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AN EXAMINATION OF THE PRE- AND POSTSYNAPTIC α-ADRENOCEPTORS INVOLVED IN NEUROEFFECTOR TRANSMISSION IN RABBIT AORTA AND PORTAL VEIN

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1 The α-adrenoceptor agonists, clonidine and xylazine, reduced and the α-antagonists, yohimbine and rauwolscine, increased the stimulation-evoked tritium overflow from rabbit aorta and portal vein pre-incubated with [^{3}H]-noradrenaline.

2 Based on an order of agonist potency of clonidine > xylazine > phenylephrine and antagonist potency of rauwolscine = yohimbine > prazosin, the presynaptic receptor mediating these effects is of the α_2-type.

3 In the aorta, stimulation-evoked contractions were abolished by prazosin (0.1 μM) and potentiated by rauwolscine and yohimbine in concentrations that increased the stimulation-evoked overflow of tritium.

4 In the portal vein, prazosin was less potent in reducing, and rauwolscine and yohimbine failed to potentiate, the stimulation-evoked contraction.

5 In experiments in which tissues were pre-exposed to phenoxybenzamine (30 nM) to block some of the postsynaptic α-receptors, rauwolscine in concentrations that increased stimulation-evoked tritium overflow, reduced the evoked contraction in the portal vein but not in the aorta.

6 It is concluded that presynaptic α_2-autoreceptors are present in both tissues and that the postsynaptic α-receptors which mediate nerve stimulation-evoked contractions are α_1 in the aorta but a mixture of α_1 and α_2 in the portal vein.

Introduction

There are several sites at which α-adrenoceptor agonists or antagonists can act to influence sympathetic neuroeffector transmission. Apart from the postsynaptic α-receptors, presynaptic α-receptors are now recognized targets for both agonists and antagonists, and these latter receptors may play a physiological role in the modulation of transmitter release (Starke, 1977; Gillespie, 1980; Langer, 1981). Presynaptic α-autoreceptors have been found wherever they have been sought, except for a few apparent discrepancies (e.g. Kalsner & Chan, 1979). Pre- and postsynaptic α-receptors differ in their sensitivity to drugs (Starke, 1972) and this has led to a subclassification into α_1-postsynaptic and α_2-presynaptic receptors (Langer, 1974). Recent evidence obtained *in vivo* now suggests that a contraction-mediating receptor resembling the α_2-receptor can occur postsynaptically on smooth muscle cells (Drew & Whiting, 1979; Docherty & McGrath, 1980; Madjar, Docherty & Starke, 1980).

The purpose of the present study was to examine the pre- and postsynaptic α-adrenoceptors involved in neurotransmission in two tissues which have been previously neglected, the rabbit aorta and portal vein. In these tissues the contractile response to exogenous α-agonists is mediated almost exclusively by α_1-receptors (Docherty & Starke, 1981a); however, since receptors close to the noradrenaline-secreting varicosities may contribute little to the overall response to an exogenous agonist, such agonist responses give no information about the postsynaptic receptors which mediate nerve stimulation-evoked contractions.

Some of these results have been presented in abstract form (Docherty & Reichenbacher, 1981; Docherty & Starke, 1981b).

Methods

The descending aorta and the portal vein were obtained from rabbits of either sex (2–2.5 kg); the aorta was cut spirally into a strip approximately 3 × 30 mm, and the portal vein was cut longitudinally. Tissues were mounted vertically in an organ bath (70 ml) and

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connected to an isometric tension transducer under tension of 4 g. Tissues were incubated or superfused with Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.6, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, KH_2PO_4 1.2, glucose 11.1, ascorbic acid 0.28 and disodium edetate (Na_2EDTA) 0.03. The solution was equilibrated at 37°C with a mixture of 95% O_2 and 5% CO_2 and contained, except during incubation with [^{3}H]-noradrenaline, cocaine (30 μM), corticosterone (40 μM) and propranolol (4 μM) to block neuronal and extraneuronal amine uptake and β-adrenoceptors, respectively. In some experiments, as stated in the results section, cocaine was omitted from the superfusion medium.

Antagonist drugs used were rauwolscine (selective α_2-antagonist; Weitzell, Tanaka & Starke, 1979), yohimbine (α_2-antagonist, less selective than rauwolscine; Weitzell *et al.*, 1979) and prazosin (selective α_1-antagonist Cambridge, Davey & Massingham, 1977); agonists were phenylephrine (selective α_1-agonist Starke, Endo & Taube, 1975), and clonidine and xylazine (selective α_2-agonists, Docherty & McGrath, 1980).

