

## ORIGINAL ARTICLE

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## Effects of avitriptan, a new 5-HT<sub>1B/1D</sub> receptor agonist, in experimental models predictive of antimigraine activity and coronary side-effect potential

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**Abstract** Several acutely acting antimigraine drugs, including ergotamine and sumatriptan, have the ability to constrict porcine arteriovenous anastomoses as well as the human isolated coronary artery. These two experimental models seem to serve as indicators, respectively, for the therapeutic and coronary side-effect potential of the compounds. Using these two models, we have now investigated the effects of avitriptan (BMS-180048; 3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-*N*-methyl-*1H*-indole-5-methanesulfonamide monofumarate), a new 5-HT<sub>1B/1D</sub> receptor agonist. In anaesthetized pigs, avitriptan (10, 30, 100 and 300  $\mu\text{g}\cdot\text{kg}^{-1}$ ) decreased the total carotid blood flow by exclusively decreasing arteriovenous anastomotic blood flow; capillary blood flow was increased. The mean  $\pm$  SEM i.v. dose of avitriptan eliciting a 50% decrease (ED<sub>50</sub>) in the porcine carotid arteriovenous anastomotic blood flow was calculated to be  $76 \pm 23 \mu\text{g}\cdot\text{kg}^{-1}$  ( $132 \pm 40 \text{ nmol}\cdot\text{kg}^{-1}$ ) and the highest dose (300  $\mu\text{g}\cdot\text{kg}^{-1}$ ) produced a  $72 \pm 4\%$  reduction. In recent comparative experiments (DeVries et al. 1996), the mean  $\pm$  SEM ED<sub>50</sub> (i.v.) of sumatriptan in decreasing carotid arteriovenous anastomotic blood flow was  $63 \pm 17 \mu\text{g}\cdot\text{kg}^{-1}$  ( $158 \pm 43 \text{ nmol}\cdot\text{kg}^{-1}$ ), with a reduction of  $76 \pm 4\%$  by 300  $\mu\text{g}\cdot\text{kg}^{-1}$ , i.v. Both avitriptan (pD<sub>2</sub>:  $7.39 \pm 0.09$ ; E<sub>max</sub>:  $13.0 \pm 4.5\%$  of the contraction to 100 mM K<sup>+</sup>) and sumatriptan (pD<sub>2</sub>:  $6.33 \pm 0.09$ ; E<sub>max</sub>:  $15.5 \pm 2.3\%$  of the contraction to 100 mM K<sup>+</sup>) contracted the human isolated coronary artery. The above results suggest that avitriptan should be able to abort migraine headaches in patients, but may exhibit sumatriptan-like effects on coronary arteries. Initial clinical studies have demonstrated the therapeutic action of the drug in acute migraine.

**Key words** Antimigraine drugs · Arteriovenous anastomoses · Avitriptan · BMS-180048 · Carotid artery · Human · Human coronary artery · Migraine · Pig · Sumatriptan

### Introduction

Sumatriptan, which is the first member of a completely new class of compounds designated as 5-HT<sub>1</sub>-like (or 5-HT<sub>1D</sub>) receptor agonists (Humphrey et al. 1988, 1990; Saxena and Ferrari 1992), is very effective in aborting migraine headaches (The Subcutaneous Sumatriptan International Study Group 1991; Ferrari and Saxena 1993). The drug constricts large cranial and extracranial blood vessels (see Saxena and Tfelt-Hansen 1993), including porcine carotid arteriovenous anastomoses (Den Boer et al. 1991a, 1992) and the vasoconstrictor effects, susceptible to methiothepin but not to ketanserin, are mediated via the 5-HT<sub>1</sub>-like receptor (Humphrey et al. 1988, 1990; Saxena et al. 1986; Hoyer et al. 1994). Evidence is now emerging that the vascular 5-HT<sub>1</sub>-like receptor may be identical to the recombinant 5-HT<sub>1B/1D</sub> (formerly designated as 5-HT<sub>1D $\beta$</sub>  and 5-HT<sub>1D $\alpha$</sub> , respectively; see Hartig et al. 1996) receptors, but most probably the 5-HT<sub>1B</sub> receptor. Thus, the mRNA for the 5-HT<sub>1B</sub>, but not that for 5-HT<sub>1D</sub>, receptor is present in cranial blood vessels (Hamel et al. 1993) and the vasoconstrictor effect of sumatriptan, which has a high affinity for 5-HT<sub>1B/1D</sub> receptors (Peroutka and McCarthy 1989; Beattie et al. 1994), is antagonized by GR127935, a selective 5-HT<sub>1B/1D</sub> receptor antagonist (Skingle et al. 1993; Clitherow et al. 1994; De Vries et al. 1996).

