Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs

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1 Vasoconstriction of carotid arteriovenous anastomoses may be involved in the therapeutic action of acutely acting antimigraine agents, including the triptans and ergot alkaloids. While $5\text{-HT}_{1B/1D}$ receptors mediate the effect of triptans, ergotamine and dihydroergotamine also interact with α-adrenoceptors. In the present study, we investigated the potential role of α_1- and α_2-adrenoceptors in mediating vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs.

2 Ten minute intracarotid infusions of phenylephrine (1, 3 and 10 μg kg^{-1} min^{-1}) or BHT 933 (3, 10 and 30 μg kg^{-1} min^{-1}) produced dose-dependent decreases in total carotid and arteriovenous anastomotic conductances; no changes were observed in the capillary fraction.

3 The carotid vascular effects of phenylephrine and BHT 933 were selectively abolished by prazosin (100 μg kg^{-1}, i.v.) and rauwolscine (300 μg kg^{-1}, i.v.), respectively. The responses to phenylephrine and BHT 933 were not affected by the selective $5\text{-HT}_{1B/1D}$ receptor antagonist GR127935 (500 μg kg^{-1}, i.v.).

4 These results show that both α_1- and α_2-adrenoceptors can mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. Since vasoconstrictor activity in this *in vivo* model is predictive of anti-migraine activity, an agonist activity at particularly the α_2-adrenoceptor subtypes, in view of their less ubiquitous nature, could provide migraine abortive potential. Thus, the present results may aid further understanding of the mode of action of some current anti-migraine agents and may eventually be helpful in the development of future treatment in migraine.

Introduction

Vasodilatation of carotid arteriovenous anastomoses may be involved in the pathophysiology of migraine headache (Heyck, 1969; Saxena, 1995). In line with this proposal, it has previously been shown that several acutely acting anti-migraine agents, including the ergots (ergotamine and dihydroergotamine) and triptans, potently vasoconstrict porcine carotid arteriovenous anastomoses predominantly *via* $5\text{-HT}_{1B/1D}$ receptors (De Vries *et al.*, 1999; Villalón *et al.*, 1999). Interestingly, the carotid vasoconstrictor effect of ergot alkaloids involves, in addition to $5\text{-HT}_{1B/1D}$ receptors, unidentified receptor mechanisms (De Vries *et al.*, 1998, 1999). Since the ergot derivatives display high affinity at α-adrenoceptors (Leysen, 1985), their therapeutic efficacy may be partly explained by an action mediated *via* these receptors. Indeed, we recently reported that canine external carotid vasoconstriction by ergot alkaloids also involves α-adrenoceptors (Villalón *et al.*, 1999). In this context, the possible involvement of α-adrenoceptors in the porcine carotid

vasculature has been hampered, mainly on the basis that porcine carotid arteriovenous anastomoses were described to be insensitive to sympathetic nerve stimulation or intracarotid infusions of noradrenaline (Verdouw *et al.*, 1984). This discrepancy is striking since in conscious pigs arteriovenous anastomoses are under a vasoconstrictor sympathetic tone (Hales, 1974; Van Woerkens *et al.*, 1990; Den Boer *et al.*, 1993). In fact, sympathetic nerve stimulation as well as exogenously administered noradrenaline causes α-adrenoceptor-mediated vasoconstriction of arteriovenous anastomoses in the hind limb of several species (Folkow & Sivertsson, 1964; Spence *et al.*, 1972; Hales, 1974; Baker *et al.*, 1978; Hales *et al.*, 1982).

In the light of the above, the present study was designed to investigate the potential role of α_1- and α_2-adrenoceptors in the vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. For this purpose, we made use of the selective agonists, phenylephrine (α_1) and BHT 933 (6-ethyl-5,6,7,8-tetrahydro-4H-oxazolo[4,5-d]azepin-2-amine dihydro-chloride; α_2), and the selective antagonists, prazosin (α_1), rauwolscine (α_2) (see Ruffolo *et al.*, 1991; Piascik *et al.*, 1996),

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in a well-defined *in vivo* model predictive of anti-migraine activity (Saxena, 1995; De Vries *et al*., 1999). In addition, GR127935 (N-[methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide was used to exclude the possible role of 5-HT$_{1B/1D}$ receptors (Clitherow *et al*., 1994; De Vries *et al*., 1996; Skingle *et al*., 1996). The results obtained may provide useful information concerning the mode of action of some currently used anti-migraine agents and, possibly, new avenues for the development of anti-migraine agents.

Methods

General

After an overnight fast, 40 domestic pigs (Yorkshire × Landrace; 10–14 kg) were anaesthetized with azaperone (120 mg, i.m.), midazolam hydrochloride (5 mg, i.m.) and sodium pentobarbitone (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35–7.48; pCO$_2$: 35–48 mmHg; pO$_2$: 100–120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbitone (20 mg kg^{-1} h^{-1}). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy (see below), leads to an increase in heart rate and vasodilatation of arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbitone-anaesthetized pigs (70–80% of carotid blood flow) than in those under fentanyl/thiopental anaesthesia (~19% of carotid blood flow) (Den Boer *et al*., 1993). A high basal carotid arteriovenous anastomotic flow is particularly useful for investigating the effects of drugs that vasoconstrict arteriovenous anastomoses.

