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Characterization of α_1-adrenoceptors mediating vasoconstriction to noradrenaline and nerve stimulation in the isolated perfused mesentery of rat

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1 The objective of this study was to investigate the α_1-adrenoceptor subtype(s) mediating vasoconstrictor responses to perfused and neuronally-released noradrenaline (NA) in the isolated perfused mesentery preparation of rat.

2 Isolated mesenteric preparations (with gut attached) from male Sprague Dawley rats (250–300g) were perfused via the superior mesenteric artery with oxygenated Krebs solution at approximately 6 ml min^{-1}. The effects of antagonists on vasoconstrictor responses to either perfused (±)-NA or periarterial nerve stimulation (70 V, 2 ms pulse width, 10 s train) were determined.

3 Vasoconstrictor responses to perfused NA were antagonized by prazosin ($pA_2 = 9.3 \pm 0.1$), WB4101 ($pA_2 = 9.6 \pm 0.1$), 5-methyl urapidil (5-MU: $pA_2 = 9.0 \pm 0.1$), (+)-niguldipine (insurmountable) and spiperone ($pA_2 = 7.7 \pm 0.1$). The insurmountable nature of the antagonism by (+)-niguldipine (0.1 nM) was greatly reduced by co-perfusion with prazosin (10 nM). Chloroethylclonidine (CEC: 100 μM for 20 min, followed by 40 min washout) caused an approximate twofold increase in the EC_{50} for (±)-NA and reduced the maximum response by approximately 25%. Pre-treatment of tissues with CEC (100 μM as above) did not significantly alter affinity estimates for prazosin ($pA_2 = 9.2 \pm 0.1$), WB4101 ($pA_2 = 9.3 \pm 0.1$) or 5-MU ($pA_2 = 8.7 \pm 0.2$). Vasoconstrictor responses to periarterial nerve stimulation were antagonized by WB4101 > 5-MU > prazosin >> spiperone. CEC (100 μM as above) reduced nerve-stimulated responses by approximately 50%.

4 The affinity estimates for the various antagonists studied suggest that vasoconstrictor responses to both exogenous and neuronally-released NA are mediated via the same α_1-adrenoceptor subtype. The pharmacological profile most resembles the 'classical' α_{1A}-adrenoceptor, which, in turn, appears to be a rat homologue of the cloned bovine α_{1C}-adrenoceptor.

Keywords: Vasoconstriction; α_{1A}-adrenoceptors; α_{1C}-adrenoceptors; isolated perfused mesentery; prazosin; 5-methylurapidil; WB4101; (+)-niguldipine; (±)-noradrenaline

Introduction

The original sub-division of α_1-adrenoceptors, using operational and radioligand binding techniques, was into two subtypes: α_{1A}-adrenoceptors and α_{1B}-adrenoceptors. WB4101, 5-methylurapidil (5-MU) and (+)-niguldipine exhibit approximately 10, 70 and 100 fold selectivity for the α_{1A}-adrenoceptor subtype versus the α_{1B}-adrenoceptor subtype (Morrow & Creese, 1986; Gross *et al.*, 1988; Boer *et al.*, 1989). Compounds selective for the α_{1B}-adrenoceptor include spiperone (Michel *et al.*, 1989), risperidone (Sleight *et al.*, 1993) and the irreversible antagonist, chloroethylclonidine (CEC; Han *et al.*, 1987).

More recently, molecular biological studies have led to the cloning and expression of α_{1B}-, α_{1C}- and α_{1D}-adrenoceptor subtypes (Coteccia *et al.*, 1988; Schwinn *et al.*, 1990; Lomasney *et al.*, 1991; Perez *et al.*, 1991). However, the relationship between these cloned receptors and the pharmacological classification outlined above has been the subject of debate. To date, the cloned hamster α_{1B}-adrenoceptor appears to match closely the pharmacologically defined α_{1B}-adrenoceptor. The cloned bovine α_{1C}-adrenoceptor appears to correspond to the α_{1A}-adrenoceptor in rat (high affinity for WB4101, (+)-niguldipine and 5-MU). In this regard, mRNA for the α_{1C}-adrenoceptor has recently been reported in several rat tissues (Rokosh *et al.*, 1994). The pharmacological equivalent of the cloned α_{1D}-adrenoceptor remains to be determined. However, the cloned α_{1D}-adrenoceptor may equate with binding sites exhibiting high affinity for WB4101 but low affinity for 5-MU and (+)-niguldipine (see Ford *et al.*, 1994).