The introduction of sumatriptan in migraine therapy has prompted several pharmaceutical companies to synthesize and evaluate new compounds with binding affinity and potential agonist activity at 5-HT<sub>1B/1D</sub> receptors (for a review, see Saxena and Ferrari 1996). One such compound currently undergoing clinical evaluation is avitriptan (BMS-180048), which is a methoxypyrimidinyl-piperazi-

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**Table 1** Binding affinities ( $pK_i$  values) of avitriptan and sumatriptan at 5-HT receptors

	5-HT <sub>1A</sub> Rat	5-HT <sub>1B</sub> Rat	5-HT <sub>1B</sub> Human	5-HT <sub>1D</sub> Human	5-HT <sub>2</sub> Rat	5-HT <sub>3</sub> Rat
Avitriptan	7.15	7.44	7.68	8.36	5.68	<6.0
Sumatriptan	7.00	7.57	7.54	8.14	<5.0	<5.0

Data from Yocca et al. (1995) and Bristol-Myers Squibb files, except for sumatriptan on rat receptors (Peroutka and MacCarthy 1989)

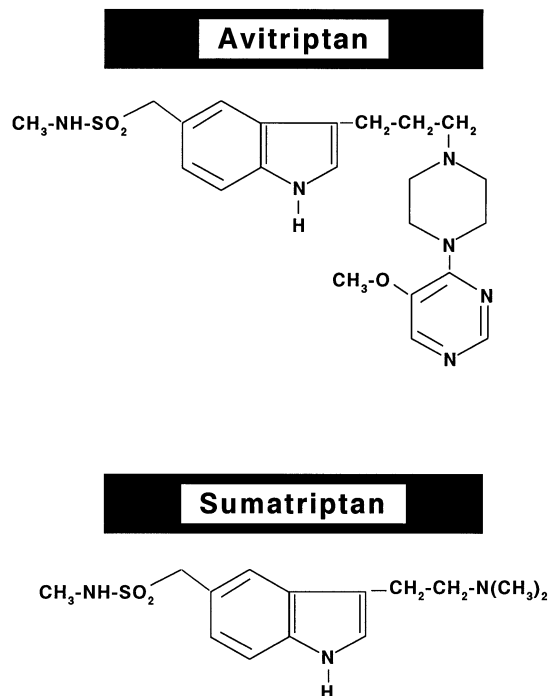
nyl analogue of sumatriptan, synthesized by Bristol-Myers Squibb, Wallingford, CT, USA (Fig. 1). The binding profile of avitriptan is comparable to that of sumatriptan and avitriptan also has a selective affinity for 5-HT<sub>1B/1D</sub> receptors, without distinguishing between the 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> subtypes (Table 1; Yocca et al. 1995). In the present investigation, we have studied the effects of avitriptan in experimental models predictive of therapeutic activity in migraine (constriction of carotid arteriovenous anastomoses in anaesthetized pigs; Saxena 1990, 1995) and coronary side effects (constriction of human isolated coronary artery; Connor et al. 1989; Chester et al. 1990; Bax et al. 1993; Bax and Saxena 1993). In the latter model, sumatriptan was simultaneously used for a direct comparison, whereas data obtained in the first model was compared with that obtained with sumatriptan in a series of experiments (De Vries et al. 1996) performed around the same time.

## Materials and methods

### Systemic and carotid haemodynamics in anaesthetized pigs

**General.** After an overnight fast, 14 pigs (Yorkshire × Landrace; 10–15 kg) were anaesthetized with azaperone (160 mg, i.m.), midazolam hydrochloride (5 mg, i.m.) and metomidate (200 mg, i.v.), intubated and connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35–7.48; pCO<sub>2</sub>: 35–48 mmHg; pO<sub>2</sub>: 100–120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium at 20 mg·kg<sup>-1</sup>·h<sup>-1</sup>. With this anaesthetic regimen, arteriovenous anastomotic blood flow is considerably higher than that in pigs in a conscious state or under thiopentone anaesthesia (Den Boer et al. 1993).

Catheters were placed in the inferior vena cava via the left femoral vein for the administration of drugs and in the aortic arch via the left femoral artery for the measurement of arterial blood pressure (P23 Dc pressure transducer; Statham, Hato Rey, Puerto Rico) and the withdrawal of arterial blood for determining blood gases (ABL-510, Radiometer, Copenhagen, Denmark). The common carotid arteries, external jugular veins and vagus nerves were identified and both vagi and the accompanying cervical sympathetic nerves were cut between two ligatures. Another catheter was placed in the right external jugular vein for the withdrawal of venous blood samples, while the right common carotid artery was dissected free and a needle was inserted against the direction of blood flow for the administration and uniform mixing of radioactive microspheres. Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (7P4 Grass Instrument Company, Quincy, Mass., USA) triggered by ECG signals.



**Fig. 1** Chemical structures of avitriptan and sumatriptan

Arterial blood pressure, heart rate and carotid blood flow were continuously monitored on a model 7 Grass polygraph. Body temperature was kept at about 37°C and the animals were continuously infused with saline to compensate for fluid losses during the experiment.

