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Competitive antagonism of α_1-adrenoceptor mediated pressor responses in the rat mesenteric artery

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(−)-Noradrenaline-mediated pressor responses in the rat mesenteric artery preparation are blocked by α_1-adrenoceptor antagonists, BE2254 and prazosin with pA_2 values of 8·59 and 8·52 respectively. The α_2-adrenoceptor selective antagonist rauwolscine, does not influence responses to (−)-noradrenaline in concentrations up to 10 μM. This rank order is consistent with an α_1-adrenoceptor mediated effect. Shifts in the (−)-noradrenaline dose-response curve produced by BE2254 and prazosin were parallel and there was no significant effect on the observed maximal response. Slope values from Schild plots were not significantly different from unity indicating that these antagonists were behaving as classical competitive antagonists.

Noradrenaline-induced pressor responses in the rat mesenteric artery preparation appear to be particularly sensitive to low concentrations of the α_1-adrenoceptor antagonist, prazosin (Adeagbo 1980; Downing et al 1983). Antagonism appears non-competitive, since maximal responses to noradrenaline are depressed at concentrations of prazosin which shift the noradrenaline dose-response curve to the right. The reason for this non-competitive antagonism is not readily apparent. Recent evidence suggests that a β-adrenoceptor mediated depressor mechanism may also operate in the rat mesenteric artery (Borkowski & Porter 1983). It it possible that the response to noradrenaline observed in untreated mesenteric artery preparations may be a balance between the actions of noradrenaline on both α- and β-adrenoceptors.

The purpose of the present work was to evaluate the activity of several selective α-adrenoceptor antagonists against noradrenaline-induced pressor responses in the rat mesenteric artery. The characterization of the α-adrenoceptor was performed in the presence of a β-adrenoceptor antagonist, and neuronal and extraneuronal uptake blockers to preclude the possibility of these mechanisms modulating the observed noradrenaline responses.

Methods

Female hooded Wistar rats (185–210 g) were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.). The mesentery was excised as described by McGregor (1965) and perfused as described by Coupar & McLennan (1978). The preparation was perfused (2 ml min^{-1}, 37 °C) with Krebs-Henseleit solution (NaCl 6·87; KCl 0·4; $MgSO_4$ $7H_2O$ 0·14; $NaH_2PO_4.2H_2O$ 0·18; $NaHCO_3$ 2·1; $CaCl_2$ 0·28 and D(+)-glucose 2·0 g $litre^{-1}$), which also contained propranolol (1 μM), desmethylimipramine (0·5 μM) and normetanephrine (1 μM).

The preparation was allowed to equilibrate for 30 min before the addition of (−)-noradrenaline. Changes in perfusion pressure produced by (−)-noradrenaline were recorded via a Statham P23DC pressure transducer connected to a Grass Model 7C polygraph. Pressor responses were produced by perfusing the preparation with a Krebs-Henseleit solution with increasing concentrations of (−)-noradrenaline. Each concentration of (−)-noradrenaline was perfused through the tissue for 10 s, the responses being separated by 4 min intervals. In the calculation of results, the dose delivered was calculated as the total amount (in nmol) of (−)-noradrenaline infused over this 10 s period.

When assessing the actions of α-adrenoceptor antagonists, one control curve to (−)-noradrenaline was constructed and this was repeated at 30 min intervals after the addition of increasing concentrations of the antagonist to the perfusion medium. Responses were calculated as increases in perfusion pressure (mmHg) and then converted to a percentage of the maximal response obtained in the first control curve. pA_2 values were calculated according to the method described by Arunlakshana & Schild (1959).

Results

(−)-Noradrenaline produced a dose-dependent increase in perfusion pressure as shown in Fig. 1, with an EC50 of 7·46 ± 1·2 (mean ± s.e.m., n = 25) nmol. The maximal increase in perfusion pressure obtained was 152 ± 7 mmHg above the basal perfusion pressure of 32 ± 4 mmHg (mean ± s.e.m., n = 25). To assess the reproducibility of (−)-noradrenaline responses over the time-course of the experiments, control experiments were performed in which (−)-noradrenaline dose-response curves were constructed at 30 min intervals. It was found that (−)-noradrenaline dose-response curves were superimposable for up to at least 4 times (2 h).

The α-adrenoceptor antagonists BE2254 (2[β-(4-hydroxyphenyl)ethyl-amino methyl]-tetralone), prazosin and phentolamine blocked responses to (−)-noradrenaline. The rank order of potency was: BE2254 = prazosin > phentolamine (Table 1). In all three cases, (−)-noradrenaline dose-response curves were shifted to the right without any significant reduction in the

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Table 1. pA_2 values together with the slope values from Schild plots (Arunlakshana & Schild 1959) for four α-adrenoceptor antagonists in the rat mesentery preparation. Values given are the mean ± s.e.

