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A61603-induced vasoconstriction in porcine carotid vasculature: involvement of a non-adrenergic mechanism

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Abstract

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

Keywords: A61603; α_1-Adrenoceptor; Arteriovenous anastomoses; Carotid artery; Migraine; Vasoconstriction

1. Introduction

There seems to be little doubt that the headache phase of migraine is associated with dilatation of cranial blood vessels. Indeed, sumatriptan as well as all 'second-generation' triptans potently constrict human isolated cranial arteries as well as carotid arteriovenous anastomoses in anaesthetised animals, mainly via the 5-HT_{1B} receptor (De Vries et al., 1996; Saxena and Tfelt-Hansen, 2000). In an attempt to explore new avenues for the development of antimigraine agents, we recently reported that phenylephrine and 6-ethyl-5,6,7,8-tetrahydro-4*H*-oxazolo [4,5-*d*]azepin-2-amine dihydrochloride (BHT933) constrict carotid arteriovenous anastomoses in anaesthetised pigs via α_1- and α_2-adrenoceptors, respectively (Willems et al., 1999). Subsequent studies suggest that the phenylephrine-induced response is mediated by the α_{1A}- and α_{1B}-adrenoceptor subtypes, but not by the α_{1D} subtype (Willems et al., 2000).

To confirm the involvement of α_{1A}-adrenoceptors, in the present study we studied the effects of a potent and selective α_{1A}-adrenoceptor agonist, *N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methane sulphonamide (A61603) (see Table 1, Knepper et al., 1995; Docherty, 1998), on regional carotid blood flow in anaesthetised pigs. The response to A61603 was characterised by using selective α-adrenoceptor antagonists, 5-methylurapidil (α_{1A}), prazosin (α_1) and a combi-

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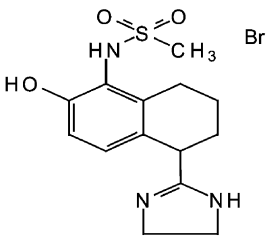
Table 1
Chemical structure and binding affinity (pK_i), potency (pEC_{50}) and intrinsic activity (i.a.), of A61603 at several cloned human receptor subtypes (Craig et al., 1997)
At other receptor subtypes ($H_{1/2}$, $D_{1/2/3}$, 5-$HT_{1/2/7}$ or β), $pK_i < 5.5$.
pEC_{50} was determined in phosphoinositol breakdown assay.
ND, not determined.

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

nation of prazosin (α_1) and rauwolscine (α_2). Similarly, the effects of *N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl)[1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate (GR127935; 5-$HT_{1B/1D}$), ketanserin (5-HT_2, α_1) and methiothepin (5-$HT_{1/2}$), in doses sufficient to block their respective receptors (see Bom et al., 1988; Villalón et al., 1995; Willems et al., 1999), were also investigated. Surprisingly, the results suggest that A61603 constricts porcine arteriovenous anastomoses by a non-adrenergic mechanism.

2. Materials and methods

2.1. General

After an overnight fast, 33 domestic pigs (Yorkshire × Landrace, female, 10–14 kg) were anaesthetised with azaperone (120 mg, i.m.), midazolam hydrochloride (5 mg, i.m.) and sodium pentobarbital (600 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds, 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 mm Hg; pO_2: 100–120 mm Hg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (20 mg kg^{-1} h^{-1}). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy (see below), leads to an increase in heart rate and dilatation of carotid arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal carotid arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbital-anaesthetised pigs (70–80% of carotid blood flow; present results) than in conscious (< 5% of carotid blood flow; Van Woerkens et al., 1990) or fentanyl/thiopental anaesthetised pigs (~ 19% of carotid blood flow; Den Boer et al., 1993). A high basal carotid arteriovenous anastomotic blood flow is particularly useful for investigating the effects of drugs that constrict these 'shunt' vessels.

A catheter was placed in the inferior vena cava via the left femoral vein for infusion of vehicle (distilled water), the antagonists and sodium pentobarbital. Another catheter was placed in the aortic arch via the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, the right common carotid artery was dissected free and bilateral vagosympathectomy was performed in order to prevent a possible influence via baroreceptor reflexes on A61603-induced carotid vascular responses. Two hub-less needles, each connected to a polyethylene tube, used for the administration of radioactive microspheres and A61603, respectively, were inserted into the right common carotid artery against the direction of blood flow for uniform mixing.