**Distribution of carotid blood flow.** The distribution of common carotid blood flow was determined with  $15 \pm 1$  (S.D.)  $\mu$ m diameter microspheres labelled with either <sup>141</sup>Ce, <sup>113</sup>Sn, <sup>95</sup>Nb, <sup>103</sup>Ru or <sup>46</sup>Sc (NEN Company, Dreieich, Germany). For each measurement a suspension of about 200 000 microspheres, labelled with one of the isotopes, was mixed and injected into the carotid artery. At the end of the experiment, the animal was killed and the heart, kidneys, lungs and the different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 5–10 min in a  $\gamma$ -scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows for discriminating the different isotopes. All data were processed by a set of specially designed programs (Saxena et al. 1980), using a personal computer.

The fraction of carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivities by the total common carotid blood flow at the time of the injection of microspheres. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping via carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of carotid blood flow (Johnston and Saxena 1978; Saxena and Verdouw 1982).

**Experimental protocol.** The experiments were started after a stabilization period of about 1 h and baseline values of heart rate, mean arterial blood pressure, carotid blood flow and its distribution as well as arterial and jugular venous blood gases were measured. The animals were then divided into two groups receiving slow i.v. injections of either avitriptan (cumulative doses: 10, 30, 100 and 300  $\mu\text{g}\cdot\text{kg}^{-1}$ ;  $n = 7$ ) or physiologic saline (0.5, 1, 2 and 4 ml;  $n = 7$ ) over a 3–4 min period. The variables were reassessed about ten min after the end of each injection.

#### Human isolated coronary artery

**Tissue preparation.** As described previously (Bax et al. 1993), the right epicardial coronary artery was obtained (via the Rotterdam Heart Valve Bank, Bio Implant Services/Eurotransplant Foundation) from 7 heart beating organ donors, who died of non-cardiac disorders (3 cerebrovascular accident, 2 head trauma, 2 cerebral hypoxia; 4 male, 3 female; age 1–53 years with mean  $\pm$  SEM  $37.4 \pm 6.7$  years) less than 24 h before the tissue was brought to the laboratory. The hearts were stored at 0–4°C in a sterile organ protecting solution (UW, Euro-Collins or HTK-Brettschneider; see Ploeg et al. 1992) immediately following 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: NaCl 118 mM, KCl 4.7 mM,  $\text{CaCl}_2$  2.5 mM,  $\text{MgSO}_4$  1.2 mM,  $\text{KH}_2\text{PO}_4$  1.2 mM,  $\text{NaHCO}_2$  25 mM and glucose 8.3 mM; pH 7.4. The vessel was cut into rings of approximately 4 mm of length and suspended on stainless steel hooks in 15 ml organ baths containing the Krebs bicarbonate solution, aerated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  and maintained at 37°C. Vessel segments containing macroscopically visible atherosclerotic lesions were not used in the present study. The segments were allowed to equilibrate for at least 30 min and washed every 15 min. Changes in tension were recorded using a Harvard isometric transducer. Preparations were stretched to a stable pre-tension of 20 mN. The tissue was exposed to  $\text{K}^+$  (30 mM) twice. Subsequently, the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) after precontraction with prostaglandin  $\text{F}_{2a}$  ( $\text{PGF}_{2a}$ , 1  $\mu\text{M}$ ). After washout, the tissue was exposed to  $\text{K}^+$  100 mM to determine the maximal contractile response to  $\text{K}^+$ . The tissue was then allowed to equilibrate in the Krebs solution for a further 30 min.

**Determination of agonist potency.** After equilibration, a cumulative concentration response curve was obtained with either avitriptan or sumatriptan. The responses were expressed as increase of tension (mN) as well as percentage of  $\text{K}^+$  (100 mM)-induced contractions. Curves were obtained in a paired, parallel experimental set-up.

Where more vessel segments from one heart donor were used for avitriptan or sumatriptan, the curves were averaged for the agonist tested and considered in further analysis as one curve. Curves covering the full sigmoidal range were analysed by means of a computerized curve fitting technique (DeLean et al. 1978) to obtain  $E_{\text{max}}$  (maximal response) and  $\text{pD}_2$  (negative logarithm of the molar concentration of an agonist needed to reach half of its  $E_{\text{max}}$ , i.e.  $-\log EC_{50}$ ) values, which were averaged for the respective agonists.

#### Ethical approval

The protocols for the two parts of the investigation were approved by the joint Ethical Committees of the Erasmus University Rotterdam and the University Hospital Rotterdam 'Dijkzigt', dealing with the use of animals and humans in scientific experiments.

#### Data presentation and statistical analysis

All data have been expressed as means  $\pm$  SEM. In the haemodynamic study, the significance of the changes (from baseline values) induced by the different doses of avitriptan (or vehicle) was evaluated with Duncan's new multiple range test, once an analysis of variance (ran-

domized block design) had revealed that the samples represented different populations. The changes caused by avitriptan (10, 30, 100 or 300  $\mu\text{g}\cdot\text{kg}^{-1}$ ) were compared with those in the vehicle group by using Student's unpaired *t*-test. The dose of avitriptan eliciting a 50% decrease ( $ED_{50}$ ) in arteriovenous anastomotic blood flow was calculated using linear regression analysis. In the isolated coronary artery studies, the significance of difference between the  $E_{\text{max}}$  and  $\text{pD}_2$  values of sumatriptan and avitriptan was calculated by Student's paired *t*-test. Statistical significance was accepted at  $P < 0.05$  (two-tailed).

