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α_1-Adrenoceptor subtypes mediating contractions of the rat mesenteric artery

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Abstract

The α_1-adrenoceptor subtype(s) mediating contractions of the rat mesenteric artery were investigated using the agonists methoxamine, cirazoline, P7480 (*N*-(4-pyridinyl)-1*H*-indol-1-amine) and subtype-selective antagonists including BMY 7378 (8-(-2(-4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9,dione dihydrochloride). pA_2 or apparent pK_B values of antagonists against methoxamine contractions correlated best with its pK_i values at the cloned α_{1b}-(0.88), with cirazoline, antagonists affinities correlated equally well with those at α_{1a}-(0.79) or the α_{1b}-(0.81) while with P7480 antagonist affinities correlated best with the α_{1d}-adrenoceptor subtype (0.94). The low affinity estimate for 5-methylurapidil (7.5) against the α_{1a}-selective cirazoline suggests an α_{1A}-subtype mediating contraction is unlikely. Shallow Schild plot slopes of subtype selective antagonists against all three agonists are consistent with heterogeneity of α_1-adrenoceptors. P7480 (putative α_{1D}-adrenoceptor-selective) acts primarily at this subtype and at another which is more likely to be an α_{1B}- than an α_{1A}-adrenoceptor. The results with both agonists and antagonists are consistent with contractions of the rat mesenteric artery being mediated via the α_{1D}- and possibly α_{1B}-adrenoceptor.

Keywords: α_1-Adrenoceptor subtype; Methoxamine; Cirazoline; P7480; Mesenteric artery, rat; BMY 7378

1. Introduction

α_1-Adrenoceptors are a heterogeneous population of G-protein coupled receptors. Three α_1-adrenoceptors have been cloned and their corresponding tissue subtypes have been identified (Hieble et al., 1995). Contraction of a number of smooth muscles is thought to be mediated via the α_{1A}-adrenoceptor subtype (vas deferens, portal vein and human prostate) or the α_{1B}-adrenoceptor subtype (rat spleen; Aboud et al., 1993; Burt et al., 1995, 1998; Marshall et al., 1995, 1996). However, the functional subtype of α_1-adrenoceptors present in rat blood vessels is controversial (Aboud et al., 1993; Kong et al., 1994; Orsetti and Distillo, 1994; Saussy et al., 1996; Van der Graaf et al., 1996; Stam et al., 1999). The α_1-adrenoceptor mediating contraction to phenylephrine in the rat mesenteric artery as well as the aorta and pulmonary artery is primarily of the α_{1D}-adrenoceptor subtype although data with selective antagonists suggested the involvement of a heterogeneous population of adrenoceptors (Hussain and Marshall, 1997). However, the identity of the second receptor subtype was unclear.

Recent reports have suggested that certain classes of agonists show α_1-adrenoceptor subtype selectivity. For example, methoxamine, a phenylethylamine, has been shown to be about 20-fold more potent in human embryonic kidney 293 cells at activating α_{1a}- than α_{1b}- or α_{1d}-adrenoceptor mediated [^{3}H] inositol phosphate formation (Minneman et al., 1994). Furthermore, imidazoline compounds such as oxymetazoline and cirazoline have been shown to be more selective for the α_{1a}-adrenoceptor for increasing both inositol phosphates and intracellular calcium (Minneman et al., 1994; Horie et al., 1995). On the other hand, noradrenaline (a catecholamine) and phenylephrine (a phenylethylamine) show some selectivity for the α_{1d}-adrenoceptor subtype (Minneman et al., 1994; Yang et al., 1997). Most recently P7480 a metabolite of besipirdine, was suggested to be at least 30-fold selective for the α_{1D}-adrenoceptor (Hubbard et al., 1997).

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In the present study, the activity of eight α_1-adrenoceptor agonists including methoxamine, cirazoline and P7480, was assessed on the rat mesenteric artery and compared with that on the rat aorta and pulmonary artery. Furthermore, the rat mesenteric artery was selected for further investigation with antagonists as it was here that the greatest separation in the affinity of BMY 7378 was observed between the two α_1-adrenoceptors which mediated contraction (Hussain and Marshall, 1997). Antagonist affinity estimates were obtained against cirazoline and methoxamine to test the involvement of α_{1A}-adrenoceptor in mediating contraction of the mesenteric artery. In addition, the α_1-subtype selectivity of P7480 was tested using a range of subtype selective antagonists including the α_{1A}-adrenoceptor-selective antagonist 5-methylurapidil and the α_{1D}-adrenoceptor-selective antagonist BMY 7378.