The key role of α_1-adrenoceptors in the control of vascular resistance is well-established, and the subdivision of α_1-adrenoceptors described above has led to the pharmacological characterization of NA-mediated responses in a number of vascular preparations from a variety of species (Tian *et al.*, 1990; Oriowo & Ruffolo, 1992). However, a large component of vascular resistance lies at the level of the arterioles and relatively few studies have characterized α_1-adrenoceptor-mediated responses in these specific vessels (but see Kong *et al.*, 1994). The aim of the present study was to characterize pharmacologically the α_1-adrenoceptor subtype(s) mediating NA-induced vasoconstrictor responses in the isolated perfused arterial mesenteric bed of rat. A preliminary account of these studies has been presented previously (Williams & Clarke, 1994).

Methods

Rat perfused isolated mesentery preparation

Fasted male Sprague Dawley rats (300–350g) were anaesthetized (sodium pentobarbitone, 60 mg kg^{-1}, i.p.) and the abdominal cavity opened. Heparin (6 mg in 0.25 ml saline) was administered via the femoral vein. After freeing the superior mesenteric artery from surrounding connective tissue the vessel was cannulated with a PE-90 polyethylene (Intramedic) cannula, which was secured with cotton ties. The

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mesenteric artery was immediately perfused with Krebs solution to clear blood from the mesenteric vascular bed. Following surgical removal of the blood vessels and intestine from the rat, the contents of the intestine were removed by flushing with Krebs solution at room-temperature.

Preparations were placed on a gauze pad in a water-jacketed petri dish, and perfused with warm (37°C) oxygenated Krebs solution at a rate of approximately 6.0–6.5 ml min^{-1}. Perfusion pressure was measured with a pressure transducer and recorded on a Graphtec chart recorder. Perfusate was removed from the dish with a vacuum line.

The perfusate contained the following (mM):- Na^+ 143.5, K^+ 6.0, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 128.3, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, glucose 11.0.

Ascorbate 100 μM (to minimize oxidation of NA), cocaine 30 μM. corticosterone 30 μM (to block neuronal and extraneuronal uptake of NA respectively), indomethacin 10 μM (to inhibit prostanoid production), propranolol 1 μM (to block β-adrenoceptors) and idazoxan 300 nM (to block α_2-adrenoceptors) were also included except where indicated in Results. pH (7.4) was maintained by gassing with 95% O_2, 5% CO_2.

Vasoconstrictor responses to perfused NA

Non-cumulative concentration-response curves (CRCs) were obtained to NA by perfusing preparations with Krebs solution containing agonist until a peak vasoconstrictor response (measured as an increase in perfusion pressure) was obtained; at this point the perfusing solution was switched to one free of agonist. The perfusion pressure was allowed to return to baseline before addition of the next ascending concentration of agonist.

Studies with antagonists were performed by adding antagonist to the perfusate following construction of the first CRC. Preparations were perfused with antagonist for 1 h prior to, and then during construction of a second CRC. In experiments where the effect of CEC was studied, CEC was perfused for 20 min, followed by a 40 min washout. Preparations receiving only vehicle for the antagonist were used to control for time-dependent shifts in the CRCs to agonist.

Estimates of antagonist affinity (pA_2) from single concentration studies were made from the fold increase in the EC_{50} values to NA (concentration-ratio, CR) as follows: $pA_2 = -\log\{[\text{antagonist}]/(CR-1)\}$. In experiments in which more than one concentration of antagonist was studied, resultant data were subjected to Schild analysis (Arunlakshana & Schild, 1959): Schild slopes not significantly different from 1 were constrained to 1 for the estimation of pA_2 values.

