Br. J. Pharmac. (1985), **86**, 335–339

No evidence for differences between pre- and post-junctional α_2-adrenoceptors

J.R. Docherty[1] & L. Hyland

Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2, Ireland

1 We have examined the pre- and post-junctional effects of a series of α-adrenoceptor agonists and antagonists at α_2-adrenoceptors in the pithed rat preparation and the human isolated saphenous vein.

2 In the pithed rat, there was no difference in relative agonist and antagonist potencies between pre- and post-junctional α_2-adrenoceptors but the absolute potencies of antagonists differed: antagonists were more potent prejunctionally.

3 In the human saphenous vein, the α_2-adrenoceptor antagonist yohimbine had pre- and post-junctional actions over the same concentration range.

4 We have no evidence to suggest differences between pre- and post-junctional α_2-adrenoceptors: differences in absolute antagonist potencies in the pithed rat may be due to non-equilibrium conditions.

Introduction

In the periphery, it is now well established that two types of α-adrenoceptor exist, the α_1- and α_2-subtypes, and that both subtypes can be present postjunctionally on vascular smooth muscle (Docherty & McGrath, 1980a; Constantine *et al.*, 1980) or prejunctionally on adrenergic nerve terminals (Kobinger & Pichler, 1980; Docherty, 1984). However, some authors have suggested that two subtypes of α-adrenoceptor are insufficient to explain all findings, and in particular that prejunctional and postjunctional α_2-adrenoceptors may differ (Kobinger & Pichler, 1980; Hicks, 1981), although De Jonge *et al.* (1981) have suggested that these differences may be small.

The present study arose from investigations in the pithed rat preparation in which postjunctional α_2-receptor-mediated pressor responses were characterized (Docherty & McGrath, 1980a,b): the major problem had been the low post-junctional potency of the α_2-adrenoceptor antagonist yohimbine. We can now report that the relatively poor postjunctional potency of α_2-adrenoceptor antagonists in the pithed rat preparation does not appear to be due to differences between pre- and post-junctional α_2-adrenoceptors. There is no evidence to suggest a further subdivision of α_2-adrenoceptors as yet.

The choice of antagonist drugs was based on their selectivities for α_1- or α_2-adrenoceptors: prazosin as α_1-adrenoceptor selective, phentolamine and phenoxybenzamine as relatively non-selective, and yohimbine, rauwolscine and Wy 26392 (Lattimer *et al.*, 1982) as α_2-adrenoceptor selective antagonists (see Stark, 1981; Docherty, 1984).

Some of these results have been published in abstract form (Docherty, 1983).

Methods

Pithed rat preparation

Male Wistar rats (250–300 g) were pithed by the method of Gillespie *et al.* (1970) and ventilated with 100% O_2 at a rate of 60 per min. Heart rate was extracted from carotid arterial pressure and drugs were injected into the jugular vein. The pithing rod was used as an electrode positioned at T_1 to stimulate the cardio-accelerator nerves with a single stimulus pulse (0.5 ms, supramaximal voltage) every 2 min.

Dose-response curves to α-adrenoceptor agonists were constructed from the effects of increasing doses (10 fold increments) administered at 5 min intervals. Prejunctional effects of agonists were assessed as the inhibition of the cardio-acceleration to a single stimulus and postjunctional effects were assessed as the peak pressor response. In interaction experiments,

[1]Correspondence.

antagonist drugs were administered intravenously 10 min before starting the agonist dose-response curve, except in some experiments involving phenoxybenzamine in which the antagonist was administered intraperitoneally 24 h before pithing, as stated in results. Potency of agonists was expressed as a prejunctional ID_{50} (dose producing 50% inhibition of the cardio-acceleration to a single stimulus) and a postjunctional ED_{50} (dose producing a rise in diastolic blood pressure of 45 mmHg or 60 mmHg, which are approximately 50% of maximum pressor response for α_2- and α_1-adrenoceptor agonists, respectively) and the effects of antagonists were assessed as the shift produced in agonist potency.

