapter 9

Summary, general discussion, and implications for future research

Summary of the thesis

Belonging to the academic thesis: *"Characterisation of α-adrenoceptor subtypes in the carotid vasculature: possible implications to migraine therapy"*

This thesis mainly focusses on the possible role of α-adrenoceptor mediating vasoconstriction in the carotid vasculature of anaesthetised pigs and dogs. For this purpose we investigated the possible involvement of α_1- and α_2-adrenoceptors and their respective subtypes (α_{1A}, α_{1B}, α_{1D} and α_{2A}, α_{2B}, α_{2C}), using selective agonists and antagonists. Since vasoconstriction in these models seems predictive for antimigraine activity, we made a correlation between the outcome of the present studies and their possible relevance to migraine therapy. However, these implications (see below) need further validation in humans.

Chapter 1 summarises the current pharmacological aspects of α–adrenoceptors in general, that of α_1- and α_2-adrenoceptors and their respective subtypes. Furthermore, the potential therapeutic application of drugs interacting with α-adrenoceptors, with special emphasis of their possible role in migraine therapy, has been discussed briefly.

α_1-Adrenoceptors are mainly located postsynaptically on smooth muscles and stimulation of these receptors causes vasoconstriction of vascular and non-vascular tissues, a response mainly produced by an increase in inositol triphosphate (IP_3) turnover and cellular Ca^{2+}-levels. Even though the α_1-adrenoceptor subtypes are regarded pharmacologically different, comparable pharmacological characteristics have been reported for these receptors (Table 1.1). The distribution of specific α_1-adrenoceptor subtypes in the peripheral and central vasculature and their possible role in the regulation of physiological responses, such as blood pressure, has been a matter of great interest. However, this has been hampered due to a controversy in the relationship between the pharmacologically defined (native) subtypes and the cDNA (recombinant) clones. Following cloning of human α_1-adrenoceptor subtypes and the development of subtype-selective ligands (see Table 1.2), it is now generally agreed

that the three subtypes (α_{1A}, α_{1B} and α_{1D}) can be distinguished pharmacologically in various tissues. Because of the fact that specific α_1-adrenoceptor subtypes play an important role in the regulation of blood pressure and prostate tone, several pharmaceutical companies have developed selective ligands as putative therapeutic agents (e.g. treatment of hypertension or benigh prostatic hypertrophy). Several lines of evidence suggest the existence of some other α_1-adrenoceptor subtypes (e.g. α_{1L}), however, these putative receptors lack full characterisation based on *structural* (structure of receptor), *operational* (functional responses of ligands/receptor) and *transductional information* (signal transductional pathways).

Already since the 1970's, it is known that α_2-adrenoceptors are located both pre- and postsynaptically (see Table 1.3). Presynaptic α_2-adrenoceptors can regulate neurotransmitter (e.g. noradrenaline) release, which is mediated by α_{2A}- and/or α_{2C}-adrenoceptor subtypes, depending on species and tissue. Even though stimulation of postsynaptic α_2-adrenoceptors has been known to produce vasopressor responses, little is known about the individual contribution of the three known α_2-adrenoceptors subtypes (α_{2A}, α_{2B} and α_{2C}). Nevertheless, it has been reported that α_{2B}-adrenoceptors mediate vasopressor responses in anaesthetised mice, whereas α_{2C}-adrenoceptors mediate noradrenaline-induced contraction of human isolated saphenous vein. Common difficulties experienced in distinguishing specific α_2-adrenoceptor subtype(s) mediating a physiological response include diversity in signal-transduction mechanisms, co-expression of these subtypes and the lack of highly subtype-selective ligands (especially antagonists). Recently, several moderately selective antagonists have been developed (see Table 1.2), which may help resolve these matters. Eventually, this information may lead to the development of novel and/or better (fewer side effects) therapeutic agents acting directly at specific α_2-adrenoceptor subtypes.

Since it has been shown that vasoconstriction in the carotid vasculature seems predictive for antimigraine activity, the possibility of α-adrenoceptors as a new avenue for migraine therapy has been discussed. The following chapters are designed to elucidate this matter in greater detail.

Chapter 2 addresses the question whether α-adrenoceptors mediate porcine carotid vasoconstriction, with special emphasis on carotid arteriovenous anastomoses. To

elucidate this matter in more detail, we selectively stimulated α_1- and α_2-adrenoceptors by, respectively, phenylephrine (α_1-adrenoceptor agonist) and BHT933 (α_2-adrenoceptor agonist), which were administered *via* the common carotid artery. Without causing major systemic haemodynamic changes, both agonists produced dose-dependent vasoconstriction of carotid arteriovenous anastomoses without affecting the distribution of carotid blood flow to the different cranial tissues. Prior treatment of the animals with prazosin (α_1-adrenoceptor antagonist) and rauwolscine (α_2-adrenoceptor antagonist) selectively abolished the vascular effects of phenylephrine and BHT933, respectively.

These results clearly show that both α_1- and α_2-adrenoceptors can mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs. This knowledge could be helpful in developing future therapy for migraine.

Chapter 3 sets out to further characterise α_1-adrenoceptor subtypes that mediate vasoconstriction in the carotid arterial bed of anaesthetised pigs. We analysed the effects of selective antagonists (5-methylurapidil, L-765,314 and BMY7378, respectively) at α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors on the carotid arteriovenous anastomotic vasoconstriction produced by intracarotid infusions of phenylephrine in anaesthetised pigs. 5-Methylurapidil and BMY7378 (both partially) and L-765,314 (completely) attenuated this response, in doses (300 and 1000 $\mu g\,kg^{-1}$) sufficient to block their respective receptors. It must be noted that the effect produced by the highest dose of BMY7378 is likely to be due to α_{1B}-adrenoceptor blockade.

These results suggest the involvement of both α_{1A}- and α_{1B}-adrenoceptors in the phenylephrine-induced carotid vasoconstriction, while that of α_{1D}-adrenoceptors is limited (if any). The α_{1B}-adrenoceptor may turn out to be an interesting target for future antimigraine drugs, especially when considering that these receptors, in contrast to the α_{1A}-adrenoceptor subtype, do not seem to be involved much in the vasoconstriction of the peripheral blood vessels. Indeed, our results show that the vasopressor effect caused by intravenous bolus injections of phenylephrine is mediated by α_{1A}-adrenoceptors, but is not affected by α_{1B}-adrenoceptor blockade, which is in agreement with results obtained in other species, including man.

Chapter 4 addresses the receptors involved in the total carotid and arteriovenous anastomotic vasoconstriction produced by intracarotid infusions of the α_{1A}-adrenoceptor agonist A61603, using the same experimental set up as the described in the previous two chapters. Except for A61603 (α_{1A}), selective agonists at the different α_1-adrenoceptor subtypes are currently unavailable. As described in the previous chapter, α_{1A}-adrenoceptors mediate phenylephrine-induced vasoconstriction in the systemic and carotid circulation of anaesthetised pigs. In order to verify this hypothesis, we decided to investigate the vascular effects of A61603 in this model. A61603 produced a dose-dependent vasopressor response, either by an intracarotid or an intravenous route of administration, accompanied by a dose-dependent carotid vasoconstriction. Unexpectedly, 5-methylurapidil (α_{1A}-antagonist) did not attenuate the carotid vascular effects to A61603, whereas it slightly attenuated the observed vasopressor response after intravenous bolus injections of A61603. Furthermore, other antagonists at known vasoconstrictor receptors, i.e. prazosin (α_1), a combination of prazosin and rauwolscine (α_2), phentolamine (α), GR127935 (5-$HT_{1B/1D}$) or ketanserin (5-HT_2) alone or in combination, also did not produce major changes in above-mentioned responses. Exclusion of these receptors in these responses is important when considering that methiothepin, a 5-$HT_{1/2}$ antagonist, partially attenuated the carotid vasoconstriction and abolished the vasopressor responses produced by A61603.

Taken together, the present results show that A61603 does not behave as a potent and selective α_{1A}-adrenoceptor agonist in the pig and that the vasoconstriction of porcine carotid arteriovenous anastomoses by A61603 is primarily mediated by a novel methiothepin-sensitive receptor/mechanism. A selective agonist at this novel receptor, without causing major systemic haemodynamic changes (e.g. hypertension), could be successful in the therapy of migraine.

Chapter 5 assesses the antimigraine potential of the newly developed α-adrenoceptor agonist S19014 by analysing its systemic and carotid haemodynamic effects in anaesthetised pigs. S19014 displays high (nanomolar) affinity for α_1- and α_2-adrenoceptors and produces an α-adrenoceptor-mediated vasoconstriction of several isolated blood vessels. Intravenous administration of S19014 produced a small, short-lasting hypertension accompanied by a long-lasting, dose-dependent

vasoconstriction of carotid arteriovenous anastomoses in anaesthetised pigs. Whereas treatment of the animals with prazosin was ineffective, rauwolscine markedly attenuated the carotid vasoconstrictor response and produced a rightward shift of the S19014-induced vasopressor response curve. Subsequent studies showed that S19014 produced only minor changes in the distribution of cardiac output in anaesthetised pigs, which suggests few vascular side-effects when tested in humans.

