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Neurochemical Evidence for Two Types of Presynaptic Alpha$_2$-Adrenoceptors*

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Neurochemical and pharmacological evidence has been obtained that noradrenergic varicosities (in mouse and rat vas deferens) and cholinergic varicosities (in the Auerbach's plexus) contain heterogenous alpha$_2$-adrenoceptors through which the release of [^{3}H]noradrenaline and [^{3}H]acetylcholine can be modulated. The quantitative data also support the hypothesis that different noradrenaline and xylazine sensitive alpha$_2$-adrenoceptors are present prejunctionally in the vas deferens and Auerbach's plexus preparations. Prazosin, although it has a presynaptic inhibitory effect on alpha$_2$-adrenoceptors of noradrenergic axon terminals, has no effect on cholinergic axon terminals. These data suggest that there are two different types of alpha$_2$-adrenoceptors at the presynaptic axon terminals.

KEY WORDS: Prazosin; [^{3}H]Noradrenaline release; presynaptic alpha$_2$-adrenoceptors; dissociation of alpha$_2$-adrenoceptors.

INTRODUCTION

The alpha$_2$-adrenoceptor, which is present at both pre-and postjunctional sites, has been identified in various organs and tissues (1–4). Although it has been reported that there are no differences pharmacologically, between pre- and postsynaptic alpha$_2$-adrenoceptors (3) or between receptors in different tissues (5) there is evidence that alpha$_2$-adrenoceptors have more than one binding site. Ligand binding studies have demonstrated both high- and low- affinity binding of [^{3}H]clonidine (6–9) and functional differences have been reported for the action of clonidine and noradrenaline on the alpha$_2$-adrenoceptor mediated response in the rat vas deferens (10). Differences have also been observed between clonidine and alpha-methylnoradrenaline activity at presynaptic receptors in occipital cortex (11). Mottram (12) has reported that yohimbine exerts a differential antagonistic activity against clonidine and alpha-methylnoradrenaline induced inhibition of the twitch response to field stimulation in the rat vas deferens. The ability of drugs to modify the binding properties of alpha adrenoceptor binding sites was earlier investigated (13). They not only furnished evidence for the existence of separate binding sites for imidazoline and phenethylamine compounds within the same receptor but showed that phenethylamine agonists promote the binding of [^{3}H]dihydrazapetine whereas imidazoline antagonists inhibit it (14). An apparent dissociation of presynaptic alpha$_2$-adrenoceptor binding sites has been demonstrated to occur in the presence of prazosin (15). In the presence of 1 μM prazosin yohimbine failed to antagonize the inhibitory effect of 1-noradrenaline (1-NA) on electrically stimulated contractions of rat vas deferens but did antagonize the effect of alpha$_2$-adrenoceptor agonist, xylazine, in

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a dose related manner. Prazosin was also demonstrated to be a weak competitive antagonist of xylazine (pA_2 = 5.96). These investigations have now been extended to other tissues, with the result that evidence has been obtained that the properties of alpha$_2$-adrenoceptors may be different in different tissues.

EXPERIMENTAL PROCEDURE

Rat Vas Deferens. Rats of CFY strain weighing 160–190 g were used. The vasa were dissected and mounted in Krebs solution as described before (16). An initial tension of 1 g was applied to each. Field stimulation (square wave pulses of 1 msec duration, 0,10 Hz frequency) of supramaximal intensity (10 V/cm) was applied by a Biostim (Eltron, Budapest). The vasa were equilibrated for 1 hour under continous stimulation and were washed every 15–20 min.

Mouse Vas Deferens. Mice of the CFLP strain weighing 25–35 g were used. The vasa deferentia were dissected and cleaned by "stripping" and expulsion of seminal contents. The initial tension was 0.1 g.

Longitudinal Muscle Strip Preparation of Guinea-Pig Ileum. Guinea pig ileal longitudional muscle preparations with Auerbach's plexus attached were prepared as described before (17). In order to ascertain that pure longitudinal muscle-Auerbach plexus preparations which did not preserve any circular muscle were obtained, the preparations were checked with a magnifying glass. Field stimulation (square wave pulses of 1 msec duration, 0.1 Hz frequency) of supramaximal voltage (10 V/cm) was used.