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

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

2.2. Distribution of total common carotid blood flow

The distribution of total common carotid blood flow was determined with 15.5 ± 0.1 μm (S.D.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbital and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in 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 right common carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the common carotid blood flow at the time of the injection of microspheres, labelled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping via carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the total common carotid blood flow (Saxena, 1995). Vascular conductance (10^{-2} ml min^{-1} mm Hg^{-1}) was calculated by dividing blood flow (ml min^{-1}) by mean arterial blood pressure (mm Hg) multiplied by 100.

2.3. Experimental protocol

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

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

2.4. Data presentation and statistical analysis

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

2.5. Drugs

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

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

3. Results

3.1. Systemic and carotid haemodynamic variables after different antagonists

Baseline values of these variables in the 33 pigs used in the present investigation were: mean arterial blood pressure, 94 ± 3 mm Hg; heart rate, 101 ± 3 beats min^{-1}; total

Table 2
Absolute values in mean arterial blood pressure, heart rate and total carotid blood flow before and after i.v. administration of vehicle and various antagonists used in anaesthetised pigs
$P < 0.05$ vs. the vehicle-treated group.

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

[a]The corresponding values of total carotid conductance 'Before' and 'After' were not significantly different ($P > 0.05$) for any of the treatments and are not shown in the table.

common carotid blood flow, 133 ± 7 ml min^{-1} and total common carotid conductance, 144 ± 8 10^{-2} ml min^{-1} mm Hg^{-1}. No significant differences were observed between the values of haemodynamic variables collated before and after the administration of the vehicle (distilled water) or different antagonists used in this study (Table 2).

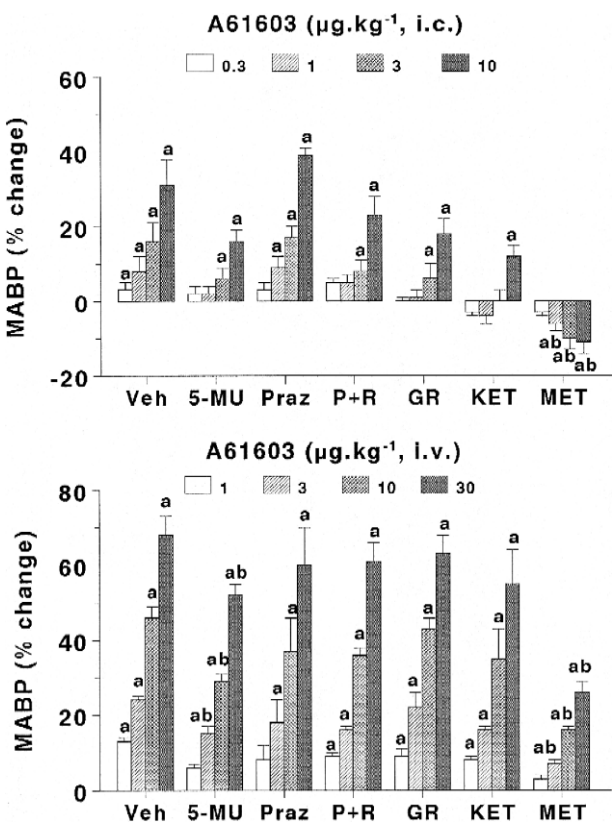


Fig. 1. Changes in mean arterial blood pressure (MABP) following i.c. (upper panel) or i.v. (lower panel) administration of A61603 in anaesthetised pigs treated i.v. with any of the following: vehicle (Veh; 5 ml distilled water), 5-methylurapidil (5-MU; 1000 $\mu g\ kg^{-1}$), prazosin (Praz; 100 $\mu g\ kg^{-1}$), a combination of prazosin and rauwolscine (P + R; 100 and 300 $\mu g\ kg^{-1}$, respectively), GR127935 (GR; 500 $\mu g\ kg^{-1}$), ketanserin (KET; 500 $\mu g\ kg^{-1}$) or methiothepin (MET; 3000 $\mu g\ kg^{-1}$). Data are presented as mean ± S.E.M. a: $P < 0.05$ vs. baseline; b: $P < 0.05$ vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