Concentration/contractile-response curves

Tissues were initially incubated for 1 h with changes of bathing fluid every 15 min. A single cumulative concentration-response curve was obtained per tissue, with 0.5 log unit increments in concentration until a maximum response was reached. Concentrations of agonist producing 20% of maximum response were interpolated from individual cumulative log concentration-response curves, and the mean postsynaptic EC_{20} calculated for each agonist. To obtain the intrinsic activity of an agonist relative to phenylephrine, concentration-response curves were obtained to phenylephrine and the other agonist, separated by a 2 h interval, in the same tissue; the order of agonist administration was reversed each day. The maximum response obtained to the other agonist was expressed as a percentage of the maximum response to phenylephrine.

Isotope experiments

Tissues were incubated for 1 h in 1.5 ml medium containing [^{3}H]-noradrenaline (2 μM, specific activity 4.5 Ci/mmol), and were then mounted vertically in an organ bath between parallel platinum electrodes and superfused with [^{3}H]-noradrenaline-free medium at a rate of 2 ml/min. The superfusate was collected in 3 or 6 min fractions. For electrical stimulation, rectangular pulses of 0.3 ms duration and 200 mA current strength were delivered to the tissue at frequencies of 2 or 8 Hz. Following an initial 8 Hz stimulation period of 3 min (S_1; after 126 min of superfusion), five or six 3 min periods of stimulation (2 or 8 Hz) were applied after 153 (S_2), 174 (S_3), 195 (S_4), 216 (S_5), 237 (S_6) and, in some experiments, 258 min (S_7) of superfusion. Test drugs were infused, where appropriate, into the superfusion stream in two increasing concentrations from 15 min before S_4 and S_6 (group I protocol, Figure 1), or in three increasing concentrations from 15 min before S_4, S_5 and S_6 (group II protocol, Figure 3). The rate of infusion of test drug or, in control experiments, of vehicle was 16 μl/min.

In some experiments, following preincubation with [^{3}H]-noradrenaline, tissues were exposed in an organ bath to medium containing phenoxybenzamine (30 nM) for 10 min. After washout, tissues were superfused and experiments were continued as previously described.

At the end of the experiments, tissues were solubilized in 1 ml Soluene (Packard) and the radioactivity in superfusate samples and tissues was determined by liquid scintillation counting.

The basal outflow of tritium was expressed as a fractional rate, i.e. the outflow of total tritiated compounds per min was expressed as a fraction of the tritium content of the tissue at the time of collection. To quantify the effects of a drug on basal outflow of tritium, the fractional rate of efflux in the 3 min before stimulation in the presence of the drug (S_4, S_5, S_6 or S_7) was divided by the fractional rate of efflux in the 3 min before S_3 and expressed as a percentage of the equivalent ratio obtained in control experiments without the test drug. Where a drug concentration altered the basal outflow by more than 10%, the evoked overflow of tritium was not quantified.

The stimulation-evoked overflow of total tritium was calculated by subtraction of the basal overflow and was expressed as a percentage of the tritium content of the tissue at the onset of the respective stimulation period (Borowski, Starke, Ehrl & Endo, 1977). To quantify the effects of a drug on stimulation-evoked overflow of tritium, the evoked overflow in the presence of the drug (S_4, S_5, S_6 or S_7) was divided by the overflow evoked by S_3, and expressed as a percentage of the equivalent ratio obtained in control experiments without the test drug. Only one set of control experiments was necessary for experiments with Group I protocol, but six sets of control experiments were required for experiments with Group II protocol due to differences in stimulation frequency etc.

The following drugs were used: clonidine hydrochloride (Böhringer, Ingelheim); cocaine hydrochloride (Merck, Darmstadt); corticosterone (Fluka, Buchs); phenoxybenzamine hydrochloride (Röhm Pharma, Darmstadt); (−)-phenylephrine hydrochloride (Böhringer, Ingelheim); prasozin hyd-

rochloride (Pfizer, Karlsruhe); (±)-propranolol hydrochloride (Rhein-Pharma, Heidelberg); rauwolscine hydrochloride (Roth, Karlsruhe); yohimbine hydrochloride (Merck, Darmstadt) and xylazine base (Bayer, Leverkusen). Drugs were dissolved in distilled water, except corticosterone (ethanol), phenoxybenzamine (tartaric acid, 1 mM) and xylazine (HCl, 100 mM).

Results are expressed as means ± s.e.mean, with n the number of experiments. Significance of differences was calculated by Student's t test.