These results show that mainly α_2-adrenoceptors mediate the S19014-induced vasoconstriction of porcine carotid arteriovenous anastomoses and the short-lasting hypertension. For this reason, S19014 could be effective in aborting migraine attacks, however, further studies have to be conducted to evaluate the clinical relevance of potential side-effects (hypertension) in humans. In this context, the development and evaluation of other, more subtype-selective, potent and efficacious (full agonists) α_2-adrenoceptor agonists are awaited with great interest.

Chapter 6 investigates the possible external carotid effects of adrenaline and noradrenaline and the receptors involved in anaesthetised dogs. It has been known for several decades that the endogenous catecholamines, adrenaline and noradrenaline produce their vascular effects *via* α- (vasoconstriction) and β-adrenoceptors (vasodilatation). Whereas the effects of serotonin (5-HT) and its receptors in the canine external carotid vasculature has been well characterised pharmacologically, little is known about the effects of these catecholamines in this vascular bed. Consecutive 1-min intracarotid infusions of either adrenaline or noradrenaline produced equipotent, dose-dependent external carotid vasoconstriction, without affecting heart rate or blood pressure. These responses, being mimicked by phenylephrine (α_1-adrenoceptor agonist) and BHT933 (α_2-adrenoceptor agonist), remained unaffected by administration of saline. Treatment of the animals with phentolamine (non-selective α-adrenoceptor antagonist) revealed a vasodilator component to adrenaline and noradrenaline, which was subsequently abolished by propranolol (non-selective β-adrenoceptor antagonist). Similarly, rauwolscine partly attenuated the external carotid vascular effects of both adrenaline and noradrenaline, whereas subsequently administration of prazosin abolished these effects and revealed vasodilator responses to these sympathomimetic agents. In line with these findings, rauwolscine markedly attenuated the responses to BHT933 without affecting those to

phenylephrine; likewise, prazosin abolished the responses to phenylephrine without affecting those to BHT933.

These data show that (*i*) both adrenaline and noradrenaline produce effects in the external carotid vasculature of anaesthetised dogs; (*ii*) the doses of prazosin (100 $\mu g\,kg^{-1}$) and rauwolscine (300 $\mu g\,kg^{-1}$) are sufficient to block α_1- and α_2-adrenoceptors, respectively; and (*iii*) adrenaline and noradrenaline exert their external carotid vascular effects *via* α_1-, α_2- and β-adrenoceptors, mediating vasoconstriction and vasodilatation, respectively.

Chapter 7 describes the pharmacological characterisation of α_1- and α_2-adrenoceptor subtypes mediating external carotid vasoconstriction in anaesthetised dogs. As shown in the previous chapter, both α_1- and α_2-adrenoceptor are operative in mediating external carotid vasoconstriction in anaesthetised dogs. For this purpose, we analysed the effects of several selective antagonists at the different α_1- (5-methylurapidil, L-765,314 and BMY7378) and α_2-adrenoceptor subtypes (BRL44408, imiloxan and MK912) on the external carotid vasoconstrictor effects produced by consecutive intracarotid infusions of phenylephrine and BHT933 in anaesthetised dogs. Whereas L-765,314 (α_{1B}) was ineffective, 5-methylurapidil (α_{1A}) and BMY7378 (α_{1D}), alone or in combination, attenuated the effects to phenylephrine; the antagonists did not affect the external carotid vascular effects to BHT933. Similarly, whereas imiloxan (α_{2B}) was ineffective, BRL44408 (α_{2A}) and MK912 (α_{2C}), alone or in combination, attenuated the effects to BHT933; the vasoconstrictor effects of phenylephrine remained unaffected.

These results suggest that both α_{1A}- and α_{1D}-adrenoceptors mediate the external carotid effects of phenylephrine, whereas the involvement of α_{1B}-adrenoceptors is limited (if any), while those to BHT933 are mediated by α_{2A}- and α_{2C}-adrenoceptors; the involvement of α_{2B}-adrenoceptors seems also limited. Moreover, a selective agonist at any of these specific receptor subtypes, which is currently unavailable, could be efficacious in the treatment of migraine. It must be noted that even though A61603 is regarded to be a selective α_{1A}-adrenoceptor agonist, it also displays moderate affinity at α_2-adrenoceptors and other, yet unknown, receptors (see Chapter 4).

Chapter 8 investigates the external carotid vascular effects of the known antimigraine agent isometheptene and the possible mechanisms of action, using the same experimental set-up as described in Chapters 6 and 7. Intracarotid infusions of isometheptene produced a dose-dependent vasoconstriction in the external carotid vascular bed on anaesthetised dogs, devoid of any systemic haemodynamic changes. Treatment of these animals with the monoamine depletor reserpine markedly attenuated the decrease in external carotid blood flow produced by isometheptene. Whereas prazosin (α_1-adrenoceptor antagonist) was ineffective in control and reserpine-treated dogs, treatment with rauwolscine (α_2-adrenoceptor antagonist) produced a marked attenuation in control animals and complete blockade of this response in rauwolscine-treated, reserpinised dogs. Further investigation into the specific α_2-adrenoceptor subtypes, using selective antagonists, showed that BRL44408 (α_{2A}) and MK912 (α_{2C}) markedly attenuated this response, while imiloxan (α_{2B}) was ineffective; SB224289 (5-HT_{1B}) and BRL15572 (5-HT_{1D}), in doses sufficient to block their respective receptors, were also ineffective against this response. Taken together, from the present results we can conclude that intracarotid infusion of isometheptene produces a cranio-selective vasoconstriction in the canine external carotid vascular bed, *via* both direct (***α_2-adrenoceptor-mediated***) and indirect (*tyramine-like*) mechanisms. Furthermore, the carotid vasoconstrictor effect produced by isometheptene is mainly mediated by α_{2A}- and α_{2C}-adrenoceptors, while the involvement of α_1-, α_{2B}-adrenoceptors or 5-$HT_{1B/1D}$ receptors seem unlikely.

General discussion

Introduction of sumatriptan and the second generation triptans in migraine therapy

Even though the underlying mechanisms for the pathophysiology of migraine attacks are (still) not completely understood, many studies have shown that serotonin and its receptors play an important role in the therapy of this disorder. Ever since the success of sumatriptan (GR43175) in the treatment of acute migraine attacks[see 215, 280, 281, 282], it became clear that 5-HT_1 receptors might serve as a prospective target for the development of antimigraine agents. Several lines of evidence suggest that sumatriptan owes its antimigraine action to vasoconstriction of dilated extracerebral, intracranial blood vessels and inhibition of neuropeptide (e.g. CGRP and substance P) release from trigeminal nerve endings by activation of 5-HT_1 receptors[216]. Based

on different *in vitro* and *in vivo* studies, using the potent and selective 5-$HT_{1B/1D}$ receptor antagonist GR127935[see 215, 216], it has been established that mainly 5-$HT_{1B/1D}$ receptors mediate these responses.

Despite its great utility in migraine management, sumatriptan has several limitations, i.e. low oral bioavailability, high headache recurrence and contra-indication in patients with coronary artery disease[216, 228]. Over the years, many pharmaceutical companies have developed derivatives of sumatriptan, known as *second generation triptans*, for example eletriptan (Pfizer), zolmitriptan (Zeneca), naratriptan (GlaxoWelcome Ltd.), frovatriptan (SmithKline Beecham Pharmaceuticals/Vanguard Medica), almotriptan (Almirall Prodesfarma), rizatriptan (Merck Sharp & Dohme Laboratories) and donitriptan (Centre de Recherche Pierre Fabre). Even though these compounds display different pharmacodynamic or pharmacokinetic properties with respect to the affinity at 5-$HT_{1B/1D}$ receptors, bioavailability or lipophilicity (i.e. central penetration), their therapeutic efficacy in migraine are comparable[215]. Pharmacological, molecular and immunocytochemical investigations[see 168, 178, 217, 283] suggest that mainly the 5-HT_{1B} receptor is involved in the sumatriptan-induced vasoconstriction of several cranial and extracranial blood vessels, while 5-HT_{1D} and/or 5-HT_{1F} receptors mediate the pre-synaptic inhibition of the trigemino-vascular inflammatory response. Recently, it has been shown that the selective 5-HT_{1D} receptor agonist PNU109791 was devoid of any carotid vasoconstrictor effects in anaesthetised cats[284], pigs (De Vries *et al.*, unpublished observations) or dogs (Villalon *et al.*, unpublished observations). Therefore, the introduction of potent and selective 5-HT_{1B} receptor agonists for the treatment of migraine is awaited with great interest.

