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### Publication status and date:

Published: 22/12/1999

### Document Version

Publisher's PDF, also known as Version of record

### Citation for the published version (APA):

Maassen van den Brink, A. (1999). *Coronary side effects of antimigraine drugs: from patient to receptor*. [Doctoral Thesis, Erasmus University Rotterdam]. Erasmus Universiteit Rotterdam (EUR).

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# Coronary Side Effects of Antimigraine Drugs From Patient to Receptor

The Netherlands Heart Foundation is gratefully acknowledged for their financial support of the work presented in this thesis (research grant 93.146)

**ISBN 90-9013290-2**

**NUGI 741/746**

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CORONARY SIDE EFFECTS OF  
ANTIMIGRAINE DRUGS

FROM PATIENT TO RECEPTOR

CORONAIRE BIJWERKINGEN VAN  
ANTIMIGRAINE GENEESMIDDELEN

VAN PATIËNT TOT RECEPTOR

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR

AAN DE ERASMUS UNIVERSITEIT ROTTERDAM

OP GEZAG VAN DE RECTOR MAGNIFICUS

PROF. DR. P.W.C. AKKERMANS M.A.

EN VOLGENS HET BESLUIT VAN HET COLLEGE VOOR PROMOTIES

DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP

WOENSDAG 22 DECEMBER 1999 OM 11.45 UUR

DOOR

ANTOINETTE MAASSEN VAN DEN BRINK

GEBOREN TE DELFT

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Financial support by the Netherlands Heart Foundation and the Dr. Saal van Zwanenbergstichting for the publication of this thesis is gratefully acknowledged.

Financial support of the following institutions and companies is gratefully acknowledged:

Anglo-Dutch Migraine Association, AstraZeneca, Centre Recherche Pierre Fabre, Glaxo Wellcome Inc., Institut de Recherches Internationales Servier, Janssen-Cilag B.V., Merck Sharpe & Dohme B.V., Novartis Pharma A.G., Pfizer Ltd., Vanguard Medica Ltd.,

*Aan Peter*



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# Chapter 1

## Migraine and Antimigraine Drugs for the Acute Treatment of Migraine

### 1.1 Migraine

Migraine is a paroxysmal neurological disorder, which occurs in 6% of males and 15-18% of females, with the highest prevalence between the ages of 25 and 55 years<sup>1,2</sup>. Attacks consist of moderate or severe headache, associated with nausea, vomiting, photo- and phonophobia<sup>3</sup>. The headache lasts 4 to 72 hours and increases with physical activity. The migraine attack may be resolved by sleep during or after the headache<sup>4</sup>. In about 15% of patients (*migraine with aura*), an aura may precede the migraine headache within about one hour. The aura usually consists of visual symptoms such as fortifications, scotoma or hemianopsia, but may also be sensory (paresthesia), motor- (weakness, paresis) or speech-related (dysarthria, aphasia)<sup>1</sup>.

To study migraine scientifically, there is a clear need for uniform criteria to determine whether a patient is suffering from a migraine headache. In 1988, the International Headache Society (IHS) provided such criteria<sup>5</sup> (see Table 1.1 for migraine without and with aura).

### 1.2 Migraine pathophysiology

Migraine attacks *per se* are not necessarily an abnormal feature, considering that anyone may experience one or two migraine attacks in life. Only when someone experiences recurrent attacks (five or more attacks of migraine without aura or two or more attacks of migraine with aura<sup>5</sup>), he or she is classified as a migraine patient.

**Table 1.1** IHS classification and diagnostic criteria for migraine without aura and migraine with aura<sup>5</sup>

---

### **Migraine without aura**

- A. At least five attacks fulfilling B-D
- B. Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated)
- C. Headache has at least two of the following characteristics:
  - 1. Unilateral location
  - 2. Pulsating quality
  - 3. Moderate or severe intensity
  - 4. Aggravation by walking upstairs or similar routine physical activity
- D. During headache at least one of the following:
  - 1. Nausea and/or vomiting
  - 2. Photophobia and phonophobia
- E. At least one of the following:
  - 1. History, physical and neurological examinations do not suggest associated head trauma, vascular or non-vascular intracranial disorders, exposure to or withdrawal from (toxic) substances, non-cephalic infection, metabolic disorders or cranial or facial disorders
  - 2. History and/or physical and neurological examinations do suggest such disorder, but is ruled out by appropriate investigations
  - 3. Such disorder is present, but migraine attacks do not occur for the first time in close temporal relation to the disorder

### **Migraine with aura**

- A. At least two attacks fulfilling B
  - B. At least three of the following four characteristics:
    - 1. One or more fully reversible aura symptoms indicating focal cerebral cortical and/or brain stem dysfunction
    - 2. At least one aura symptom develops over more than 4 minutes, or two or more symptoms occur in succession
    - 3. No aura symptom lasts more than 60 minutes. If more than one aura symptom is present, accepted duration is proportionally increased
    - 4. Headache follows aura with a free interval of less than 60 minutes. (It may also begin before or simultaneously with the aura)
  - C. At least one of the following:
    - 1. History, physical and neurological examinations do not suggest associated head trauma, vascular or non-vascular intracranial disorders, exposure to or withdrawal from (toxic) substances, non-cephalic infection, metabolic disorders or cranial or facial disorders
    - 2. History and/or physical and neurological examinations do suggest such disorder, but is ruled out by appropriate investigations
    - 3. Such disorder is present, but migraine attacks do not occur for the first time in close temporal relation to the disorder
-

Thus, migraine patients probably have a decreased threshold for developing migraine attacks. Genetic abnormalities, possibly ion channel dysfunction<sup>6</sup>, seem to act as predisposing factors for migraine attacks, but other factors such as hormonal changes and relaxation after stress (e.g. migraine attacks during the weekend) may also contribute<sup>7</sup>. Although the initiation of migraine attacks has extensively been studied, the exact mechanism has not yet been identified. Recently, it was demonstrated with positron emission tomography that the brain stem is activated during migraine attacks, and that this activation persists even after amelioration of the headache by sumatriptan<sup>8,9</sup>. This finding suggests that there may be a 'migraine generator', possibly located in the brain stem.

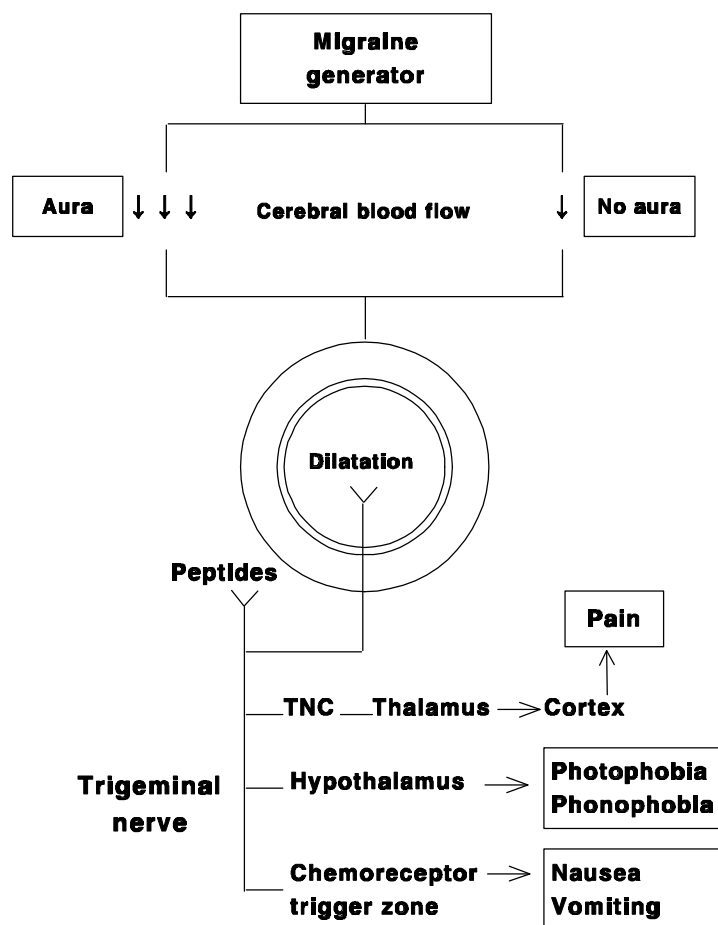
### *Migraine aura*

As mentioned above, the migraine aura is experienced by about 15% of migraine patients. The aura usually consists of visual symptoms (fortifications, scotoma, hemianopsia), but may also be sensory (paresthesia), motor- (weakness, paresis) or speech-related (dysarthria, aphasia). The aura may be caused by 'cortical spreading depression', a short-lasting depolarisation wave starting in the occipital cortex and moving across the cortex at a speed of 3-5 mm/min, followed by a depression of neuronal activity<sup>10</sup> which induces a regional reduction in cerebral blood flow. Although cortical spreading depression has been demonstrated in animal models<sup>11</sup>, definite evidence that this mechanism also applies to humans is lacking. However, the concept of cortical spreading depression fits well with clinical observations in patients with migraine aura such as oligemia preceding over the cortex at a speed of 2-3 mm/min<sup>12</sup> and visual disturbances propagating at the same rate.

### *Headache phase*

The reduced cerebral blood flow in the aura phase is followed by the headache phase, which is characterised by a vasodilatation of cranial extracerebral large arteries and arteriovenous anastomoses (e.g. in the dura mater, base of the skull and scalp). This vasodilatation probably may be assigned to increased neuronal innervation of the blood vessels. Vasodilator peptides that may be involved include neurokinin A,

substance P, calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP). In addition, nitric oxide (NO) may be involved in the vasodilatation during a migraine attack<sup>13</sup>. Dilated blood vessels may well be responsible for the pulsating headache during a migraine attack. The cranial vasodilatation activates perivascular afferent terminals of the trigeminal sensory nerve that may then also release neuropeptides, continuing or intensifying the attack. Axonal conduction transmits nociceptive information towards the trigeminal nucleus caudalis and higher brain centres such as thalamus and hypothalamus for the registration of pain, photophobia, phonophobia and nausea (Figure 1.1).



**Figure 1.1** Diagram showing putative changes in migraine. Based on Saxena<sup>14</sup> and De Vries *et al.*<sup>15</sup>. TNC, trigeminal nucleus caudalis.

### 1.3 Drugs for the acute treatment of migraine

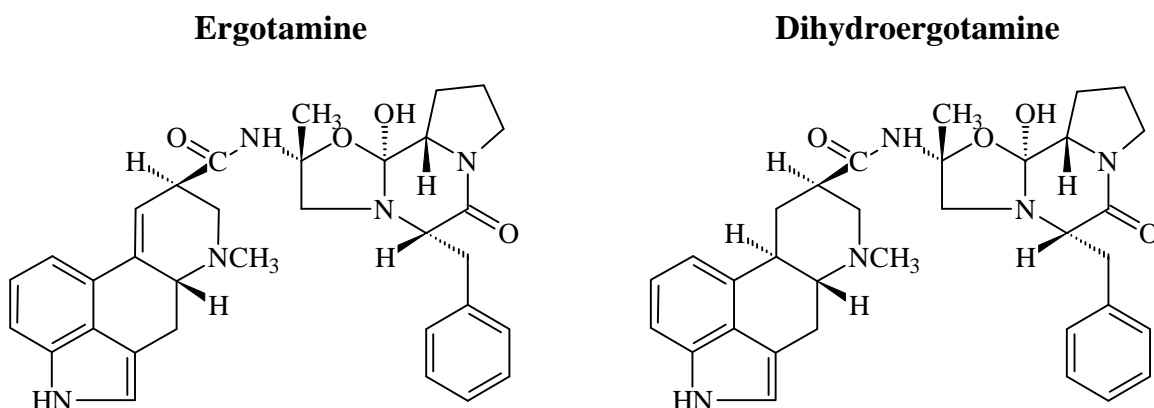
Mild to moderate migraine attacks may be treated by non-specific drugs such as analgesics and rapidly absorbable NSAIDs such as aspirin, ibuprofen and paracetamol<sup>3,16</sup>. Antiemetic compounds such as metoclopramide and domperidone are able to speed up gastric emptying and may thus, when taken early during a migraine attack, improve the absorption of other drugs<sup>3,16</sup>. The combination of aspirin and metoclopramide has proven to be highly effective in the treatment of migraine<sup>17</sup>. This chapter will focus on specific drugs for the acute treatment of migraine attacks, which are often used for the treatment of moderate to severe migraine attacks. The specific drugs include the ergot alkaloids ergotamine and dihydroergotamine and 5-HT<sub>1B/1D</sub> receptor agonists (triptans), from which sumatriptan has been extensively studied. Some new triptans (zolmitriptan, naratriptan and rizatriptan) have been recently marketed and some others (eletriptan, almotriptan and frovatriptan) are expected to be marketed in near future.

#### *Ergot alkaloids*

For decades, ergot alkaloids have been the only specific drugs for the acute treatment of migraine. Although these drugs are widely used, their efficacy has been poorly demonstrated by controlled clinical trials<sup>18,19</sup>. Ergotamine and dihydroergotamine (Figure 1.2) are vasoconstrictors, but they also inhibit perivascular inflammation in animals<sup>20</sup>. Ergotamine and, to a lesser extent, dihydroergotamine, may induce many side effects such as nausea, vomiting, vertigo, gastric symptoms, dry mouth, restlessness and, as will be discussed in Chapter 2, chest symptoms. In addition, incidental overdose or chronic overuse may induce ergotism, a rare but severe generalised vasospasm causing cyanosis, necrosis and infarctions of the heart and brain<sup>21</sup>. More frequent are ergot-dependent headaches, which may occur when the ergots are taken more often than once per day per week<sup>3,22</sup>. The high occurrence of side effects is probably due to the fact that ergotamine and dihydroergotamine display affinity for a large number of receptors, among which  $\alpha$ -adrenoceptors, dopamine



receptors and 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1F</sub> and 5-HT<sub>2</sub> receptors<sup>21,23</sup> (Pauwels, personal communication). Ergots have a low oral and rectal bioavailability and the clinical response is not related to the plasma concentration of the drug<sup>24,25</sup>, which is due to the slow washout of these compounds from their receptor biophase<sup>25,26</sup>.



**Figure 1.2** Chemical structures of the ergot alkaloids ergotamine and dihydroergotamine.

### *Sumatriptan*

The development of the 5-HT<sub>1</sub> receptor agonist sumatriptan<sup>27</sup> was prompted by the observations that administration of 5-HT could abort migraine attacks<sup>28</sup> and that ergotamine and methysergide elicit selective carotid vasoconstriction<sup>29,30</sup>. Further evidence that 5-HT is involved in the pathophysiology of migraine was provided by the finding that urinary excretion of 5-Hydroxyindole-3-acetic acid, the metabolite of 5-HT, increased during the headache phase<sup>31</sup> with concomitant decreased levels of platelet 5-HT<sup>32</sup>. Whereas some reports suggest that sumatriptan owes its action to presynaptic action only, inhibiting neuropeptide release and thus neurogenic inflammation<sup>20</sup>, sumatriptan may well owe its therapeutic action to its vasoconstrictor properties<sup>33</sup>. Sumatriptan was designed to act selectively as a vasoconstrictor at 5-HT<sub>1</sub> receptors in cranial blood vessels, but the drug also acts on 5-HT<sub>1</sub> receptors located in peripheral human blood vessels. Sumatriptan has affinity for the 5-HT<sub>1B</sub>,

5-HT<sub>1D</sub>, 5-HT<sub>1F</sub> and, although less, for the 5-HT<sub>1A</sub> receptors (Table 1.2).

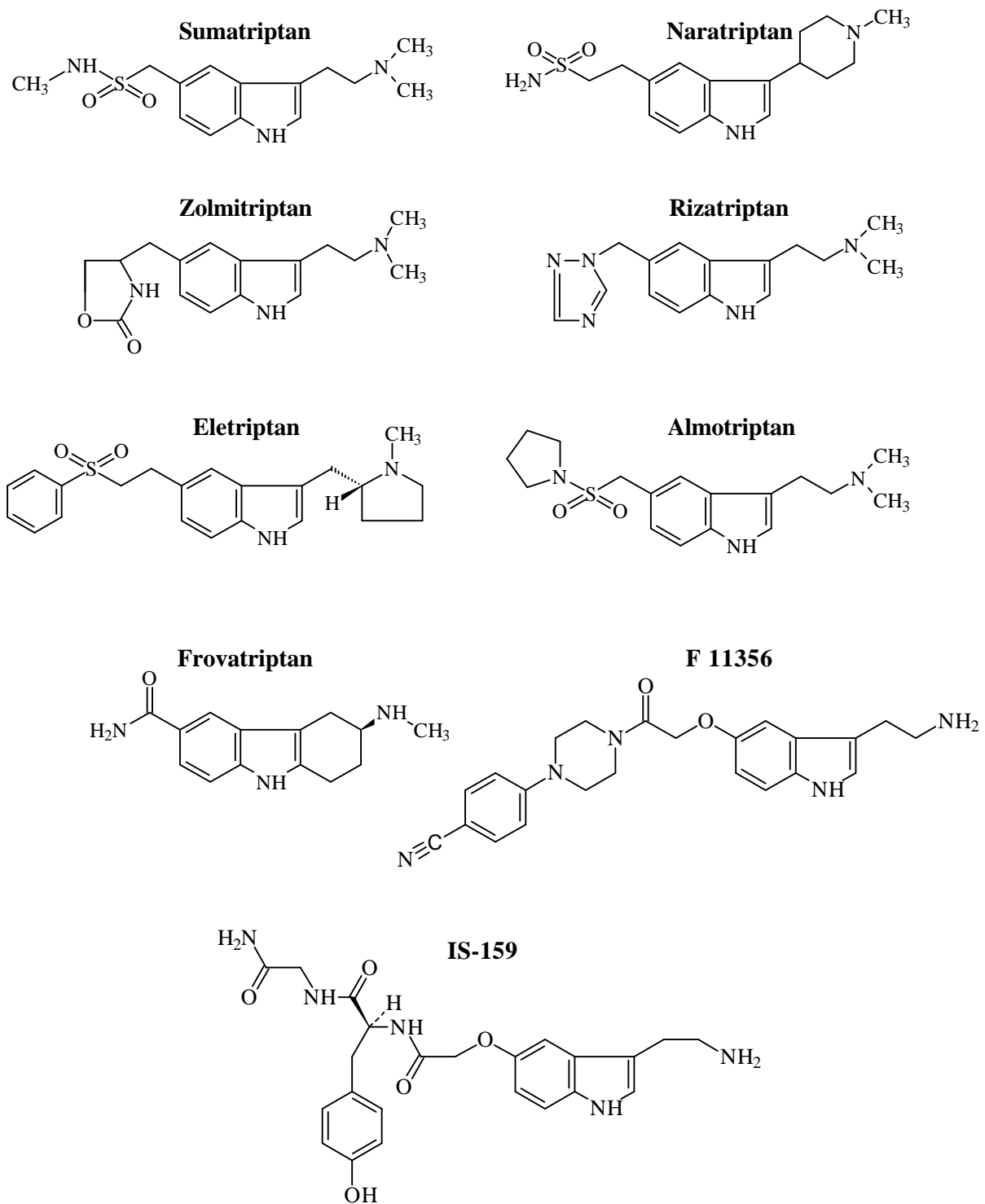
The discovery of the relatively selective 5-HT<sub>1</sub> receptor agonist sumatriptan was a major improvement in the acute treatment of migraine. The drug is highly effective and is generally well tolerated<sup>34,35</sup>. However, sumatriptan also has some shortcomings such as low oral bioavailability (14%)<sup>36,37</sup> and recurrence of the headache within 24 hours after initial headache relief in up to 40% of patients with initial good response<sup>38-41</sup>. Furthermore, the drug is contraindicated in patients with coronary artery disease because of its potential to constrict coronary arteries (this thesis). These shortcomings of sumatriptan, in addition to the excellent sales potentials in migraine, have prompted several pharmaceutical companies to develop 5-HT<sub>1B/1D</sub> receptor agonists which should be at least as effective as sumatriptan, but devoid of its shortcomings.

### *Second generation triptans*

Currently, six triptans are marketed (zolmitriptan, naratriptan, rizatriptan) or are expected to be launched in near future (eletriptan, almotriptan, frovatriptan). The new 5-HT<sub>1B/1D</sub> receptor agonists F 11356<sup>42</sup> and IS-159<sup>43</sup> are still in earlier phases of investigation. The triptans are all indole derivatives with chemical structures similar to sumatriptan (Figure 1.3). Standardised data on clinical efficacy are still limited and will therefore not be discussed in detail. In short, the newer triptans may have a faster onset of action, they may induce less recurrence of the headache and their improved oral pharmacokinetics may contribute to a more consistent response compared to oral sumatriptan. This latter does not apply to IS-159, which is a peptide and therefore not orally available, but is intended to be marketed as a nasal spray<sup>43</sup>.

As shown in Table 1.2, the second-generation triptans display slightly higher affinities at the 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors than sumatriptan<sup>44-48</sup>. With the exception of rizatriptan, almotriptan and to some extent sumatriptan, all other triptans, particularly zolmitriptan, have a high affinity at the 5-HT<sub>1A</sub> receptor. Similarly, the compounds have high affinity for the 5-HT<sub>1F</sub> receptor, except for rizatriptan and F 11356. A high degree of selectivity is observed over the other 5-HT receptors, although some drugs (sumatriptan, zolmitriptan, eletriptan, frovatriptan and possibly

Migraine and antimigraine drugs



**Figure 1.3** Chemical structures of sumatriptan and 'second generation' triptans.

**Table 1.2** Receptor binding properties (pK<sub>i</sub> values) of sumatriptan and second-generation triptans at 5-HT receptors. pK<sub>i</sub> values at 5-HT<sub>2C</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub>, 5-HT<sub>5A</sub>, 5-HT<sub>5B</sub> and 5-HT<sub>6</sub> receptors are not mentioned since these are <6.0 (except for eletriptan at 5-HT<sub>6</sub> receptor, pK<sub>i</sub>=6.28<sup>49</sup>). No affinity data are available on IS-159; therefore this compound was not included in the table.

	Sumatriptan	Zolmitriptan	Naratriptan	Rizatriptan	Eletriptan	Almotriptan	Frovatriptan	F 11356
5-HT <sub>1A</sub>	5.96 <sup>49</sup>	6.64 <sup>49</sup>	7.12 <sup>49</sup>	6.37 <sup>49</sup>	7.35 <sup>49</sup>	6.3 <sup>47</sup>	7.3 <sup>48</sup>	7.60 <sup>51</sup>
	6.43 <sup>50</sup>	9.20 <sup>45</sup>	7.58 <sup>44</sup>					
	6.90 <sup>44</sup>							
5-HT <sub>1B</sub>	7.37 <sup>49</sup>	7.69 <sup>49</sup>	8.09 <sup>49</sup>	6.86 <sup>49</sup>	8.00 <sup>49</sup>	8.0 <sup>47</sup>	8.6 <sup>48</sup>	9.81 <sup>51</sup>
	7.82 <sup>50</sup>	8.30 <sup>45</sup>	8.70 <sup>46</sup>	7.74 <sup>53</sup>	7.82 <sup>52</sup>		8.23 <sup>52</sup>	
	8.50 <sup>52</sup>	9.08 <sup>52</sup>	9.32 <sup>52</sup>	8.14 <sup>52</sup>				
5-HT <sub>1D</sub>	8.04 <sup>49</sup>	8.88 <sup>49</sup>	8.30 <sup>46</sup>	7.88 <sup>49</sup>	8.94 <sup>49</sup>	8.0 <sup>47</sup>	8.4 <sup>48</sup>	10.21 <sup>51</sup>
	8.46 <sup>50</sup>	9.20 <sup>45</sup>	8.41 <sup>49</sup>	8.63 <sup>52</sup>	8.82 <sup>52</sup>		8.52 <sup>52</sup>	
	8.68 <sup>52</sup>	9.66 <sup>52</sup>	9.16 <sup>52</sup>					
5-HT <sub>1E</sub>	5.79 <sup>49</sup>	7.73 <sup>49</sup>	7.69 <sup>49</sup>	6.77 <sup>49</sup>	7.25 <sup>49</sup>	ND	<6.0 <sup>48</sup>	5.94 <sup>51</sup>
	5.80 <sup>50</sup>							
5-HT <sub>1F</sub>	6.30 <sup>54</sup>	6.21 <sup>54</sup>	7.37 <sup>54</sup>	5.27 <sup>54</sup>	7.72 <sup>52</sup>	ND	7.0 <sup>48</sup>	5.47 <sup>51</sup>
	7.78 <sup>52</sup>	7.20 <sup>45</sup>	8.18 <sup>49</sup>	6.81 <sup>49</sup>	7.99 <sup>49</sup>		7.20 <sup>52</sup>	
	7.86 <sup>50</sup>	7.54 <sup>49,52</sup>	8.40 <sup>52</sup>	6.86 <sup>52</sup>				
	7.88 <sup>49</sup>							
5-HT <sub>2A</sub>	<5.5 <sup>49</sup>	<5.5 <sup>49</sup>	<5.5 <sup>49</sup>	<5.5 <sup>49</sup>	<5.5 <sup>49</sup>	ND	<5.3 <sup>48</sup>	6.47 <sup>51</sup>
5-HT <sub>2B</sub>	ND	7.19*	ND	6.59*	ND	ND	ND	ND
5-HT <sub>7</sub>	5.86 <sup>49</sup>	7.02 <sup>49</sup>	<5.5 <sup>49</sup>	5.73 <sup>49</sup>	6.70 <sup>49</sup>	<6.5 <sup>47</sup>	6.70 <sup>48</sup>	6.43 <sup>51</sup>

\* P. Gupta, personal communication; † P.J. Pauwels, personal communication

**Table 1.3** pEC<sub>50</sub> values of contraction to sumatriptan and second-generation triptans in human blood vessels. No data are available on F 11356 and IS-159; therefore these compounds were not included in the table.

	Sumatriptan	Zolmitriptan	Naratriptan	Rizatriptan	Eletriptan	Almotriptan	Frovatriptan
Basilar artery	6.93 <sup>55</sup>					5.46	7.86 <sup>55</sup>
Middle meningeal artery	7.15 <sup>56</sup> 7.28 <sup>57</sup>			7.05 <sup>56</sup>	7.30 <sup>57</sup>	7.52 <sup>47</sup>	
Saphenous vein	6.14 <sup>58</sup>				5.91 <sup>58</sup>		
Coronary artery	6.10 <sup>26</sup> 6.70 <sup>45</sup> 6.14 <sup>46</sup> 6.20 <sup>59</sup>	6.32 <sup>26</sup> 7.30 <sup>45</sup>	6.77 <sup>26</sup> 6.77 <sup>46</sup>	6.35 <sup>26</sup> 5.99 <sup>59</sup>	5.69 <sup>57</sup>		7.38 <sup>60</sup>

almotriptan and F 11356) display affinity at the 5-HT<sub>7</sub> receptor, which mediates smooth muscle relaxation<sup>61,62</sup>. Finally, it is worth mentioning that F 11356 displays a rather high affinity at the 5-HT<sub>2A</sub> receptor<sup>51</sup>.

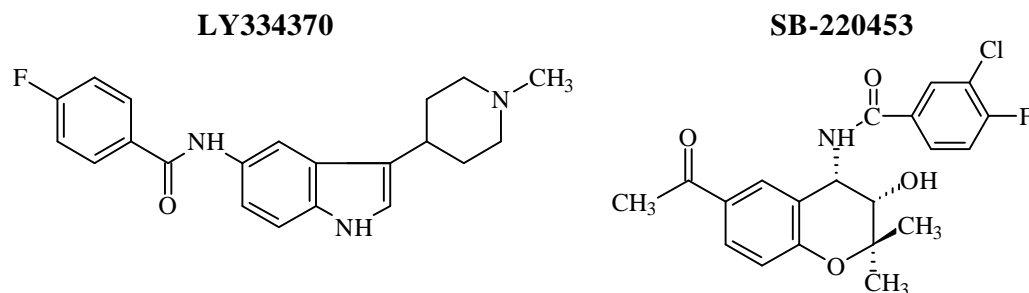
The second-generation triptans contract human blood vessels with a potency and maximal effect that is similar to that of sumatriptan<sup>26,45-47,55-60</sup> (Table 1.3). Whereas sumatriptan does not cross the intact blood-brain barrier<sup>63</sup>, the newer 5-HT<sub>1B/1D</sub> receptor agonists such as zolmitriptan, naratriptan, rizatriptan and eletriptan, which are more lipophilic than sumatriptan, are able to penetrate the blood-brain barrier and bind to trigeminal neurones in the nucleus caudalis in the brain stem and upper cervical cord<sup>64-66</sup>. It is not yet clear whether the central action of these compounds contributes to its therapeutic action.

In summary, all second-generation triptans seem to display pharmacodynamic properties not substantially different from sumatriptan. Most probably, the main differences between these compounds will be determined by their different pharmacokinetics. Since all triptans induce blood vessel contraction, it is to be expected that their ability to contract coronary arteries, eventually leading to myocardial ischaemia, will be similar<sup>26</sup>.

### *Beyond triptans*

Vasoconstrictor activity is not desirable in view of coronary artery constriction, which may consequently occur after the use of vasoconstrictor agents. Several approaches are being followed to develop antimigraine drugs devoid of vasoconstrictor action. These novel compounds have a low affinity for the 5-HT<sub>1B</sub> receptor, which seems to be responsible for vasoconstriction<sup>67,68</sup>. However, it is not yet clear whether vasoconstriction is necessary for a drug to be active in migraine. Recently the endothelin antagonist bosentan<sup>69</sup>, the NK-1 receptor antagonist lanepitant<sup>70</sup>, the selective 5-HT<sub>1D</sub> receptor agonist PNU-142633<sup>71</sup> and CP122,288<sup>72</sup>, which are all devoid of vasoconstrictor action, but potently inhibit plasma-protein extravasation in the rat, proved not to be effective in the treatment of migraine attacks. All currently available antimigraine drugs for the acute treatment of migraine possess the ability to contract blood vessels.

The 5-HT<sub>1F</sub> receptor agonist LY334370 (pK<sub>i</sub>: 6.9, 6.9 and 8.8 at 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub> and 5-HT<sub>1F</sub> receptor, respectively<sup>73</sup>, Figure 1.4) potently inhibits dural plasma protein extravasation<sup>73,74</sup>, but is devoid of vasoconstrictor activity in the rabbit saphenous vein<sup>75</sup>. This compound has recently been shown to be clinically effective in the treatment of migraine<sup>76</sup>, albeit at high doses of 60 and 200 mg. Unfortunately, it has not yet been demonstrated that the high plasma concentrations that are reached after these doses of LY334370 are devoid of vasoconstrictor activity in human blood vessels. The absence of vasoconstriction by LY334370 would suggest that 5-HT<sub>1B</sub> receptor-induced vasoconstrictor activity may not be required for antimigraine activity. This would, however, have no bearing on the importance of the 5-HT<sub>1B</sub> receptor in mediating the therapeutic action of the triptans, as illustrated by the clinical efficacy of alniditan<sup>77</sup>, a potent 5-HT<sub>1B/1D</sub> receptor agonist with a low affinity for the 5-HT<sub>1F</sub> receptor<sup>50</sup>.



**Figure 1.4** Chemical structures of LY334370 and SB-220453, potential antimigraine drugs possibly without vasoconstrictor properties.

A new therapeutic approach not involving vasoconstriction is attempted with the novel benzopyran SB-220453<sup>78</sup> (Figure 1.4). The compound has no significant affinity at 5-HT<sub>1B/1D</sub> receptors, nor does it show any activity in a large number of assays at other receptors, ion channels and enzymes<sup>78</sup>. SB-220453 exhibits a high affinity for a selective, but not yet characterised binding site in the human brain<sup>79</sup> and may be active in the treatment of migraine via blockade of vasodilator responses

initiated by spreading depression. A study on the contractile action in human blood vessels is described in Chapter 7. Results of the clinical efficacy of SB-220453 are awaited with interest.

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## Chapter 2

### Coronary Side Effects of Antimigraine Drugs

#### 2.1 Epidemiology of chest symptoms after ergot alkaloids and sumatriptan

The ergot alkaloids ergotamine and dihydroergotamine and the indole derivative sumatriptan are in widespread use for the acute treatment of migraine attacks. Besides the occurrence of ergotism and 'ergot headaches' associated with the use of ergot alkaloids<sup>1</sup>, one of the most important side effects of the acutely acting antimigraine drugs is the occurrence of chest symptoms in a proportion of patients. In contrast with the newer antimigraine drugs, the side effects of ergotamine and dihydroergotamine, as well as their efficacy<sup>1,2</sup>, have not systematically been studied in placebo-controlled trials. However, the use of ergotamine and dihydroergotamine has frequently been associated with substernal chest pain and discomfort<sup>3,4</sup> and even myocardial infarction<sup>3,5-10</sup> and sudden death<sup>11</sup>. In view of these problems, the pharmacologically more selective and, thus, probably safer indole derivative sumatriptan was developed. Side effects after sumatriptan have been well documented in clinical trials<sup>12,13</sup>. Chest symptoms, which include chest pressure, tightness and pain, sometimes mimicking pectoral angina, occur in up to 15% of patients consistently<sup>12-14</sup>. In addition, there are various reports on myocardial infarction associated with the use of sumatriptan<sup>15-22</sup>. Studies investigating risk factors for chest symptoms after the use of sumatriptan resulted in conflicting conclusions<sup>13,23</sup>. These studies are probably hampered by the fact that there often is no clear, uniform distinction between chest symptoms such as heavy arms or anxiety and specific more angina pectoris-like symptoms such as chest pain and radiating pain. The aetiology of the specific angina-like symptoms may differ from that of the more general symptoms. Determinants for the occurrence of chest symptoms may include young age, hypertension, general complaints of

abdominal pain, a family history of myocardial infarction and Raynaud's phenomenon<sup>23</sup>, although others could not confirm this, except for young age<sup>13</sup>. It is conceivable that the pharmacokinetics of sumatriptan in patients with and without chest symptoms differ; in patients with chest symptoms sumatriptan may be more rapidly absorbed than in patients without these side effects, thus leading to higher plasma concentrations and more side effects. However, this is unlikely since patients with chest symptoms lack other indicators of rapid and high plasma rise such as greater efficacy<sup>13</sup>. Another factor predisposing for the occurrence of chest symptoms may be a genetic difference between patients with and without chest symptoms. This is also unlikely since the DNA sequence encoding the receptor proteins of the 5-HT<sub>1B</sub> and 5-HT<sub>1F</sub> receptors, two of the most probable pharmacological targets of sumatriptan<sup>24</sup>, do not differ between patients with and without chest symptoms<sup>25,26</sup> (see also Chapters 11 and 12). The current chapter will focus on the origin of chest symptoms after sumatriptan and other acutely acting antimigraine drugs, summarise the information available about the mechanisms involved in coronary artery contraction to antimigraine drugs and discuss the improvements which may be achieved by novel drugs.

## **2.2 Chest pain after alkaloids and sumatriptan: cardiac versus non-cardiac origin**

Although several studies have been performed on the origin of chest symptoms after sumatriptan, the mechanism responsible for this side effect has not yet been fully elucidated. Chest symptoms may well be caused by constriction of coronary arteries, although there are several arguments against coronary artery constriction as a sole cause for chest symptoms. Firstly, in patients with chest symptoms, electrocardiographic (ECG) abnormalities are frequently absent<sup>27-29</sup>. Although in incidental cases myocardial infarction is not accompanied by ECG changes<sup>30</sup>, it is indeed unlikely that this exceptional condition applies to many of the patients with chest symptoms following the use of antimigraine drugs. Secondly, chest symptoms after sumatriptan are not related to the presence of cardiovascular disease; in one



study even an opposite relationship was observed<sup>13</sup>. However, as suggested by an *in vitro* study<sup>31</sup> (see also Chapter 9), human coronary artery contraction to sumatriptan is most probably more pronounced in patients *without* cardiovascular disease than in patients *with* diseased coronary arteries, thus allowing some debate about the conclusion mentioned above<sup>13</sup>.

Several non-cardiac mechanisms, which may cause chest symptoms, include spasms or constriction of oesophagus<sup>32,33</sup> or intercostal muscle<sup>34</sup>, pulmonary vasoconstriction<sup>35,36</sup> or bronchoconstriction<sup>37</sup>. Oesophageal spasm resulting from administration of sumatriptan has indeed been demonstrated, albeit at supratherapeutic doses<sup>32</sup>. The spasms were related to the occurrence of chest symptoms, and none of the subjects had ECG abnormalities. Others, however, have linked oesophageal spasms to throat tightness, instead of chest symptoms<sup>38</sup>. Concerning pulmonary vasoconstriction, sumatriptan causes a vasopressor response in the systemic as well as pulmonary arterial circulations<sup>39-42</sup>, suggesting that pulmonary artery constriction may also cause chest symptoms in some patients. Indeed, 5-HT<sub>1B/1D</sub> receptors, a most probable target of sumatriptan, have been demonstrated in human pulmonary arteries and veins<sup>40</sup>. The suggestion that sumatriptan may induce bronchoconstriction<sup>37</sup> was challenged after detailed analysis of a large number of clinical trials<sup>43,44</sup>.

Notwithstanding the evidence mentioned above, there are several pointers that, in at least a proportion of patients with chest symptoms after sumatriptan or other antimigraine drugs, coronary artery constriction is responsible for this side effect. Firstly, ergotamine has a long history of inducing adverse reactions related to coronary artery constriction such as myocardial infarction<sup>3,5-9</sup> and even sudden death<sup>11</sup>. Dihydroergotamine, although perhaps less frequent than ergotamine, has also been associated with myocardial infarction<sup>45</sup>. Sumatriptan, which was developed in an attempt to create a pharmacologically more selective and thus cardiovascular safer alternative for ergotamine, has also been associated with myocardial infarction in a number of cases<sup>15-22</sup>. In some incidental cases, episodes of chest pain after ergotamine<sup>3,9,46</sup> or sumatriptan<sup>47</sup> were accompanied by ECG changes.

In addition to these clinical data, coronary artery constriction has been observed after ergotamine, dihydroergotamine, sumatriptan and other antimigraine drugs both

*in vivo*<sup>39,48,49</sup> and *in vitro*<sup>50-55</sup>. A study on the myocardial perfusion in healthy female migraine patients showed no decrease by sumatriptan<sup>56</sup>, whereas a similar study with ergotamine showed a substantial decrease in myocardial blood flow in response to ergotamine<sup>57</sup>. The fact that myocardial perfusion was not affected by sumatriptan is not necessarily in contrast with the study of MacIntyre and colleagues who reported the coronary artery diameter decreased in response to sumatriptan<sup>39</sup>. It may well be that the coronary arteries were constricted to a level not affecting myocardial perfusion. In healthy coronary arteries, coronary blood flow remains unchanged until the arterial lumen is compromised by >80%<sup>58</sup>.

### **2.3 Mechanisms involved in coronary artery contraction to ergot alkaloids and sumatriptan**

As discussed above, constriction of the coronary artery is most probably responsible for the development of chest symptoms in at least a proportion of patients. It has not yet been elucidated which factors determine the degree of coronary artery constriction in response to sumatriptan and other antimigraine drugs.

#### *In vitro studies on human isolated coronary artery*

There are several reports on the degree of human isolated coronary artery contraction to sumatriptan under various conditions. Sumatriptan induced a higher contraction, relative to potassium, in arteries obtained from patients with ischemic heart disease than in non-diseased arteries<sup>52</sup>. In the same study, contraction to sumatriptan was the highest in arterial segments located directly distal to an atheromatous lesion<sup>52</sup>. Another study on coronary artery segments obtained from a patient with variant angina reported a supersensitive response to sumatriptan, mediated by the 5-HT<sub>1B</sub> receptor<sup>59</sup>. This report, which only involved one patient, was merely based on the low threshold for coronary artery contraction to sumatriptan; the pEC<sub>50</sub> (6.5) and E<sub>max</sub> (28% of contraction to 40 mM K<sup>+</sup>) of sumatriptan were not abnormal in this study<sup>59</sup>. Coronary arteries from patients with variant angina may display an impaired production of nitric oxide (NO)<sup>60,61</sup>, although others did not observe this<sup>62</sup>. The

observation of increased sensitivity of coronary artery contraction to sumatriptan in variant angina<sup>59</sup> may thus be in line with a report on the pulmonary artery, where sumatriptan was more potent in vessel segments treated with the NO synthase inhibitor L-nitro-L-arginine methylester (L-NAME) than in control segments. Contractions of internal mammary artery to sumatriptan were reported to be slightly increased in hypertensive subjects<sup>63</sup>, although the authors did not make any statement of the endothelial quality in these vessels.

In contrast with the studies mentioned above, others observed no relation between the response to 5-HT mediated via 5-HT<sub>1</sub> receptors and the presence of atheromatous lesions<sup>53,64</sup>. Other data on human isolated coronary contraction to sumatriptan do not indicate a more potent contraction to sumatriptan when the endothelium was mechanically removed<sup>51</sup>. Moreover, a post hoc study on the relation between human isolated coronary artery contraction to sumatriptan and endothelial quality showed that the pEC<sub>50</sub> of sumatriptan was not related to the endothelial quality; the maximal contraction to sumatriptan was even decreased in arteries with impaired endothelial function<sup>31</sup> (see also Chapter 9). Summarised, no conclusive statement about the role of the endothelium in sumatriptan-induced contraction of the human coronary artery can be made.

Several studies have reported that the contractile response to sumatriptan may be augmented by thromboxane A<sub>2</sub><sup>51,65-67</sup>, which is present in blood platelets but is also endogenously produced by the coronary artery<sup>51</sup>. Thromboxane A<sub>2</sub> may well contribute to coronary vasospasm in response to antimigraine drugs<sup>68-70</sup>. Most strikingly, coronary sinus levels of thromboxane B<sub>2</sub>, the stable metabolite of thromboxane A<sub>2</sub>, were markedly increased just prior to provocation with ergonovine maleate in patients who responded with coronary vasospasm, as opposed to patients without this reaction<sup>70</sup>. Moreover, the amounts of ergonovine maleate needed to induce coronary spasm were inversely correlated with thromboxane B<sub>2</sub> levels in the coronary sinus, and in almost all patients the coronary spasticity was attenuated after treatment with a thromboxane A<sub>2</sub> synthase inhibitor reducing coronary sinus thromboxane B<sub>2</sub> levels<sup>70</sup>. The mechanism of the augmentation of contraction to sumatriptan by thromboxane A<sub>2</sub> is not yet clear, but does not seem to depend on

increased basal tension only because contraction to sumatriptan may be augmented by concentrations of agonist inducing only a threshold contraction<sup>71</sup> that does not increase basal tension. Similarly, augmented levels of phosphatidyl-inositol 4,5-biphosphate (PIP<sub>2</sub>) can not explain the augmentation of contraction to sumatriptan, as was suggested earlier<sup>72</sup>, because the vasoconstrictor peptide endothelin-1 does not augment human coronary artery contraction to sumatriptan<sup>51</sup>, while it acts via the same second messenger as thromboxane A<sub>2</sub><sup>73</sup>.