Antagonist	pA_2	Slope	Antagonist concentration range (M)	n
BE2254	8·59 ± 0·06	1·02 ± 0·05	10^{-9}–10^{-7}	5
Prazosin	8·52 ± 0·15	1·10 ± 0·04	10^{-8}–10^{-7}	4
Phentolamine	7·11 ± 0·09	1·05 ± 0·04	10^{-7}–10^{-5}	5
Rauwolscine	inactive	—	10^{-7}–10^{-5}	4

maximal responses. Also the slopes of the Schild plots were not significantly different from unity, indicating all behave as classical competitive antagonists. The mean dose-response curves for (−)-noradrenaline in the absence and presence of increasing concentrations of prazosin are shown in Fig. 1A.

Rauwolscine, an α_2-selective antagonist (Weitzell et al 1979) did not affect (−)-noradrenaline dose-response curves up to a concentration of 10 μM. The mean curves to (−)-noradrenaline in the absence and presence of increasing concentrations of rauwolscine are shown in Fig. 1B.

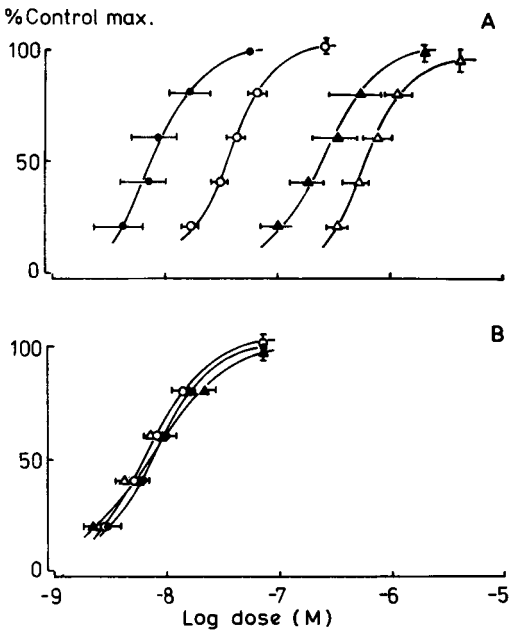

FIG. 1. Mean dose-response curves for noradrenaline in the absence (●) and in the presence of either prazosin (10^{-8} (○), 5×10^{-8} (▲), or 10^{-7} (△) M panel (A) or rauwolscine (10^{-7} (○), 10^{-6} (▲) or 10^{-5} (△) M; panel B). Mean curves were constructed by calculating the mean dose for a given percentage response. Responses in the presence of the α-adrenoceptor antagonists were expressed as a percentage of the maximal response obtained to the first (control) dose-response curve. Error bars represent the s.e. of the mean for 3 to 5 separate determinations.

Discussion

This study has confirmed that (−)-noradrenaline induced pressor responses are mediated via α_1-adrenoceptors. Thus BE2254 and prazosin, both of which are α_1-selective (Doxey et al 1977; Heinz & Hofferber 1980), were potent antagonists of (−)-noradrenaline responses. Phentolamine (non-selective) was also a potent antagonist. Conversely, rauwolscine, an α_2-selective antagonist, did not affect (−)-noradrenaline pressor responses at concentrations up to 10 μM. This rank order of potency (i.e. BE2254 = prazosin > phentolamine ≫ rauwolscine) is consistent with an α_1-adrenoceptor mediated action of (−)-noradrenaline in this preparation. However, there are certain dissimilarities between these results and others reported in the literature.

Firstly it has been reported that prazosin, in low concentrations ($10^{-9} - 5 \times 10^{-8}$ M) can depress the maximal responses observed to (−)-noradrenaline (Adeagbo 1980; Downing et al 1983) as well as shifting the dose-response curve to the right. This is in disagreement with this work which showed prazosin acting as a classical competitive antagonist. In previous studies (Downing et al 1983) and in the present work, precautions were taken to block both β-adrenoceptors and neuronal uptake. The most obvious differences are that in previous studies noradrenaline was delivered as a bolus dose to the mesentery obtained from male Wistar rats. However, in this study a 10 s infusion of the mesentery from female Hooded Wistar rats was used. These differences may account for the discrepant results.