A catheter was placed in the inferior vena cava *via* the left femoral vein for infusion of antagonists and sodium pentobarbitone. Another catheter was placed in the aortic arch *via* the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, the right common carotid artery and the external jugular vein were dissected free and the accompanying vagosympathetic trunks were cut between two ligatures in order to prevent a possible influence *via* baroreceptor reflexes on agonist-induced carotid vascular responses. The right external jugular vein was catheterized for withdrawal of venous blood samples for determining blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Two hub-less needles, each connected to a polyethylene tube, were inserted into the right common carotid artery and used for agonist (phenylephrine or BHT 933) infusion and radioactive microspheres injection, respectively. The microspheres were injected against the direction of blood flow for uniform mixing.

Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram

signals. Arterial blood pressure, heart rate and right common carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept around 37°C and the animal was continuously infused with saline to compensate for fluid loss.

Distribution of carotid blood flow

The distribution of common carotid blood flow was determined with 15.5±0.1 *μ*m (s.d.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, U.S.A.). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbitone and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 10 min in a *γ*-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120–167, KeV, ^{113}Sn: 355–435 KeV, ^{103}Ru: 450–548 KeV, ^{95}Nb: 706–829 KeV and ^{46}Sc: 830–965 KeV). All data were processed by a set of specially designed programs (Saxena *et al*., 1980). The fraction of carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the total common carotid blood flow at the time of the injection of the microspheres labelled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping *via* carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an index of the arteriovenous anastomotic fraction of the common carotid blood flow (Saxena & Verdouw, 1982). Vascular conductance (10^{-2} ml min^{-1} mmHg^{-1}) was calculated by dividing blood flow (ml min^{-1}) by blood pressure (mmHg), multiplied by hundred.

Experimental protocol

After a stabilization period of about 60 min, baseline values of heart rate, mean arterial blood pressure, common carotid blood flow and its distribution, as well as arterial and jugular venous blood gases were measured. Thereafter, the animals were divided into four groups receiving i.v. infusions (0.5 ml min^{-1} for 10 min) of either distilled water (vehicle; $n=14$), prazosin (100 *μ*g kg^{-1}; $n=10$), rauwolscine (300 *μ*g kg^{-1}; $n=10$) or GR127935 (500 *μ*g kg^{-1}; $n=6$). After a waiting period of 15 min, all variables were reassessed. Subsequently, each group was divided into two subgroups receiving 10-min intracarotid infusions (0.1 ml min^{-1}) of phenylephrine (cumulative doses: 1, 3, and 10 *μ*g kg^{-1} min^{-1}) or BHT 933 (cumulative doses: 3, 10 and 30 *μ*g kg^{-1} min^{-1}). The number of animals in the different groups receiving phenylephrine and BHT 933 infusions were: vehicle ($n=7$ each), prazosin ($n=7$ and 3, respectively), rauwolscine ($n=3$ and 7, respectively) and GR127935 ($n=3$ each). All variables were collated again 10 min after the start of each agonist infusion.

It may be pointed out here that GR127935, which is a potent and selective 5-HT$_{1B/1D}$ receptor antagonist (Clitherow *et al*., 1994; De Vries *et al*., 1996; Skingle *et al*., 1996), was used to rule out the remote possibility that the effects of phenylephrine and/or BHT 933 were mediated *via* 5-HT$_{1B/1D}$

receptors. The effectiveness of the blockade of 5-HT$_{1B/1D}$ receptors by GR127935 was confirmed by evaluating decreases in the total carotid blood flow and conductance by sumatriptan (De Vries *et al.*, 1996). Sumatriptan (30, 100 and 300 μg kg^{-1}, i.v.; every 10 min over a period of 5 min each) was administered in animals treated with GR127935 after a near complete recovery from the effects of the last dose of phenylephrine or BHT 933 had been achieved ($\sim$90 min).

Data presentation and statistical analysis

All data have been expressed as the mean $\pm$ s.e.mean. The per cent changes from baseline (i.e. after treatment with vehicle, prazosin, rauwolscine and GR127935) values caused by the different doses of phenylephrine or BHT 933 within each group of animals were calculated. A two-way repeated measures ANOVA with Bonferroni's correction (SigmaStat 1.0, Jandel Corporation, Chicago, IL, U.S.A.) was used to establish whether these changes were statistically significant ($P<0.05$, two-tailed) when compared with the baseline in each group as well as with the corresponding agonist dose in the vehicle-treated group.

Drugs

Apart from the anaesthetics azaperone (Stresnil®; Janssen Pharmaceuticals, Beerse, Belgium), midazolam hydrochloride (Dormicum®; Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and sodium pentobarbitone (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: prazosin hydrochloride (Bufa Chemie b.v., Castricum; The Netherlands), GR127935, sumatriptan succinate (both from GlaxoWellcome, Ware, Herts, U.K.; courtesy: Dr H.E. Connor), phenylephrine hydrochloride, BHT 933 and rauwolscine dihydrochloride (all from Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands). Finally, heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of blood in the catheters. All drugs were dissolved in distilled water. For prazosin, rauwolscine and GR127935 a short period of heating was needed. The doses of the drugs refer to their respective salts.