Apart from the *degree* of coronary artery constriction, also the *duration* of constriction may determine safety of a drug. The ergot alkaloids, ergotamine and dihydroergotamine, diffuse slowly from the receptor biophase<sup>74</sup>, which is in accordance with the sustained coronary artery contraction after washout of these drugs<sup>50</sup>. Also in the clinical situation, the effects of ergotamine and dihydroergotamine sustain much longer than is to be expected from their plasma concentration profiles<sup>74-76</sup>.

#### *Coronary artery constriction in patients*

Beside information obtained from basic *in vitro* studies, it is important to investigate cardiac events following antimigraine drugs in clinical cases to obtain information about the mechanisms involved. The use of ergot alkaloids as well as sumatriptan has led to myocardial infarction in patients with demonstrated obstructive coronary artery lesions<sup>10,18,21</sup>. In these patients, who have a limited coronary artery reserve<sup>58</sup>, even a small coronary artery contraction may cause myocardial ischaemia<sup>77</sup>. However, many of the patients who experienced myocardial infarction after ergotamine or sumatriptan had no obstructive coronary artery disease, but had a history of chest pain suggestive of variant angina<sup>5,17</sup> or had no demonstrated cardiovascular abnormalities<sup>6-9,16,19</sup>. Probably, in these patients the antimigraine drugs caused a vasospasm of the coronary artery, in the absence of any abnormal atheromatous lesions<sup>78,79</sup>. This vasospasm may be a reaction to the antimigraine drugs *per se*, or may have developed in association with other mediators like thromboxane A<sub>2</sub>.

Migraine has been suggested to be part of a generalised vasospastic disorder, thus being related to other pathological conditions such as Prinzmetal's angina and

Raynaud's phenomenon. Such a relation would predispose migraine patients to develop coronary vasospasms<sup>80-83</sup>, especially in response to vasoconstrictive antimigraine medication. However, others did not observe a relationship between migraine, Raynaud's phenomenon and Prinzmetal's angina<sup>84,85</sup>. One of the terms previously used to describe the occurrence of chest symptoms, as a major constituent of migraine, is 'precordial migraine'. Whereas currently cardiovascular disease is a contraindication to antimigraine drugs with vasoconstrictor properties, some years ago a migraine patient with chest pain could be deliberately treated with ergotamine. This is illustrated by the following case history, described by O.W. Sacks in 1970<sup>86</sup>:

'[The patient], a 61-year-old woman, had had attacks of classical migraine since adolescence. .... One of the symptoms, during severe attacks, is a feeling of painful tightness in the chest, accompanied by the radiation of pain to the left scapula, and down the left arm: it generally lasts for two to three hours. The chest pain .... is diminished, in company with its other accompanying symptoms, by ergotamine.'

## **2.4 Novel antimigraine drugs; any improvement?**

Second generation sumatriptan-like antimigraine drugs are all aimed at achieving a better oral bioavailability compared to sumatriptan (thus potentially generating a higher efficacy and longer duration of action), and reduced coronary vasoconstrictor activity<sup>87</sup>.

In patients undergoing cardiac catheterisation, coronary artery diameter was not affected by naratriptan<sup>88</sup> (1.5 mg s.c., a dose reaching plasma concentrations similar to that obtained with a therapeutic 2.5 mg p.o. dose<sup>89</sup>) or eletriptan<sup>90</sup> (3.33 µg/kg/min infusion i.v., reaching plasma concentrations similar to that obtained with a therapeutic 40 mg dose<sup>90</sup>). In contrast, coronary artery diameter decreased after avitriptan<sup>91</sup> (0.39 or 0.56 mg/kg/h infusion i.v.), in a similar manner as after sumatriptan<sup>39,48</sup> (6 mg, s.c., or 10 mg, i.v.). The fact that naratriptan and eletriptan behaved different than avitriptan and sumatriptan is most probably explained by the

lower doses of naratriptan<sup>88</sup> and eletriptan<sup>90</sup> that were used compared to avitriptan<sup>91</sup> and sumatriptan<sup>39,48</sup>.

*In vitro* studies investigating human coronary artery contraction to a number of antimigraine drugs which are already in use for some years (sumatriptan, ergotamine, dihydroergotamine, methysergide and its active metabolite methylergometrine<sup>92</sup>) and novel 'second generation' sumatriptan-like antimigraine drugs (naratriptan, zolmitriptan, rizatriptan, avitriptan and eletriptan) will be described in Chapters 5 and 6. Frovatriptan induced a similar maximal contraction in the human isolated coronary artery as sumatriptan<sup>93</sup>. Clinical data on the side effects of the novel drugs are difficult to compare. Thus, no conclusions about differences between the triptans can be drawn from clinical studies yet.

Some of the new 5-HT<sub>1B/1D</sub> receptor agonists such as zolmitriptan<sup>94</sup> and eletriptan<sup>95</sup> have been described to behave as partial agonists relative to sumatriptan at rabbit<sup>94</sup> and canine saphenous vein<sup>95</sup>, respectively. This property could have clinical implications since partial agonists may exhibit tissue-selective pharmacological effects<sup>96</sup>. However, it is not yet clear whether the partial agonism demonstrated in animal tissues will be similar in human blood vessels, and whether this translates into a favourable side-effect profile.

Another approach for the development of safe acutely acting antimigraine drugs is the development of drugs which are devoid of any vasoconstrictor property and owe their therapeutic efficacy to an alternative mechanism (see Chapter 1). It remains to be seen whether such drugs will be effective in the treatment of migraine at doses not inducing vasoconstriction via 5-HT<sub>1B/1D</sub> receptors.

## **2.5 Summary and conclusion**

Although alternative mechanisms may play a role in a proportion of patients, there is overwhelming evidence that, at least in some patients, chest symptoms following antimigraine drug therapy are due to coronary artery spasm. It is not yet clear which factors determine the degree of coronary artery constriction to antimigraine drugs. In

a proportion of patients antimigraine drugs may, possibly in association with endogenous thromboxane A<sub>2</sub>, cause variant angina-like coronary vasospasm, although in patients with healthy coronary arteries, myocardial ischaemia remains unlikely<sup>97</sup>. In contrast, in patients with pre-existing coronary artery lesions who have only a limited coronary reserve<sup>58</sup>, a small coronary artery contraction may be sufficient to induce cardiac ischaemia. It is not yet clear whether drugs that lack vasoconstrictor property, which most likely will be devoid of coronary side effects, will be able to abolish migraine attacks.

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## Chapter 3

# 5-Hydroxytryptamine Receptors Mediating Contraction of the Human Coronary Artery

### 3.1 Effects of 5-hydroxytryptamine on the human coronary artery

When 5-HT is infused in the coronary artery of healthy subjects it induces dose-dependent increases in blood flow due to vasodilatation<sup>1,2</sup>, which are potentiated in the presence of the 5-HT<sub>2</sub> receptor antagonist ketanserin<sup>2</sup>. This relaxation has been attributed to activation of 5-HT<sub>1</sub>(-like) receptors located on the endothelial surface, mediating release of endothelium-derived relaxing factor in the form of nitric oxide or a nitrosoderivative yielding nitric oxide<sup>3-5</sup>. In patients with coronary artery atherosclerosis<sup>1,2,6</sup> or in patients undergoing coronary angioplasty<sup>7,8</sup>, serotonin reduces the coronary artery diameter. This reduction in diameter is prevented by ketanserin in proximal arteries<sup>2,7</sup>, and to some extent in distal arteries<sup>6</sup>. Coronary arteries are also known to constrict in response to the selective 5-HT<sub>1</sub> receptor agonist sumatriptan<sup>9</sup>, providing evidence that 5-HT<sub>1</sub> receptors may also induce vasoconstriction. The presence of functional endothelium may be one of the factors determining the net effect of serotonergic agonists<sup>10-14</sup> (see also Chapter 9).

Taken together, the results from the studies mentioned above suggest that in healthy subjects, as well as in patients with coronary artery disease, 5-HT induces vasoconstriction via ketanserin-sensitive 5-HT<sub>2</sub> receptors as well as via ketanserin-insensitive 5-HT<sub>1</sub> receptors. In addition, in healthy subjects, there seems to be a dilating, ketanserin-resistant receptor, which has been suggested to be of the 5-HT<sub>1</sub>(-like) type. However, it seems likely that this receptor is the 5-HT<sub>7</sub> receptor<sup>15</sup>, which has been demonstrated to mediate relaxation in, for example, canine coronary arteries<sup>16</sup>. The loss of functional endothelium in diseased arteries results in a loss of

5-HT receptor-mediated vasodilatation. Most strikingly, the dilating effect of 5-HT has not been observed in human coronary arteries *in vitro*<sup>17,18</sup>, although it has been demonstrated in animal experiments<sup>19,20</sup>.

In the human isolated coronary artery, 5-HT<sub>2</sub> receptors generally induce more contraction than 5-HT<sub>1</sub> receptors<sup>18,21-24</sup>. The contribution of contraction mediated by 5-HT<sub>1</sub> receptors is highly variable, and in some cases the contraction mediated by 5-HT<sub>1</sub> receptors predominates over that mediated by 5-HT<sub>2</sub> receptors<sup>25</sup>. The large degree of variability of human isolated coronary artery contraction mediated by the 5-HT<sub>1</sub> receptor has been assigned to, at least partly, endogenous production thromboxane A<sub>2</sub><sup>26</sup>. Indeed, augmentation of contraction to 5-HT by a thromboxane A<sub>2</sub> analogue is mediated by 5-HT<sub>1</sub> receptors rather than by 5-HT<sub>2</sub> receptors<sup>17</sup>.

The relevance of 5-HT in coronary artery spasm is still debated. Based on the lack of effect of ketanserin in the treatment of patients with pure vasospastic angina<sup>27-29</sup>, it was argued that 5-HT does not play a role in the aetiology of Prinzmetal's angina. However, the authors did not entertain the possibility that 5-HT<sub>1</sub> receptor-mediated constriction causes vasospasms in these patients. Later, it was indeed suggested that in patients with variant angina, coronary artery constriction is mainly mediated by the 5-HT<sub>1</sub> receptor<sup>30</sup>. Thus, it seems that in some conditions, e.g. after percutaneous transluminal angioplasty<sup>7,8</sup> or in variant angina, the contraction induced by 5-HT is augmented, possibly due to an increased 'crosstalk' with other substances such as thromboxane A<sub>2</sub> under these conditions<sup>31,32</sup>. As described above, the augmentation of contraction to 5-HT by other substances may mainly be assigned to the 5-HT<sub>1</sub> receptor<sup>17</sup>.

In summary, under basal physiological conditions, 5-HT<sub>2</sub> receptor-mediated contraction seems to be more pronounced than contraction mediated by 5-HT<sub>1</sub> receptors. Under exceptional, possibly pathophysiological conditions such as variant angina, 5-HT<sub>1</sub> receptor-mediated contraction may become important than 5-HT<sub>2</sub> receptor-mediated contraction. This may also have implications for the relevance of coronary artery constriction in response to antimigraine drugs.



### 3.2 5-Hydroxytryptamine receptors mediating coronary artery contraction to antimigraine drugs

Whereas for many years human contractile vascular 5-HT receptors were divided into 5-HT<sub>1</sub>(-like) and 5-HT<sub>2</sub> receptors<sup>33</sup>, currently much interest is focused on the precise nature of the human contractile 5-HT<sub>1</sub> receptors. Possible candidates for the contractile 5-HT<sub>1</sub> receptor in the human isolated coronary artery are the 5-HT<sub>1B</sub> (formerly 5-HT<sub>1DB</sub><sup>34</sup>), 5-HT<sub>1D</sub> (formerly 5-HT<sub>1D $\alpha$</sub> <sup>34</sup>) and 5-HT<sub>1F</sub><sup>35</sup> receptors. The precise nature of the contractile 5-HT<sub>1</sub> receptor in the human coronary artery has been investigated using functional and, since some years, molecular techniques.

#### *Functional experiments*

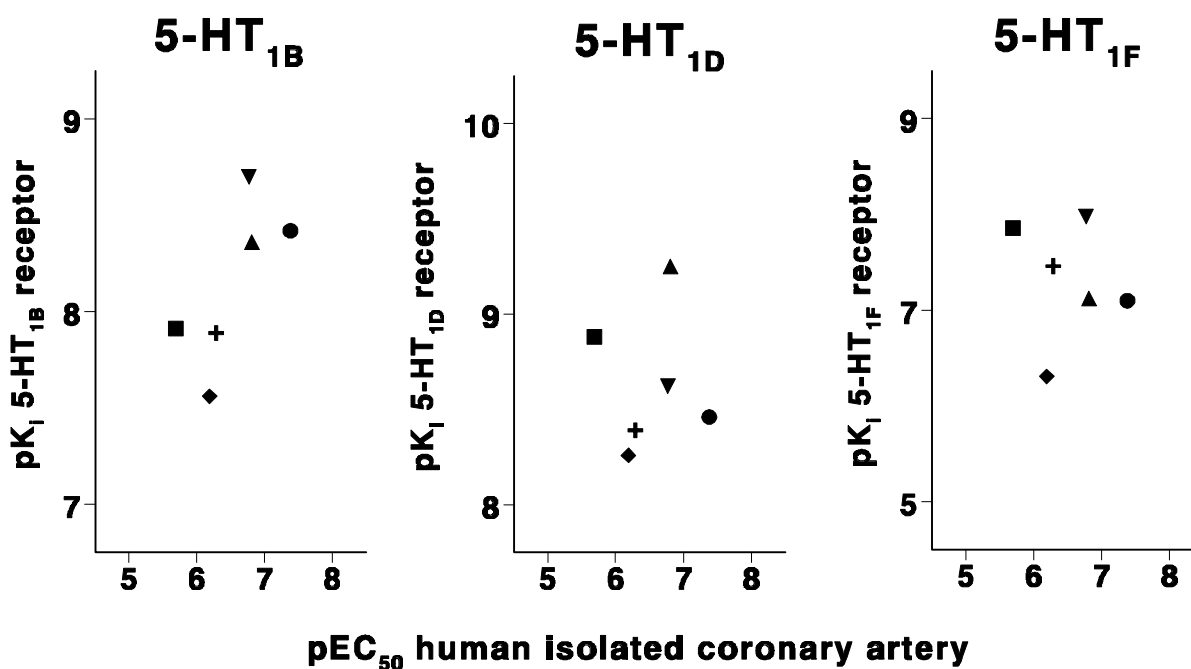
The antimigraine drugs, ergotamine and dihydroergotamine, display affinity for a large number of receptors, including  $\alpha$ -adrenoceptors, dopamine receptors and 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1F</sub> and 5-HT<sub>2</sub> receptors<sup>36,37</sup> (Pauwels, personal communication). It has not yet fully been elucidated which of these receptors contribute to human coronary artery contraction to the ergot alkaloids, although at least 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors are likely to contribute to this contraction. More studies have been performed on receptors mediating human isolated coronary artery contraction to novel antimigraine drugs. Sumatriptan, a 5-HT derivative, was developed to obtain a pharmacologically more selective and thus cardiac safer alternative for ergotamine. Sumatriptan displays affinity for the 5-HT<sub>1B</sub> (pK<sub>i</sub> 7.4-7.8<sup>37,38</sup>), 5-HT<sub>1D</sub> (pK<sub>i</sub> 8.0-8.5<sup>37,38</sup>) and 5-HT<sub>1F</sub> (pK<sub>i</sub> 7.9<sup>37,38</sup>) receptors, all of which may theoretically mediate contraction of the human coronary artery by inhibition of cAMP<sup>39</sup>. However, there are several arguments, which do not support the contention that the 5-HT<sub>1F</sub> receptor mediates coronary artery contraction. Firstly, 5-carboxamidotryptamine (5-CT) is more potent than sumatriptan in inducing coronary artery contraction (pEC<sub>50</sub> 7.0<sup>23</sup> versus 6.3<sup>18,24,26</sup>, whereas it displays a lower affinity for the 5-HT<sub>1F</sub> receptor (pK<sub>i</sub> 6.1 and 7.6, respectively<sup>35</sup>). However, 5-CT, in our own studies, was less potent than sumatriptan in inducing human isolated coronary artery contraction (Chapter 10), and others also have reported a relatively

low potency for 5-CT in the human isolated coronary artery ( $pEC_{50}$  6.3<sup>40</sup>). Secondly, the selective 5-HT<sub>1F</sub> receptor agonist, LY344864<sup>41</sup>, does not contract rabbit saphenous vein<sup>42</sup> and human cerebral arteries<sup>43</sup>, which may suggest that this compound is also ineffective in the human isolated coronary artery<sup>44</sup>. However, the contractile effects of a selective 5-HT<sub>1F</sub> receptor agonist should be investigated in the human isolated coronary artery before the involvement of the 5-HT<sub>1F</sub> receptor in human isolated coronary artery contraction can definitely be excluded.

To determine whether the 5-HT<sub>1B</sub> or 5-HT<sub>1D</sub> receptor mediates contraction of the human isolated coronary artery, Kaumann and colleagues<sup>25</sup> investigated whether ketanserin antagonised contraction elicited by 5-HT<sub>1</sub> receptors. Since sumatriptan-induced contractions were not affected by ketanserin, in a concentration (1  $\mu$ M) high enough to block 5-HT<sub>1D</sub> receptors, the authors concluded that the 5-HT<sub>1</sub> receptor mediating coronary artery contraction is likely to be the 5-HT<sub>1B</sub> receptor. Finally, the selective 5-HT<sub>1D</sub> receptor agonist PNU-109291 does not induce contraction of human cerebral arteries<sup>43</sup>. Although the coronary and cerebral arteries resemble in their responses<sup>44</sup>, it is obvious that definite conclusions can only be based on results obtained in the human coronary artery.

Another approach to determine the receptor(s) involved in human isolated coronary artery contraction is to study the correlation between the affinity of agonists for a receptor ( $pK_i$  values) with the potency of the agonists to induce contraction in the human isolated coronary artery ( $pEC_{50}$ ). Based on the data summarised in Tables 1.2 and 1.3 (Chapter 1), the potencies of the compounds were not significantly correlated with either the 5-HT<sub>1B</sub> (Pearson's  $r$ : 0.71,  $p$ : 0.11), 5-HT<sub>1D</sub> (Pearson's  $r$ : 0.00,  $p$ : 0.99) or 5-HT<sub>1F</sub> (Pearson's  $r$ : -0.12,  $p$ : 0.81) receptor (Figure 3.1). There are several explanations for this lack of significant correlation with any of these receptors. Firstly, it is possible that these receptors do not mediate contraction of the human isolated coronary artery and that contraction is mediated by other receptors. Secondly, the affinities and potencies of the compounds studied were too close to each other to reveal an underlying correlation. This could well be, since there was a tendency for a correlation between the  $pEC_{50}$  in human isolated coronary artery and the  $pK_i$  values for the 5-HT<sub>1B</sub> receptor. Finally, potencies of agonists, used in the present analysis,

may be expected to correlate less with the binding affinity than potencies of antagonists<sup>45</sup>.



**Figure 3.1** Scatterplots showing pEC<sub>50</sub> values of sumatriptan (+), zolmitriptan (□), naratriptan (△), rizatriptan (▽), eletriptan (●) and frovatriptan (●) in the human isolated coronary artery, correlated to the pK<sub>i</sub> values of these compounds on the 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub> and 5-HT<sub>1F</sub> receptor. When more than one pEC<sub>50</sub> or pK<sub>i</sub> value was listed in Table 1.2 and 1.3 (Chapter 1), the mean was calculated. For none of the receptors, there was a significant correlation between pK<sub>i</sub> and pEC<sub>50</sub> values.

### *Molecular studies*

Over the last few years, new molecular techniques have enabled the detection of receptors and their messenger RNA (mRNA) in tissues. Using reverse transcriptase polymerase chain reaction (RT-PCR), mRNA coding for the 5-HT<sub>1B</sub><sup>46,47</sup>, 5-HT<sub>1F</sub><sup>47</sup> and 5-HT<sub>2A</sub><sup>47</sup> receptor has clearly been demonstrated in the human coronary artery, while mRNA coding for the 5-HT<sub>1A</sub><sup>47</sup> or 5-HT<sub>1D</sub><sup>47</sup> receptor is weakly expressed. Whereas

**Figure 3.2** Immunohistochemical staining of human coronary artery. Immunoreactivity is visualised in black and background staining is shown in grey. Panel upper right shows that 5-HT<sub>1D</sub>-immunoreactivity was not detected on the vessel wall. Panel upper left shows that dense 5-HT<sub>1B</sub>-immunoreactivity was detected on smooth muscle cells (anti  $\alpha$ -actin immunostaining, shown in panel lower left) and faint on endothelial cells (Ulex europeas immunostaining, panel lower right). Figure adapted from Nilsson *et al.*<sup>47</sup>

in one study, mRNA coding for the 5-HT<sub>7</sub> receptor was clearly present<sup>48</sup>, others reported only a weak expression of 5-HT<sub>7</sub> receptor mRNA<sup>47</sup>. A study on a coronary artery of a patient suffering from variant angina demonstrated mRNA for 5-HT<sub>1B</sub> and the 5-HT<sub>2A</sub> receptors, but not for the 5-HT<sub>1A</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub>, 5-HT<sub>1F</sub>, 5-HT<sub>2B</sub> or 5-HT<sub>2C</sub> receptor<sup>49</sup>. In cultured endothelial cells of human coronary artery, mRNA for the 5-HT<sub>1B</sub> and 5-HT<sub>2B</sub> receptors was demonstrated<sup>50</sup>. Unfortunately, studies investigating mRNA can only demonstrate that the genomic DNA is transcribed to mRNA in a certain tissue, but do not ensure the presence of the receptor protein.

An elegant approach to demonstrate receptor protein is the use of immunohistochemistry, which also provides information on the anatomical localisation of the receptors. Using selective polyclonal antibodies against 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors<sup>51</sup>, the presence of 5-HT<sub>1B</sub>, but not 5-HT<sub>1D</sub> receptors was demonstrated in the smooth muscle layer of the coronary artery<sup>47,52</sup>. Only few 5-HT<sub>1B</sub> receptors, and no 5-HT<sub>1D</sub> receptors, were detected in the endothelial layer.

Summarising results from functional and molecular studies, it seems that the 5-HT<sub>1</sub> receptor mediating human coronary artery contraction is likely to be the 5-HT<sub>1B</sub> receptor. Yet, there was no clear correlation between potency of several 5-HT<sub>1</sub> receptor agonists to contract the human isolated coronary artery and their affinity for the 5-HT<sub>1B</sub> receptor, thus leaving the possibility of other receptors being involved in coronary artery contraction.

Contraction of human cranial arteries, the potential pharmacological target of sumatriptan and other 'triptans'<sup>53,54</sup>, also seems to be mediated by the 5-HT<sub>1B</sub> receptor<sup>43,55</sup>. As long as the therapeutic efficacy of acutely acting antimigraine drugs is mediated via the 5-HT<sub>1B</sub> receptor, these antimigraine drugs will, most likely, also induce contraction of the human coronary artery. It remains to be shown that compounds, which do not contract cranial arteries via 5-HT<sub>1B</sub> receptors, are indeed effective in the treatment of migraine. Such drugs may be devoid of adverse coronary side effects.

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## **Chapter 4**

### **Aims of the Thesis**

Based on the questions that were posed in the previous chapters, the objective of this thesis was to investigate the coronary side-effect potential to the 'golden standard' sumatriptan and other antimigraine drugs. For this purpose, the following objectives were defined:

1. To determine the coronary side-effect potential of several current and prospective antimigraine drugs, and to compare this with that of sumatriptan (see Part 2 of the thesis).
2. To investigate which factors determine the magnitude of human isolated coronary artery contraction to sumatriptan (see Part 3 of the thesis).
3. To characterise the 5-HT receptors involved in human isolated coronary artery contraction to sumatriptan (see Part 4 of the thesis).
4. To assess whether there is a genetic basis for the occurrence of chest symptoms after the use of sumatriptan (see Part 5 of the thesis).

## Chapter 5

### Coronary Side-Effect Potential of Current and Prospective Antimigraine Drugs

**Summary** - The antimigraine drugs ergotamine and sumatriptan may cause angina-like symptoms, possibly resulting from coronary artery constriction. We compared the coronary vasoconstrictor potential of a number of current and prospective antimigraine drugs (ergotamine, dihydroergotamine, methysergide and its metabolite methylergometrine, sumatriptan, naratriptan, zolmitriptan, rizatriptan, avitriptan). Concentration-response curves to the antimigraine drugs were constructed in human isolated coronary artery segments to obtain the maximum contractile response ( $E_{\max}$ ) and the concentration eliciting 50% of  $E_{\max}$  ( $EC_{50}$ ). The  $EC_{50}$  values were related to maximum plasma concentrations ( $C_{\max}$ ) reported in patients, obtaining  $C_{\max}/EC_{50}$  ratios as an *index* of coronary vasoconstriction occurring in the clinical setting. For the compounds from which data on plasma protein binding were available to us, we also corrected for the amount of drug bound to plasma proteins. Furthermore, we studied the duration of contractile responses after washout of the acutely acting antimigraine drugs to assess their disappearance from the receptor biophase. Compared to sumatriptan, all drugs were more potent (lower  $EC_{50}$  values) in contracting the coronary artery but had similar efficacies ( $E_{\max}$  <25% of  $K^+$ -induced contraction). The  $C_{\max}$  of avitriptan was 7- to 11-fold higher than its  $EC_{50}$  value, whereas those of the other drugs were <40% of their respective  $EC_{50}$  values. After correction for plasma protein binding, the free  $C_{\max}/EC_{50}$  ratio for avitriptan was in the same range as for the other compounds. The contractile responses to ergotamine and dihydroergotamine persisted even after repeated washings, but those to the other drugs declined rapidly after washing. All current and prospective antimigraine drugs contract the human coronary artery *in vitro*, but in view of low efficacy, these drugs are unlikely to cause myocardial ischemia at therapeutic plasma

Based on: MaassenVanDenBrink A, Reekers M, Bax WA, Ferrari MD, Saxena PR. Coronary side-effect potential of current and prospective antimigraine drugs. *Circulation*. 1998;98:25-30.

concentrations in healthy subjects. In patients with coronary artery disease, however, these drugs must remain contraindicated. The sustained contraction by ergotamine and dihydroergotamine seems to be an important disadvantage compared to sumatriptan-like drugs.

## **5.1 Introduction**

Sumatriptan, a 5-hydroxytryptamine (5-HT) derivative with agonist activity at 5-HT<sub>1B/1D</sub> receptors, is highly effective in aborting attacks of migraine and cluster headache. The drug is generally well tolerated. However, up to 15% of patients consistently report chest symptoms, including chest pressure, tightness and pain, often mimicking pectoral angina<sup>1-3</sup>. Although extracardiac mechanisms have been invoked<sup>4</sup>, chest symptoms may well be caused by coronary vasoconstriction, which has been observed after sumatriptan both *in vivo*<sup>5</sup> and *in vitro*<sup>6-8</sup>. In some cases, the use of sumatriptan, like that of ergotamine<sup>9-11</sup>, was even associated with myocardial infarction<sup>12,13</sup> and cardiac arrest<sup>14</sup>. 'Second generation' sumatriptan-like antimigraine drugs are all aimed at, besides achieving high efficacy and long duration of action, avoiding coronary vasoconstrictor activity<sup>15</sup>.

The present study deals with two major issues in clinical practice, namely i) do new antimigraine compounds cause less coronary artery constriction than sumatriptan? and ii) is sumatriptan better than ergot derivatives in this respect? Obviously, these questions cannot be easily answered by clinical trials. We have therefore employed a pharmacological approach using the human isolated coronary artery to determine the *potency* (sensitivity) and *efficacy* (magnitude) of the contractile responses to sumatriptan and other current (ergotamine, dihydroergotamine, methysergide and its active metabolite methylergometrine<sup>16</sup>) as well as new antimigraine drugs (naratriptan<sup>17</sup>, zolmitriptan<sup>18,19</sup>, rizatriptan<sup>20</sup> and avitriptan<sup>21</sup>). Results were related to the respective maximum plasma concentrations (C<sub>max</sub>) reported in patients.

Since sumatriptan-induced contractions of coronary arteries show substantial variability, both within and between studies<sup>22,23</sup>, we used a 'parallel' experimental

design involving segments from the same coronary artery. These coronary arteries were obtained from organ donors who died of causes unrelated to cardiac diseases and, therefore, may potentially represent the population treated with antimigraine drugs.

## 5.2 Patients and methods

### *Preparation of tissue*

Right epicardial coronary arteries were obtained from 14 'heart beating' organ donors (7 male, 7 female; age 7-61 years) who died of non-cardiac disorders (11 cerebrovascular accident, 3 head trauma) less than 24 hours before the tissue was taken to the laboratory. Hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services / Eurotransplant Foundation) after removal of the aortic and pulmonary valves for transplantation purposes. The hearts were stored at 0°C to 4°C in a sterile organ-protecting solution immediately after circulatory arrest. After arrival in the laboratory, the right coronary artery was removed and placed in a cold, oxygenated Krebs bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>2</sub> 25 and glucose 8.3; pH 7.4. Vessels were cut into rings approximately 4 mm long, suspended on stainless steel hooks in 15 ml organ baths containing the Krebs bicarbonate solution, aerated with 95% O<sub>2</sub> / 5% CO<sub>2</sub>, and maintained at 37°C. Vessel segments containing distinct, macroscopically visible atherosclerotic lesions were not used.

### *Experimental protocol*

After equilibration for at least 30 min and wash every 15 min, changes in tissue force were recorded using a Harvard isometric transducer. The vessel segments, stretched to a stable force of about 15 mN, were exposed to 30 mM K<sup>+</sup> twice, and the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) after pre-contraction with prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>, 1 μM). The tissue was washed and then exposed to 100 mM K<sup>+</sup>. The data obtained with PGF<sub>2α</sub>, substance P

### *Antimigraine drugs in the human isolated coronary artery*

and 100 mM K<sup>+</sup> were averaged for each coronary artery. Subsequently, the vessel segments were washed again and, following a 30 min equilibration period, two series of experiments were performed.

#### *Concentration response curves and relation with clinical plasma concentrations*

In the first series of nine experiments, a concentration-response curve was constructed with the different compounds (ergotamine, dihydroergotamine, methysergide, methylergometrine, sumatriptan, naratriptan, rizatriptan, avitriptan and zolmitriptan, as well as 5-HT used as a 'marker'). In some cases, concentration response curves to sumatriptan were obtained in duplicate, which were averaged and regarded as one curve in further analysis. As described earlier in detail<sup>23</sup>, contractile responses were expressed as percentage of the contraction induced by 100 mM K<sup>+</sup> in the respective segments and the data were analysed to obtain in each case values of E<sub>max</sub> (maximum effect) and EC<sub>50</sub> (concentration eliciting 50% of *its own* E<sub>max</sub>). The E<sub>max</sub> and EC<sub>50</sub> values represent, respectively, the *efficacy* and *potency* of a drug in eliciting a response (in this case, coronary artery contraction). Thus, the lower the EC<sub>50</sub> of a drug, the more likely it is to cause coronary vasoconstriction at lower plasma concentrations; the E<sub>max</sub> is obviously only of importance when a drug is present in high enough concentrations, as dictated by its potency. To assess the *capacity* of various agonists to contract the human coronary artery during clinical use in migraine, we calculated the ratio between the reported C<sub>max</sub> after administration of clinically effective doses (Table 5.1) and the EC<sub>50</sub> value of the compounds in contracting the human isolated coronary artery. For compounds from which data on plasma protein binding were available to us, also *free* (=plasma protein *unbound*) C<sub>max</sub>/EC<sub>50</sub> ratios were calculated. Free C<sub>max</sub> values were used because the fraction of drug which is bound to plasma proteins only serves as a reservoir and is pharmacologically inactive<sup>24-26</sup>. A high C<sub>max</sub>/EC<sub>50</sub> ratio indicates that the plasma concentration of the drug is high enough to contract the human coronary artery in the *clinical situation*. The magnitude of this contraction will be dictated by the E<sub>max</sub> of the drug.



### *Duration of action*

In a second series of 5 experiments, the duration of action of the acutely acting antimigraine drugs (ergotamine, dihydroergotamine, sumatriptan, naratriptan, rizatriptan, avitriptan and zolmitriptan) was compared. For this purpose, contractions of coronary artery segments were elicited with a single concentration of these drugs (2 times  $EC_{50}$ , as determined in the first series of experiments) and the time to reach a stable contraction was noted. The segments were then washed twice every 15 min and contractions remaining after each wash were noted for a total period of 90 min.

### *Analysis of data*

Differences between  $EC_{50}$  and  $E_{max}$  values of sumatriptan and other compounds as well as between contractions remaining after each wash (every 15 min) were evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations. The  $E_{max}$  of the compounds tested was correlated with the relaxant response to substance P obtained in the individual coronary arteries (Pearson's correlation coefficient).  $P$  values of  $\leq 0.05$  were considered to indicate significant differences. All data in the text and illustrations are presented as means  $\pm$  s.e.mean.

### *Ethical approval*

The Ethical Committee of the Erasmus University Medical Centre Rotterdam approved this study.

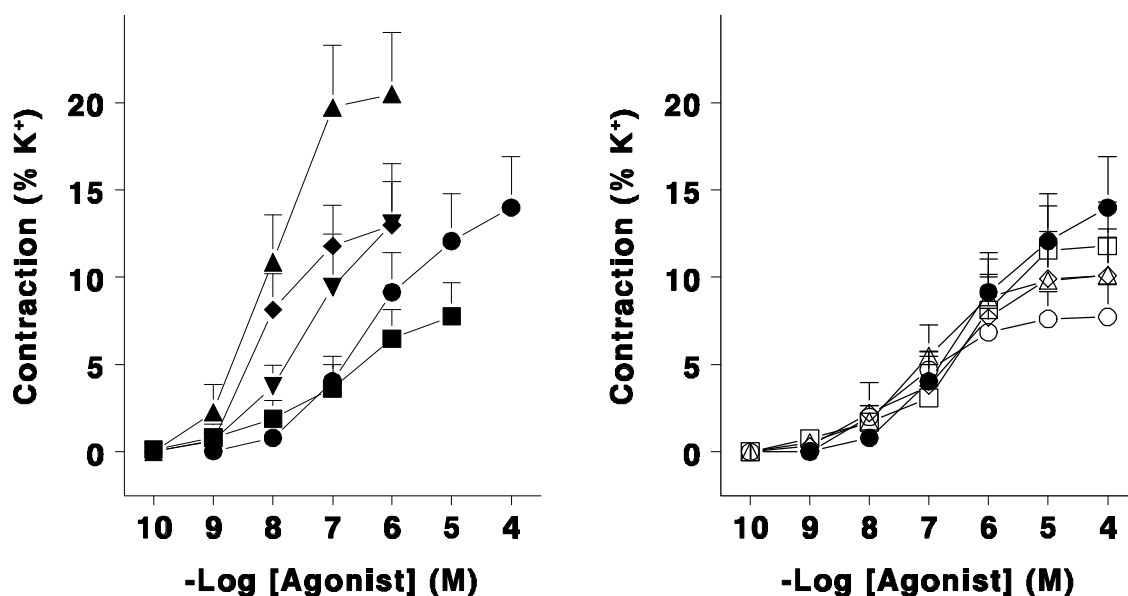
## **5.3 Results**

### *Basic properties of the preparations: effects of substance P and potassium*

All coronary artery segments, obtained from 14 hearts, relaxed after substance P (1 nM), the response amounting  $61 \pm 8\%$  of the precontraction ( $35 \pm 2$  mN) to  $PGF_{2\alpha}$  (1  $\mu$ M). Contraction to 100 mM  $K^+$  was  $47 \pm 3$  mN.

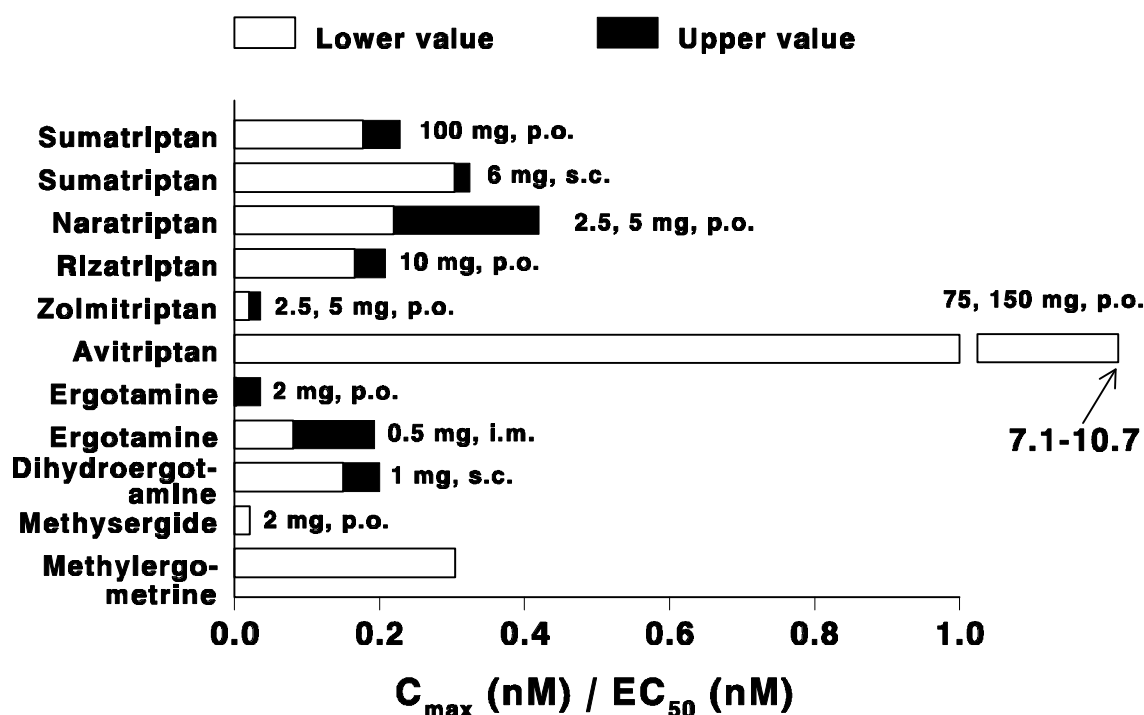
*Concentration response curves and relation with substance P response and clinical plasma concentrations*

Concentration response curves obtained in 9 coronary arteries with the various compounds investigated are shown in Figure 5.1, while the derived values of EC<sub>50</sub> (nM) and E<sub>max</sub> (% of contraction elicited by 100 mM K<sup>+</sup>) are shown in Table 5.1. As reported earlier<sup>22,23</sup>, the contractile effect of sumatriptan on the isolated human coronary artery showed a considerable variability; the EC<sub>50</sub> and E<sub>max</sub> values ranged from, respectively, 117-2042 nM and 2.3-27.0% of the response to 100 mM K<sup>+</sup>.



**Figure 5.1** Concentration-response (expressed as % of response to 100 mM K<sup>+</sup>) curves in human isolated coronary arteries ( $n=9$ ) obtained with current (left panel: ergotamine,  $\blacksquare$ ; dihydroergotamine,  $\bullet$ ; methysergide,  $\blacktriangle$ ; and its metabolite methylergometrine,  $\square$ ) and prospective (right panel: zolmitriptan,  $\square$ ; rizatriptan,  $\triangle$ ; naratriptan,  $\circ$ ; and avitriptan,  $\diamond$ ) antimigraine drugs compared with sumatriptan ( $\bullet$ ). Data are mean $\pm$ s.e.mean.

The  $EC_{50}$  values of all compounds, in particular ergotamine, dihydroergotamine and methylergometrine, were significantly lower than that of sumatriptan. The  $E_{max}$  of 5-HT was significantly higher, but those of the other compounds did not differ significantly from that of sumatriptan. However, it may be noted that the  $E_{max}$  of other triptan derivatives is about half that of ergotamine.

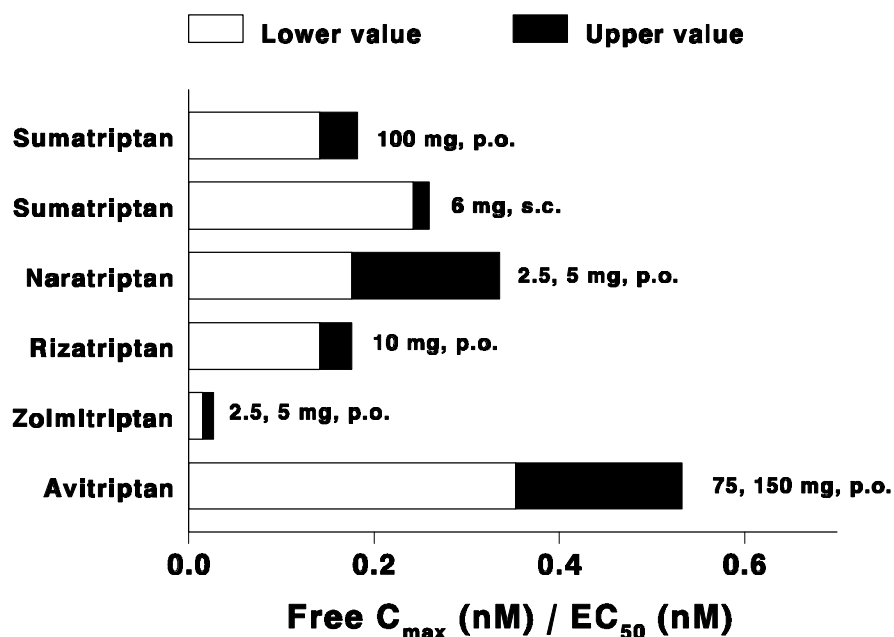


**Figure 5.2** Relationship between the reported  $C_{max}$  concentration in patients and the  $EC_{50}$  values of the various antimigraine compounds in contracting the human isolated coronary artery. The clinical doses and the mode of administration associated with  $C_{max}$  values (see Table 5.1 for references) are indicated in each case, methylergometrine (measured after methysergide, 2 mg p.o.). Where lower and upper values are mentioned, they indicate the reported range. Note that a  $C_{max}/EC_{50}$  ratio of 1 indicates that, if the same conditions were applicable in patients as in the present *in vitro* experiments, the drug would elicit 50% of its maximum contraction of the coronary artery. In view of the use of  $C_{max}$  data from the literature, the  $C_{max}/EC_{50}$  ratios of the different drugs were not subjected to statistical analysis.