#### Compounds

Apart from the anaesthetics, azaperone, metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), midazolam hydrochloride (Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and pentobarbitone sodium (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: prostaglandin  $\text{F}_{2a}$  (Tris salt) and substance P acetate (both purchased from Sigma Chemical Co., St. Louis, Mo., USA); sumatriptan succinate (Glaxo Group Research, Ware, Herts, UK), avitriptan (BMS-180048; 3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-*N*-methyl-1*H*-indole-5-methanesulfonamide monofumarate; Bristol-Myers Squibb, Wallingford, Conn., USA) and heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) to prevent clotting of the catheters. For in vitro experiments, all compounds (substance P, prostaglandin  $\text{F}_{2a}$ , sumatriptan and avitriptan) were dissolved in distilled water, while for in vivo experiments avitriptan was dissolved in physiological saline and all doses refer to the salt.

## Results

### Systemic and carotid haemodynamics in anaesthetized pigs

#### Systemic haemodynamics

Heart rate decreased moderately, but significantly, in pigs treated with the highest 3 doses of either physiological saline (1, 2 and 4 ml) or avitriptan (30, 100 and 300  $\mu\text{g}\cdot\text{kg}^{-1}$ , i.v.). However, the decrease in heart rate with avitriptan was significantly more than that with the corresponding dose of saline (Table 2). Mean arterial blood pressure decreased slightly in both saline (highest 2 doses) and avitriptan (highest dose) groups. The change in the mean arterial blood pressure with avitriptan was not significantly different from that in the saline group (Table 2).

#### Arterio-jugular venous oxygen saturation difference

Avitriptan elicited an increase in the arterio-jugular venous oxygen saturation difference with the two highest doses (Table 2).

#### Carotid haemodynamics

The total carotid blood flow and its arteriovenous anastomotic fraction were significantly decreased by the 3 highest doses of avitriptan; the capillary fraction increased significantly with the 2 highest doses. Treatment with physiological saline did not produce any significant change in the distribution of carotid blood flow (Fig. 2).

**Table 2** Absolute values of heart rate, mean arterial blood pressure and difference in arterial and jugular venous oxygen saturation at baseline and after cumulative doses of avitriptan ( $n = 7$ ) and its vehicle (physiological saline;  $n = 7$ ). Percent changes from baseline in each variable is given in brackets

Pretreatment	Avitriptan ( $\mu\text{g kg}^{-1}$ )				
	Baseline	10	30	100	300
Heart rate (beats $\text{min}^{-1}$ )					
Saline (control) <sup>a</sup>	84 $\pm$ 3	83 $\pm$ 3 (-2 $\pm$ 1)	81 $\pm$ 3 (-4 $\pm$ 1) <sup>b</sup>	80 $\pm$ 3 (-5 $\pm$ 2) <sup>b</sup>	78 $\pm$ 3 (-7 $\pm$ 2) <sup>b</sup>
Avitriptan	90 $\pm$ 4	85 $\pm$ 5 (-6 $\pm$ 2)	79 $\pm$ 5 (-12 $\pm$ 3) <sup>b,c</sup>	77 $\pm$ 5 (-14 $\pm$ 4) <sup>b,c</sup>	75 $\pm$ 5 (-17 $\pm$ 4) <sup>b,c</sup>
Mean arterial blood pressure (mmHg)					
Saline	98 $\pm$ 6	95 $\pm$ 6 (-3 $\pm$ 1)	94 $\pm$ 5 (-4 $\pm$ 2)	88 $\pm$ 7 (-11 $\pm$ 4) <sup>b</sup>	86 $\pm$ 7 (-13 $\pm$ 3) <sup>b</sup>
Avitriptan	93 $\pm$ 5	91 $\pm$ 4 (-2 $\pm$ 2)	90 $\pm$ 5 (-2 $\pm$ 4)	88 $\pm$ 5 (-4 $\pm$ 6)	80 $\pm$ 5 (-13 $\pm$ 6) <sup>b</sup>
Arteriovenous difference in oxygen saturation (%)					
Saline	6.7 $\pm$ 1.3	7.5 $\pm$ 2.0 (4 $\pm$ 12)	7.0 $\pm$ 1.7 (3 $\pm$ 12)	7.9 $\pm$ 1.9 (23 $\pm$ 23)	9.2 $\pm$ 1.7 (52 $\pm$ 24)
Avitriptan	4.4 $\pm$ 0.5	5.1 $\pm$ 1.2 (19 $\pm$ 31)	6.9 $\pm$ 1.1 (62 $\pm$ 27)	9.5 $\pm$ 1.9 (122 $\pm$ 51) <sup>b</sup>	12.5 $\pm$ 2.5 (182 $\pm$ 70) <sup>b</sup>