Ethical approval

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

Results

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

As shown in Table 1, the administration of vehicle (5 ml of distilled water) did not produce any change in systemic or carotid haemodynamic variables. On the other hand, prazosin (100 μg kg^{-1}, i.v.), rauwolscine (300 μg kg^{-1}, i.v.) and GR127935 (500 μg kg^{-1}, i.v.) decreased mean arterial blood pressure by 7 ± 2, 9 ± 3 and $13\pm2\%$, respectively, which only in the case of rauwolscine was accompanied by a significant decrease in arteriovenous anastomotic blood flow by $18\pm5\%$. Additionally, rauwolscine increased capillary blood flow and conductance by $24\pm7\%$ and $38\pm9\%$, respectively. These effects of rauwolscine were noticed in several tissues (ear, skin, muscle, bones, salivary gland, fat and tongue; data not shown). No other significant changes were noticed with prazosin, rauwolscine or GR127935.

Systemic haemodynamic changes by phenylephrine and BHT 933

Systemic haemodynamic variables measured before and after the two agonists in animals treated with vehicle, prazosin, rauwolscine or GR127935 are shown in Table 2. In animals treated with vehicle, phenylephrine elicited an immediate and dose-dependent increase in heart rate by up to $29\pm6\%$. This increase was not affected by treatment with prazosin, rauwolscine or GR127935. BHT 933 did not cause any change in heart rate. Moreover, no changes in mean arterial blood pressure were observed after infusions of phenylephrine or BHT 933.

In vehicle-treated animals, both phenylephrine and BHT 933 showed a trend to increase the difference between arterial and jugular venous oxygen saturation (A-VSO$_2$) by up to 1036 ± 325 and $254\pm140\%$, respectively (Table 2). However, statistical significance was achieved only with the highest dose of phenylephrine. The phenylephrine-induced response was selectively antagonized by treatment with prazosin.

Carotid haemodynamic responses to phenylephrine and BHT 933

Absolute values of total carotid vascular conductance and its arteriovenous anastomotic and capillary fractions before and

Table 1 Effects of vehicle. prazosin, rauwolscine or GR127935 on heart rate (beats min^{-1}), mean arterial blood pressure (mmHg), A-VSO$_2$ (%) and total carotid, AVA and capillary blood flow (ml min^{-1}) and conductance (10^{-2} ml min^{-1} mmHg^{-1}) in anaesthetized pigs ($n=40$)

Variable	*Vehicle* (i.v.; n=14) *Before*	*After*	*Prazosin* (100 μg kg^{-1}; i.v.; n=10) *Before*	*After*	*Rauwolscine* (300 μg kg^{-1}, i.v.; n=10) *Before*	*After*	*GR127935* (500 μg kg^{-1}, i.v.; n=6) *Before*	*After*
Heart rate	102 ± 3	100 ± 2	103 ± 4	100 ± 4	101 ± 4	100 ± 4	97 ± 2	94 ± 3
Mean arterial blood pressure	96 ± 2	93 ± 2	98 ± 2	90 ± 2^a	91 ± 2	82 ± 3^a	102 ± 2	88 ± 2^a
A-VSO$_2$	11 ± 3	11 ± 3	5 ± 2	6 ± 2	7 ± 3	9 ± 2	7 ± 3	7 ± 2
Total carotid blood flow	117 ± 8	116 ± 7	135 ± 6	125 ± 6	124 ± 12	109 ± 10	125 ± 20	108 ± 14
Total carotid conductance	121 ± 7	124 ± 6	139 ± 6	139 ± 7	137 ± 14	133 ± 12	124 ± 22	123 ± 15
AVA blood flow	87 ± 8	87 ± 7	106 ± 8	98 ± 7	97 ± 12	78 ± 10^a	97 ± 17	77 ± 13
AVA conductance	90 ± 7	93 ± 7	109 ± 8	109 ± 8	107 ± 12	94 ± 11	96 ± 17	87 ± 14
Capillary blood flow	30 ± 2	29 ± 2	28 ± 4	27 ± 3	26 ± 3	32 ± 2^a	28 ± 7	31 ± 5
Capillary conductance	31 ± 2	32 ± 4	29 ± 4	30 ± 3	29 ± 3	39 ± 3^a	28 ± 8	35 ± 5

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

after infusions of phenylephrine and BHT 933 in the four groups of animals treated with vehicle, prazosin, rauwolscine or GR127935 are shown in Figures 1 and 2, respectively. In vehicle-treated animals, both phenylephrine and BHT 933 caused dose-dependent decreases in total carotid conductance (maximum change: 74 ± 4 and $59 \pm 4\%$, respectively). These decreases were solely due to changes in arteriovenous anastomotic conductances, since capillary (nutrient) vascular conductance remained unchanged. Phenylephrine-induced changes were absent in animals treated with prazosin, but not in those treated with rauwolscine or GR127935 (Figure 1). On the contrary, BHT 933-induced effects were absent in animals treated with rauwolscine, but not in those treated with prazosin or GR127935 (Figure 2).

In Figure 3 per cent changes (from baseline) observed with phenylephrine and BHT 933 in carotid arteriovenous anastomotic conductance have been compared in the four groups of animals. It is clearly shown that prazosin and rauwolscine selectively blocked the decreases in carotid arteriovenous anastomotic conductance induced by, respectively, phenylephrine and BHT 933. The responses to the two agonists were not affected in animals treated with the potent and selective $5\text{-HT}_{1B/1D}$ receptor antagonist GR127935.