Human saphenous vein

Human saphenous veins were obtained from coronary artery bypass grafts of male patients (aged 38–58 years). Tissues were cut spirally into strips approximately 3 mm wide and 20 mm long, placed between platinum electrodes in organ baths and superfused at 37°C at a rate of 2 ml min^{-1} in Krebs-Henseleit solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, (+)-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, ascorbic acid 0.28, tetrasodium EDTA 0.03. Tissues were attached to myograph transducers under 1 g tension for isometric tension recordings.

In all experiments, tissues were stimulated 5 or 6 times (S_1–S_6) for 3 min at a frequency of 5 Hz at intervals of 30 min, beginning after 2 h of superfusion. Yohimbine, Wy 26392 or distilled water vehicle was added to the superfusion stream at a rate of 16 μl min^{-1} in three or four cumulative concentrations beginning 18 min before the third stimulation (S_3). The effects of yohimbine, Wy 26392 or vehicle on the stimulation-evoked contraction were expressed in terms of an IC_{30} (concentration causing a 30% reduction in the evoked response).

In isotope experiments, tissues were preincubated for 1 h in 1 ml medium containing [^{3}H]-noradrenaline (specific activity 39 Ci $mmol^{-1}$) before being superfused with [^{3}H]-noradrenaline-free Krebs-Henseleit solution. Effluent samples were collected in 3 ml aliquots and at the end of the experiment tissues were made soluble in 2 ml of tissue solvent. A volume of 1 ml of superfusate or dissolved tissue was added to 9 ml of liquid scintillation solution (Liquiscint) and counted in a liquid scintillation counter. The stimulation-evoked overflow of tritium was calculated by subtraction of the basal outflow, and was expressed as a fractional rate (i.e. the evoked overflow during a given stimulation period was expressed as a fraction of the tritium content of the tissue at the onset of that stimulation period). The effects of yohimbine on the stimulation-evoked overflow of tritium were expressed as an EC_{30} (concentration causing 30% potentiation of the stimulation-evoked overflow).

Drugs

Amidephrine hydrochloride (gift: Bristol-Myers); cirazoline hydrochloride (gift: Synthelabo); clonidine hydrochloride (Boehringer); phenoxybenzamine hydrochloride (gift: Smith, Kline & French); phentolamine mesylate (Ciba); prazosin hydrochloride (gift: Pfizer); rauwolscine hydrochloride (Roth); Wy 26392 (N-methyl-N-[1,3,4,6,7,11, α-hexahydro-2H-benzo-[a]-quinolizin-2/3yl] propan-1-sulphonamide-hydrochloride) gift: Wyeth); xylazine hydrochloride (gift: Bayer); yohimbine hydrochloride (Sigma).

Drugs were dissolved in distilled water except phenoxybenzamine (tartaric acid 1 mM) and dilutions were administered in saline (0.9% NaCl w/v) except for prazosin (distilled water).

Statistics

Differences between groups in agonist ED_{50} or ID_{50} values or antagonist EC_{30} or IC_{30} values were compared by a Student's t test for unpaired data. Values are expressed as mean ± s.e.mean or geometric mean and 95% confidence limits.

Results

Pithed rat preparation

In pithed rats, resting diastolic blood pressure (DBP) was 28.9 ± 1.4 mmHg ($n = 14$) and resting heart rate was 287 ± 7 min^{-1} ($n = 14$). Single pulse stimulation produced a cardio-acceleration of 24.5 ± 0.9 min^{-1} ($n = 60$). Antagonist drugs caused a transient inhibition of the stimulation-evoked cardio-acceleration but the response had recovered by 10 min (just before beginning agonist dose-response curves), except in the case of rauwolscine (10 mg kg^{-1}) when the response was still 80.4 ± 7.7% of control ($P < 0.05$, different from effects of saline).

The pre- and post-junctional potencies of a series of α-adrenoceptor agonists are shown in Table 1. A useful criterion for selecting the best α_2-adrenoceptor agonist is to take the one whose potency pre- and postjunctionally is not altered by the α_1-adrenoceptor antagonist prazosin in a high dose (1 mg kg^{-1}): this was true especially for xylazine and to a lesser extent for clonidine (Table 1). Xylazine was used as the agonist of choice in interaction experiments with antagonists.