Recording of Muscle Contractions. Muscle contractions were recorded by means of a force displacement transducer coupled to a Radelkis (Budapest, Hungary) pen recorder. Krebs solution which was used with all preparations was gassed with 5% CO_2 in oxygen. Unless otherwise stated the preparations were stimulated by supramaximal field stimulation at a frequency of 0.1 Hz.

Determination of Agonist Activity (Potency). Agonists were added to the bathing fluid in a cumulative manner. A period of 3–5 min was allowed to elapse between successive increases in concentration to enable the full inhibitory effect of the agonist to develop. The inhibitory effect of each concentration of agonist on the twitch response was expressed as a percentage change and the concentration required to produce 50% inhibition of the twitch response was calculated graphically (IC_{50}).

Determination of Alpha$_2$-Adrenoceptor Antagonist Potency. Because tissues were observed to recover rapidly from maximally effective inhibitory concentrations of 1-NA and xylazine after washout it was possible to study the interaction of agonists and antagonists in the same preparation. 1-NA or xylazine dose ratios were determined from the concentrations producing 50% inhibition of the twitch response in the presence and absence of antagonist (yohimbine). The common logarithms of the dose ratio-1 (DR-1) were plotted against the negative logarithm of concentrations of yohimbine (Schild plot). The logarithm of the antagonist receptor affinity, the pA_2 value, and the slope of regression were calculated by means of a computer program.

[^{3}H]Acetylcholine Release From Guinea-Pig Ileum Longitudinal Muscle Strip. The longitudinal muscle strip from guinea-pig ileum was mounted in an organ bath of 2.0 ml maintained at 35°C. The preparation was incubated in Krebs solution containing 1.44×10^5 Bq/ml methyl-[^{3}H] choline chloride (spec. activity 550 GBq/mmol, Amersham International) and 10^{-6} mol/l cold choline chloride. The bath was gassed with 95% O_2 + 5% CO_2 during both the incubation and collection periods. During the 30-min preincubation the preparation was continously stimulated at 1 Hz (1.0 msec, and supramaximal voltage). After the preincubation period the bath was washed three times with normal Krebs solution. The tissue was perfused at a rate of 1 ml/min with Krebs solution equilibrated with 95% O_2 and 5% CO_2. Perfusion fluid was collected in separate fractions every 5 min after perfusing for 60 min to wash the tissues. At the start of the collection of the 3rd (S_1) and 10th (S_2) samples, tissues were stimulated (1.0 Hz, 1.0 msec, supramaximal voltage) (18). Since the electrical stimulation releases almost exclusively acetylcholine (ACh) (19–21), and not choline no attempt was made to separate labelled choline from ACh.

[^{3}H]Noradrenaline Release From Mouse Vas Deferens. Pairs of vasa deferentia prepared from CFLP mice weighing 25–30 g were incubated with 1-(7, 8) [^{3}H]noradrenaline (^{3}H-NA) (490 KBq/ml, spec. activity 355 GBq/mmol, Amersham) for 45 min at 35°C in Krebs solution gassed with 95% O_2 + 5% CO_2 containing 3×10^{-4} M ascorbic acid and 3×10^{-5}M Na_2 EDTA.

After 45 min, the vasa were transferred to a 2 ml organ bath and superfused at a steady rate of 3 ml/min for 120 min with Krebs solution containing ascorbic acid (3×10^{-4} M) Na_2 EDTA (3×10^{-5} M) and prednisolone (2.7×10^{-6} M) at 35°C. In order to inhibit monoamine oxidase, nialamide (100 mg/kg) was administered subcutaneously two hours prior to experiments. The first 120 min effluent was discarded and 3 min fractions were subsequently collected (perfusion rate, 1 ml/min). The content of radioactivity was measured before ($1.62 \pm 0.10 \times 10^6$ Bq g^{-1}, Mean ± SEM, n = 25) and after the perfusion. That 90.5 ± 4.2% of the total radioactivity in the tissue was present as NA (means ± SEM of six observations) was confirmed by high pressure liquid chromatography combined with scintillation spectrometry. The release of tritium was expressed as the fractional release rate (3 min^{-1}); i.e., the release was expressed as a percentage of radioactivity in the tissue at the time when the release was measured. The fractional release was calculated with a computing program on a Hewlett Packard 41 CV.