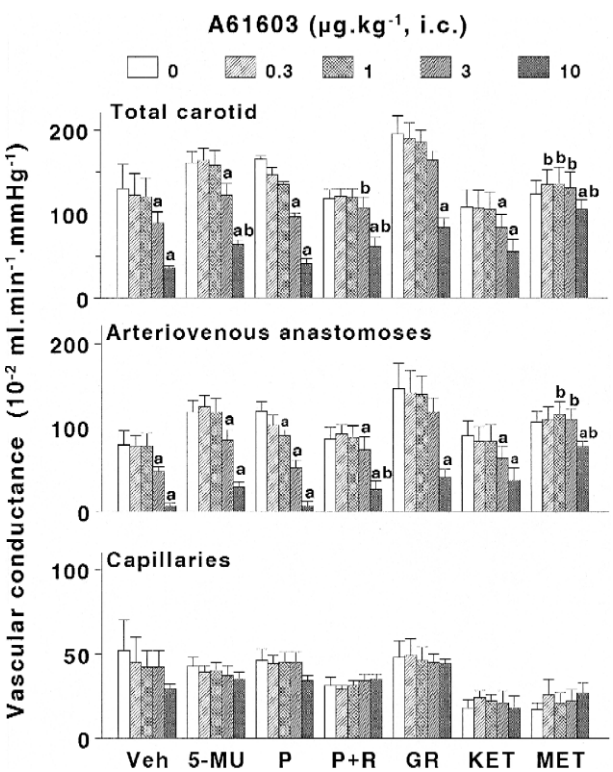


Fig. 2. Total carotid, arteriovenous anastomotic and capillary vascular conductances before and after i.c. administration of A61603 in anaesthetised pigs treated i.v. with any of the following: vehicle (Veh; 5 ml distilled water), 5-methylurapidil (5-MU; 1000 $\mu g\ kg^{-1}$), prazosin (Praz; 100 $\mu g\ kg^{-1}$), a combination of prazosin and rauwolscine (P + R; 100 and 300 $\mu g\ kg^{-1}$, respectively), GR127935 (GR; 500 $\mu g\ kg^{-1}$), ketanserin (KET; 500 $\mu g\ kg^{-1}$) or methiothepin (MET; 3000 $\mu g\ kg^{-1}$). Data are presented as mean ± S.E.M. a: $P < 0.05$ vs. baseline; b: $P < 0.05$ vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

3.2. Systemic haemodynamic responses to A61603

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

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

3.3. Carotid haemodynamic responses to A61603

Absolute values of total carotid, arteriovenous anastomotic and capillary conductances before and after i.c. infusions of A61603 (0.3–10 μg kg^{-1}) in the seven groups of animals are shown in Fig. 2. In animals treated with vehicle, A61603 produced a dose-dependent decrease in total carotid conductance. This effect was restricted to the carotid arteriovenous anastomotic fraction, since the capillary fraction remained unmodified. The A61603-induced changes were clearly attenuated in animals treated with methiothepin, but not in those treated with prazosin, GR127935 or ketanserin. 5-Methylurapidil only attenuated the decrease in the total carotid blood flow by the highest dose of A61603. The treatment with prazosin and rauwolscine combination affected the A61603-induced decreases in the total carotid (highest two doses) and its carotid arteriovenous anastomotic fraction (highest dose).

Fig. 3 compares decreases in carotid arteriovenous anastomotic blood flow by A61603 as percent changes from baseline values in control pigs (vehicle treatment) and in pigs treated with the different antagonists. It can be observed that the responses to A61603 were clearly antagonised by methiothepin and only slightly by the combination of prazosin and rauwolscine; 5-methylurapidil, prazosin, GR127935 and ketanserin were ineffective.