All values are presented as means  $\pm$  SEM. <sup>a</sup> The doses of saline, corresponding to avitriptan doses, were 0.5, 1, 2 and 4 ml. <sup>b</sup> Significant difference ( $P < 0.05$ ) from the baseline value; <sup>c</sup> % change from baseline significantly different ( $P < 0.05$ ) from that in the saline group

**Fig. 2** Effect of avitriptan (upper panels) and physiological saline (lower panels) on the total carotid blood flow and its arteriovenous anastomotic (AVAs) and nutrient (capillary) fractions in anaesthetized pigs. All values are presented as means  $\pm$  SEM. \*,  $P < 0.05$  vs baseline

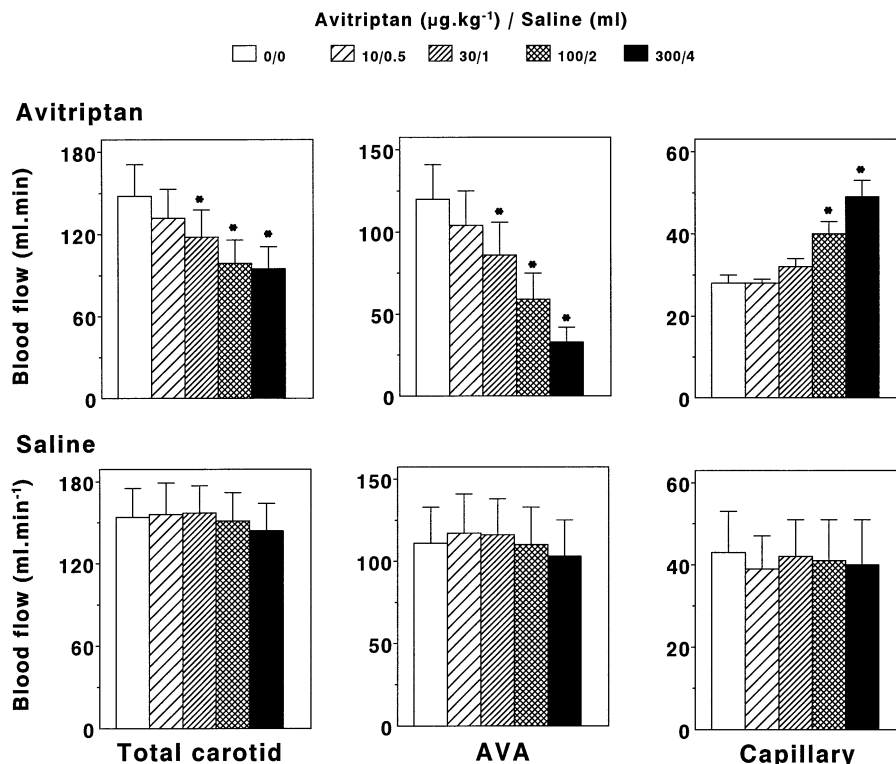
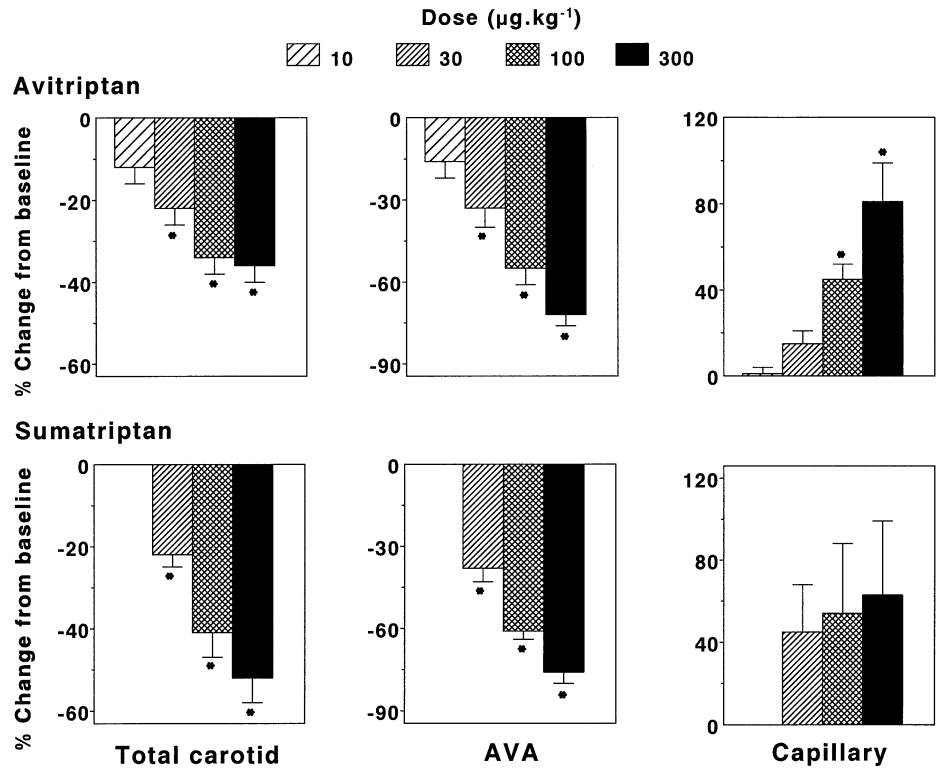