2. Material and methods

2.1. Measurements of isometric tension changes

Male Sprague–Dawley rats (350–450 g) were stunned and killed by cervical dislocation. Arterial rings were denuded of endothelium (by gentle abrasion with a serrated file) and suspended in 10 ml organ baths containing Krebs solution of the following composition (mM): Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128, HCO_3^- 25, HPO_4^{2-} 1.2, SO_4^{2-} 1.2, and D-glucose 11, maintained at 37°C and gassed with 95% O_2/5% CO_2. The Krebs solution also contained cocaine (10^{-5} M), rauwolscine (10^{-7} M) and propranolol (10^{-7} M) to block uptake$_1$ and inhibit α_2- and β-adrenoceptors, respectively. In addition, in experiments with the rat pulmonary artery β-oestradiol (10^{-5} M) was also added to inhibit extra-neuronal uptake (uptake$_2$). Preliminary experiments showed that β-oestradiol on its own shifted the phenylephrine concentration–contraction curve to the right in the aorta and mesenteric artery (results not shown) and therefore it was excluded from the Krebs solution when these tissues were used.

Initially a concentration of phenylephrine producing a sub-maximal contraction (70–85% of maximum) was given to the rat thoracic aorta (10^{-7} M), mesenteric artery (3×10^{-7} M) and pulmonary artery (3×10^{-8} M). Once the response had stabilised (10–15 min) acetylcholine (10^{-6} M) was added to assess the integrity of the endothelium. If the contractions to phenylephrine were not maintained or relaxation ($>5\%$ of the phenylephrine induced tone) to acetylcholine was observed, the tissues were discarded.

Tissues were washed and left to recover for 30 min except for the pulmonary artery, which needed a longer time interval (40 min). Stable contraction to all α_1-adrenoceptor agonists permitted the use of cumulative concentration–response curves in all tissues. A concentration–effect curve to noradrenaline was performed before the tissues were washed to baseline (for 30 min) and left to recover for a further 30 min prior to repeating the concentration–response curve to a test agonist. In some experiments the effect of glacial acetic acid (0.001%) on noradrenaline contractions was studied.

Antagonist studies were carried out with some agonists in the mesenteric artery. After an initial concentration–response curve to methoxamine or cirazoline the tissues were washed for 1 or 1.5 h, respectively. In some tissues, two consecutive curves to either methoxamine or cirazoline (without antagonist) were carried out to assess the reproducibility of agonist effects. Other tissues were incubated with a concentration of antagonist for 30 min before a concentration–effect curve to the agonist was repeated.

The effect of P7480 was difficult to reverse after the initial concentration–response curve and after 2 h the tone of most tissues had not returned to baseline. Even in cases where the wash out was thought to be successful, tissues often contracted spontaneously before the second curve was repeated. Therefore, only a single concentration–response curve to P7480 was carried out in a given tissue. The following experimental protocol was used: after the stability test, phenylephrine was washed out and KCl (80 mM) was added and left in contact with the tissues for 15–20 min. After washing out for a further 45 min, tissues were either incubated with an antagonist or left untreated for 30 min before the concentration–response curve to P7480 was carried out.

Preparation of all stock solutions and their subsequent dilution were made using distilled water except for prazosin which was made up in ethanol while P7480 was dissolved in distilled water with 10% glacial acetic acid. The agonists were prepared fresh each day whereas a stock solution (10^{-2} M) of the antagonists was stored frozen in aliquots and thawed and diluted fresh daily.