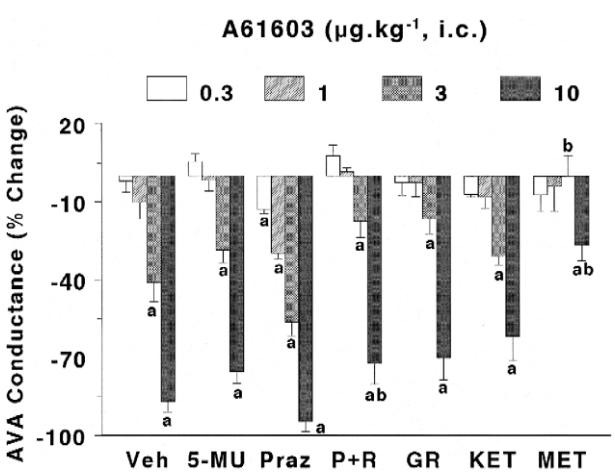


Fig. 3. Changes in carotid arteriovenous anastomotic (AVA) conductance following i.c. administration of A61603 in anaesthetised pigs treated i.v. with any of the following: vehicle (Veh; 5 ml distilled water), 5-methylurapidil (5-MU; 1000 μg kg^{-1}), prazosin (Praz; 100 μg kg^{-1}), a combination of prazosin and rauwolscine (P + R; 100 and 300 μg kg^{-1}, respectively), GR127935 (GR; 500 μg kg^{-1}), ketanserin (KET; 500 μg kg^{-1}) or methiothepin (MET; 3000 μg kg^{-1}). Data are presented as mean ± S.E.M. a: $P < 0.05$ vs. baseline; b: $P < 0.05$ vs. the response produced by the corresponding dose of A61603 in the vehicle-treated group.

4. Discussion

4.1. Consideration of known receptors that mediate carotid vasoconstriction

A number of studies have shown that sumatriptan produces vasoconstriction in the carotid vasculature of several species via GR127935-sensitive 5-HT$_{1B/1D}$ receptors; these species include the dog (Villalón et al., 1996), pig (De Vries et al., 1998) and rabbit (Choppin and O'Connor, 1996; De Vries et al., 1997). It is now known that these receptors mediating vasoconstriction are of the 5-HT$_{1B}$ subtype (for references, see De Vries et al., 1999; Villalón et al., 1999a). In line with this proposal, the therapeutic efficacy of sumatriptan in migraine can be explained by carotid vasoconstriction mediated by the 5-HT$_{1B}$ receptor. Furthermore, the canine external carotid vasoconstriction by ergotamine and dihydroergotamine involves 5-HT$_{1B/1D}$ receptors as well as α-adrenoceptors (Villalón et al., 1999b). Since the ergots display reasonable affinity at α-adrenoceptors (Leysen, 1985), their carotid vasoconstrictor effects in the pig may also be explained by these receptors. In this respect, we recently showed that: (i) both α_1- and α_2-adrenoceptors mediate constriction of porcine carotid arteriovenous anastomoses (Willems et al., 1999); and (ii) these α_1-adrenoceptors belong to α_{1A} and α_{1B} subtypes, but not the α_{1D} subtype (Willems et al., 2000).

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

4.2. Pharmacological profile of A61603

A61603 (Table 1) is a tetrahydro-1-napthyl imidazoline derivative that has been reported to show potent α_{1A}-adrenoceptor-agonist properties (Knepper et al., 1995; Docherty, 1998). As described previously (Knepper et al.,

1995; Meyer et al., 1996), A61603 is 35-fold more potent at human cloned α_{1a}- than at α_{1b}- or α_{1d}-adrenoceptors in radioligand binding studies and 100- to 300-fold more potent than noradrenaline and phenylephrine in isolated canine prostate strips and rat vas deferens (α_{1A}-adrenoceptors). In contrast, A61603 is only 40-fold more potent than phenylephrine at α_{1B}-adrenoceptors (rat spleen) and 35-fold less potent at α_{1D}-adrenoceptors (rat aorta) (Knepper et al., 1995; Meyer et al., 1996). Although the compound displays low affinity ($pK_i < 6$) for other receptors, it has a reasonable affinity and agonist property at α_2-adrenoceptor subtypes (see Table 1; Craig et al., 1997). In anaesthetised dogs, A61603 increases intra-urethral as well as diastolic arterial blood pressure (Knepper et al., 1995). In agreement with the latter, A61603 produces pressor responses in conscious rats at 50- to 100-fold lower doses than those of phenylephrine, and tamsulosin (α_{1A}-adrenoceptor antagonist) causes a marked shift of the A61603-induced response curve (Knepper et al., 1995).

4.3. Possible involvement of a novel mechanism?

A61603 produced a dose-dependent increase in blood pressure when administered by either i.c. (0.3–10 μg kg^{-1}) or i.v. (1–30 μg kg^{-1}) routes (Fig. 1). In view of the high affinity of A61603 at the α_{1A}-adrenoceptor (Table 1) and the important role of α-adrenoceptors in the regulation of vascular tone (see review by Vargas and Gorman, 1995), it was surprising that the hypertensive response to A61603 was little affected by 5-methylurapidil, prazosin or a combination of prazosin and rauwolscine. On the other hand, A61603-induced pressor response was markedly attenuated (i.v.) or even converted to hypotension (i.c.) by methiothepin.