Figure 4 shows the effects of phenylephrine and BHT 933 on tissue vascular conductances in animals treated with vehicle. Neither phenylephrine nor BHT 933 induced any change in vascular conductance in the ear, skin, salivary gland, fat and tongue. On the other hand, phenylephrine decreased vascular conductance in bone, eye and dura mater. These phenylephrine-induced conductance decreases were absent in animals treated with prazosin (data not shown). In vehicle-treated animals (Figure 4), but not in rauwolscine-treated animals (data not shown), BHT 933 increased muscle and brain vascular conductances. The responses to the two agonists were similar in GR127935-treated animals as in animals treated with vehicle (data not shown).

Table 2 Changes in heart rate (beats min^{-1}), mean arterial blood pressure (mmHg) and A-VSO$_2$ (%) induced by intracarotid phenylephrine or BHT 933 in anaesthetized pigs treated i.v. with vehicle (5 ml), prazosin (100 $\mu g\ kg^{-1}$), rauwolscine (300 $\mu g\ kg^{-1}$) or GR127935 (500 $\mu g\ kg^{-1}$)

| | Phenylephrine ($\mu g\ kg^{-1}\ min^{-1}$) | | | | BHT 933 ($\mu g\ kg^{-1}\ min^{-1}$) | | | |
Treatment group	Baseline*	1	3	10	Baseline*	3	10	30
Heart rate								
Vehicle	95 ± 2	99 ± 3	106 ± 3	123 ± 5^a	105 ± 4	104 ± 4	105 ± 4	104 ± 4
Prazosin	98 ± 5	99 ± 5	103 ± 5	115 ± 6^a	96 ± 5	96 ± 5	95 ± 5	95 ± 4
Rauwolscine	91 ± 2	91 ± 3	93 ± 2	111 ± 3^a	102 ± 6	101 ± 5	101 ± 6	99 ± 5
GR127935	94 ± 5	96 ± 6	101 ± 8	118 ± 15^a	96 ± 3	95 ± 3	92 ± 4	93 ± 3
Mean arterial blood pressure								
Vehicle	96 ± 2	97 ± 3	96 ± 4	99 ± 5	89 ± 2	93 ± 3	92 ± 3	94 ± 3
Prazosin	86 ± 3	84 ± 3	83 ± 3	79 ± 3	92 ± 2	94 ± 3	92 ± 4	93 ± 4
Rauwolscine	83 ± 1	82 ± 1	82 ± 2	81 ± 3	79 ± 3	78 ± 3	78 ± 3	79 ± 3
GR127935	86 ± 1	83 ± 1	82 ± 1	84 ± 1	90 ± 1	89 ± 1	85 ± 3	83 ± 5
A-VSO$_2$								
Vehicle	6 ± 2	7 ± 2	13 ± 3	39 ± 6^a	17 ± 4	21 ± 4	24 ± 4	32 ± 2
Prazosin	4 ± 1	4 ± 1	4 ± 1	7 ± 1^b	11 ± 4	20 ± 8	25 ± 6	29 ± 3
Rauwolscine	5 ± 1	6 ± 3	8 ± 2	22 ± 8	12 ± 3	12 ± 2	14 ± 2	14 ± 3
GR127935	6 ± 2	11 ± 1	15 ± 5	33 ± 9	7 ± 4	14 ± 6	25 ± 9	30 ± 6

All values have been presented as the means $\pm$ s.e.mean. *, Values after administration of vehicle, prazosin, rauwolscine or GR127935. $^a P < 0.05$ vs baseline (ANOVA); $^b P < 0.05$ vs vehicle group (ANOVA). The number of animals receiving phenylephrine and BHY 933 in the vehicle, prazosin, rauwolscine and GR127935 group were: phenylephrine (7, 7, 3 and 3, respectively); BHT 933 (7, 3, 7 and 3, respectively).
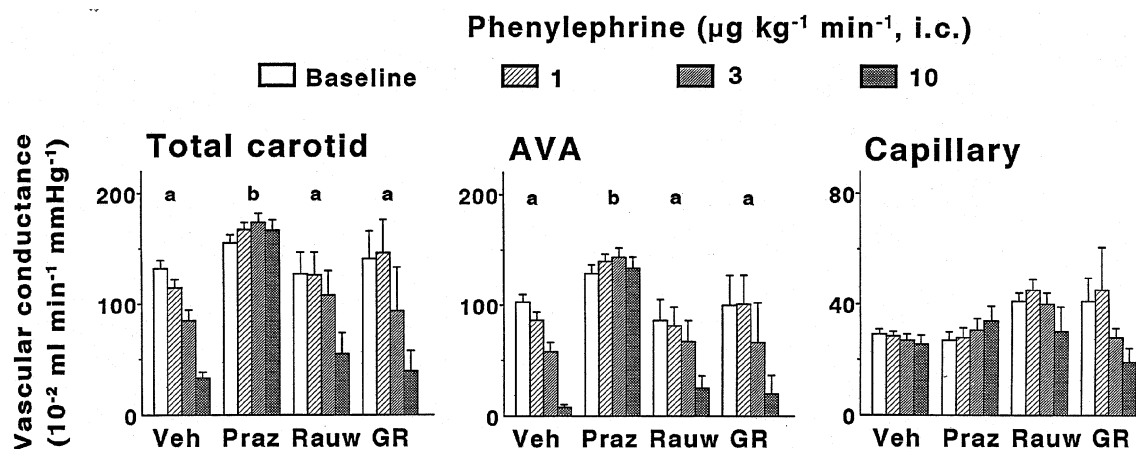