Figure 3 presents the % changes produced by the different doses of avitriptan (upper panels) in the carotid blood flow and its distribution into arteriovenous anastomotic and capillary fractions; the changes observed with sumatriptan in recent experiments using the same experimental model (data from De Vries et al. 1996; lower panels) are presented for comparison. The  $\text{ED}_{50}$  of avitriptan in decreasing arteriovenous anastomotic blood flow was calculated to be  $76 \pm 23 \mu\text{g}\cdot\text{kg}^{-1}$  ( $132 \pm 40 \text{ nmol}\cdot\text{kg}^{-1}$ ) and with the highest dose ( $300 \mu\text{g}\cdot\text{kg}^{-1}$ ) the decrease amounted to be  $72 \pm 4\%$ . Sumatriptan also decreased the total carotid and arteriovenous anastomotic blood flows, but the increase in the capillary blood flow was not statistically significant (De Vries et al. 1996). The  $\text{ED}_{50}$  of su-

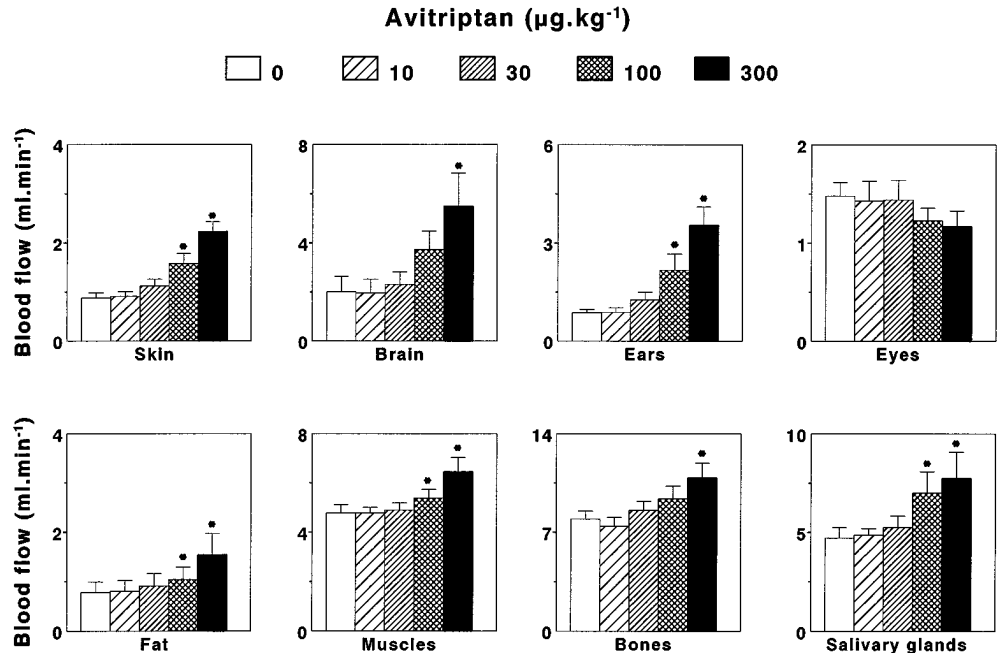
matriptan in decreasing arteriovenous anastomotic blood flow was  $63 \pm 17 \mu\text{g}\cdot\text{kg}^{-1}$  ( $158 \pm 43 \text{ nmol}\cdot\text{kg}^{-1}$ ) and the highest dose used ( $300 \mu\text{g}\cdot\text{kg}^{-1}$ ) decreased arteriovenous anastomotic blood flow by  $76 \pm 4\%$ . Thus, the two compounds, avitriptan and sumatriptan were both equipotent and equi-effective in decreasing porcine carotid arteriovenous anastomotic blood flow.

The changes in the distribution of carotid blood flow to the head tissues by avitriptan are depicted in Fig. 4. Avitriptan ( $\geq 100 \mu\text{g}\cdot\text{kg}^{-1}$ ) increased the fraction of carotid blood flow distributed to the skin, brain, ears, fat, muscles, bones and salivary glands; the fraction distributed to the eye did not change significantly.

