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The role of several α_1- and α_2-adrenoceptor subtypes mediating vasoconstriction in the canine external carotid circulation

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1 It has recently been shown that both α_1- and α_2-adrenoceptors mediate vasoconstriction in the canine external carotid circulation. The present study set out to identify the specific subtypes (α_{1A}, α_{1B} and α_{1D} as well as α_{2A}, α_{2B} and α_{2C}) mediating the above response.

2 Consecutive 1 min intracarotid infusions of phenylephrine (α_1-adrenoceptor agonist) and BHT933 (α_2-adrenoceptor agonist) produced dose-dependent decreases in external carotid blood flow, without affecting mean arterial blood pressure or heart rate.

3 The responses to phenylephrine were selectively antagonized by the antagonists, 5-methylurapidil (α_{1A}) or BMY7378 (α_{1D}), but not by L-765,314 (α_{1B}), BRL44408 (α_{2A}), imiloxan (α_{2B}) or MK912 (α_{2C}). In contrast, only BRL44408 or MK912 affected the responses to BHT933.

4 The above results support our contention that mainly the α_{1A}, α_{1D}, α_{2A} and α_{2C}-adrenoceptor subtypes mediate vasoconstriction in the canine external carotid circulation.
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Abbreviations: BHT933, 6-ethyl-5, 6, 7, 8-tetrahydro-4H-oxazolo[4, 5-d]azepin-2-amine dihydrochloride; BMY7378, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5] decane-7,9-dione dihydrochloride; BRL44408, 2-[2H-(1-methyl-1,3-dihydroisoindole)methyl]-4,5-dihydroimidazole; 5-HT, 5-hydroxytryptamine; i.c., intracarotid; i.v., Intravenous; L-765,314, 4-Amino-2-[4-[1-(benzyloxycarbonyl)-2(S)-[[(1,1-dimethylethyl) amino] carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline; MK912, (2S,12bS)-1′3′-dimethylspiro(1,3,4,5′,6,6′,7,12b-octahydro-2H-benzo[b]furo[2,3-a]quinazoline)-2,4′-pyrimidin-2′-one (L-657743)

Introduction

Although the underlying mechanisms for the initiation of a migraine attack are poorly understood, the clinical efficacy of several acute anti-migraine agents can be explained by their ability to produce a cranio-selective vasoconstriction (Verheggen *et al*., 1998; De Vries *et al*., 1999a; Saxena & Tfelt-Hansen, 2000). This has been shown for the triptans (e.g. sumatriptan) and the ergots (ergotamine and dihydroergotamine) in dogs as well as pigs (Den Boer *et al*., 1991; De Vries *et al*., 1998a; Villalón *et al*., 1999; Saxena & Tfelt-Hansen, 2000). In this respect, the carotid vasoconstrictor responses to sumatriptan are exclusively mediated by serotonin 5-HT_{1B} receptors (De Vries *et al*., 1998a,b), whilst those to the ergots seem to involve both 5-HT_{1B} and α_2-adrenoceptors (Villalón *et al*., 1999).

Since both α_1- and α_2-adrenoceptors mediate the vasoconstriction to adrenaline and noradrenaline in the canine external carotid circulation (Willems *et al*., 2001), the present study was designed to identify the specific subtypes (α_{1A}, α_{1B} and α_{1D} as well as α_{2A}, α_{2B} and α_{2C}) mediating the above response. For this purpose, the external carotid vascular bed vasoconstrictor responses to the α-adrenoceptor agonists, phenylephrine (α_1) and BHT933 (α_2), were analysed before and after administration of selective antagonists at α_1- and α_2-adrenoceptor subtypes (see Table 1). The results obtained may open new avenues for the development of future anti-migraine agents.

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Methods

General

Experiments were carried out in a total of 42 dogs (16–30 kg) not selected for breed or sex. The animals were anaesthetized with an intravenous (i.v.) bolus injection of sodium pentobarbitone (30 mg kg^{-1}) and additional amounts (1 mg kg^{-1}, i.v.) were provided when required throughout the experiment. All dogs were intubated with an endotraceal tube and artificially respired with room air; for this purpose, a Palmer ventilation pump was used at a rate of 20 strokes min^{-1} and a stroke volume of 13–16 ml kg^{-1}, as previously established by Kleinman & Radford (1964). Catheters were placed in the inferior vena cava *via* a femoral vein for the administration of antagonists and in the aortic arch *via* a femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of blood pressure.