Vasoconstrictor responses to periarterial nerve stimulation

Vasoconstrictor responses were elicited by electrical stimulation of mesenteric nerves with a bipolar stimulating electrode placed around the mesenteric artery. Frequency-response curves (FRCs) were constructed by applying 10 s trains of 0.3 to 100 Hz (in threefold steps) at 70 V, 2 ms pulse duration. Trains were applied at 6 min intervals and peak vasoconstrictor responses measured. The perfusion fluid was identical to that described above, except that cocaine and corticosterone were omitted. This step was taken to maintain the physiological characteristics of the neuroeffector junctions. Blockade of NA reuptake could conceivably lead to 'spill-over' and perhaps to neuromuscular transmission via 'extra-synaptic' receptors.

In order to minimize the possible involvement of a purinergic component in the response to electrical stimulation purinoceptors were desensitized with 1 μM α,β-methylene ATP, a purinoceptor agonist (Kasakov & Burnstock, 1983). α,β-Methylene ATP was perfused 15 min prior to, and during the construction of each frequency-response curve. α,β-Methylene ATP produced a transient vasoconstrictor response, with perfusion pressure returning to baseline values after approximately 5 min. Under these conditions, initial studies showed that the vasoconstrictor response to nerve stimulation was inhibited completely by the adrenergic neurone blocking drug, guanethidine (1 μM), and by a high concentration of prazosin (1 μM) (data not shown).

The effects of antagonists on the frequency-response relationship were determined by perfusing the preparation with antagonist for 1 h prior to construction of a second frequency-response curve. The effects of CEC on the response to nerve stimulation were determined by perfusing the antagonist for 20 min or 40 min following the first FRC, followed by a 40 min washout and construction of a second FRC.

Chemicals

Drugs were obtained from the following sources: cocaine, idazoxan, 5-MU, (+)-niguldipine, spiperone, WB4101 (2-(2,6-dimethoxyphenoxyethyl)-aminoethyl-1,4-benzodioxane), and chloroethylclonidine (Research Biochemicals Inc., Natick, MA, U.S.A.), (±)-NA, corticosterone, prazosin and α,β-methylene ATP (Sigma Chemical Co., St. Louis, MO, U.S.A.). Solutions were prepared in deionised water or dimethylsulphoxide (5-MU, (+)-niguldipine, spiperone, prazosin and corticosterone).

Results

Vasoconstrictor responses to perfused NA

Perfused (±)-NA caused a concentration-dependent increase in perfusion pressure, with a maximum response typically in the range 80–120 mmHg, and an EC_{50} of approximately 5–10 μM NA. In time control experiments the second CRC to NA typically showed a small (approximately twofold) leftward shift, with little change in maximum response. The α_1-adrenoceptor antagonists, prazosin (Figure 1), WB4101, 5-MU and spiperone produced concentration-dependent rightward shifts in the concentration-response curve to NA. pA_2 estimates obtained by Schild analysis are shown in Table 1 (Schild slopes did not differ significantly from 1). The selective α_{1A}-adrenoceptor antagonist, (+)-niguldipine (Boer *et al.*, 1989), at subnanomolar concentrations, potently and insurmountably antagonized NA responses (Figure 2). At a concentration of 0.3 nM, (+)-niguldipine reduced the maximum response to NA to approximately 10% of control values. When perfused together with prazosin (10 nM), the ability of niguldipine (0.1 nM) to suppress the maximum response to NA was greatly diminished (Figure 3). The L-type calcium channel antagonist, nitrendipine (1 μM), was without effect on the CRC to NA (data not shown).

CEC at 1 μM was without effect on the CRC to NA: higher concentrations of CEC, (10 μM and 100 μM), produced a small rightward shift (approximately twofold after correction for the temporal shift) accompanied by a one-third reduction at 100 μM CEC in the maximum response to NA (Figure 4). Exposure of tissues to 100 μM CEC for 20 min (plus 40 min washout) prior to the first CRC did not significantly affect affinity estimates for prazosin (30 nM), WB4101 (10 nM) or 5-MU (10 nM) (Table 1).