Control pre- and post-junctional dose-response curves for xylazine are shown in Figure 1, together with dose-response curves for xylazine in the presence of

Table 1 Pre- and post-junctional potencies of agonists assessed in the absence and presence of prazosin (1 mg kg^{-1}) and the shift in potencies produced by prazosin

Agonist	*Prejunctional ID_{50}*			*Postjunctional ED_{50}*		
	Control	*After prazosin*	*Dose-ratio*	*Control*	*After prazosin*	*Dose-ratio*
Xylazine	4.8 (3.2–8.9)	8.7 (3.6–20.9)	NS	219 (117–407)	282 (178–447)	NS
Clonidine	0.76 (0.17–3.3)	1.4 (0.79–2.6)	NS	15.8 (3.3–75.8)	47.8 (15.1–120)	NS
Cirazoline	2.6 (1.7–3.3)	38.0 (21.9–66.1)	14.8	7.9 (2.9–21.9)	851 (457–1580)	107
Amidephrine	89.1 (44.7–178)	759 (182–3160)	8.5	47.9 (24.0–95.5)	7240 (3160–16500)	151

Values are means and 95% confidence limits (μg kg^{-1}) from at least 4 experiments. The agonist dose-ratio was obtained by dividing the mean ID_{50} or ED_{50} in the presence of prazosin by the mean value in the absence of prazosin. NS indicates that there was no significant change ($P > 0.05$) in agonist potency.

the antagonists prazosin, phenoxybenzamine, yohimbine, rauwolscine, phentolamine and Wy 26392. The shifts in xylazine potency produced by the antagonists are shown in Table 2. The α_1-adrenoceptor selective antagonist prazosin had no significant effect on the pre- or post-junctional potency of xylazine, but all other antagonists caused a greater shift in pre- than post-junctional potency of xylazine. The effects of yohimbine (1 mg kg^{-1}) against clonidine were examined and it produced a larger shift in pre- than post-junctional potency (56 fold and 11.3 fold respectively).

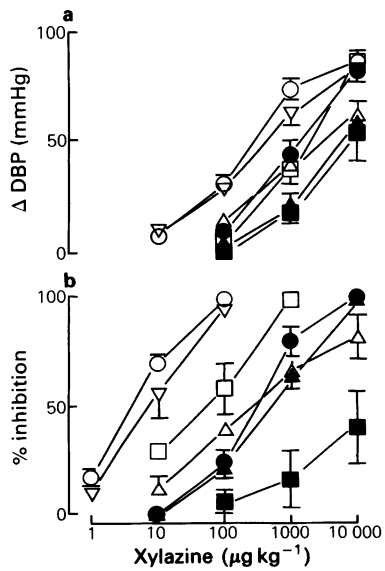

Figure 1 The postjunctional pressor (changes in diastolic blood pressure) (a) and the prejunctional cardioinhibitory (b) responses obtained to the α_2-adrenoceptor agonist xylazine in the pithed rat and the antagonism of these effects by α-adrenoceptor antagonists. Symbols: control (○); prazosin (1 mg kg^{-1}) (▽); Wy 26392 (1 mg kg^{-1}) (□); phenoxybenzamine (10 mg kg^{-1}) (△); yohimbine (1 mg kg^{-1}) (●); phentolamine (1 mg kg^{-1}) (▲); rauwolscine (10 mg kg^{-1}) (■). Vertical bars indicate s.e.mean, and each value is the mean from at least 4 experiments.