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

Table 1 Binding affinity constants (pK_i) for cloned human α_1- and α_2-adrenoceptor subtypes

Antagonist	α_{1a}	α_{1b}	α_{1d}	α_{2a}	α_{2b}	α_{2c}
5-Methylurapidil	9.0[i]	7.5[i]	7.9[I]	6.2[h]	6.4[h]	6.9[h]
	9.1[a]	7.4[a]	8.0[a]			
L-765,314	6.4[b]	8.7[b]	7.5[b]	n.d.	n.d.	n.d.
BMY7378	6.6[c]	7.2[c]	9.4[c]	5.1[c]*	5.1[c]*	5.1[c]*
	6.8[j]	7.0[j]	9.0[j]			
BRL44408	n.d.	n.d.	n.d.	8.2[d]	6.2[d]	6.8[d]
				7.6[e]	6.0[e]	6.4[e]
Imiloxan	<4[g]	<4[g]	<4[g]	5.8[e]	6.9[e]	6.0[e]
				6.5[f]	7.2[f]	6.8[f]
MK912	n.d.	n.d.	n.d.	8.9[d]	8.9[d]	10.2[d]
				9.1[f]	9.1[f]	10.2[f]

Data taken from [a]Shibata *et al.* (1995); [b]Patane *et al.* (1998); [c]Goetz *et al.* (1995); [d]Uhlen *et al.* (1994); [e]Devedjian *et al.* (1994); [f]Jasper *et al.* (1998); [g]Michel & Whiting (1981) (pA_2 value for α_1-adrenoceptors; no affinity data is available on specific α_1-adrenoceptor subtypes); [h]Hieble *et al.* (1995); [i]Saussy *et al.* (1996); [j]Craig *et al.* (1997); *Value for rat receptor; n.d., not determined.

(DAP) arterial pressures: MAP = DAP + (SAP-DAP)/3. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co., Quincy, MA, U.S.A.) triggered from the blood pressure signal. The common carotid artery was dissected free and the corresponding internal carotid and occipital arteries were ligated. Bilateral cervical vagosympathectomy was systematically performed in order to prevent possible baroreceptor reflexes produced by the intracarotid infusions of phenylephrine or BHT933. These compounds were administered into the carotid artery by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.) with a catheter inserted into the right cranial thyroid artery. Thereafter, an ultrasonic flow probe (4 mm R-Series) connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, NY, U.S.A.) was placed around the common carotid artery and the flow through this artery was considered as the external carotid blood flow (Villalón *et al.*, 1993). Blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The body temperature of the animals was maintained between 37–38°C.

Experimental protocol

After the animals ($n=42$) had been in a stable haemodynamic condition for at least 60 min, baseline values of mean blood pressure, heart rate and external carotid blood flow were determined. After collecting these data, the animals were divided into two groups. The first group ($n=36$) received consecutive intracarotid infusions (1 ml min^{-1}; during 1 min) of phenylephrine (0.3, 1, 3 and 10 μg min^{-1}) and BHT933 (3, 10, 30 and 100 μg min^{-1}). At this point, the dogs were subdivided into six subgroups ($n=6$ each) and the effects produced by the above infusions of phenylephrine and BHT933 were elicited again after i.v. treatment with each dose of either: (1) 5-methylurapidil (100 and 300 μg kg^{-1}); (2) L-765,314 (100 and 300 μg kg^{-1}; (3) BMY7378 (100 and 300 μg kg^{-1}); (4) BRL44408 (300 and 1000 μg kg^{-1}); (5) imiloxan (300 and 1000 μg kg^{-1}) or (6) MK912 (100 and 300 μg kg^{-1}).

The second group ($n=6$) was subdivided into two subgroups ($n=3$). The first subgroup received consecutive intracarotid infusions of phenylephrine (0.3, 1, 3 and 10 min^{-1}) as previously described. Then, the responses elicited by these infusions of phenylephrine were elicited again after i.v. administration of 5-methylurapidil (100 μg kg^{-1}) and, subsequently, BMY7378 (100 μg kg^{-1}). Likewise, the second subgroup received consecutive intracarotid infusions of BHT933 (3, 10, 30 and 100 μg min^{-1}) and the responses produced were elicited again after i.v. administration of BRL44408 (1000 μg kg^{-1}) and, subsequently, MK912 (100 μg kg^{-1}).

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

Data presentation and statistical analysis

All data in the text, figures and tables are presented as mean ± s.e.mean. The peak changes in external carotid blood flow were expressed as per cent change from baseline. The difference between the variables within one group of animals was compared using an analysis of variance (randomized block design) followed by the Student-Newman-Keuls test (Steel & Torrie, 1980). Statistical significance was accepted at $P<0.05$ (two-tailed).

Drugs

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

Results

Systemic and carotid haemodynamic effects of the different treatments

Baseline values of heart rate, mean arterial blood pressure and external carotid blood flow in the 42 dogs were, respectively, 131 ± 4 beats min^{-1}, 132 ± 3 mmHg and 134 ± 9 ml min^{-1}. The systemic and carotid haemodynamic

values before and 15–25 min after i.v. administration of the different compounds are shown in Table 2. None of the compounds produced significant changes under these conditions ($P>0.05$). However, immediately after its administration, BRL44408 (1000 μg kg^{-1}) produced a transient, though significant, increase (35±10%) in mean blood pressure (from 127±13 to 170±14 mmHg; $n=6$). This vasopressor effect, which was not accompanied by significant changes in heart rate and external carotid blood flow or conductance (data not shown), returned to baseline values after 25 min. In contrast, no immediate haemodynamic changes were observed in the other subgroups.