Animal model based on shunt hypothesis

As described in Chapter 1, several studies[see 169, 170, 175] show that vasodilatation of cranial blood vessels may play an important role in the pathophysiology of migraine. Based on this, the animal model used in the present studies has proved predictive for antimigraine activity[161]. This animal model, together with the *microsphere method*[197], enables us to determine the distribution of porcine carotid blood flow into different cranial tissues (such as skin, fat, muscles, etc.) and carotid

arteriovenous anastomoses. As described by Saxena[161], arteriovenous anastomoses are large precapillary communications between arteries and veins and are present in most structures (see Chapter 1). Under normal physiological conditions, these shunt vessels are under a sympathetic vasoconstrictor tone, shunting less than 3% of the total carotid blood flow[174]. Opening of carotid arteriovenous anastomoses will result that a large portion of carotid blood flow is shunted back to the venous side, thereby bypassing the cephalic tissues, which may explain the facial pallor of patients during a migraine attack. Based on the work of Heyck (1969), it has been proposed that carotid arteriovenous anastomoses are dilated during the headache phase of a migraine attack and that compounds that constrict these shunt vessels may have antimigraine activity[161, 285]. To date, all acutely acting antimigraine agents, such as the triptans (e.g. sumatriptan) or ergots (ergotamine or dihydroergotamine) produce a potent reduction in the porcine carotid blood flow, exclusively by constricting arteriovenous anastomoses[see 167, 215, 216].

Besides the role of specific 5-HT receptors in migraine treatment, also other mechanisms that may play an important role in the pathogenesis of a migraine attack have been investigated using this *in vivo* shunt model. For example, Van Gelderen *et al.* have shown that nitric oxide plays an important role in the shunting of arterial blood through carotid arteriovenous anastomoses[286, 287]. These results may suggest that a selective nitric oxide synthase inhibitor could be successful as prophylactic antimigraine agent, preventing the proposed vasodilatation of extracerebral, intracranial blood vessels in a migraine attack.

Résumé of the results and conclusions

The present investigation was conducted to investigate whether α-adrenoceptors can mediate vasoconstriction in the carotid circulation of both anaesthetised pigs and dogs and, if so, which subtypes are involved. For this reason, we explored the possibility of α-adrenoceptors as a novel avenue for the development of acutely acting antimigraine agents, with fewer side-effects than those current available (e.g. the triptans or ergots). We investigated the possible vasoconstrictor role of α_1- and α_2-adrenoceptors in the porcine (arteriovenous anastomoses) and canine (external) carotid circulation. For this purpose, we tested the carotid vascular effects of selective agonists (i.e. phenylephrine and BHT933, respectively) and antagonists

(prazosin and rauwolscine, respectively), at these receptors in the carotid circulation of these species. In order to avoid any systemic haemodynamic changes, for example an increase in blood pressure, we administered the agonists by slow and local, intracarotid infusions. As described in Chapters 2 (pigs) and 6 (dogs), it turned out that both α_1- and α_2-adrenoceptors mediate vasoconstriction in this vascular bed in both species. Further investigation described in Chapter 3 revealed that both α_{1A}- and α_{1B}-adrenoceptors mediate carotid arteriovenous anastomotic vasoconstriction in anaesthetised pigs. Interestingly, in anaesthetised dogs, the phenylephrine-induced carotid vasoconstriction was mediated by α_{1A}- and α_{1D}-adrenoceptors; the α_{1B}-adrenoceptor antagonist L-765,314 was ineffective. Taking into consideration that both *in vivo* models are predictive for antimigraine activity [see 161, 162, 167, 177, 215], this discrepancy in the involvement of α_{1B}-adrenoceptors in porcine and canine carotid vasoconstriction may be explained by a species difference. Since these results have been obtained from animal models, the possible usefulness of a selective α_{1B}-adrenoceptor agonist (currently unavailable) in the treatment of migraine obviously needs to be validated by clinical studies in humans. The potential unwanted side effects mediated by α_{1A}-adrenoceptors (e.g. blood pressure changes) limits the possible use of α_{1A}-adrenoceptor agonists in migraine therapy. In order to verify this hypothesis, we investigated the effects of intravenous (possible side-effects) and intracarotid (therapeutic efficacy) administration of the selective α_{1A}-adrenoceptor agonist A61603 (see Chapter 4). Even though, A61603 potently constricted porcine carotid arteriovenous anastomoses, it also produced a pressor response after both routes of administration. It was found that the A61603-induced vasoconstriction of these shunt vessels was mediated by novel methiothepin-sensitive receptors, unrelated to α-adrenoceptors or 5-HT receptors. In Chapter 5, we assessed the antimigraine potential of the newly developed α-adrenoceptor agonist S19014, using the porcine *in vivo* model. It has been shown that S19014 produced a moderate vasoconstriction of porcine carotid arteriovenous anastomoses, accompanied by a short-lasting hypertension; α_2-adrenoceptors mediated both responses. These results suggest that S19014 could be effective in migraine therapy, however, the clinical relevance of observed hypertension requires further study, and that a potent and selective agonist at α_2-adrenoceptors or its subtypes, which are also currently

unavailable, could be effective in migraine therapy. For example, clonidine (α_2-adrenoceptor agonist) is one of such compounds that has been used for many decades in the treatment of migraine[288-290]. The latter has been substantiated by the fact that α_2-adrenoceptors can mediate carotid vasoconstriction in anaesthetised pigs (see Chapter 2) and dogs (Chapter 6). For this reason, it would be very interesting to know which specific subtype (α_{2A}, α_{2B} and α_{2C}) can mediate vasoconstriction of porcine carotid arteriovenous anastomoses; this was, however, not investigated in the present investigation. On the other hand, both α_{2A}- (slightly) and α_{2C}-adrenoceptors (predominantly) mediate the external carotid vasoconstriction in anaesthetised dogs, produced by intracarotid infusions of BHT933 (see Chapter 7). Moreover, the external carotid vascular effects of the acutely acting antimigraine agent isometheptene were also mediated by these receptors, whereas the involvement of α_1-adrenergic or 5-$HT_{1B/1D}$ receptors seemd unlikely, if any (see Chapter 8).

The results described in this thesis clearly show that: (*i*) both α_1- and α_2-adrenoceptors can mediate vasoconstriction in the carotid circulation of anaesthetised pigs and dogs; (*ii*) α_{1A}- and α_{1B}-adrenoceptors mediate the phenylephrine-induced porcine carotid vasoconstriction, while α_{1A}- and α_{1D}-adrenoceptors mediate this response in anaesthetised dogs; (*iii*) the carotid haemodynamic effect of the α_{1A}-adrenoceptor agonist A61603 involves unknown, methiothepin-sensitive receptors in anaesthetised pigs, unrelated to α-adrenoceptors or 5-HT receptors; (*iv*) the newly developed α-adrenoceptor agonist S19014 could be effective in the treatment of acute migraine attacks; (*v*) α_{2A}- and α_{2C}-adrenoceptors mediate carotid vasoconstriction induced by BHT933 in anaesthetised dogs; (*vi*) the antimigraine agent isometheptene produces external carotid vasoconstriction *via* $\alpha_{2A/C}$-adrenoceptors. Based on these results, it seems of interest to develop potent and selective agonists at the different α_1- and α_2-adrenoceptor subtypes in order to verify the present results (which are mainly based on antagonists) and for their possible clinical implication in migraine therapy. Potential side-effects (e.g. hypertension) of these specific subtype-selective agonists requires extensive investigation before they could be useful in migraine therapy. Additionally, *in vitro* experiments assessing their contractile properties in isolated blood vessels of importance in migraine therapy (e.g. middle meningeal or temporal artery) may also be very useful.

Implications for future research

Although the present studies offer a better insight in the existence of α_1- and α_2-adrenoceptors subtypes eliciting vasoconstriction within the carotid arterial bed, several questions still remain.

1. *Which specific α_2-adrenoceptor subtype mediates vasoconstriction of porcine carotid arteriovenous anastomoses?* As shown in Chapter 2, both α_1- and α_2-adrenoceptors mediate vasoconstriction in this vascular bed. Only the involvement of α_1-adrenoceptor subtype has been investigated in the pigs.

2. *Are α_1- and/or α_2-adrenoceptor subtypes involved in the porcine carotid vasoconstriction elicited by ergotamine and dihydroergotamine?* The vasoconstriction of porcine carotid arteriovenous anastomoses by these ergot alkaloids was attenuated, but not completely blocked by the 5-$HT_{1B/1D}$ receptor antagonist GR127935[181], thereby revealing a GR127935-insensitive vasoconstrictor component. Since the ergots show high affinity for both α_1- and α_2-adrenoceptors, it could be that these receptors play a role in the carotid vasoconstrictor effects to these antimigraine agents. Indeed, in anaesthetised dogs, it has been shown that besides 5-$HT_{1B/1D}$ receptors, also α_2-adrenoceptors mediate the external carotid vasoconstrictor effects produced by ergotamine as well as dihydroergotamine[177].