Human saphenous vein

Field stimulation of the human saphenous vein at a frequency of 5 Hz for 3 min produced a contraction of 0.85 ± 0.20 g ($n = 12$) and an evoked overflow of tritium of 1.22 ± 0.35% of tissue tritium ($n = 8$). Basal outflow of tritium was 0.33 ± 0.08% of tissue tritium

Table 2 The shifts in the pre- and post-junctional potencies of xylazine produced by α-adrenoceptor antagonists

Antagonist	*Xylazine dose-ratio*	
	Prejunctional	*Postjunctional*
Prazosin (1 mg kg^{-1})	NS	NS
Phenoxybenzamine (10 mg kg^{-1}, i.p. 24 h)	3.8	NS
Phenoxybenzamine (10 mg kg^{-1})	66	8.3
Yohimbine (1 mg kg^{-1})	63	5.0
Phentolamine (1 mg kg^{-1})	102	21
Wy 26392 (1 mg kg^{-1})	27	6.0
Rauwolscine (10 mg kg^{-1})	> 1000	26

The xylazine dose-ratio was obtained by dividing the mean ID_{50} or ED_{50} of xylazine in the presence of the antagonist by the mean value in the absence of antagonists. NS indicates that there was no significant change ($P > 0.05$) in agonist potency in the presence of the antagonist.

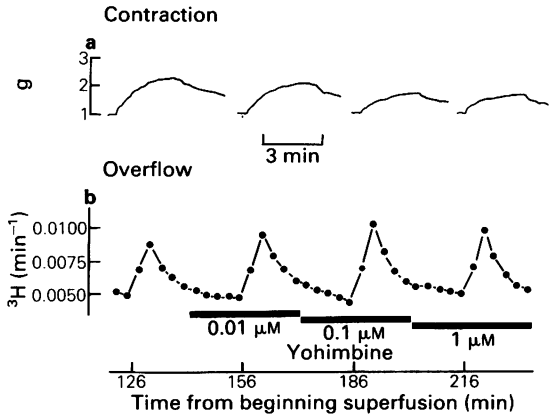

Figure 2 Effects of the α_2-adrenoceptor antagonist yohimbine on a human isolated saphenous vein which was preincubated with [^{3}H]-noradrenaline and superfused in [^{3}H]-noradrenaline-free medium. In (b) the ^{3}H-outflow is expressed as a fraction of the ^{3}H-content of the tissue (min^{-1}). (a) Original recordings of the isometric contractions obtained in response to field stimulation: responses are, from left to right, control response, and responses in the presence of yohimbine (0.01, 0.1 and 1 μM). Note that the time scales differ in (a) and (b). The tissue was stimulated 5 times (S_1–S_5) at 5 Hz for 3 min (S_1 not shown) at intervals of 30 min and yohimbine was infused in 3 concentrations beginning 18 min before S_3, S_4 and S_5 (shown by thick horizontal bars in b).

per min ($n = 8$). The α_2-antagonist yohimbine (0.01–1 μM) did not significantly alter the basal outflow of tritium, as compared with the effects of vehicle, but reduced the stimulation-evoked contraction and potentiated the stimulation-evoked overflow (see Figure 2). Maximum potentiation of stimulation-evoked overflow was to 145.5 ± 7.6% of control ($n = 4$), achieved by yohimbine (0.1 μM) and this concentration reduced the stimulation-evoked contraction to 51.4 ± 2.2% of control ($n = 7$). Although yohimbine (1 μM) produced a further reduction in the stimulation-evoked contraction to 44.0 ± 3.7% of control, this may be due, in part, to α_1-antagonism by yohimbine in this high concentration. Since yohimbine in concentrations of up to 0.1 μM produced approximately 50% increases and decreases in stimulation-evoked overflow and contraction, respectively, we chose to compare pre- and post-junctional potencies of yohimbine in terms of concentrations producing 30% increases or decreases (EC_{30} or IC_{30}). The EC_{30} and IC_{30} values of yohimbine were similar (Table 3). Wy 26392, 0.1 μM, reduced the stimulation-evoked contraction to 46.3 ± 5.3% of control ($n = 5$), and Wy 26392, 1 μM, produced a small further reduction to 38.5 ± 7.5% of control. The postjunctional IC_{30} of Wy 26392 was similar to that of yohimbine (Table 3), as was also true of their postjunctional potencies in the pithed rat (Table 2).