2.2. Analysis of data

Results for all α_1-adrenoceptor agonists were expressed as a percentage of the maximum of the noradrenaline contractions (first curve). For the antagonist studies, the results were calculated as a percentage of the maximum response of the first concentration–effect curve to either methoxamine or cirazoline. However, the results with P7480 in the antagonist studies were normalised by comparison with the contraction to KCl (80 mM, 100%) in the same tissue. All responses were plotted graphically as means from at least four separate experiments with vertical bars representing standard error of mean (S.E.M.). When error bars do not appear on the figure, this is because they are small and fall within the dimension of the symbols. Curves were fitted to all the data by non-linear regression using either Inplot or Prism (GraphPAD software San Diego, CA, USA) to calculate pEC_{50} values ($-\log$ of the EC_{50} values). In all cases 50% of the maximum for each

concentration–response curve was used to calculate the EC_{50} value. The EC_{50} value in the presence and absence of antagonist in a single tissue was used to determine the concentration-ratio. In the case of P7480 curves, the concentration ratios for antagonists were calculated by comparing the EC_{50} value from the concentration–effect curve in the presence of an antagonist with the mean EC_{50} value from control curves to P7480 (without antagonist).

pA_2 values and Schild slopes were calculated by the method of Arunlakshana and Schild (1959). Where the slope of the Schild plot appeared to be significantly different from one, an apparent affinity estimate was obtained using the equation $pK_B = \log(\text{concentration ratio} - 1) - \log$ [molar antagonist concentration]. The lowest concentration of antagonist shifting the curve to the right was used for this calculation. The pA_2 values or pK_B estimates obtained for the antagonists in the rat mesenteric artery were plotted against their average pK_i values calculated from measurements using the expressed cloned subtypes (data from the literature). Linear regression was used to correlate the pK_i and pA_2/pK_B values.

A paired *t*-test was used to assess the significance of differences between (a) pEC_{50} values for the control and repeat curves, (b) increases in the maximum responses, between control curves and that in the presence of an antagonist. A *P*-value < 0.05 was taken to indicate a statistically significant difference. Statistical analysis was performed using either Instat or Prism (GraphPAD software, San Diego, CA, USA).

2.3. Chemicals

Prazosin hydrochloride and WB4101 (2(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4 benzodioxane hydrochloride) were donated by Pfizer Central Research, Kent. Cocaine hydrochloride, propranolol hydrochloride and β-oestradiol, were obtained from Sigma. 5-Methylurapidil, benoxathian hydrochloride, rauwolscine hydrochloride and BMY 7378 dihydrochloride (8-(-2(-4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-8-azaspiro(4,5)decane-7,9,dione dihydrochloride) were obtained from RBI. P7480 (*N*-(4-pyridinyl)-1*H*-indol-1-amine) was donated by Hoechst Marion Roussell.

3. Results

3.1. Effect of α_1-adrenoceptor agonists

All agonists contracted the rat mesenteric artery, aorta and pulmonary artery in a concentration dependent manner. Methoxamine, phenylpropanolamine, oxymetazoline, amidephrine and P7480 were partial agonists in the rat mesenteric artery (Fig. 1a). The rank order of potency of agonists on this tissue was as follows: noradrenaline = cirazoline > P7480 > phenylephrine > methoxamine > oxymetazoline > amidephrine > phenylpropanolamine.