In the nine hearts where concentration response curves to the antimigraine agents were constructed, the correlation between the  $E_{max}$  of the drugs and the coronary

artery relaxation to substance P (1 nM) after precontraction with PGF<sub>2α</sub> (1 μM) was assessed. With none of the drugs, Pearson's correlation coefficient yielded significant *P* values (data not shown).

Figure 5.2 depicts the ratio between the reported plasma C<sub>max</sub> obtained after administration of a clinically effective dose (see Table 5.1) and the EC<sub>50</sub> value of the compounds in contracting the human isolated coronary artery. The data show that, compared to that of 100 mg oral sumatriptan, the C<sub>max</sub>/EC<sub>50</sub> ratios of avitriptan (75 and 150 mg oral) were higher, those of ergotamine (2 mg oral) and zolmitriptan (2.5 and 5 mg oral) lower, and those of the other compounds (naratriptan, rizatriptan and methysergide measured as its active metabolite methylergometrine) were in the same range. The range of C<sub>max</sub>/EC<sub>50</sub> ratios obtained with parenteral ergotamine (0.5 mg, i.m.) and dihydroergotamine (1 mg, s.c.) were not much different from that with parenteral sumatriptan (6 mg, s.c.). Figure 5.3 shows the free, i.e. plasma protein unbound, C<sub>max</sub>/EC<sub>50</sub> ratios for the compounds from which data on plasma protein binding were available to us (Table 5.1).



**Figure 5.3** Relationship between the reported free (plasma protein unbound) C<sub>max</sub> concentration in patients and the EC<sub>50</sub> values of the various antimigraine compounds in contracting the human isolated coronary artery (Table 5.1). Lower and upper values indicate the reported range.

**Table 5.1**  $E_{\max}$  (maximum contraction expressed as % of effect caused by 100 mM  $K^+$ ) and  $EC_{50}$  (concentration eliciting 50% of  $E_{\max}$ , nM) values in the human isolated coronary artery of 5-HT and antimigraine drugs, together with their therapeutic dose and  $C_{\max}$  (maximum plasma concentration) in patients.

	$EC_{50}$ (nM)	$E_{\max}$ (% $K^+$ )	Dose (mg) mode of administration	$C_{\max}$ (nM)	PPB (%)
5-HT	334±99*	58±8*	...	...	...
Sumatriptan	803±197	14±3	100 p.o. <sup>2,27</sup> 6 s.c. <sup>2,27</sup>	142-183 <sup>2,27</sup> 244-261 <sup>2,27</sup>	14-21 <sup>28</sup>
Naratriptan	171±36*	10±2	2.5, 5 p.o. <sup>29</sup>	38, 71 <sup>29</sup>	20‡
Rizatriptan	448±88*	10±3	10 p.o. <sup>30,31</sup>	74-93 <sup>31</sup>	14‡
Zolmitriptan	476±87*	12±3	2.5, 5 p.o. <sup>18,32</sup>	9,17 <sup>18</sup>	25‡
Avitriptan	89±16*	8±2	75, 150 p.o. <sup>21</sup>	628, 948 <sup>21</sup>	95§
Ergotamine	17±7*	21±4	2 p.o. 0.5 i.m. <sup>33</sup>	0.03-0.62 <sup>33,34</sup> 1.38-3.27 <sup>33</sup>	ND
Dihydroergotamine	20±10*	13±3	0.75, 1 i.m./s.c. <sup>35</sup>	3, 4 <sup>35</sup>	ND
Methysergide†	243±98*	8±2	2 p.o. <sup>16</sup>	5 <sup>16</sup>	ND
Methylergometrine†	46±14*	13±4	...	14 <sup>16</sup>	ND

\* Significantly different from sumatriptan ( $P < 0.05$ )

† Not used in acute migraine therapy

‡ A.D. McHarg, personal communication

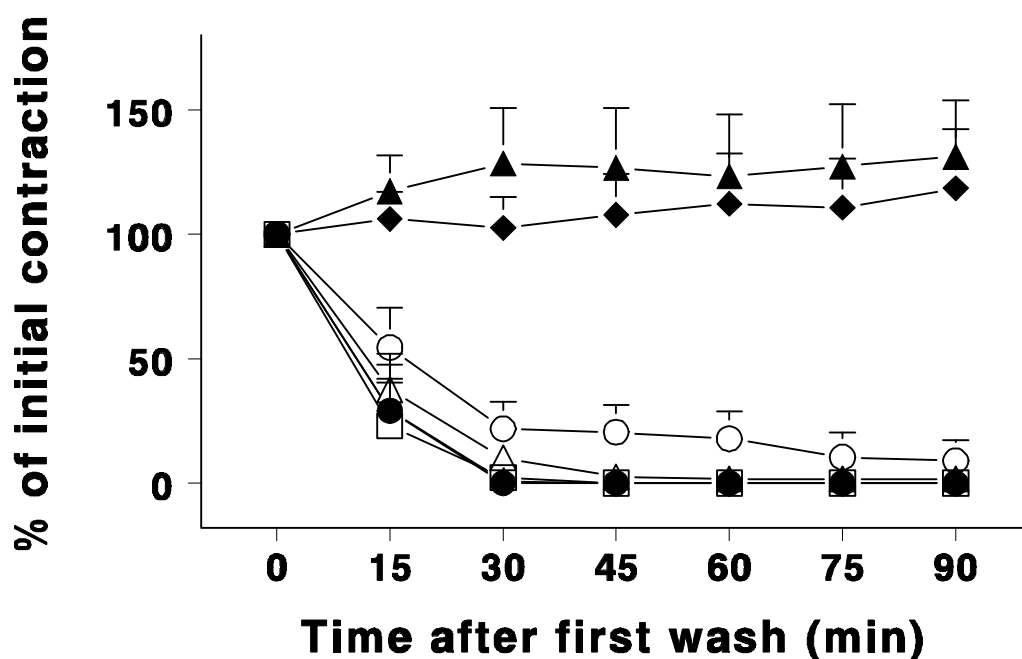
§ F.D. Yocca, personal communication

PPB, plasma protein binding; ND, no data available

### *Duration of action*

Contractions to a single concentration (2 times  $EC_{50}$ ) of the acutely acting antimigraine drugs were elicited in 5 coronary arteries. The peak stable contraction (% of 100 mM  $K^+$ ) and the time required to reach the peak contraction with the

different compounds were: sumatriptan  $15\pm 4\%$  ( $3\pm 2$  min), naratriptan  $10\pm 3\%$  ( $3\pm 1$  min), zolmitriptan  $21\pm 15\%$  ( $4\pm 7$  min), rizatriptan  $22\pm 16\%$  ( $4\pm 2$  min), avitriptan  $17\pm 12\%$  ( $5\pm 1$  min), ergotamine  $26\pm 7\%$  ( $24\pm 13$  min) and dihydroergotamine  $27\pm 11\%$  ( $18\pm 10$  min). The effects of repeated washings on the peak contractions elicited by each drug are presented in Figure 5.4. The data show that the contractile responses to ergotamine and dihydroergotamine were sustained over the 90-min period ( $P < 0.05$  versus sumatriptan), whereas those to sumatriptan, naratriptan, rizatriptan, avitriptan and zolmitriptan nearly completely disappeared after the second wash 30 min later.



**Figure 5.4** Effect of repeated washings (3 times every 15 minutes) on contractions of human coronary arteries ( $n=5$ ) elicited by acutely acting antimigraine drugs (ergotamine, ; dihydroergotamine, ; sumatriptan, ; zolmitriptan, ; rizatriptan, ; naratriptan, ; and avitriptan, ). All drugs were administered once at a concentration 2 times their  $EC_{50}$  (see Table 5.1). Data are mean $\pm$ s.e.mean.

## 5.4 Discussion

### *Human coronary artery contraction in vitro*

As reported earlier *in vitro*<sup>6,8,36</sup> and *in vivo*<sup>37,38</sup>, 5-HT induced contraction of the human isolated coronary artery. 5-HT was more efficacious (higher  $E_{\max}$  value) than sumatriptan, due to a more prominent action mediated via 5-HT<sub>2</sub> receptors<sup>6,8,36,38</sup>.

With respect to the antimigraine compounds, the results of our study show that all drugs, but in particular ergotamine, dihydroergotamine and methylergometrine (metabolite of methysergide<sup>16</sup>), were more potent (lower  $EC_{50}$  values) than sumatriptan in contracting the human isolated coronary artery. Although the  $E_{\max}$  of ergotamine tended to be somewhat higher than that of sumatriptan, we observed no statistically significant differences between  $E_{\max}$  values of sumatriptan and any of the other antimigraine drugs. Our findings are in agreement with a recent report on zolmitriptan<sup>19</sup>, but they appear to be at variance with earlier studies claiming that rizatriptan is only half as effective as sumatriptan in contracting the human isolated coronary artery<sup>39,40</sup>. Admittedly, data obtained in human *post mortem* tissues can vary between experiments due to a variety of factors, which are difficult to discern. However, the coronary arteries used in these studies<sup>39,40</sup> were endothelium-denuded and obtained from hearts from patients undergoing cardiac transplantation. In contrast, the coronary arteries in the present experiments were obtained from organ donors who died of non-cardiac causes and the endothelium was not removed. Thus, our population may better reflect the population likely to be treated with 5-HT<sub>1B/1D</sub> receptor agonists.

### *Coronary artery contraction and relaxation to substance P*

It is suggested that relaxation to substance P is a measure of the functional integrity of the endothelium and could be related to underlying coronary artery disease<sup>41,42</sup>. Since there was no significant correlation between the  $E_{\max}$  of the compounds and relaxant response to substance P in the coronary arteries used in the present investigation, it would appear that the contractile effect of the antimigraine drugs is not increased by underlying coronary artery disease. We concede that the present study, with few data

### *Antimigraine drugs in the human isolated coronary artery*

points at the outer sides of the range of the relaxant response to substance P, may not be particularly suitable for such an analysis. However, even in a larger analysis (62 donor hearts), we did not find an inverse, but in fact a positive, correlation between the  $E_{\max}$  of sumatriptan and the magnitude of substance P relaxation in the human coronary artery<sup>43</sup>.

#### *Coronary artery contraction at therapeutic plasma concentrations*

We calculated the ratio between the clinically-effective plasma  $C_{\max}$  and  $EC_{50}$  values of the different antimigraine compounds to estimate the degree of coronary vasoconstriction to be expected during therapeutic use (see Figure 5.2). Except for avitriptan, the plasma  $C_{\max}$  values of all drugs tested remain below 40% and in the case of zolmitriptan, ergotamine (2 mg oral) and methysergide (but note, not its metabolite methylergometrine) even below 10% of their  $EC_{50}$  values. After correction for plasma protein binding, the (free)  $C_{\max}/EC_{50}$  ratio for avitriptan falls in the same range as for the other compounds (Figure 5.3). Thus, therapeutic doses of the antimigraine drugs investigated will cause only little coronary artery constriction *in vivo*, and are unlikely to decrease coronary artery blood flow, which remains unchanged until the arterial lumen is compromised by more than 80%<sup>44</sup>. Recently, using positron emission tomography it was shown that sumatriptan did not affect myocardial perfusion in healthy migraineurs at therapeutic plasma concentrations<sup>45</sup>. In contrast, in patients with pre-existing coronary artery lesions, having only a limited coronary reserve<sup>44</sup>, even a small coronary artery contraction that may occur with plasma concentrations encountered during clinical use could be enough to cause myocardial ischemia. A similar phenomenon may also be observed in patients with 'variant' angina pectoris, who have increased coronary artery sensitivity to 5-HT<sup>38</sup>. It has been reported that the contractile effect of sumatriptan on the human isolated coronary artery is potentiated by thromboxane  $A_2$ <sup>23,46</sup> and is inhibited by aspirin as well as the thromboxane receptor antagonist, SQ30741<sup>23</sup>. Thus, exaggerated production of such substances locally may augment coronary artery contractions to similar antimigraine drugs *in vivo*.



*Additional factors involved in coronary artery constriction in patients*

Other factors involved in the contraction of coronary arteries *in vivo* are slow diffusion from the receptor biophase and the formation of active metabolites. Slow diffusion from the receptor biophase, which has been reported for ergot derivatives<sup>47</sup>, is in accordance with our findings concerning the sustained response to both ergotamine and dihydroergotamine despite repeated washings (Figure 5.4). Also in the clinical situation, it is known that the effects of ergotamine and dihydroergotamine sustain much longer than is to be expected from their plasma concentration profiles<sup>47-49</sup>. Indeed, substernal chest pain and discomfort suggestive of myocardial infarction have repeatedly been described with ergot preparations, particularly ergotamine<sup>10,50</sup>. Dihydroergotamine<sup>51</sup>, methysergide<sup>16</sup> and possibly ergotamine<sup>50</sup> form active metabolites, which, as is clearly the case with methysergide (see results with methylethergometrine), may also cause coronary artery constriction. Sumatriptan<sup>28</sup> and naratriptan<sup>52</sup> do not have active metabolites, but zolmitriptan forms an *N*-desmethyl derivative, which is approximately twice as potent as the parent compound in causing vasoconstriction<sup>53</sup>. Also rizatriptan forms a pharmacologically active *N*-desmethyl derivative<sup>54</sup>. We do not know whether active metabolites are formed by avitriptan.

**In conclusion**, all current and prospective antimigraine drugs investigated contract the human coronary artery *in vitro*. Therapeutic plasma concentrations of the drugs do not reach levels likely to cause myocardial ischemia in individuals with normal coronary circulation. However, like sumatriptan, all antimigraine compounds investigated, including the newer drugs, must remain contraindicated in patients with coronary artery disease. The sustained coronary artery contraction induced by ergotamine and dihydroergotamine is an important disadvantage compared to the sumatriptan-like drugs.

## 5.5 References

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## Chapter 6

### **Eletriptan shows craniovascular selectivity for human isolated meningeal arteries over peripheral vessels – a comparative study with sumatriptan**

**Summary** - Eletriptan is a novel 5-HT<sub>1B/1D</sub> receptor agonist with proven efficacy in the acute treatment of migraine with or without aura. The objective of this study was to compare the craniovascular selectivity of eletriptan with sumatriptan in blood vessels predictive of therapeutic efficacy (i.e., human isolated middle meningeal artery) and adverse coronary side effects (human isolated coronary artery and human isolated saphenous vein). Coronary artery tissue was obtained from nine heart-beating organ donors who died of non-cardiac causes, middle meningeal artery segments were obtained from patients ( $n=11$ ) undergoing craniotomy and human saphenous vein tissue was obtained from patients ( $n=9$ ) undergoing coronary artery bypass surgery. For each vessel segment, concentration-response curves to eletriptan and sumatriptan were constructed to obtain the maximum contractile response ( $E_{max}$ ) and the concentration eliciting 50% of  $E_{max}$  ( $EC_{50}$ ). The contraction that is likely to be induced at the maximal free plasma concentration ( $C_{max}$ ) was determined by calculating  $C_{max}/EC_{50}$  ratios and by interpolation of the concentration-response curves. Eletriptan and sumatriptan induced concentration-dependent contractions of middle meningeal artery, coronary artery and saphenous vein. The potency of eletriptan and sumatriptan was higher in middle meningeal artery than in coronary artery (86- and 30-fold, respectively) or saphenous vein (66- and 25-fold, respectively). Moreover, eletriptan was less potent than sumatriptan in the coronary artery, whereas both compounds had similar potency in middle meningeal artery and saphenous vein. The  $E_{max}$  of eletriptan and sumatriptan was similar within tissues. The predicted contractions by eletriptan (40 mg and 80 mg) and sumatriptan (100 mg) at free  $C_{max}$  were similar in middle meningeal artery, whereas in coronary artery and saphenous vein they

Based on: MaassenVanDenBrink A, Van den Broek RWM, De Vries R, Bogers AJJC, Avezaat CJJ, Saxena PR. Eletriptan shows craniovascular selectivity for human isolated meningeal arteries over peripheral vessels - a comparative study with sumatriptan. *Submitted*.

appeared to be lower for 40 mg eletriptan than for sumatriptan. Both eletriptan and sumatriptan contract middle meningeal artery at therapeutic plasma concentrations more than coronary artery. Although both drugs remain contraindicated in patients with coronary artery disease, they have a limited propensity to cause adverse coronary side effects in patients with healthy coronary arteries.

## **6.1 Introduction**

The 5-HT<sub>1B/1D</sub> receptor agonist sumatriptan has been shown to be effective in the treatment of migraine attacks<sup>1-3</sup>. However, from a therapeutic perspective, sumatriptan has several limitations, including low oral bioavailability, variable absorption, short half-life, and some reports of coronary artery constriction. These limitations have prompted the development of several new 5-HT<sub>1B/1D</sub> receptor agonists with improved clinical and pharmacological profiles. Eletriptan appears to have several advantages over sumatriptan with regard to its pharmacological and pharmacokinetic characteristics. Eletriptan has a higher affinity for the human recombinant 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptor (pK<sub>i</sub> 8.0 and 8.9, respectively) than sumatriptan (pK<sub>i</sub> 7.4 and 8.0, respectively)<sup>4</sup>. Eletriptan has also been shown to be more rapidly and consistently absorbed from the gastrointestinal tract than sumatriptan<sup>5,6</sup> and has a longer plasma half-life<sup>5</sup>. These features of eletriptan may contribute to its superior efficacy versus sumatriptan in comparative clinical trials<sup>7</sup>.

Eletriptan is effective in preclinical models that are believed to be predictive of clinical effect. For example, eletriptan effectively reduces carotid arteriovenous anastomotic blood flow in anaesthetised pigs<sup>8</sup> and reverses established plasma protein extravasation in anaesthetised rats with a potency and efficacy equivalent to sumatriptan<sup>9</sup>. Eletriptan has also been shown to exhibit an improved selectivity compared to sumatriptan in reducing carotid artery blood flow when compared with coronary artery diameter and femoral arterial blood flow in the anaesthetised dog<sup>9</sup>. *In vitro* studies show that eletriptan elicits a potent and concentration-dependent contraction of the dog isolated saphenous vein and basilar artery, where it acts as a partial agonist<sup>10</sup>.



Vasoconstriction of the large cranial and extracranial blood vessels is considered to be a putative mechanism of the antimigraine action of sumatriptan<sup>2,3</sup>. Indeed, sumatriptan has been shown to potently contract the human isolated middle meningeal artery<sup>11,12</sup>. Thus, contraction of the human isolated middle meningeal artery by eletriptan may be indicative of its therapeutic efficacy in migraine patients. Similarly, contraction of the human isolated coronary artery<sup>13</sup>, and possibly also of the saphenous vein<sup>14</sup>, to antimigraine drugs may predict the cardiovascular safety profile of the triptans in migraine patients without cardiovascular artery disease.

In the present study, we determined the potency ( $EC_{50}$ ) and efficacy ( $E_{max}$ ) of eletriptan and sumatriptan in contracting the human isolated middle meningeal artery, the coronary artery, and the saphenous vein. Contractions to 5-HT were also studied as controls. As described previously<sup>13</sup>, we related our findings to the therapeutic free plasma concentrations of eletriptan and sumatriptan in migraine patients.

## 6.2 Patients and methods

### *Tissue preparation; human isolated middle meningeal artery*

Middle meningeal arteries were obtained from 11 patients (6 male, 5 female; age 26-73 years; mean age $\pm$ s.e.mean: 50 $\pm$ 5 years) undergoing craniotomy at the neurosurgical unit of the University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. During operation, a part of the skull is temporarily removed to obtain access to the brain. Usually a small redundant portion of a branch of the middle meningeal artery is found attached to the dural sheath covering the removed piece of the skull. After careful removal from the dura, the artery was placed in a plastic tube filled with ice-cold (0-4°C) physiological saline and immediately transported to the laboratory. After arrival at the laboratory, the middle meningeal artery was placed in a cold, oxygenated Krebs buffer solution of the following composition: 119 mM NaCl, 4.7 mM KCl, 1.3 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub> and 11.1 mM glucose; pH 7.4. To avoid spontaneous contractions during the experiment, the cyclo-oxygenase inhibitor indomethacin

### *Craniovascular selectivity of eletriptan*

(0.1  $\mu\text{M}$ ) was added to the Krebs solution. In the experiments where relaxation to eletriptan and sumatriptan was studied after a precontraction with 5-HT, the Krebs solution was enriched with a cocktail of antagonists and re-uptake inhibitors (atropine, mepyramine, mesulergine, prazosin and imipramine: all 0.1  $\mu\text{M}$ ; corticosterone: 10  $\mu\text{M}$ ). The artery was cleaned from connective tissue, and no attempt was made to remove the endothelium.

#### *Tissue preparation; human isolated coronary artery*

The right epicardial coronary artery was obtained from nine heart beating organ donors who died of non-cardiac disorders less than 24 h before the tissue was taken to the laboratory (6 cerebrovascular accident, 3 trauma of the head; 6 male, 3 female; age 5-57 years; mean age $\pm$ s.e.mean: 37 $\pm$ 6 years). The hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services Foundation / Eurotransplant Foundation) after removal of the aortic and pulmonary valves for homograft valve transplantation. The hearts were stored at 0-4°C in a sterile organ protecting solution (UW, EuroCollins, or HTK-Bretschneider) immediately following circulatory arrest. After arrival in the laboratory, the right coronary artery was removed and placed in a cold, oxygenated Krebs buffer solution of the following composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub> and 8.3 mM glucose; pH 7.4. Vessel segments containing macroscopically visible atherosclerotic lesions were not used.

#### *Tissue preparation; human isolated saphenous vein*

Leftover human saphenous vein was obtained postoperatively from nine patients (7 male, 2 female; age 60 - 78 years; mean age $\pm$ s.e.mean: 69 $\pm$ 2 years) undergoing coronary bypass surgery at the cardiothoracic surgical unit of the University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. The tissue was immediately placed in cold (0-4°C) physiological saline and transported to the laboratory within 15 min. After arrival at the laboratory, the vein was cleaned of connective tissue and placed in a cold, oxygenated Krebs buffer solution of the same composition as used for coronary artery.

*Isometric tension measurements*

Vessels were cut into ring segments of approximately 3-4 mm length and suspended on stainless steel hooks in organ baths (10 ml for middle meningeal artery, 15 ml for coronary artery and saphenous vein), containing Krebs buffer solution as described above for each tissue. The buffer was aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and was maintained at 37°C. The segments were allowed to equilibrate for at least 30 min and were washed every 15 min. Changes in tension were measured with an isometric force transducer (EMKA Technology, Paris, France: middle meningeal artery; Harvard, South Nattick, Massachusetts, U.S.A: coronary artery and saphenous vein) and were recorded with IOX 1.103 software (EMKA Technology, Paris, France: middle meningeal artery) or on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria: coronary artery and saphenous vein). Preparations were stretched to a stable pretension of about 4 mN for middle meningeal artery, 15 mN for coronary artery or 10 mN for saphenous vein.

*Experimental protocols; human isolated middle meningeal artery*

Vessel segments were exposed two to three times to 0.1 μM prostaglandin F<sub>2α</sub> (K<sup>+</sup> was avoided as it frequently increased basal tone; unpublished observations) to 'prime' the tissue stable contractions. Subsequently, the segments were contracted with 1 μM prostaglandin F<sub>2α</sub> and endothelial functional integrity was assessed by observing relaxation to substance P (10 nM). Contractions were expressed as a percentage of contraction to 1 μM prostaglandin F<sub>2α</sub>. After a 30-min incubation period, concentration response curves were constructed with eletriptan, sumatriptan and 5-HT (in all cases 0.1 nM-100 μM). Where enough segments could be obtained from one artery, experiments were performed in a paired parallel manner (i.e. all compounds were tested in different segments obtained from the same artery; *n*=4). Otherwise, experiments were performed in a non-parallel manner (three arteries, resulting in *n*=1 for eletriptan, sumatriptan and 5-HT).

*Experimental protocols; human isolated coronary artery and saphenous vein*

Segments were exposed to  $K^+$  (30 mM) twice. After precontraction with prostaglandin  $F_{2\alpha}$  (1  $\mu$ M), the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) in coronary artery or to bradykinin (1  $\mu$ M) in saphenous vein (substance P is nearly inactive in saphenous vein). Following washout, the tissue was exposed to  $K^+$  (100 mM) to determine the maximal contractile response to  $K^+$ . After a 30-min incubation period, concentration response curves to eletriptan, sumatriptan and 5-HT (all 1 nM-100  $\mu$ M) were constructed in a paired, parallel set-up. The contractions were expressed as a percentage of contraction to 100 mM  $K^+$ .

*Relation with clinical plasma concentrations*

The  $E_{max}$  and  $EC_{50}$  represent the efficacy and the potency of a drug, respectively, in eliciting a response. Thus, the lower the  $EC_{50}$  of a drug, the more likely it is to cause vasoconstriction at lower plasma concentrations; the  $E_{max}$  is only of importance when a drug is present in high enough concentrations, as dictated by its potency (see MaassenVanDenBrink *et al.*<sup>13</sup>). To assess the ability of eletriptan and sumatriptan to contract the blood vessels during clinical use, we calculated the ratio between the maximal free (corrected for protein binding) plasma concentration (free  $C_{max}$ ) of these drugs after the clinically used oral dose (eletriptan: 40 mg and 80 mg, sumatriptan: 100 mg)<sup>15-18</sup> and the  $EC_{50}$  value of the compounds. In addition, we determined the contraction that would occur at free  $C_{max}$ . Free  $C_{max}$  values were used because the fraction of drug that is bound to plasma proteins only serves as a reservoir and is pharmacologically inactive<sup>19-21</sup>.

*Data analysis and presentation*

The concentration response curves obtained with eletriptan, sumatriptan and 5-HT were analysed using GraphPad software (GraphPad software Inc., San Diego, California, U.S.A.) to determine  $EC_{50}$  values. In case a concentration response curve did not reach a plateau, the contraction in response to the highest concentration was considered as  $E_{max}$ . Contraction occurring at free  $C_{max}$  was calculated by interpolation

of the individual concentration response curves, using a sigmoidal function (SlideWrite Plus, Advanced Software Inc., Encinitas, California, U.S.A.).

EC<sub>50</sub> values were transformed into pEC<sub>50</sub> values ( $-\log EC_{50}$ ) before statistical analyses to obtain a normal distribution. The EC<sub>50</sub> values of the different compounds obtained in the three blood vessels were compared using analysis of variance, followed by Tukey's post hoc test (*between* tissue unpaired comparison). Analysis of variance, followed by Tukey's post hoc test was also used to compare the EC<sub>50</sub> and E<sub>max</sub> values of the compounds (*within* tissue comparison). Analysis of variance was performed in a paired manner for coronary artery and saphenous vein and in an unpaired manner for middle meningeal artery. The relaxation of middle meningeal artery observed with eletriptan and sumatriptan was compared using unpaired t-test.

For analysis on the predicted contraction to eletriptan and sumatriptan at free C<sub>max</sub>, the mean of the reported C<sub>max</sub><sup>22,23</sup> and plasma protein binding<sup>24</sup> values of sumatriptan was used. In view of use of these data from literature, free C<sub>max</sub>/EC<sub>50</sub> ratios and predicted contractions occurring at free C<sub>max</sub> were not subjected to statistical analysis.

Data are presented as mean±s.e.mean in figures. In all cases statistical significance was assumed when  $P < 0.05$ .

### *Compounds*

Eletriptan hydrogen bromide and sumatriptan succinate (extracted from tablets) were a gift from Pfizer Limited (Sandwich, Kent, U.K.). Prostaglandin F<sub>2α</sub> (tris salt), substance P acetate, bradykinin acetate and 5-hydroxytryptamine creatinine sulphate (serotonin; 5-HT) were purchased from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Indomethacin hydrochloride was obtained from the pharmacy of University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. Indomethacin was dissolved in DMSO and further diluted in distilled water. All other compounds were dissolved in distilled water.

*Ethical approval*

The Ethical Committee of the Erasmus University Medical Centre Rotterdam approved this study.

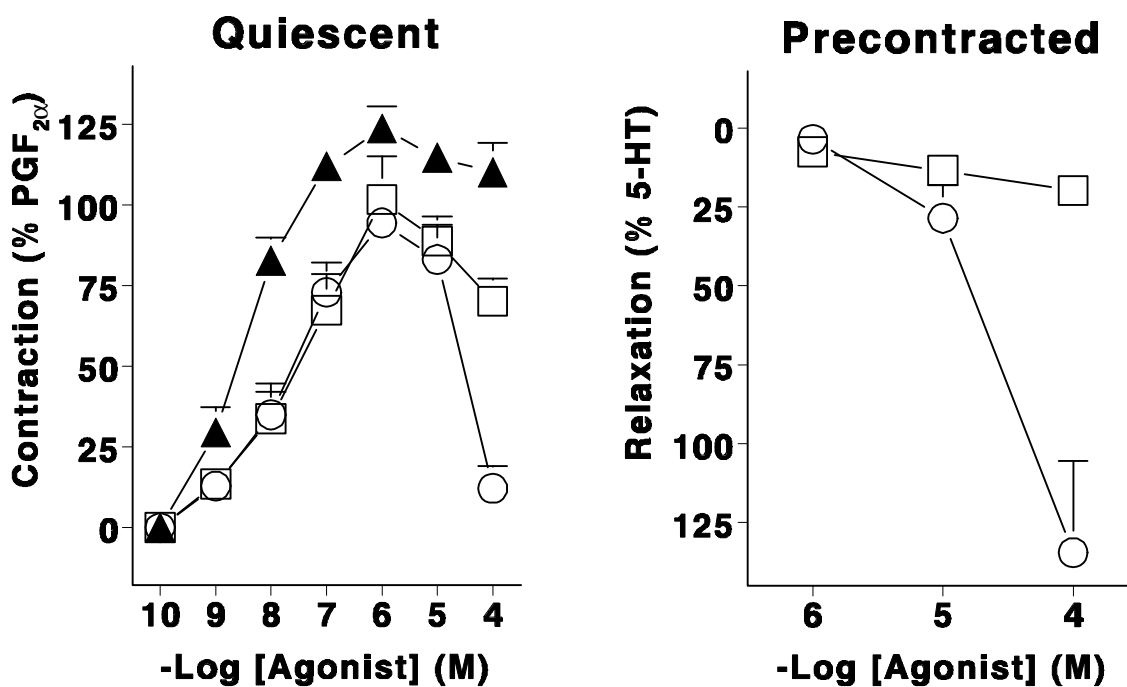
### **6.3 Results**

*Basic contractile properties*

In vessel segments of middle meningeal artery, relaxation to substance P (10 nM) amounted to  $40\pm 9\%$  of precontraction with  $1\ \mu\text{M}$  prostaglandin  $F_{2\alpha}$  ( $10\pm 2\ \text{mN}$ ,  $n=11$ ). Coronary artery segments relaxed in response to substance P (1 nM), the response amounting to  $48\pm 16\%$  of precontraction ( $30\pm 4\ \text{mN}$ ) induced by prostaglandin  $F_{2\alpha}$  ( $1\ \mu\text{M}$ ,  $n=9$ ). Coronary artery contraction to  $100\ \text{mM}\ \text{K}^+$  was  $43\pm 12\ \text{mN}$ . In segments of saphenous vein, relaxation to bradykinin ( $1\ \mu\text{M}$ ) was  $42\pm 7\%$  of precontraction ( $10\pm 3\ \text{mN}$ ) induced by  $1\ \mu\text{M}$  prostaglandin  $F_{2\alpha}$  ( $n=9$ ). Contraction to  $100\ \text{mM}\ \text{K}^+$  was  $17\pm 4\ \text{mN}$ .

*Contractile responses to eletriptan, sumatriptan and 5-HT;  
human isolated middle meningeal artery*

As shown in Figure 6.1 (*left panel*), eletriptan, sumatriptan and 5-HT contracted the human isolated middle meningeal artery in a concentration-dependent manner up to a concentration of  $1\ \mu\text{M}$ . Higher concentrations of the agonists relaxed the artery; at  $100\ \mu\text{M}$  the relaxant response was  $11\pm 7\%$  (5-HT),  $31\pm 7\%$  (sumatriptan) or  $87\pm 9\%$  (eletriptan) of their individual maximal contraction. The  $E_{\text{max}}$  and  $EC_{50}$  of the contractile response to 5-HT, sumatriptan and eletriptan did not differ significantly (Table 6.1).

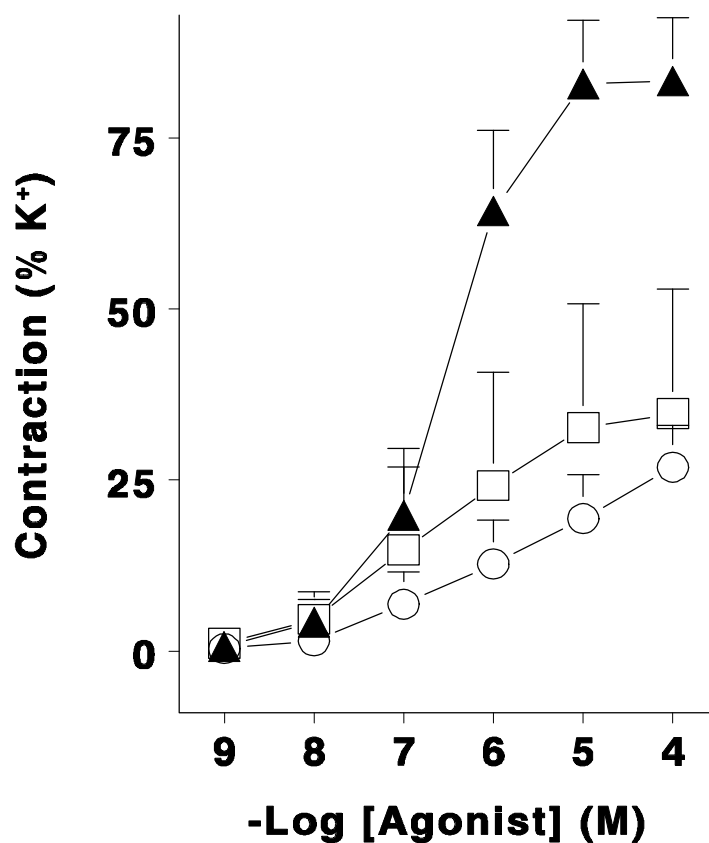


**Figure 6.1** Human middle meningeal artery. *Left panel:* Contractile responses ( $n=5$ ) to eletriptan (□), sumatriptan (○) and 5-HT (▲). *Right panel:* Relaxant responses ( $n=4$ ) to eletriptan (□) and sumatriptan (○) in vessel precontracted with 100  $\mu$ M 5-HT.

To further investigate the relaxant responses to sumatriptan and eletriptan, the compounds were studied after precontraction with 5-HT (100  $\mu$ M). As shown in Figure 6.1 (*right panel*), the relaxation by eletriptan ( $134 \pm 29\%$  of precontraction with 100  $\mu$ M 5-HT) was significantly higher than that by sumatriptan ( $20 \pm 5\%$ ).

*Contractile responses to eletriptan, sumatriptan and 5-HT;  
human isolated coronary artery*

Eletriptan, sumatriptan and 5-HT all caused concentration-dependent contractions of coronary artery (Figure 6.2). The  $EC_{50}$  of eletriptan and sumatriptan was significantly higher (i.e. the potency was significantly lower) than that of 5-HT, whereas the  $E_{max}$  of these compounds was significantly smaller than that of 5-HT. Further, the  $EC_{50}$  of eletriptan was significantly smaller than that of sumatriptan (Table 6.1).

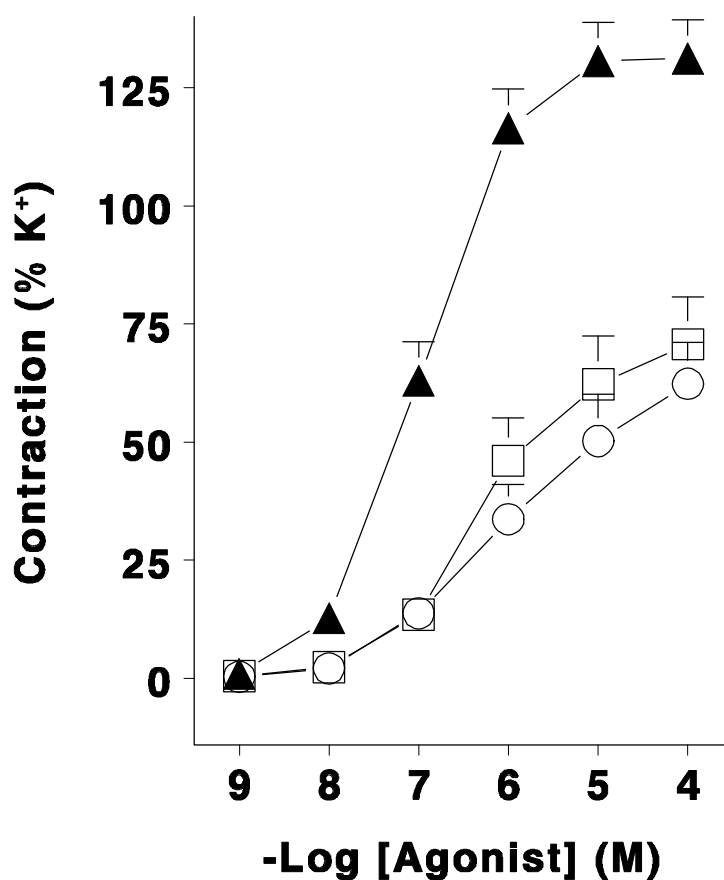


**Figure 6.2** Contractile responses to eletriptan (▲), sumatriptan (□) and 5-HT (○) in the human coronary artery ( $n=9$ ).

*Contractile responses to eletriptan, sumatriptan and 5-HT;  
human isolated saphenous vein*

Eletriptan, sumatriptan and 5-HT all caused concentration-dependent contractions of saphenous vein (Figure 6.3). The  $EC_{50}$  of both eletriptan and sumatriptan was significantly higher than that of 5-HT, whereas the  $E_{max}$  of these compounds was significantly lower than that of 5-HT (Table 6.1).





**Figure 6.3** Contractile responses to eletriptan (▲), sumatriptan (□) and 5-HT (○) in the human saphenous vein (n=9).

*Comparison of contractile responses between blood vessels*

Both eletriptan and sumatriptan were significantly more potent (i.e. lower  $EC_{50}$ ) in middle meningeal artery than in coronary artery or saphenous vein (Table 6.1); this selectivity (i.e.  $EC_{50}$  middle meningeal artery divided by  $EC_{50}$  coronary artery or saphenous vein) was 86-fold and 66-fold, respectively for eletriptan and 30-fold and 25-fold, respectively for sumatriptan. 5-HT was also significantly more potent in middle meningeal artery than in coronary artery or saphenous vein. In addition, 5-HT was more potent in saphenous vein than in coronary artery (Table 6.1). Because  $E_{max}$  values between different tissues may differ depending on the internal standard used (prostaglandin  $F_{2\alpha}$  or  $K^+$ ), the  $E_{max}$  values were not compared *between* tissues.

**Table 6.1** EC<sub>50</sub> and E<sub>max</sub> values of eletriptan, sumatriptan and 5-HT for the contraction of human blood vessels.

	Middle meningeal artery (n=5)		Coronary artery (n=9)		Saphenous vein (n=9)	
	EC <sub>50</sub> (nM)	E <sub>max</sub> (% PGF <sub>2α</sub> )	EC <sub>50</sub> (nM)	E <sub>max</sub> (% K <sup>+</sup> )	EC <sub>50</sub> (nM)	E <sub>max</sub> (% K <sup>+</sup> )
Eletriptan	50±28	98±6	4299±1624 <sup>b,c</sup>	27±6	3299±1635 <sup>c</sup>	62±9
Sumatriptan	53±21	103±13	1597±465 <sup>a,c</sup>	35±18	1327±577 <sup>c</sup>	71±11
5-HT	21±11	123±7	529±166 <sup>a,b,c</sup>	83±9 <sup>a,b</sup>	138±30 <sup>a,b,c,d</sup>	130±9 <sup>a,b</sup>

<sup>a</sup>Significantly different from the EC<sub>50</sub> or E<sub>max</sub> of eletriptan in the respective tissue (i.e. *within* tissue comparison).

<sup>b</sup>Significantly different from the EC<sub>50</sub> or E<sub>max</sub> of sumatriptan in the respective tissue (i.e. *within* tissue comparison).

<sup>c</sup>Significantly different from the EC<sub>50</sub> in middle meningeal artery for the respective compound (i.e. *between* tissue comparison).

<sup>d</sup>Significantly different from the EC<sub>50</sub> in coronary artery for the respective compound (i.e. *between* tissue comparison).

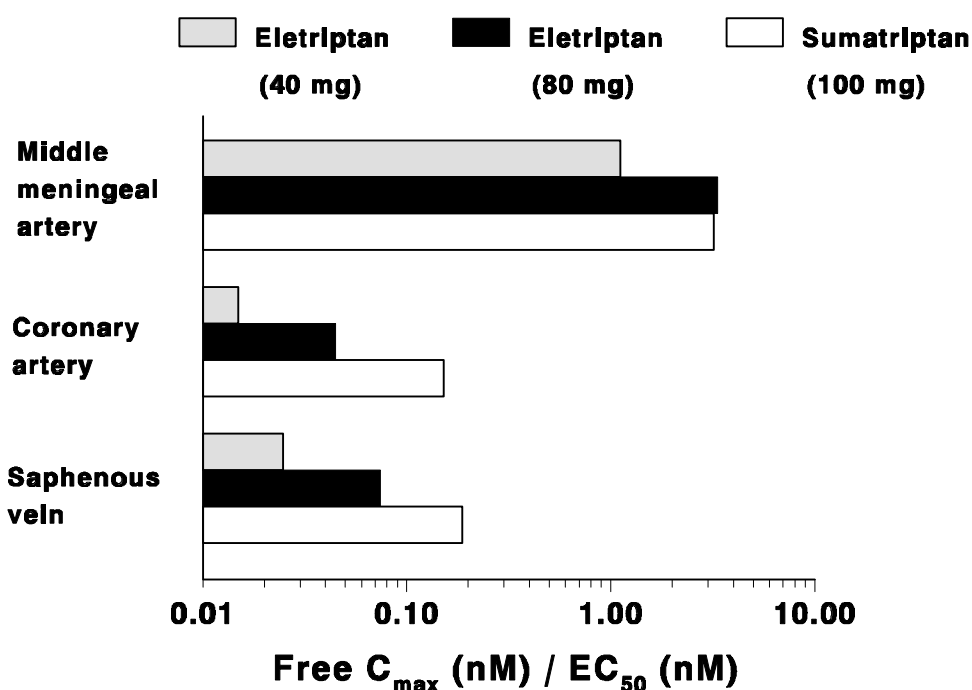
**Table 6.2**  $C_{max}$  of eletriptan and sumatriptan following oral dose, plasma protein binding values and free  $C_{max}$  for eletriptan and sumatriptan, as well as the predicted contraction occurring at free  $C_{max}$  in middle meningeal artery, coronary artery and saphenous vein.