**Fig. 3** Percent changes from baseline values by avitriptan (*upper panels*) in the total carotid blood flow and its arteriovenous anastomotic (AVAs) and nutrient (capillary) fractions in anaesthetized pigs. For comparison, data of sumatriptan (*lower panels*;  $n = 5$ ), obtained in a recent investigation (De Vries et al. 1996), have been included. All values are presented as means  $\pm$  SEM \*,  $P < 0.05$  vs baseline



**Fig. 4** Effect of avitriptan on the distribution of carotid blood flow to different cranial tissues in anaesthetized pigs. All values are presented as means  $\pm$  SEM, \* $P < 0.05$  vs baseline



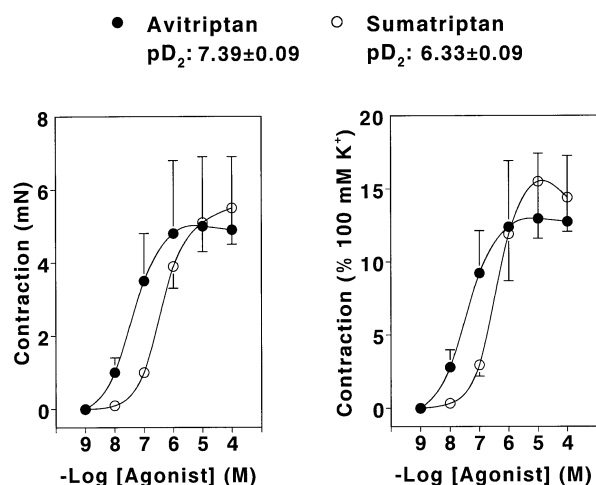
**Human isolated coronary artery**

*Effect of substance P and K<sup>+</sup>*

Coronary vessel segments relaxed to substance P (1 nM) after precontraction with PGF<sub>2 $\alpha$</sub>  (1  $\mu$ M) with  $82.5 \pm 9.8\%$  of the contractile responses to PGF<sub>2 $\alpha$</sub> . K<sup>+</sup> (100 mM) caused a mean contractile response of  $40.9 \pm 7.4$  mN.

*Effect of sumatriptan and avitriptan*

As shown in Fig. 5, the human isolated coronary artery contracted in a concentration-dependent manner to both sumatriptan ( $pD_2$ :  $6.33 \pm 0.09$ ;  $E_{max}$ :  $5.5 \pm 1.0$  mN or  $15.5 \pm 3.9\%$  of contractions to 100 mM K<sup>+</sup>) and avitriptan ( $pD_2$ :  $7.39 \pm 0.09$ ;  $E_{max}$ :  $5.0 \pm 1.9$  mN or  $13.0 \pm 4.5\%$  of contractions to 100 mM K<sup>+</sup>). The  $pD_2$  of avitriptan was



**Fig. 5** Human isolated coronary artery. Contractile responses to sumatriptan (8 segments from 7 donors) and avitriptan (11 segments from the same 7 donors), expressed both in mN (left panel) and as % of the response to 100 mM K<sup>+</sup> (right panel)

significantly higher than that of sumatriptan, but the  $E_{max}$  of the two drugs did not differ significantly. Both drugs appeared to elicit contractions at the same pace.

## Discussion

### Systemic haemodynamic changes

Although in the control (saline) experiments heart rate was decreased, the decreases in heart rate by avitriptan ( $-12 \pm 3\%$ ,  $-14 \pm 4$  and  $-17 \pm 4\%$  after 30, 100 and 300  $\mu\text{g}\cdot\text{kg}^{-1}$ , respectively) were more than in saline experiments. A similar bradycardic effect has also been noticed with sumatriptan (Feniuk et al. 1989; Den Boer et al. 1991a, 1992; DeVries et al. 1996). The mechanism involved in the decrease in heart rate by sumatriptan or avitriptan is not clear, but it may be related to presynaptic inhibition of sympathetic neurons (Humphrey et al. 1988, 1990) or central 5-HT<sub>1A</sub> receptor activation (Dreteler et al. 1989; Saxena and Villalón 1990; see Table 1). However, in any case, bradycardia following the use of sumatriptan in patients seems to be of little clinical relevance (Saxena and Tfelt-Hansen 1993) and the same seems to be true for avitriptan, which is undergoing clinical trials (Goldstein 1995).

Significantly, the fact that avitriptan did not produce changes in mean arterial blood pressure implies that, like sumatriptan (Humphrey et al. 1988, 1990; Den Boer et al. 1991a), the drug has a more selective vasoconstrictor action on cranial blood vessels than ergotamine, which elicits a hypertensive response (Saxena and DeVlaam-Schluter 1974; Den Boer et al. 1991b).

### Carotid haemodynamic changes

In the past we have reported that no significant changes in the carotid haemodynamics are observed after treatment with saline (e.g. Saxena and Verdouw 1982; Den Boer et al. 1991a). The same was the case in the present experiments with saline (see Fig. 2). On the other hand, like sumatriptan (Perren et al. 1989; Den Boer et al. 1991a; DeVries et al. 1996), avitriptan elicited a reduction in the total carotid blood flow, which was exclusively due to the decrease in its arteriovenous anastomotic fraction; the capillary fraction distributed to several head tissues in fact increased slightly. In conformity with the reduction of arteriovenous anastomotic blood flow, avitriptan significantly increased the arterio-jugular venous oxygen saturation difference with the 2 highest doses. The vasoconstrictor effect of avitriptan on porcine carotid arteriovenous anastomoses were similar to those observed with sumatriptan in the same experimental model (Fig. 3; Den Boer et al. 1991a; DeVries et al. 1996); the ED<sub>50</sub> values ( $132 \pm 40$  and  $158 \pm 43$   $\text{nmol}\cdot\text{kg}^{-1}$ , i.v., respectively) and the maximal decreases ( $72 \pm 4$  and  $76 \pm 4\%$ , respectively, with 300  $\mu\text{g}\cdot\text{kg}^{-1}$ , i.v.) in the case of the two drugs were close to each other.