3. *Are α_2-adrenoceptors a better target for future migraine therapy over α_1-adrenoceptors?* Based on the involvement of α_2-adrenoceptors in the carotid vasoconstriction produced by ergotamine (in dogs), dihydroergotamine (in dogs), S19014 (in pigs, Chapter 4) and isometheptene (in dogs, Chapter 8), it is likely that an α_2-adrenoceptor subtype-selective agonist may be more useful in the treatment of migraine than an α_1-adrenoceptor subtype agonist. Additionally, since α_2-adrenoceptors distribution is less widespread as compared to α_1-adrenoceptors, an antimigraine agent acting at specific α_2-adrenoceptor subtypes may cause fewer side effects than an α_1-adrenoceptor agonist. This

question should be investigated in more detail by developing and evaluating potent and selective (subtype-selective) α_2-adrenoceptor agonists.

4. *Is there a difference in receptors mediating carotid vasoconstriction in anaesthetised dogs and pigs?* It has been known that intracarotid infusions of either buspirone[200] or isometheptene (see Chapter 8) elicits vasoconstriction in the canine external carotid vasculature, while buspirone (Villalón *et al.*, unpublished observations) and isometheptene (Willems *et al.*, unpublished observations) are ineffective in the porcine carotid circulation to produce these carotid vasoconstrictor effects. This feature can well be explained by the involvement of different mechanisms (e.g. receptors), probably due to a species variation, and requires further investigation.

5. *Are there other possibilities for novel migraine therapy?*
   - *Calcitonin gene-related peptide (CGRP) antagonists.* The innervation of the cranial vessels by the trigeminal nerve, the trigeminovascular system, has been the subject of major studies in view of its possible role in the mediation of some aspects of migraine[see 214]. Since stimulation of the trigeminal ganglion in humans leads to facial pain and flushing and associated release of powerful neuropeptide vasodilator substances (e.g. SP, CGRP, VIP, NPY; for full names, see list of abbreviations), their release has been investigated in humans[291]. It has been shown that during a migraine attack, jugular venous blood levels of CGRP were elevated, while that of the others remained unaltered[292]. Moreover, treatment of these patients with acute antimigraine agents like dihydroergotamine or sumatriptan reduced this elevated CGRP plasma level[292, 293]. In addition, a selective CGRP antagonist, h-CGRP(8-37), markedly reduced trigeminal-evoked cerebral vasodilatory responses[291], showing the possible importance of CGRP antagonists as potential prophylactic antimigraine agents. In this context, a potent and chemically stable CGRP receptor antagonist, BIBN4096BS has been developed[294]. It would be very interesting to evaluate the effects of BIBN4096BS against vasodilator effects of endogenous CGRP, released either by capsaicin[see 295] or trigeminal nerve

stimulation, on porcine arteriovenous anastomotic blood flow. The effects of BIBN4096B may also be investigated *in vitro* models, relevant for migraine.

- *5-HT_7 receptor antagonists.* Indirect evidence has demonstrated that the 5-HT_7 receptor mediates cranial vessel vasodilatation in dogs[296-298] and, possibly, pigs as well[181]. It would be very interesting to investigate the blocking effects of the recent developed potent and selective 5-HT_7 receptor antagonist (SB258719) on the 5-HT-induced vasodilatation (after 5-$HT_{1/2}$ receptor blockade) within the carotid circulation of anaesthetised dogs and pigs.
- *In vitro experiments.* Besides *in vivo* experiments, it would be interesting to know whether potent and selective agonist at specific α_1- and α_2-adrenoceptor subtypes produce contraction of human isolated blood vessels and to correlate this with the experiments gathered from *in vivo* animal experiments and clinical data.

6. *Which receptors mediate the 'uroselectivity of α_{1A}-adrenoceptor antagonists?* Several lines of evidence have shown that α_{1A}-adrenoceptor agonists and antagonists are useful in the treatment of urinary incontinence[72] and benign prostatic hypertrophy[see 79, 129, 299], respectively. Since the α_{1A} is the main α_1-adrenoceptor subtype regulating systemic resistance and blood pressure changes[44], uroselective α_{1A}-adrenoceptor antagonists that do not cause hypotension may be more useful in the treatment of the benign prostatic hypertrophy. Several animal models have been developed where the effects of α_1-adrenoceptor antagonists on blood pressure and urethral pressure have been measured simultaneously. Using these animal models, diverse compounds have been developed (e.g. RWJ-38063[79]) that show high selectivity at cloned human α_{1A}-adrenoceptors and markedly attenuate phenylephrine-induced urethral pressure, without causing any blood pressure changes. One likely explanation for this discrepancy could be the involvement of another $\alpha_{1(A)}$-adrenoceptor subtype, sensitive to these α_{1A}-adrenoceptor antagonists, but which is not involved in the regulation of blood pressure. A possible candidate for these receptors could be one of the novel human α_{1A}-adrenoceptor isoforms[40], since Daniels *et al.*[41] have shown that these isoforms display α_{1L}-adrenoceptor pharmacology in

functional studies. Further investigation into the existence of other α_1-adrenoceptors, in particular that of the contentious α_{1L}-adrenoceptors, may turn out to be of importance for the treatment of a variety of disorders, including benign prostatic hypertrophy.

Chapter 10

Samenvatting in het Nederlands; Summary in Dutch

Behorende bij het proefschrift: *"Karakterisering van α-adrenoceptoren in het halsslagader vaatbed: mogelijk implicaties voor migraine therapie"*

Dit proefschrift behandelt voornamelijk de mogelijk rol van α-adrenoceptoren in de vasoconstrictie in het halsslagader (carotid) vaatbed van zowel genarcotiseerde varkens als honden. Met dit in ogenschouw hebben we de mogelijke betrokkenheid van zowel α_1- en α_2-adrenoceptoren, maar ook van de respectievelijke subtypen (α_{1A}, α_{1B}, α_{1D} en/of α_{2A}, α_{2B}, α_{2C}), onderzocht in dit vaatbed, gebruikmakend van verschillende selectieve agonisten en antagonisten. Omdat vasoconstrictie in deze experimentele modellen voorspelbaar is voor antimigraine activiteit, hebben we de resultaten van de beschreven studies proberen te correleren met eventuele therapeutische mogelijkheden in de behandeling van migraine. Echter, deze mogelijke implicaties moeten worden gevalideerd in mensen.

Hoofdstuk 1 probeert de huidige farmacologische aspecten van α-adrenoceptoren in het algemeen, van α_1- en α_2-adrenoceptoren en de respectievelijke subtypen samen te vatten. Bovendien worden de therapeutische toepassingen van geneesmiddelen die interacties aangaan met α-adrenoceptoren in het kort behandeld, met speciale aandacht voor de behandeling van migraine.

α_1-Adrenoceptoren zijn voornamelijk post-synaptisch gelokaliseerd, bijvoorbeeld op glad spier weefsel. Activatie van deze receptoren geeft aanleiding tot vasoconstrictie van vasculaire en niet-vasculaire weefsel, een effect dat voornamelijk wordt veroorzaakt door een verhoogde inositol trifosfaat (IP_3) productie en cellulaire calcium concentratie. Alhoewel de α_1-adrenoceptor subtypen als farmacologisch verschillende receptoren worden beschouwd, zijn er echter vergelijkbare farmacologische karakteristieken gerapporteerd, zoals weergegeven in Tabel 1.1. De distributie van specifieke α_1-adrenoceptor subtypen in zowel perifeer als centraal vasculatuur en de eventuele betrokkenheid van deze receptoren in de regulatie van verschillend fysiologische effecten, bijvoorbeeld bloeddrukregulatie, heeft de

afgelopen jaren veel aandacht gekregen. Het in kaart brengen hiervan was echter voor vele jaren bemoeilijkt door een discrepantie in de relatie tussen de farmacologisch bepaalde receptoren en de gekloneerde subtypen. Sinds 1995 wordt het algemeen aangenomen dat de drie oorspronkelijke subtypen (α_{1A}, α_{1B} en α_{1D}) farmacologisch kunnen worden gescheiden en vertonen gelijkwaardigheid met de gekloneerde en de functionele subtypen in de verschillende weefsels. Deze consensus was voornamelijk bereikt door het kloneren van de humane α_1-adrenoceptor subtypen en het testen van meer subtype-selectieve liganden (zie Tabel 1.2). Doordat specifieke α_1-adrenoceptor subtypen een belangrijke rol spelen in de tonus regulatie van vasculaire en niet-vasculaire weefsels, zijn verscheidene farmaceutische bedrijven al jaren bezig om meer selectieve agonisten/antagonisten van deze subtypen te ontwikkelen, met als doel deze op de markt te brengen als geneesmiddel. Verschillende studies hebben het mogelijke bestaan van additionele α_1-adrenoceptor subtypen aangetoond, bijvoorbeeld α_{1L}-adrenoceptoren. Daar deze receptoren nog niet volledig zijn gekarakteriseerd, zoals dit tegenwoordig is gebaseerd op zowel structurele, operationele en transductionele informatie, kunnen ze nog niet als apart worden beschouwd.