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

Intracarotid infusions of phenylephrine (α_1-adrenoceptor agonist; 0.3–10 μg min^{-1}; Figure 1) and BHT933 (α_2-adrenoceptor agonist; 3–100 μg min^{-1}; Figure 2) produced dose-dependent decreases in external carotid blood flow (maximal per cent changes were −64±5 and −68±4%, respectively). These responses to phenylephrine and BHT933, which were not accompanied by significant changes in heart rate or blood pressure (not shown), have been previously demonstrated to remain unaffected after two i.v. bolus injections of physiological saline (Willems *et al*., 2001).

Figures 1 and 2 also show the effects of subsequent i.v. administration of the antagonists, 5-methylurapidil (α_{1A}), L-765,314 (α_{1B}), BMY7378 (α_{1D}), BRL44408 (α_{2A}), imiloxan (α_{2B}) or MK912 (α_{2C}) on the external carotid vasoconstrictor responses produced by the above agonists. The vasoconstrictor responses to phenylephrine remained unaffected after L-765,314 (up to a dose of 300 μg kg^{-1}), but were significantly attenuated after 5-methylurapidil or BMY7378 (both 100 μg kg^{-1}) (maximal responses: −35±10 and −42±5%, respectively; Figure 1, upper panel). The blockade produced by 100 μg kg^{-1} of these antagonists was maximal, since a higher dose (300 μg kg^{-1}) of either antagonist did not produce a further blockade (maximal responses: −24±4 and −48±7%, respectively; Figure 1, upper panel). Similarly, a higher dose of L-765,314 (1000 μg kg^{-1}) did not affect phenylephrine-induced vascular responses (data not shown). It is worth noting that the blockade produced by the above antagonists was specific, as their corresponding doses did not significantly modify the external carotid vasoconstrictor responses to BHT933 (Figure 2, upper panel).

Moreover, the vasoconstrictor responses to BHT933 remained unaffected after imiloxan (up to 1000 μg kg^{-1}), but were significantly attenuated after BRL44408 (1000 μg kg^{-1}) or MK912 (100 μg kg^{-1}) (maximal responses: −56±5 and −37±4%, respectively; Figure 2, lower panel). The blockade produced by 100 μg kg^{-1} of MK912 was maximal, since a higher dose (300 μg kg^{-1}) did not produce a further blockade (maximal response: −28±6%). It should be highlighted that the antagonism produced by BRL44408 or MK912 (at the doses mentioned above) was specific, as they did not significantly modify the external carotid vasoconstrictor responses to phenylephrine (Figure 1, lower panel). Based on its affinity profile at the different α_2-adrenoceptor subtypes (see Table 1), we did not test a higher dose of BRL44408.

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

Figure 3 (left panel) shows that the vasoconstrictor responses to phenylephrine were significantly attenuated by BMY7378 (100 μg kg^{-1}), as previously observed (Figure 1, upper panel). It is noteworthy that the subsequent administration of 5-methylurapidil (100 μg kg^{-1}) produced a further blockade of the phenylephrine induced responses over the one previously produced by BMY7378 (Figure 3, left panel). By analogy, Figure 3 (right panel) shows that the responses to BHT933 were significantly attenuated by BRL44408 (1000 μg kg^{-1}), as previously shown (Figure 2, lower panel). The subsequent administration of MK912 (100 μg kg^{-1}) produced a further blockade of the responses to BHT933 over the one previously produced by BRL44408 (Figure 3, right panel).

Discussion

General

According to the International Union of Pharmacology Subcommittee on Nomenclature for Adrenoceptors, α-adrenoceptors have been divided into α_1- and α_2-adrenoceptors with subdivisions into α_{1A}, α_{1B} and α_{1D} and α_{2A}, α_{2B} and α_{2C} subtypes, respectively (Hieble *et al*., 1995; Hieble &

Table 2 Absolute values of heart rate, mean arterial blood pressure and external carotid blood flow and conductance in anaesthetized dogs before and after i.v. treatment with 5-methylurapidil (300 μg kg^{-1}), L-765,314 (300 μg kg^{-1}), BMY7378 (300 μg kg^{-1}), BRL44408 (1000 μg kg^{-1}), imiloxan (300 μg kg^{-1}) or MK912 (300 μg kg^{-1})