[^{3}H]Noradrenaline Release From Rabbit Pulmonary Artery. The experiments were carried out on strips of rabbit main pulmonary artery and the release of radioactivity (^{3}H-NA) and ^{3}H-metabolites) was measured as described for mouse vas deferens. The tissue was stimulated at 2 Hz (360 shocks) every 27 minutes (S_1; S_2 and S_3).

Determination of [^{3}H]Noradrenaline From the Released Radioactivity Using HPLC Combined With Radiochemical Detection. The release of ^{3}H-NA was determined with a Biotronik HPLC-ED system (Biotronik Wissentschaftliche Gerate, GmbH, Frankfurt am Main, West Germany) as described earlier (22).

Noradrenaline Uptake. Synaptosomal NA uptake was determined by the method of Komiskey et al. (23) with some modifications. Dissected hypothalamus was homogenated in ice-cold 0.32 M sucrose containing 1 mM nialamide and centrifuged for 10 min at 900 g. The supernatant was used for the NA uptake assay.

The reaction mixture consisted of Krebs-Ringer phosphate buffer, 0.2 ml of tissue suspension and varying concentrations of drugs and labeled NA in a final volume of 3 ml. Incubations were

carried out at 37°C and 0°C for 6 min. The cold incubation served as a blank. Incubation was stopped by filtering the reaction mixtures under vacuum through Millipore filters (pore size 0.45 μm). Filters were washed with 5 ml aliquots of buffers which contained 10 μM cold NA. Radioactivity was determined by liquid scintillation counting. High affinity NA uptake was defined as the difference between NA taken up at 37°C and 0°C and was expressed as percentage of control.

Drugs Used. The following drugs were used: xylazine (Bayer), 1-noradrenaline hydrogen tartrate (Fluka AG), nifedipine (Sigma), yohimbine HCl (Chinoin), prazosin HCl (Pfizer). The radioactive materials were purchased from Amersham International.

Statistical Analysis. One way ANOVA followed by Dunn-test and Student's t test were used. For the tests logarithm transformed values were used, in the tables means ± SE values are presented.

RESULTS

1. Effect on Twitch Responses of Guinea-Pig Ileum Longitudinal Muscle Strip and Rat Vas Deferens. The alpha$_2$-adrenoceptor agonist xylazine and 1-NA reduced the stimulation-evoked contraction of the guinea-pig longitudinal muscle strip and rat vas deferens. IC_{50} values and values of intrinsic activity were calculated from the dose-response curves and summarized in Table I. There were significant differences in the potencies of xylazine and 1-NA in rat vas deferens and the longitudinal muscle strip. Rat vas deferens was much more sensitive to xylazine than to 1-NA.

In the longitudinal muscle strip preparation (Figure 1) yohimbine, over the concentration range of 0.1–10 μM, produced a concentration dependent parallel shift to the right in the dose response curve of 1-NA (pA_2 = 7.19 ± 0.15). When xylazine was used as the agonist, yohimbine did not produce a parallel shift in the dose-response curve (pA_2 = 7.37 ± 0.18). Schild plots for yohimbine against 1-NA or xylazine indicated that blockade of 1-NA was competitive (slope = 1.16 ± 0.1), but that of xylazine was noncompetitive (slope = 0.42 ± 0.05). When xylazine was applied to the longitudinal muscle preparation the maximal DR possible did not exceed 9.2 and was achieved at 200 μM concentration of yohimbine.