Figure 1 Effects of 10-min intracarotid infusions of phenylephrine on total carotid, arteriovenous anastomotic (AVA) and capillary conductances in anaesthetized pigs treated i.v. with vehicle (Veh; $n=7$), prazosin (Praz; 100 $\mu g\ kg^{-1}$; $n=7$), rauwolscine (Rauw; 300 $\mu g\ kg^{-1}$; $n=3$) or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

Since in the present experiments mean arterial blood pressure was not affected by intracarotid infusion phenylephrine or BHT 933 (Table 2), the above described agonist-induced changes in vascular conductances were qualitatively and quantitatively similar to those observed in blood flow (data not shown).

Carotid vascular responses to sumatriptan in animals treated with GR127935

As reported earlier (De Vries *et al.*, 1996), sumatriptan (30, 100 and 300 μg kg^{-1}, i.v.) did not decrease either total carotid blood flow (% changes: -4 ± 3, -5 ± 3 and -6 ± 3, respectively) or conductance (% changes: 8 ± 4, 5 ± 4 and 3 ± 3, respectively) in animals treated with GR127935. In several previous publications from our laboratory (for references, see De Vries *et al.*, 1998), we have shown that these doses of sumatriptan consistently cause dose-dependent decreases in the total carotid conductance. For example, the changes in the carotid vascular conductance reported by De Vries (1996) with 30, 100 and 300 μg kg^{-1}, i.v. of sumatriptan in vehicle-treated pigs ($n=5$) were -22 ± 4, -42 ± 5 and -49 ± 7, respectively.
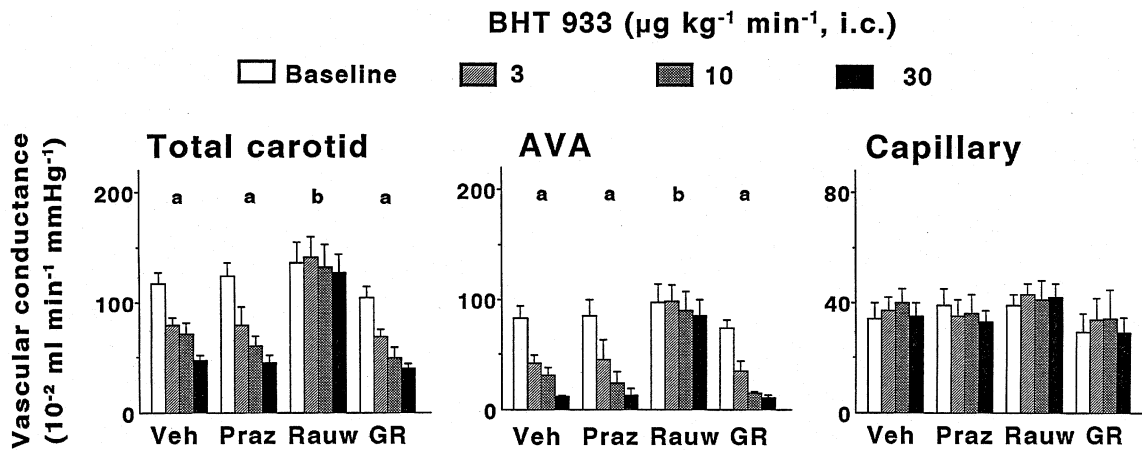

Figure 2 Effects of 10-min intracarotid infusions of BHT933 on total carotid, arteriovenous anastomotic (AVA) and capillary conductances in anaesthetized pigs treated i.v. with either vehicle (Veh; $n=7$), prazosin (Praz; 100 μg kg^{-1}; $n=3$), rauwolscine (Rauw; 300 μg kg^{-1}; $n=7$) or GR127935 (GR; 500 μg kg^{-1} $n=3$). All data are presented as mean$\pm$s.e.mean. $^aP<0.05$ vs baseline (ANOVA); $^bP<0.05$ vs vehicle group (ANOVA).
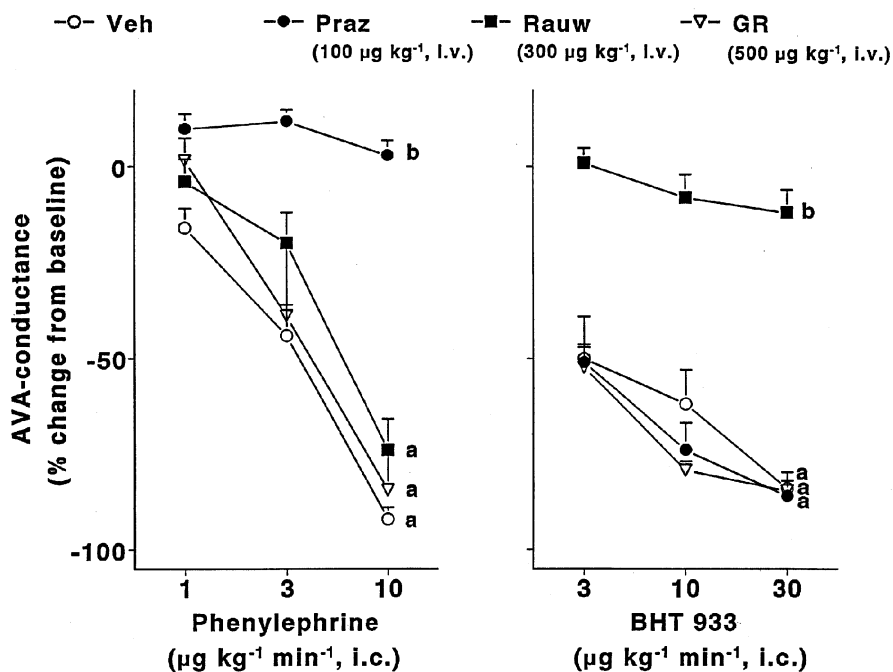