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

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

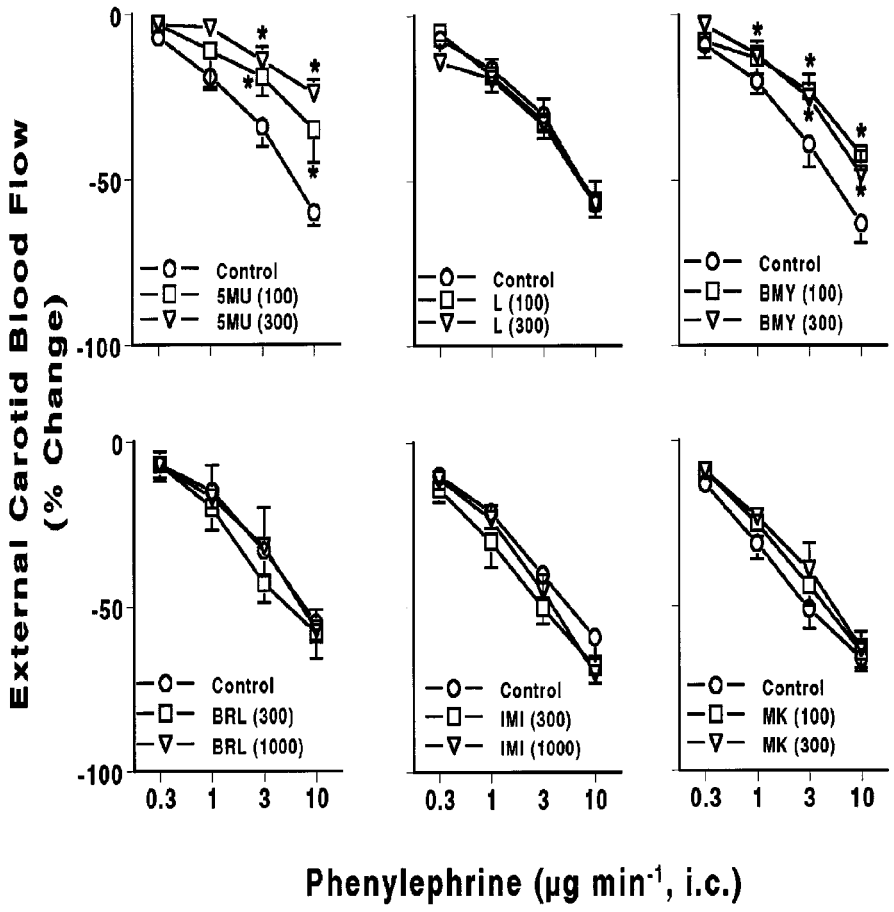

Figure 1 The effect of subsequent i.v. administration of 5-methylurapidil (5MU; 100 and 300 μg kg^{-1}), L-765,314 (L; 100 and 300 μg kg^{-1}), BMY7378 (BMY; 100 and 300 μg kg^{-1}), BRL44408 (BRL; 300 and 1000 μg kg^{-1}), imiloxan (IMI; 300 and 1000 μg kg^{-1}) or MK912 (MK; 100 and 300 μg kg^{-1})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

Ruffolo, 1996; Docherty, 1998; Brodde & Michel, 1999; Langer, 1999). We recently demonstrated that both α_1- and α_2-adrenoceptors mediate vasoconstrictor responses in the porcine (Willems *et al*., 1999) as well as canine (Willems *et al*., 2001) carotid arterial bed. The present study set out to identify which α_1- and α_2 -adrenoceptor subtypes mediate this latter vasoconstrictor response, employing antagonists with a moderate to high subtype selectivity: 5-methylurapidil (α_{1A}), L-765,314 (α_{1B}), BMY7378 (α_{1D}), BRL44408 (α_{2A}), imiloxan (α_{2B}) and MK912 (α_{2C}); Table 1. Our results show that mainly α_{1A}- and α_{1D}-adrenoceptors mediate the canine external carotid vasoconstrictor responses to phenylephrine, whereas those to BHT933 are mainly mediated by α_{2A}- and α_{2C}-adrenoceptors. Admittedly, the differences in antagonist potency observed against phenylephrine- and BHT933-induced vascular responses may be partly due to differences in the metabolism of antagonists or by affinity differences between canine and human α_1- and α_2-adrenoceptor subtypes; however, these data are not available at present.

Systemic and carotid haemodynamic effects of the different antagonists

Apart from the α_{2A}-adrenoceptor antagonist, BRL44408, none of the antagonists used in this investigation produced any significant haemodynamic response. Even with BRL44408, the immediate increase in blood pressure was short lasting. This effect may have been due to a direct activation of vascular α_1-adrenoceptors, as shown with other α_2-adrenoceptor antagonists in anaesthetized rats (Vayssettes-Courchay *et al*., 1996). Alternatively, such a vasopressor effect could also be explained by a blockade of prejunctional α_2-adrenoceptors leading to an enhancement of neuronal release of noradrenaline (Hoffman & Lefkowitz, 1996).