2. Effect on [^{3}H]Noradrenaline Release From Mouse Vas Deferens. In 65 experiments the fractional release of radioactivity at rest was 0.191 ± 0.02% per 5 min (2060.8 ± 196.4 Bq/g per 5 min). When the tissue was stimulated (S_2 and S_3) the percentages of total radioactivity released were 0.54 ± 0.01% (n = 25) and 0.52 ± 0.01% (n = 17) and the S_2/S_1 and S_3/S_2 ratios were 0.96 ± 0.08 and 0.98 ± 0.03, respectively. Xylazine (0.1 μM) and 1-NA (10 μM) significantly reduced the release of radioactivity (Table II) but yohimbine (1 μM) alone enhanced the release (S_3/S_2 = 4.80 ± 0.11, Table II) and prevented the effect of xylazine and 1-NA. Prazosin (2.5 μM) increased the release such that the S_3/S_2 ratio rose from 0.98 ± 0.03 to 2.80 ± 0.23 ($p < 0.01$) and it also antagonized the effect of xylazine. When prazosin and yohimbine were present together in the perfusion fluid the release of radioactivity (S_3/S_2 = 5.55 ± 0.15) was significantly higher in comparison with either the control (S_3/S_2 = 0.98 ± 0.03 $p < 0.01$) or the prazosin treated tissue (S_3/S_2 = 2.80 ± 0.23 $p < 0.05$). However yohimbine did not antagonize the inhibitory effect of 1-NA when prazosin was also present (S_3/S_2 = 0.11 ± 0.01) but the effect of xylazine was completely prevented (S_3/S_2 = 5.79 ± 0.77). These data are consistent with our previous observations (15)

Table I. Different potencies of xylazine and 1-noradrenaline on twich tension of rat vas deferens and longitudinal muscle strip of guinea-pig ileum

Drug	Rat vas deferens [1]		Ileal long. m. strip.[2]		ileum IC_{50} / vas def. IC_{50}
	IC_{50}[3] (μM) potency	E_{max} intrinsic activity[4]	IC_{50} (μM) potency	E_{max} intrinsic activity[4]	
1-Noradrenaline	1.67 ± 0.77[5] (4)	88.4 ± 5.6 (4)	2.5 ± 0.5 (8)	76.1 ± 7.4 (8)	1.5
Xylazine	0.05 ± 0.005 (9)	95.2 ± 4.2 (9)	3.7 ± 0.7 (8)	85.9 ± 4.4 (8)	74.0

Mean ± SEM, Number of experiments is in brackets. Exposure time, 20 min.
[1,2] 0.1 Hz stimulation (for details see Experimental Procedure).
[3] IC_{50} the concentration that produces a half maximal inhibition.
[4] Intrinsic activity (%), maximal effect determined from dose-response curve.
[5] In order to avoid the postjunctional effect of exogenous 1-noradrenaline nifedipin (1 μM) was added into the organ bath. When the potency of 1-NA in the presence of 10 μM prazosin was estimated a similar IC_{50} value 1.95 ± 0.56 μM (n = 6) (Vizi et al. 1983) was found.