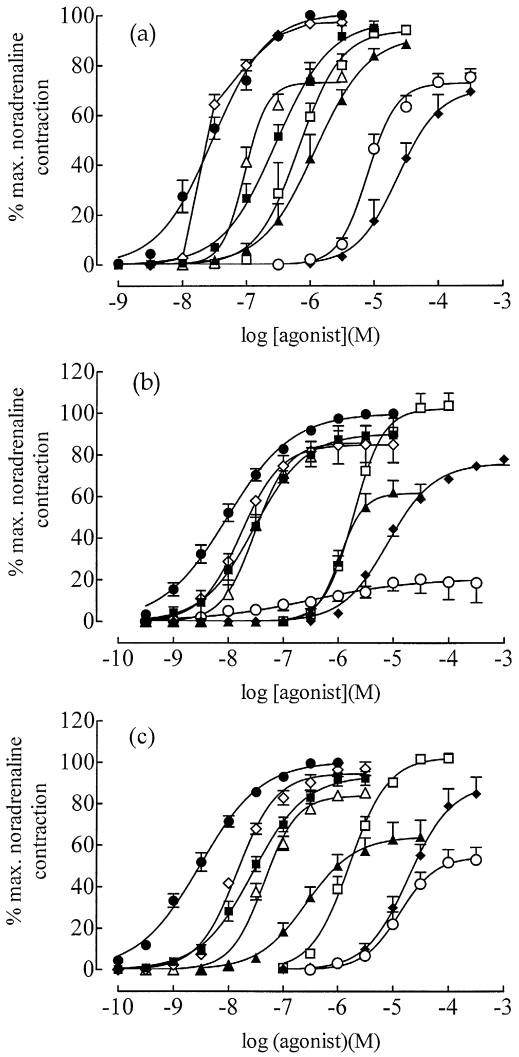

Fig. 1. Concentration–response curves to phenylephrine (■), methoxamine (□), phenylpropanolamine (♦), oxymetazoline (▲), cirazoline (◇), amidephrine (○) and P7480 (△) in the rat mesenteric artery (a) thoracic aorta (b), and pulmonary artery (c). Agonist effects are given as percentage of the maximum response to noradrenaline (●). Results are expressed as mean ± S.E.M of at least four different experiments.

Phenylpropanolamine, oxymetazoline, and amidephrine were also partial agonists in the aorta and pulmonary artery (Fig. 1b and c). In addition, phenylephrine and P7480 were partial agonists in the pulmonary artery. The rank order of potency of agonists in the aorta and pulmonary artery were roughly similar with noradrenaline being the most potent while phenylpropanolamine and amidephrine were the least potent. While the pEC_{50} values for most agonists were similar across the three tissues, phenylephrine was 10-fold less potent on the rat mesenteric artery compared with the other two tissues and amidephrine was more potent on the rat thoracic aorta. The maximum responses produced by each of the agonists

Table 1
Comparison of antagonist pA_2 or pK_B values on rat mesenteric artery with their published pK_i values calculated with measurements using cloned receptor subtypes

Antagonists	pK_B (±s.e.m) or pA_2^a on rat mesenteric artery				pK_i on cloned α_1-adrenoceptor subtypes[b]		
	Phenylephrine[c]	Methoxamine	Cirazoline	P7480	α_{1a}	α_{1b}	α_{1d}
Prazosin	9.9[a]	9.1 ± 0.1	9.0 ± 0.1	9.4 ± 0.1	9.2 ± 0.2	9.6 ± 0.2	9.4 ± 0.2
WB4101	9.8[a]	8.7 ± 0.1	9.2[a]	8.7 ± 0.1	9.5 ± 0.3	8.2 ± 0.1	9.2 ± 0.1
5-MU	7.9[a]	7.0 ± 0.1	7.5[a]	7.0 ± 0.1	8.8 ± 0.1	6.8 ± 0.3	7.3 ± 0.3
Benoxathian	8.8[a]	8.6 ± 0.1	8.5 ± 0.1	n.d	9.0	7.8	8.7
Indoramin	7.2[a]	n.d	n.d	6.9 ± 0.1	8.2 ± 0.3	7.3 ± 0.1	6.8 ± 0.2
BMY 7378	8.8[a]	7.9 ± 0.1	7.3[a]	8.4 ± 0.1	6.6	7.2	9.4

[b]Data are mean values from, Faure et al., 1994, Forray et al., 1994, Kenny et al., 1994a,b, Testa et al., 1994 and Goetz et al., 1995. S.E.M. are not listed for compounds with only one or two values, n.d not determined.
[c]Data for phenylephrine are from Hussain and Marshall, 1997.

(compared with noradrenaline) were not too different across the three tissues except for amidephrine. The maximum response for this agonist varied from approximately 20% of that of noradrenaline in the thoracic aorta to about 75% in the rat mesenteric artery.

In the mesenteric artery the concentration of glacial acetic acid equivalent to that added to organ baths with dilutions of P7480 did not alter the concentration–response curve to noradrenaline (pEC_{50} 7.32 ± 0.08, mean ± S.E.M., and 7.42 ± 0.10 for the first and second curves, respectively).