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

The blockade produced by 5-methylurapidil and BMY7378 (both 100 μg kg^{-1}) of the responses to phenylephrine, being maximal as a higher dose (300 μg kg^{-1}) did not produce a further blockade, was selective as these compounds failed to antagonize the responses to BHT933. These results favour a predominant involvement of α_{1A}- and α_{1D}-adrenoceptors. Admittedly, since 5-methylurapidil shows a reasonable affinity at α_{1A}- and α_{1D}-adrenoceptors (pK_i: 9.0 and 7.9, respectively), we cannot categorically exclude a possible blockade of phenylephrine-induced responses resulting from antagonism of both receptors. However, the fact that 5-methylurapidil produced a further blockade of the responses

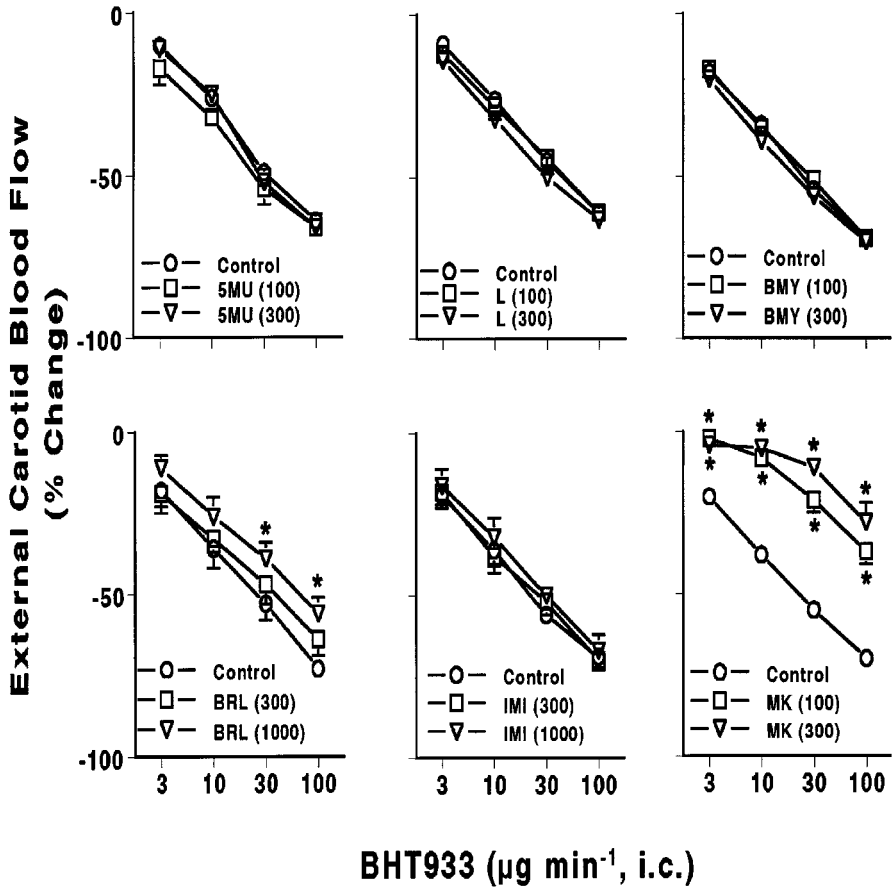

Figure 2 The effect of subsequent i.v. administration of 5-methylurapidil (5MU; 100 and 300 μg kg^{-1}), L-765,314 (L; 100 and 300 μg kg^{-1}), BMY7378 (BMY; 100 and 300 μg kg^{-1}), BRL44408 (BRL; 300 and 1000 μg kg^{-1}), imiloxan (IMI; 300 and 1000 μg kg^{-1}) or MK912 (MK; 100 and 300 μg kg^{-1}) on the external carotid vasoconstrictor responses produced by consecutive 1 min intracarotid (i.c.) infusions of BHT933 in anaesthetized dogs. *$P<0.05$ vs corresponding dose in control curve.

to phenyleprhine after a high dose of BMY7378 suggests the additional role of α_{1A}-adrenoceptors. Thus, after BMY7378 and 5-methylurapidil (both 100 μg kg^{-1}) the maximal response to phenlephrine (-20 ± 8%; Figure 3) did not significantly differ from that after prazosin (maximal response: -11 ± 3%, Willems *et al*., 2001). In the light of these findings, the role of α_{1B}-adrenoceptors seems questionable, a view consistent with the fact that L-765,314 (up to 1000 μg kg^{-1}; data not shown) did not affect the responses to phenylephrine.

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

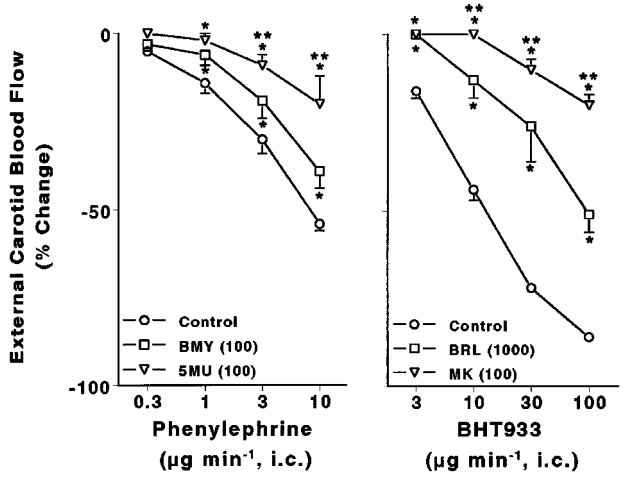

Figure 3 The effect of subsequent i.v. administration of: (i) BMY7378 (BMY; 100 μg kg^{-1}) and 5-methylurapidil (5MU; 100 μg kg^{-1}) on the external carotid vasoconstrictor responses to intracarotid (i.c.) infusions of phenylephrine (left panel); or (ii) BRL44408 (BRL; 1000 μg kg^{-1}) and MK912 (MK; 100 μg kg^{-1}) on the vasoconstrictor responses to i.c. infusions of BHT933 (right panel) in anaesthetized dogs. *$P<0.05$ vs corresponding dose of agonist in control curve. **$P<0.05$ vs corresponding dose of agonist after administration of the first antagonist (i.e. BMY7378 in the left panel or BRL44408 in the right panel).