Results

The experimental protocol used in isotope experiments (group I) is shown in Figure 1. Electrical stimulation at a frequency of 2 Hz for 3 min produced a contraction and an increase in the outflow of tritium in both tissues. In the example shown from the portal vein (Figure 1), prazosin (0.1 μM) was infused for two stimulation periods, beginning after S_3, and caused a marked reduction in the stimulation-evoked contraction without any effect on stimulation-evoked overflow. Prazosin (1 μM) caused a further reduction in the stimulation-evoked contraction but increased the basal outflow of tritium. The effects of a given drug concentration were qualitatively similar at each of the two stimulation periods, but all data were evaluated using the response obtained during the first stimulation period in the presence of the drug concentration (S_4 or S_6).

Control values for basal efflux, stimulation-evoked overflow and stimulation-evoked contraction from experiments in which no test drug was given are shown in Table 1. Values for S_3 are shown, and also the ratios (S_4/S_3 etc) which were used to calculate drug effects as '% of control' in experiments with the protocol of group I.

Concentration-response curves were constructed for the effects of the antagonists yohimbine and prazosin and the agonists clonidine, xylazine and phenylephrine on the stimulation-evoked overflow of tritium in aorta and portal vein. In both tissues, yohimbine (0.01–10 μM) caused a concentration-dependent increase in stimulation-evoked overflow

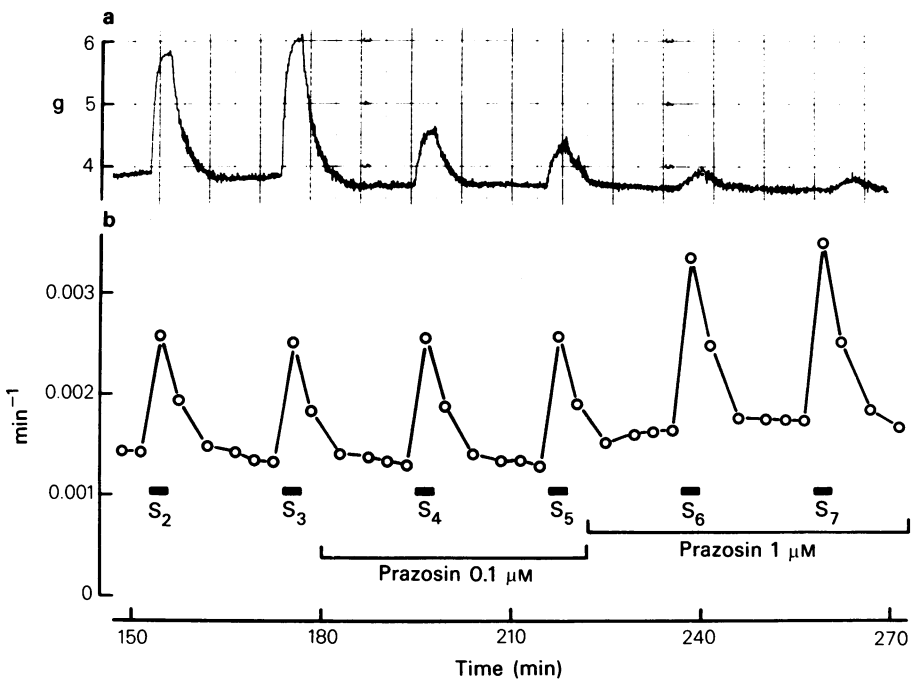

Figure 1 Effects of prazosin (0.1 and 1 μM) on an isolated portal vein which, following incubation with [^{3}H]-noradrenaline, was superfused with [^{3}H]-noradrenaline-free medium. The abscissa scale shows time after beginning superfusion (min). The superfusate was collected in 3 or 6 min samples, and the ^{3}H-outflow is expressed as a fraction of the ^{3}H content of the tissue (min^{-1}, (b)); (a) shows an original isometric tension recording. The tissue was stimulated 7 times, once at 8 Hz for 3 min (not shown) and 6 times at 2 Hz for 3 min at the points indicated (S_2–S_7). Prazosin (0.1 μM) was infused beginning 15 min before S_4, and prazosin (1 μM) was infused beginning 15 min before S_6.