Sinds het begin van de jaren zeventig is het al bekend dat α_2-adrenoceptoren zowel pre- als post-synaptisch zijn gelokaliseerd. Pre-synaptische α_2-adrenoceptoren kunnen de afgifte van verschillende transmitters, bijvoorbeeld noradrenaline, reguleren. Zeer recentelijk is het aangetoond dat dit effect voornamelijk wordt gemedieerd door α_{2A}- en/of α_{2C}-adrenoceptors, afhankelijk van de te onderzoeken species en/of weefsel. Alhoewel het bekend is dat stimulatie van post-synaptische α_2-adrenoceptoren een verhoging van de bloeddruk veroorzaakt, is de informatie over de specifieke distributie van de verschillende α_2-adrenoceptor (α_{2A}, α_{2B} en α_{2C}) subtypen schaars. Desalniettemin is het bekend dat α_{2B}-adrenoceptoren een belangrijke rol spelen in de bloeddrukregulatie van genarcotiseerde muizen, terwijl contractie van de humaan geïsoleerde *saphena vein* wordt gemedieerd door voornamelijk α_{2C}-adrenoceptoren. Verscheidene problemen voor het ontrafelen van welke specifieke α_2-adrenoceptor subtypen een bepaald fysiologische effect mediëren zijn de overeenkomstig signaal transductie mechanismen, de aanwezigheid van verschillende subtypen en het gebrek aan zeer selectieve antagonisten. Recentelijk zijn er selectieve antagonisten van de verschillende α_2-adrenoceptor subtypen

ontwikkeld (zie Tabel 1.3), waardoor dit hopelijk mogelijk wordt gemaakt. Uiteindelijk zal deze informatie kunnen leiden tot de ontwikkeling van andere, mogelijkerwijs betere, antimigraine middelen. De studies, beschreven in de volgende hoofdstukken, zijn uitgevoerd om deze mogelijkheid nauwkeurig te onderzoeken.

Hoofdstuk 2 onderzoekt of α-adrenoceptoren inderdaad vasoconstrictie kunnen mediëren in de halsslagader (carotid) vasculatuur van het genarcotiseerde varken, met de nadruk op de carotid arteriovenous anastomoses. Verscheidene aanwijzingen bewogen ons om dit onderzoeken, gebaseerd op zowel *in vitro* als *in vivo* studies. Een belangrijke reden waardoor deze studie gehinderd werd, was de ongevoeligheid van carotid arteriovenous anastomoses voor sympathisch zenuwstimulatie of exgogeen toegevoegde (intracarotid infusie) van noradrenaline in genarcotiseerde varkens. Om dit nader te onderzoeken diende we phenylephrine (α_1-adrenoceptor agonist) en BHT933 (α_2-adrenoceptor agonist) *via* de halsslagader (lokale toevoeging) toe om de respectievelijke receptoren selectief te stimuleren. Beide agonisten veroorzaakten een dosis-afhankelijke vasoconstrictie van carotid arteriovenous anastomoses. Terwijl de bloeddruk niet veranderde tijdens of na de toediening van deze agonisten, veroorzaakte phenylephrine echter een β-adrenoceptor gemedieerde tachycardia; BHT933 veroorzaakte geen hartritme veranderingen. Bovendien bleef de distributie van de totale halsslagader bloeddoorstroming naar de verschillende craniële weefsels onveranderd door beide agonisten. De vasculaire effecten van phenylephrine en BHT933 waren afwezig na behandeling van de dieren met, respectievelijk, prazosin (α_1-adrenoceptor antagonist; 100 $\mu g\,kg^{-1}$) en rauwolscine (α_2-adrenoceptor antagonist; 300 $\mu g\,kg^{-1}$).

Deze resultaten geven op een duidelijke manier weer dat zowel α_1- als α_2-adrenoceptoren vasoconstrictie in de halsslagader vasculaire circulatie kunnen mediëren, wat nuttige informatie kan zijn voor de ontwikkeling van toekomstige antimigraine middelen.

Hoofdstuk 3 beschrijft de farmacologische karakterisering van α_1-adrenoceptor subtypen die zijn betrokken bij de phenylephrine-geïnduceerde halsslagader vasoconstrictie van het genarcotiseerde varken, gebaseerd op het vorige hoofdstuk. Gebruikmakend van hetzelfde experimentele model, hebben we de effecten onderzocht van selectieve antagonisten van α_{1A}-, α_{1B}- en α_{1D}-adrenoceptor subtypen op de carotid arteriovenous anastomotic vasoconstrictie veroorzaakt door phenylephrine. Zowel 5-methylurapidil (α_{1A}), L-765,314 (α_{1B}) als een hoge dosering van BMY7378 (α_{1D}) veroorzaakte een verminderde vasoconstrictie door phenylephrine, in doseringen voldoende om de respectievelijke α_1-adrenoceptor subtypen te blokkeren. Belangrijk om op te merken is dat het verminderde effect na BMY7378 waarschijnlijk wordt veroorzaakt door blokkade van α_{1A}- en/of α_{1B}-adrenoceptoren; de betrokkenheid van α_{1D}-adrenoceptoren is onwaarschijnlijk.

De resultaten van deze studie suggereren de betrokkenheid van zowel α_{1A}- als α_{1B}-adrenoceptoren in de phenylephrine-geïnduceerde halsslagader vasoconstrictie, terwijl dat van α_{1D}-adrenoceptoren onwaarschijnlijk is. De α_{1B}-adrenoceptor zou een interessante kandidaat kunnen zijn als mogelijk doel voor toekomstige antimigraine middelen, daar de betrokkenheid van deze receptoren in de vasoconstrictie van perifere bloedvaten miniem is. De resultaten laten zien dat de dosis-afhankelijke hypertensie, geproduceerd door intraveneus toegediende phenylephrine, is gemedieerd door α_{1A}-adrenoceptoren en niet door α_{1B}- of α_{1D}-adrenoceptoren, wat overeen komt met studies uitgevoerd in andere species, alsmede in de mens.

Hoofdstuk 4 onderzoekt welke receptoren betrokken zijn bij de halsslagader vasoconstrictie, geproduceerd door intracarotid infusie van de α_{1A}-adrenoceptor agonist A-61603 in genarcotiseerde varkens, gebruikmakend van hetzelfde model als beschreven in afgelopen twee hoofdstukken. Behalve A61603 (α_{1A}), zijn selectieve agonisten voor de verschillende α_1-adrenoceptoren momenteel niet beschikbaar. Zoals beschreven in het vorige hoofdstuk, wordt de halsslagader vasoconstrictie door phenylephrine mede gemedieerd door α_{1A}-adrenoceptors. Om deze hypothese te testen, besloten we om de vasculaire effecten van A61603 in dit model te testen. A61603 produceerde een verhoging van de bloeddruk, zowel na intracarotid als intraveneuze toediening, gepaard gaand met dosis-afhankelijke halsslagader vasoconstrictie na intracarotid toediening. De halsslagader vasculaire effecten van

A61603 werden niet verminderd door de selectieve α_{1A}-adrenoceptor antagonist 5-methylurapidil (1000 $\mu g\,kg^{-1}$), terwijl het de verhoogde bloeddruk door intraveneus toegediende A61603 in mindere mate verzwakte. Bovendien, andere antagonisten voor bekende vasoconstrictieve receptoren, namelijk prazosin (α_1), rauwolscine (α_2), phentolamine (α), GR127935 (5-$HT_{1B/1D}$) of ketanserin (5-HT_2), individueel of in combinatie, waren ook ineffectief om deze vasculaire effecten te verminderen. Uitsluiting van al deze receptoren is belangrijk wanneer men beseft dat methiothepine, een 5-$HT_{1/2}$ antagonist, deze halsslagader (partieel) en systemisch (volledig) haemodynamische effecten verminderde.

Samengevat laten deze resultaten zien dat de carotid arteriovenous anastomotic vasconstrictie door intracarotid infusies van A61603 in genarcotiseerde varkens is gemedieerd door onbekende en methiothepin-gevoelige receptoren, niet gerelateerd aan α-adrenerge of 5-HT receptoren. Overeenstemmend, α_{1A}-adrenoceptoren en nog onbekend, methiothepin-gevoelige receptoren mediëren de A61603-geïnduceerde hypertensie. Een selectieve agonist voor deze receptor, zonder systemisch haemodynamische effecten, zou effectief kunnen zijn voor de behandeling van migraine.

Hoofdstuk 5 stelt de antimigraine effectiviteit van de α-adrenoceptor agonist S19014 vast door de potentiëel systemische en halsslagader haemodynamische effecten van intraveneuze administratie van S19014 in genarcotiseerde varkens te onderzoeken. S19014 heeft hoge affiniteit voor zowel α_1- als α_2-adrenoceptoren en functionele studies hebben aangetoond dat S19014 contractie veroorzaakt van humaan en dierlijk geïsoleerde bloedvaten (Verbeuren *et al.,* niet gepubliceerde observaties). Intraveneuze toediening van S19104 veroorzaakte een kleine en kortstondige bloeddrukverhoging, vergezeld gaand met een langdurige en dosis-afhankelijke vasoconstrictie van carotid arteriovenous anastomoses in genarcotiseerde varkens. Terwijl behandeling van de dieren met prazosin (100 $\mu g\,kg^{-1}$) ineffectief was, veroorzaakte voorbehandeling met rauwolscine (300 $\mu g\,kg^{-1}$) een aanzienlijke vermindering van de halsslagader vasoconstrictoire effecten en veroorzaakte een rechtsverschuiving van de bloeddrukverhoging respons-curve geproduceerd door S19014.