Accordingly, after BRL44408 (100 μg kg^{-1}) and MK912 (100 μg kg^{-1}) the maximal response to BHT933 ($-20\pm3\%$; Figure 3) did not significantly differ from that after rauwolscine (maximal response: $-15\pm3\%$; Willems *et al*., unpublished observation). Considering the above, the role of α_{2B}-adrenoceptors seems unlikely, a view reinforced by the fact that imiloxan (up to 1000 μg kg^{-1}) failed to block the responses to BHT933.

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

As reviewed by Vargas & Gorman (1995) as well as Docherty (1998), a number of studies shows that both α_1- and α_2-adrenoceptor subtypes can mediate smooth muscle contraction. Some of the pharmacological preparations employed include rat vas deferens (α_{1A}) (Burt *et al*., 1998), rat aorta (α_{1D}) (Hussain & Marshall, 1997), porcine common digital artery (α_{2A}) (Blaylock & Wilson, 1995) and dog saphenous vein (α_{2C}) (MacLennan *et al*., 1997). Other blood vessels suggest a functional coexistence of α_{1A}- and α_{1D}-adrenoceptor subtypes, e.g. rat renal artery (Villalobos-Molina *et al*., 1997). The α_{1B} and α_{2B} subtypes, although not involved in canine external carotid vasoconstriction, seem to mediate constriction of the rabbit cutaneous resistance arteries (α_{1B}) (Smith *et al*., 1997) and the rat kidney (α_{2B}) (Docherty, 1998).

In a number of tissues, admittedly, α_2-adrenoceptors contribute to a predominantly α_1-adrenoceptor mediated response (Docherty, 1998). However, to the best of our knowledge, the present study seems to be one of the first to show *in vivo* the functional role of specific α_1 (α_{1A} and α_{1D})- and α_2 (α_{2A} and α_{2C})-adrenoceptor subtypes mediating vasoconstriction in a vascular preparation (the canine external carotid bed).

Possible clinical implications

To date, all acutely-acting anti-migraine agents, including the triptans and the ergots alkaloids, produce a potent vasoconstriction in the carotid circulation of dogs (Saxena, 1972; Villalón *et al*., 1995; 1999; De Vries *et al*., 1998a) and pigs (Saxena, 1995; De Vries *et al*., 1998b; 1999b). In contrast to the well-established role of serotonin 5-HT$_{1B}$ receptors in the carotid vasoconstrictor effects of the triptans (De Vries *et al*., 1998a; 1999b), the ergots seem to involve both 5-HT$_{1B}$ and α_2-adrenoceptors (Villalón *et al*., 1999). However, irrespective to the mechanisms involved, a selective vasoconstriction within the carotid circulation is an important property of antimigraine drugs. Therefore, we submit that the development of selective agonists at α_{1A}, α_{1D}, α_{2A} and α_{2C}-adrenoceptor subtypes may have potential therapeutic usefulness in the treatment of migraine.

Conclusion

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

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References

BLAYLOCK, N.A. & WILSON, V.G. (1995). Pharmacological characterization of noradrenaline-induced contractions of the porcine isolated palmar lateral vein and palmar common digital artery. *Br. J. Pharmacol.,* **114,** 694 – 702.

BRODDE, O.E. & MICHEL, M.C. (1999). Adrenergic and muscarinic receptors in the human heart. *Pharmacol. Rev.,* **51,** 651 – 690.

BURT, R.P., CHAPPLE, C.R. & MARSHALL, I. (1998). α_{1A}-adrenoceptor mediated contraction of rat prostatic vas deferens and the involvement of ryanodine stores and Ca^{2+} influx stimulated by diacylglycerol and PKC. *Br. J. Pharmacol.,* **123,** 317 – 325.

CRAIG, D.A., FORRAY, C.C., GLUCHOWSKI, C. & BRANCHEK, T.A. (1997). Use of α_{1A}-selective adrenoceptor agonists for the treatment of urinary incontinence. In *United States Patent (5,610,174)*. USA: Synaptic Pharmaceutical Corporation, Paramus, N.J.

DE VRIES, P., SANCHEZ-LOPEZ, A., CENTURION, D., HEILIGERS, J.P.C., SAXENA, P.R. & VILLALÓN, C.M. (1998a). The canine external carotid vasoconstrictor 5-HT$_1$ receptor: blockade by 5-HT$_{1B}$ (SB224289), but not by 5-HT$_{1D}$ (BRL15572) receptor antagonists. *Eur. J. Pharmacol.,* **362,** 69 – 72.

