Br. J. Pharmac. (1983), **78**, 655–657

An investigation of presynaptic α-adrenoceptor subtypes in the pithed rat heart

James R. Docherty

Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St Stephen's Green, Dublin 2, Ireland

1 The presynaptic cardio-inhibitory and postsynaptic pressor responses to the α-adrenoceptor agonists xylazine and cirazoline, and the interaction with the antagonists yohimbine and prazosin, were examined in the pithed rat.

2 Evidence was found to suggest that, as well as the already established pre- and postsynaptic α_2- and postsynaptic α_1-receptors, presynaptic α_1-receptors are present.

Introduction

When it became clear that there were two major groups of peripheral α-adrenoceptor, there was an initial subclassification into α_1- postsynaptic and α_2-presynaptic adrenoceptors (Langer, 1974). It is now known that α_2-adrenoceptors are not restricted to nerve terminals but are also present on vascular smooth muscle cells (Drew & Whiting, 1979; Docherty, MacDonald & McGrath, 1979; Timmermans, Kwa & van Zwieten, 1979), and this and other observations have led to a more general subclassification independent of receptor location (Starke & Langer, 1979). However, it has generally been assumed that presynaptic α-adrenoceptors are exclusively, or at least predominantly, of the α_2-type, although one study suggests that α_1-receptors may also be present (Kobinger & Pichler, 1980). The object of the present investigation was to examine presynaptic α-adrenoceptors in the pithed rat heart, and to demonstrate that, as well as α_2-receptors, α_1-receptors are present.

Methods

Male Wistar rats (225–275 g) were pithed by the method of Gillespie, MacLaren & Pollock (1970) and respired with 100% O_2, 1 ml/100 g per beat, at a rate of 60/min. Heart rate was extracted from carotid arterial pressure, and the right jugular vein was used for drug injections.

Cardioaccelerator responses were obtained to single pulse (0.05 ms, supramaximal voltage) stimulation of the sympathetic outflow at T_1 every 2 min (Docherty & McGrath, 1980). Cumulative dose-response curves were obtained to each agonist: presynaptic effects were assessed as the inhibition of the cardioaccelerator response to a single pulse, measured 5 min after drug injection; postsynaptic effects were assessed as the rise in diastolic blood pressure measured 5 min after injection (Docherty & McGrath, 1980). In interaction experiments, the antagonist was injected intravenously 10 min before beginning the agonist dose-response curve. Agonist ID_{50} values (dose producing 50% inhibition of the cardioaccelerator response to a single stimulus pulse) and ED_{50} values (dose producing a rise in diastolic pressure of 50 mmHg) were obtained from each individual experiment. Differences in ID_{50} or ED_{50} values between groups were compared by Student's *t* test for unpaired data.

Drugs used were cirazoline hydrochloride (gift: Synthelabo, Paris); prazosin hydrochloride (gift: Pfizer, Sandwich); yohimbine hydrochloride (Sigma, Poole); xylazine hydrochloride (gift: Bayer, Leverkusen). Drug stocks were prepared in distilled water and drug dilutions were administered in 0.9% w/v NaCl solution (saline), except for prazosin (distilled water).

Results

In pithed rats basal heart rate was in the range of 280–330 min^{-1}, and resting diastolic pressure was 30–40 mmHg. High concentrations of agonists ($>$ 1 mg/kg) produced marked falls in heart rate, but

Table 1 Effects of α-adrenoceptor antagonists on the cardio-inhibitory and pressor responses to the agonists xylazine and cirazoline

	Presynaptic ID_{50}		*Postsynaptic ED_{50}*	
	Xylazine	*Cirazoline*	*Xylazine*	*Cirazoline*
No antagonist	23 (5.5–99)	5.5 (2.5–12)	940 (430–2100)	29 (21–41)
Yohimbine (100 μg/kg)	/	33* (20–53)	/	38 (16–92)
Yohimbine (1 mg/kg)	1200* (380–3700)	61* (47–79)	4700* (3500–6500)	190* (51–700)
Prazosin (100 μg/kg)	/	35* (19–66)	/	420* (240–740)
Prazosin (1mg/kg)	36 (15–88)	99* (26–370)	1000 (430–2500)	>1000*
Yohimbine (0.5 mg/kg) + Prazosin (0.5 mg/kg)	/	1200* (840–1700)	/	>1000*