	Oral dose	$C_{max}$ (nM)	Plasma protein binding (%)	Free $C_{max}$ (nM)	Predicted contraction at free $C_{max}$ (range)		
					Middle meningeal artery (% $PGF_{2\alpha}$ )	Coronary artery (% $K^+$ )	Saphenous vein (% $K^+$ )
Eletriptan	40 mg	213*	83-88*	30	51	4	7
	80 mg	643 <sup>25</sup>		90	73	7	13
Sumatriptan	100 mg	142-183 <sup>22,23</sup>	14-21 <sup>24</sup>	112-157	74	16	17

\*A.D. McHarg, personal communication.

*Predicted contraction at therapeutic plasma concentrations*

The maximal plasma concentrations ( $C_{max}$ ) attained at therapeutic doses of eletriptan and sumatriptan were corrected for plasma protein binding to obtain free  $C_{max}$  values. As described in detail earlier<sup>13</sup>, we calculated the ratio between the reported free plasma  $C_{max}$  after administration of a clinically effective dose (see Table 6.2) and the  $EC_{50}$  value of the compounds in contracting the human isolated middle meningeal and coronary artery and saphenous vein (Figure 6.4). The data show that the  $C_{max}/EC_{50}$  ratios in middle meningeal artery are considerably higher than in coronary artery or saphenous vein. Whereas in middle meningeal artery the  $C_{max}/EC_{50}$  ratio of eletriptan (40 and 80 mg) and sumatriptan (100 mg) are similar, this ratio seems to be smaller for eletriptan in coronary artery and saphenous vein.



**Figure 6.4** Relationship between the reported  $C_{max}$  concentration in patients (see Table 6.2) and  $EC_{50}$  values of eletriptan (40 and 80 mg) and sumatriptan (100 mg) in contracting human isolated middle meningeal artery, coronary artery and saphenous vein. Note that a free  $C_{max}/EC_{50}$  ratio of 1 indicates that, if same conditions were applicable in patients as in present *in vitro* experiments, the drug would elicit 50% of its maximum contraction. In view of use of  $C_{max}$  values from literature, data were not subjected to statistical analysis.

In addition to the analysis described above, we determined the contraction occurring at free  $C_{\max}$  by interpolation of the concentration response curves (see 'data analysis'). At free  $C_{\max}$ , the predicted contractions of middle meningeal artery to eletriptan (40 mg and 80 mg) and sumatriptan (100 mg) were similar. In contrast, the predicted contraction of coronary artery and saphenous vein for the 40-mg eletriptan dose seem to be smaller than that for sumatriptan (Table 6.2).

## 6.4 Discussion

### *Contraction to eletriptan and sumatriptan*

In the present study, we investigated contraction elicited by eletriptan and sumatriptan in human isolated blood vessels predictive of therapeutic efficacy (middle meningeal artery) and coronary side-effect potential (coronary artery, saphenous vein). Both eletriptan and sumatriptan contracted these blood vessels in a concentration-dependent manner and exhibited selectivity for contracting the human isolated middle meningeal artery relative to the coronary artery (86- and 30-fold, respectively) as well as saphenous vein (66- and 25-fold, respectively). Whereas there was no difference in potency between eletriptan and sumatriptan in middle meningeal artery or saphenous vein, eletriptan was less potent than sumatriptan in coronary artery. This is in accordance with another study at our laboratory, where eletriptan was significantly less potent than sumatriptan in contracting the human isolated coronary artery<sup>26</sup>.

It is to be noted that, compared to sumatriptan, eletriptan elicited a more marked relaxation of middle meningeal artery (Figure 6.1). This relaxant response may be mediated by the 5-HT<sub>7</sub> receptor<sup>27-29</sup>, since eletriptan has a higher affinity for the 5-HT<sub>7</sub> receptor than sumatriptan ( $pK_i$ : 6.7 and 5.9, respectively<sup>4</sup>). On the other hand, eletriptan (and sumatriptan) did not relax coronary artery, where, at least, the 5-HT<sub>7</sub> receptor mRNA has been located<sup>30,31</sup>. Irrespective of the mechanism involved, the relaxant response to eletriptan in middle meningeal artery is not clinically relevant because it was observed at a concentration of 100  $\mu$ M, which is over a 1000-fold

higher than its therapeutic plasma concentration (see Table 6.2). In middle meningeal artery, contraction to eletriptan and sumatriptan reached the same  $E_{\max}$  as contraction to 5-HT, whereas in coronary artery and saphenous vein the maximum contraction to 5-HT was 2- to 3-fold higher than that of eletriptan and sumatriptan. This confirms that in middle meningeal artery no or few 5-HT<sub>2</sub> receptors are present<sup>12,32,33</sup>, in contrast to coronary artery and saphenous vein where contraction to 5-HT is mainly mediated by 5-HT<sub>2</sub> receptors<sup>34-36</sup>. The potency rank order of the compounds obtained with coronary artery and saphenous vein was similar, suggesting that the human isolated saphenous vein may serve as a model for human coronary artery contraction to antimigraine drugs.

*Predicted contraction at therapeutic plasma concentrations*

Both eletriptan and sumatriptan had a significantly lower  $EC_{50}$  (higher potency) in middle meningeal artery than in coronary artery and saphenous vein. This higher potency in middle meningeal artery is favourable for antimigraine drugs, conferring selectivity for cerebral over coronary blood vessels. Indeed, the predicted contraction by eletriptan and sumatriptan at free  $C_{\max}$  was high in middle meningeal artery, while contraction of coronary artery and saphenous vein was generally low for both compounds (Table 6.2). The comparison of the magnitude of contraction in middle meningeal artery versus coronary artery and saphenous vein is hampered by the fact that contractions in middle meningeal artery are expressed as percentage of contraction to 1  $\mu$ M prostaglandin  $F_{2\alpha}$  ( $K^+$  was avoided as it frequently increased basal tone), while in coronary artery and saphenous vein they are expressed as percentage of contraction to  $K^+$  (100 mM). However, the contraction to 1  $\mu$ M prostaglandin  $F_{2\alpha}$  in coronary artery and saphenous vein was about 60-70% of the contraction obtained with 100 mM  $K^+$  (see Results, basic contractile properties). Thus, the use of prostaglandin  $F_{2\alpha}$ - or  $K^+$ -induced contraction for reference most probably does not account for the high efficacy of eletriptan and sumatriptan in contracting middle meningeal artery.

The predicted coronary artery contraction at free  $C_{\max}$  may be lower for 40 mg eletriptan than for sumatriptan (100 mg), whereas there seems to be no difference in

predicted middle meningeal artery contraction (see Table 6.2). This data is supported by a recent study in patients without significant obstructive coronary artery disease who underwent diagnostic cardiac catheterisation. Eletriptan was infused at 3.33 µg/kg/minute (that resulted in a mean free  $C_{max}$  equivalent to that observed after an oral dose of 40 mg) and it produced no significant changes in mean proximal, middle, or distal coronary artery diameters<sup>37</sup>. Thus, it appears that the 40-mg dose of eletriptan has been chosen to reduce therapeutic burden, including coronary side effects, yet retaining similar therapeutic efficacy as 100 mg sumatriptan or 80 mg eletriptan. Obviously, clinical experience over a long period may confirm or refute this assumption.

In any case, the predicted coronary artery contraction to both doses of eletriptan as well as sumatriptan, naratriptan, rizatriptan and zolmitriptan (present results and MaassenVanDenBrink et al.<sup>13</sup>), is such that myocardial ischaemia is unlikely to occur in patients with healthy coronary arteries. In contrast, in patients with pre-existing coronary artery lesions who have only a limited coronary reserve<sup>38</sup>, even a small coronary artery contraction could lead to myocardial ischaemia. A similar phenomenon may also be observed in patients with "variant" angina pectoris, who have increased coronary artery sensitivity to 5-HT<sup>39</sup>.

**In conclusion**, eletriptan and sumatriptan contract middle meningeal artery more potently and more effectively than coronary artery or saphenous vein. At therapeutic plasma concentrations, both drugs contract middle meningeal artery more than coronary artery. While both drugs have a limited propensity to cause adverse coronary side effects in patients with healthy coronary arteries, they remain contraindicated in patients with coronary artery disease.

**Acknowledgements** - The authors want to express their sincere gratitude to the neurosurgical and cardiothoracic surgical units of the University Hospital 'Dijkzigt', Erasmus University Medical Centre Rotterdam, as well as to the Rotterdam Heart

Valve Bank (Bio Implant Services Foundation / Eurotransplant Foundation) for supplying the human tissues. This study was partly supported by Pfizer Limited, U.K.

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## Chapter 7

# **The Potential Antimigraine Compound SB-220453 does not Contract Human Isolated Blood Vessels or Myocardium; a Comparison with Sumatriptan**

**Summary** - The mechanistically novel benzopyran derivative SB-220453, which is undergoing clinical evaluation in migraine, exhibits a high affinity for a selective, but not yet characterised binding site in the human brain. It inhibits nitric oxide release and cerebral vasodilatation following cortical spreading depression as well as carotid vasodilatation induced by trigeminal nerve stimulation in the cat. The aim of our study was to investigate the contractile properties of SB-220453 on a number of human isolated blood vessels (coronary artery, saphenous vein and middle meningeal artery) as well as atrial and ventricular cardiac trabeculae. While sumatriptan induced marked contractions in all blood vessels investigated, SB-220453 was devoid of any effect. In atrial and ventricular cardiac trabeculae, neither SB-220453, nor sumatriptan displayed a positive or negative inotropic effect. Since SB-220453 did not contract the middle meningeal artery, we conclude that potential antimigraine effects are not mediated via a direct cerebral vasoconstriction. The lack of activity of SB-220453 in coronary artery, saphenous vein and cardiac trabeculae demonstrates the compound is unlikely to display adverse cardiac side effects.

Based on: MaassenVanDenBrink A, Van den Broek RWM, De Vries R, Upton N, Parson AA, Saxena PR. The potential antimigraine compound SB-220453 does not contract human isolated blood vessels or myocardium; a comparison with sumatriptan. *Submitted*.

## 7.1 Introduction

Sumatriptan, an indole sulfonamide with agonist activity at 5-HT<sub>1B/1D</sub> receptors, is highly effective in aborting attacks of migraine and cluster headache. The drug is generally well tolerated, but up to 15% of patients consistently report chest symptoms, including chest pressure, tightness and pain, often mimicking angina pectoris<sup>1-3</sup>. Although extracardiac mechanisms have been invoked<sup>4</sup>, chest symptoms may well be caused by coronary vasoconstriction, which has been observed after sumatriptan both *in vivo*<sup>5</sup> and *in vitro*<sup>6-9</sup>. In some cases, the use of sumatriptan, like that of ergotamine<sup>10,11</sup>, was even associated with myocardial infarction<sup>12,13</sup> and cardiac arrest<sup>14</sup>. 'Second generation' sumatriptan-like antimigraine drugs are aimed at, in addition to achieving high efficacy and long duration of action, avoiding coronary vasoconstrictor activity<sup>15</sup>. However, these drugs also contract human isolated coronary artery and seem to have a similar coronary side-effect potential as sumatriptan<sup>9,16,17</sup>.

Due to concerns about cardiac side effects, it would be highly desirable to develop antimigraine drugs that act via a mechanism not involving 5-HT<sub>1B/1D</sub> receptors. Indeed, the mechanistically novel benzopyran SB-220453<sup>18</sup> has no significant affinity at 5-HT<sub>1B/1D</sub> receptors, nor does it show any activity in a large number of receptor-, ion channel- and enzyme-assays<sup>18</sup>. SB-220453 exhibits a high affinity for a selective, but structurally unknown binding site in the human brain<sup>19</sup> and may be active in the treatment of migraine via blockade of excessive cortical excitability. SB-220453 inhibits neurogenic inflammation in rat brain meninges<sup>18</sup>, nitric oxide release associated with cortical spreading depression as well as carotid vasodilatation induced by trigeminal nerve stimulation in the cat<sup>19,20</sup>. In the present study, we investigated the effects of SB-220453 on a number of human isolated blood vessels (coronary artery, saphenous vein and middle meningeal artery) as well as human isolated atrial and ventricular cardiac trabeculae. Sumatriptan was used for comparison.

## 7.2 Patients and methods

### *Preparation of tissue; human isolated coronary artery*

Right epicardial coronary arteries were obtained from six heart beating organ donors (2 male, 4 female; age 37-63 years), who died of non-cardiac disorders (5 of cerebrovascular accident, 1 head trauma) less than 24 h before the tissue was taken to the laboratory. Hearts, provided by the Rotterdam Heart Valve Bank (Bio Implant Services / Eurotransplant Foundation) after removal of the aortic and pulmonary valves for transplantation purposes, were stored at 0°C to 4°C in a sterile organ-protecting solution immediately after circulatory arrest. After arrival at the laboratory, the right coronary artery was removed and placed in a cold, oxygenated Krebs buffer solution of the following composition (mM): NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25 and glucose 8.3; pH 7.4. The artery was kept overnight before the experiment.

On the following day, the artery was cut into segments of 3-4 mm length, excluding distinct, macroscopically visible atherosclerotic lesions. The vessel segments were mounted in 15-ml organ baths filled with oxygenated Krebs buffer solution at 37°C. After equilibration for at least 30 min and a wash every 15 min, the vessel segments were stretched to a stable tension of about 15 mN. Changes in tissue tension were measured using an isometric transducer (Harvard, South Nattick, Massachusetts, USA) and recorded on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria).

### *Preparation of tissue; human isolated saphenous vein*

Human saphenous veins were obtained postoperatively from four patients (2 male, 2 female; 68-79 years) undergoing coronary bypass surgery. The tissue was immediately placed in cold saline and was brought to the laboratory within 15 min. Subsequently, the vein was cleaned of connective tissue and placed in a cold, oxygenated Krebs buffer solution (for composition, see above). After overnight storage, the vein was cut into segments of 3-4 mm length. The vessel segments were mounted in 15-ml organ baths filled with oxygenated Krebs buffer solution at 37°C.

After equilibration for at least 30 min and a wash every 15 min, the vessel segments were stretched to a stable tension of about 10 mN. Contractions were measured with an isometric transducer (Harvard, South Natick, Massachusetts, USA) and recorded on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria).

*Preparation of tissue; human isolated middle meningeal artery*

Human middle meningeal arteries were obtained from ten patients (3 male, 7 female; 30-71 years) undergoing craniotomy during neurosurgical procedures. In such patients, a part of the skull is temporarily removed to gain access to the brain and a small redundant portion of a branch of the middle meningeal artery is usually found attached to the dural sheath covering the removed piece of the skull. After careful removal from the dura mater, this arterial piece was placed in cold saline and brought to the laboratory immediately. Upon arrival at the laboratory, the artery was cleaned of connective tissue and was placed in cold oxygenated Krebs buffer solution of the following composition (mM): NaCl 119, KCl 4.7, CaCl<sub>2</sub> 1.25, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25 and glucose 11.1; pH 7.4. Vessel segments of 4 mm length were and mounted in 10-ml organ baths filled with oxygenated Krebs buffer solution at 37°C. After equilibration for at least 30 min and a wash every 15 min, the vessel segments were stretched to a stable tension of about 4 mN. Contractions of the artery were measured with an isometric force displacement transducer and recorded using the IOX 1.103 software (both: EMKA Technology, Paris, France).

To prevent prostaglandin synthesis, the cyclo-oxygenase inhibitor indomethacin (0.1 µM) was added to the Krebs solution. The experiments were performed within 2 h after surgery.

*Preparation of tissue; human atrial and ventricular cardiac trabeculae*

As described above (coronary artery section), hearts were obtained from five heart beating organ donors (2 male, 3 female; age 36-57 years), who died of non-cardiac disorders (all cerebrovascular accident). Immediately after arrival at the laboratory, right atrial and left ventricular trabeculae of approximately 1 mm thickness were carefully dissected and mounted in a 15-ml organ bath in the same Krebs buffer

solution as used for the coronary artery and saphenous vein. The trabeculae were paced at 1 Hz using electrical field stimulation (5 ms, 15-20 V) delivered by a Grass S6 Square Wave Stimulator (Quincy, MA, USA). Resting tension was set to 7.5 mN and 20 mN for atrial and ventricular tissue, respectively. Changes in contraction were recorded with a Harvard force transducer (South Nattick, MA, USA) on a flatbed recorder (Servogor 124, Goerz, Neudorf, Austria). The preparation was allowed to stabilise during 1 h with a wash every 15 min<sup>21</sup>.

*Experimental protocol; human isolated coronary artery and saphenous vein*

Segments were exposed to K<sup>+</sup> (30 mM) twice. The functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM, coronary artery) or bradykinin (1 μM, saphenous vein, substance P is nearly inactive in this blood vessel) after precontraction with prostaglandin F<sub>2α</sub> (1 μM). After washout, the tissue was exposed to K<sup>+</sup> (100 mM) to determine the maximal contractile response to K<sup>+</sup>. After a 30-min incubation period, a concentration response curve to sumatriptan (dissolved in distilled water or dimethylsulfoxide; DMSO) or SB-220453 (dissolved in DMSO) was constructed. In four of the experiments in coronary artery, a concentration response curve to these agonists was also constructed after a 30-min incubation with the stable thromboxane A<sub>2</sub> analogue U46619 (3-10 nM, the lowest concentration, determined in half logarithmic steps, eliciting a contraction ≥10% of K<sup>+</sup>-induced contraction). A paired parallel set-up (i.e. all compounds were tested in different segments obtained from the same artery) was used for coronary artery experiments. In the saphenous vein, a paired parallel crossover design was used (i.e. similar as in coronary artery, but, in addition, a second concentration response curve was constructed to SB-220453 after sumatriptan, or to sumatriptan after SB-220453 after a 30-60 min washout period). Contractions were expressed as a percentage of contraction to 100 mM K<sup>+</sup>.

*Experimental protocol; human isolated middle meningeal artery*

Since the addition of K<sup>+</sup> frequently increased basal tone (unpublished observations), vessel segments were exposed to prostaglandin F<sub>2α</sub> (0.1 μM) two to three times to



'prime' the tissue for stable contractions. Subsequently, the segments were contracted with prostaglandin F<sub>2α</sub> (1 μM) and the functional integrity of endothelium was assessed by observing relaxation to substance P (10 nM). Due to the limited number of vessel segments that could be obtained from one patient, middle meningeal artery experiments were performed in an unpaired design, i.e. concentration response curves to sumatriptan (dissolved in distilled water) or SB-220453 (dissolved in DMSO) were constructed in different arterial segments, which were in most cases obtained from different patients. In addition, a concentration response curve to sumatriptan (dissolved in DMSO) was constructed following a 30-60 min washout period after the concentration response curve to SB-220453. Contractions were expressed as a percentage of contraction to 1 μM prostaglandin F<sub>2α</sub>.

*Experimental protocol; atrial and ventricular cardiac trabeculae*

A concentration response curve to noradrenaline (10 nM - 10 μM) was constructed to verify the viability of the tissue. Trabeculae yielding less than 0.25 mN response to 10 μM noradrenaline were excluded from further analysis. After washout, a concentration response curve to sumatriptan (dissolved in distilled water or DMSO) or SB-220453 (dissolved in DMSO) was constructed. In four out of five experiments, concentration response curves were also obtained after precontraction with noradrenaline (10 μM). Since this precontraction was not always stable for a period long enough to allow construction of a complete concentration response curve (1 nM - 100 μM) to the agonists, not all concentrations were studied in these experiments. Changes in contraction were expressed as percentage of increase in contraction to 10 μM noradrenaline).

*Compounds*

Sumatriptan succinate was a kind gift from GlaxoWellcome (Dr. H.E. Connor, Ware, Hertfordshire, UK). SB-220453 ((-)-*cis*-6-acetyl-4*S*-(3-chloro-4-fluorobenzoylamino)3,4-dihydro-2,2-dimethyl-2*H*-benzo[*b*]pyran-3*S*-ol) was kindly provided by SmithKline Beecham (Dr. A.A. Parsons, Harlow, Essex, UK). U46619 (9,11-dideoxy-11α,9α-epoxy, methanoprostaglandin F<sub>2α</sub>), prostaglandin F<sub>2α</sub> (Tris

salt), bradykinin acetate, substance P acetate and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Indomethacin was obtained from the pharmacy of Erasmus University Medical Centre Rotterdam (Rotterdam, The Netherlands). The chemicals used for the Krebs buffer solutions were purchased from Merck (Darmstadt, Germany).

Sumatriptan was dissolved in distilled water or, where indicated, in DMSO and further diluted in distilled water. Indomethacin and SB-220453 were dissolved in DMSO and further diluted in distilled water. The other compounds were dissolved in distilled water.

#### *Analysis of data*

Concentration response curves analyzed using GraphPad software (GraphPad software Inc., San Diego, California, USA) to determine pEC<sub>50</sub> values (negative logarithm of the concentration eliciting 50% of the maximal contractile response, E<sub>max</sub>). When a plateau in the concentration response curve was not reached, the response observed with the highest concentration used (100 μM) was considered as E<sub>max</sub>.

At 100 μM, SB-220453 and sumatriptan (dissolved in DMSO) induced a small relaxation of the blood vessels, which was not easily quantifiable. This relaxant response, which was also observed with equivalent amounts of the solvent DMSO, has been ignored. For the experiments in the presence of U46619, the precontraction induced by U46619 was subtracted from the concentration response curves obtained with the agonists.

All data in the text and illustrations are presented as mean±s.e.mean, with *n* representing the number of different subjects or, when specifically mentioned, the number of vessel segments or trabeculae. Differences between pEC<sub>50</sub> and E<sub>max</sub> values of the compounds were evaluated with Tukey's test, once an analysis of variance (ANOVA) for paired (coronary artery, saphenous vein, myocardial trabeculae) or unpaired data (middle meningeal artery) had revealed that the samples represented different populations. Values of *P*<0.05 were considered to indicate significant differences.

### *Ethical approval*

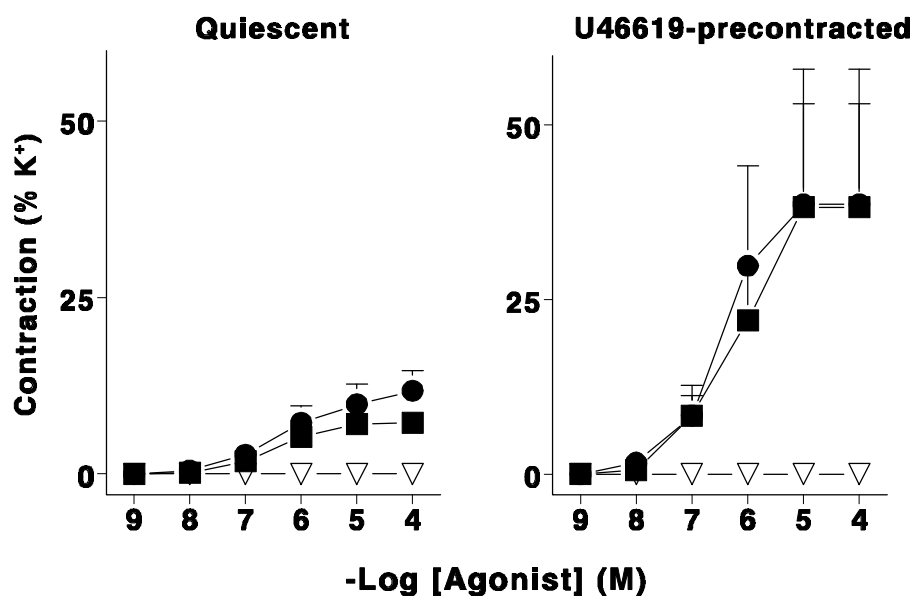
The Ethical Committee of the Erasmus University Medical Centre Rotterdam approved this study.

## **7.3 Results**

### *Human isolated coronary artery*

All coronary artery segments relaxed after substance P (1 nM). The response amounted to  $48 \pm 15\%$  (range: 9-107%) of the precontraction ( $22 \pm 6$  mN) to prostaglandin  $F_{2\alpha}$  (1  $\mu$ M). Contraction to 100 mM  $K^+$  was  $55 \pm 6$  mN ( $n=6$ ).

Sumatriptan induced a concentration-dependent contraction, which was independent of the solvent (distilled water and DMSO) used;  $E_{\max}$ :  $12 \pm 3\%$  and  $7 \pm 1\%$  of  $K^+$ -induced contraction, respectively,  $pEC_{50}$ :  $6.0 \pm 0.2$  and  $6.4 \pm 0.2$ , respectively. SB-220453 induced no response (Figure 7.1, *left panel*). After precontraction with U46619 ( $17 \pm 3\%$  of  $K^+$ -induced contraction), the  $E_{\max}$  to sumatriptan was substantially augmented in two out of the four experiments. However, the mean  $E_{\max}$  ( $39 \pm 24\%$  for sumatriptan dissolved in distilled water and  $38 \pm 17\%$  for sumatriptan dissolved in DMSO) was, as reported previously<sup>22</sup>, not significantly different from values in these four experiments in the absence of U46619 ( $16 \pm 3\%$  for sumatriptan dissolved in distilled water and  $9 \pm 1\%$  for sumatriptan dissolved in DMSO). The  $pEC_{50}$  of sumatriptan in the presence of U46619 ( $6.7 \pm 0.3$  and  $6.6 \pm 0.3$  for sumatriptan dissolved in distilled water and DMSO, respectively) was also not significantly increased, when compared to those in the absence of U46619 ( $6.2 \pm 0.3\%$  for sumatriptan dissolved in distilled water and  $6.5 \pm 0.2\%$  for sumatriptan dissolved in DMSO). Similar to the experiments performed on quiescent arteries, SB-220453 failed to contract the coronary artery in the presence of U46619 (Figure 7.1, *right panel*).

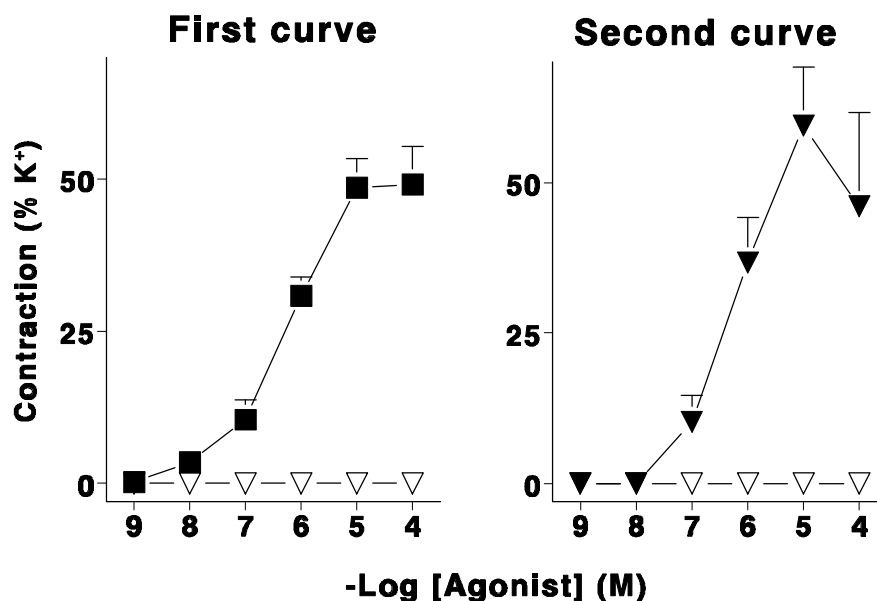


**Figure 7.1** Concentration response curves in human isolated coronary arteries to sumatriptan (dissolved in distilled water,  $\bullet$  or DMSO,  $\circ$ ) and SB-220453 ( $\nabla$ , dissolved in DMSO). Experiments were performed in the absence of U46619 (*left panel*,  $n=6$ ) or after precontraction with U46619 (3-10 nM; *right panel*,  $n=4$ , except sumatriptan dissolved in distilled water  $n=3$ ). The precontraction induced by U46619 was  $17\pm 3\%$ ,  $n=15$  vessel segments.

#### *Human isolated saphenous vein*

Saphenous vein segments relaxed after bradykinin (1  $\mu\text{M}$ ), the response amounting to  $49\pm 24\%$  (range: 10-107%) of the precontraction ( $6\pm 3$  mN) induced by prostaglandin  $\text{F}_{2\alpha}$  (1  $\mu\text{M}$ ). Contraction to 100 mM  $\text{K}^+$  was  $10\pm 2$  mN ( $n=4$ ).

In all experiments, sumatriptan (dissolved in DMSO) induced a concentration-dependent contraction ( $E_{\text{max}}$ :  $52\pm 5\%$ ,  $\text{pEC}_{50}$ :  $6.3\pm 0.1$ ). SB-220453 did not induce a contraction in any of the concentrations used (Figure 7.2, *left panel*). The concentration response curves to sumatriptan that were constructed after the concentration response curve to SB-220453 did not differ from those constructed before SB-220453 ( $E_{\text{max}}$ :  $59\pm 10\%$ ;  $\text{pEC}_{50}$ :  $6.2\pm 0.2$ ). Also after the concentration response curve to sumatriptan, SB-220453 failed to contract the saphenous vein (Figure 7.2, *right panel*).

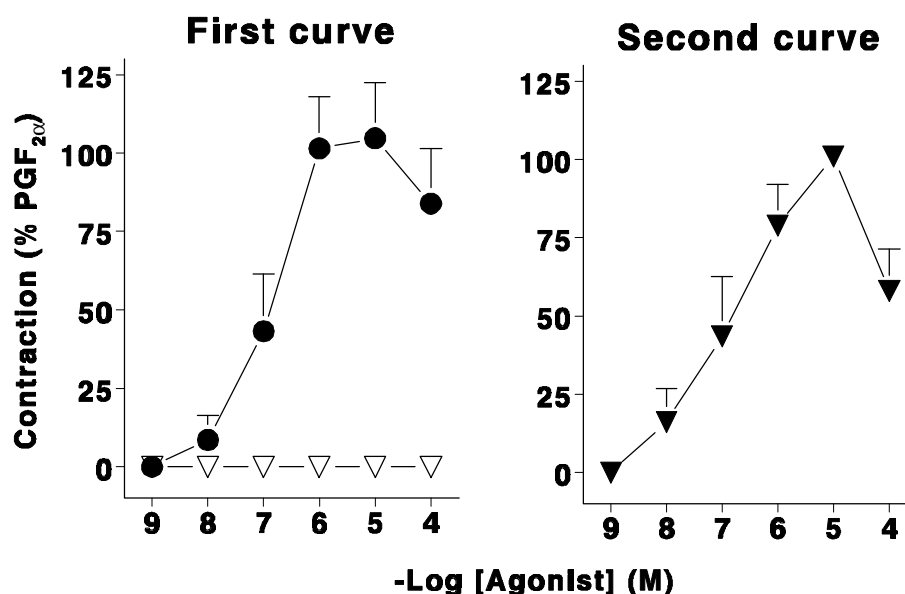


**Figure 7.2** Concentration response curves in human isolated saphenous vein to sumatriptan ( ) and SB-220453 ( ; both dissolved in DMSO;  $n=4$ ). Whereas sumatriptan induced a concentration-dependent contraction in all experiments, SB-220453 had no effect (*left panel*). The second concentration response curves (*right panel*) to sumatriptan after SB-220453 ( ) as well as to SB-220453 after sumatriptan ( ;  $n=4$ ) did not differ from the first curve.

#### *Human isolated middle meningeal artery*

Middle meningeal artery segments relaxed to substance P (10 nM) with  $44\pm 7\%$  (range: 19-76%) of the precontraction ( $8\pm 2$  mN) induced by prostaglandin  $F_{2\alpha}$  (1  $\mu$ M,  $n=10$ ).

Sumatriptan (dissolved in distilled water) induced a concentration-dependent contraction in all experiments ( $E_{\max}$ :  $105\pm 18\%$ ,  $pEC_{50}$ :  $6.9\pm 0.2$ ), whereas SB-220453 induced no contraction (Figure 7.3, *left panel*). After construction of the concentration response curves to SB-220453, the contraction to sumatriptan (dissolved in DMSO) did not differ significantly from the that to sumatriptan dissolved in distilled water ( $E_{\max}$ :  $101\pm 2\%$ ;  $pEC_{50}$ :  $6.8\pm 0.4$ , Figure 7.3, *right panel*).



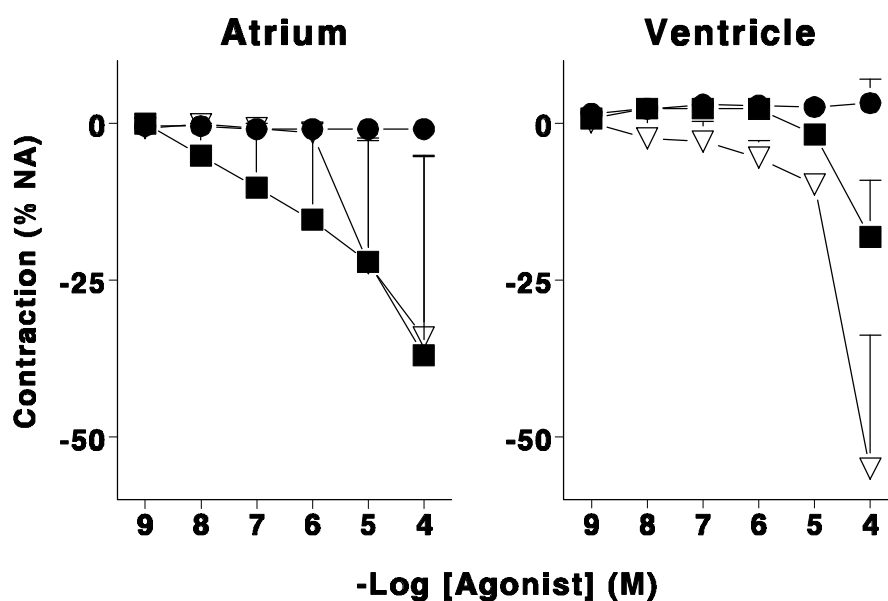
**Figure 7.3** Concentration response curves in human isolated middle meningeal artery to sumatriptan (dissolved in distilled water,  $\bullet$ ;  $n=5$ ) and SB-220453 ( $\nabla$ , dissolved in DMSO;  $n=4$ ; *left panel*). The second concentration response curves (*right panel*) to sumatriptan (dissolved in DMSO) after SB-220453 ( $\blacktriangledown$ ,  $n=4$ ) did not differ from the first curve.

#### *Human atrial and ventricular cardiac trabeculae*

As we reported earlier<sup>21</sup>, baseline contractile force was significantly lower in the atrial ( $0.64 \pm 0.13$  mN,  $n=17$  trabeculae) than in ventricular ( $3.04 \pm 0.45$  mN,  $n=20$  trabeculae) tissue. In both tissues, noradrenaline (10 nM - 10  $\mu$ M) increased contractile force in a concentration-dependent manner. After exposure to 10  $\mu$ M noradrenaline, the force of contraction increased to  $2.43 \pm 0.36$  mN ( $n=17$  trabeculae) and  $5.02 \pm 0.43$  mN ( $n=20$  trabeculae) in the atrial and ventricular trabeculae, respectively.

Neither SB-220453, nor sumatriptan displayed a positive inotropic effect on atrial and ventricular trabeculae (Figure 7.4). At concentrations  $\geq 10$   $\mu$ M, SB-220453 and sumatriptan (dissolved in DMSO) induced a negative inotropic effect in both atrial (sumatriptan:  $37 \pm 32\%$  of contraction to 10  $\mu$ M noradrenaline, SB-220453:  $34 \pm 29\%$ ) and ventricular (sumatriptan:  $18 \pm 9\%$ , SB-220453:  $55 \pm 21\%$ ) trabeculae, which were not different for these compounds. This negative inotropic effect may be assigned to

the solvent DMSO, since it was not observed with sumatriptan dissolved in distilled water (Figure 7.4). After a precontraction with noradrenaline (10  $\mu$ M) also, none of the compounds induced a positive inotropic effect, while a negative effect on contractility was observed at concentrations  $\geq 10$   $\mu$ M of SB-220453 and sumatriptan dissolved in DMSO (data not shown).



**Figure 7.4** Concentration response curves in human isolated atrial (*left panel*,  $n=3-5$ ) and ventricular (*right panel*,  $n=5$ ) trabeculae to sumatriptan (dissolved in distilled water,  $\bullet$  or  $\square$ ) and SB-220453 ( $\circ$ ,  $\triangle$ , dissolved in DMSO). NA, Noradrenaline.

## 7.4 Discussion

### *Human isolated blood vessels*

SB-220453 did not induce any significant contraction of the human isolated coronary artery, saphenous vein or middle meningeal artery. In contrast, sumatriptan, investigated in parallel, produced marked contractions. Since contractions to some agonists are 'unmasked' or augmented in the presence of increased tension<sup>7,22</sup>, we also

investigated the coronary artery contraction in the presence of the thromboxane A<sub>2</sub> analogue U46619. Indeed, the contraction to sumatriptan was augmented in two of the coronary arteries investigated (in accordance with our previous findings<sup>22</sup>), but even in these precontracted segments, SB-220453 failed to elicit any contraction.

In all blood vessels, a slight relaxation was observed at the highest concentration of SB-220453 and sumatriptan (both dissolved in DMSO), but not with sumatriptan dissolved in distilled water. Therefore, this relaxation may be assigned to the solvent DMSO. However, despite this relaxant response, SB-220453 did not affect the concentration response curve to sumatriptan (see Figures 7.2 and 7.3).

It is known that the presence or absence of functional endothelium can influence contractile responses in blood vessels<sup>23,24</sup>. In our study, the endothelial quality of the blood vessels varied, as is illustrated by the relaxation to substance P (coronary artery: 9-107%, middle meningeal artery: 19-76%) or bradykinin (saphenous vein: 10-107%). Because in none of the blood vessels SB-220453 induced any contraction, our results suggest that the lack of response to these compounds is not dependent on the quality of the endothelium.

#### *Atrial and ventricular cardiac trabeculae*

Apart from a negative inotropic effect associated with the solvent DMSO, no inotropic response was observed with SB-220453 or sumatriptan in both atrial and ventricular cardiac trabeculae. Sumatriptan did not display any effect on cardiac trabecular contractility despite expression of both 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> mRNA in human atrium and ventricle<sup>25</sup>. However, the present results are consistent with the observed lack of hemodynamic effects of sumatriptan in patients, where no negative or positive inotropic effects were demonstrated<sup>26</sup>. These studies are also in accordance with *in vitro* studies performed on guinea pig<sup>27,28</sup> and rabbit<sup>29</sup> cardiac tissue. In fact, the role of 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors on the human heart is still poorly understood<sup>30</sup>, although the 5-HT<sub>1D</sub> receptor has been reported to mediate inhibition of noradrenaline release in human atrium<sup>31</sup>.



**In conclusion**, our results do not provide any evidence for human blood vessel contraction or altered myocardial contractility in response to SB-220453. Since SB-220453 did not contract the middle meningeal artery, we conclude that potential therapeutic efficacy is independent of cerebral vasoconstriction. SB-220453 did not contract coronary artery, saphenous vein or cardiac trabeculae, and is therefore likely to be devoid of adverse cardiovascular side effects observed with serotonergic agonists.

**Acknowledgements** - The authors want to express their sincere gratitude to the neurosurgical and cardiothoracic surgical units of the University Hospital 'Dijkzigt', Erasmus University Medical Centre Rotterdam, as well as to the Rotterdam Heart Valve Bank (Bio Implant Services Foundation / Eurotransplant Foundation) for supplying the human tissues.

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## Chapter 8

# Augmented Contraction of the Human Isolated Coronary Artery by Sumatriptan: a Possible Role for Endogenous Thromboxane

**Summary** - The antimigraine drug, sumatriptan, contracts the human coronary artery and, in some patients, elicits chest symptoms (e.g. pressure and pain), particularly after subcutaneous administration. We studied the effects of the thromboxane A<sub>2</sub> (TxA<sub>2</sub>) analogue, U46619 and endothelin-1 on contractile responses to sumatriptan in the human isolated coronary artery as well as the role of endogenously produced TxA<sub>2</sub> and endothelin-1 in contractions evoked by sumatriptan. In the presence of U46619 (1 and 3 nM), mean concentration response curves to sumatriptan in the human coronary artery were shifted vertically due to the initial contraction by U46619, but when this initial contraction was subtracted from the response to sumatriptan, no significant augmentation was observed. However, analysis of the degree of augmentation in individual arterial segments revealed that the augmentation was variable and related inversely to the E<sub>max</sub> of sumatriptan in the absence of U46619 ( $r=0.78$  and  $0.81$  for 1 and 3 nM, respectively;  $P<0.05$ ). Treatment with the TxA<sub>2</sub> receptor antagonist, SQ30741 (100 nM), or incubation of vessel segments with aspirin (10  $\mu$ M), significantly reduced responses to sumatriptan; in aspirin-treated vessel segments, SQ30741 failed to decrease further the contractions to sumatriptan. The decrease in E<sub>max</sub> of sumatriptan by both SQ30741 and aspirin correlated significantly with the E<sub>max</sub> of sumatriptan without SQ30741 ( $r=0.74$ ;  $P<0.01$ ) or aspirin ( $r=0.94$ ;  $P<0.01$ ). In aspirin-treated vessel segments, responses to sumatriptan were significantly augmented in the presence of U46619 (3 nM;  $P<0.05$ ). The specificity of SQ30741 was demonstrated by its ability to antagonise coronary artery

Based on: MaassenVanDenBrink A, Bax WA, Ferrari MD, Zijlstra FJ, Bos E, Saxena PR. Augmented contraction of the human isolated coronary artery by sumatriptan: a possible role for endogenous thromboxane. *Br J Pharmacol.* 1996;104:855-862.

contractions to U46619 (pA<sub>2</sub>: 7.54±0.30), but not endothelin-1. Similarly, incubation with aspirin (10 µM) did not affect contractile responses to endothelin-1, but significantly reduced TxA<sub>2</sub> production in coronary artery segments as judged by a decrease in thromboxane B<sub>2</sub> (TxB<sub>2</sub>) from 4.77±0.98 to 1.38±0.36 ng g<sup>-1</sup> 2 h<sup>-1</sup>. Endothelin-1 (1 nM) did not significantly augment contractions to sumatriptan; there was also no relationship between the degree of augmentation and the control E<sub>max</sub> of sumatriptan in the absence of endothelin-1. Furthermore, unlike SQ30741 or aspirin, a high concentration (100 nM) of the non-selective ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist, SB 209670, failed to affect contractile responses to sumatriptan. However, SB 209670 potently antagonised coronary artery contractions induced by endothelin-1 with a pA<sub>2</sub> of 8.84±0.32. Compared to the control vascular segments, endothelial denudation did not reduce TxA<sub>2</sub> production (with endothelium: 2.56±1.38 versus without endothelium: 12.32±4.94 ng TxB<sub>2</sub> g<sup>-1</sup> 2 h<sup>-1</sup>), suggesting that the production of TxA<sub>2</sub> production is not confined to the endothelium. The sumatriptan-induced contractions were also not affected by endothelial denudation. The results of the present study suggest that endogenously produced TxA<sub>2</sub> enhances contractions to sumatriptan in the human isolated coronary artery. Such a mechanism may play a role in causing chest symptoms after sumatriptan by potentiating coronary vascular contraction by sumatriptan *in vivo*.