Figure 3 Per cent changes in arteriovenous anastomotic (AVA) conductance induced by 10-min intracarotid infusions of phenylephrine or BHT933 in anaesthetized pigs treated i.v with vehicle (Veh), prazosin (Praz; 100 μg kg^{-1}), rauwolscine (Rauw; 300 μg kg^{-1}) or GR127935 (GR; 500 μg kg^{-1}). All data are presented as mean$\pm$s.e.mean. $^aP<0.05$ vs baseline (ANOVA); $^bP<0.05$ vs vehicle group (ANOVA). The number of animals receiving phenylephrine and BHT933 in the vehicle, prazosin, rauwolscine and GR127935 group were: phenylephrine (7, 7, 3 and 3, respectively); BHT933 (7, 3, 7 and 3, respectively).
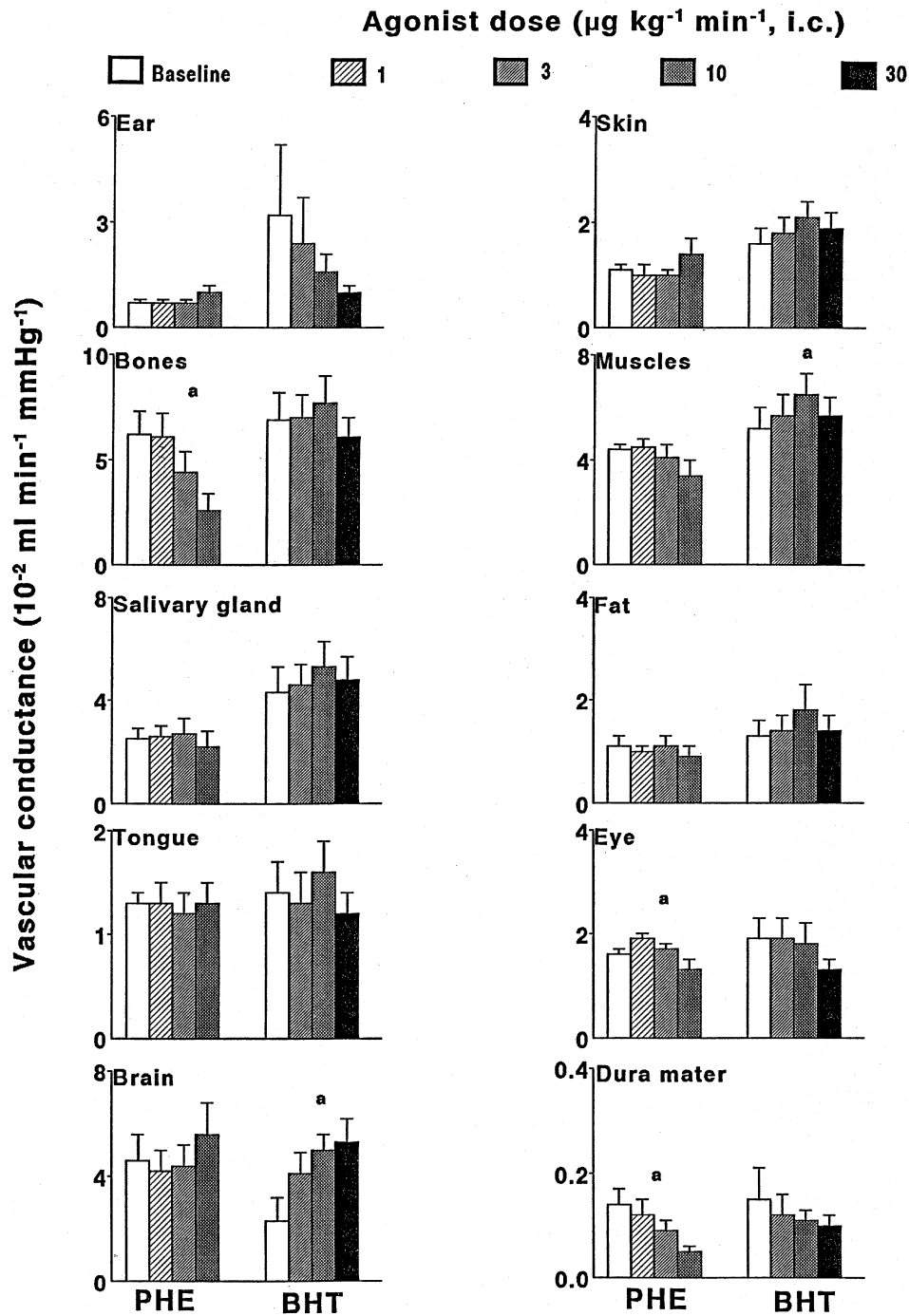
Figure 4 The effect of 10-min intracarotid infusions of phenylephrine (PHE) or BHT933 (BHT) on the distribution of total carotid conductance in different ipsilateral cranial tissues in anaesthetized pigs treated with vehicle ($n = 7$ each). All data are presented as mean ± s.e.mean. [a]$P < 0.05$ vs baseline (ANOVA); [b]$P < 0.05$ vs vehicle group (ANOVA).