Table 1 Control values obtained in aorta and portal vein for basal tritium outflow, stimulation-evoked tritium overflow and stimulation-evoked contraction

	S_3	S_4/S_3	S_5/S_3	S_6/S_3
Aorta				
Basal outflow	0.0020 ± 0.0002 min^{-1}	0.94 ± 0.01	0.93 ± 0.02	0.91 ± 0.02
Evoked overflow	0.71 ± 0.15%	1.00 ± 0.02	1.00 ± 0.03	0.97 ± 0.05
Evoked contraction	1.00 ± 0.23 g	1.09 ± 0.02	1.12 ± 0.03	1.11 ± 0.03
Portal vein				
Basal outflow	0.0018 ± 0.0002 min^{-1}	0.92 ± 0.01	0.86 ± 0.01	0.80 ± 0.01
Evoked overflow	0.58 ± 0.10%	1.01 ± 0.02	1.03 ± 0.04	1.11 ± 0.02
Evoked contraction	1.15 ± 0.26 g	1.12 ± 0.03	1.24 ± 0.06	1.32 ± 0.06

Basal outflow, stimulation-evoked overflow and stimulation-evoked contraction obtained for the period S_3 are given as fractional rate, % of tissue tritium and g, respectively. Values obtained for S_4 etc. are expressed as a fraction of the respective S_3 value. Values are expressed as mean ± s.e. Aorta, $n = 6$; portal vein $n = 5$.
In absolute terms, basal tritium outflow immediately before S_3 was 3400 ± 500 and 2700 ± 300 d/min per min, and stimulation-evoked tritium overflow was 12700 ± 3600 and 8600 ± 1000 d/min for the period S_3, in aorta and portal vein, respectively.

of tritium, whereas prazosin at concentrations of up to 0.1 μM had no effect (Figure 2). Higher concentrations of prazosin (1–10 μM) significantly increased the basal efflux of tritium. In both tissues the agonist order of potency in reducing the stimulation-evoked tritium overflow was clonidine, xylazine and, least potent, phenylephrine. Phenylephrine (10 μM) significantly increased the basal efflux of tritium.

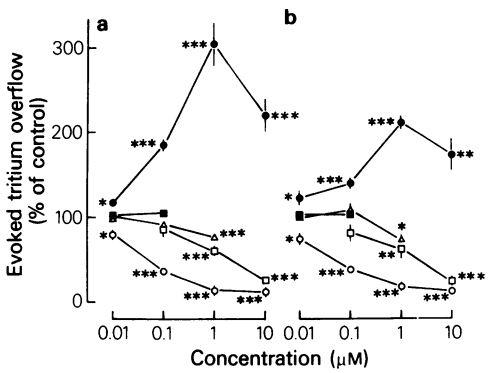

Figure 2 Effects of agonists and antagonists on the overflow of tritium in response to sympathetic nerve stimulation at a frequency of 2 Hz for 3 min in (a) aorta and (b) portal vein. After incubation with [^{3}H]-noradrenaline, the tissues were superfused with [^{3}H]-noradrenaline-free medium. Test drugs were infused in two increasing concentrations beginning 15 min before S_4 and S_6, respectively, and the ratios S_4/S_3 and S_6/S_3 were measured. All ratios were then expressed as a percentage of the respective ratios obtained in control experiments. Symbols: yohimbine (●); prazosin (■); clonidine (○); xylazine (□); phenylephrine (△). Each point is the mean of at least 4 experiments; vertical lines show s.e.mean. Asterisks indicate significant differences from controls: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

The agonists clonidine and xylazine also reduced the stimulation-evoked contraction over the same concentration ranges at which they reduced the stimulation-evoked overflow of tritium. These agonists themselves caused a contraction at higher concentrations (xylazine did not cause a contraction in the aorta in the concentrations examined) which complicated interpretation of results (not shown). Phenylephrine (0.01 μM) potentiated the stimulation-evoked contraction in the aorta but not portal vein and higher concentrations caused a contraction in both tissues.

Presynaptic IC_{20} values (concentration which reduced stimulation-evoked tritium overflow by 20%) for agonists were obtained from the data shown in Figure 2 and are summarized in Table 2. Table 2 also contains postsynaptic EC_{20} values (concentration producing 20% of maximum contraction to that agonist) which were obtained in separate experiments from concentration/contractile-response curves. The order of postsynaptic potency was phenylephrine ≈ clonidine > xylazine. Clonidine and xylazine were partial agonists at the smooth muscle α-receptors, with intrinsic activities relative to phenylephrine (assigning a value of 1 to phenylephrine) of 0.76 ± 0.11, 0.30 ± 0.14 in the aorta and 0.38 ± 0.14, 0.32 ± 0.11 in the portal vein for clonidine and xylazine, respectively. As shown by the IC_{20pre}/EC_{20post} ratios, clonidine and xylazine had clear preference for presynaptic receptors and phenylephrine had clear postsynaptic preference. Xylazine had greater presynaptic preference than clonidine in the aorta, but had similar preference in the portal vein, due to an increased postsynaptic potency.