Deze resultaten tonen aan dat voornamelijk α_2-adrenoceptoren de S19014-geïnduceerde halsslagader vasoconstrictie en de hypertensie mediëren. S19014 zou een effectief antimigraine medicijn kunnen zijn, daar dit model predicatief is voor antimigraine activiteit. Echter, additionele studies moeten worden uitgevoerd om de klinische relevantie van eventueel ongewenste bijwerkingen, bijvoorbeeld op de bloeddruk, in mensen te bepalen. Om deze redenen wordt de ontwikkeling en evaluatie van meer subtype-selectieve α_2-adrenoceptor agonisten met veel interesse tegemoet gezien.

Hoofdstuk 6 onderzoekt de potentiële external (externe) halsslagader effecten van adrenaline en noradrenaline en de mogelijk betrokken receptoren in genarcotiseerde honden. Het is al lang bekend dat de endogene catecholamines (adrenaline en noradrenaline), die worden vrijgegeven na stimulatie van het sympathische zenuwstelsel, vele vasculaire effecten veroorzaken *via* zowel α- (voornamelijk vasoconstrictie) als β-adrenoceptoren (voornamelijk vasodilatatie). Terwijl de vasculaire effecten van serotonine (5-HT) en de betrokken receptoren in het external halsslagader vaatbed in genarcotiseerd honden farmacologisch grotendeels zijn gekaraktiseerd, is er echter weinig bekend over de effecten van deze catecholamines in dit vaatbed. Achtereenvolgende één minuut infusies van adrenaline en noradrenaline in de external carotid arterie produceerde een equipotent en dosis-afhankelijke external halsslagader vasoconstrictie, zonder enig effect op hartritme of bloeddruk. Deze cranio-selectieve vasoconstrictoire effecten, die werden gesimuleerd door phenylephrine (α_1-adrenoceptor agonist) en BHT933 (α_2-adrenoceptor agonist), werden niet beïnvloed door intraveneuze toediening van fysiologische saline. Behandeling van de honden met de niet-selectieve α-adrenoceptor antagonist phentolamine openbaarde een vasodilatoire component, die was verdwenen na voorbehandeling met de niet-selectieve β-adrenoceptor antagonist propranolol. Voorbehandeling met rauwolscine verminderde de external halsslagader vasoconstrictoire effecten van zowel adrenaline en noradrenaline gedeeltelijk, die volkomen waren verdwenen na achtereenvolgende toediening van prazosin. Na blokkade van α_1- en α_2-adrenoceptoren, door toediening van rauwolscine en prazosin, werd er wederom een external halsslagader vasodilatatie waargenomen. In overeenstemming met deze resultaten, waren de external halsslagader

vasoconstrictoire effecten van BHT933 afwezig na voorbehandeling met rauwolscine, zonder die van phenylephrine te beïnvloeden; de vasculaire effecten van phenylephrine waren afwezig na voorbehandeling met prazosin; prazsoin had geen effect op de vasoconstrictieve effecten van BHT933.

Deze resultaten tonen aan dat: (*i*) zowel adrenaline als noradrenaline vasculaire effecten in de external halsslagader vasculatuur in genarcotiseerde honden produceren; (*ii*) de dosis van prazosin (100 $\mu g\,kg^{-1}$) en rauwolscine (300 $\mu g\,kg^{-1}$) voldoende zijn voor de blokkade van, respectievelijk, α_1- en α_2-adrenoceptoren; en (*iii*) adrenaline en noradrenaline produceren vasoconstrictie *via* zowel α_1- als α_2-adrenoceptoren en vasodilatatie *via* β-adrenoceptoren in dit vaatbed.

Hoofdstuk 7 Beschrijft de farmacologische karakterisering van de verschillende α_1- en α_2-adrenoceptor subtypen die vasoconstrictie in het external halsslagader vaatbed van genarcotiseerde honden mogelijkerwijs kunnen mediëren. Zoals beschreven in het vorige hoofdstuk, zijn zowel α_1- als α_2-adrenoceptoren operatief *in vivo* door vasoconstrictie te mediëren in dit vaatbed. Met dit doel voor ogen, analyseerde we de effecten van verschillende selectieve antagonisten van de verschillende α_1- (5-methylurapidil, L-765,314 en BMY7378) en α_2-adrenoceptor subtypen (BRL44408, imiloxan en MK912) op de vasoconstrictoire effecten van phenylephrine (α_1) en BHT933 (α_2) in dit model. Terwijl L-765,314 (α_{1B}) ineffectief was, verminderden 5-methylurapidil (α_{1A}) en BMY 7378 (α_{1D}), alleen of in combinatie, de effecten van phenylephrine zonder die van BHT933 te beïnvloeden. Terwijl imiloxan (α_{2B}) ineffectief was, verminderde BRL44408 (α_{2A}) en MK912 (α_{2C}), alleen of in combinatie, de effecten van BHT933 zonder enige effecten op die geproduceerd door phenylephrine.

Deze resultaten suggereren dat: (*i*) zowel α_{1A}- als α_{1D}-adrenoceptoren de external halsslagader vasoconstrictoire effecten van phenylephrine mediëren, terwijl de betrokkenheid van α_{1B}-adrenoceptoren miniem is; (*ii*) de BHT933-geïnduceerde external halsslagader vasoconstrictoire effecten zijn gemedieerd door zowel α_{2A}- als α_{2C}-adrenoceptoren, terwijl de betrokkenheid van α_{2B}-adrenoceptoren miniem is; en (*iii*) een selectieve agonist van een specifieke $\alpha_{1A/D}$- of $\alpha_{2A/C}$-adrenoceptor subtype zal effectief zijn in de behandeling van migraine. Desalniettemin zullen eventuele

mogelijke ongewenste bijwerkingen van een dergelijke α-adrenoceptor subtype-selectieve agonist, met name bloeddrukverhoging, alvorens in ogenschouw moeten worden genomen. Belangrijk om te vermelden is dat ondanks A61603 wordt beschouwd als een potent en selectieve α_{1A}-adrenoceptor agonist, het ook middelmatige affiniteit (en activiteit) vertoont voor α_2-adrenoceptoren en andere, nog onbekende, receptoren (zie Hoofdstuk 4).

Hoofdstuk 8 onderzoekt de mogelijke external halsslagader vasoconstrictoire effecten van het bekende antimigraine middel isometheptene en de mogelijke betrokkenheid van α_1- en/of α_2-adrenoceptoren, gebruikmakend van hetzelfde experimentele model als beschreven in Hoofdstukken 6 en 7. Achtereenvolgende intracarotid toediening van isometheptene produceerde een dosis-afhankelijke vasoconstrictie in dit vaatbed, zonder enige effecten op hartritme of bloeddruk. Dit vasoconstrictieve effect was aanzienlijk verminderd in honden die waren voorbehandeld met de monoamine depletor reserpine. Terwijl prazosin ($100\ \mu g\ kg^{-1}$) ineffectief was in zowel onbehandelde als reserpine-behandelde dieren, was het effect van isometheptene aanzienlijk verminderd in rauwolscine ($300\ \mu g\ kg^{-1}$) dieren en afwezig in dieren behandeld met reserpine en rauwolscine. Bovendien, SB224289 (5-HT_{1D}) en BRL15572 (5-HT_{1D}) waren ineffectief om het vasoconstrictieve effect van isometheptene te verminderen. Deze resultaten laten duidelijk zien dat isometheptene halsslagader vasoconstrictie in genarcotiseerde honden kan veroorzaken, die wordt gemedieerd door zowel indirekte (zogenaamde *tyramine-like effect*) als directe (α_2-adrenoceptor-gemedieerd) mechanismen, terwijl α_1-adrenerge en 5-$HT_{1B/1D}$ receptoren niet betrokken zijn bij dit effect. Vervolgens werd er door middel van het testen van verschillende subtype-selectieve antagonisten (BRL44408, imiloxan en MK912) onderzocht door welke specifieke α_2-adrenoceptor subtypen de external halsslagader vasculaire effecten van isometheptene worden gemedieerd. Daar BRL44408 en MK912, alleen of in combinatie, de effecten van isometheptene verminderde, terwijl imiloxan ineffectief was, suggereert dat α_{2A}- en α_{2C}-adrenoceptors de external halsslagader vasculaire effecten van isometheptene mediëren, terwijl de betrokkenheid van het α_{2B}-adrenoceptor subtype onwaarschijnlijk is.