Discussion

General

Several *in vitro* studies show that both α_1- and α_2-adrenoceptors mediate vascular effects in carotid vessels, including those of the dog (Kawai *et al.*, 1988) and pig (Ohgushi *et al.*, 1993). Nevertheless, very few studies have investigated whether these receptors are operative in the carotid circulation *in vivo*. Verdouw *et al.* (1984) reported that intracarotid bolus injections of noradrenaline elicited short-lasting and phentolamine-sensitive decreases in carotid and arteriovenous anastomotic conductance in pigs, whereas intracarotid infusions of

noradrenaline were devoid of carotid vasoconstriction. Notwithstanding, the mechanisms involved in this response to noradrenaline were not further analysed, particularly in terms of different subtypes of α-adrenoceptors. Apart from the implications discussed below, the present study clearly shows that both α_1- and α_2-adrenoceptors can mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs.

Systemic and carotid haemodynamic effects of vehicle, prazosin and rauwolscine

Prazosin and rauwolscine produced moderate decreases in mean arterial blood pressure, most likely due to their blocking

properties at, respectively, α_1- and α_2-adrenoceptors (Massingham & Hayden, 1975; Hoffman & Lefkowitz, 1996; Terrón *et al.*, 1996). Importantly, no changes in carotid or arteriovenous anastomotic conductance were observed after treatment with prazosin or rauwolscine. This observation seems to contradict the results of Den Boer *et al.* (1993), who reported that prazosin causes a potent vasodilatation of carotid arteriovenous anastomoses. However, in contrast to their study where fentanyl/thiopentane was employed as anaesthetic, we used sodium pentobarbitone, which attenuates sympathetic tone and potently vasodilates carotid arteriovenous anastomoses (see also the Method section). Interestingly, rauwolscine, but not prazosin, increased capillary conductance, which was noticed in the ear, skin, muscle, bones, salivary gland, fat and tongue. Although we do not have a clear explanation, it may be that α_2-adrenoceptors mainly maintain vascular tone in these cranial tissues. Alternatively, rauwolscine may have activated 5-HT$_{1B/1D}$ receptors, at which the compound displays partial agonist property (Shimamoto *et al.*, 1993). 5-HT$_{1B/1D}$ receptors can indeed mediate vasodilatation in porcine coronary arteries (Schoeffter & Hoyer, 1990) and cranial tissues (De Vries *et al.*, 1996).

Systemic haemodynamic effects of phenylephrine and BHT 933

No changes in blood pressure or heart rate were observed after BHT 933. In contrast, phenylephrine produced a moderate increase in heart rate, which remained essentially unchanged in animals treated with prazosin, rauwolscine or GR127935. Since this tachycardia was absent after propranolol (500 μg kg^{-1}, i.v., $n=3$; unpublished observations), it would appear that phenylephrine has a direct action at cardiac β-adrenoceptors, as shown earlier (Bassett *et al.*, 1968).

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

Intracarotid infusions of phenylephrine and BHT 933 dose-dependently decreased total carotid conductance, which in both cases was exclusively caused by vasoconstriction of carotid arteriovenous anastomoses. Consistent with the closure of arteriovenous anastomoses (Saxena, 1987), both phenylephrine and BHT 933 showed a trend towards increasing A-VSO$_2$; this was however only significant after phenylephrine. The activity of the above agonists implies, but does not categorically prove, that α_1- and α_2-adrenoceptors are involved.

The involvement of α_1-adrenoceptors in the vasoconstriction of carotid arteriovenous anastomoses is strengthened by the finding that prazosin completely blocked the effects of phenylephrine. It should be noted that in the dose employed prazosin acted selectively at α_1-adrenoceptors, since the drug did not modify BHT 933-induced carotid vasoconstriction. α_1-Adrenoceptors also mediate vasoconstriction within the canine external carotid vascular bed (Terrón *et al.*, 1996) and seem to maintain vascular tone in porcine carotid arteriovenous anastomoses (Den Boer *et al.*, 1993). On similar grounds, the fact that rauwolscine completely antagonized BHT 933-induced responses indicates that α_2-adrenoceptors also mediate carotid arteriovenous anastomotic vasoconstriction. This conclusion is substantiated by previous results demonstrating that clonidine vasoconstricts porcine carotid arteriovenous anastomoses (Verdouw *et al.*, 1984) and that α_2-adrenoceptors partly mediate canine external carotid vasoconstriction by ergotamine and dihydroergotamine (Villalón *et al.*, 1999).