Table 3 The prejunctional EC_{30} and postjunctional IC_{30} values of yohimbine and Wy 26392 in the human saphenous vein

	Prejunctional EC_{30} (nM)	*Postjunctional* IC_{30} (nM)
Yohimbine	10.0 (2.3–42.6)	13.8 (6.3–30.2)
Wy 26392	—	13.2 (3.2–55.0)

EC_{30} = concentration producing 30% potentiation of stimulation-evoked overflow and IC_{30} = concentration producing 30% inhibition of stimulation-evoked contraction. Values are means and 95% confidence limits (nM) from at least 4 experiments.

Discussion

The object of this study was to determine whether prejunctional α_2-adrenoceptors differ pharmacologically from postjunctional α_2-adrenoceptors by examining relative and absolute potencies of agonist and antagonist drugs in two preparations: the pithed rat and the human isolated saphenous vein.

In the pithed rat, in the presence of prazosin to remove α_1-adrenoceptor mediated actions, the relative pre- and post-junctional potencies of agonists were similar, in the order clonidine > xylazine > cirazoline > amidephrine. It is inappropriate to compare the absolute pre- and post-junctional potencies of an agonist determined from such different effects as pressor responses and cardio-inhibition, especially as the potency assessed on the latter is largely dependent on the stimulation frequency employed (see Docherty & McGrath, 1980a). The order of antagonist potency, against the α_2-adrenoceptor agonist xylazine in the pithed rat, was similar pre- and post-junctionally. However, each antagonist had greater absolute potency against the prejunctional than against the postjunctional actions of xylazine. Under equilibrium conditions, antagonist potency against an agonist should depend only on the receptor subtype present. The simplest explanation for the difference in pre- and post-junctional absolute antagonist potencies is that equilibrium conditions are not obtained in the pithed rat preparation and we do not know either the agonist or the antagonist concentrations at the receptors. This is especially important when comparing effects of drugs at receptors in areas with different blood flow, as for example in the heart and peripheral blood vessels.

We have recently found that the human isolated saphenous vein allows pre- and post-junctional α_2-

adrenoceptors to be examined under near equilibrium conditions. The postjunctional receptors mediating field stimulation-evoked contractions in human saphenous vein are predominantly α_2- and the prejunctional receptors are classically α_2-adrenoceptors (Docherty & Hyland, 1985). Yohimbine (0.01 μM) significantly altered both the stimulation-evoked overflow of tritium in tissues pre-incubated with [^{3}H]-noradrenaline and the stimulation-evoked isometric contraction. Hence, under conditions *in vitro* near to equilibrium, it is clear that pre- and post-junctional α_2-adrenoceptors are similar in terms of the potency of the α_2-adrenoceptor antagonist yohimbine. As supporting evidence, Wy 26392 had similar postjunctional potency (present data) and rauwolscine had similar prejunctional potency (Göthert *et al.*, 1984) to yohimbine in human saphenous vein; in other tissues yohimbine has similar α_2-adrenoceptor potency to Wy 26392 (Lattimer *et al.*, 1982) and rauwolscine (Weitzell *et al.*, 1979). Absolute antagonist potencies would probably also be similar pre- and post-junctionally in the pithed rat if the concentration of antagonist at the receptors were measured given that a whole series of antagonists had similar relative potencies pre- and post-junctionally. Previous accounts of differences between pre- and post-junctional α_2-adrenoceptors were from experiments carried out *in vivo* (Kobinger & Pichler, 1980; De Jonge *et al.*, 1981; Hicks, 1981); no such differences have been found *in vitro* (present results and Skärby, 1984).

In conclusion, we have no reason to suggest, based on the present data, that prejunctional α_2-adrenoceptors differ from postjunctional α_2-adrenoceptors. Differences found *in vivo* may reflect non-equilibrium conditions. It is suggested that relative rather than absolute antagonist potencies may be a more meaningful guide to receptor subclassification under the non-equilibrium conditions existing *in vivo*.

This work was supported by the research funds of the Royal College of Surgeons in Ireland and by the Medical Research Council of Ireland. L.H. is an MRC(I) scholar.

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(*Received February 12, 1985.*
Revised June 19, 1985.
Accepted June 26, 1985.)