Chapter 11

Appendix

Acknowledgements (Dankwoord)

Admittedly, the first (and in some cases the only) part of one's thesis is the acknowledgements, however, being unaware of the underlying reason(s). One of the great features of acknowledgements is that one can take off their scientific coat and express one's thoughts in an understandable language to those for which one is indebted.

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Thanks ALL,

Eddyman (Edwin Willems)

About the author

Edwin Willems was born in Lelystad (Zuidelijke Ijsselmeerpolders, The Netherlands) on 22nd of December 1971. He graduated from high school (HAVO) in 1989, where after he finished the Higher Laboratory School (HLO; Ing. degree) at the Hogeschool van Amsterdam (The Netherlands, with Organic Chemistry as his specialisation) in 1994.

The same year, he started his study Pharmacochemistry at the faculty of Chemistry of the Vrije Universiteit in Amsterdam. During this time his major research focussed on several pharmacological and physiological aspects of the histamine H_3 receptor in different rat brain areas, under the supervision of Dr. Alexandra Alves-Rodrigues, Dr. Rob Leurs and Prof. Dr. Henk Timmerman, where he also worked as research assistant for six months more. Additionally, he worked for six months at the PANUM in Denmark under the supervision of Dr. Ulrich Knigge and Prof. Dr. Jørgen Warberg, investigating the possible involvement of histaminergic and adrenergic receptors in the release of several peptides (ACTH, PRL and AVP) in rats.

In 1997, he obtained his master's degree (Drs.) and initiated his PhD-project under the supervision of Prof. Dr. P.R. Saxena at the Erasmus University in Rotterdam, The Netherlands. During this period he had the opportunity to work in the laboratory of Prof. Dr. Carlos M. Villalón (Departemento de Farmacología y Toxicología, México D.F., México) to perform experiments in dogs.

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List of abbreviations

^{o}C	: Degrees Celsius.
γ	: Gamma (radiation).
^{3}H	: Tritium-radiolabelled hydrogen.
µg	: Microgram (10^{-6} g).
5-HT	: 5-Hydroxytryptamine (serotonin).
5-methylurapidil	: 5-Methyl-6[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl] amino]-1,3-dimethyluracil.
A61603	: N-[5-(4,5-dihydro-1H-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulphonamide.
A131701	: 3-[2-((3aR,9bR)-cis-6-methoxy-2,3,3a,4,5,9b-hexahydroxy-1H-benisoindol-2-yl)ethyl]pyrido-[3',4':4,5]thieno[3,2-d] pyrimidine-2,4(1H,3H)-dione.
ANOVA	: Analysis of variance.
ARC239	: 2-[2[4-(O-methoxyphenyl)piperazin-1-yl]ethyl]-4,4-dimethyl-1,3-(2 H,4H)-isoquinolimedione.
AVA	: Arteriovenous anastomotic (arteriovenous anastomoses).
$AVSO_2$	: Difference between arterial and jugular-venous oxygen saturation.
BHT920	: 5-Allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-d]azepine dihydrochloride.
BHT933	: 6-Ethyl-5,6,7,8-tetrahydro-4H-oxazolo[4,5-d] azepin-2-amine dihydrochloride.
BMY7378	: 8-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5 decane-7,9- dione dihydrochloride.
BPH	: Benigh prostatic hyperplasia (hypertrophy).
BRL 41992	: 1,2-Dimethyl-2,3,9,13b-tetrahydro-1H-ibenzo[c,f]imidazol[1,5-a]azepine.
BRL44408	: 2-[2H-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazole.
CA	: Chicago (USA).
Ca^{2+}	: Calcium(II).
cAMP	: Cyclic adenosine monophosphate.
cDNA	: Complementary deoxyribonucleic acid.
Ce	: Cerium.
CEC	: Chloroethylclonidine.
CGRP	: Calcitonin gene-related peptide.
CHO	: Chinese Hamster Ovary.
CINVESTAV (IPN)	: Center of Research and Advanced Studies (Instituto Politecnico Nacional).
CRW	: Centraal Research Werkplaats.
D	: Dopamine (of Dosis in pD_2 of ED_{50}).
DAP	: Diastolic blood pressure.
ECG	: Electrocardiography.
e.g.	: For example.
et al.	: and colleagues.
F12640	: 4-(4-(2-[3-(2-aminoethyl)-1H-indol-5-yloxyl]-acethyl)-piperazin-1-yl)-benzonitril mesylate.
G	: G-protein.
GR127935	: N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide hydrochloride.
GTP	: Guanosine triphosphate.
H	: Histamine.
H^{+}	: Hydrogen(I).
HBr	: Hydrobromide.
HCl	: Hydrochloride.
HEK 293	: Human embryonic kidney cells.
HV 723	: α-Ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)-amino)-propyl)-benzene acetonitrile fumarate.
I	: Imidazoline.
i.a.	: Intrinsic activity.
i.c.	: Intracarotid.
i.e.	: Namely.
IL	: Illinois (USA).
i.m.	: Intramuscular.
Imiloxan	: RS-21361-193.
IP_3	: Inositol triphosphate.
IUPHAR	: International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification.
i.v.	: Intravenous.
K^{+}	: Potassium(I)

K_B	: The equilibrium dissociation constant (mol litre^{-1}) for a competitive antagonist.
KeV	: Kilo electro-volt (radioactive γ-radiation).
kg	: Kilogram (10^3 g).
L-765,314	: 4-Amino-2-[4-[1-(benzyloxycarbonyl)-2(S)-[[(1,1- dimethylethyl) amino] carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline.
M	: Molar concentration (mol litre^{-1}).
MA	: Massachusetts (USA).
MAP	: Mean arterial blood pressure.
mg	: Milligram (10^{-3} g).
min	: minutes.
ml	: millilitre.
MK912	: (2S,12bS)-1'3'-dimethylspiro(1,3,4,5',6,6',7,12b-octahydro-2H-benzo[b]furo[2,3-a]quinazoline)-2,4'-pyrimidin-2'-one (L-657743).
mmHg	: Millimetre mercury (pressure).
MO	: Montana (USA).
*m*RNA	: Messenger RNA.
n	: Number of animals used.
Na^+	: Sodium(I).
Nb	: Niobium.
NEN	: New English Nuclear.
ng	: Nanogram (10^{-9} g).
NPY	: Neuropeptide Y.
NY	: New York.
P	: Probability.
pCO_2	: Negative logarithm to base 10 of the carbon-dioxide (CO_2) concentration.
pEC_{50}	: Negative logarithm to base 10 of an agonist concentration eliciting half the maximum effect.
pH	: Negative logarithm of base 10 of the hydrogen (H) concentration.
PI	: Phosphoinositol.
pK_i	: Negative logarithm of a concentration of a competing ligand in a competition assay that would occupy 50% of the receptors if no radioligand would be present.
pO_2	: Negative logarithm of oxygen (O_2) concentration.
RBI	: Research Biochemicals International (SIGMA-Aldrich).
Rec 15/2739	: 8-3-[4-(2-Methoxyphenyl)-1-piperazinyl]propylcarbamoyl)-3-methyl-4oxo-22-phenyl-4H-1-benzopyran dihydrochloride.
RS 10753	: N-[2-(2-cyclopropyl methoxy phenoxy)ethyl]5-chloro-α,α-dimethyl-1H-indole-3-ethanamine hydrochloride.
Ru	: Ruthenium.
RWJ-38063	: N-(2-{4-[2-methylethoxy)phenyl]piperazinyl}ethyl)-2-(2-oxopiperidyl)acetamide.
S19014	: Servier compound.
SAP	: Systolic blood pressure.
Sc	: Scandium.
s.d.	: Standard deviation.
SDZ NVI 085	: (-)-(4aR,10aR)-3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2H-naphth[2,3b]-1,4-oxazine.
SEM	: Standard error of the mean.
SKF 104078	: 6-Chloro-9—[(methyl-2-butenyl)oxyl]-3-methyl-1H-2,3,4,5-tetrahydro-3-bezazepine.
SKF 104856	: 2-Vinyl-7-chloro-3,4,5,6-tetrahydro-4-methylthienol[4,3,2 ef][3]benzazepine.
Sn	: Stannum.
SNAP 5089	: 2,6-Dimethyl-4-(4-nitrophenyl)1,4-dihydropyridine-3,5-dicarboxylic acid-N[3-(4,4-diphenylpiperidine-1-yl)propyl]amide-methyl ester.
SP	: Substance P.
UK	: United Kingdom.
UK 14304	: 6-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline tartrate.
USA	: United Stated of America.
VIP	: Vasoactive intestinal polypeptide.
vs.	: Versus.
WB 4101	: 2-(2',5'-Dimethoxyphenoxyethyl) aminoethyl-1,4-benzodioxan.

General terms and definitions in quantitative pharmacology

Table. General terms and definitions in quantitative pharmacology, according to the IUPHAR[12, 300].