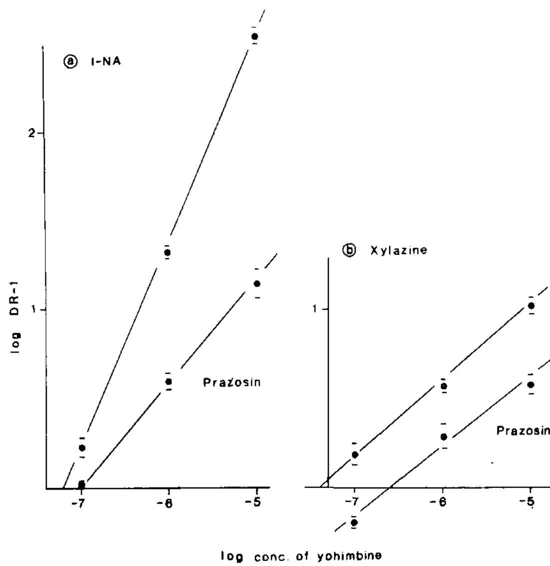

Fig. 1. Schild plot for the antagonism of l-noradrenaline (a) and xylazine (b) by yohimbine in the absence and presence of 1 μM) prazosin. The common logarithm of DR-1 of antagonist is plotted against negative logarithm. The horizontal intercept, corresponding to DR = 2, gives pA_2. Longitudinal muscle strip of guinea-pig ileum, 0.1 Hz supramaximal field stimulation. Log dose ratios were calculated from the rightward shift of the dose-response curves made in the absence and presence of yohimbine (20 min exposure time). For details see Experimental Procedure (a) l-Noradrenaline as agonist. The regression of the curve; y = −1.17 ± 0.10 × +8.39 ± 0.56, pA_2 = 7.19 ± 0.15. In the presence of 1 μM prazosin, y = −0.59 ± 0.28 × +4.10 ± 1.64, pA_2 = 6.99 ± 0.58. The slopes do not differ from the theoretical one ($p > 0.1$ in both cases). However, they differ from each other ($p < 0.05$). (b) Xylazine as agonist. The regrassion of the curve: y = −0.42 ± 0.05 × +3.09 ± 0.28, pA_2 = 7.37 ± 0.18. In the presence of prazosin y = −0.40 ± 0.06 × +2.61 ± 0.34, pA_2 = 6.56 ± 0.15. The slopes differ from the theoretical one ($p <$ 0.01 in both cases). However, they do not differ significantly (p > 0.1) from each other.

on twitch contractions of rat vas deferens. The effect of xylazine (0.1 μM), which alone reduced stimulation evoked release of radioactivity (Figure 2) was abolished in the presence of prazosin (S_3/S_2 = 0.90 ± 0.13, n = 4). This value does not differ significantly from that obtained in experiments where prazosin alone (2.5 μM) was present during S_2 and S_3 stimulation periods (S_3/S_2 = 0.95 ± 0.08, n = 4) (Figure 2). In a different series of experiments, the effect of prazosin was studied on the spontaneous release of radioactivity. Prazosin (2.5 μM) not only enhanced the stimulation-evoked release but significantly increased the spontaneous release of radioactivity (Figure 2) as well.

At concentrations of 2.5 and 10 μM, prazosin enhanced the spontaneous release in a concentration dependent manner; it increased by a factor of 1.8 ± 0.1 (n = 4) and 6.5 ± 0.41 (n = 4) respectively.

High-pressure liquid chromatography combined with radiochemical detection was used to provide direct evidence that the increase in radioactivity released by prazosin was mainly due to ^{3}H-NA and not to ^{3}H-metabolites. At rest 31.9 ± 2.8% of the total radioactivity was identified as ^{3}H-NA and 53.6 ± 4.8% of the total radioactivity released by prazosin was found to be ^{3}H-NA. When electrical field stimulation was applied, 52.6 ± 4.7% of the radioactivity was ^{3}H-NA and in the presence of 2.5 μM prazosin, this proportion was increased to 65.2 ± 5.3%. These data indicate that prazosin enhances primarily the release of NA. In our experiments prazosin, in concentrations of as little as 0.1 μM, has been shown to act also as an antagonist at prejunctional alpha$_2$-adrenoceptors in that it enhances significantly the release of NA (S_3/S_2 = 1.25 ± 0.04, n = 4). This action would thus to some extent mask its inhibitory effect mediated at postjunctional alpha$_1$-adrenoceptors and may account for the resistance of the twitch response to prazosin (24).

3. Effect on [^{3}H]Noradrenaline Release From Rabbit Pulmonary Artery. In 6 experiments the resting fractional release of tritium was 0.22 ± 0.02% per 3 min. Both the resting and the stimulation-evoked release were significantly enhanced by 2.5 μM prazosin (Table III). At the threshold concentration (0.25 μM), prazosin produced a slight but significant increase in the release of radioactivity. The pA_2 value for inhibition of contractions of the pulmonary artery by prazosin when l-NA was used as an agonist was 8.54 ± 0.08 (y = 11.36 ± 0.51 − 1.52 ± 0.07x). When the effect of prazosin against endogenous NA was studied it was found that concentrations as low as 0.19 ± 0.012 nM inhibited by 50% the responses of the artery to field stimulation (2Hz, 360 shocks, contraction size = 788 ± 26 mNewton, n = 23). These findings indicate that the ratio between the pre-and postsynaptic activity of prazosin is high and the inhibitory effect of prazosin mediated at postjunctional alpha$_1$-adrenoceptors was not masked by its presynaptic actions.