Similar results were obtained with respect to the carotid haemodynamics. As shown in Fig. 2, A61603 (0.3–10 μg kg^{-1}, i.c.) produced a dose-dependent decrease in the porcine carotid blood flow, exclusively due to a constriction of carotid arteriovenous anastomoses. This selective carotid vasoconstriction was apparently maximal because a higher dose of A61603 (30 μg kg^{-1}) did not produce an additional decrease in total carotid conductance (maximal change: 80 ± 4%; $n = 6$).

The A61603-induced constriction of carotid arteriovenous anastomoses was, unexpectedly, resistant to blockade by the potent and selective α_{1A}-adrenoceptor antagonist 5-methylurapidil (Hieble and Ruffolo, 1996; Zhong and Minneman, 1999). Since prazosin was also ineffective in attenuating this response, it seems plausible to conclude that α_1-adrenoceptors do not play an important role. As mentioned before, both α_1- and α_2-adrenoceptors can mediate vasoconstriction in the porcine carotid arterial bed (Willems et al., 1999). For this reason and for the fact that A61603 also has affinity for α_2-adrenoceptors (see Table 1, Craig et al., 1997), we applied a combination of prazosin and rauwolscine to investigate the possible involvement of α_2-adrenoceptors. The combination of prazosin and rauwolscine produced only a slight attenuation in the A61603-induced constriction of carotid arteriovenous anastomoses, which implies, at most, a limited involvement of α_2-adrenoceptors. Similarly, the fact that GR127935 as well as ketanserin did not significantly modify this response excludes the possible involvement of 5-$HT_{1B/1D}$ and 5-HT_2 receptors, respectively. Although A61603 shows only a low affinity at these receptors (see Table 1; Craig et al., 1997), the exclusion of 5-$HT_{1B/1D}$ receptors is of interest, considering the affinity of benzylimidazoline derivatives related in structure to A61603 at 5-$HT_{1B/1D}$ receptors (Law et al., 1998). As reported elsewhere, the involvement of 5-HT_{1F} receptors in the carotid vasoconstriction of pigs and dogs (external) carotid vascular bed has been categorically excluded (see De Vries et al., 1998; Villalón et al., 1999a). Moreover, an endothelium-dependent vasoconstriction via the release of pro-constrictor cyclo-oxygenase products (Rosenblum and Nelson, 1988; Seager et al., 1992) also seems unlikely, based on the lack of effect of indomethacin (3000 μg kg^{-1}, i.v.; data not shown) on A61603-induced decrease in total carotid conductance. Similarly, a combination of indomethacin, prazosin, rauwolscine, GR127935 and ketanserin, at the doses previously mentioned, also failed to attenuate the decreases in total carotid conductance produced by i.c. infusions of A61603 (data not shown).

Methiothepin displays high affinity at 5-$HT_{1/2}$ receptors (Hoyer et al., 1994) as well as $\alpha_{1/2}$ adrenoceptors (Leysen, 1985). Therefore, we decided to test methiothepin against the A61603-induced constriction of arteriovenous anastomoses in this porcine model. It may be noted that a relatively high dose of methiothepin (3000 μg kg^{-1}) was required to abolish sumatriptan-induced carotid vasoconstriction in anaesthetised dogs and pigs (Den Boer et al., 1991; Villalón et al., 1995, 1999a); while a lower dose (1000 μg kg^{-1}) was ineffective. As shown in Figs. 2 and 3, treatment of the animals with methiothepin (3000 μg kg^{-1}) markedly attenuated the A61603-induced vasoconstriction in the porcine carotid vascular bed. Since all currently known vasoconstrictor receptors/mechanisms ($\alpha_{1/2}$-adrenoceptors, 5-$HT_{1B/1D}$, 5-HT_2 and eicosanoid receptors) had already been excluded (see above), this latter finding implies the involvement of another, possibly novel mechanism in the constriction of carotid arteriovenous anastomoses by A61603. Because this in vivo animal model is predictive for antimigraine activity (Saxena, 1995), this novel mechanism could be a potential new target for the development of antimigraine agents in the future. Admittedly, as an antimigraine drug, such an agonist must be devoid of systemic vasoconstrictor properties.

5. Conclusion

The present results show that A61603 does not behave as a potent and selective α_{1A}-adrenoceptor agonist in the

pig and that the constriction of porcine carotid arteriovenous anastomoses by A61603 is primarily mediated by a novel non-adrenergic mechanism.

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