Postsynaptic IC_{50} values (concentrations of antagonist that reduced the stimulation-evoked contraction by 50%) of prazosin, obtained by linear

Table 2 Pre- and postsynaptic potencies of the agonists clonidine, xylazine and phenylephrine in (a) aorta and (b) portal vein

Drug	*Presynaptic IC_{20}* (nM)	*Postsynaptic EC_{20}* (nM)	$\frac{IC_{20pre}}{EC_{20post}}$
(a) Aorta			
Clonidine	7.9	650	0.012
Xylazine	180	74000	0.0024
Phenylephrine	550	200	2.8
(b) Portal vein			
Clonidine	6.0	220	0.027
Xylazine	130	7600	0.017
Phenylephrine	720	230	3.1

The presynaptic IC_{20} is the concentration of agonist that reduces stimulation-evoked tritium overflow by 20%. The postsynaptic EC_{20} is the concentration of agonist producing 20% of maximum contraction. $n = 4-17$.

regression analysis of the data in Figure 2, were 5.1 nM and 132 nM in aorta and portal vein respectively. Hence, prazosin was approximately 25 times more potent at reducing stimulation-evoked contractions in the aorta than in the portal vein.

The latter finding suggested differences between the aorta and portal vein in the postsynaptic receptors which mediate nerve stimulation-evoked contractions, so that additional experiments were carried out to examine this possibility, employing a different protocol. In these experiments the highly selective α_2-antagonist, rauwolscine, was employed and its effects were compared with those of prazosin. The experimental protocol (group II) is shown in Figure 3, with an example from the aorta. Electrical stimulation at a frequency of 2 Hz for 3 min produced a contraction and an increase in the outflow of tritium. After two stimulation periods, rauwolscine (0.01 μM) was infused for one stimulation period without a clear-cut effect. Rauwolscine (0.1 μM) potentiated

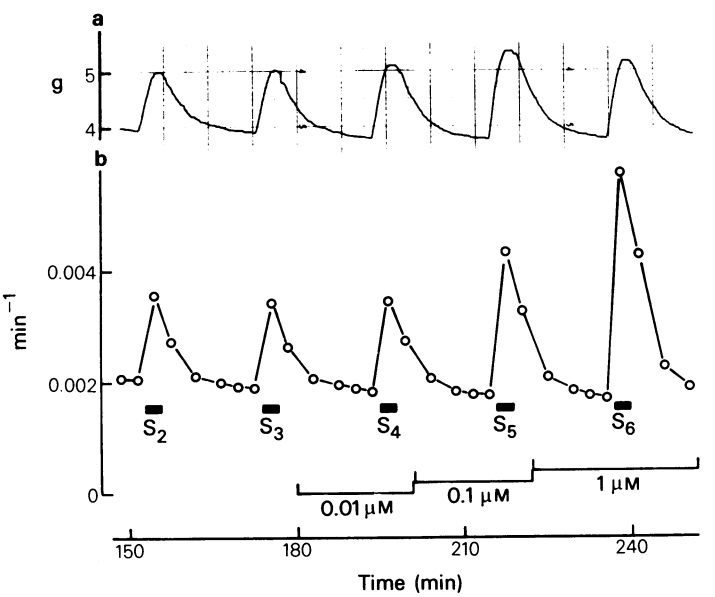

Figure 3 Effects of increasing concentrations of rauwolscine on an aortic strip which, following incubation with [^{3}H]-noradrenaline, was superfused with [^{3}H]-noradrenaline-free medium. The abscissa scale shows time after beginning superfusion (min). The superfusate was collected in 3 or 6 min samples, and the ^{3}H-overflow was expressed as a fraction of the ^{3}H-content of the tissue (min^{-1}, b); (a) shows an original isometric tension recording. The trissue was stimulated 6 times, once at 8 Hz for 3 min (not shown) and 5 times at 2 Hz for 3 min at the points indicated (S_2–S_6). Rauwolscine 0.01, 0.1 and 1 μM were infused beginning 15 min before the start of S_4, S_5 and S_6 respectively.

the stimulation-evoked contraction, and caused a marked increase in the stimulation-evoked overflow of tritium. Rauwolscine (1 μM) further increased the stimulation-evoked overflow of tritium. In these experiments the ratios S_4/S_3, S_5/S_3 and S_6/S_3 were calculated and employed for quantitative evaluation of results.

Control values for basal efflux, stimulation-evoked overflow and stimulation-evoked contraction from experiments in which no test drug was given were similar to those of Table 1.