## **8.1 Introduction**

The antimigraine drug sumatriptan, a 5-HT<sub>1B/1D</sub> receptor agonist<sup>1,2</sup>, causes chest symptoms (e.g. pressure and pain) in up to 5% of patients in clinical trials<sup>3-6</sup>. Although extracardiac mechanisms such as increased oesophageal contractions<sup>7</sup> may be involved, sumatriptan has been reported to cause myocardial infarction<sup>8</sup> due to coronary artery constriction, observed both *in vivo*<sup>9</sup> and *in vitro*<sup>10-13</sup>. The response to sumatriptan in human isolated coronary arteries exhibits a high degree of variability<sup>14</sup>, which is not directly related to underlying disease, age or atherosclerosis<sup>10,14</sup>. In the human coronary artery<sup>12,15</sup> as well as the guinea-pig iliac<sup>16</sup> and rabbit renal<sup>17</sup>, iliac<sup>18</sup> and femoral<sup>19</sup> arteries, prior exposure to a number of substances enhances responses to sumatriptan by 'unmasking' contractions elicited via stimulation of 5-HT<sub>1</sub> receptors, presumed to be similar or identical to the 5-HT<sub>1B/1D</sub> type. In particular, this holds true for the TxA<sub>2</sub> mimetic U46619<sup>12,15</sup>, but endothelin-1 has also been reported to augment contractions to

5-hydroxytryptamine (5-HT)<sup>20</sup>. However, to our knowledge, no study has been performed on augmentation of sumatriptan-induced contractions of the human coronary artery by endothelin-1.

Kaumann *et al.*<sup>14</sup> have reported that sumatriptan-induced contractions in the human isolated artery show a wide variability. We hypothesised that endogenous production of TxA<sub>2</sub> by the vessel segments may play a role in variability of responses to sumatriptan and may be responsible for chest symptoms in some patients after sumatriptan. Indeed, saliva concentrations of TxA<sub>2</sub> were increased in migraine attacks<sup>21</sup> and plasma concentrations of TxA<sub>2</sub> and 5-HT were increased in myocardial infarction and unstable angina<sup>22-24</sup>. Therefore, synergistic effects of 5-HT receptor agonists and TxA<sub>2</sub> may be important in these conditions.

In the present study, we investigated augmentation of sumatriptan-induced contractions of the human isolated coronary artery by U46619. Also, the possibility of involvement of endogenously produced TxA<sub>2</sub> in the contraction to sumatriptan was studied by investigating the response to sumatriptan in the presence of a TxA<sub>2</sub> receptor antagonist, SQ30741<sup>25</sup> or the cyclo-oxygenase inhibitor, aspirin. The production of the TxA<sub>2</sub> metabolite, thromboxane B<sub>2</sub> (TxB<sub>2</sub>) by coronary arteries was investigated in both untreated and aspirin-treated coronary artery segments. Lastly, we studied augmentation of sumatriptan-induced contractions by endothelin-1 and investigated the involvement of endogenously produced endothelin-1 by studying the influence of the ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist SB 209670<sup>26</sup> on the contraction to sumatriptan.

## 8.2 Patients and methods

### *Tissue preparation*

Right epicardial coronary arteries were obtained from 45 heart beating organ donors who died of non-cardiac disorders less than 24 hours before the tissue was taken to the laboratory (23 trauma, 17 cerebrovascular accident, 4 cerebral hypoxia, 1 hypoglycaemia; 24 male, 21 female; age 1-55 years). In addition, one heart was obtained from a patient undergoing heart transplantation (female suffering from

cardiomyopathy, age 63 years). The hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services/Eurotransplant Foundation) after removal of the heart valves for transplantation purposes and stored at 0-4°C in a sterile organ-protecting solution immediately after circulatory arrest. After arrival in the laboratory, the right epicardial coronary artery was removed and placed in a cold, oxygenated Krebs bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25 and glucose 8.3; pH 7.4. Vessel segments containing macroscopically visible atherosclerotic lesions were excluded from the study. In some experiments, the endothelium was removed with a cotton swab attached to a forceps. Unless mentioned otherwise, the results refer to experiments with endothelium-intact vessel segments. The study was approved by the Ethical Committee of the Erasmus University Medical Centre Rotterdam.

#### *Functional experiments*

Coronary artery segments of approximately 4 mm were suspended on stainless steel hooks in 15 ml organ baths aerated with 95% O<sub>2</sub> / 5% CO<sub>2</sub>, and were maintained at 37°C. Vessel segments were allowed to equilibrate for at least 30 min, and were washed every 15 min. Changes in tension were recorded with a Harvard isometric transducer. The vessel segments, stretched to a stable tension of about 15 mN, were exposed to 30 mM K<sup>+</sup> twice. Subsequently, the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) after pre-contraction with prostaglandin F<sub>2α</sub> (1 μM). The tissue was washed and exposed to 100 mM K<sup>+</sup>. After another wash and a 30 min equilibration period, U46619, endothelin-1, SQ30741, SB 209670 or in each case vehicle was added, 30 min before a concentration-response curve was constructed. Contractile responses were expressed as percentage of the contraction induced by 100 mM K<sup>+</sup>.

To assess the influence of endogenously produced TxA<sub>2</sub> on the response to sumatriptan, vessel segments were kept overnight in Krebs solution containing aspirin (10 μM) before the experiment. Control segments were kept in Krebs solution without aspirin. For the aspirin-treated vessel segments, the experiments were performed in



Krebs solution containing 10  $\mu\text{M}$  aspirin, which did not change the pH of the Krebs solution.

#### *Measurement of endogenously produced $\text{TxA}_2$*

Production of  $\text{TxA}_2$  by human isolated coronary arteries was measured in oxygenated Krebs solution (segment length 1-2 cm in 2 ml during a 2 h period) at 37°C. For aspirin-treated vessel segments, the incubation was performed in Krebs solution containing aspirin (10  $\mu\text{M}$ ). After 2 h, a sample of the Krebs solution was removed and indomethacin (30  $\mu\text{M}$ ) was added to stop cyclo-oxygenase activity. The samples were centrifuged at 570 g (20°C) for 20 min and the supernatant was stored at -20°C until assay. The concentration of  $\text{TxB}_2$ , the stable metabolite of  $\text{TxA}_2$ , was measured by radioimmunoassay<sup>27,28</sup> and expressed as ng  $\text{TxB}_2$  per g wet weight of tissue.

#### *Data analysis*

All data are presented as mean $\pm$ s.e.mean. Concentration response curves were analysed using the logistic function described by De Lean *et al.*<sup>29</sup> to obtain pEC<sub>50</sub> (-log EC<sub>50</sub>) values. To analyse differences between concentration-response curves to sumatriptan obtained in the presence or absence of either U46619 or endothelin-1, responses to sumatriptan were corrected for the increase in basal tone by the two agents. Differences between the corrected concentration-response curves (in the presence of U46619 or endothelin-1) and the control concentration-response curve were examined by analysis of variance (ANOVA) for repeated measures. The degree of augmentation by U46619 or endothelin-1 was defined as the ratio of the  $E_{\text{max}}$  of sumatriptan in the presence of either U46619 or endothelin-1 (corrected for the basal contraction by these agents) and that in the absence of these compounds (control  $E_{\text{max}}$ ). Correlation coefficients were calculated according to Pearson<sup>30</sup>. The degree of variation in a series of experiments under different experimental conditions was expressed by subtracting the contraction in an individual segment from the mean contraction in each series. These values were divided by the mean response to correct for the magnitude of the response. Mean values of the three highest agonist concentrations were used to test differences between experimental conditions with the Wilcoxon signed rank test. The pA<sub>2</sub> values of

antagonists were determined by Schild analysis<sup>31</sup>. Differences in TxA<sub>2</sub> production, relaxation to substance P, contraction to 100 mM K<sup>+</sup>, and differences between pEC<sub>50</sub> values were analysed using a paired *t* test. *P* values of ≤0.05 were considered to indicate significant differences. Data analyses were performed with the SPSS (SPSS Inc., Chicago, Illinois, U.S.A.) statistical program.

#### *Compounds used in this study*

Sumatriptan was a gift from Glaxo Wellcome (Dr. H.E. Connor, Ware, Hertfordshire, U.K.); U46619 (9, 11 – dideoxy - 11 $\alpha$ , 9 $\alpha$  - epoxy - methanoprostaglandin F<sub>2 $\alpha$</sub> ), prostaglandin F<sub>2 $\alpha$</sub>  (Tris salt), substance P acetate and aspirin (acetylsalicylic acid) were purchased from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.); endothelin-1 was purchased from Neosystem Laboratoire (Strasbourg, France); SQ30741 (1S-[1 $\alpha$ ,2 $\alpha$ (Z),3 $\alpha$ ,4 $\alpha$ ]-7-[3-[[[(1-oxoheptyl)amino]acetyl]amino]methyl]-7-oxabicyclo-[2.2.1]hept-2-yl]-5-heptenoic acid) was a gift from Bristol-Myers Squibb (Princeton, NJ, U.S.A.); SB 209670 (( $\pm$ )-(1S,2R,3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)-indane-2-carboxylic acid) was a gift from SmithKline Beecham Pharmaceuticals (Dr. E.H. Ohlstein, King of Prussia, Pennsylvania, U.S.A.). Indomethacin was obtained from the pharmacy of University Hospital Rotterdam 'Dijkzigt' (Rotterdam, The Netherlands). [5,6,8,9,11,12,14,15(n)-<sup>3</sup>H]-thromboxane B<sub>2</sub> (specific activity: 8.21 TBq mmol<sup>-1</sup>; Batch B65) was purchased from Amersham (Little Chalfont, Buckinghamshire, U.K.); TxB<sub>2</sub> antiserum was purchased from PerSeptive Diagnostics (Cambridge, U.K.).

### **8.3 Results**

#### *Basic properties of the preparations: effects of substance P and potassium*

Endothelium-intact human coronary artery segments relaxed after addition of substance P (1 nM), the response amounting to 84±5% of pre-contraction (26.7±2.1 mN) induced by prostaglandin F<sub>2 $\alpha$</sub>  (1  $\mu$ M; *n*=41). The vessel segments responded to 100 mM K<sup>+</sup> with a contractile response of 34.3±2.3 mN (*n*=41). Vasorelaxation by substance P

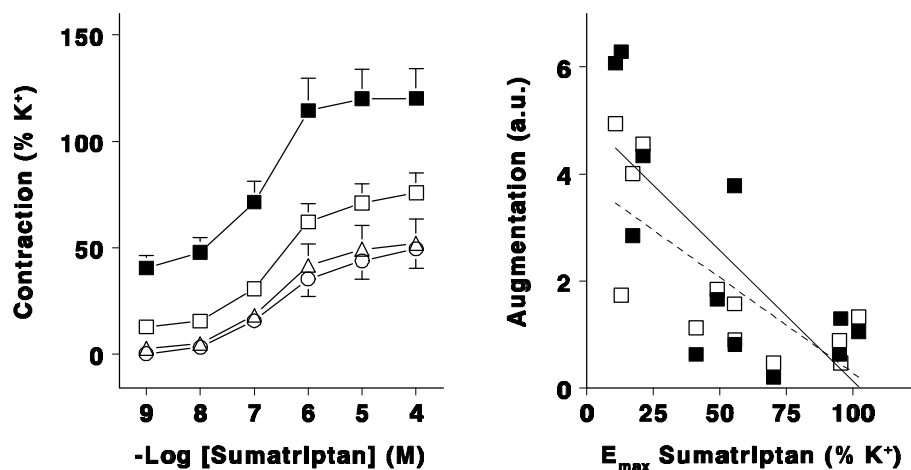
was related inversely to the age of the heart donors (Pearson's  $r=0.40$ ,  $P<0.01$ ; endothelium-intact vessel segments). On the other hand, the contractile responses to  $K^+$  (100 mM) and prostaglandin  $F_{2\alpha}$  (1  $\mu$ M) were not related to donors' age, nor was there any relationship between the relaxation by substance P or contraction by  $K^+$  and the cause of death or sex of the donors.

Removal of the endothelium resulted in a non-significant reduction of the contractile response to prostaglandin  $F_{2\alpha}$  (1  $\mu$ M; without endothelium= $21.9\pm 3.3$  mN versus with endothelium= $28.3\pm 2.6$  mN), but it reduced significantly the relaxant response to substance P (without endothelium= $9\pm 4\%$  versus with endothelium= $79\pm 9\%$ ;  $P<0.0001$ ;  $n=7$ ) as well as the contractile response to 100 mM  $K^+$  (without endothelium= $22.7\pm 3.7$  mN versus with endothelium= $31.2\pm 3.3$  mN;  $P<0.005$ ;  $n=7$ ).

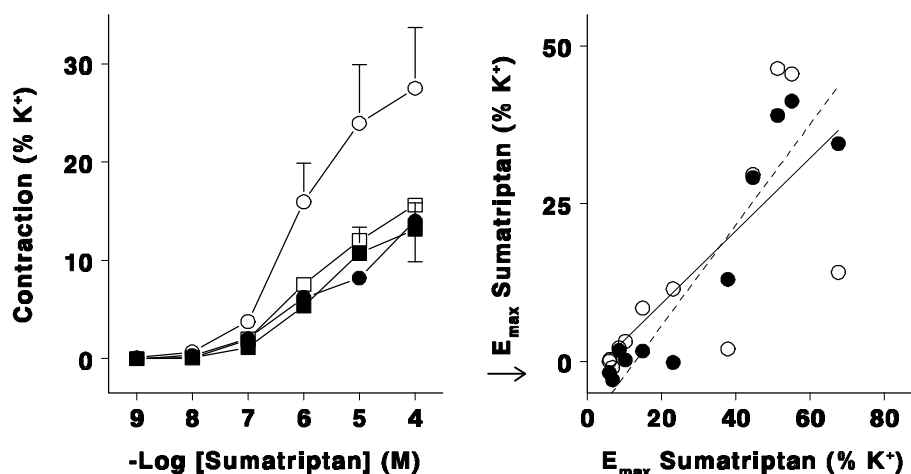
Incubation with aspirin (10  $\mu$ M) did not affect the contractile response to prostaglandin  $F_{2\alpha}$  (1  $\mu$ M;  $26.4\pm 2.6$  mN versus  $28.9\pm 2.4$  mN for control) or the relaxant response to substance P ( $68\pm 7\%$  of contraction to prostaglandin  $F_{2\alpha}$  versus  $73\pm 7\%$  for control segments;  $n=17$ ).

#### *Augmentation of the responses to sumatriptan*

In the presence of U46619 (1 and 3 nM), concentration-response curves to sumatriptan were shifted to a higher level due to the initial contraction associated with U46619 (Figure 8.1, left panel). When this initial contraction was subtracted from the response generated by sumatriptan in the presence of U46619 to allow calculation of the degree of augmentation (see 'data analysis' in the method section), concentration-response curves to sumatriptan in the presence of U46619 were not significantly different from the control curve. However, further analysis of the degree of augmentation in individual arterial segments revealed that the augmentation was variable and related inversely to the  $E_{\max}$  of sumatriptan in the absence of U46619 (Figure 8.1, right panel); correlation coefficients were 0.78 (1 nM U46619;  $P<0.01$ ) and 0.81 (3 nM U46619;  $P<0.01$ ).



**Figure 8.1** *Left panel:* Contractile responses to sumatriptan (expressed as % of the response to 100 mM  $K^+$ ) in the absence (□, control) or presence of U46619 (■, 0.3 nM; △, 1 nM; ▴, 3 nM; n=12-13). *Right panel:* Augmentation of the contractile response to sumatriptan by U46619 (■, solid line, 3 nM; □, broken line, 1 nM) plotted against the control (without U46619)  $E_{max}$  of sumatriptan. The augmentation by U46619, expressed in arbitrary units (a.u.) was calculated as the ratio of  $E_{max}$  of sumatriptan in the presence of U46619 (corrected for the basal contraction by U46619) and that in the absence of U46619 (control  $E_{max}$ ).



**Figure 8.2** *Left panel:* Contractile responses to sumatriptan (expressed as % of the response to 100 mM  $K^+$ ) in the absence (○, □) or presence (●, ▴) of SQ30741 (100 nM) in vessel segments with (filled symbols) or without (open symbols) incubation with aspirin (10  $\mu$ M). *Right panel:* Attenuation of the  $E_{max}$  of sumatriptan by SQ30741 (100 nM, ●; solid line) or aspirin (10  $\mu$ M, ○; broken line) plotted against the control  $E_{max}$  of sumatriptan (i.e. in the absence of SQ30741 and aspirin). The attenuations by SQ30741 and aspirin were positively correlated to the control  $E_{max}$  of sumatriptan (Pearson's  $r=0.74$  and  $0.84$ , respectively;  $P<0.01$ ;  $n=12$  each).

When experiments in which the  $E_{\max}$  for sumatriptan was  $\leq 50\%$  of the potassium-induced contraction were analysed separately, a significant augmentation of the response to sumatriptan was found in the presence of both 1 nM and 3 nM U46619 ( $n=6$ ,  $P<0.01$ ).

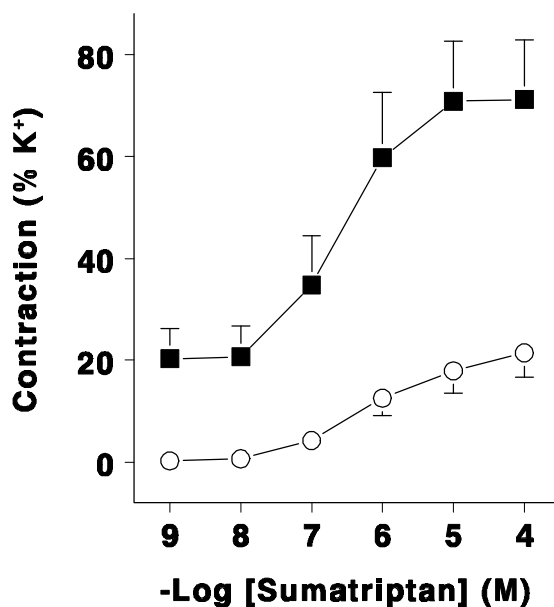
The  $pEC_{50}$  value of sumatriptan ( $6.5\pm 0.1$ ;  $n=13$ ) was not significantly changed in the presence of U46619 (0.3 nM:  $6.6\pm 0.1$ ; 1 nM:  $6.6\pm 0.2$ ; 3 nM:  $6.9\pm 0.1$ ).

#### *Endogenous production of $TxA_2$ and contractile responses to sumatriptan*

In view of the inverse relationship between the degree of augmentation of sumatriptan responses by U46619 and the  $E_{\max}$  of sumatriptan, we investigated whether endogenously produced  $TxA_2$  may have already augmented the response to sumatriptan, thus decreasing the margin for further augmentation by U46619 in segments with higher endogenous  $TxA_2$  production. In order to test this possibility, we performed experiments using the  $TxA_2$  receptor antagonist, SQ30741 and the cyclo-oxygenase inhibitor, aspirin.

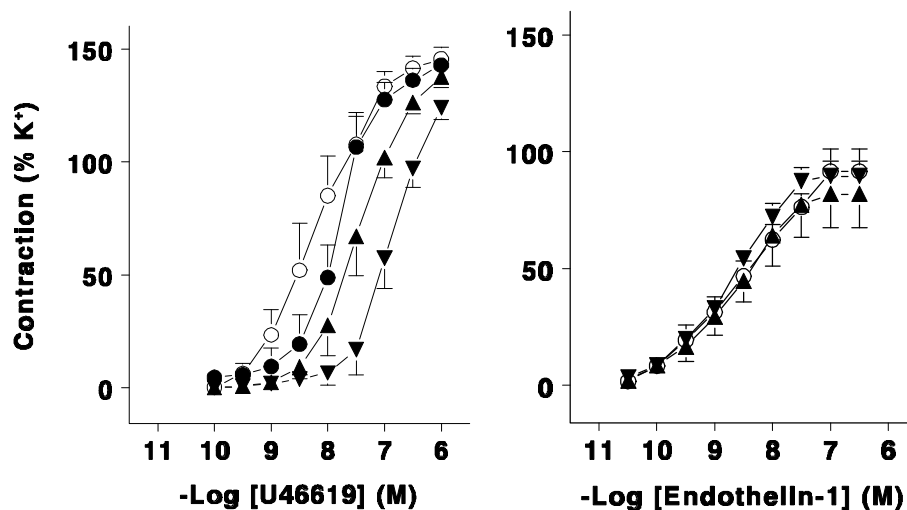
Responses to sumatriptan (in the absence of U46619) were significantly attenuated by 100 nM SQ30741 ( $E_{\max}$ :  $14\pm 4\%$  versus  $28\pm 6\%$  in controls;  $n=12$ ). Incubation with aspirin (10  $\mu$ M; overnight) also resulted in a decreased response to sumatriptan ( $E_{\max}$ :  $16\pm 2\%$  versus  $28\pm 6\%$  in controls;  $n=12$ ), but in these vessel segments SQ30741 did not further decrease sumatriptan-induced contractions ( $E_{\max}$ :  $13\pm 3\%$ ; Figure 8.2, left panel). The attenuation of the maximal response to sumatriptan by SQ30741 as well as aspirin was significantly correlated to the control  $E_{\max}$  of sumatriptan ( $r=0.74$  and  $0.94$  for SQ30741 and aspirin, respectively;  $P<0.01$  for both; Figure 8.2, right panel). The variability in the concentration-response curves was significantly decreased (for calculation: see 'data analysis';  $P<0.05$ ) in the presence of SQ30741 or after treatment with aspirin, indicating that a source of variability had been eliminated under these conditions (s.e.mean / mean response for three highest sumatriptan concentrations: control, 0.241; SQ30741, 0.165; aspirin, 0.111). In addition, as shown in Figure 8.3, responses to sumatriptan were significantly augmented by 3 nM U46619 ( $P<0.05$ ;  $n=7$ ) in aspirin-treated vessel segments, which produce decreased amounts of  $TxA_2$  (see below). However, the

production of  $\text{TxA}_2$  in aspirin-untreated vessel segments did not correlate with the  $E_{\text{max}}$  values of sumatriptan ( $r=0.14$ ;  $n=11$ ).



**Figure 8.3** Contractile responses to sumatriptan (expressed as % of the response to 100 mM  $\text{K}^+$ ) in the absence (○, control) or presence (■) of 3 nM U46619 in vessel segments incubated with 10  $\mu\text{M}$  aspirin ( $n=7$  each). The responses to sumatriptan were significantly augmented by U46619 ( $P<0.05$ ).

The potency and selectivity of the  $\text{TxA}_2$  receptor antagonist SQ30741 was studied by comparing concentration-response curves for U46619 and endothelin-1 in the absence or presence of several concentrations (30-300 nM) of SQ30741 (Figure 8.4). Treatment of the vessel segments with SQ30741 shifted the dose-response curves of U46619 in a parallel manner to the right, but did not affect those to endothelin-1. When data of the experiments with U46619 were represented in a Schild plot, the calculated  $\text{pA}_2$  value was  $7.54\pm 0.30$  ( $n=5$ ) with a slope ( $1.39\pm 0.15$ ) not differing from unity, indicating a competitive antagonism.



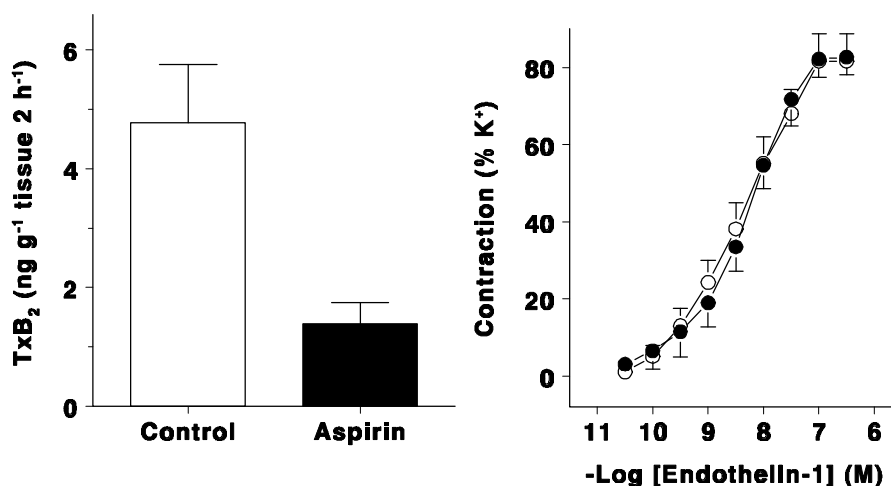
**Figure 8.4** Contractile responses to U46619 (*left panel*;  $n=5$ ) and endothelin-1 (*right panel*;  $n=4$ ), both expressed as % of the contraction induced by 100 mM  $K^+$ , in the absence (○, control) or presence of the  $TxA_2$  receptor antagonist, SQ30741 (●, 30 nM; ▲, 100 nM; ▼, 300 nM). The responses to U46619 were inhibited by SQ30741 ( $pA_2: 7.54 \pm 0.30$ ), while those to endothelin-1 were not modified by SQ30741.

Incubation of human coronary artery segments with aspirin (10  $\mu$ M overnight) led to a decreased production of  $TxA_2$ . As shown in Figure 8.5 (left panel), the concentration of  $TxB_2$  (the stable metabolite of  $TxA_2$ ) in the control and aspirin-incubated segments were, respectively,  $4.77 \pm 0.98$  and  $1.38 \pm 0.36$  ng  $g^{-1} 2 h^{-1}$  ( $n=12$  each;  $P < 0.005$ ). The potency as well as the efficacy of endothelin-1 in the aspirin-incubated vessel segments ( $pEC_{50}: 8.35 \pm 0.14$ ;  $E_{max}: 82 \pm 4.6\%$  100 mM  $K^+$ ) was not different from that in the control segments ( $pEC_{50}: 8.44 \pm 0.17$ ;  $E_{max}: 82 \pm 7.2\%$  100 mM  $K^+$ ), indicating that the contractile characteristics of the vessel were not altered by aspirin (Figure 8.5, right panel).

#### *Localisation of endogenous $TxA_2$ production*

In an attempt to determine whether endogenously produced  $TxA_2$  was derived from the endothelium or other parts of the vascular tissue, a comparison was made between the

production of  $\text{TxA}_2$  as well as the responses to sumatriptan in endothelium-intact and endothelium-denuded human coronary artery segments. Measurements of  $\text{TxA}_2$  production in endothelium-denuded vessel segments revealed a non-significant change in the levels of  $\text{TxB}_2$  compared to the vessels where the endothelium was not removed ( $12.32 \pm 4.94$  versus  $2.56 \pm 1.38$  ng  $\text{TxB}_2$   $\text{g}^{-1}$   $2 \text{ h}^{-1}$ , respectively;  $n=8$ ;  $P>0.05$ ). Similarly, there was no significant change in sumatriptan-induced contractile responses in the endothelium-denuded vessel segments ( $E_{\text{max}}$ :  $20 \pm 6\%$  in endothelium-intact versus  $37 \pm 14\%$  in endothelium-denuded segments;  $P>0.05$ ;  $n=7$ ).



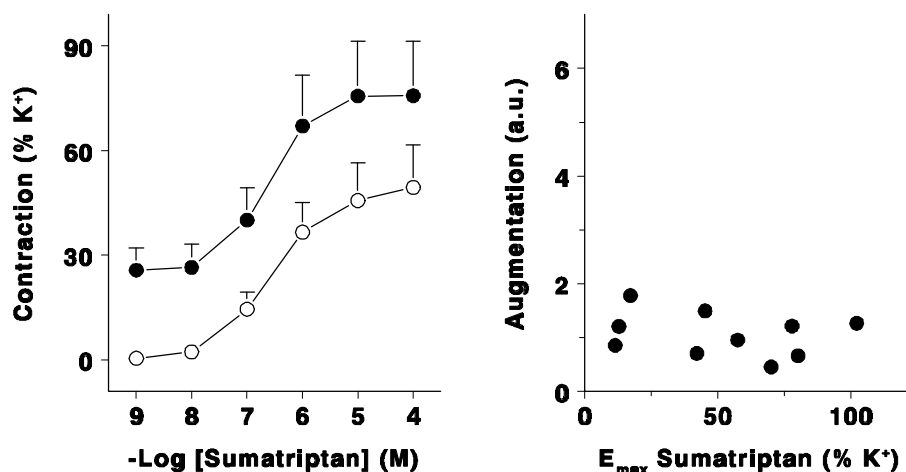
**Figure 8.5** *Left panel:* Endogenous production of  $\text{TxA}_2$ , expressed in terms of its stable metabolite  $\text{TxB}_2$  (ng  $\text{TxB}_2$   $\text{g}^{-1}$   $2 \text{ h}^{-1}$ ), in human coronary artery segments after incubation with (filled bar) or without (open bar) aspirin ( $10 \mu\text{M}$ ).  $\text{TxA}_2$  production was significantly decreased in vessel segments incubated with aspirin ( $P<0.005$ ;  $n=12$ ). *Right panel:* Contractile responses to endothelin-1 in human coronary artery segments after incubation in the presence ( ) or absence ( ) of aspirin ( $10 \mu\text{M}$ ). Incubation with aspirin did not affect the concentration-response curve to endothelin.

#### *Involvement of endothelin-1 in the responses to sumatriptan*

In the presence of endothelin-1 ( $1 \text{ nM}$ ), the concentration-response curve to sumatriptan yielded higher contractions due to the initial contraction associated with endothelin-1; higher concentrations of endothelin-1 could not be used due to phasic contractions in the



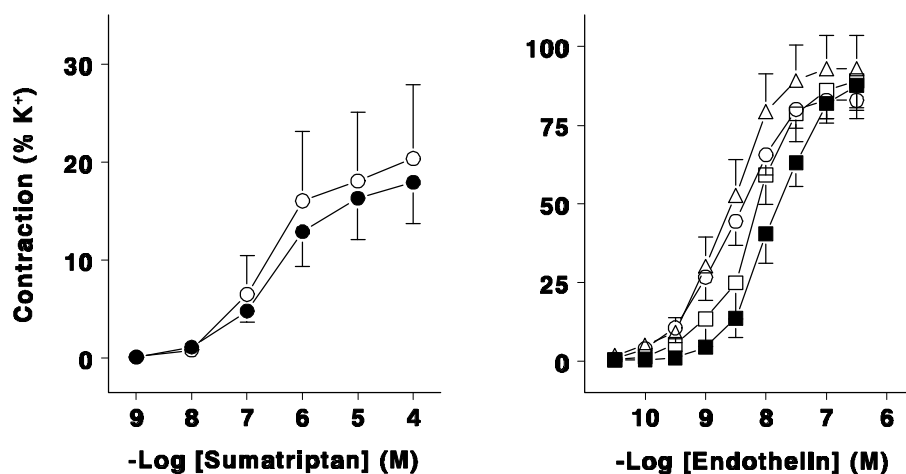
majority of vessels. As found with U46619 (see above), the concentration-response curve to sumatriptan in the presence of endothelin-1 was not significantly different from the control curve (Figure 8.6, left panel). However, unlike U46619, there was no relationship between the degree of augmentation of the response to sumatriptan and the control  $E_{\max}$  of sumatriptan, in the absence of endothelin-1 ( $r=0.26$ ;  $n=10$ ;  $P>0.05$ ; Figure 8.6, right panel).



**Figure 8.6** *Left panel:* Contractile responses to sumatriptan in the absence (○, control) or presence (●) of endothelin-1 (1 nM). Though the concentration-response curve of sumatriptan was shifted upwards by endothelin-1, there was no augmentation of the responses as can be noted by similar differences in the responses with or without endothelin-1 throughout the concentration range. *Right panel:* The degree augmentation of the contractile response to sumatriptan, expressed in arbitrary units (a.u.), by endothelin-1 (1 nM) plotted against the control (without endothelin-1)  $E_{\max}$  of sumatriptan. Note that the augmentation by endothelin-1 was much less than with U46619 (Figure 8.1) and that there was no significant correlation between the augmentation and the control  $E_{\max}$  ( $r=0.26$ ;  $P>0.05$ ;  $n=10$ ).

Concentration response curves of sumatriptan and endothelin-1 were also generated in the absence or presence SB 209670, a potent non-selective  $ET_A/ET_B$  receptor antagonist. A high concentration of SB 209670 (100 nM) failed to affect the concentration-response curve of sumatriptan ( $E_{\max}$ :  $18\pm 4.2\%$  versus  $20\pm 7.5\%$  for control;  $n=9$ ; Figure 8.7, left panel), thus providing little evidence for a role of endothelin-1 in the contractile response to sumatriptan. As expected, SB 209670 shifted

the concentration-response curves of endothelin-1 to the right in a parallel manner (Figure 8.7, right panel). The estimated pA<sub>2</sub> value was 8.84±0.32 (*n*=6) and the slope (0.86±0.23) did not differ from unity, indicating a competitive antagonism.



**Figure 8.7** Contractile responses to sumatriptan (*left panel*; *n*=9) and endothelin-1 (*right panel*; *n*=6), both expressed as % of the contraction induced by 100 mM K<sup>+</sup>, in the absence (○, control) or presence of the non-selective ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist SB 209670 (□, 0.3 nM; ◻, 1 nM; ◼, 3 nM; and/or ◼, 100 mM). Contractile responses to sumatriptan were not affected by SB 209670, but those to endothelin-1 were competitively inhibited (pA<sub>2</sub>: 8.84±0.32).

## 8.4 Discussion

### *Augmentation of the responses to sumatriptan by U46619*

In the present study, the response of the human isolated coronary artery to sumatriptan was not significantly augmented in the presence of the TxA<sub>2</sub> analogue, U46619 (0.3-3 nM). This seems to be at variance with earlier reports of Chester *et al.*<sup>12</sup> and Cocks *et al.*<sup>15</sup>, but, in both these studies, the control E<sub>max</sub> of sumatriptan response was less than 8% of the K<sup>+</sup>-induced contraction. In contrast, several other studies reported a

higher  $E_{\max}$  of sumatriptan (40% of contraction to 90 mM  $K^+$ , Chester *et al.*, 1990; 23% of contraction to 100 mM  $K^+$ , Bax *et al.*, 1993; 11% of contraction to 0.1  $\mu$ M U46619, Connor *et al.*, 1989). When, in our study, experiments with a relatively low control  $E_{\max}$  of sumatriptan ( $\leq 50\%$  of  $K^+$ -induced contractions) were analysed separately, a significant augmentation of the response to sumatriptan was found in the presence of both 1 nM and 3 nM of U46619. The inverse relation between the  $E_{\max}$  of sumatriptan and the degree of augmentation suggested that, in arteries with a high  $E_{\max}$  of sumatriptan, the response may already have been augmented by an endogenous factor, possibly  $TxA_2$ . Differing endogenous production of this factor in the various studies could explain the variation in the obtained control  $E_{\max}$  of sumatriptan. Also within a study, variable responses to sumatriptan have been reported<sup>14</sup>, but no explanation was forwarded.

#### *Role of endogenous production of $TxA_2$ in the contractile response to sumatriptan*

The hypothesis that endogenously produced  $TxA_2$  may be involved in the response to sumatriptan was investigated in two different ways. Firstly, the  $TxA_2$  receptor was blocked by the use of a  $TxA_2$  receptor antagonist, SQ30741. Secondly, the formation of endogenous  $TxA_2$  was inhibited by incubation of the tissues with the cyclo-oxygenase inhibitor, aspirin. The findings that (i) sumatriptan-induced contractions were decreased to the same extent by SQ30741 and aspirin, (ii) no further decrease was noticed with SQ30741 in aspirin-treated vessel segments (Figure 8.2, left panel) and (iii) contractile responses to sumatriptan were augmented by U46619 in aspirin-incubated vessel segments, suggest that an endogenously produced cyclo-oxygenase product, most probably  $TxA_2$ , augments the contractile response to sumatriptan. The positive correlation between the attenuation of the response to sumatriptan by SQ30741 or aspirin and the control  $E_{\max}$  of sumatriptan also supports this contention. Further evidence is provided by the fact that the variability of concentration-response curves of sumatriptan was decreased by both SQ30741 and aspirin; a decreased variability suggests that a source of variation, most likely the presence of differing concentrations of  $TxA_2$ , was reduced. However, in contrast, the  $E_{\max}$  of sumatriptan did not correlate with the concentrations of  $TxB_2$  measured in the medium incubating coronary artery segments

not treated with aspirin. There are several factors which may have confounded the relationship between the sumatriptan  $E_{\max}$  and endogenous TxA<sub>2</sub> production: (i) although these two variables were determined in two segments of the same coronary artery, the functional ( $E_{\max}$  sumatriptan) and biochemical (TxB<sub>2</sub>) assays had to be performed under different conditions; as described in detail in the method section, vessel segments (4 mm length in 15 ml Krebs solution) for functional assays were stretched to a force of about 15 mN and exposed to K<sup>+</sup> (three times), prostaglandin F<sub>2α</sub> and substance P and washed several times during a period of about 5 h before constructing the sumatriptan curve, whereas the biochemical assays were performed in unstretched, untreated and unwashed vessel segments (1-2 cm length in 2 ml Krebs solution in order to keep the TxB<sub>2</sub> concentration above the detection limit) during 2 h, (ii) whereas the  $E_{\max}$  of sumatriptan is likely to be influenced by TxA<sub>2</sub> concentrations prevailing at that time, the measured TxB<sub>2</sub> concentrations represent cumulative TxA<sub>2</sub> production over a 2 h period, and (iii) as may be expected from earlier investigations (e.g.<sup>16,17,32</sup>), it is possible that a certain threshold concentration of TxA<sub>2</sub> augments the contraction to sumatriptan, without a linear relationship between the amount of endogenous TxA<sub>2</sub> and the potentiation of the response to sumatriptan.

It is conceivable that incubation with aspirin may have affected the ability of the smooth muscle cells to contract and, thus, reduced contractile responses to sumatriptan. However, in such a case, it may be expected that the TxA<sub>2</sub> receptor antagonist SQ30741 would have the same effect whether the vessel segments were previously incubated with aspirin or not. Clearly, this was not the case. Furthermore, the response to endothelin-1 was not attenuated by incubation of vessel segments with aspirin. Direct evidence for the fact that TxA<sub>2</sub> was indeed produced by the human isolated coronary artery and that this production was inhibited by aspirin was furnished by the measurements of TxB<sub>2</sub> in the medium surrounding the vessel segments (Figure 8.5, left panel). Since mechanical removal of the endothelium did not decrease (if anything, tended to increase) the formation of TxB<sub>2</sub> as well as sumatriptan-induced vessel contractions, it would appear that endogenous TxA<sub>2</sub> production is not, or not exclusively, located in vascular endothelium. Although the formation of TxA<sub>2</sub> has been reported to be restricted to the endothelium in the human internal mammary artery<sup>33</sup>, the media and adventitia of

human arteries (gastric, mesenteric, splenic, and renal) have also been reported to produce  $\text{TxA}_2$ <sup>34</sup>.

#### *Role of endothelin-1 in the responses to sumatriptan*

In our study, endothelin-1 did not augment sumatriptan-induced contractions of the human coronary artery. Detailed analysis of the responses in individual vascular segments, in contrast to the experiments with U46619, did not reveal any relationship between the degree of augmentation by endothelin-1 and the magnitude of the control  $E_{\text{max}}$  of sumatriptan. It must be pointed out that we could use only 1 nM endothelin-1, since a higher concentration (3 nM) caused phasic contractions in the majority of experiments, thus precluding the possibility of obtaining a stable concentration-response curve of sumatriptan. However, with the concentration used (1 nM), the initial contraction of the human coronary artery by endothelin-1 was more than that by 1 nM U46619 (compare Figures 8.1 and 8.6), which significantly augmented sumatriptan responses in vessels with low  $E_{\text{max}}$ . Although Yang *et al.*<sup>20</sup> have observed that concentrations of 1 nM and, even, 0.3 nM of endothelin-1 potentiate responses to 5-HT, others, in a larger series of experiments, were unable to confirm such a augmentation<sup>35</sup>. In addition, unlike the  $\text{TxA}_2$  antagonist SQ30741, the response to sumatriptan was not influenced by SB 209670, which in the present investigation, as well as in an earlier study<sup>26</sup>, was shown to be a potent inhibitor of endothelin-1-induced contractions of the human isolated coronary artery. These observations suggest that endothelin-1 did not induce a significant augmentation of the responses to sumatriptan and that the augmentation by U46619 was not merely dependent on increased basal force or augmented levels of  $\text{PIP}_2$ , as suggested by MacLennan *et al.*<sup>36</sup>. Another argument pleading against the possibility that augmentation may be regulated via increased basal tension only, is the fact that responses to sumatriptan in, for example, the guinea-pig iliac artery are augmented by U46619 in threshold concentrations hardly causing any contraction (Sahin-Erdemli *et al.*<sup>16</sup>; our own unpublished observations).

As mentioned above, endothelin-1 may augment contractile responses to 5-HT in the human isolated coronary artery<sup>20</sup>. By contrast, U46619 augments the responses to sumatriptan, to an even higher extent than it was found to augment the responses to

5-HT<sup>12,32</sup>. Whereas the augmentation of the response to 5-HT by U46619 seems to be regulated most profoundly by the 5-HT<sub>1B/1D</sub> receptor subtype, augmentation of the response to 5-HT by endothelin-1 could be mediated by other 5-HT receptor subtypes, not involved in the contraction to sumatriptan (see<sup>19</sup>).