Vasoconstrictor responses to periarterial nerve stimulation

Figure 5 shows the effect of increasing concentrations of prazosin on the frequency-response curve to peri-arterial nerve stimulation. In order to quantify and compare the inhibitory effects of antagonists, the area under the second frequency-response curve (in the presence of antagonist) was

expressed as a percentage of the area under the first frequency-response curve in the absence of antagonist. These data are represented in Figure 6, which shows that WB4101, 5-MU, prazosin and spiperone (respective order of potency) concentration-dependently inhibited the neuronal stimulation-induced vasoconstrictor response. As with prazosin, all drugs tested caused a reduction in the maximal response to stimulation, suggesting that increased stimulation frequencies liberate concentrations of endogenous agonist below those required to surmount the antagonism.

Figure 7 shows the effect of exposure to increasing concentrations of CEC (1 μM–100 μM) on the vasoconstrictor response to nerve stimulation. Employing a 20 min exposure time to CEC, 1 μM of the alkylating agent did not affect the frequency-response curve; higher concentrations of 10 μM and 100 μM CEC reduced the response to nerve stimulation to 79 ± 7% and 46 ± 5% of the first curve respectively (mean ± s.e.mean, $n = 7-12$ separate experiments). Although increasing the time of exposure of CEC to 40 min did not further reduce responses to nerve stimulation at a CEC concentration of 10 μM (64 ± 8% of area under first curve), further inhibition was observed with 100 μM CEC (19 ± 1% of area under first curve).

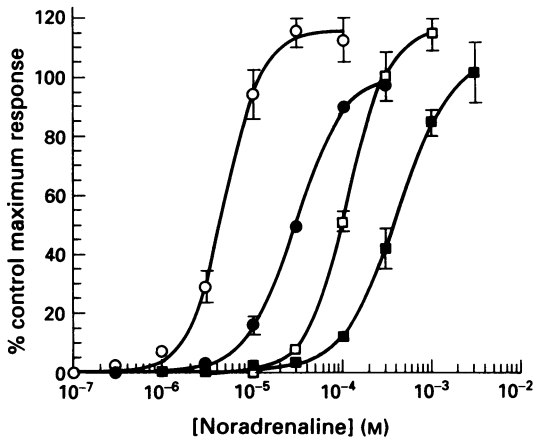

Figure 1 Antagonism by prazosin of vasoconstrictor responses to perfused noradrenaline (NA) in the isolated perfused mesentery preparation of rat. Prazosin (or vehicle) was perfused for 1 h prior to construction of the second concentration-response curve: (○) vehicle (●) prazosin 3 nM; (□) prazosin 10 nM; (■) prazosin 30 nM. Data shown are second concentration-response curves and give mean ± s.e.mean from 4 separate experiments.

Table 1 Antagonist affinity estimates versus (±)-noradrenaline-mediated vasoconstrictor responses in the isolated perfused mesentery of rat

Antagonist	pA_2	*Schild slope* (95% CL)	pA_2 (CEC-treated)
Prazosin	9.3 ± 0.1	0.96 (0.67–1.25)	9.2 ± 0.1
WB4101	9.6 ± 0.1	1.29 (0.75–1.83)	9.3 ± 0.1
5 MU	9.0 ± 0.1	1.24 (0.66–1.76)	8.7 ± 0.2
Niguldipine	US (0.03–0.3 nM)		ND
Spiperone	7.7 ± 0.1	1.13 (0.83–1.25)	ND

Antagonist affinities in preparations treated previously with CEC (100 μM, 20 min) are calculated from shifts using single antagonist concentrations (see text for concentrations used). US = insurmountable. ND = not determined. CEC, chloroethylclonidine; 5-MU, 5-methyl urapidil. Data are mean ± s.e.mean from 3–6 separate experiments.