Concentration-response curves were constructed for the effects of the antagonists rauwolscine and prazosin on the stimulation-evoked contraction and stimulation-evoked overflow of tritium in aorta and portal vein (Figure 4, filled symbols). In the aorta, rauwolscine (0.01–1 μM) increased the stimulation-evoked overflow of tritium and rauwolscine (0.1–1 μM) potentiated the stimulation-evoked contraction; prazosin (0.1 μM) had no effect on the stimulation-evoked overflow of tritium, yet prazosin (0.001 μM) reduced and prazosin (0.1 μM) abolished the stimulation-evoked contraction (Figure 4a). In the portal vein, rauwolscine (0.01–1 μM) potentiated

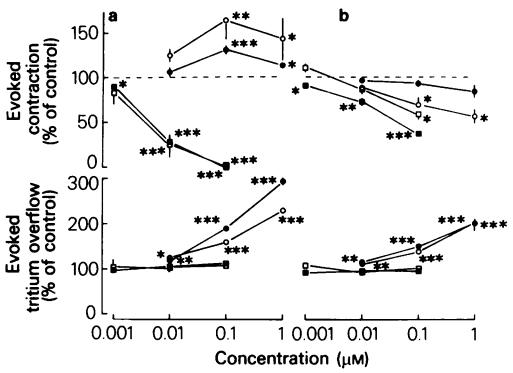

Figure 4 Effects of rauwolscine and prazosin on the responses of (a) aorta and (b) portal vein to sympathetic nerve stimulation at a frequency of 2 Hz for 3 min. After incubation with [^{3}H]-noradrenaline, the tissues were superfused with [^{3}H]-noradrenaline-free medium. Drugs were infused in three increasing concentrations beginning 15 min before S_4, S_5 and S_6, and the ratios S_4/S_3, S_5/S_3 and S_6/S_3 were measured. All ratios were then expressed as a percentage of the respective ratios obtained in control experiments. Top panel, stimulation-evoked contraction; bottom panel, stimulation-evoked overflow of tritium. Symbols: rauwolscine (●, ○); prazosin (■, □). Filled symbols: experiments without phenoxybenzamine; open symbols: tissues were exposed to phenoxybenzamine (30 nM) for 10 min before beginning superfusion. Each point is the mean of at least 4 experiments; vertical lines show s.e.mean. Asterisks indicate significant differences from controls: $*P<0.05$; $**P<0.01$; $***P<0.001$.

the stimulation-evoked overflow of tritium but failed to increase the stimulation-evoked contraction. Prazosin up to 0.1 μM had no effect on the stimulation-evoked overflow of tritium, but in the concentration range 0.001–0.1 μM prazosin reduced and only at 10 μM (not shown) almost abolished the stimulation-evoked contraction (Figure 4b). Yohimbine (0.01–1 μM) had similar effects to those of rauwolscine, and yohimbine (10 μM) reduced the stimulation-evoked contraction in both tissues (not shown).

Further experiments were carried out employing the protocol of group II in which tissues were pre-exposed to phenoxybenzamine (30 nM) to block irreversibly some of the α_1-adrenoceptors. Control values for basal efflux and stimulation evoked overflow were not significantly different from values obtained in experiments without phenoxybenzamine pre-exposure, but the stimulation-evoked contraction was significantly decreased (S_3 values: aorta, 0.44 ± 0.04 g; portal vein, 0.40 ± 0.1 g, $n = 4$ each). In the aorta under these conditions, the pre- and post synaptic effects of prazosin were unchanged and those of rauwolscine were qualitatively similar to those obtained in experiments without pre-exposure to phenoxybenzamine (Figure 4a, open symbols). In the portal vein the presynaptic effects of rauwolscine and prazosin were unaffected by phenoxybenzamine pre-exposure, but rauwolscine, in the concentration range which increased the stimulation-evoked overflow of tritium, decreased the evoked contraction and became approximately equipotent with prazosin (Figure 4b).

Further experiments were carried out on the portal vein using stimulation at a frequency of 8 Hz for 3 min, with a protocol otherwise identical to that of Figure 3 (group II) except that cocaine was omitted from the superfusion medium in some experiments. Control values (S_3) for basal outflow, stimulation-evoked overflow and stimulation-evoked contraction were 0.0017 ± 0.0002 min^{-1}, 1.60 ± 0.20%, and 3.29 ± 0.50 g, with cocaine and 0.0018 ± 0.0002 min^{-1}, 2.05 ± 0.15%, and 3.20 ± 0.31 g, without cocaine, respectively. Rauwolscine (0.01–1 μM) had identical pre- and postsynaptic effects whether cocaine was present or not, causing an increase in evoked tritium overflow and no significant effect on the evoked contraction (Figure 5). In further experiments employing phenoxybenzamine (30 nM) pre-exposure, rauwolscine (1 μM) significantly reduced the stimulation-evoked contraction whether or not the medium contained cocaine. The only effect this phenoxybenzamine pre-exposure had on control values (S_3) was to reduce the stimulation-evoked contraction to 2.00 ± 0.21 g and 1.61 ± 0.22 g with cocaine present or absent from the medium, respectively.