Effects of agonists are expressed as dose (μg/kg) producing 50% inhibition of the cardioaccelerator response to stimulation (ID_{50}) or a rise in diastolic blood pressure of 50 mmHg (ED_{50}); effects were measured 5 min after injection of the agonist. Values shown are geometric mean and 95% confidence limits, obtained from linear regression analysis of cumulative dose-response curves, and are the mean of at least 4 experiments.
*Denotes response to agonist significantly different from response to agonist in absence of antagonist (Student's *t* test, $P<0.05$).

heart rate was near pre-injection level at 5 min, when nerve-mediated responses were measured, except in the case of cirazoline ($28\pm5\,min^{-1}$ below pre-injection). In some animals, cirazoline (1 mg/kg) was fatal. Low doses of cirazoline produced small rises in heart rate and the tachycardia was prevented by prazosin (100 μg/kg) but not by yohimbine (1 mg/kg).

Single pulse stimulation produced a cardioacceleration of $24\pm1.4\,min^{-1}$ ($n=28$). Antagonist drugs caused an initial large inhibition of the cardioacceleration, but the response had recovered by 1 min prior to agonist injection to $95\pm1.6\%$, $89\pm4.7\%$, $87\pm6.5\%$ and $91\pm5.4\%$ of control after yohimbine (0.1 and 1 mg/kg) and prazosin (0.1 and 1 mg/kg), respectively ($n=4$–14, in all cases not significantly different from saline effects). The combination of yohimbine (0.5 mg/kg) and prazosin (0.5 mg/kg) significantly reduced the single pulse response to $83\pm3.9\%$ of control ($P<0.05$ when compared to saline effects).

Xylazine and cirazoline produced dose-dependent pressor responses and dose-dependent inhibition of the cardioacceleration to a single stimulus pulse. In the presence of yohimbine (1 mg/kg), the dose-response curves for each agonist were shifted to the right with ID_{50} and ED_{50} values significantly different from controls, although the shift in ID_{50} was greater for xylazine (Table 1). Prazosin (1 mg/kg) did not affect responses to xylazine but produced large shifts in the ID_{50} and ED_{50} of cirazoline. Against cirazoline, prazosin and yohimbine were approximately equi-effective presynaptically, even at the lower concentration of 100 μg/kg, but the combination of yohimbine (0.5 mg/kg) and prazosin (0.5 mg/kg) produced a significantly greater shift in the ID_{50} than either drug alone in a dose of 1 mg/kg ($P<0.01$ in both cases) (Table 1).

Discussion

It is now established that pressor responses in the pithed rat are mediated by both α_1- and α_2-adrenoceptors (see Docherty & McGrath, 1980). In the present experiments, the pressor response to the α_2-agonist xylazine (Docherty & McGrath, 1980) was antagonized by yohimbine but not by prazosin, and the pressor response to the α_1-agonist cirazoline (Roach, Lefevre & Cavero, 1978) was antagonized by prazosin and to a lesser extent by yohimbine. However, whereas the presynaptic inhibitory effects of xylazine were antagonized by yohimbine but not by prazosin, the presynaptic inhibitory effects of cirazoline were antagonized by yohimbine and prazosin to a similar extent. The nature of the presynaptic actions of cirazoline is clarified by the fact that the combination of yohimbine and prazosin together was more potent than either drug alone, confirming that cirazoline acted on both α_1- and α_2-adrenoceptors.

The existence of presynaptic α_1-receptors in the

pithed rat heart has been suggested previously (Kobinger & Pichler, 1980). The present results confirm and extend those findings, but whereas Kobinger and Pichler concluded that pre- and postsynaptic α_1-receptors (likewise α_2) differed from each other, the present data are explicable without the necessity of a further subdivision of α-adrenoceptors. Cirazoline was predominantly an α_1-agonist postsynaptically but a mixed agonist presynaptically, which suggests that α_1-receptors are a relatively smaller proportion of the receptor population presynaptically than postsynaptically. In agreement with this, yohimbine caused a greater shift presynaptically than postsynaptically in the dose-response curve of xylazine, and prazosin caused a greater shift postsynaptically than presynaptically against cirazoline; differences in the linkage between receptor activation and response may also contribute.