3.2. Effect of α_1-adrenoceptor antagonists on methoxamine contractions of the rat mesenteric artery

Methoxamine produced reproducible contractions of the mesenteric artery (pEC_{50} 5.86 ± 0.04 (1.10 ± 0.10 g maximum tension) and 5.96 ± 0.09 (1.16 ± 0.10 g maximum tension) for first and second curves, respectively, $n = 4$). Prazosin (3×10^{-9} M) and WB4101 (10^{-8} M) produced a rightward shift of the methoxamine concentration–response (Table 1). 5-Methylurapidil produced a rightward shift of the concentration–effect curve with a decrease in the maximum response with increasing antagonist concen-

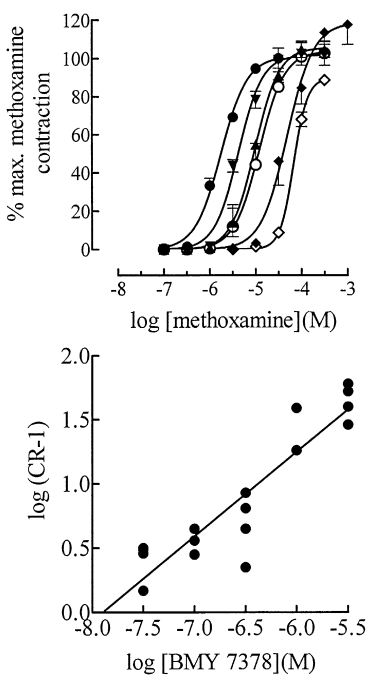

Fig. 2. Concentration–response curves and Schild plot for the antagonism of methoxamine induced contractions of the rat mesenteric artery by BMY 7378. Control (●), plus BMY 7378 3×10^{-8} M (▼), 10^{-7} M (▲), 3×10^{-7} M (○), 10^{-6} M (♦) and 3×10^{-6} M (◇). Each plot represents the mean±S.E.M. of at least 4 separate experiments. The Schild plot was constructed using the concentration ratios from individual experiments.

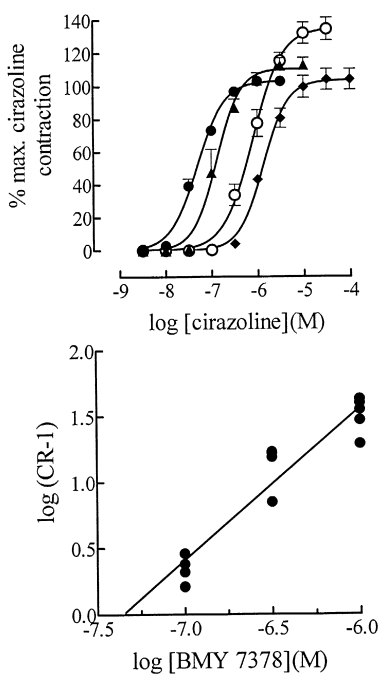

Fig. 3. Concentration–response curves and Schild plot for the antagonism of cirazoline induced contractions of the rat mesenteric artery by BMY 7378. Control (●), plus BMY 7378 10^{-7} M (▲), 3×10^{-7} M (○) and 10^{-6} M (♦). Each plot represents the mean±S.E.M. of at least four separate experiments. The Schild plot was constructed using the concentration ratios from individual experiments.

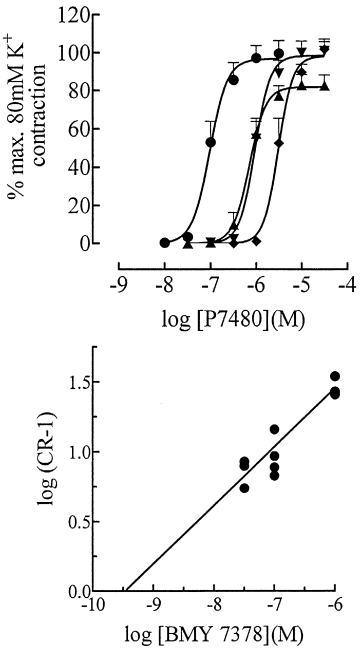

Fig. 4. Concentration–response curves and Schild plot for the antagonism of P7480 induced contractions of the rat mesenteric artery by BMY 7378. Control (●), plus BMY 7378 3×10^{-8} M (▼), 10^{-7} M (▲) and 10^{-6} M (♦). Each plot represents the mean ± S.E.M. of at least four separate experiments. The Schild plot was constructed using the concentration ratios from individual experiments.

tration (3×10^{-7} M–3×10^{-6} M 5-methylurapidil; Table 1). Benoxathian (3×10^{-8} M) produced a rightward shift of the concentration–response curve (Table 1). BMY 7378 behaved in a manner inconsistent with simple competitive antagonism (pA_2 7.9, Schild plot slope 0.66 ± 0.07, mean ± S.E.M; Table 1; Fig. 2).