DE VRIES, P., VILLALÓN, C.M. & SAXENA, P.R. (1999a). Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. *Eur. J. Pharmacol.,* **375,** 61 – 74.

DE VRIES, P., VILLALÓN, C.M., HEILIGERS, J.P.C. & SAXENA, P.R. (1998b). Characterization of 5-HT receptors mediating constriction of porcine carotid arteriovenous anastamoses; involvement of 5-HT$_{1B/1D}$ and novel receptors. *Br. J. Pharmacol.,* **123,** 1561 – 1570.

DE VRIES, P., WILLEMS, E.W., HEILIGERS, J.P.C., VILLALÓN, C.M. & SAXENA, P.R. (1999b). Investigations of the role of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors in the sumatriptan-induced constriction of porcine carotid arteriovenous anastomoses. *Br. J. Pharmacol.,* **127,** 405 – 412.

DEN BOER, M.O., HEILIGERS, J.P. & SAXENA, P.A. (1991). Carotid vascular effects of ergotamine and dihydroergotamine in the pig: no exclusive mediation via 5-HT$_1$-like receptors. *Br. J. Pharmacol.,* **104,** 183 – 189.

DEVEDJIAN, J.C., ESCLAPEZ, F., DENIS-POUXVIEL, C. & PARIS, H. (1994). Further characterization of human α_2-adrenoceptor subtypes: [^{3}H]RX821002 binding and definition of additional selective drugs. *Eur. J. Pharmacol.,* **252,** 43 – 49.

DOCHERTY, J.R. (1998). Subtypes of functional α_1- and α_2-adrenoceptors. *Eur. J. Pharmacol.,* **361,** 1 – 15.

GOETZ, A.S., KING, H.K., WARD, S.D., TRUE, T.A., RIMELE, T.J. & SAUSSY, JR, D.L. (1995). BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptors. *Eur. J. Pharmacol.,* **272,** R5 – R6.

HIEBLE, J.P., BONDINELL, W.E. & RUFFOLO, R.J. (1995). α- and β-adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. *J. Med. Chem.,* **38,** 3415 – 3444.

HIEBLE, J.P. & RUFFOLO, JR, R.R. (1996). Subclassification and nomenclature of α_1- and α_2-adrenoceptors. *Prog. Drug. Res.,* **47,** 81 – 130.

HOFFMAN, B.B. & LEFKOWITZ, R.J. (1996). Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. eds. Hardman, J.G., Limbird, L.E., Molinoff, P.B, Ruddon, R.W. & Goodman Gilman, A. pp. 199–248. New York, NY, USA: McGraw-Hill.

HUSSAIN, M.B. & MARSHALL, I. (1997). Characterization of α_1-adrenoceptor subtypes mediating contractions of phenylephrine in rat thoracic aorta, mesenteric artery and pulmonary artery. *Br. J. Pharmacol.,* **122,** 849–858.

JASPER, J.R., LESNICK, J.D., CHANG, L.K., YAMANISHI, S.S., CHANG, T.K., HSU, S.A., DAUNT, D.A., BONHAUS, D.W. & EGLEN, R.M. (1998). Ligand efficacy and potency at recombinant α_2 adrenergic receptors: agonist-mediated [^{35}S]GTPgammaS binding. *Biochem. Pharmacol.,* **55,** 1035–1043.

KLEINMAN, L.I. & RADFORD, E.P. (1964). Ventilation standards for small mammals. *J. Appl. Physiol.,* **19,** 360–362.

LANGER, S.Z. (1999). History and nomenclature of α_1-adrenoceptors. *Eur. Urol.,* **36,** 2–6.

MACLENNAN, S.J., LUONG, L.A., JASPER, J.R., TO, Z.P. & EGLEN, R.M. (1997). Characterization of α_2-adrenoceptors mediating contraction of dog saphenous vein: identity with the human α_{2A} subtype. *Br. J. Pharmacol.,* **121,** 1721–1729.

MICHEL, A.D. & WHITING, R.L. (1981). 2-(2-imidazolyl methyl)-1,4-benzodioxans, a series of selective α_2-adrenoceptor antagonists. *Br. J. Pharmacol.,* **74,** 225P.

PATANE, M.A., SCOTT, A.L., BROTEN, T.P., CHANG, R.S.L., RANSOM, R.W., DISALVO, J., FORRAY, C. & BOCK, M.G. (1998). 4-Amino-2-[4-[1-(benzyloxycarbonyl)-2(S)-[[(1,1-dimethylethyl)-amino]carbonyl]-piperazinyl]-6, 7-dimethoxyquinazoline (L-765,314): a potent and selective α_{1b} adrenergic receptor antagonist. *J. Med. Chem.,* **41,** 1205–1208.