**In conclusion**, our results suggest that endogenously produced TxA<sub>2</sub> is able to enhance the response to the 5-HT<sub>1B/1D</sub> receptor agonist sumatriptan in the human isolated coronary artery. This finding could have implications for the synergistic action of 5-HT and TxA<sub>2</sub> in vasospastic diseases, since not only platelet-derived, but also locally produced TxA<sub>2</sub> in the blood vessel wall can augment contractile responses to 5-HT. In addition, although conceivable, it remains to be seen if a similar mechanism exaggerating sumatriptan-induced coronary artery contractions also operates in migraine patients complaining of chest symptoms. In such a case, a prior treatment of patients with aspirin may be able to suppress chest symptoms after sumatriptan, in at least some patients.

**Acknowledgements** - The authors wish to express their gratitude to the Rotterdam Heart Valve Bank (Bio Implant Services/Eurotransplant Foundation) for supplying us with the human heart tissue. The authors also thank Ms. H.J. Brinks for the measurements of TxB<sub>2</sub>. The statistical advice of Dr. Th. Stijnen, Dept. of Epidemiology and Biostatistics, is sincerely acknowledged. This study was supported by the Netherlands Heart Foundation, grant no. 93.146.

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## Chapter 9

### Human Isolated Coronary Artery Contraction to Sumatriptan; a Post Hoc Analysis

**Summary** - A post hoc analysis was performed on concentration response curves to sumatriptan in 62 human isolated coronary arteries. We determined whether donor-related clinical characteristics (age, sex, cause of death) and properties of the coronary artery (functional endothelial integrity, muscle mass) were related to the potency and efficacy of sumatriptan in contracting the human isolated coronary artery. The efficacy of sumatriptan was inversely related to the functional integrity of the vessel endothelium. Thus, contrary to expectation, coronary artery constriction to sumatriptan seems to be more pronounced in patients with non-diseased coronary arteries where the endothelium is intact. Nevertheless, in view of the high coronary reserve in these patients myocardial ischemia after the use of sumatriptan is unlikely to occur, whereas in patients with coronary artery disease even a small contraction may be deleterious.

Based on: MaassenVanDenBrink A, Bax WA, Ramrattan NN, Ferrari MD, Saxena PR. Human isolated coronary artery contraction to sumatriptan: a post hoc analysis. *Cephalalgia*. 1999;19:651-654.

## **9.1 Introduction**

The selective 5-HT<sub>1B/1D</sub> receptor agonist sumatriptan has proved a highly effective and generally well tolerated drug in migraine therapy<sup>1</sup>. However, up to 15% of patients in clinical trials<sup>2</sup> and up to 42% of patients in clinical practice<sup>3</sup> report chest related symptoms mimicking pectoral angina. Although extracardiac mechanisms have been invoked<sup>4</sup>, the chest symptoms may be explained by coronary artery constriction to sumatriptan, which has been observed both *in vivo*<sup>5</sup> and *in vitro*<sup>6-9</sup>. In rare cases, the use of sumatriptan has even been associated with myocardial infarction<sup>10-13</sup> and cardiac arrest<sup>14</sup>. Risk factors for chest symptoms after the use of sumatriptan are unknown: determinants may include young age, hypertension and a family history of myocardial infarction<sup>15</sup>, although others could not confirm this, except for young age<sup>3</sup>.

Experiments on isolated blood vessels may provide important information as to the mechanism involved. In the human isolated coronary artery, contraction to sumatriptan may vary considerably<sup>6,7</sup>, possibly partly due to augmentation of the contraction to sumatriptan by endogenously produced thromboxane A<sub>2</sub><sup>6</sup>. In addition, the contraction to sumatriptan may be larger in segments adjacent to an atheromatous lesion compared to those distal to the lesion<sup>16</sup>. However, others found no relation between the contraction to sumatriptan and underlying disease, age or atherosclerosis<sup>6,7</sup>.

Over the past several years, we have studied contractions to sumatriptan in a large number of human isolated coronary arteries under identical conditions. We present here a post hoc analysis to determine whether donor-related clinical characteristics (age, sex, cause of death) or properties of the coronary artery segment (functional integrity of the endothelium or muscle mass) were related to the degree of contraction to sumatriptan.

## 9.2 Patients and methods

### *Tissue preparation*

From our database of experiments on sumatriptan in the human isolated coronary artery, we selected experiments performed on hearts obtained from donors aged 18 years and older. Hearts obtained from younger subjects were excluded because patients younger than 18 years are unlikely to be treated with sumatriptan. Right epicardial coronary arteries were obtained from 62 heart beating organ donors who had died of non-cardiac disorders less than 24 hours before the tissue was taken to the laboratory (20 trauma, 36 cerebrovascular accident, 6 hypoxia; 34 male, 28 female, age 19-63 years, mean age 42 years). The hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services Foundation / Eurotransplant Foundation) after removal of the heart valves for transplantation and were stored at 0-4°C in a sterile organ protecting solution immediately following circulatory arrest. After arrival in the laboratory, the right epicardial coronary artery (inner diameter 2-3 mm, outer 3-4 mm) was removed and placed in a cold, oxygenated Krebs buffer solution of the following composition (mM): NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25 and glucose 8.3; pH 7.4. Vessel segments containing macroscopically visible atherosclerotic lesions were excluded from the study. The ethical committee of the Erasmus University Medical Centre Rotterdam approved the study.

### *Functional experiments*

As described previously in detail<sup>6</sup>, coronary artery segments of approximately 4 mm length were suspended between stainless steel hooks in 15-ml organ baths aerated with 95% O<sub>2</sub> / 5% CO<sub>2</sub> at a temperature of 37°C. After an equilibration period of at least 30 min with a wash every 15 min, vessel segments were stretched to a tension of 15 mN. After exposure to 30 mM K<sup>+</sup> twice, the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) after pre-contraction with prostaglandin F<sub>2α</sub> (1 μM). The tissue was washed and exposed to 100 mM K<sup>+</sup>. After another wash and a 30 min equilibration period, a

concentration-response curve to sumatriptan (1 nM - 100  $\mu$ M) was constructed. Subsequently, the segments were discarded.

#### *Data analysis*

All data are presented as mean $\pm$ s.e.mean. Univariate analysis, followed by multiple regression analysis was used to relate the characteristics of donors (age, sex and cause of death — encoded as trauma, cerebrovascular accident or hypoxia) as well as those of coronary arteries (endothelial integrity, measured as relaxation to substance P) with the efficacy and potency of sumatriptan on the coronary artery. The efficacy of sumatriptan was denoted by the maximum contraction ( $E_{\max}$ ) and the potency by the negative logarithm of the concentration needed to elicit 50% of the  $E_{\max}$  (i.e.  $^{-10}\log EC_{50}$  or  $pEC_{50}$ ).  $pEC_{50}$  values were determined using the logistic function described by De Lean *et al.*<sup>17</sup>. An increase in drug response may be characterised by an increase in  $E_{\max}$  and/or  $pEC_{50}$ .

A *P* value of 0.05 or less was considered statistically significant. Data analyses were performed with the SPSS statistical program (SPSS Inc., Chicago, Illinois, U.S.A.).

### **9.3 Results**

#### *Basic properties of the preparations: effects of substance P and potassium*

The mean contractile response to 100 mM  $K^+$  was  $42\pm 2$  mN. The mean relaxant response to substance P (1 nM) was  $61\pm 5$  % of precontraction ( $31\pm 4$  mN) induced by  $PGF_{2\alpha}$ .

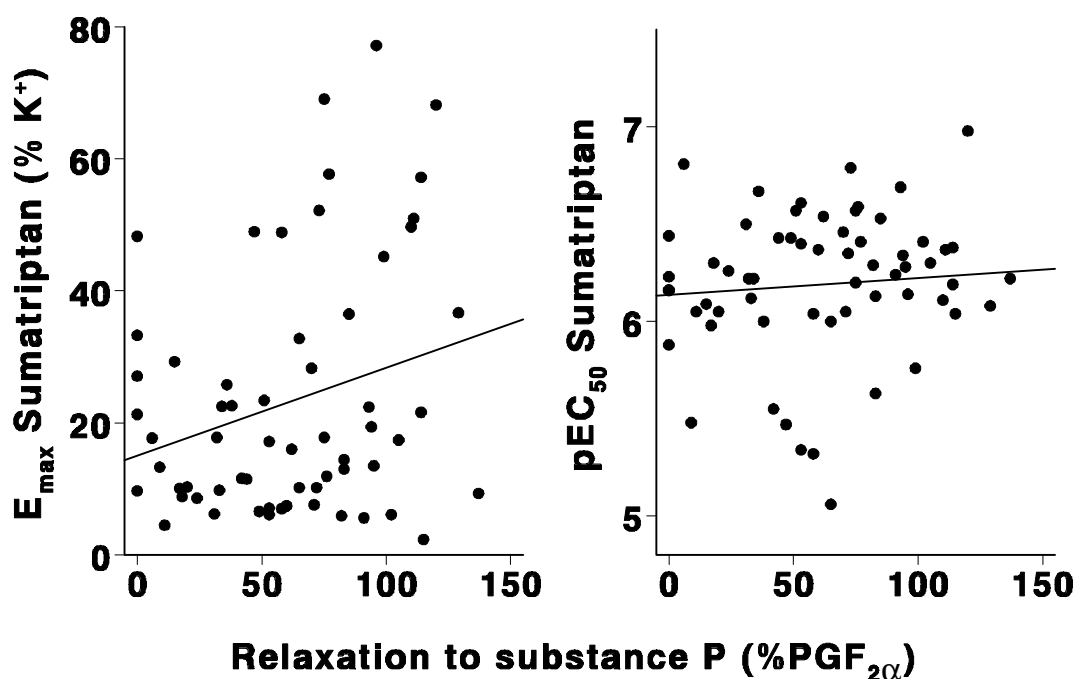
#### *Contraction to sumatriptan: relationship with characteristics from the organ donor and the endothelium*

The  $E_{\max}$  of sumatriptan was  $9\pm 1$  mN, corresponding to  $27\pm 3\%$  of  $K^+$ -induced contraction. The  $pEC_{50}$  of the response to sumatriptan was  $6.27\pm 0.05$ . Univariate analysis showed that the  $E_{\max}$  of sumatriptan, expressed as percentage contraction to

$K^+$ , was positively related to the  $E_{\max}$  of sumatriptan, expressed in mN ( $r=0.81$ ,  $p<0.001$ ). Contraction to  $K^+$  decreased with age ( $r=-0.52$ ,  $p<0.001$ ), but was not related with the relaxation to substance P.

Univariate analysis showed a significant relationship between the  $E_{\max}$  of sumatriptan (expressed as % contraction to  $K^+$ ) and relaxation to substance P, used as a marker for endothelial function (Pearson's  $r=0.263$ ,  $p=0.040$ ; Figure 9.1, left panel). The  $E_{\max}$  of sumatriptan was not related to age, sex or the cause of death of the organ donor. Multiple regression analysis also showed a positive relation between the  $E_{\max}$  of sumatriptan and relaxation to substance P ( $\beta=0.286$ ,  $p=0.033$ , Table 9.1).

Univariate analysis and multiple regression analysis revealed no significant relationship between the  $pEC_{50}$  of sumatriptan and relaxation to substance P (Figure 9.1, right panel) or age, sex, or cause of death of the organ donor (Table 9.1).



**Figure 9.1** *Left panel:*  $E_{\max}$  values of sumatriptan plotted against the relaxation to substance P (1 nM), expressed as percentage of precontraction induced by prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ , 1  $\mu$ M). Both parameters are significantly related to each other (Pearson's  $r=0.263$ ,  $p=0.040$ ). *Right panel:*  $pD_2$  values of sumatriptan plotted against the relaxation to substance P. No significant relationship between these values was observed (Pearson's  $r=0.136$ ,  $p=0.30$ ).

**Table 9.1**  $\beta$ - and P-values obtained with multivariate analysis on the  $E_{\max}$  and  $pEC_{50}$  of sumatriptan in the human isolated coronary artery.

	$E_{\max}$		$pEC_{50}$	
	$\beta$	<i>P</i>	$\beta$	<i>P</i>
Age	0.030	NS	0.060	NS
Sex	0.137	NS	0.012	NS
Cause of death	0.044	NS	0.003	NS
Substance P	0.286	0.033	0.145	NS

NS, not significant; Substance P, relaxation to substance P.

#### 9.4 Discussion

Contraction of the human isolated coronary artery to sumatriptan shows a high degree of variability<sup>6,7</sup>. In the present study we assessed whether this variability could be related to characteristics of the patient or the vessel wall.

The  $pEC_{50}$  of sumatriptan-induced contraction was not related to any of the characteristics of the patient or the artery. The unaltered  $pEC_{50}$ , which is a parameter associated with the affinity of sumatriptan for its receptor, indicates that the 5-HT<sub>1B/1D</sub> receptor subtype is unlikely to be changed with decreasing functional integrity of the endothelium.

In contrast, to our surprise we found that the  $E_{\max}$  of sumatriptan-induced contraction increased with increasing functional integrity of the endothelium, as denoted by the relaxation to substance P<sup>18</sup>. This relationship suggests that the efficiency of the receptor-effect coupling or the number of receptors is reduced in vessels with decreased endothelial integrity. Although the relationship between the  $E_{\max}$  and the relaxation to substance P was not extremely strong ( $p=0.033$  in multivariate analysis), our findings firmly illustrate that impaired functional quality of the endothelium does not *increase* the response to sumatriptan. In keeping with our findings, receptor protein of the 5-HT<sub>1B</sub> receptor, the most probable pharmacological

target of sumatriptan, was recently demonstrated mainly on smooth muscle cells of the coronary artery, and only faintly on the endothelium<sup>19</sup>. Furthermore, clinical studies most remarkably have shown no consistent<sup>15</sup> or an even opposite<sup>3</sup> relation between sumatriptan-induced chest symptoms and the existence of cardiovascular disease.

Our study has some potential shortcomings. Firstly, we analysed, post hoc, experiments performed during a period of several years. However, all experiments were performed according to an identical protocol and all information needed to perform valid analyses was documented. Secondly, one could argue that the smaller contraction to sumatriptan in vessels with impaired endothelial quality might be due to a generalised decrease in contractile capacity of diseased arteries. However, as mentioned in the 'Results' section, contraction to potassium chloride, an indicator of the contractile capacity of a blood vessel, was not related to the relaxation to substance P. Moreover, we expressed the contraction to sumatriptan as a percentage of contraction to potassium chloride and thus took into account any decrease in contractile capacity of the blood vessel related to endothelial dysfunction.

In addition to our experiments on sumatriptan, we recently investigated human isolated coronary artery contraction to several second-generation triptans (zolmitriptan, naratriptan and rizatriptan) which are all, like sumatriptan, 5-HT<sub>1B/1D</sub> receptor agonists. These compounds were slightly more potent than sumatriptan in contracting the human isolated coronary artery. This is in accordance with their somewhat higher affinity at 5-HT<sub>1B/1D</sub> receptors. The maximal contraction to these compounds was equal to that of sumatriptan<sup>20</sup>. Therefore, we expect that the E<sub>max</sub> of these newer triptans may be related to the endothelial quality of the coronary artery in a similar manner as is presented here for sumatriptan.

**In conclusion**, cardiovascular risk factors associated with endothelial dysfunction are not expected to increase coronary artery contraction to sumatriptan. Thus, coronary artery constriction to sumatriptan may be more pronounced in patients with non-diseased coronary arteries. Since the coronary reserve in these patients is larger than in patients with pre-existing coronary artery lesions, myocardial ischemia is unlikely to occur in this group.



In contrast, in patients with coronary artery disease even a small contraction may induce cardiac ischemia. Therefore, sumatriptan (and other triptans to date) remains contraindicated in patients with coronary artery disease.

## **9.5 References**

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## Chapter 10

### Human Isolated Coronary Artery Contraction to Sumatriptan Characterised by the Selective 5-HT<sub>1B/1D</sub> Receptor Antagonist GR55562

**Summary** - The antimigraine drug sumatriptan contracts the human coronary artery both *in vivo* and *in vitro*. Because sumatriptan has been associated with cardiac side effects, it is important to characterise the receptor involved in sumatriptan-induced coronary artery contraction. Using the agonists sumatriptan and 5-carboxamidotryptamine (5-CT) and the selective 5-HT<sub>1B/1D</sub> receptor antagonist GR55562, we have investigated the involvement of 5-HT<sub>1B/1D</sub> receptors in the contraction of the human isolated coronary artery. Contractions to sumatriptan (pEC<sub>50</sub>: 6.1±0.2, maximal effect: 21±4% of 100 mM K<sup>+</sup>-induced contraction) were competitively antagonised by GR55562. The pA<sub>2</sub> of GR55562 (7.41±0.16) was in accord with its reported affinity at the human 5-HT<sub>1B</sub> receptor. Since the contractions to 5-CT did not reach a maximum with the highest concentration used (10 µM), pEC<sub>50</sub> values could not be calculated for Schild analysis. However, using the pEC<sub>10%K<sup>+</sup></sub> values (negative logarithm of the concentration needed to induce 10% of the contraction to 100 mM K<sup>+</sup>), GR55562 proved a less potent antagonist against 5-CT than against sumatriptan. These results show that sumatriptan contracts the human isolated coronary artery via 5-HT<sub>1B/1D</sub> receptors, most probably the 5-HT<sub>1B</sub> subtype. 5-CT may contract the human isolated coronary artery, at least partly, via a novel, yet to be characterised, receptor.

Based on: MaassenVanDenBrink A, Reekers M, Bax WA, Saxena PR. Human isolated coronary artery contraction to sumatriptan characterised by the selective 5-HT<sub>1B/1D</sub> receptor antagonist GR55562. *Submitted*.

## 10.1 Introduction

The human coronary artery contracts in response to the antimigraine drug sumatriptan both *in vivo*<sup>1</sup> and *in vitro*<sup>2-4</sup>. Since sumatriptan has been associated with myocardial infarction<sup>5,6</sup> and cardiac arrest<sup>7</sup> in exceptional cases, it is important to characterise the receptors involved in sumatriptan-induced contraction of the human coronary artery. More knowledge about the receptor mediating coronary artery contraction to sumatriptan may contribute to the development of antimigraine drugs devoid of coronary vasoconstrictor activity.

Sumatriptan displays affinity for the 5-HT<sub>1B</sub> (pK<sub>i</sub>: 7.8-8.0), 5-HT<sub>1D</sub> (pK<sub>i</sub>: 8.1-8.5) and the 5-HT<sub>1F</sub> (pK<sub>i</sub>: 7.8-7.9) receptor<sup>8,9</sup>. Human isolated coronary artery contraction to 5-HT is mediated via both 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors<sup>2,3</sup>. The contraction mediated by the 5-HT<sub>2</sub> receptor usually exceeds that mediated by 5-HT<sub>1</sub> receptors<sup>2,3</sup>. In some cases, however, 5-HT<sub>1</sub> receptor-mediated contraction is predominant over the 5-HT<sub>2</sub> receptor-mediated contraction<sup>10</sup>. In agreement with the variable contribution of 5-HT<sub>1</sub> receptors in 5-HT-mediated contraction, the contraction to sumatriptan is subject to a high degree of variability<sup>10,11</sup>. The involvement of 5-HT<sub>1</sub> receptors in sumatriptan-induced contraction of the human isolated coronary artery has been confirmed by resistance to ketanserin and by sensitivity to methiothepin<sup>2,10</sup>. The 5-HT<sub>1</sub> receptor subtype mediating contraction to sumatriptan in the human coronary artery has been described to functionally resemble the 5-HT<sub>1B</sub> receptor<sup>10</sup>. No studies in the human isolated coronary artery, however, have yet been performed with the use of selective 5-HT<sub>1B/1D</sub> receptor antagonists, which may give a direct answer to the question which 5-HT<sub>1</sub> receptor subtype is involved in the contraction of the human isolated coronary artery to sumatriptan. Using the agonists sumatriptan and 5-carboxamidotryptamine (5-CT) and the selective 5-HT<sub>1B/D</sub> receptor antagonist, GR55562<sup>12</sup> we have investigated involvement of 5-HT<sub>1B/D</sub> receptors in the contraction of the human isolated coronary artery.

## 10.2 Patients and methods

### *General*

Right epicardial coronary arteries were obtained from 7 heart beating organ donors who died of non-cardiac disorders less than 24 hours before the tissue was taken to the laboratory (5 cerebrovascular accident, 2 trauma; 5 female, 2 male, age 38 to 56 years). As described previously<sup>11</sup>, the hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services / Eurotransplant Foundation) after explantation of the aortic and pulmonary valves for transplantation purposes. The hearts were stored at 0-4°C in a sterile organ protecting solution immediately after circulatory arrest. Coronary artery segments of approximately 4 mm length were suspended between stainless steel hooks in 15-ml organ baths containing Krebs bicarbonate solution (composition in mM: NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25 and glucose 8.3; pH 7.4; 37°C) aerated with 95% O<sub>2</sub> / 5% CO<sub>2</sub>. Contraction was recorded isometrically. Vessel segments with macroscopically visible atherosclerotic lesions were not used.

### *Experimental protocol*

Coronary artery segments of approximately 4 mm length were mounted on stainless steel hooks in 15-ml organ baths containing oxygenated Krebs solution kept at a temperature of 37°C. Vessel segments were allowed to equilibrate for at least 30 min and were washed every 15 min throughout the experiment prior to the addition of antagonist. The rings were stretched to a stable tension of approximately 15 mN before exposure to K<sup>+</sup> (30 mM) twice. Subsequently, the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) after precontraction with prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>; 1 μM). After washout, the segments were exposed to 100 mM K<sup>+</sup>. Following a washout and equilibration period of another 30 min, all segments were incubated for 30 min with vehicle or GR55562 (30 nM - 3 μM). Subsequently, a concentration response curve to sumatriptan or 5-CT was constructed. Contractile responses were expressed as percentage of the

contraction to 100 mM K<sup>+</sup>. All experiments were conducted in a paired parallel set-up for each agonist.

#### *Data analysis*

Concentration response curves were analysed by a curve fitting function using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, U.S.A.) to obtain pEC<sub>50</sub> (negative logarithm of the concentration eliciting half maximal effect, i.e.  $-\log EC_{50}$ ) values. Since concentration response curves to 5-CT did not reach a maximum, pEC<sub>50</sub> values could not be obtained. To study the shift of the concentration response curves by GR55562 without the use of pEC<sub>50</sub> values, we calculated the negative logarithm of the concentration of agonist needed to induce 10% of K<sup>+</sup>-induced contraction (pEC<sub>10%K<sup>+</sup></sub>), corresponding to about 50% of the maximum contraction induced by sumatriptan. Curves were fitted to the overall mean data ( $n=5-6$ ). Differences between groups were tested by analysis of variance followed by Dunnett's post-hoc test. P-values < 0.05 were considered to indicate significant differences. All data are represented as mean±s.e.mean.

#### *Compounds*

Sumatriptan and GR55562 (3-[3-(dimethylamino)propyl]-4-hydroxy-N-[4-(4-pyridinyl) phenyl]benzamide) were a gift from GlaxoWellcome, Stevenage, Ware, Herts, U.K. (Courtesy: Dr. H.E. Connor). Prostaglandin F<sub>2α</sub> (tris salt) and substance P acetate were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. 5-CT (5-carboxamidotryptamine maleate) was purchased from Research Biochemicals International, Natick, MA, U.S.A. All compounds were dissolved in distilled water.

#### *Ethical approval*

The Ethical Committee of the Erasmus University Medical Centre Rotterdam approved this study.

### 10.3 Results

#### *Basic contractile properties*

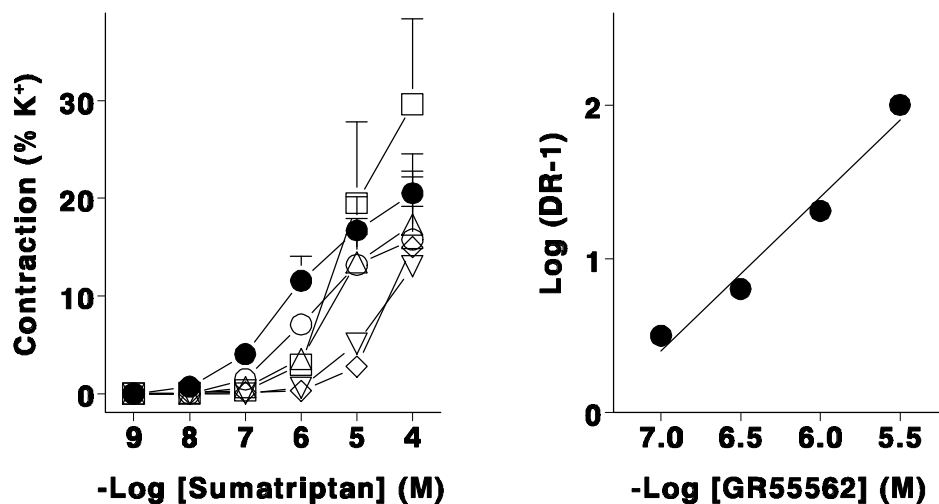
Coronary artery segments relaxed after addition of substance P (1 nM), the response amounting to  $47 \pm 13\%$  of the precontraction induced by prostaglandin  $F_{2\alpha}$  (1  $\mu\text{M}$ ,  $n=7$ ). The vessel segments responded to 100 mM  $\text{K}^+$  with a contractile response of  $34 \pm 5$  mN.

#### *Sumatriptan and GR55562*

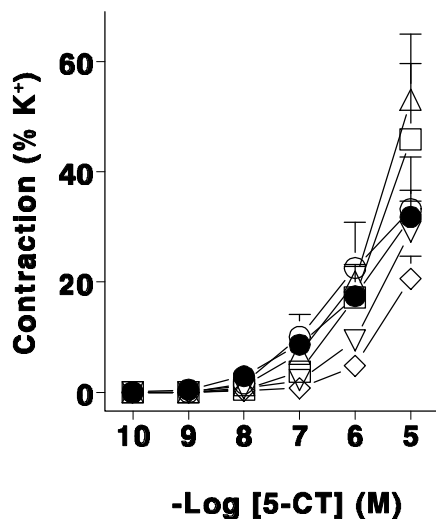
Sumatriptan induced a concentration-dependent contraction (Figure 10.1, *left panel*,  $n=5-6$ ). The maximal contraction to sumatriptan in the absence of GR55562 was  $21 \pm 4\%$  of 100 mM  $\text{K}^+$ -induced contraction, the  $\text{pEC}_{50}$  was  $6.07 \pm 0.42$  (Table 10.1). GR55562 shifted the concentration response curve to sumatriptan to the right, as illustrated by the decreased  $\text{pEC}_{50}$  and  $\text{pEC}_{10\% \text{K}^+}$  values in the presence of GR55562 ( $\geq 100$  nM, Table 10.1). Schild analysis of the concentration response curves in the presence of various concentrations of GR55562 (Figure 10.1, *right panel*) revealed a slope of  $1.00 \pm 0.12$  and  $\text{pA}_2$  of  $7.41 \pm 0.16$ .

#### *5-CT and GR55562*

5-CT induced concentration-dependent contraction of the coronary artery segments (Figure 10.2,  $n=5$ ). At the highest concentration of 5-CT (10  $\mu\text{M}$ ), the response was  $32 \pm 5\%$  of  $\text{K}^+$ -induced contraction. Because the curves did not reach a plateau with the concentrations of 5-CT used, it was not possible to obtain  $\text{pEC}_{50}$  values. The  $\text{pEC}_{50}$  value of the control curve was  $< 6.44 \pm 0.23$ . In contrast with sumatriptan, the contraction to 5-CT seemed to be less antagonised by low concentrations of GR55562 (see Figure 10.2 and Table 10.1).



**Figure 10.1** *Left panel:* Concentration response curves obtained with sumatriptan in the human isolated coronary artery in the absence (□, control) or presence of the 5-HT<sub>1B/1D</sub> receptor antagonist GR55562 (○, 30 nM; △, 100 nM; ◇, 300 nM; ●, 1 μM; ■, 3 μM). *Right panel:* Schild plot representing the antagonism by GR55562 of the contractions of the human isolated coronary artery induced by sumatriptan. The slope of the Schild plot was 1.00±0.12 with the corresponding pA<sub>2</sub> of GR55562 being 7.41±0.16.



**Figure 10.2** Concentration response curves obtained with 5-CT in the human isolated coronary artery in the absence (□, control) or presence of the 5-HT<sub>1B/1D</sub> receptor antagonist GR55562 (○, 30 nM; △, 100 nM; ◇, 300 nM; ●, 1 μM; ■, 3 μM).



**Table 10.1** pEC<sub>50</sub> and pEC<sub>10%K<sup>+</sup></sub> values of sumatriptan and 5-CT in the absence (control) or presence of the 5-HT<sub>1B/1D</sub> receptor antagonist GR55562.

GR55562	Sumatriptan (n=5-6)		5-CT (n=5)	
	pEC <sub>50</sub>	pEC <sub>10 %K<sup>+</sup></sub>	pEC <sub>50</sub>	pEC <sub>10 %K<sup>+</sup></sub>
0 (Control)	6.07±0.42	6.14±0.29	<6.44±0.23	6.76±0.13
30 nM	5.84±0.35	5.58±0.26	ND	6.95±0.30
100 nM	5.45±0.40	5.34±0.22*	ND	6.62±0.15
300 nM	5.99±0.32	5.46±0.15	ND	6.38±0.19
1 μM	4.73±0.39*	4.46±0.15*	ND	5.95±0.10*
3 μM	4.06±0.18*	4.33±0.14*	ND	5.55±0.11*

\* Significantly different from control.

ND Not determined (contraction did not reach a maximum at the highest concentration applied).

pEC<sub>50</sub>, Negative logarithm of the concentration eliciting half maximal effect ( $-\log EC_{50}$ ).

pEC<sub>10%K<sup>+</sup></sub> Negative logarithm of the concentration needed to induce 10% of contraction to K<sup>+</sup>.

## 10.4 Discussion

In the present study, we investigated the 5-HT receptor subtypes involved in the contractions to sumatriptan and 5-CT in the human isolated coronary artery. The 5-HT<sub>1B/1D</sub> receptor antagonist GR55562 antagonised contractions induced by sumatriptan. The pA<sub>2</sub> value of GR55562 obtained in our experiments (7.4) is in accord with its affinity at the 5-HT<sub>1B</sub> receptor determined in radioligand binding assay (pK<sub>i</sub>: 7.3) as well as in functional assay against sumatriptan-induced inhibition of cAMP production (pK<sub>B</sub>: 7.4) in cells transfected with human 5-HT<sub>1B</sub> receptor<sup>12</sup>. The pK<sub>i</sub> values in ligand binding assays obtained at 5-HT<sub>1A</sub> (5.3), 5-HT<sub>1D</sub> (6.3) and 5-HT<sub>1F</sub> (5.6) receptors and the pK<sub>B</sub> value against sumatriptan-induced inhibition of cAMP production in cells transfected with human 5-HT<sub>1D</sub> receptor (6.2) were clearly lower<sup>12</sup>.

Thus, it appears that the sumatriptan-induced contraction of the human isolated coronary artery is mediated via the 5-HT<sub>1B</sub> receptor.

5-CT was less potent ( $pEC_{50} < 6.4$ ) in contracting the human isolated coronary artery in our experiments than we expected on the basis of previous studies in the human coronary<sup>13</sup> and pulmonary<sup>14</sup> arteries as well as saphenous vein<sup>15</sup>. This was also the reason that we did not use 5-CT in concentrations higher than 10  $\mu$ M. A low potency for 5-CT-induced contraction has been described before<sup>16</sup> and may possibly be assigned to simultaneous relaxation mediated via the 5-HT<sub>7</sub> receptor<sup>17</sup>. 5-CT has a high affinity for the 5-HT<sub>7</sub> receptor (1000-fold higher than sumatriptan,  $pK_i=9.03$  and  $6.02$ , respectively) and mRNA for the 5-HT<sub>7</sub> receptor has been detected in the human coronary artery<sup>18</sup>. Because of the low potency of 5-CT, we were unable to calculate  $pEC_{50}$  values. However, we attempted to quantify the antagonism displayed by GR55562 by calculating  $pEC_{10\%K+}$  values. As can be seen in Figure 10.2 and Table 10.1, the antagonism of the contraction to 5-CT by GR55562 was, in contrast to the sumatriptan-induced contraction, restricted to the two highest concentrations (1  $\mu$ M and 3  $\mu$ M) of GR55562. The lower potency of antagonism by GR55562 on the contraction induced by 5-CT compared to sumatriptan seems to be in agreement with the reported novel recognition site for 5-CT in mammalian brain<sup>19</sup>. 5-CT and 5-HT have a 1000-fold higher affinity than sumatriptan ( $pK_i$ : 8.1, 8.0 and 5.1, respectively) at this recognition site<sup>19</sup>. Similarly, De Vries *et al.*<sup>20</sup> found that the 5-HT<sub>1B/1D</sub> receptor antagonist GR127935, which potently antagonised the sumatriptan-induced constriction of porcine carotid arteriovenous anastomoses, failed to affect the ketanserin-resistant constrictor effect of 5-HT. Thus, 5-HT may constrict porcine carotid arteriovenous anastomoses via a novel, non-5-HT<sub>1B/1D</sub> receptor<sup>20</sup>. It is possible that a part of the constriction of human isolated coronary artery by 5-CT in our experiments may be mediated by a GR55562-resistant receptor.

**In conclusion**, our results suggest that sumatriptan contracts the human isolated coronary artery via 5-HT<sub>1B/1D</sub> receptors. In view of the close resemblance of the  $pA_2$  value of GR55562 obtained in our experiments with its reported affinity at the 5-HT<sub>1B</sub> receptor, it is likely that the 5-HT<sub>1B</sub> rather than 5-HT<sub>1D</sub> subtype is involved. The

contraction by 5-CT may be, at least partly, mediated by a novel, yet to be characterised, receptor.

**Acknowledgements** - The authors want to express their gratitude to the Rotterdam Heart Valve Bank (Bio Implant Services / Eurotransplant Foundation) for supplying us with the human heart tissue. This study was supported by the Netherlands Heart Foundation, grant no. 93.146.

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## Chapter 11

### 5-HT<sub>1B</sub> Receptor Polymorphisms and Clinical Response to Sumatriptan

**Summary** - The 5-HT<sub>1</sub> receptor agonist sumatriptan is highly effective in the treatment of migraine. Some patients, however, do not respond, or experience recurrence of the headache. In addition, some patients report chest symptoms after sumatriptan. We investigated whether these different responses could be attributed to genetic diversity of the 5-HT<sub>1B</sub> receptor, which most likely mediates the therapeutic action and the coronary side effects of sumatriptan. Allele frequencies of two polymorphisms in the 5-HT<sub>1B</sub> receptor gene (G861C and T-261G) were investigated in migraine patients with consistently good response to sumatriptan ( $n=14$ ), with no response ( $n=12$ ), with recurrence of the headache ( $n=12$ ), with chest symptoms ( $n=13$ ), and in patients without chest symptoms ( $n=27$ ). Allele frequencies (G:0.74;C:0.26 at nt 861 and T:0.39;G:0.61 at nt -261) did not differ between patient groups, indicating that genetic diversity of the 5-HT<sub>1B</sub> receptor does not seem to be involved in the different clinical responses to sumatriptan.

Based on: MaassenVanDenBrink A, Vergouwe MN, Ophoff RA, Saxena PR, Ferrari MD, Frants RR. 5-HT<sub>1B</sub> receptor polymorphisms and clinical response to sumatriptan. *Headache*. 1998;38:288-291.

## **11.1 Introduction**

Migraine is a common paroxysmal neuro-vascular disorder. The selective 5-HT<sub>1</sub> receptor agonist sumatriptan is highly effective in the acute treatment of migraine attacks<sup>1-3</sup>. Up to 15% of patients, however, never respond to subcutaneous sumatriptan, and up to 40% of responders consistently experience recurrence of the headache within 24 hours after initial headache relief<sup>3-6</sup>. In addition, about 40% of patients always experience one or more "chest symptoms", including chest pressure, heaviness in arms, shortness of breath and chest pain, shortly after the use of subcutaneous sumatriptan<sup>6,7</sup>.

Comparing clinical, demographic and pharmacokinetic characteristics of consistent responders and non-responders, as well as of patients who always experience headache recurrence or chest symptoms and those who never do so, yielded only few and relatively insignificant differences<sup>6,8-10</sup>. For the risk of chest symptoms, determinants such as low age, hypertension, general complaints of abdominal pain, and a family history of myocardial infarction have been suggested<sup>11</sup>, but this could not be confirmed by others<sup>7</sup>.

Besides demographic, clinical, and pharmacokinetic characteristics, genetic factors may also contribute to inter-patient differences in clinical responses to drugs or hormones<sup>12-14</sup>. For sumatriptan, differences in clinical responses may well be explained by differences in one of the 5-HT<sub>1</sub> receptor subtypes, the pharmacological target of sumatriptan<sup>15,16</sup>. Sumatriptan displays high affinities for 5-HT<sub>1B</sub> (former 5-HT<sub>1DB</sub>), 5-HT<sub>1D</sub> (former 5-HT<sub>1D $\alpha$</sub> ) and, although tenfold less, for 5-HT<sub>1F</sub> receptors<sup>4</sup>. Vasoconstriction of cranial arteries mediated by 5-HT<sub>1B</sub> receptors is believed to be, at least partly, important in the mechanism of anti-migraine action of sumatriptan<sup>4</sup>. Recently, mRNA for the 5-HT<sub>1B</sub> receptor was demonstrated in human cerebral arteries<sup>17</sup>.

The 5-HT<sub>1B</sub> receptor gene is localised on chromosome 6q13-q15<sup>18,19</sup>. This intronless gene, with a coding sequence of 1959 nucleotides, encodes a protein of 390 amino acids. The receptor, which contains seven transmembrane regions, is negatively coupled to adenylyl cyclase. Two common polymorphisms in the 5-HT<sub>1B</sub>

receptor gene (G861C<sup>19</sup> and T-261G<sup>20</sup>, numbering starting with the first nucleotide of the initiating methionine codon which is designated +1) have been described. Beside these two common polymorphisms, some rare mutations have been reported, i.e. G-511T<sup>20</sup> and a deletion of bp -179 and -178<sup>20</sup>. In addition to these silent mutations, a T124G transversion leading to an amino acid exchange (Phe6Cys) has been reported in one subject<sup>20</sup>.

In the present study, we investigated whether differences in the clinical response to sumatriptan were associated with the two common polymorphisms in the 5-HT<sub>1B</sub> receptor gene (G861C<sup>19</sup> and T-261G<sup>20</sup>). We, therefore, studied the frequency of the polymorphisms in responders and non-responders to sumatriptan, as well as in migraine patients who experience recurrence of the headache or chest symptoms after sumatriptan.

## 11.2 Patients and methods

### *Patients and statistical analysis*

Forty unrelated migraine patients (35 female, 5 male; age 20-69 years) from the out-patient database of the Department of Neurology of Leiden University Medical Centre, were included in this study. All patients fulfilled the International Headache Society criteria<sup>21</sup>. Patients were divided into five groups according to their clinical response to 6 mg subcutaneous (s.c.) sumatriptan: *i) Responders* were defined as patients who had headache relief within 2 hours after 6 mg s.c. sumatriptan in at least 4 out of 5 migraine attacks and who experienced headache recurrence within 24 hours in less than 1 out of 5 successfully treated attacks; *ii) Patients with headache recurrence* were defined as those who responded to s.c. sumatriptan in at least 4 out of 5 migraine attacks, but in whom the headache recurred within 24 hours in at least 4 out of 5 successfully treated attacks; *iii) Non-responders* were defined as patients who had headache relief in no more than one out of 5 migraine attacks treated with s.c. sumatriptan, or in none of 3 consecutively treated migraine attacks; *iv) Patients with chest symptoms* (chest pressure, heaviness in arms, shortness of breath, or chest pain)



had treated at least 3 migraine attacks with s.c. sumatriptan and had experienced one or more chest symptoms in each of these attacks (the use of the minimal number of 3 rather than 5 attacks in the latter groups emerged from practical reasons since patients with severe adverse events or no response usually refrained from continuing sumatriptan use); and v) *Patients without chest symptoms*, derived from the other groups, were compared with patients with chest symptoms. Clinical and demographic data of the patients are summarised in Table 11.1.

**Table 11.1** Demographic data of the study groups

	<i>n</i>	Sex (M/F)	Mean age (range)
Responders	14	1M, 13 F	49 (23-69)
Recurrence	12	1M, 11F	50 (34-66)
Non-responders	12	3M, 9F	44 (20-60)
Chest symptoms	13	0M, 13F	48 (23-59)
No chest symptoms	27	5M, 22F	47 (20-69)
Total (all patients)*	40	5M, 35F	47 (20-69)

\* Most patients were included in several (non mutually excluding) groups, e.g. patients in the group with chest symptoms could also be included in the groups with responders. M and F refer to male and female, respectively; age is expressed in years.

For both polymorphisms, the allele frequency in patients was compared with the allele frequency in a (historical) reference group consisting of 26 Dutch subjects (male, all 35 years of age<sup>22</sup>). The study was approved by the local ethics committee of Leiden University Medical Centre and informed consent was obtained from all patients.

In statistical analyses, responders, non-responders, and patients with recurrence of the headache were compared with each other. Patients with chest symptoms were compared with patients without chest symptoms. Based on the Hardy-Weinberg equilibrium, allele frequencies of different groups were compared by Chi-square analysis. For differences in allele frequencies between groups, 95% confidence intervals were calculated, followed by a post hoc power analysis to check whether

sufficient patients were investigated. *P* values <0.05 were considered to indicate significant differences.

#### *5-HT<sub>1B</sub> receptor gene polymorphisms*

##### *PCR and HincII RFLP nt 861*

Genomic DNA was isolated according to the method of Miller *et al.*<sup>23</sup>. PCR was carried out in a 30 ml volume containing 60 mM Tris-HCl (pH=8.5), 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.5 mM MgCl<sub>2</sub>, 10% DMSO, 100 ng genomic DNA, 200 mM of each dNTP, 15 pmol of the forward primer 5-HT1B5 (5'-GAAACAGACGCCCAACAGGAC-3'), 15 pmol of the reverse primer 5-HT1B6 (5'-CCAGAAACCGCGAAAGAAGAT-3'), and 0.25 U Supertrooper Taq polymerase (HT Biotechnology Ltd.). PCR was carried out in a PCR-100<sup>TM</sup> apparatus (MJ Research Inc.). Following an initial denaturation of 5 min at 94°C, samples were amplified for 35 cycles, each consisting of 45 sec at 94°C, 45 sec at 62°C, and 90 sec at 72°C. After a final extension step of 10 min at 72°C, a 548 bp fragment was obtained. 5 ml of PCR product was incubated with 3 U of the restriction enzyme *HincII* (Pharmacia) in 1x One-Phor-All buffer (Pharmacia) at 37°C. For alleles of the wild type (G at nt 861), digestion of the 5-HT1B5/5-HT1B6 fragment (548 bp) with *HincII* resulted in two fragments of 452 bp and 96 bp. The G6C substitution at nt 861 resulted in an additional restriction site, splitting the 452 bp fragment into 309 bp and 143 bp fragments. The digested PCR products were separated on a 3% agarose gel.