3.3. Effect of a_1-adrenoceptor antagonists on cirazoline contractions of the rat mesenteric artery

Cirazoline produced reproducible contractions of the mesenteric artery (pEC_{50} 7.24 ± 0.06 (0.88 ± 0.07 g maximum tension) and 7.23 ± 0.06 (0.97 ± 0.07 g maximum tension) for first and second curves, respectively, $n=4$). Prazosin (10^{-8} M), WB4101 (Schild slope 1.06 ± 0.12), 5-methylurapidil (Schild slope 1.00 ± 0.13) and benoxathian (3×10^{-8} M) antagonised cirazoline contractions (for pA_2 and apparent pK_B values see Table 1). BMY 7378 produced rightward shifts of the concentration–response curve to cirazoline (pA_2 7.3, Schild slope 1.15 ± 0.11; Fig. 3). An increase in the maximum cirazoline contraction was seen in the presence of 3×10^{-7} M BMY 7378 (Table 1).

3.4. Effect of α_1-adrenoceptor antagonists on P7480 contractions of the rat mesenteric artery

P7480 produced contractions of the rat mesenteric artery with a pEC_{50} of 7.02 ± 0.06 ($n=5$, maximum contraction 1.13 ± 0.06 g tension which was 99.6 ± 6.8% of K^+ contraction). Prazosin (3×10^{-9} M), WB4101 (10^{-8} M), 5-methylurapidil (3×10^{-7} M) and indoramin (10^{-6} M) produced a rightward shift of the concentration–response curve to P7480 (Table 1). WB4101 and 5-methylurapidil did not alter the maximum response to P7480, but in the presence of indoramin, it was decreased by 15%. Against P7480, BMY 7378 had a pA_2 value of 9.5 and a shallow Schild plot slope (0.42 ± 0.05; Fig. 4). An apparent pK_B value was therefore calculated for BMY 7378 using the lowest concentration that produced a shift (3×10^{-8} M; Table 1).

Correlations of the antagonist functional affinity estimates against methoxamine contractions were highest against pK_i values from the α_{1b}-subtype and lowest with those from the α_{1a}-clone. Intermediate values for both correlation coefficient and slopes were obtained with the α_{1d}-adrenoceptor (Table 2). Antagonist affinities obtained against cirazoline contractions correlated equally well with affinities of these antagonists on both the α_{1a}- and the α_{1b}-adrenoceptor subtypes whereas a much lower correlation was obtained with the α_{1d}-clone (Table 2). Affinities of antagonists for P7480 responses correlated best with that at the α_{1d}-subtype. A relatively high correlation was also obtained against the α_{1b}-clone whereas low values for

Table 2
Correlation coefficients and slopes of the correlation plots for antagonist pA_2 or pK_B values against methoxamine, cirazoline and P7480 contractions of the rat mesenteric artery with their pK_i calculated from measurements using cloned subtypes

α_1-Subtype	Phenylephrine[a]		Methoxamine		Cirazoline		P7480	
	(r)	slope	(r)	slope	(r)	slope	(r)	slope
α_{1a}	0.34	0.40 ± 0.46	0.39	0.55 ± 0.74	0.79	1.06 ± 0.48	0.21	0.20 ± 0.59
α_{1b}	0.68	0.61 ± 0.27	0.88	1.16 ± 0.35	0.81	1.02 ± 0.43	0.84	0.86 ± 0.32
α_{1d}	0.88	0.82 ± 0.18	0.79	0.85 ± 0.38	0.45	0.46 ± 0.53	0.94	1.09 ± 0.23

[a] Data for phenylephrine are taken from Hussain and Marshall, 1997. Data for the cloned subtypes are mean values from, Faure et al., 1994, Forray et al., 1994, Kenny et al., 1994a,b, Testa et al., 1994; Goetz et al., 1995 and Saussy et al., 1996. S.E.M. are not listed for compounds with only one or two values.

both the correlation coefficient and slope was obtained with the α_{1a}-adrenoceptor subtype (Table 2).