Secondly, evidence has been provided (Fiotakis & Pipili 1983) which suggests that an α_2-adrenoceptor mediated dilator response may operate in the rat mesenteric vascular bed. This conclusion was drawn from the finding that rauwolscine potentiated submaximal responses to both noradrenaline and phenylephrine. Again, this was not apparent in the present work. Rauwolscine failed to affect (−)-noradrenaline responses even at high concentrations. The lack of activity of rauwolscine is somewhat surprising, since previous work has shown that this compound is a potent antagonist (pA_2 = 7·8) of α_1-adrenoceptor mediated constrictor responses produced by (−)-adrenaline in guinea-pig spleen strips (McPherson, unpublished observations). A species/tissue difference however, may account for this finding.

Despite these obvious differences, the results of the present study indicate that under controlled experimental conditions, (−)-noradrenaline pressor responses in the rat mesentery preparation are mediated through α_1-adrenoceptors. In addition, prazosin, BE2254 and phentolamine all behave as classical competitive antagonists of these responses.

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Comparison between in-vivo and in-vitro tissue-to-plasma unbound concentration ratios ($K_{p,f}$) of quinidine in rats

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The comparison between in-vivo and in-vitro tissue-to-plasma concentration ratio of drug unbound ($K_{p,f}$) has been made using quinidine as a model for weak basic drugs. In-vitro $K_{p,f}$-values were calculated from the binding data to tissue homogenates determined by equilibrium dialysis. In-vivo $K_{p,f}$-values were calculated from the tissue distribution data after intravenous administration of quinidine, by considering the difference in the unbound concentration between plasma and the tissues produced by the pH difference across the cell membrane. It was concluded that the extensive tissue distribution of quinidine observed in-vivo may be explained by tissue binding and the pH-difference across the cell membrane in most tissues.

The recent development of physiological pharmacokinetic models has made it possible to predict quantitatively the distribution and elimination of drugs in various species (Himmelstein & Lutz 1979). One of the parameters required to develop these models is the tissue-to-plasma partition coefficient (K_p), which is defined as the ratio of the drug concentration in the tissue to that in the venous plasma. We have succeeded in estimating K_p-values of ethoxybenzamide from in-vitro binding studies using plasma and tissue homogenates, suggesting that in-vitro binding data can be used to estimate the in-vivo tissue distribution (Lin et al 1982).

Weak basic drugs such as quinidine, imipramine and propranolol show characteristic tissue distributions with respect to their extensive tissue distribution and marked differences in K_p-values among tissues (Bickel et al 1975; Biarchetti et al 1980; Harashima et al 1983). In our previous study, we determined the tissue distribution of quinidine after its intravenous administration to rats and found a good correlation of the K_p-values for quinidine and propranolol or imipramine in various tissues suggesting that there exists a common mechanism for tissue distribution among these basic drugs (Harashima et al 1983).

For the present study, we selected quinidine as a model drug for weak basic drugs and compared the tissue binding determined in in-vitro studies with those obtained in in-vivo (Harashima et al 1983).

Methods

Adult Wistar (Nihon Seibutsu Zairyo, Tokyo, Japan) male rats, 250–300 g were used. Five rats were decapitated and each tissue excised, pooled and frozen at −20 °C until study. 17% tissue homogenates were prepared in 0·01 M phosphate buffer containing 0·15 M KCl (pH 7·0) and were predialysed for 2 days at 4 °C against the same phosphate buffer to remove the substances which affect the fluorometric determination of quinidine. The tissue binding was determined by equilibrium dialysis at 37 °C for 4 h using semimicrocells and a semipermeable membrane (Spectrapor membrane, Spectrum Medical Industries Inc., CA) against 0·01 M phosphate buffer containing 0·15 M KCl (pH 7·0). The initial concentration of quinidine in each homogenate was 2·0 μg ml^{-1}. Heat-treated homogenates were prepared placing them in boiling water (100 °C) for 15 min. n-Butanol-treated homogenates were prepared by the method of Ishitani et al (1977). Briefly, 2 ml of 17% liver homogenate was extracted with water-saturated n-butanol by shaking for 30 min. Treated homogenates were predialysed against the phosphate buffer for 2 days at 4 °C. Subcellular distribution of quinidine in the liver was determined by the method of Schneck et al (1977). 17% liver homogenate was prepared in 0·05 M Tris buffer containing 0·25 M sucrose (pH 7·0) at 4 °C. Quinidine was spiked into the homogenate at the initial concentration of 1·74 μg ml^{-1} and then was incubated for 10 min at 4 °C. Four subcellular fractions, nuclei and cell debris, mitochondria, microsomes, and cytosol were obtained by the

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