Discussion

In addition to perfused NA, the present study has used endogenous, neuronally-released NA in an attempt to further characterize the α_1-adrenoceptor subtype(s) mediating vasoconstrictor responses in the isolated perfused mesenteric arterial bed of rat. Characterization of responses to nerve stimulation is of importance for two reasons. First, differential location of α_1-adrenoceptor subtypes, synaptic versus extrasynaptic, might lead to responses having distinctly different pharmacological profiles. Secondly, characterization of nerve-mediated responses may provide some insight into the role of α_1-adrenoceptor subtypes in the physiological maintenance and control of blood pressure. This is especially so as peripheral resistance, particularly in the mesenteric circulation, is a major determinant of systemic blood pressure.

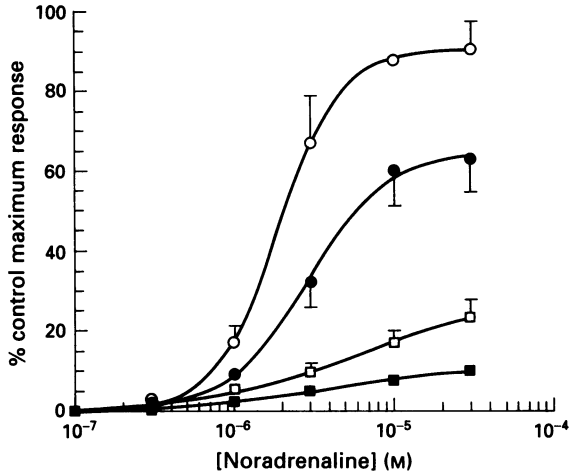

Figure 2 Antagonism by (+)-niguldipine of vasoconstrictor responses to perfused noradrenaline in the isolated perfused mesentery preparation of rat. (+)-Niguldipine (or vehicle) was perfused for 1 h prior to construction of the second concentration response curve: (○) vehicle; (●) niguldipine 0.03 nM; (□) niguldipine 0.1 nM; (■) niguldipine 0.3 nM. Data shown are second concentration-response curves and give mean ± s.e.mean from 4 separate experiments.

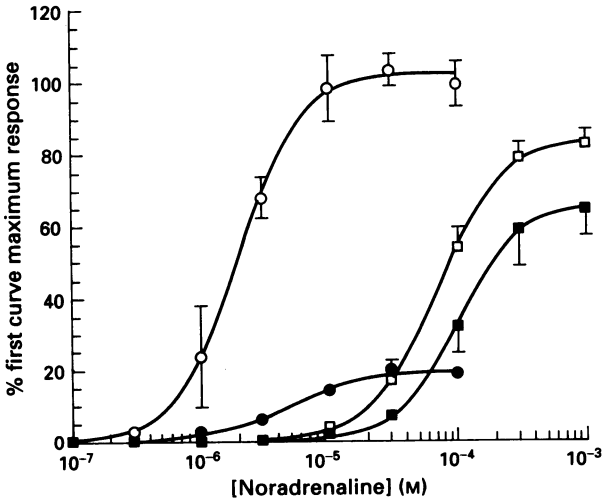

Figure 3 Effect of (+)-niguldipine (0.1 nM) or vehicle, perfused for 1 h in the presence or absence of prazosin (10 nM), on vasoconstrictor responses to perfused noradrenaline in the isolated perfused mesentery preparation of rat: (○) vehicle; (●) niguldipine 0.1 nM; (□) prazosin 10 nM; (■) prazosin 10 nM + niguldipine 0.1 nM. Data shown are second concentration-response curves and give mean ± s.e.mean from 4 separate experiments.