Furthermore, prazosin selectively attenuated the phenylephrine-induced increases in A-VSO$_2$.

Taken together, the results of the present study clearly show that activation of both α_1- and α_2-adrenoceptors results in a vasoconstriction of carotid arteriovenous anastomoses. Interestingly, our results seem in contradiction with earlier experiments in pigs, where intracarotid infusions of noradrenaline did not induce carotid vasoconstriction, but rather increased total carotid and arteriovenous anastomotic blood flow (Verdouw *et al.*, 1984). The suggestion that the absence of noradrenaline-induced vasoconstrictor effect was due to the putative α_1-blocking properties of the anaesthetic azaperone (Den Boer *et al.*, 1993) seems not to be the case, as we used similar anaesthetic regimen as Verdouw *et al.* (1984). A more likely explanation could be that noradrenaline, when continuously and slowly infused, also induces a β-adrenoceptor-mediated vasodilator effect in the carotid circulation, which negates carotid vasoconstriction. Interestingly, Cohen & Coffman (1981) have provided evidence for the presence of β-adrenergic vasodilator mechanism in human digital arteriovenous shunts.

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

As pointed out earlier, 5-HT$_{1B/1D}$ receptors mediate the potent and selective vasoconstriction of carotid arteriovenous anastomoses caused by acutely acting anti-migraine drugs in the pig (for references, see Saxena, 1995; De Vries *et al.*, 1999). One could therefore argue that a part of the phenylephrine- and/or BHT 933-induced carotid vasoconstriction might also involve 5-HT$_{1B/1D}$ receptors. This possibility can however be discounted as treatment of the animals with the potent and selective 5-HT$_{1B/1D}$ receptor antagonist GR127935 (Clitherow *et al.*, 1994; Pauwels, 1996; Skingle *et al.*, 1996) did not modify carotid vascular haemodynamic changes induced by either phenylephrine or BHT 933 (see Figures 1–3).

Importantly, the dose of GR127935 used in this study (500 μg kg^{-1}, i.v.) completely blocks sumatriptan-induced decreases in total carotid (and arteriovenous anastomotic) blood flow and conductance (De Vries *et al.*, 1996). This was apparently also the case in our present experiments where no changes in total carotid blood flow and conductance were observed with sumatriptan in animals treated with GR127935. From these data we can exclude the possibility that 5-HT$_{1B/1D}$ receptors play a role in the phenylephrine- or BHT 933-induced vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs.

Effects of phenylephrine and BHT 933 on cranial tissue conductances

Although the effects of α-adrenoceptor stimulation have been extensively studied on isolated blood vessels, the use of intracarotid injection of radiolabelled microspheres allowed us to study these effects in the different cranial tissues *in vivo*. Stimulation of prazosin-sensitive α_1-adrenoceptors resulted in vasoconstriction in bones, dura mater and, to a lesser extent, in the eye, whereas BHT 933 was devoid of tissue vasoconstrictor effects. In fact, BHT 933 produced vasodilatation in brain vasculature, as also reported after clonidine (Verdouw *et al.*, 1984). This may be due to an interaction with endothelial vasodilator α_2-adrenoceptors as previously reported in other blood vessels (Miller & Vanhoutte, 1985; Angus *et al.*, 1986; Ohgushi *et al.*, 1993). Indeed, the increase in brain vascular conductance was absent in the rauwolscine-treated group.

Possible clinical implications

Lastly, we would like to consider the possible clinical relevance of the vasoconstriction of the carotid arteriovenous anastomoses induced by phenylephrine and BHT 933. It has been suggested that vasodilatation of these 'shunt' vessels may play an important role in the pathophysiology of migraine (Heyck, 1969; Saxena, 1995). Indeed, to date all migraine abortive agents vasoconstrict carotid arteriovenous anastomoses in different animal species (for references see Saxena, 1995; Saxena *et al.*, 1997). While carotid arteriovenous anastomoses cannot be directly investigated in humans, sumatriptan has been shown to vasoconstrict arteriovenous anastomoses in the human forearm (Van Es *et al.*, 1995). In addition, ergotamine, dihydroergotamine, clonidine and isometheptene, all of which produce vasoconstriction in the carotid vascular bed in dogs

and pigs (Spierings & Saxena, 1980; Verdouw *et al.*, 1984; De Vries *et al.*, 1998; Villalón *et al.*, 1999), interact with α-adrenoceptors. At least, a part of the therapeutic effect of these drugs may be related to their agonist action at these receptors. Thus, our results demonstrating the role of α_1- and α_2-adrenoceptor in mediating vasoconstriction of carotid arteriovenous anastomoses imply that selective agonists at, particularly, α_2-adrenoceptors (in view of the less ubiquitous nature of α_2-adrenoceptors compared to α_1-adrenoceptors) should have potential anti-migraine properties. In this connection, further studies, which fall beyond the scope of the present investigation, will be required to ascertain the specific subtypes of α_1- (α_{1A}, α_{1B}, α_{1D}) and α_2 (α_{2A}, α_{2B}, α_{2C}) adrenoceptors mediating the vasoconstrictor response mentioned above. Eventually, these results may be helpful in the development of anti-migraine agents in the future.

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