SAUSSY, JR, D.L., GOETZ, A.S., QUEEN, K.L., KING, H.K., LUTZ, M.W. & RIMELE, T.J. (1996). Structure activity relationships of a series of buspirone analogs at α_1-adrenoceptors: further evidence that rat aorta α_1-adrenoceptors are of the α_{1D} subtype. *J. Pharmacol. Exp. Ther.,* **278,** 136–144.

SAXENA, P.R. (1972). The effects of antimigraine drugs on the vascular responses by 5-hydroxytryptamine and related biogenic substances on the external carotid bed of dogs: possible pharmacological implications to their antimigraine action. *Headache,* **12,** 44–54.

SAXENA, P.R. (1995). Cranial arteriovenous shunting, an *in vivo* animal model for migraine. In *Experimental headache models*. eds. Olesen, J. & Moskowitz, M.A. pp. 189–198. Philadelphia, USA: Lippincott-Raven Publishers.

SAXENA, P.R. & TFELT-HANSEN, P. (2000). Triptan, 5-$HT_{1B/1D}$ receptor agonists in the acute treatment of migraine. In *The Headaches*. eds. Olesen, J., Tfelt-Hansen, P. & Welch, K.M.A. pp. 411–438. Philadelphia: Lippincott Williams & Wilkins.

SHIBATA, K., FOGLAR, R., HORIE, K., OBIKA, K., SAKAMOTO, A., OGAWA, S. & TSUJIMOTO, G. (1995). KMD-3213, a novel, potent, α_{1A}-adrenoceptor-selective antagonist: characterization using recombinant human α_{1A}-adrenoceptors and native tissues. *Mol. Pharmacol.,* **48,** 250–258.

SMITH, K., MACMILLAN, J.B. & MCGRATH, J.C. (1997). Investigations of α_1-adrenoceptor subtype mediating vasoconstriction in rabbit cutaneous resistance arteries. *Br. J. Pharmacol.,* **122,** 825–832.

STEEL, R.G.D. & TORRIE, J.H. (1980). *Principles and procedures of statistics. A biomedical approach (2nd edition)*. Tokyo, Japan: McGraw-Hill Kogakusha Ltd.

UHLEN, S., PORTER, A.C. & NEUBIG, R.R. (1994). The novel α_2-adrenergic radioligand [^{3}H]-MK912 is α_{2C} selective among human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors. *J. Pharmacol. Exp. Ther.,* **271,** 1558–1565.

VARGAS, H.M. & GORMAN, A.J. (1995). Vascular α_{1A} adrenergic receptor subtypes in the regulation of arterial pressure. *Life Sci.,* **57,** 2291–2308.

VAYSSETTES-COURCHAY, C., BOUYSSET, F., CORDI, A.A., LAUBIE, M. & VERBEUREN, T.J. (1996). A comparative study of the reversal by different α_2-adrenoceptor antagonists of the central sympatho-inhibitory effect of clonidine. *Br. J. Pharmacol.,* **117,** 587–593.

VERHEGGEN, R., HUNDESHAGEN, A.G., BROWN, A.M., SCHINDLER, M. & KAUMANN, A.J. (1998). 5-HT_{1B} receptor-mediated contractions in human temporal artery: evidence from selective antagonists and 5-HT receptor mRNA expression. *Br. J. Pharmacol.,* **124,** 1345–1354.

VILLALOBOS-MOLINA, R., LOPEZ-GUERRERO, J.J. & IBARRA, M. (1997). α_{1D}- and α_{1A}-adrenoceptors mediate contraction in rat renal artery. *Eur. J. Pharmacol.,* **322,** 225–227.

VILLALÓN, C.M., DE VRIES, P., RABELO, G., CENTURIÓN, D., SÁNCHEZ-LÓPEZ, A. & SAXENA, P.R. (1999). Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: a role of 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. *Br. J. Pharmacol.,* **126,** 385–394.

VILLALÓN, C.M., RAMIREZ-SAN JUAN, E., CASTILLO, C., CASTILLO, E., LOPEZ-MUNOZ, F.J. & TERRÓN, J.A. (1995). Pharmacological profile of the receptors that mediate external carotid vasoconstriction by 5-HT in vagosympathectomized dogs. *Br. J. Pharmacol.,* **116,** 2778–2784.

VILLALÓN, C.M., TERRÓN, J.A. & HONG, E. (1993). Role of 5-HT_1-like receptors in the increase in external carotid blood flow induced by 5-hydroxytryptamine in the dog. *Eur. J. Pharmacol.,* **240,** 9–20.

WILLEMS, E.W., TRION, M., DE VRIES, P., HEILIGERS, J.P.C., VILLALÓN, C.M. & SAXENA, P.R. (1999). Pharmacological evidence that α_1- and α_2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. *Br. J. Pharmacol.,* **127,** 1263–1271.

WILLEMS, E.W., VALDIVIA, L.F., RAMIREZ-SAN JUAN, E., SAXENA, P.R. & VILLALÓN, C.M. (2001). Pharmacological identification of the major subtypes of adrenoceptors involved in the canine external carotid vasoconstrictor effects of adrenaline and noradrenaline. *Life Sci.,* (in press).

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