##### *PCR and BsmalI RFLP nt -261*

PCR was carried out in a 30 ml volume containing 60 mM Tris-HCl (pH=9.5), 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgCl<sub>2</sub>, 10% DMSO, 100 ng genomic DNA, 200 mM of each dNTP, 15 pmol of the forward primer 1DB2-F (5'-GCTCAGCCTCAAGCAACTG-3'), 15 pmol of the reverse primer 1DB2-RM (5'-CTCTCCACACCGGGTCTGAG-3'), and 1.2 U AmpliTaq DNA polymerase (Perkin Elmer). PCR was carried out in a PCR-100<sup>TM</sup> apparatus (MJ Research Inc.). After an initial denaturation of 5 min at 94°C, samples were amplified for 40 cycles, each consisting of 30 sec at 94°C, 30 sec at 57°C, and 30 sec at 72°C. This was followed by a final extension step of 10 min at

72°C. A 162 bp fragment was obtained. 5 ml of PCR product was incubated with 5 U of the restriction enzyme *Bsm*I (Biolabs) in NEBuffer 3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 1 mM DTT, pH=7.9; Biolabs) containing 100 mg/ml BSA, at 55°C. The wild type 1DB2-F/1DB2-RM fragment (162 bp, T at nt -261) is not cut by *Bsm*I. However, in alleles with the T6G substitution at nt -261 of the 5-HT<sub>1B</sub> receptor gene, a restriction site for *Bsm*I is created, resulting in two fragments of 146 bp and 16 bp. The digested PCR products were separated on a 8% polyacryl amide gel.

### **11.3 Results**

#### *Patient characteristics*

Patient groups did not differ significantly in age and gender distribution (Table 11.1), nor in age of onset of migraine (mean±s.e.mean: 21±12 years), duration of migraine history (26±2 years), and attack frequency (2.7±0.3 attacks/month). The number of attacks treated with s.c. sumatriptan was lower in non-responders than in responders and patients with recurrence of the headache (6±1 vs. 72±21 and 67±26, resp.;  $P<0.001$ ), and tended to be lower in patients with chest symptoms (36±15 vs. 54±15 for patients without chest symptoms). The mean number of attacks treated with s.c. sumatriptan was 48±11 for all patients.

#### *5-HT<sub>1B</sub> receptor gene polymorphisms*

##### *HincII RFLP nt 861*

Allele frequencies of the patients and reference group are listed in Table 11.2. Genotypes of all patient groups were in Hardy-Weinberg equilibrium. No significant differences in allele frequencies were observed between the different groups. The mean allele frequency of all patients (G: 0.74; C:0.26) and reference subjects (G: 0.69; C:0.31) did not differ from that found by Lappalainen *et al.*<sup>19</sup> in Finnish and American subjects.

*BsmI* RFLP nt -261

Allele frequencies of the patients and reference subjects are listed in Table 11.2. In all groups, genotypes were in Hardy-Weinberg equilibrium. Allele frequencies did not differ between responders, patients with recurrence, non-responders, patients with or without chest symptoms, and reference subjects. The allele frequencies of patients with chest symptoms (T: 0.27; G: 0.73) and reference subjects (T:0.35; G:0.65) were different from the allele frequency found by Nöthen *et al.*<sup>20</sup> (T:0.51; G:0.49;  $\chi^2=7.97$ ,  $P=0.019$  for patients with chest symptoms, and  $\chi^2=6.61$ ,  $P=0.037$  for reference subjects).

**Table 11.2** Allele frequencies for the *HincII* RFLP (G→C substitution at nt 861) and the *BsmI* RFLP (T→G substitution at nt -261) of patients with different clinical responses to sumatriptan.

	G861C		T-261G	
	G	C	T	G
Responders	0.79	0.21	0.46	0.54
Recurrence	0.75	0.25	0.38	0.63
Non-responders	0.71	0.29	0.38	0.63
Chest symptoms	0.81	0.19	0.27	0.73
No chest symptoms	0.70	0.30	0.44	0.56
Total (all patients)*	0.74	0.26	0.39	0.61
Reference subjects	0.69	0.31	0.35	0.65

\* Most patients were included in several (non mutually excluding) groups, e.g. patients in the group with chest symptoms could also be included in the groups with responders.

*Power of the study*

For both polymorphisms, 95% confidence intervals (c.i.) were calculated for the difference in allele frequencies between patient groups. For the G861C polymorphism, allele frequency differences between groups, derived from the data

listed in Table 11.2, ranged from 3% (c.i. -20 to 27%) up to 10% (c.i. -9 to 30%). For the T-261G polymorphism, allele frequency differences ranged from 0% (c.i. -27 to 27%) up to 18% (c.i. -4 to 40%). A post hoc power analysis showed that, for both polymorphisms, our patient groups were sufficiently large to detect an allele frequency difference of 30% ( $\beta=0.80$ ), which we consider to be a clinically relevant difference.

#### **11.4 Discussion**

In the present study, we investigated whether differences in the clinical response to s.c. sumatriptan were associated with different polymorphisms of the 5-HT<sub>1B</sub> receptor gene. We studied association with two recently described common polymorphisms, which do not change the amino acid sequence of the 5-HT<sub>1B</sub> receptor, but are located in the non-coding domain of the receptor gene<sup>20</sup>, or are silent substitutions, i.e. encoding the same amino acid as in the wild type<sup>19</sup>. Such polymorphisms may affect translation through the secondary structure or stability of the mRNA, or may serve as associated markers through linkage disequilibrium with other functional mutations, which affect drug-receptor interaction. Indeed, silent receptor polymorphisms have been shown to be associated with altered drug response or disease. For example, the T102C polymorphism in the 5-HT<sub>2A</sub> receptor was more frequent among patients not responding to the antidepressant clozapine<sup>14</sup>. Similarly, the A1166C genotype in the angiotensin II type 1 receptor seems to be associated with hypertension<sup>24</sup> and greater coronary vasoconstriction<sup>25</sup>.

In the present study, allele frequencies of the G861C and T-261G polymorphisms were not different in the five patient groups, suggesting that mutations in the 5-HT<sub>1B</sub> receptor gene do not play a pivotal role in the clinical response to sumatriptan. Although our patients were clinically well defined, it is possible that we falsely missed differences due to the relatively small number of patients in the different groups. However, in view of the confidence intervals and the power analysis, we feel that if one of the polymorphisms were to play a major role in

determining the clinical response to sumatriptan, this should have been revealed in our study.

For the *Bsm*I RFLP at nt -261, the allele frequencies of the patients with chest symptoms and the reference subjects differed from those in the study population of Nöthen and colleagues<sup>20</sup>, which consisted of healthy unrelated, probably German subjects. Since the allele frequency of the reference subjects fitted perfectly with the frequency found in our patients, the discrepancies in allele frequencies are most likely due to slight ethnic differences between the different study populations. Allele frequencies of the population used by Lappalainen *et al.*<sup>19</sup>, whose population consisted of healthy and alcoholic Finnish and American subjects, did not differ from the allele frequencies in the present study.

Besides the 5-HT<sub>1B</sub> receptor, 5-HT<sub>1D</sub> and 5-HT<sub>1F</sub> receptors may also be present in human cerebral arteries, and could therefore be involved in the clinical response to sumatriptan. Alternatively, the therapeutic effect of sumatriptan may be mediated by mechanisms other than vasoconstriction, leaving open the possibility for involvement of receptor subtypes other than the 5-HT<sub>1B</sub> receptor. Finally, sporadic mutations in the 5-HT<sub>1B</sub> receptor gene may not have been detected in the present study. However, in view of the fact that non-response, recurrence of the headache, or chest symptoms after sumatriptan occur in a considerable proportion of patients, it is unlikely that these responses are associated with a rare mutation in the majority of patients. In conclusion, our results indicate that genetic diversity of the 5-HT<sub>1B</sub> receptor is not involved in different clinical responses to sumatriptan.

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## Chapter 12

### **Chromosomal Localisation of the 5-HT<sub>1F</sub> Receptor Gene: No Evidence for Involvement in Response to Sumatriptan in Migraine Patients**

**Summary** - The 5-HT<sub>1F</sub> receptor, which is present in both human vascular and neuronal tissue, may mediate the therapeutic effect and/or side effects of sumatriptan. We investigated the chromosomal localisation of the 5-HT<sub>1F</sub> receptor gene and the relation between eventually existing polymorphisms and the clinical response to sumatriptan in migraine patients. The 5-HT<sub>1F</sub> receptor gene was localised using a monochromosomal mapping panel, followed by a radiation reduced hybrid mapping and fluorescent in situ hybridisation. The results of these techniques show that the 5-HT<sub>1F</sub> receptor gene is localised on chromosome 3p12. We investigated the presence of polymorphisms by single strand conformation polymorphism (SSCP) analysis in 14 migraine patients who consistently responded well to sumatriptan, 12 patients who consistently experienced recurrence of the headache after initial relief, 12 patients with no response to sumatriptan, and in 13 patients who consistently experienced chest symptoms after the use of sumatriptan. No polymorphisms were detected in any of the patients. We therefore conclude that genetic diversity of the 5-HT<sub>1F</sub> receptor gene is most probably not responsible for the variable clinical response to sumatriptan.

Based on: MaassenVanDenBrink A, Vergouwe MN, Ophoff RA, Naylor SL, Dauwse HG, Saxena PR, Ferrari MD, Frants RR. Chromosomal localization of the 5-HT<sub>1F</sub> receptor gene: no evidence for involvement in response to sumatriptan in migraine patients. *Am J Med Genetics*. 1998;77:415-420.

## **12.1 Introduction**

Migraine is a common paroxysmal neurological disorder, which consists of attacks with moderate to severe headache, associated with nausea, vomiting, photo- and phonophobia<sup>1</sup>. Sumatriptan, a 5-HT<sub>1</sub> (5-HT=5-hydroxytryptamine=serotonin) receptor agonist, is highly effective in the acute treatment of migraine attacks<sup>2-4</sup>. However, up to 15% of patients consistently do not respond to subcutaneous sumatriptan, and up to 40% of responders consistently experience recurrence of the headache within 24 hours after initial headache relief<sup>4-7</sup>. In addition, about 40% of patients always experience one or more chest symptoms, including chest pressure or chest pain, heaviness in arms and shortness of breath, shortly after the use of subcutaneous sumatriptan<sup>7,8</sup>.

Comparing clinical, demographic and pharmacokinetic characteristics of consistent responders and non-responders, as well as of patients who always experience headache recurrence or chest symptoms and those who never do so, yielded only few and relatively insignificant differences. For example, patients with chest symptoms were slightly younger than patients without chest symptoms, and more often female than male. Also, headache recurrence was more frequent in patients with more severe attacks and longer untreated attack duration<sup>7,9-11</sup>. Although it was not confirmed by the study mentioned above<sup>8</sup>, others suggested determinants such as young age, hypertension, general complaints of abdominal pain, and a family history of myocardial infarction for the development of chest symptoms<sup>12</sup>.

Besides demographic, clinical, and pharmacokinetic characteristics, genetic factors may contribute to inter-patient differences in clinical responses to drugs or hormones<sup>13-15</sup>. For sumatriptan, differences in clinical responses may well be explained by different alleles of the 5-HT<sub>1</sub> receptor subtypes, the presumed clinically relevant pharmacological target of sumatriptan<sup>16</sup>. Sumatriptan displays high affinity for the 5-HT<sub>1B</sub> (former 5-HT<sub>1DB</sub>), 5-HT<sub>1D</sub> (former 5-HT<sub>1D $\alpha$</sub> ) and, although tenfold less, for the 5-HT<sub>1F</sub> receptor<sup>16,17</sup>. Recently, we have shown that genetic variation in the gene encoding the 5-HT<sub>1B</sub> receptor, one of the receptors possibly mediating the therapeutic action and coronary side-effects of sumatriptan, is not associated with the

variation in the clinical effects of this drug<sup>18</sup>. Another candidate receptor for the mediation of the clinical effects of sumatriptan is the 5-HT<sub>1F</sub> receptor<sup>19</sup>. This receptor is present in human neuronal as well as vascular tissue<sup>20</sup>, which are both considered to be a possible target for sumatriptan<sup>21</sup>. Presently, a selective 5-HT<sub>1F</sub> receptor agonist is under investigation as a potential anti-migraine compound<sup>22</sup>. On the other hand, the compound alniditan, which is effective in the acute treatment of migraine attacks<sup>23</sup>, displays only a low affinity for the 5-HT<sub>1F</sub> receptor<sup>24</sup>, arguing against the fact that the 5-HT<sub>1F</sub> receptor necessarily should be involved in the therapeutic action of anti-migraine drugs.

The intronless 5-HT<sub>1F</sub> receptor gene encodes for a receptor protein consisting of 366 amino acids, containing seven membrane-spanning domains<sup>19</sup>. The chromosomal localisation of the 5-HT<sub>1F</sub> receptor gene has not been reported until now. The 5-HT<sub>1F</sub> receptor gene was screened for mutations in a population consisting of schizophrenic patients, bipolar patients, and healthy controls<sup>25</sup>. The gene contains three rare sequence variants, namely a silent A→T transversion at bp 785 (third position of codon 261), a silent C→T transition at bp 530 (third position codon 176), and a C→T transition in position -78 upstream from the start codon. The frequency of these sequence variants ranged from 1 to 4%<sup>25</sup>. These sequence variants were not related to the presence of the diseases<sup>25</sup>. Nevertheless, it is feasible that sequence variants of the 5-HT<sub>1F</sub> receptor gene are involved in the clinical response to drugs such as sumatriptan.

In the present study, we established the chromosomal localisation of the 5-HT<sub>1F</sub> receptor gene. Furthermore, we investigated the presence of mutations in the 5-HT<sub>1F</sub> receptor gene in subgroups of migraine patients who display different clinical responses (improvement - no improvement; recurrence - no recurrence; chest symptoms - no chest symptoms) to sumatriptan.

## **12.2 Patients and methods**

### *Patients and statistical analysis*

In the present study, we included forty unrelated migraine patients (35 female, 5 male; age 20-69 years), all fulfilling the criteria of the International Headache Society<sup>1</sup>, from the out-patient clinic database of the Department of Neurology of the Leiden University Medical Centre. Patients were divided into five groups according to their clinical response to 6 mg subcutaneous (s.c.) sumatriptan: *i*) Responders were defined as patients who had headache relief within 2 hours after sumatriptan in at least 4 out of 5 migraine attacks and who experienced headache recurrence within 24 hours in less than 1 out of 5 successfully treated attacks. *ii*) Patients with headache recurrence responded to s.c. sumatriptan in at least 4 out of 5 migraine attacks, followed by recurrence of the headache within 24 hours in at least 4 out of 5 successfully treated attacks. *iii*) Non-responders were defined as patients who had headache relief in not more than one out of 5 migraine attacks treated with s.c. sumatriptan, or in none of 3 consecutively treated migraine attacks. *iv*) Patients with chest symptoms had treated at least 3 migraine attacks with s.c. sumatriptan and had experienced one or more chest symptoms in each of these attacks. The use of the minimal number of 3 rather than 5 attacks in the latter groups emerged from practical reasons: patients with severe adverse events or no response usually refrained from continuing sumatriptan use. *v*) Patients without chest symptoms, derived from the other groups, were compared with patients with chest symptoms. Clinical and demographic data of the patients are listed in Table 12.1.

### *Chromosomal localisation of the 5-HT<sub>1F</sub> receptor gene; PCR mapping*

A PCR reaction amplifying the whole coding sequence of the 5-HT<sub>1F</sub> receptor gene was performed on a human monochromosomal mapping panel (UK HGMP Resource Centre) and on a panel of somatic cell hybrids previously defined<sup>26,27</sup>. The monochromosomal panel and the somatic cell hybrids were used as a template for a PCR reaction using the primers 5HT<sub>1F</sub>1F and 5HT<sub>1F</sub>4R (Table 12.2) resulting in a 1.3 kb fragment. The PCR was performed in a total volume of 30 µl containing

**Table 12.1** Demographic data of the study groups.

	<i>n</i>	Sex (M/F)	Mean age (range)
Responders	14	1M, 13 F	49 (23-69)
Recurrence	12	1M, 11F	50 (34-66)
Non-responders	12	3M, 9F	44 (20-60)
Chest symptoms	13	0M, 13F	48 (23-59)
No chest symptoms	27	5M, 22F	47 (20-69)
Total (all patients)*	40	5M, 35F	47 (20-69)

\* Most patients were included in several (non mutually excluding) groups, e.g. patients in the group with chest symptoms could also be included in the groups with responders. M and F refer to male and female, respectively; age is expressed in years.

15 pmol of each primer, 1 U of AmpliTaq (Perkin Elmer Cetus), 0.2 mM of each dNTP and a reaction buffer with 60 mM Tris-HCl, 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgCl<sub>2</sub>, pH 10 (30 sec 94°C, 30 sec 59°C and 1 min 72°C for 33 cycles).

#### *Chromosomal localisation of the 5-HT<sub>1F</sub> receptor gene; FISH mapping*

The 1.3 kb PCR fragment (5HT<sub>1F</sub>1F and 5HT<sub>1F</sub>4R), cloned into the pCR<sup>TM</sup> vector (Invitrogen) and the chromosome specific library, pBS3<sup>28</sup> (kindly provided by Dr. J.W. Gray), were labelled by nick translation<sup>29</sup> with biotin-11-dATP (Gibco BRL) and digoxigenin-11-dUTP, respectively. The probes were simultaneously hybridised to metaphase chromosomes from normal human lymphocytes and visualised as previously described<sup>30</sup>. Slides were analysed on a Leitz DM-RBE microscope equipped for fluorescence microscopy and mounted with a Photometrics Series 200, KAF1400 CCD camera. Image acquisition and processing was performed on a Power Macintosh 7100, using the IP Lab Spectrum Multiprobe software.

#### *Mutation analysis; polymerase chain reaction (PCR)*

A primary PCR was performed in a reaction volume of 30 µl containing 15 pmol of each primer, 1 U of AmpliTaq (Perkin Elmer Cetus), 0.2 mM of each dNTP and a reaction buffer with 60 mM Tris-HCl, 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.5 mM MgCl<sub>2</sub>, pH 8.5;

### *5-HT<sub>1F</sub> receptor and clinical response to sumatriptan*

100 ng genomic DNA was subjected to 34 cycles of amplification (30 sec 94°C, 30 sec 60°C and 30 sec 72°C). Four sets of primers were chosen to produce four overlapping fragments (354 - 414 bp) covering the whole coding region of the 5-HT<sub>1F</sub> receptor gene (Table 12.2). Primary PCR products were radioactively labelled by a second PCR performed in a 15 µl reaction volume containing 1 µl primary PCR product, 7.5 pmol of each primer, 0.2 mM dTTP, dGTP, dATP, 0.002 mM dCTP and 0.7 µCi [ $\alpha$ -<sup>32</sup>P]-dCTP (3000 Ci/mmol; Amersham), 0.5 U AmpliTaq (Perkin Elmer Cetus), 10% DMSO and buffer as for the primary PCR. Denaturation was performed at 94°C for 45 sec, annealing at 62°C for 45 sec, followed by elongation at 72°C for 1 min and 30 sec for a total of 13 cycles.

#### *Mutation analysis; single-strand conformation polymorphism (SSCP) analysis*

Subsequent to the secondary PCR, the four fragments were subjected to restriction enzyme digestions to yield shorter fragments ( $\leq$ 300 bp) suitable for SSCP analysis. Digestion was performed by adding 5 µl mixture containing a specific buffer and 3 U restriction enzyme, directly to the secondary PCR product (15 µl) for 3 hours at a temperature optimal for the restriction enzyme (Table 12.2).

The digested fragments were diluted 1 to 15 in loading buffer (47% formamide, 0.05% SDS, 15 mM EDTA, 0.005% bromophenol blue and 0.005% xylene cyanol) and denatured at 94°C for 5 min. 3 µl of the denatured DNA was loaded per lane on a 8% polyacrylamide gel with 10% glycerol and 1xTBE. Electrophoresis was carried out for 7 hours at 27 W constant power at room temperature. Gels were dried and exposed to X-ray film (Kodak X-AR).

**Table 12.2** Primer sequences, fragment sizes and used restriction enzymes.

Primer	Primer sequence	Fragment size (bp)	Nucleotide position (5'-3')*	Restriction enzyme	Fragment size after digestion (bp)
5-HT <sub>1F</sub> 1F	5'-AAAACCTTCAATCTGAACCTCA-3'	358	-186-172	<i>Bst</i> XI	99, 259
5-HT <sub>1F</sub> 1R	5'-GCTGGATGGTGCAGCTTCCG-3'				
5-HT <sub>1F</sub> 2F	5'-TCGCTGCAATTATTGTGACC-3'	414	134-547	<i>Hinf</i> I	142, 272
5-HT <sub>1F</sub> 2R	5'-TGGTGGAAACAATGTGGTTCG-3'				
5-HT <sub>1F</sub> 3F	5'-TCCTCTATTCTGGAGGCACC-3'	354	471-825	<i>Rsa</i> I	86, 286
5-HT <sub>1F</sub> 3R	5'-CTCATGCTTGAATTCAGACC-3'				
5-HT <sub>1F</sub> 4F	5'-GCACAGTGAGAAGTCTCAGG-3'	366	788-1153	<i>Rsa</i> I	69, 297
5-HT <sub>1F</sub> 4R	5'-GTTATTCCTCCCCTCAAAAACC-3'				

\* Numbering of nucleotide position starts with the first nucleotide of the initiating methionine codon (+1).

## 12.3 Results

### *Localisation of the 5-HT<sub>1F</sub> receptor gene; PCR mapping*

Based on the PCR with the human monochromosomal mapping panel, the 5-HT<sub>1F</sub> receptor gene was found to be localised on chromosome 3 (results not shown). Further investigation was performed using a radiation reduced hybrid mapping panel containing fragments of chromosome 3. One of the cell lines in this panel, GM11750, resulted in a discordant, negative PCR result (Figure 12.1). We have, however, occasionally detected deletions in this cell line before, and considering the results of the other cell lines, the gene should be localised on the short arm of chromosome 3, region H (region 3p14.1 to 3p12), which is located close to the centromere (Figure 12.1, for detailed description of regions, see Leach *et al.*<sup>26</sup>).

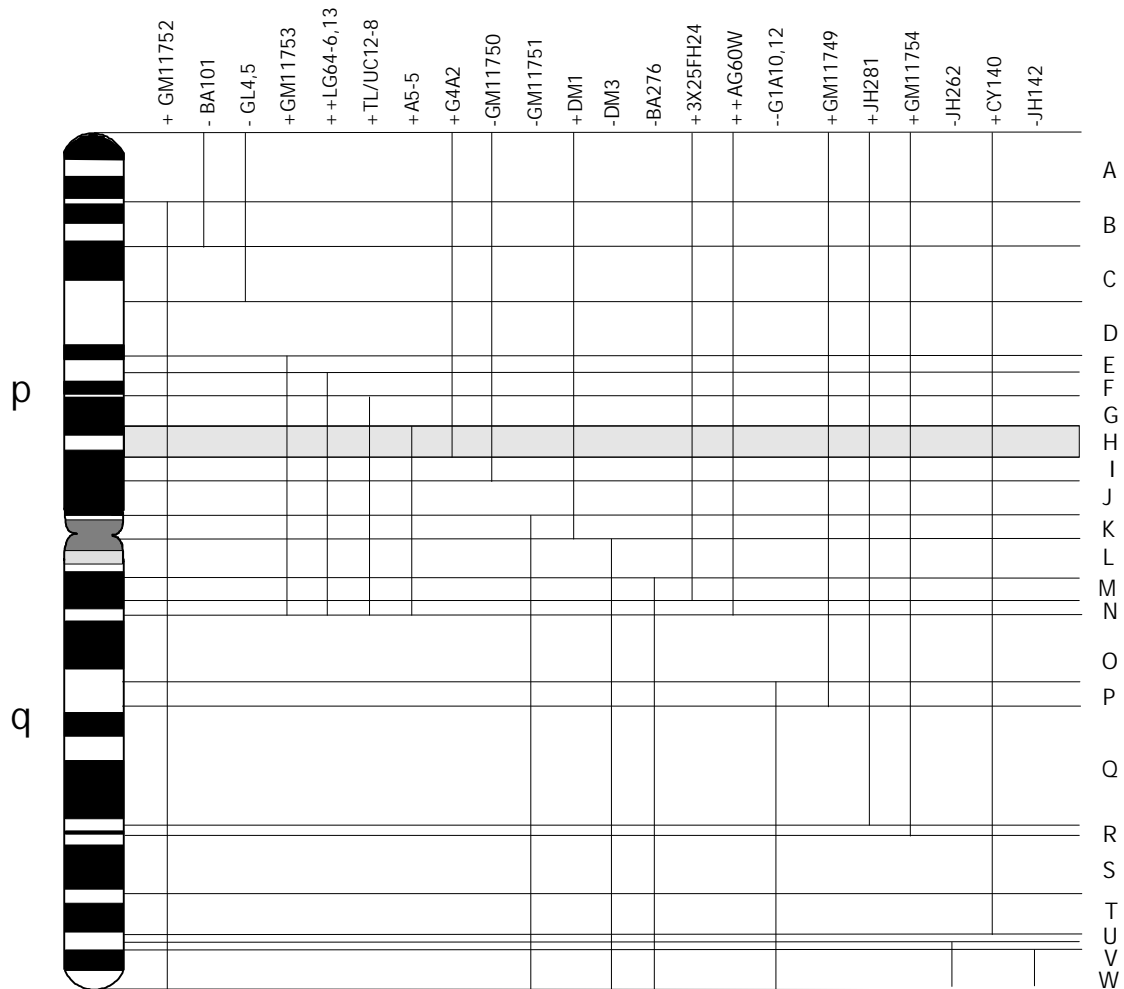
### *Localisation of the 5-HT<sub>1F</sub> receptor gene; FISH mapping*

The 1.3 kb fragment (5HT<sub>1F</sub>1F - 5HT<sub>1F</sub>4R) was hybridised *in situ* to human metaphase chromosomes. Analysis of chromosomes with a positive hybridisation signal showed the fragment of the 5-HT<sub>1F</sub> receptor gene to be localised on region p12.1 of chromosome 3 (Figure 12.2).

### *Mutation analysis; single-strand conformation polymorphism (SSCP) analysis*

The coding region of the gene was screened for sequence variation by SSCP. To achieve high sensitivity, the four overlapping fragments were further reduced in size by restriction enzyme digestion prior to SSCP. In all eight DNA fragments, no polymorphism was observed neither in any of the migraine groups nor in the control samples.





**Figure 12.1** Map of chromosome 3, obtained after a PCR covering the whole coding region of the 5-HT<sub>1F</sub> receptor gene on a panel of somatic cell hybrids. The fragments of the hybrids are represented by the vertical lines. Positive PCRs are indicated by plus signs, negative PCRs with minus signs preceding the hybrid names. From the above results, it is concluded that the 5-HT<sub>1F</sub> receptor gene is located in region H, corresponding to region 3p14.1 to 3p12. For details about this method, see Leach et al.<sup>26</sup>.

**Figure 12.2** Metaphase chromosome spread from human lymphocytes simultaneously hybridised with a 1.3-kb fragment covering the whole coding region of the 5-HT<sub>1F</sub> receptor gene labelled with digoxigenin and visualised with FITC (green) and a chromosome 3 specific library labelled with biotin and visualised with Texas Red (red), showing hybridisation signals on both chromosomes 3, on the long arms, close to the centromere. Chromosomes were counterstained with DAPI (blue). More precise localisation was obtained on extended chromosomes and by making use of DAPI banding (shown on extended chromosome, bottom right hand corner) to 3p12.1.

## **12.4 Discussion**

In the present study, we investigated the chromosomal localisation and the presence of polymorphisms of the 5-HT<sub>1F</sub> receptor gene. The precise physiological role of the 5-HT<sub>1F</sub> receptor is not known yet, but it is conceivable that this receptor, which is present both in human neuronal and vascular tissue<sup>20</sup>, is involved in the pathophysiology or therapeutics of migraine. The therapeutic action and the coronary side-effects of the anti-migraine drug sumatriptan could also be mediated via the

5-HT<sub>1F</sub> receptor<sup>22</sup>; sumatriptan has a high affinity for the 5-HT<sub>1F</sub> receptor<sup>19</sup>, and has been shown to bind to putative 5-HT<sub>1F</sub> receptor binding sites<sup>31</sup>.

Our results show that the 5-HT<sub>1F</sub> receptor gene is located on chromosome 3p12. We defined this localisation using three different approaches, which resulted in concordant conclusions. The coding region of the 5-HT<sub>1F</sub> receptor was screened by SSCP analysis. The sensitivity of this method is not fully 100%<sup>32</sup>, but this limitation was partially overcome by the use of overlapping PCR fragments, increasing the SSCP sensitivity. Nevertheless, we did not show polymorphisms among the groups of migraine patients with different clinical responses to sumatriptan, arguing against genetic diversity of the 5-HT<sub>1F</sub> receptor gene as a major determinant of the clinical response to sumatriptan. Admittedly, our relatively small number of subjects could have allowed the negligence of sporadic mutations like those reported by Shimron-Abarbanell et al.<sup>25</sup>, which had a frequency of 1 to 4%. However, taking into account that we did not detect any mutation in any of the patients in our study, it is highly unlikely that rare mutations were to play a pivotal role in the clinical response to sumatriptan. Although genetic variation surrounding the coding region of the 5-HT<sub>1F</sub> receptor gene such as promotor or enhancer sequences may be associated with the clinical response to sumatriptan via regulation of expression of the 5-HT<sub>1F</sub> receptor, we think, in conclusion, that genetic diversity of the 5-HT<sub>1F</sub> receptor is not responsible for the variable clinical response to sumatriptan.

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# Chapter 13

## General Discussion

### 13.1 Human isolated coronary artery contraction to antimigraine drugs

As discussed in this thesis, antimigraine drugs such as ergotamine, dihydroergotamine and sumatriptan cause cardiac side effects. In view of these side effects, it is important to characterise the contraction of the human coronary artery to current and potential antimigraine drugs. All currently available antimigraine drugs for the acute treatment of migraine attacks contract the human isolated coronary artery. We demonstrated that ergotamine, dihydroergotamine and the prophylactic drug methysergide as well as its metabolite methylergometrine contract the human isolated coronary artery<sup>1</sup>. In addition, we observed contraction induced by the 5-HT<sub>1B/1D</sub> receptor agonists sumatriptan<sup>1</sup>, naratriptan<sup>1</sup>, zolmitriptan<sup>1</sup>, rizatriptan<sup>1</sup>, avitriptan<sup>1</sup>, eletriptan<sup>2</sup>, BMS181885<sup>3</sup>, GMC2021<sup>4</sup> and S20749<sup>5</sup>. Others have studied human isolated coronary artery contraction to rizatriptan<sup>6,7</sup> and frovatriptan<sup>8</sup>.

Obviously, it is of interest to compare the contraction to these compounds as observed in the different studies<sup>1-8</sup> in a quantitative manner. Unfortunately, there are several factors hampering a straightforward comparison of the contraction induced by these compounds in various studies.

#### *Difficulties in interpreting data on human isolated coronary artery contraction to antimigraine drugs obtained from various in vitro studies*

When comparing coronary artery contraction induced by antimigraine drugs in different studies, one should be aware of the following complicating factors. Firstly, the heart donors and, consequently, the endothelial quality of coronary arteries vary in different studies. The hearts used in the experiments described in this thesis were all

obtained from heart beating organ donors who died of non-cardiac causes. No attempt was made to remove the endothelium from the vessel. These hearts most probably better reflect hearts from patients using antimigraine medication than hearts obtained from patients undergoing heart transplantation, which were used by others<sup>6-8</sup>. Indeed, in the vast majority of patients undergoing heart transplantation these drugs would have been contraindicated because of coronary artery disease. In addition, in some studies the endothelium was removed from the coronary artery<sup>6-8</sup>.

Secondly, a mere methodological problem is that contraction in the various studies is expressed in different manners (as percentage of contraction to 100 mM<sup>1</sup> or 45 mM<sup>6,7</sup> K<sup>+</sup> or to 10 μM 5-HT in the presence of 1 μM ketanserin<sup>8</sup>). Unfortunately, the authors do not always report the absolute value of the contractions expressed in mN, which would have facilitated a comparison of data expressed in different manners.

Finally, and perhaps most importantly, contraction of the human isolated coronary artery to sumatriptan shows a high degree of variability<sup>9,10</sup>. Whereas in some studies sumatriptan hardly induced a contraction<sup>11,12</sup>, other studies report a contraction to sumatriptan that is clearly present<sup>6,8,13-15</sup>, and sometimes is even of a similar magnitude as that to 5-HT<sup>9</sup>. Also in the studies described in this thesis, which were all performed on the same type of tissue (coronary arteries obtained from heart beating organ donors) following the same protocol, the mean contraction to sumatriptan varied from 12-28 % of contraction to 100 mM K<sup>+</sup>. Contraction in individual segments had a much wider range (2-125 % of contraction to 100 mM K<sup>+</sup>). Just the type of heart used or the quality of the endothelium (Chapter 9) can not explain the variability of contractile responses to sumatriptan. As described in Chapter 8, one of the factors contributing to this variability is endogenous production of a thromboxane A<sub>2</sub>-like substance by the coronary artery. However, also in studies where the cyclo-oxygenase inhibitor indomethacin, which inhibits the synthesis of thromboxane A<sub>2</sub>, was added to the buffer solution, the response to sumatriptan was highly variable<sup>8</sup>.



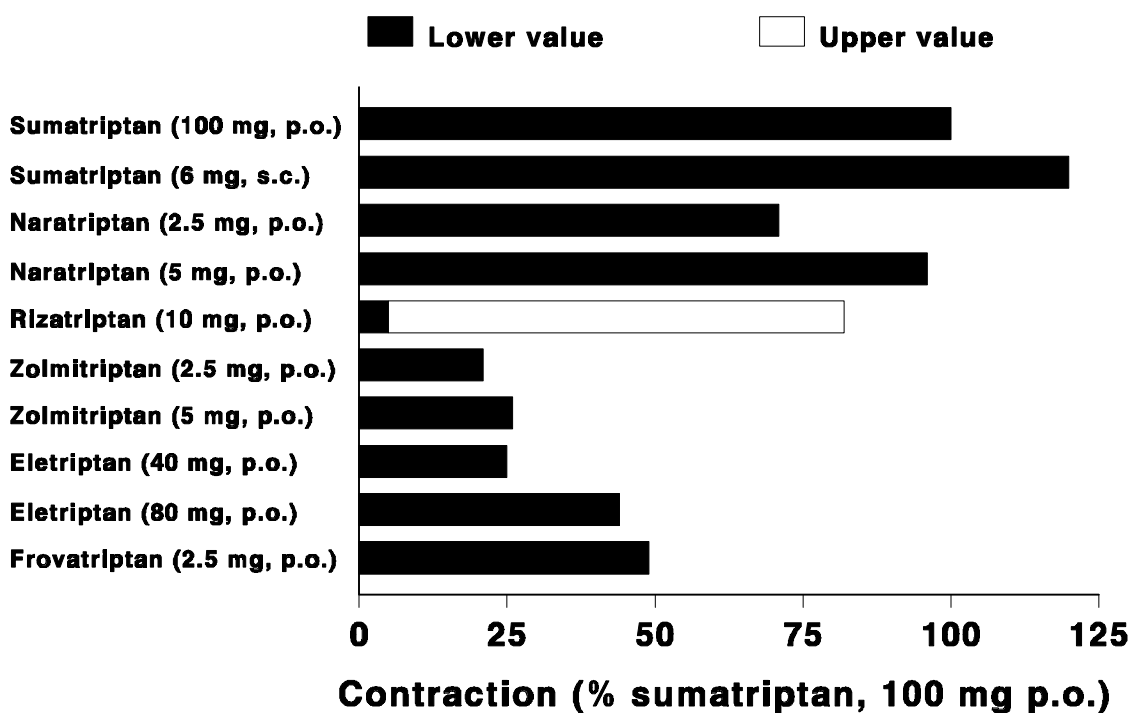
The factors described above imply that data on contraction to novel antimigraine compounds are of limited value without comparison to the 'golden standard' sumatriptan in *paired* experiments in adjacent segments of the same artery.

#### *Coronary artery contraction to novel triptans compared to sumatriptan*

Notwithstanding the difficulties in interpreting data from various studies, we attempted to compare human isolated coronary artery contraction to a number of 5-HT<sub>1B/1D</sub> agonists, which are already marketed or are being evaluated in clinical trials. Coronary artery contraction at therapeutic maximum plasma concentrations ( $C_{\max}$ ) was calculated by interpolation of the concentration response curves using a sigmoidal function. We corrected  $C_{\max}$  values for the fraction of plasma protein bound drug because the fraction of drugs that is bound to plasma proteins only serves as a reservoir and is pharmacologically inactive<sup>16-18</sup>. Contractions were expressed as percentage of the predicted contraction to sumatriptan (100 mg p.o.) in the respective study (Table 13.1, Figure 13.1). All compounds are predicted to contract the human isolated coronary artery following administration of a clinically effective dose. Contraction induced by zolmitriptan, eletriptan and, possibly, rizatriptan and frovatriptan, may be somewhat lower than that of sumatriptan. However, for zolmitriptan and rizatriptan additional contraction may be induced by the pharmacologically active *N*-desmethyl metabolites<sup>19,20</sup>. In conclusion, human coronary artery contraction elicited by the novel triptans will be similar to or marginally smaller than that elicited by sumatriptan. Probably, the same will hold true for other 5-HT<sub>1B/1D</sub> receptor agonists that have not yet been investigated in the human isolated coronary artery.

In addition to the contraction induced by the drugs *per se*, contraction to sumatriptan, and most probably also to the other triptans, may be augmented by the presence of other compounds. As pointed out in Chapter 8, contraction to sumatriptan may be augmented by thromboxane A<sub>2</sub> that is endogenously produced by the human coronary artery<sup>10</sup>. However, also other compounds, such as phenylephrine, norepinephrine, histamine, angiotensin II and prostaglandin F<sub>2 $\alpha$</sub>  have been described to augment contraction to sumatriptan in blood vessels from various species<sup>21</sup>.

As mentioned in Chapter 2, zolmitriptan<sup>22</sup> and eletriptan<sup>23</sup> behave as partial agonists relative to sumatriptan in rabbit<sup>22</sup> and canine<sup>23</sup> saphenous vein, which may theoretically lead to reduced coronary artery contraction<sup>24</sup>. Since these agonists display a maximal contractile effect similar to that of sumatriptan in the human isolated coronary artery (Chapters 5 and 6), these compounds are unlikely to have a reduced coronary side-effect burden compared to sumatriptan based on partial agonistic properties. In conclusion, differences in coronary artery contraction induced by various triptans are likely to be related to different doses of these drugs, rather than to different pharmacodynamic properties.



**Figure 13.1** Predicted contraction of the human isolated coronary artery at therapeutic free  $C_{max}$ , i.e. at the protein-unbound fraction of the maximum therapeutic plasmaconcentration, expressed as percentage of contraction elicited after 100 mg sumatriptan p.o. (100%). Closed bars represent the lower value, open bars represent the upper value. Contraction to sumatriptan, obtained as a reference, was always derived from the same study as the respective compound (for sumatriptan s.c., naratriptan and zolmitriptan:<sup>1</sup>; rizatriptan:<sup>1,6,7</sup>; eletriptan:<sup>2</sup>; frovatriptan<sup>8</sup>). Because data from literature were used, no statistics were performed.

**Table 13.1**  $C_{\max}$  of sumatriptan, naratriptan, rizatriptan, zolmitriptan, eletriptan and frovatriptan following therapeutic doses, as well as their plasma protein binding values and free  $C_{\max}$ .

Compound	Dose, mode of administration	$C_{\max}$ (nM)	Plasma Protein Binding (%)	Free $C_{\max}$ (nM)
Sumatriptan	100 mg, p.o. <sup>25,26</sup>	142-183 <sup>25,26</sup>	14-21 <sup>27</sup>	112-157
	6 mg, s.c. <sup>25,26</sup>	244-261 <sup>25,26</sup>		193-224
Naratriptan	2.5, p.o.	38 <sup>28</sup>	20*	30
	5 mg, p.o.	71 <sup>28</sup>		57
Rizatriptan	10 mg, p.o.	74-93	14*	64-80
Zolmitriptan	2.5, p.o.	9 <sup>29,30</sup>	25*	7
	5 mg, p.o.	17 <sup>29,30</sup>		13
Eletriptan	40 mg, p.o.	213*	85*	32
	80 mg, p.o.	643 <sup>31</sup>		96
Frovatriptan	2.5 mg, p.o.	29	15†	25

\*A.D. MacHarg, personal communication

†P. Buchan, personal communication

### 13.2 Limitations of the model

This thesis, except for Chapters 11 and 12, deals with contraction of the human isolated coronary artery to antimigraine drugs. Experiments were performed in organ baths, where segments of coronary artery were suspended for isometric tension measurements. Extrapolation of results obtained from *in vitro* studies to the clinical situation obviously introduces uncertainties. Further, our model has some specific limitations.

Firstly, we investigated the right epicardial coronary artery in all studies. This artery is an appropriate choice for the study of coronary vasospasms, since these tend to occur in epicardial conduit arteries, converting it into a major resistance vessel

impeding myocardial blood flow<sup>32</sup>. Moreover, the right epicardial coronary artery is more frequently involved in coronary artery spasm than the left anterior descending artery, the circumflex artery or the main trunk<sup>33-35</sup>. However, it is of interest to study whether antimigraine drugs induce a similar contraction in distal coronary arteries as in proximal sections. Whereas the ketanserin-resistant coronary artery constriction to 5-HT has been suggested to be larger in distal parts of the coronary artery<sup>36</sup>, it was smaller in a study performed with the 5-HT<sub>1B/1D</sub> receptor agonist avitriptan<sup>37</sup>. Comparative *in vitro* studies on proximal and distal coronary arteries may shed more light on the distribution of 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors in these blood vessels.