4. Effect on [^{3}H]Acetylcholine Release From Ileal Longitudinal Muscle Strips. While prazosin in a concentration of 2.5 μM enhanced the release of ^{3}H-NA, it failed to affect the release of [^{3}H]acetylcholine. The S_2/S_1 ratios in the absence (0.68 ± 0.08, n = 4) and in the presence of prazosin (0.71 ± 0.06, n = 4) did not differ significantly (p

Table II. Interaction of prazosin and yohimbine. Effect of alpha$_2$-adrenoceptor agonists (xylazine and 1-noradrenaline) on tritium release from mouse vas deferens preloaded with [^{3}H]noradrenaline

	[^{3}H]noradrenaline release, S_3/S_2					
	Control			Prazosin 2.5 μM		
	Mean ± SE A	Yohimbine, 1 μM Mean ± SE B	Significance P A:B	Mean ± SE C	Yohimbine 1 μM Mean ± SE D	Significance P C:D
1. Control	0.98 ± 0.03 (n = 17)	4.80 ± 0.11 (n = 4)	<0.01	2.80 ± 0.23 (n = 10)	5.55 ± 0.15 (n = 4)	<0.05
2. Xylazine, 0.1 μM	0.42 ± 0.05 (n = 3)	3.05 ± 0.18 (n = 3)	<0.01	2.10 ± 0.11 (n = 4)	5.79 ± 0.77 (n = 3)	<0.01
3. 1-Noradrenaline 10 μM	0.12 ± 0.02 (n = 6)	3.23 ± 0.11 (n = 4)	<0.01	0.65 ± 0.17 (n = 4)	0.11 ± 0.01 (n = 4)	<0.01
Significance $p < 0.01$	1:2 and 1:3 2:3			1:3 and 2:3		

Prazosin 25 min, yohimbine 20 min, xylazine and 1-noradrenaline 10 min before S_3 stimulation (1 Hz, 180 shocks) were added into the perfusion fluid.
Significance (One-way of ANOVA, F = 115.768, Degree of freedom 11.54).

> 0.5). In addition, prazosin (2.5 μM) did not influence the inhibitory effect of 1 μM xylazine. The S_2/S_1 ratio was 0.26 ± 0.02 (n = 4) when xylazine alone was applied and 0.31 ± 0.04 (n = 4) in the presence of both xylazine and prazosin.

5. *Effect on [^{3}H]Noradrenaline Uptake.* Recently it was reported that inhibition of neuronal uptake of NA reduced the effect of imidazolines (e.g. clonidine) to decrease noradrenaline release

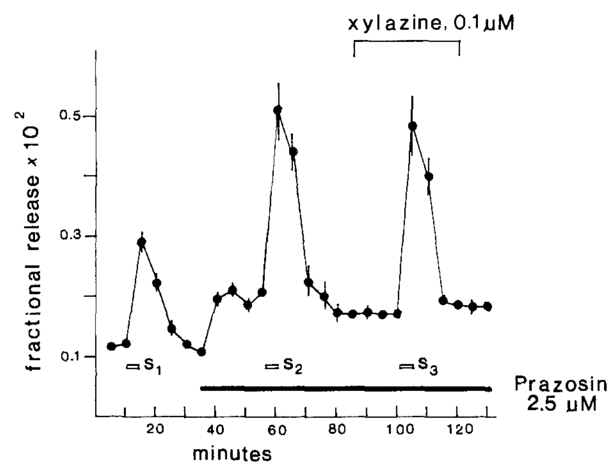

Fig. 2. Effect of prazosin (2.5 μM) on the release of radioactivity from mouse vas deferens loaded with [^{3}H]noradrenaline. Fractional release of radioactivity. Average of four identical experiments. Mean ± SEM. Field stimulation, 1 Hz 180 shocks. Content of radioactivity, 1,303,840 ± 145,600 Bq/g. (S_2/S_1 = 2.10 ± 0.11, S_3/S_2 = 0.90 ± 0.13). Note that prazosin enhances the spontaneous release, S_2/S_1 ratio, and prevents the inhibitory effect of xylazine.