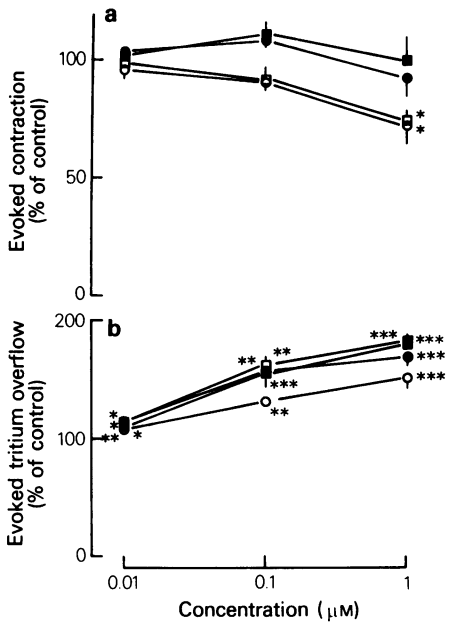

Figure 5 Effects of rauwolscine on the responses of the portal vein to sympathetic nerve stimulation at a frequency of 8 Hz for 3 min. After incubation with [^{3}H]-noradrenaline, the tissues were superfused with [^{3}H]-noradrenaline-free medium containing cocaine (■, □) or without cocaine (●, ○). Rauwolscine was infused in three increasing concentrations beginning 15 min before S_4, S_5 and S_6, and the ratios S_4/S_3, S_5/S_3 and S_6/S_3 were measured. All ratios were then expressed as a percentage of the respective ratios obtained in the relevant control experiments. (a) Stimulation-evoked contraction; (b) stimulation-evoked overflow of tritium. In some experiments, tissues were exposed to phenoxybenzamine (30 nM) for 10 min before beginning superfusion. Filled symbols, effects of rauwolscine in the absence of phenoxybenzamine: open symbols, effects of rauwolscine in the presence of phenoxybenzamine. Each point is the mean of at least 4 experiments; (vertical lines show s.e.mean). Asterisks indicate significant differences from controls: $*P<0.05$; $**P<0.01$; $***P<0.001$.

Discussion

From the evidence that the agonists clonidine and xylazine reduced, and that the antagonists yohimbine and rauwolscine increased the stimulation-evoked overflow of tritium (presumably by blockade of endogenous feedback inhibition by transmitter noradrenaline), it can be concluded that presynaptic α-autoreceptors are present in rabbit aorta and portal vein. This demonstration adds two more to the list of tissues in which the presynaptic α-receptor has been found. The α-autoreceptor theory is now well established (see Gillespie, 1980; Langer, 1981; Starke, 1981b); the few contradictory findings can possibly be explained by peculiarities in the experimental procedures (e.g. Kalsner & Chan 1979, Angus & Korner 1980).

The presynaptic α-receptors were the prototype α_2-receptors (Starke, 1972; Langer, 1981; Starke, 1981a). In the present study, phenylephrine, which was at least equipotent with clonidine at postsynaptic α-receptors, was less potent than clonidine by approximately two orders of magnitude in reducing the stimulation-evoked overflow of tritium. Likewise, the α_1-antagonist, prazosin, did not increase the stimulation-evoked overflow of tritium even in concentrations (0.1 μM) that abolished stimulation-evoked contractions in the aorta; in contrast, the α_2-selective antagonists, yohimbine and rauwolscine, increased the stimulation-evoked overflow at concentrations of 0.01 μM. These data are consistent with the presence of a single population of presynaptic receptors in these two tissues, which based on agonist and antagonist potencies is α_2 (see Starke, 1981a).

For calculation of relative potencies of agonists at pre- and postsynaptic α-receptors, the ratio IC_{20pre}/EC_{20post} was obtained. It should be noted that these two terms differ: the presynaptic IC_{20} value is not the concentration that produced 20% of the maximum inhibitory effect, but rather the concentration that produced a 20% reduction in the response (see Starke, 1981a). Figure 2 shows that the maximum inhibitory effect was obtainable only for clonidine and perhaps xylazine, but not for phenylephrine. Although this convention may cause some distortion of potency ratios, it allows a useful comparison between drugs.