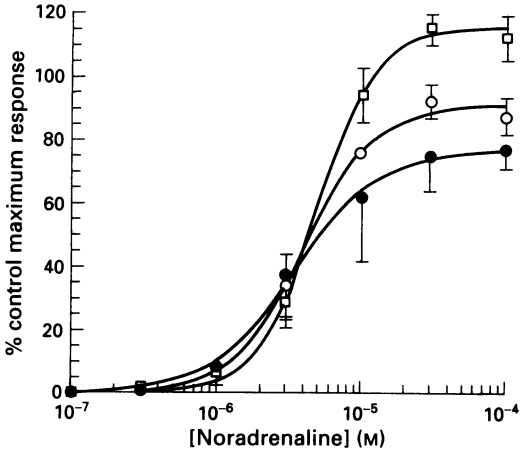

Figure 4 Effect of exposure to chloroethylclonidine (CEC) on vasoconstrictor responses to perfused noradrenaline (NA) in the isolated perfused mesentery preparation of rat. CEC (or vehicle) was perfused for 20 min (with 40 min washout) following construction of the first concentration-effect curve to NA: (□) vehicle; (○) CEC 10 μM (●) CEC 100 μM. Data shown are second concentration-response curves and give mean ± s.e.mean from 3-4 separate experiments.

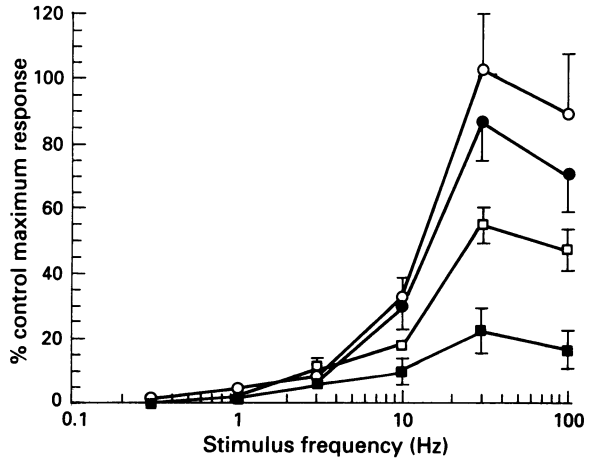

Figure 5 Effect of prazosin on vasoconstrictor responses to periarterial nerve stimulation in the isolated perfused mesentery preparation of rat (see text for stimulation parameters): (○) vehicle; (●) prazosin 0.3 nM; (□) prazosin 1.0 nM; (■) prazosin 3.0 nM. Data shown are second frequency-response curves and give mean ± s.e.mean from 4 separate experiments.

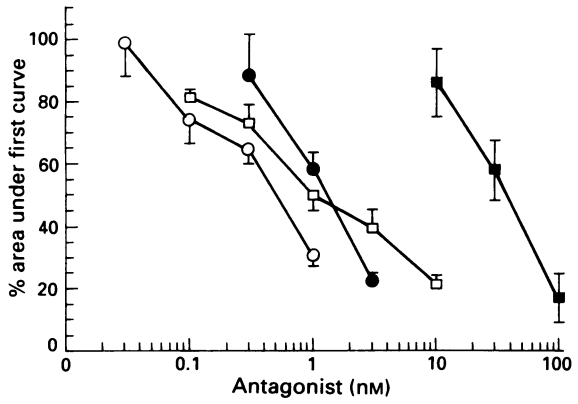

Figure 6 Inhibition by WB4101 5-methyl urapidil (5-MU), prazosin and spiperone of vasoconstrictor responses to periarterial nerve stimulation in the isolated perfused mesentery preparation of rat (see text for stimulation parameters): (○) WB4101; (●) prazosin; (□) 5-MU; (■) spiperone. Data shown are mean ± s.e.mean from 3–5 separate determinations.

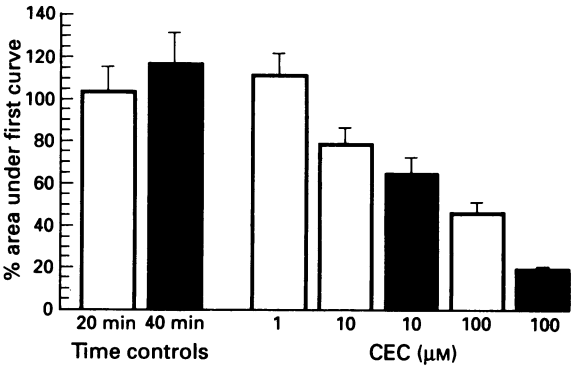

Figure 7 Effect of exposure to chloroethylclonidine (CEC) on vasoconstrictor responses to periarterial nerve stimulation in the isolated perfused mesentery preparation of rat (see text for stimulation parameters). CEC was perfused for 20 min (open columns) or 40 min (solid columns) following construction of the first frequency-response curve. A 40 min washout period was allowed before construction of a second frequency-response curve. Data shown are mean ± s.e.mean, from 4–12 separate experiments.