Table III. Effect of prazosin on tritium-release from rabbit pulmonary artery loaded with [^{3}H]noradrenaline

	[^{3}H]noradrenaline release	
	Resting, S_{p3}/S_{p2}	Stimulation evoked, S_3/S_2 (2 Hz 360 shocks)
1. —	0.93 ± 0.15 (5)	0.98 ± 0.05 (5)
2. Prazosin, 0.25 μM	1.06 ± 0.08 (3)	1.34 ± 0.03 (3)
3. Prazosin, 2.5 μM	1.46 ± 0.21 (5)	2.20 ± 0.42 (5)
Significance^x, $p < 0.01$ One-way of ANOVA	1:3	1:2 and 1:3

without affecting the inhibition produced by catecholamines (10, 25). It is also known that uptake-inhibitors (e.g. imipramine) enhance the amount of NA released. In order to determine whether prazosin inhibits NA uptake its effect on a hypothalamic synaptosomal preparation was compared with that of desmethylimipramine. Although desmethylimipramine inhibited the uptake (IC_{50} = 9.2 ± 0.4 × 10^{-9} M, n = 3) prazosin in the concentrations used in this study had only a slight effect on the uptake ($IC_{50} > 10^{-5}$ M, n = 3).

DISCUSSION

It is generally accepted that differences between receptors could be based exclusively upon

the relative potencies of agonist and relative affinity for antagonists (3). In this study evidence has been obtained that

1. Xylazine, a relatively pure alpha$_2$-adrenoceptor agonist in comparison to 1-NA is more effective on rat vas deferens than ileal longitudinal muscle strip (Table I).
2. Yohimbine is a competitive antagonist of the effect of 1-NA but not of xylazine on the twitch response of field stimulated longitudinal muscle strip.
3. Yohimbine failed to antagonize the effect of 1-NA on ^{3}H-NA release when prazosin was also present but the inhibitory effect of xylazine was completely prevented.
4. Prazosin completely antagonized the effect of xylazine on ^{3}H-NA release but had no effect on release of ^{3}H-ACh from cholinergic axon terminals also equipped with inhibitory alpha-adrenoceptors (17, 26).

In this study prazosin, like yohimbine, showed an affinity for prejunctional alpha$_2$-adrenoceptors as judged by measuring the stimulation-evoked release of ^{3}H-NA from mouse vas deferens and from rabbit pulmonary artery where it enhanced the release of ^{3}H-NA in response to stimulation. It also inhibited the antagonistic effect of yohimbine on NA action and antagonized the effect of xylazine on ^{3}H-NA release but had no effect on cholinergic axon terminals.

Enhancement of the spontaneous release of radioactivity from both mouse vas deferens and rabbit pulmonary artery by prazosin proved to be concentration dependent. This finding is consistent with those others (27, 28) who also showed that prazosin released radioactivity from tissues which had been preloaded with [^{3}H]NA. That the radioactivity released by prazosin was due to release of ^{3}H-NA was confirmed by HPLC combined with radiochemical detection. Whatever the mode of action of prazosin, it seems feasible that its site of action is located prejunctionally, on the varicosity. In this respect our data are in agreement with those of others (29), who also showed that prazosin had a presynaptic effect, and provide neurochemical evidence for the effect of prazosin on axon terminals (30, 31).

The present study further supports the contention that there is a dissocation of presynaptic alpha$_2$-adrenoceptors (10–13, 15, 32, 33). Our data indicate that noradrenergic varicosities (in mouse and rat vas deferens) and cholinergic varicosities (in the Auerbach's plexus) contain heterogeneous alpha$_2$-adrenoceptors through which the release of [^{3}H]noradrenaline and [^{3}H]acetylcholine can be modulated. The quantitative data also support the hypothesis that different NA and xylazine sensitive alpha$_2$-adrenoceptors are present prejunctionally in the vas deferens and Auerbach plexus preparations (Table I and Figure 1). In summary, neurochemical and pharmacological evidence has been obtained that the presynaptic alpha$_2$-adrenoceptors controlling NA or ACh release are not homogeneous and prazosin although it has a presynaptic inhibitory effect on alpha$_2$-adrenoceptors of noradrenergic axon terminals, it has no effect on cholinergic axon terminals.

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