A second limitation of our studies is that drugs in the organ bath set-up reach both intraluminal and extraluminal cells. The situation may be different *in vivo*, where only the intraluminal side of blood vessels is directly exposed to drugs. However, an important advantage of the experimental set-up used in the present experiments is that large number of coronary artery segments (up to sixteen obtained from one heart) may be studied in parallel. Experiments on perfused coronary artery segments may reveal whether intraluminal exposure to drugs differs from exposure to drugs on both intra- and extraluminal side as in our organ bath.

Finally, the hearts used by us were stored up to 24 hours before they reached our laboratory. Although storage was at a temperature of 0-4°C in a sterile, organ protecting solution (UW, Eurocollins or HTK-Bretschneider), we can not exclude that this storage may have damaged the tissue, or may have induced certain enzymes. Several arguments suggest that storage under the conditions described above does not harm the tissue to any great extent. 1. Endothelial function (quantified as relaxation to substance P) in our studies was similar to that in coronary arteries obtained from patients with cardiomyopathy which were studied directly after explantation of the heart<sup>38,39</sup>. 2. We mimicked the storage procedure followed with the human hearts by storing porcine hearts for 24 hours in the same way, as the human donor hearts were stored. After storage, the porcine coronary arteries displayed the same basic contractile properties and relaxation to substance P as coronary arteries investigated immediately after explantation of the heart<sup>40</sup>.

Despite the limitations mentioned above, experiments on the human isolated coronary artery provide the possibility for more detailed studies than would ever be possible in experiments performed on patients *in vivo*. Or, as S. Kalsner stated in one of his reviews<sup>41</sup>: 'The *in vitro* study of large epicardial coronary vessels is sure to be a rich and fruitful area for further study, and with reasonable hope, it may provide us with the solution to a major facet of cardiac disease'.

### 13.3 Implications for future research

#### *Extension and improvement of the model*

As described above, it would be of interest to compare the pharmacological properties of distal portions of coronary artery with those of the epicardial coronary artery, which has been studied in this thesis. Also, experiments on perfused coronary artery segments may reveal whether intraluminal exposure to antimigraine drugs differs from exposure to drugs on both intra- and extraluminal side as in an organ bath.

#### *Augmentation of contraction to antimigraine drugs by other compounds*

As described in this thesis, amplification of coronary artery contraction by endogenous mediators such as thromboxane A<sub>2</sub> may be important in the development of myocardial ischaemia in response to antimigraine drugs (Chapter 8). It seems obvious that contraction to other triptans will be augmented in a similar manner, because these compounds display affinity for the 5-HT<sub>1B/1D</sub> receptors (Chapter 1), which most probably mediated human coronary artery contraction to sumatriptan. However, other receptors may be involved in contraction to, e.g., eletriptan<sup>42</sup>. In addition, variability of contraction to sumatriptan was considerably larger than variability of contraction to eletriptan<sup>2</sup> or frovatriptan<sup>8</sup>, making it tempting to speculate that, possibly, augmentation of contraction is not similar for all triptans. It seems therefore justified to investigate augmentation of contraction to novel triptans by endogenous mediators such as thromboxane A<sub>2</sub>.

## *General discussion*

### *Study of diseased coronary artery*

In conditions such as variant angina or severe atherosclerosis, contraction mediated via several receptors may be affected or increased. The combination of functional and immunohistochemical experiments will provide more knowledge on altered expression of receptors and their functional role in coronary artery disease.

### *Receptor characterisation*

Chapter 10 describes a study on the 5-HT receptor subtype involved in coronary artery contraction to sumatriptan using the 5-HT<sub>1B/1D</sub> receptor antagonist GR55562. Presently, novel and more selective antagonists are available for the 5-HT<sub>1B</sub> (SB224289) and 5-HT<sub>1D</sub> (BRL15572) receptor subtypes<sup>43</sup>. The 5-HT receptors, as well as other receptors mediating blood vessel contraction (e.g.  $\alpha$ -adrenergic receptors) in coronary artery should be studied in detail and should be compared with those in cranial arteries. Receptors mediating contraction in cranial, but not in coronary arteries apparently provide new avenues to develop antimigraine drugs, which may be devoid of adverse coronary side effects.

### *Genetics*

As described in Chapters 11 and 12, chest symptoms after sumatriptan are most probably not caused by genetic variability of the 5-HT<sub>1B</sub> or 5-HT<sub>1F</sub> receptors. It is not known whether other genetic factors may predispose some patients for the development of chest symptoms after antimigraine medication. Chapter 8 describes augmentation of coronary artery contraction by thromboxane A<sub>2</sub>. Since the amount of thromboxane A<sub>2</sub> did not correlate with the augmentation of the contraction to sumatriptan (see Chapter 8), it is conceivable that a mutation in the thromboxane A<sub>2</sub> receptor gene is involved in augmented coronary artery response to sumatriptan. Also genes that are thought to be related to coronary vasospasm, such as the endothelial nitric oxide synthase (eNOS) gene<sup>44</sup> and a gene linked to HLA region HLA-DR2<sup>45</sup> may be involved in the occurrence of chest symptoms. Variability of these genes may not only be studied on DNA obtained from patients with chest symptoms after antimigraine drugs, but also on DNA obtained from hearts from which coronary artery

contraction is studied *in vitro*. Possibly, variation in one of these genes will be related to contraction to antimigraine drugs.

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## Chapter 14

### Summary - Samenvatting

#### 14.1 Summary

**Chapter 1** discusses the clinical symptoms and diagnostic criteria of migraine. The pathophysiology of migraine has not yet fully been elucidated, but most probably both neuronal and vascular changes are involved in the development of migraine attacks. All currently available specific drugs for the acute treatment of migraine possess the ability to contract cerebral and extracerebral blood vessels. Whereas this characteristic seems to bestow therapeutic activity, it is not yet clear whether drugs devoid of vasoconstrictor action are effective in acute migraine therapy.

Ergot alkaloids such as ergotamine and dihydroergotamine are in clinical use for many years. The development of sumatriptan, a 5-HT<sub>1B/1D</sub> receptor agonist, was a major improvement in the treatment of migraine. The drug is highly effective and is generally well tolerated. Currently, a number of new triptans are marketed and some others are to be launched in the near future. The pharmacodynamic properties of these novel triptans are generally similar to those of sumatriptan. Presently, compounds that may be effective in migraine, but are devoid of vasoconstrictor action, are in early stages of development.

**Chapter 2** reviews the epidemiology of chest symptoms as a side effect of sumatriptan. Although several different mechanisms may be responsible for chest symptoms, it is apparent that chest symptoms after sumatriptan and other antimigraine drugs are caused by coronary artery constriction in at least a proportion of patients. Indeed, a number of cases of myocardial infarction have been reported after the use of ergot alkaloids as well as sumatriptan. Although antimigraine drugs are unlikely to

cause myocardial ischaemia in patients with healthy coronary arteries, even a small contraction may be sufficient to cause cardiac ischaemia in patients with reduced coronary flow reserve due to atherosclerotic coronary arteries. In some exceptional cases, antimigraine drugs, possibly in association with endogenous mediators such as thromboxane A<sub>2</sub>, may cause variant angina-like coronary vasospasm in patients without obstructive coronary artery lesions. Coronary artery contraction to novel 5-HT<sub>1B/1D</sub> receptor agonists will be described in this thesis.

**Chapter 3** provides an overview of 5-HT receptors mediating contraction of the human coronary artery. Under normal, physiological conditions, the major portion of 5-HT-induced contraction of the coronary artery is mediated by 5-HT<sub>2</sub> receptors. Under some conditions, possibly in the presence of endogenous mediators such as thromboxane A<sub>2</sub>, or in hypersensitive blood vessels from patients with variant angina, contraction mediated by 5-HT<sub>1</sub> receptors may predominate over contraction elicited via 5-HT<sub>2</sub> receptors. The precise nature of the 5-HT<sub>1</sub> receptor mediating coronary vasoconstriction has been studied following both functional and molecular approaches. Most probably, this 5-HT<sub>1</sub> receptor in the human coronary artery is of the 5-HT<sub>1B</sub> subtype. Since the 5-HT<sub>1B</sub> receptor also seems to mediate the contraction of cranial and extracranial arteries, antimigraine drugs eliciting their therapeutic effects via this mechanism will have some propensity for coronary side effects. It remains to be seen whether compounds devoid of vasoconstrictor action prove effective in the acute treatment of migraine.

The aims of the thesis, described in **Chapter 4**, were (i) to determine the coronary side-effect potential of several current and prospective antimigraine drugs, and to compare this with that of sumatriptan, (ii) to investigate which factors determine the magnitude of human isolated coronary artery contraction to sumatriptan, (iii) to characterise the 5-HT receptors involved in human isolated coronary artery contraction to sumatriptan, and (iv) to assess whether there is a genetic basis for the occurrence of chest symptoms after the use of sumatriptan.

In **Chapter 5**, we compared the coronary side-effect profile of current and prospective antimigraine drugs (ergotamine, dihydroergotamine, methysergide, sumatriptan, naratriptan, zolmitriptan, rizatriptan and avitriptan). All drugs were more potent (lower  $EC_{50}$  values) than sumatriptan, but had a similar efficacy ( $E_{max} < 25\%$  of contraction induced by 100 mM  $K^+$ ). Except avitriptan, the  $EC_{50}$  of the other drugs was much higher than their maximum plasma concentration observed in patients following therapeutic doses. After correction for the fraction of drug bound to plasma proteins, avitriptan was in the same range as the other triptans. In view of the low contraction induced at therapeutic plasma concentrations, these drugs are unlikely to cause myocardial ischaemia in healthy subjects, but in patients with coronary artery disease myocardial ischaemia may result. Unlike the triptans, the coronary artery contraction by ergotamine and dihydroergotamine remained unabated after repeated washings. This seems to be an important disadvantage with these ergot alkaloids.

In **Chapter 6**, we investigated contraction to eletriptan and sumatriptan in blood vessels predictive of their clinical efficacy (human isolated middle meningeal artery) and adverse coronary side effects (human isolated coronary artery and human isolated saphenous vein). The derived  $EC_{50}$  and  $E_{max}$  values were related to the clinical plasma concentration of the drugs. Eletriptan and sumatriptan induced concentration-dependent contractions of middle meningeal artery, coronary artery and saphenous vein. The potency of eletriptan and sumatriptan was higher (lower  $EC_{50}$ ) in middle meningeal artery than in coronary artery or saphenous vein. In coronary artery the  $EC_{50}$  of eletriptan was higher than that of sumatriptan, whereas the compounds were equipotent in middle meningeal artery and saphenous vein. The  $E_{max}$  of eletriptan and sumatriptan was also similar within tissues. The predicted contractions by sumatriptan (100 mg p.o.) and eletriptan (40 mg and 80 mg p.o.) at free  $C_{max}$  were similar in middle meningeal artery, but in coronary artery and saphenous vein they appear to be lower for 40 mg eletriptan than for sumatriptan. In conclusion, both eletriptan and sumatriptan contract middle meningeal artery at therapeutic plasma concentrations more than coronary artery. While both drugs have a limited propensity

to cause adverse coronary side effects in patients with healthy coronary arteries, they must remain contraindicated in patients with coronary artery disease.

The benzopyran derivative SB-220453, which is undergoing clinical evaluation in migraine, exhibits a high affinity for a selective, but not yet characterised binding site in the human brain. SB-220453 inhibits nitric oxide release and cerebral vasodilatation following cortical spreading depression as well as carotid vasodilatation induced by trigeminal nerve stimulation in the cat. The aim of the investigation reported in **Chapter 7** was to study whether SB-220453 contracts human isolated blood vessels (coronary artery, saphenous vein and middle meningeal artery) or atrial and ventricular cardiac trabeculae. While sumatriptan induced marked contractions in all blood vessels investigated, SB-220453 was devoid of any effect. In atrial and ventricular cardiac trabeculae, SB-220453 nor sumatriptan displayed any inotropic (positive or negative) effect. Since SB-220453 did not contract the middle meningeal artery, we conclude that should SB-220453 prove effective in migraine, its therapeutic efficacy, unlike that of sumatriptan, will be independent of a direct cerebral vasoconstriction. Because SB-220453 also did not contract coronary artery, saphenous vein or cardiac trabeculae, the compound is unlikely to display adverse cardiac side effects.

**Chapter 8** reports studies dealing with the effects of the thromboxane A<sub>2</sub> analogue, U46619 and endothelin-1 on contractile responses to sumatriptan in the human isolated coronary artery, as well as the role of endogenously produced thromboxane A<sub>2</sub> and endothelin-1 in contractions evoked by sumatriptan. In the presence of U46619 (1 and 3 nM), augmentation of the contraction to sumatriptan was variable and related inversely to the E<sub>max</sub> of sumatriptan in the absence of U46619. Treatment with the thromboxane A<sub>2</sub> receptor antagonist, SQ30741 (100 nM), or incubation of vessel segments with aspirin (10 µM), significantly reduced responses to sumatriptan. Endothelin-1 (1 nM) did not augment contractions to sumatriptan; there was also no relationship between the degree of augmentation and the control E<sub>max</sub> of sumatriptan in the absence of endothelin-1. Furthermore, a high concentration

(100 nM) of the ET<sub>A</sub>/ET<sub>B</sub> receptor antagonist, SB 209670, failed to affect contractile responses to sumatriptan. Our results suggest that endogenously produced thromboxane A<sub>2</sub> enhances contractions to sumatriptan in the human isolated coronary artery. Such a mechanism may play a role in causing chest symptoms after sumatriptan by augmenting coronary vascular contraction by sumatriptan *in vivo*.

A post hoc analysis on concentration response curves to sumatriptan in 62 human isolated coronary arteries is described in **Chapter 9**. We determined whether donor-related clinical characteristics (age, sex, cause of death) and properties of the coronary artery (functional endothelial integrity, muscle mass) were related to the potency and efficacy of sumatriptan in contracting the human isolated coronary artery. The efficacy of sumatriptan was inversely related to the functional integrity of the vessel endothelium. Thus, contrary to expectation, coronary artery constriction to sumatriptan seems to be more pronounced in patients with non-diseased coronary arteries where the endothelium is intact. Nevertheless, in view of the high coronary reserve in these patients myocardial ischaemia after the use of sumatriptan is unlikely to occur, whereas in patients with coronary artery disease even a small contraction may be deleterious.

In **Chapter 10**, we characterised the receptor involved in sumatriptan-induced coronary artery contraction. Using the agonists sumatriptan and 5-carboxamidotryptamine (5-CT) and the selective 5-HT<sub>1B/1D</sub> receptor antagonist GR55562, we have investigated the involvement of 5-HT<sub>1B/1D</sub> receptors in the contraction of the human isolated coronary artery. Contractions to sumatriptan were competitively antagonised by GR55562. The pA<sub>2</sub> of GR55562 (7.41±0.16) was in accord with its reported affinity at the human 5-HT<sub>1B</sub> receptor. Since the contractions to 5-CT did not reach a maximum with the highest concentration used (10 µM), pEC<sub>50</sub> values could not be calculated for Schild analysis. However, using the pEC<sub>10%K+</sub> values (negative logarithm of the concentration needed to induce 10% of the contraction to 100 mM K<sup>+</sup>), GR55562 proved a less potent antagonist against 5-CT than against sumatriptan. These results show that sumatriptan contracts the human



isolated coronary artery via 5-HT<sub>1B/1D</sub> receptors, most probably the 5-HT<sub>1B</sub> subtype. 5-CT may contract the human isolated coronary artery, at least partly, via a novel, yet to be characterised, receptor.

**Chapter 11** describes a study dealing with the role of 5-HT<sub>1B</sub> receptor polymorphisms in the clinical response to sumatriptan. Although sumatriptan is a highly effective antimigraine drug, some patients do not respond, or experience recurrence of the headache. In addition, some patients report chest symptoms after sumatriptan. We investigated whether these different responses could be attributed to genetic diversity of the 5-HT<sub>1B</sub> receptor, which most likely mediates the therapeutic action and the coronary side effects of sumatriptan. Allele frequencies of two polymorphisms in the 5-HT<sub>1B</sub> receptor gene (G861C and T-261G) were investigated in migraine patients with consistently good response to sumatriptan ( $n=14$ ), with no response ( $n=12$ ), with recurrence of the headache ( $n=12$ ), with chest symptoms ( $n=13$ ), and in patients without chest symptoms ( $n=27$ ). Allele frequencies (G:0.74;C:0.26 at nt 861 and T:0.39;G:0.61 at nt -261) did not differ between patient groups, indicating that genetic diversity of the 5-HT<sub>1B</sub> receptor does not seem to be involved in the different clinical responses to sumatriptan.

Besides the 5-HT<sub>1B</sub> receptor, the 5-HT<sub>1F</sub> receptor may mediate the therapeutic effect and/or side effects of sumatriptan. In **Chapter 12**, we investigated the chromosomal localisation of the 5-HT<sub>1F</sub> receptor gene and the relation between eventually existing polymorphisms and the clinical response to sumatriptan in migraine patients. The 5-HT<sub>1F</sub> receptor gene was localised using a monochromosomal mapping panel, followed by a radiation reduced hybrid mapping and fluorescent *in situ* hybridisation. The results using these techniques show that the 5-HT<sub>1F</sub> receptor gene is localised on chromosome 3p12. We also investigated the presence of polymorphisms by single strand conformation polymorphism (SSCP) analysis in 40 migraine patients. Fourteen patients consistently responded well to sumatriptan, 12 patients consistently experienced recurrence of the headache after initial relief by sumatriptan, 12 patients did not respond to sumatriptan and 13 patients consistently

experienced chest symptoms following the use of sumatriptan. No polymorphisms were detected in any of the patients. We therefore conclude that genetic diversity of the 5-HT<sub>1F</sub> receptor gene is most probably not responsible for the variable clinical response to sumatriptan.

## 14.2 Samenvatting

**Hoofdstuk 1** beschrijft de klinische symptomen en diagnostische criteria van migraine. De pathofysiologie van migraine is nog niet geheel ontrafeld, maar waarschijnlijk spelen zowel neuronale als vasculaire veranderingen een rol bij het ontstaan van migraine aanvallen. Alle momenteel beschikbare specifieke geneesmiddelen voor de acute behandeling van migraine aanvallen beschikken over het vermogen om cerebrale en extracerebrale bloedvaten te contraheren. Waarschijnlijk ontlenen antimigraine middelen hun therapeutisch effect aan deze eigenschap; het is nog niet duidelijk of geneesmiddelen die geen contractie van bloedvaten induceren effectief zijn in de acute behandeling van migraine.

Ergot alkaloiden zoals ergotamine en dihydroergotamine worden al sinds jaren gebruikt voor de behandeling van migraine aanvallen. De ontwikkeling van sumatriptan, een 5-HT<sub>1B/1D</sub> receptor agonist, was een grote verbetering voor de behandeling van migraine; het geneesmiddel is zeer effectief en wordt in het algemeen goed getolereerd. Onlangs is er een aantal nieuwe triptanen op de markt gebracht, en sommige andere zullen in de nabije toekomst beschikbaar zijn. De farmacodynamische eigenschappen van deze nieuwe triptanen zijn in het algemeen gelijk aan die van sumatriptan. Momenteel zijn stoffen, die wellicht effectief zijn in de behandeling van migraine maar die bloedvaten niet contraheren, in vroege stadia van ontwikkeling.

In **Hoofdstuk 2** wordt de epidemiologie van een pijnlijk of drukkend gevoel op de borst als bijwerking van sumatriptan beschreven. Hoewel verschillende mechanismen verantwoordelijk kunnen zijn voor deze bijwerkingen, is het duidelijk

dat een pijnlijk of drukkend gevoel op de borst na sumatriptan en andere antimigraine middelen in tenminste een deel van de patiënten wordt veroorzaakt door constrictie van coronair arteriën. Inderdaad zijn er enkele gevallen van myocard infarct beschreven na gebruik van ergot alkaloiden of sumatriptan. Hoewel het onwaarschijnlijk is dat antimigraine middelen myocard ischaemie zullen induceren in patiënten met gezonde coronair arteriën, kan in patiënten met een verminderde coronaire stroomreserve ten gevolge van atherosclerose een kleine contractie al tot ischaemie van het hart leiden. In enkele uitzonderlijke gevallen kunnen antimigraine middelen, wellicht in associatie met endogene mediators zoals thromboxaan A<sub>2</sub>, angina pectoris-achtige coronaire vaatspasmen induceren in patiënten zonder obstructieve coronaire laesies. Dit proefschrift beschrijft contractie van de humane coronair arterie in respons op nieuwe 5-HT<sub>1B/1D</sub> receptor agonisten.

**Hoofdstuk 3** geeft een overzicht van 5-HT receptoren die contractie van de humane coronair arterie mediëren. Onder normale, fysiologische omstandigheden, zal het grootste deel van de door 5-HT geïnduceerde contractie van de coronair arterie gemedieerd worden door 5-HT<sub>2</sub> receptoren. Onder sommige omstandigheden, wellicht in de aanwezigheid van endogene mediators zoals thromboxaan A<sub>2</sub> of in overgevoelige bloedvaten van patiënten met variante angina pectoris, zal contractie gemedieerd via 5-HT<sub>1</sub> receptoren domineren over contractie opgewekt door 5-HT<sub>2</sub> receptoren. De exacte aard van de 5-HT<sub>1</sub> receptor die contractie van de coronair arterie medieert is bestudeerd met zowel functionele als moleculaire benaderingen. Hoogstwaarschijnlijk is de 5-HT<sub>1</sub> receptor in de humane coronair arterie van het 5-HT<sub>1B</sub> subtype. Omdat de 5-HT<sub>1B</sub> receptor ook de contractie van craniale en extracraniale bloedvaten lijkt te mediëren, zullen antimigraine middelen die via dit mechanisme hun therapeutisch effect bereiken waarschijnlijk ook coronaire bijwerkingen induceren. Het is nog niet aangetoond of geneesmiddelen die bloedvaten niet contraheren effectief zijn in de acute behandeling van migraine.

De doelstellingen van de in dit proefschrift beschreven studies (zie **Hoofdstuk 4**) waren (i) het bepalen van het potentieel aan coronaire bijwerkingen van enkele

huidige en toekomstige antimigraine middelen, en dit te vergelijken met dat van sumatriptan, (ii) te onderzoeken welke factoren de grootte van contractie van de humane geïsoleerde coronair arterie in respons op sumatriptan bepalen, (iii) te karakteriseren welke 5-HT receptoren betrokken zijn bij contractie van de humane geïsoleerde coronair arterie in respons op sumatriptan, en (iv) te onderzoeken of er een genetische basis is voor het voorkomen van een pijnlijk of drukkend gevoel op de borst na gebruik van sumatriptan.

In **Hoofdstuk 5** hebben wij het potentieel aan coronaire bijwerkingen van enkele huidige en toekomstige antimigraine middelen (ergotamine, dihydroergotamine, methysergide, sumatriptan, naratriptan, zolmitriptan, rizatriptan en avitriptan) vergeleken. Alle geneesmiddelen waren potenter (hadden een lagere  $EC_{50}$  waarde) dan sumatriptan, maar hadden een soortgelijke effectiviteit ( $E_{max} < 25\%$  van contractie geïnduceerd door  $K^+$ ). Behalve voor avitriptan was de  $EC_{50}$  van de geneesmiddelen veel hoger dan hun maximale plasma concentratie gemeten in patiënten na therapeutische doses; na correctie voor de fractie geneesmiddel die aan plasma eiwitten is gebonden lag ook avitriptan in dezelfde grootte orde als de andere triptanen. Gezien de kleine contractie die geïnduceerd wordt bij therapeutische plasma concentraties is het onwaarschijnlijk dat het gebruik van deze middelen tot myocard ischaemie leidt, hoewel in patiënten met aangedane coronair arteriën wel myocard ischaemie kan voorkomen. In tegenstelling tot de triptanen bleef de contractie van de coronair arterie in respons op ergotamine en dihydroergotamine gehandhaafd na herhaald wassen. Deze aanhoudende contractie lijkt een belangrijk nadeel van deze ergot alkaloiden.

In **Hoofdstuk 6** hebben wij de contractie in respons op eletriptan en sumatriptan onderzocht in bloedvaten die indicatief zijn voor de klinische effectiviteit (humane geïsoleerde arteria meningea media) en de coronaire bijwerkingen (humane geïsoleerde coronair arterie en humane geïsoleerde vena saphena). De verkregen  $EC_{50}$  en  $E_{max}$  waarden werden gerelateerd aan de klinische plasma concentraties van de

geneesmiddelen. Eletriptan en sumatriptan induceerden concentratie afhankelijke contracties van de arteria meningea media, coronair arterie en vena saphena. De potentie van eletriptan en sumatriptan was hoger (lagere  $EC_{50}$ ) in de arteria meningea media dan in de coronair arterie of de vena saphena. De  $EC_{50}$  van eletriptan was groter dan die van sumatriptan in de coronair arterie, terwijl de  $EC_{50}$  waarden van eletriptan en sumatriptan niet verschilden in de arteria meningea media en de vena saphena. De  $E_{max}$  van eletriptan en sumatriptan was ook gelijk binnen weefsels. De voorspelde contracties opgewekt door de vrije  $C_{max}$  van sumatriptan (100 mg p.o.) en eletriptan (40 mg en 80 mg p.o.) waren gelijk in arteria meningea media, maar in de coronair arterie en de vena saphena leken de voorspelde contracties lager voor 40 mg eletriptan dan voor sumatriptan. Samenvattend contraheren zowel eletriptan als sumatriptan de arteria meningea media meer dan de coronair arterie op therapeutische plasma concentraties. Hoewel beide geneesmiddelen slechts een kleine kans hebben op schadelijke coronaire bijwerkingen in patiënten met gezonde coronair arteriën, moeten ze gecontraïndiceerd blijven in patiënten met aangedane coronair arteriën.

Het benzopyraan derivaat SB-220453, dat momenteel klinisch onderzocht wordt voor de behandeling van migraine, heeft een hoge affiniteit voor een selectieve maar nog niet gekarakteriseerde bindingsplaats in de humane hersenen. SB-220453 remt afgifte van stikstof oxide, dilatatie van cerebrale bloedvaten ten gevolge van 'cortical spreading depression' en dilatatie van de arteria carotis geïnduceerd door stimulatie van de nervus trigeminus in de kat. Het doel van de studie beschreven in **Hoofdstuk 7** was te onderzoeken of SB-220453 humane geïsoleerde bloedvaten (coronair arterie, vena saphena en arteria meningea media) of geïsoleerde atriale en ventriculaire cardiale trabekels contraheert. Terwijl sumatriptan alle onderzochte bloedvaten duidelijk contraheerde, had SB-220453 geen effect. In atriale en ventriculaire cardiale trabekels had noch sumatriptan, noch SB-220453 enig inotrop (positief of negatief) effect. Omdat SB-220453 de arteria meningea media niet contraheert concluderen wij dat, indien SB-220453 effectief blijkt te zijn in migraine, het therapeutisch effect onafhankelijk zal zijn van cerebrale vasoconstrictie, in tegenstelling tot sumatriptan. Omdat SB-220453 ook de coronair arterie, de vena

saphena en cardiale trabekels niet contraheert, is het onwaarschijnlijk dat deze stof cardiale bijwerkingen zal hebben.

**Hoofdstuk 8** beschrijft studies naar de effecten van de thromboxaan  $A_2$  analoog U46619 en endotheline-1 op de contractiele respons van sumatriptan in de humane geïsoleerde coronair arterie. Ook wordt de rol van endogeen geproduceerd thromboxaan  $A_2$  en endotheline-1 in dit hoofdstuk beschreven. In de aanwezigheid van U46619 (1 en 3 nM) was versterking van de contractie in respons op sumatriptan variabel en negatief gerelateerd aan de  $E_{max}$  van sumatriptan in de afwezigheid van U46619. Behandeling met de thromboxaan  $A_2$  receptor antagonist SQ30741 (100 nM) of incubatie van de vaatsegmenten met aspirine (10  $\mu$ M) verlaagde de contractie in respons op sumatriptan significant. Endotheline-1 versterkte de contractie op sumatriptan niet; er was ook geen relatie tussen de mate van versterking en de controle  $E_{max}$ , in de afwezigheid van endotheline-1. Verder verlaagde een hoge concentratie (100 nM) van de  $ET_A/ET_B$  receptor antagonist SB209670 de contractiele respons op sumatriptan niet. Onze resultaten suggereren dat endogeen geproduceerd thromboxaan  $A_2$  de contractie in respons op sumatriptan versterkt in de humane geïsoleerde coronair arterie. Een dergelijk mechanisme zou een rol kunnen spelen in het ontstaan van pijn op de borst na sumatriptan, door de contractie van de coronair arterie in respons op sumatriptan *in vivo* te versterken.

Een post hoc analyse van contractie in respons op sumatriptan in 62 humane geïsoleerde coronair arteriën wordt beschreven in **Hoofdstuk 9**. Wij hebben onderzocht of donor-gerelateerde kenmerken (leeftijd, geslacht, doodsoorzaak) en eigenschappen van de coronair arterie (functionele integriteit van het endotheel, spiermassa) gerelateerd waren aan de potentie en de effectiviteit van sumatriptan in het contraheren van de humane coronair arterie. De effectiviteit van sumatriptan was negatief gecorreleerd aan de functionele integriteit van het vaatendotheel. Constrictie van de coronair arterie in respons op sumatriptan is dus, tegen de verwachting in, het hevigst in patiënten met gezonde coronair arteriën, waar het endotheel intact is. Niettegenstaande onze bevindingen is het onwaarschijnlijk dat in deze patiënten met

een grote coronaire reserve het gebruik van sumatriptan tot myocard ischaemie leidt. In patiënten met atherosclerotische coronair arteriën kan zelfs een kleine contractie schadelijk zijn.

In **Hoofdstuk 10** karakteriseerden wij de receptor die betrokken is bij contractie van de coronair arterie in respons op sumatriptan. Met behulp van de agonisten sumatriptan en 5-carboxamidotryptamine (5-CT) en de selectieve 5-HT<sub>1B/1D</sub> receptor antagonist GR55562 hebben wij de betrokkenheid van 5-HT<sub>1B/1D</sub> receptoren bij contractie van de humane geïsoleerde coronair arterie onderzocht. Contracties in respons op sumatriptan werden competitief geantagoneerd door GR55562. De pA<sub>2</sub> van GR55562 (7.41±0.16) was in overeenstemming met de gerapporteerde affiniteit van deze antagonist voor de humane 5-HT<sub>1B</sub> receptor. Omdat de contractie in respons op 5-CT geen maximum bereikte met de hoogste concentratie die gebruikt werd (10 µM), kon de pEC<sub>50</sub> niet berekend worden ten behoeve van Schild analyse. Door gebruik te maken van pEC<sub>10%K+</sub> waarden (de negatieve logaritme van de concentratie benodigd voor het induceren van een contractie die 10% bedraagt van de contractie opgewekt door 100 mM K<sup>+</sup>) bleek GR55562 echter een minder potente antagonist te zijn tegen 5-CT dan tegen sumatriptan. Deze resultaten tonen aan dat sumatriptan de humane geïsoleerde coronair arterie contraheert via 5-HT<sub>1B/1D</sub> receptoren, hoogstwaarschijnlijk van het 5-HT<sub>1B</sub> subtype. 5-CT lijkt de humane geïsoleerde coronair arterie, tenminste gedeeltelijk, via een nieuwe, nog te karakteriseren receptor te contraheren.

**Hoofdstuk 11** beschrijft een studie naar de rol van 5-HT<sub>1B</sub> receptor polymorfismen in de klinische respons op sumatriptan. Hoewel sumatriptan een zeer effectief antimigraine middel is, reageren sommige patiënten niet op sumatriptan, of ervaren ze een terugkeer van de hoofdpijn. Daarnaast beschrijven sommige patiënten een drukkend of pijnlijk gevoel op de borst na gebruik van sumatriptan. Wij hebben onderzocht of deze verschillende responsen het gevolg zijn van genetische variabiliteit van de 5-HT<sub>1B</sub> receptor, die hoogstwaarschijnlijk het therapeutisch effect en de coronaire bijwerkingen van sumatriptan medieert. Alle frequenties van twee

polymorfismen in het 5-HT<sub>1B</sub> receptor gen (G861C en T-261G) zijn onderzocht in migraine patiënten met een consistent goede respons op sumatriptan ( $n=14$ ), in patiënten zonder respons ( $n=12$ ), in patiënten met een terugkeer van de hoofdpijn na sumatriptan ( $n=12$ ), in patiënten met een drukkend of pijnlijk gevoel op de borst ( $n=13$ ) en in patiënten zonder een drukkend of pijnlijk gevoel op de borst ( $n=27$ ). Allel frequenties (G:0.74;C:0.26 op nt 861 en T:0.39;G:0.61 op nt -261) verschilden niet tussen de groepen patiënten, er op duidend dat genetische diversiteit van de 5-HT<sub>1B</sub> receptor niet betrokken lijkt te zijn bij de verschillende klinische responsen op sumatriptan.

Naast de 5-HT<sub>1B</sub> receptor zou ook de 5-HT<sub>1F</sub> receptor de therapeutische werking en/of bijwerkingen van sumatriptan kunnen mediëren. In **Hoofdstuk 12** hebben wij de chromosomale lokalisatie van het 5-HT<sub>1F</sub> receptor gen en de relatie tussen eventuele polymorfismen en de klinische respons op sumatriptan in migraine patiënten onderzocht. Het 5-HT<sub>1F</sub> receptor gen is gelokaliseerd met behulp van een monochromosomaal lokalisatie panel, gevolgd door een stralings gereduceerde hybride lokalisatie en fluorescentie *in situ* hybridisatie. De resultaten verkregen met deze technieken tonen aan dat het 5-HT<sub>1F</sub> receptor gen gelokaliseerd is op chromosoom 3p12. Wij hebben ook de aanwezigheid van polymorfismen onderzocht met ‘enkel strengs conformatie polymorfisme’ (SSCP) analyse in 40 migraine patiënten. Veertien patiënten reageerden consistent goed op sumatriptan, 12 patiënten hadden altijd een terugkeer van de hoofdpijn na initieel goede respons op sumatriptan, 12 patiënten reageerden niet op sumatriptan en 13 patiënten hadden een drukkend of pijnlijk gevoel op de borst na gebruik van sumatriptan. In geen van de patiënten werden polymorfismen aangetroffen. Wij concluderen daarom dat genetische diversiteit van het 5-HT<sub>1F</sub> receptor gen hoogstwaarschijnlijk niet verantwoordelijk is voor de variatie in klinische responsen op sumatriptan.



## Acknowledgement - Dankwoord

Dit proefschrift was niet tot stand gekomen zonder de hulp van vele anderen. Bovendien hebben velen er voor gezorgd dat de tijd dat ik aan dit proefschrift heb gewerkt een erg aangename tijd was. Enkele van deze personen wil ik hier graag bij naam noemen.

Allereerst mijn promotor, Prof. dr. P.R. Saxena. Pramod, bedankt voor het vertrouwen, enthousiasme en de persoonlijke vriendschap die je me in de afgelopen jaren hebt geschonken. Ik hoop dat onze samenwerking nog lang op deze voet zal mogen doorgaan.

Mijn co-promotor, dr. M.D. Ferrari, ben ik veel dank verschuldigd voor zijn inspirerende bijdrage als clinicus. Michel, ook met jou hoop ik in de toekomst nog veel samen te zullen werken.

Dr. W.A. Bax wil ik bedanken voor het uitzetten van de grote lijnen van mijn project en zijn excellente begeleiding gedurende het begin van mijn onderzoek. Willem, ik had mij geen betere start kunnen wensen!

Het in dit proefschrift beschreven onderzoek had nooit tot stand kunnen komen zonder de medewerking van de afdelingen Thoraxchirurgie, Neurochirurgie en de Hartkleppenbank Rotterdam. Prof. dr. A.J.J.C. Bogers, Prof. dr. C.J.J. Avezaat, dr. Sander Stegmann, dr. Claes Wassenaar, Zohara Aghai, Corina van Tricht en vele chirurgen en OK-assistenten die ik hier niet bij naam kan noemen wil ik hartelijk bedanken voor hun enthousiaste medewerking aan dit onderzoek. Hoewel het beschikbaar stellen van humaan weefsel voor onderzoek soms lastig is en tijd kost, is dit materiaal onmisbaar voor deze onderzoekslijn. Ik hoop van harte onze samenwerking nog lang te kunnen continueren.

## *Publications*

Mijn dank gaat uit naar Prof. dr. A.J.J.C. Bogers, Prof. dr. P.D. Verdouw en Prof. dr. P.A. van Zwieten voor de beoordeling van mijn manuscript.

Prof. dr. R.R. Frants ben ik zeer erkentelijk voor zijn gastvrijheid op de afdeling Anthropogenetica van het Leids Universitair Medisch Centrum, waar de studies beschreven in de hoofdstukken 11 en 12 zijn uitgevoerd. Rune, Roel Ophoff, en in het bijzonder Monique Vergouwe hebben mij met raad en daad gesteund tijdens mijn periode op hun afdeling.

Een speciaal woord van dank richt ik aan de studenten Rebecca Vlagsma, Alide Tieleman, Navin Ramrattan, Marije Reekers, Mustafa Albayrak en Dianne den Boer, die allen een stimulerende en belangrijke bijdrage aan mijn onderzoek hebben geleverd. Met name Marije Reekers heeft een lange (en soms turbulente) periode op het lab meegemaakt, bedankt voor je werkinzet en gezelligheid.

Mijn kamergenoten hebben mij in de loop der jaren altijd veel gezelligheid verschaft. Twee van hen wil ik speciaal noemen. Toos van Kesteren wil ik bedanken voor het gezelschap tijdens vele potten thee, de onvergetelijke congressen en de warme vriendschap die er gelukkig nog steeds is, ook al zijn we al een tijd geen collega's meer. Ook met Inge Lankhuizen heb ik al veel thee mogen drinken. Ik ben haar zeer dankbaar voor de gezelligheid en haar onvermoeibare esthetische adviezen over dia's, dalmatiërs en dit proefschrift. Toos en Inge, ik ben erg blij dat jullie mijn paranimfen willen zijn.

Peter de Vries bedank ik voor het delen van de 'promotie-gerelateerde zaken' en voor zijn gezelschap tijdens vele congressen. Corné Tak ben ik erg erkentelijk voor zijn hulp tijdens problemen met mijn computer, en Magda Busscher-Lauw wil ik bedanken voor haar administratieve ondersteuning. Rémon van den Broek en René de Vries wil ik hartelijk bedanken voor de prettige samenwerking.

Charlotte en Frank ben ik erge dankbaar voor hun niet aflatende vriendschap in goede en minder goede tijden. Mijn 'pianojuf' Irja wil ik bedanken voor al haar geduld in de afgelopen tijd, ik hoop nu mijn proefschrift klaar is een ijverigere leerling te worden. De kaft van dit proefschrift bewijst dat je lessen ook in drukke tijden wel degelijk erg inspirerend waren!

Tenslotte wil ik mijn ouders en schoonvader bedanken voor hun altijd aanwezige liefde en ondersteuning. Een (schoon)dochter die tegelijkertijd verhuist en promoveert levert flink wat werk op! Bovenal wil ik Peter bedanken. Jij bent verreweg het beste wat ik aan mijn promotietijd op de afdeling Farmacologie heb overgehouden. Daarom is dit proefschrift aan jou opgedragen.

## **Curriculum Vitae**

The author of this thesis was born in Delft, The Netherlands, on 17 April 1971. After attending 'Stedelijke Scholengemeenschap Hugo Grotius', where she passed her secondary school exam in 1989, she studied Biomedical Sciences at Leiden University Medical Centre until 1994. During her studies, she was involved in research projects at the departments of Pharmacology (Prof. dr. E.R. de Kloet) and Physiology / Cell Biology (dr. A. Wiltink). Her final project, performed at the department of Clinical Neurophysiology, was on 'Physiological and pathological variability of magnetic evoked potentials' under supervision of dr. J.G. van Dijk. She joined the department of Pharmacology of the Erasmus University Medical Centre Rotterdam in 1994. Under the guidance of Prof. dr. P.R. Saxena and dr. M.D. Ferrari (department of Neurology, Leiden University Medical Centre) she worked on a project entitled 'Coronary side effects of antimigraine drugs; from patient to receptor'. During the project, she was a guest at the departments of Neurology and Human Genetics (Leiden University Medical Centre), where the work on 5-HT<sub>1B</sub> and 5-HT<sub>1F</sub> receptor genes in relation to clinical response to sumatriptan was performed. Since June 1999, she works as scientific investigator at the department of Pharmacology, Erasmus University Medical Centre Rotterdam. She is married to Peter van Haren.

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## **List of abbreviations**

5-CT	5-carboxamidotryptamine
5-HT	5-hydroxytryptamine
AMP	adenosine monophosphate
a.u.	arbitrary units
ANOVA	analysis of variance
bp	base pair
cAMP	cyclic AMP
c.i.	confidence interval
C <sub>max</sub>	maximum plasma concentration
DAPI	4,6-diamidino-2-phenylindole
DHE	dihydroergotamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EC <sub>50</sub>	concentration of an agonist eliciting half the maximal effect
ECG	electrocardiography
EDTA	ethylenediaminetetraactetic acid
E <sub>max</sub>	maximal effect
eNOS	endothelial nitric oxide synthase
ET	endothelin
FISH	fluorescent in situ hybridisation
FITC	fluorescein isothiocyanate
HCA	human isolated coronary artery
HLA	human leukocyte antigen
HMA	human isolated middle meningeal artery
HSV	human isolated saphenous vein
i.m.	intramuscularly
i.v.	intravenously

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K <sub>i</sub>	concentration of competing ligand in a competition assay that would occupy 50% of the receptors if no radioligand were present
L-NAME	L-nitro-L-arginine methylester
M	molar
min	minute
mm	millimeter
m/μl	milli/micro liter
mRNA	messenger RNA
N	Newton
PCR	polymerase chain reaction
pA <sub>2</sub>	the negative logarithm (- <sup>10</sup> log) of the concentration of antagonist that would produce a two-fold shift in the concentration response curve of an agonist
pEC <sub>50</sub>	the negative logarithm (- <sup>10</sup> log) of EC <sub>50</sub>
PIP <sub>2</sub>	phosphatidyl-inositol 4,5-biphosphate
PG	prostaglandin
p.o.	per os (orally)
RNA	ribonucleic acid
RT-PCR	reverse transscriptase polymerase chain reaction
s.c.	subcutaneously
sec	second
s.e.mean	standard error of the mean
SSCP	single strand conformation polymorphism
TBE	tris borate EDTA buffer
Tx	thromboxane
U	unit