In the present study the vasoconstrictor response to perfused NA was antagonized with high affinity ($pA_2 = 9.3$) by the non-subtype-selective α_1-adrenoceptor antagonist, prazosin and also by the α_{1A}-adrenoceptor-selective antagonists WB4101, 5-MU and (+)-niguldipine (Morrow & Creese, 1986; Gross *et al.*, 1988; Hanft & Gross, 1989; Boer *et al.*, 1989). The affinity estimates obtained with WB4101 and 5-MU (9.6 and 8.9 respectively), plus the apparently high affinity for (+)-niguldipine, suggest strongly that the vasoconstrictor response to perfused NA is mediated via α_{1A}-adrenoceptors (Han *et al.*, 1990). This contention is supported by the observation that the pA_2 value for spiperone (7.7), which is approximately 10 fold selective for the α_{1B}-adrenoceptor subtype (Michel *et al.*, 1989), was closest to its affinity at α_{1A}-adrenoceptors as determined in ligand binding studies (pK_i s at α_{1A} and α_{1B}-adrenoceptors of 8.14 and 9.25 respectively; Michel *et al.*, 1989).

Although (+)-niguldipine also has high affinity for L-type calcium channels (Boer *et al.*, 1989), the protective effect of co-perfusion with prazosin (10 nM), together with the lack of effect of nitrendipine (1 μM) towards NA, serve to suggest that the insurmountable antagonism by (+)-niguldipine is related to an action at α_{1A}-adrenoceptors and not to blockade of L-type channels. Insurmountable antagonism towards responses to NA may result from lack of equilibrium between a high affinity antagonist and NA, as described by Clarke *et al.* (1991) in an isolated perfused kidney preparation of rat.

A number of studies have shown that CEC, despite having similar affinity at both α_{1A}- and α_{1B}-adrenoceptors, appears to alkylate preferentially the latter subtype (Han *et al.*, 1987; Minneman *et al.*, 1988). The modest effect of even large concentrations of CEC against perfused NA in the present study and the fact that CEC (100 μM) did not alter the apparent affinity estimate for prazosin, WB4101 or 5-MU, rules out the involvement of α_{1B}-adrenoceptors.

The interpretation of results from experiments in which nerve stimulation was used to elicit vasoconstrictor responses is complicated by a number of factors, including possible drug effects on NA release (see below) and the inability to calculate absolute antagonist affinities (as synaptic concentrations of NA are not known, and parallel shifts of frequency-

response curves were not obtained). However, the pharmacological characteristics of the stimulation-induced responses in the present study are similar to the results obtained with exogenously-perfused NA, and suggest that the two types of responses are mediated by similar receptors. Thus, stimulation-induced responses were antagonized with high affinity by prazosin and by the α_{1A}-selective antagonists, WB4101 and 5-MU. Similarly, spiperone was effective only at concentrations consistent with its binding affinity for α_{1A}-adrenoceptors.