In a previous study (Roach *et al.*, 1978), cirazoline had little effect on the tachycardia produced by continuous stimulation of cardioaccelerator nerves in the pithed rat, in contrast to the present results which employed intermittent stimulation. However, continuous stimulation is an unsuitable method for the assessment of possible presynaptic inhibitory effects of an agent which itself produces a tachycardia. In preliminary experiments for the present study, cirazoline had inconsistent effects on the tachycardia to continuous stimulation at a frequency of 0.1 Hz (Author, unpublished results). Similar apparently contradictory findings have arisen with the α_1-agonist, St 587, in the pithed rat: it did not affect the tachycardia to continuous stimulation (De Jonge, Van Meel, Timmermans & Van Zwieten, 1981), but inhibited the tachycardia to intermittent stimulation (Kobinger & Pichler, 1982).

In conclusion, while the present results confirm the existence of an α_1-receptor-mediated inhibition of cardioaccelerator responses in the pithed rat, it remains to be established whether these are true presynaptic autoreceptors and whether these receptors are widespread in peripheral nerves.

This study was supported by the medical research funds of the Royal College of Surgeons in Ireland and by the M.R.C. (Ireland). Generous gifts of drugs are gratefully acknowledged.

References

DE JONGE, A., VAN MEEL, J.C.A., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1981). A lipophilic, selective alpha$_1$-adrenoceptor agonist: 2-(2-chloro-5-trifluoromethylphenylimino) imidazolidine (St 587). *Life Sci.*, **28**, 2009–2016.

DOCHERTY, J.R., MACDONALD, A. & MCGRATH, J.C. (1979). Further subclassification of α-adrenoceptors in the cardiovascular system, vas deferens and anococcygeus of the rat. *Br. J. Pharmac.*, **67**, 421–422P.

DOCHERTY, J.R. & MCGRATH, J.C. (1980). A comparison of pre- and post-junctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus muscle of the rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **312**, 107–116.

DREW, G.M. & WHITING, S.B. (1979). Evidence for two distinct types of postsynaptic α-adrenoceptor in vascular smooth muscle in vivo. *Br. J. Pharmac.*, **67**, 207–215.

GILLESPIE, J.S., MACLAREN, A. & POLLOCK, D. (1970). A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. *Br. J. Pharmac.*, **40**, 257–267.

KOBINGER, W. & PICHLER, L. (1980). Investigation into different types of post- and presynaptic alpha-adrenoceptors at cardiovascular sites in rats. *Eur. J. Pharmac.*, **65**, 393–402.

KOBINGER, W. & PICHLER, L. (1982). Presynaptic activity of the imidazolidine derivative St 587, a highly selective alpha$_1$-adrenoceptor agonist. *Eur. J. Pharmac.*, **82**, 203–206.

LANGER, S.Z. (1974). Commentary: presynaptic regulation of catecholamine release. *Biochem. Pharmac.*, **23**, 1793–1800.

ROACH, A.G., LEFEVRE, F. & CAVERO., I. (1978). Effects of prazosin and phentolamine on cardiac presynaptic alpha-adrenoceptors in the cat, dog and rat. *Clin. exp. Hypertension*, **1**, 87–102.

STARKE, K. & LANGER, S.Z. (1979). A note on terminology for presynaptic receptors. In *Presynaptic Receptors, Advances in the Biosciences*, Vol. 18, ed. Langer, S.Z., Starke, K. & Dubocovich, M.L., p. 1. Oxford: Pergamon Press.

TIMMERMANS, P.B.M.W.M., KWA, H.Y. & VAN ZWIETEN, P.A. (1979). Possible subdivision of postsynaptic alpha-adrenoceptors mediating pressor responses in the pithed rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **314**, 149–156.

(Received August 4, 1982. Revised November 4, 1982.)