Term	Suggested usage
Agonist	A ligand that binds to receptors and thereby alters the proportion of them that are in an active form, resulting in a biological response. Conventional agonists increase this proportion, while inverse agonists reduce it.
Agonist potency ratios	Quantitative agonist data are preferably gives as equi-effective molar ratios where the standard agonist = 1, defined in terms of a functional response in a particular tissue or cell type.
Antagonist	A drug that reduces the action of another drug, generally an agonist. Many act at the same active site as the agonist. Antagonists of this kind may be surmountable or insurmountable depending on the experimental conditions (see below).
Antagonist potency	Expressed as pK_B or pA_2 values from functional studies, or as IC_{50} values.
Competitive antagonism	If the agonist and antagonist form only short-lasting combinations with the receptor active site, so that equilibrium between agonist, antagonist and receptors is reached during the presence of the agonist, the antagonism will be surmountable over a wide range of concentrations (reversible competitive antagonism). In contrast, some antagonists when in close enough proximity to their binding site may form a covalent bond with it (irreversible competitive antagonism), and the antagonism becomes insurmountable when no spare receptors remain.
Concentration	It is recommended that the concentration of a drug is referred to the molar concentration, denoted by either [X] or c_X, with the formed preferred. Decimal multipliers should be indicated using either SI prefixes (e.g. mM, nM) or by powers of ten (e.g. $3x10^{-8}$ M), with the former preferred.
Desensitisation, fade, tachyphylaxis	Overlapping terms that refer to a spontaneous decline in the response to a continuous application of agonist, or to repeated applications or doses.
Dose	In some cases (e.g. in therapeutics and clinical pharmacology, in *in vivo* experiments and when tissues are perfused *in vitro*, and exposed to a bolus application of a drug), absolute drug concentrations are uncertain. It becomes more appropriate to specify the quantity of drug administration, either in mass or mol quantity. In the case of *in vivo* experiments, the quantity of the drug should be expressed per unit of animal mass (e.g. mol kg^{-1}, µg kg^{-1}).

Table continued…

EC_{50}	The molar concentration of an agonist that produces 50% of the maximal possible effect of that agonist. Other percentage values (EC_{20}, EC_{40}, etc.) can be specified.
ED_{50}	Either the dose of a drug that produces (on average) a specified all-or-none response in 50% of a test population or the dose of a drug that produces 50% of the maximal response to that drug if the response is graded.
Efficacy (e)	The concept and numerical term that expresses the way in which different agonists vary in their ability to produce a response, even though they may occupy the same proportion of receptors.
Full agonists	When a tissue has spare receptors, several agonists may be able to elicit the same maximal response, albeit at different receptor occupancies. They are said to be full agonists in that experimental situation. A full agonist in one tissue may be a partial agonist in another.
Functional assay	Pharmacological test systems in which a response can be firmly attributed to the function of a defined receptor type or subtype.
Hill equation (Concentration-Effect relation)	$E/E_{max} = [L]^{nH} / (EC_{50} + [L]^{nH})$, Where E=effect, E_{max}=maximal effect, EC_{50}=concentration that produces 50% of maximal effect, [L]=concentration of ligand (L), n_H=Hill coefficient.
IC_{50}	Either the molar concentration of an antagonist that reduces a specified response to 50% of its former value (see EC_{50}) or the molar concentration of an agent/ligand (agonist or antagonist) that causes a 50% reduction in the specific binding of a radioligand (also referred to as pK_i).
Intrinsic efficacy	See efficacy.
Inverse agonist	A ligand which by binding to receptors reduces the fraction of them in an active conformation (see agonist). This can occur if some of the receptors are in the active form even in the absence of a conventional agonist.
Ligand	A compound that acts on a receptor, either an agonist or antagonist.
Ligand affinity	Binding affinity for key ligands are given as pIC_{50} (or IC_{50}) values or expressed as pK_d (or K_d) values unless otherwise stated.

Table continued…

Maximal agonist effect (a)	The maximal effect that an agonist, whether conventional or inverse, can elicit in a given tissue under particular experimental conditions, expressed as a fraction of that produced by a full agonist acting through the same receptors under the same conditions; also referred to as intrinsic activity (though the maximal agonist effect is preferable) or efficacy.-
Modulator	A ligand that increases or decreases the action or an agonist by combining with the distinct (allosteric) site(s) on the receptor.
Non-competitive antagonism	Agonist and antagonist can be bound simultaneously; antagonist binding reduces or prevents the action of the agonist. This usage covers situations as diverse as channel block of the nicotinic acethylcholine receptor and inhibition by adrenoceptor antagonists of the response to tyramine (so-called indirect antagonism).
pA_2	The negative logarithm to base 10 of the molar concentration of an antagonist that makes it necessary to double the concentration of an agonist needed to elicit the original submaximal response[302].
Partial agonist	An agonist which in a given tissue, under specified experimental conditions, cannot elicit as large an effect (even when applied at higher concentration, so that all the receptors should be occupied) as can a full agonist acting through the same receptors. See full agonist, maximal agonist effect, and efficacy.
pEC_{50}	Negative logarithm to the base 10 of the EC_{50} of an agonist (also called pD_2).
Potency	An expression of the activity of a drug, either in terms of the concentration or amount needed to produce a defined effect, or, less acceptably, with regard to the maximal effect attainable. An imprecise term that should always be defined (see EC_{50}, IC_{50}, maximal effect. etc.)
Previous name(s)	Outdated names that exists in the literature, but which are no longer the recommended nomenclature.
Radioligands	Available radioactive-labelled ligands (agonists or antagonists, selective or non-selective).
Radioligand assays	Cell lines expressing cloned receptors, or any tissue or cell lines expressing endogenous receptors in which the radioligand is used.
Receptor	Cellular macromolecules that are concerned directly and specifically in chemical signalling between and within cells. The region of the receptor macromolecule to which endogenous ligands bind are referred to as the recognition or active site(s) of the receptor.
Receptor distribution	Central and peripheral distribution of the receptor.

Table continued…

Receptor reserve / Spare receptors	In some tissues, agonists of higher efficacy can produce a maximal effects even when a small fraction of the receptors are occupied. It is therefor possible to inactivate some of the receptors (e.g. by applying an irreversible competitive antagonist) without reducing the maximal response (although the curve relating the effect to the concentration of agonist will be shifted to the right). The tissue is said to posses spare receptors, and for a given level of response, there is a larger receptor reserve for the action of that agonist. Receptor reserve is both tissue and agonist dependent. An agent that is a partial agonist in one tissue may act as a full agonist in a second tissue with a greater receptor reserve.
Receptor type	Nomenclature for a structurally and operationally distinct receptor in a given family.
Receptor subtype	Nomenclature for a receptor with strong structural homology to other types, but with distinct operational characteristics.
Receptor code	A preliminary receptor code (RC), without the species abbreviation.
Schild plot	A graph of (r-1) against log[antagonist], where r is the concentration ratio. This should yield a straight line of unity slope if the Schild equation is obeyed[303].
Selective agonists	Agonists that are selective for the receptor type (e.g. phenylephrine at α_1-adrenoceptors); specific agonists, which are not selective between receptor types in the family, are included if useful (e.g. noradrenaline at adrenoceptors).
Selective antagonist	Antagonists that are selective for the receptor type (e.g. prazosin at α_1-adrenoceptors); specific antagonists, which are not selective between receptor types in the family, are included if useful (e.g. phentolamine at α-adrenoceptors).
Structural information	The number of transmembrane domains (TM) and amino acids (aa).
Transduction mechanism(s)	The preferred receptor signalling pathways of mechanism, when established; any identified alternative mechanism.
Tissue function(s)	The physiological response mediated by the receptor if established in whole tissue, preferably *in vivo.*

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STELLINGEN / STATEMENTS

1. Selective agonists at α_1- and α_2-adrenoceptor subtypes could provide a novel avenue for antimigraine drug development

2. The limited number of potential therapeutic applications probably does not stimulate pharmaceutical companies to develop selective α_1- and α_2-adrenoceptor subtype ligands

3. A theory is right until proven otherwise! However, if you cannot prove that something is right, try to prove it wrong and, if you fail, it is probably right (Jim Andrews)

4. Tidy desk – inactive mind, messy desk – busy mind (Jim Andrews)

5. Stress is a reflection of your incompetence to organise your professional and social life

6. Hoe zou het zijn om een vulkaan te zijn, de hele dag liggen roken en iedereen zegt dat je werkt! (Arthur Brouns)

7. Sportklimmen is de beste remedie voor nagelbijten!

8. The only lessons we learn are from the things we regret (Ed Stasium, Biohazard, 1994)

9. Het is makkelijker om iets nieuws te leren dan iets af te leren

10. Waar karate is gebasseerd op natuurlijk ronde bewegingen, het zijn de directe bewegingen die vaak effectief zijn in het dagelijks leven

11. Het waarnemen van het probleem, is meestal het probleem niet

12. We don't plan to fail, we just fail to plan (L. Verspui)

13. Patience is the art of concealing your impatience (History & Heraldry)

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