Xylazine has been reported previously to be less potent than clonidine but more selective for cardiac presynaptic receptors in the pithed rat (Docherty & McGrath, 1980). In the present work, xylazine was less potent than clonidine at presynaptic receptors in both tissues, and more selective than clonidine for presynaptic receptors in the aorta. Xylazine and clonidine had equal selectivity for presynaptic receptors in the portal vein, due to an increased potency for xylazine at postsynaptic receptors as compared to its effects on the aorta. However, the postsynaptic EC_{20} obtained for xylazine in the portal vein (7,600 nM) is still high in comparison to a value of 200 nM obtained in the rat anococcygeus muscle (authors, unpublished; see Docherty & Starke, 1981a), a tissue where postsynaptic α_2-receptors may be present.

In a previous study examining contractile responses to exogenous agonists, no clear evidence could be found for a postsynaptic α_2-receptor on the smooth muscle cells of rabbit aorta and portal vein

(Docherty & Starke, 1981a). However, the present study was carried out to examine the receptors involved in neuroeffector transmission, for which responses to exogenous agonists are an imprecise guide. In the aorta no evidence was found for an α_2-mediated contractile response to nerve stimulation, since prazosin significantly reduced the contractile response at concentrations as low as 1 nM, and rauwolscine (and yohimbine) increased the contractile response in concentrations that increased the stimulation-evoked overflow of tritium, presumably by blockade of presynaptic α_2-receptors. However, in the portal vein some evidence was found for a postsynaptic α_2-mediated contractile response since prazosin was much less potent than in the aorta at reducing the contractile response to stimulation, and rauwolscine (and yohimbine) had no effect on stimulation-evoked contractile responses in concentrations that increased the stimulation-evoked transmitter overflow. Considering the high selectivity of rauwolscine for presynaptic receptors in other tissues (see Starke, 1981a), it is surprising that the increase in transmitter overflow was not matched by an increased response.

To resolve the question, experiments were carried out in which tissues were pre-exposed to the irreversible antagonist, phenoxybenzamine, a drug that is selective for α_1-adrenoceptors (Dubocovich & Langer, 1974; Constantine & Lebel, 1980); a concentration was chosen that reduced the contractile response to stimulation by approximately two thirds. In the aorta, the phenoxybenzamine treatment changed neither pre- nor postsynaptic effects of rauwolscine and prazosin, further confirming the α_1-nature of the responses in that tissue. In the portal vein, rauwolscine now reduced the evoked contractile response to stimulation at frequencies of 2 and 8 Hz despite increasing transmitter release. Since the residual responses to 8 Hz stimulation following phenoxybenzamine pretreatment were larger than responses to 2 Hz stimulation in the absence of phenoxybenzamine, the magnitude of the stimulation-evoked contraction *per se* did not determine the effectiveness of rauwolscine.

The effectiveness of rauwolscine at reducing the contraction evoked by stimulation at frequency of 8 Hz in phenoxybenzamine pretreated tissues was unaltered when cocaine was omitted from the superfusion medium, suggesting that the rauwolscine-sensitive component of the response is mediated by receptors located in the same region as the prazosin-sensitive α_1-receptors: the synaptic region. In some blood vessels, smooth muscle α_2-receptors appear to be targets only of bloodborne agonists and not the sites of action of released transmitter (Langer, Massingham & Shepperson, 1980; Yamaguchi & Kopin, 1980). Our findings that responses to nerve stimulation but not to agonists have an α_2-component, and that the α_2-component is not due to transmitter spillover, suggest that such a differential function may not be universally true (see also Drew & Whiting, 1979; Docherty & McGrath, 1980).

Although the results can be explained most easily by assuming that a postsynaptic α_2-receptor is present in the synaptic region in the portal vein but not the aorta, we cannot rule out a nonspecific effect of rauwolscine postsynaptically but it would be surprising if this occurs only in the portal vein, and would not explain the prazosin results. The portal vein has an inhibitory nerve response (Hughes & Vane, 1967), which is still present following high concentrations of clonidine, and not potentiated by rauwolscine (Docherty & Starke, unpublished), so that this is unlikely to be important to the present results.

In conclusion, this study demonstrates that α-adrenoceptor agonists and antagonists have two sites of action by which they can influence neurotransmission in rabbit aorta and portal vein: at presynaptic receptors by which they can affect release; at postsynaptic receptors by which they can alter the contractile response. The presynaptic receptors are of the α_2-type, and the postsynaptic receptors appear to be exclusively of the α_1-type in the aorta but a mixture of α_1 and α_2 may be present in the portal vein.

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