At first sight, CEC appears to antagonize nerve-induced responses to a greater extent that those to perfused NA. However, it must be noted that for the competitive antagonists studied, a 50% reduction in response to electrical stimulation was obtained with concentrations of antagonist which approximated those producing a twofold shift in the CRC to exogenously-perfused NA. Taking this into account, the antagonism by CEC of neuronally-mediated vasoconstrictor responses is similar to its ability to antagonize responses to exogenously-perfused NA. Consideration of Figure 7 also shows a time-dependency for inhibition (alkylation) by CEC. This slow rate of alkylation, together with the lack of complete inhibition, is consistent with the reported action of CEC on the cloned α_{1C}- and α_{1D}-adrenoceptors, rather than on α_{1B}-adrenoceptors (Schwinn *et al.*, 1990; Perez *et al.*, 1991). This deduction does not imply a different adrenoceptor for nerve-induced vasoconstriction versus that elicited by exogenously-perfused noradrenaline, as we argue below that the cloned α_{1C}-adrenoceptor may equate with the functionally-defined 'classical' α_{1A}-adrenoceptor.

As a note of caution it must be pointed out that a recent publication by Bültmann & Starke (1993) concluded that CEC, acting as an 'irreversible' pre-junctional α_2-adrenoceptor agonist, significantly reduced field-stimulated [^{3}H]-NA release in an isolated vas deferens preparation from rat. The authors proposed that this action 'limits the suitability of CEC for the characterization of post-junctional α_1-adrenoceptors mediating responses to sympathetic nerve stimulation'. Although in the present study the α_2-adrenoceptor antagonist, idazoxan (300 nM) was included in the perfusion fluid, such a pre-junctional action of CEC on electrically-stimulated NA release cannot be excluded entirely.

In summary, the present study suggests that α_1-adrenoceptors mediating vasoconstrictor responses to both perfused and neuronally-released NA exhibit a pharmacological profile which is similar to that of the 'classical' α_{1A}-adrenoceptor. This study confirms and extends observations published recently by Kong *et al.* (1994) who, using the same preparation from rat, also concluded that NA-induced vasoconstriction appeared to be mediated largely via α_{1A}-adrenoceptors. This conclusion was based on a high affinity estimate for 5-MU (pA_2 value of 9.24 versus bolus doses of perfused NA) and a relatively small effect of CEC (10 μM for 1 h).

The question as to which of the currently cloned α_1-adrenoceptors most closely resembles the receptor characterized in the present study is the subject of debate, and has been addressed in a recent article from our laboratory (Ford *et al.*, 1994). Briefly, the cloned bovine α_{1C}-adrenoceptor appears to provide the closest match to the 'classical' α_{1A}-adrenoceptor, based on such criteria as high affinity for WB4101, 5-MU and (+)-niguldipine. Although expression of α_{1C}-adrenoceptor RNA in rat tissues was not detected by Schwinn *et al.* (1991), the same study also failed to detect the mRNA for the α_{1C}-adrenoceptor in bovine cortex, the tissue from which the α_{1C}-adrenoceptor was originally cloned. In a more recent report, Rokosh *et al.* (1994) have cloned a fragment of the α_{1C}-adrenoceptor from cardiac myocytes of rat, and have used an RNase protection assay to demonstrate abundant levels of mRNA for the α_{1C}-adrenoceptor in a number of rat tissues. These tissues include submaxillary gland, a tissue characterized previously in radioligand binding studies as containing only the 'classical' α_{1A}-adrenoceptor (Michel *et al.*, 1989). In addition, Forray and colleagues (1994) have reported the cloning and expression of a rat homologue of the α_{1C}-adrenoceptor which, while sharing many pharmacological characteristics of the bovine α_{1C}-adrenoceptor, is relatively insensitive to alkylation by CEC (only 19% loss of binding sites following 100 μM CEC, compared with 45% inactivation of the bovine α_{1C}-adrenoceptor). Cloning and expression of an α_{1C}-adrenoceptor from rat has also been reported by Perez *et al.* (1994): the authors concluded that the pharmacology of this receptor most resembled that of the α_{1A}-adrenoceptor.

The observations considered above suggest strongly that the α_{1A}-adrenoceptor characterized in the present study represents the rat homologue of the cloned bovine α_{1C}-adrenoceptor. Such an alignment is fully consistent with the pharmacological profile reported here. Whether the receptor should be called an α_{1A}- or α_{1C}-adrenoceptor awaits the decision of the adrenoceptor Nomenclature Committee.

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