4. Discussion

The contractile responses of the rat mesenteric artery as well as the aorta and pulmonary artery are mediated partly by the α_{1D}-adrenoceptor (Hussain and Marshall, 1997). However, the mRNA for all three cloned α_1-adrenoceptor subtypes has been found to be present in all three blood vessels using reverse transcription-polymerase chain reaction (Xu et al., 1997). In the present study, α_1-adrenoceptor agonists with different selectivities for the α_1-adrenoceptor subtypes were employed in an attempt to isolate and investigate the characteristics of the α_1-adrenoceptors mediating contractions of these vessels. Although agonists have been used often in the process of receptor characterisation, the interpretation of their effects can be difficult. Firstly, estimation of agonist selectivity is difficult because both binding and functional measurements of agonist receptor interactions are dependent on the conditions under which they are examined. In particular functional studies are complicated by differences in receptor reserve between tissues while radioligand binding studies are influenced by differential affinity states due to G-protein coupling.

The simplest hypothesis is that all three blood vessels contain a homogeneous population of α_1-adrenoceptors with a similar receptor reserve. If this was true then the subtype selective agonists might have similar relative potencies and maximum contractions across the three tissues. However, the magnitude of contraction produced by amidephrine in the aorta was low (18%) compared with that in the other vessels. While methoxamine and P7480 are full agonists in the aorta, they are only partial agonists in the mesenteric artery. Therefore, it is possible that the contraction of these vessels is mediated by more than one receptor subtype with differing distributions although alternate explanations are possible.

The receptor mediating contractions has been studied using antagonists. Firstly, the selectivity of action of methoxamine was examined using antagonists in the mesenteric artery. The relatively low affinities of 5-methylurapidil and WB4101 suggest that methoxamine contractions are unlikely to be mediated via the α_{1A}-adrenoceptor subtype. A low correlation coefficient was found between the functionally derived affinities using methoxamine and their affinities at the cloned α_{1a}-adrenoceptor, further supporting the lack of involvement of the α_{1A}-adrenoceptor subtype. However, the affinities displayed by the α_{1a}-adrenoceptor-selective antagonists are not very different from those expected at the α_{1b}-clone. Although there is an α_{1D}-subtype mediating contraction (to phenylephrine, Hussain and Marshall, 1997) in this tissue, the low affinity of BMY 7378 is inconsistent with an α_{1D}-subtype mediating contraction to methoxamine. However, the shallow Schild plot slope is consistent with receptor heterogeneity. Therefore, an α_{1B}-adrenoceptor subtype may mediate contraction to methoxamine together with another α_1-adrenoceptor subtype. However, a contribution via an α_{1A}-adrenoceptor seems unlikely. This finding in the mesenteric artery is different from that in the rat renal artery. In the latter, a high affinity for 5-methylurapidil suggested an α_{1A}-adrenoceptor mediated contraction to noradrenaline (Han et al., 1990). However, in the same tissue BMY 7378 had a low affinity against methoxamine and a 30-fold higher one against noradrenaline (Villalobos-Molina et al., 1997). These results are consistent with the presence of two α_1-adrenoceptor subtypes, α_{1A}- and α_{1D}-adrenoceptors, which are activated preferentially by methoxamine and noradrenaline respectively in the renal artery.

When cirazoline was used as the spasmogen the resulting estimates of antagoinst affinity correlated highly with the affinities of these antagonists at the α_{1a}- and α_{1b}-adrenoceptor subtypes but poorly against those on the α_{1d}-clone. Nevertheless, a relatively low affinity for the α_{1A}-adrenoceptor-selective antagonist 5-methylurapidil is not compatible with the idea that an α_{1A}-adrenoceptor is mediating the contraction. Further, the high affinity of WB4101 and an intermediate affinity of benoxathian are inconsistent with the involvement of only the α_{1B}-adrenoceptor subtype and more in keeping with a contribution from the α_{1D}-adrenoceptor (although the low affinity of BMY 7378 does not support the latter). The evidence suggests therefore that the contraction to cirazoline is unlikely to be mediated via either the α_{1D}- or the α_{1A}-subtypes. Thus, like methoxamine, it is intriguing that the highest correlation against the three cloned receptor subtypes was with the α_{1b}-adrenoceptor subtype.