Although we have made no attempt to analyse the mechanism of action involved in the porcine carotid vascular changes by avitriptan, it is reasonable to expect that, as in the case of sumatriptan (Saxena et al. 1986; DeVries et al. 1996), 5-HT<sub>1B/1D</sub> receptors may mediate the responses to avitriptan; indeed, avitriptan has a similar affinity as sumatriptan for human 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors (see Table 1). Furthermore, this compound is more potent (2.5–7 times) than sumatriptan in constricting bovine middle cerebral artery (pD<sub>2</sub>: 6.72 vs. 6.17;  $E_{max}$ : 44 vs. 50% of the response to 5-HT) and guinea-pig iliac artery (pD<sub>2</sub>: 7.83 vs. 6.96;  $E_{max}$ : 58 vs. 74% of the response to 5-HT) and these effects of avitriptan are amenable to blockade by methiothepin, but not ketanserin (Goggins et al. 1996; Unpublished data on Bristol-Myers Squibb files).

### Human isolated coronary artery

Sumatriptan has been reported to constrict the human coronary artery, both in vivo (MacIntyre et al. 1993) and in vitro (Connor et al. 1989; Chester et al. 1990; Bax et al. 1993; Bax and Saxena 1993). In the present experiments with the human isolated coronary, avitriptan also showed a contractile effect with a pD<sub>2</sub> of  $7.39 \pm 0.09$ . As in the other vascular preparations containing 5-HT<sub>1</sub>-like (5-HT<sub>1B/1D</sub>) receptors in vitro (see above), avitriptan was 11 times more potent in the human isolated coronary artery than sumatriptan (pD<sub>2</sub>:  $6.33 \pm 0.09$ ). It is known that the sumatriptan-induced contractions of the human isolated coronary artery are mediated by 5-HT<sub>1</sub>-like receptors (Connor et al. 1989; Bax et al. 1993), which may resemble the 5-HT<sub>1B</sub> receptor subtype (Kaumann et al. 1994). Since the pK<sub>i</sub> values of avitriptan and sumatriptan at the human 5-HT<sub>1B</sub> receptor (7.68 and 7.54, respectively) are similar, the

11-fold difference in the potency of the two compounds (using a paired, parallel experimental design; see methods) suggests that, apart from the 5-HT<sub>1B</sub> receptor, other 5-HT receptors (5-HT<sub>1D</sub>, 5-HT<sub>1F</sub>?) probably also participate in mediating the contraction of the human coronary artery. An analysis of this response using newer 5-HT receptor ligands may shed further light into the receptor mechanisms involved.

### Clinical perspectives

Over the years it has been shown that a number of drugs effective in aborting migraine headaches, including the ergot alkaloids, ergotamine and dihydroergotamine (Johnston and Saxena 1978; Schamhardt et al. 1979; Spierings and Saxena 1980; Den Boer et al. 1991b), sumatriptan (Den Boer et al. 1991a) as well as a number of second generation 5-HT<sub>1B/1D</sub> receptor agonists undergoing clinical evaluation in migraine (e.g., zolmitriptan, rizatriptan and avitriptan; Boulanger et al. 1995; Martin and Dixon 1995; Saxena and Ferrari 1996), are able to constrict carotid arteriovenous anastomoses, which may open up during the headache phase of migraine (see Heyck 1969; Saxena 1990, 1995; Ferrari and Saxena 1993). Since avitriptan constricted carotid arteriovenous anastomoses, it is suggested that avitriptan should also be effective in aborting headaches in migraine patients. Preliminary clinical findings indeed seem to confirm this (Goldstein et al. 1996). The results obtained in the human isolated coronary artery, however, also suggest that, like sumatriptan, avitriptan may be contra-indicated in patients with coronary artery disease.

Lastly, it may be remarked that although the experimental models that we have used seem to be predictive of therapeutic activity in migraine patients (porcine arteriovenous anastomotic constriction) as well as the coronary side-effect liability (human isolated coronary artery contraction), we cannot directly calculate and compare the 'therapeutic window' of potential antimigraine drugs in precise quantitative terms. As is quite obvious, the drug dosage, efficacy and tolerability in patients will depend on a host of factors, including the pharmacokinetic parameters (oral absorption, tissue distribution, metabolic disposition and plasma half-life), precise nature of receptors involved, severity of migraine and presence of concomitant disease processes.

In conclusion, the results of the present experiments show that avitriptan, a 5-HT<sub>1B/1D</sub> receptor agonist, causes constriction of porcine carotid arteriovenous anastomoses (suggestive of therapeutic activity in migraine) and the human isolated coronary artery (possibly indicative of coronary side-effects). Both these pharmacological effects are broadly similar to those of sumatriptan.

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