There are a number of reasons why the antagonist profiles might not fit with the α_{1a}-adrenoceptor-selectivity of these two agonists. Firstly, while the agonists may show some selectivity for the cloned subtypes they might not be as selective for tissue receptors because, for example, of differences in receptor–effector coupling. Secondly, in the absence of an α_{1A}-adrenoceptor in the blood vessel, these agonists may act on another α_1-adrenoceptor subtype. In this regard the antagonist profiles did not match that expected for the α_{1d}-adrenoceptor subtype which is known to be present (Hussain and Marshall, 1997). Thirdly, these agonists may act via more than one subtype and therefore, the antagonist affinities obtained would not match that expected at any one of the cloned α_1-adrenoceptors. A further possibility is that these agonists act on a receptor which is unlike any of the cloned α_1-adrenoceptor subtypes. To the extent that cirazoline in particular can be considered an α_{1a}-adrenoceptor-selective agonist, its antagonist pharmacology is not consistent with the functional presence of this subtype. It remains unclear whether the possible absence of an α_{1A}-adrenoceptor can be generalised to the aorta or to the pulmonary artery (Hussain and Marshall, 1997). However, while the noradrenaline evoked contraction in the rat aorta was via an α_{1D}-adrenoceptor

subtype, oxymetazoline may act via the α_{1B}-adrenoceptor in this tissue (Muramatsu et al.,1998).

A further point is that both methoxamine and cirazoline were antagonized with a high affinity by prazosin (around 9.0) in the mesenteric artery. On the other hand another α_{1A}-adrenoceptor-selective agonist, A61603 (Knepper et al., 1995), was antagonized by prazosin with a lower affinity (8.06), consistent with the presence of the putative α_{1L}-adrenoceptor in this blood vessel (Yousif et al., 1998). The reason for this difference in sensitivity to prazosin among the α_{1A}-adrenoceptor-selective agonists is unclear. The α_{1D}-adrenoceptor selective agonist P7480, like methoxamine and cirazoline, was antagonised by prazosin with a high affinity.

With P7480 as the agonist, WB4101 and BMY 7378 had a high affinity and this together with the lower values for 5-methylurapidil and indoramin are consistent with those expected at the cloned α_{1d}-adrenoceptor-subtype. However, BMY 7378 acted in a manner inconsistent with simple competitive antgonism against P7480 (Schild slope less than unity) as reported earlier for the same antagonist against phenylephrine (Hussain and Marshall, 1997). This provides further support for the idea of heterogeneity of α_1-adrenoceptors in the mesenteric artery.

Data with P7480 suggests that it has some selectivity for the α_{1D}-adrenoceptor-subtype but also acts on the second α_1-adrenoceptor in the mesenteric artery. The initial suggestion of α_{1D}-adrenoceptor-selectivity of P7480 was based partly on its ability to elicit contractions of the rat aorta (not a homogenous α_{1D}-adrenoceptor system) and partly on the 30-fold selectivity for α_1-adrenoceptors in the rat aorta compared with rat cortical α_{1A}- and α_{1B}-adrenoceptor subtypes in radioligand binding studies (Hubbard et al., 1997). Since this report Vargas et al. (1998) have suggested that P7480 has similar binding affinities on human α_{1a}-, α_{1b}- and α_{1d}-adrenoceptor subtypes although it did show a slight preference for the α_{1d}-adrenoceptor subtype in stimulating inositol phosphate production.

In conclusion, the evidence from antagonist studies is consistent with receptor heterogeneity and suggests that different agonists may activate different subtypes of α_1-adrenoceptors in the rat mesenteric artery. Thus, phenylephrine and P7480 act primarily via the α_{1D}-adrenoceptor subtype and at another, which is more likely to be an α_{1B}- than an α_{1A}-adrenoceptor. Further, the involvement of an α_{1A}-adrenoceptor subtype also appears unlikely on the basis of the results with the α_{1a}-adrenoceptor-selective agonists methoxamine and cirazoline. Therefore, in addition to the α_{1D}-adrenoceptor in the mesenteric artery, of the other two cloned receptors the α_{1B}-adrenoceptor subtype may be more likely to be functionally present.

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