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THE EFFECTS OF PRAZOSIN, PHENTOLAMINE AND PHENOXYBENZAMINE ON INHIBITORY α-ADRENOCEPTORS IN THE GUINEA-PIG ISOLATED ILEUM

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1 The relaxant effect of noradrenaline on strips of guinea-pig isolated terminal ileum was blocked by pretreatement with prazosin, phentolamine, yohimbine and phenoxybenzamine.

2 The presence of a very high concentration of noradrenaline (50 μM) during exposure to the blocking agent protected against the blocking effect of the drugs.

3 Yohimbine, prazosin and phentolamine partially protected against irreversible blockade by phenoxybenzamine.

4 Spontaneous release of acetylcholine in the unstimulated ileum was blocked by noradrenaline (0.059–5.9 μM). This effect of noradrenaline was antagonized by phentolamine (0.13–2.6 μM) and yohimbine (0.051–0.51 μM) but not by prazosin (0.53–5.3 μM) or phenoxybenzamine (4.2–42 nM). All four antagonists reversed the noradrenaline-induced relaxation of the ileum.

5 Acetylcholine output in the transmurally stimulated ileum was inhibited by noradrenaline. This effect of noradrenaline was antagonized by phentolamine and yohimbine but not by prazosin or phenoxybenzamine. The first two antagonists blocked the noradrenaline-induced inhibition of evoked twitches of the ileum while the last two had no effect.

6 The results show (a) that prazosin has no effect on presynaptic α-adrenoceptors located on cholinergic nerve endings in the guinea-pig ileum and (b) that prazosin, phentolamine and phenoxybenzamine act on the same subgroup of postsynaptic α-adrenoceptors on the smooth muscle of the guinea-pig ileum.

Introduction

Prazosin is a relatively new antihypertensive agent which is believed to act, at least in part, by blocking postsynaptic α-adrenoceptors in vascular smooth muscle (Constantine, McShane, Scriabine & Hess, 1973; Cavero, 1976; Drew & Whiting, 1979). Prazosin differs from phentolamine in having an action that is selective at the postsynaptic α-adrenoceptors in many tissues (Wood, Phelan & Simpson, 1975; Cavero, Lefevre & Roach, 1977; Doxey Smith & Walker, 1977). In the guinea-pig isolated distal ileum, prazosin antagonizes the relaxation induced by stimulating the periarterial sympathetic nerves or by exogenously applied noradrenaline but has no effect on the inhibition of transmurally evoked twitches of the guinea-pig ileum induced by sympathetic stimulation or noradrenaline. Phentolamine, on the other hand, antagonizes the relaxation of the unstimulated ileum as well as the inhibition of electrically evoked twitches of the ileum induced by endogenous or exogenous noradrenaline (Fagbemi & Salako, 1980). These results were interpreted to mean (a) that prazosin and phentolamine react with the same subgroup of postsynaptic α-adrenoceptors on the smooth muscle of the guinea-pig ileum and (b) that prazosin antagonizes the postsynaptic α-adrenoceptors located on the smooth muscle but not the receptors on the cholinergic nerve endings whereas phentolamine antagonizes both groups of receptors (Fagbemi & Salako, 1980). However, presynaptic α-adrenoceptor blocking properties have been demonstrated for prazosin in the heart of the dog (Roach, Lefevre & Cavero, 1978; Constantine, Weeks & McShane, 1978; Cavero, Dennis, Lefevre-Borg, Perrot, Roach & Scatton, 1979), and two distinct types of postsynaptic α-adrenoceptors, one of them prazosin-insensitive, have been demonstrated in vascular smooth muscle (Drew & Whiting, 1979). Drew (1978) and Wikberg (1978) have also shown that the presynaptic α-adrenoceptors on cholinergic nerve endings in the guinea-pig ileum are similar to those located postsynaptically. We have therefore further compared the actions of prazosin and phentolamine on the α-adrenoceptors in the guinea-pig ileum by carrying out receptor protection-type experiments and acetylcholine release studies.

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Methods

Guinea-pigs of either sex weighing 350 to 500 g and bred locally in the departmental animal house were used. They were killed by a blow to the head followed by exsanguination. The experiments were performed on the terminal portion of the ileum after the 10 cm nearest to the ileo-caecal junction had been discarded because of the presence of excitatory α-adrenoceptors near the ileo-caecal junction (Munro, 1953). A segment of the ileum, 4–6 cm long was mounted vertically in an organ bath under a tension of 1 g and changes in tension were recorded with a Devices strain gauge transducer connected to a Devices F-132 physiopolygraph. The bath fluid was aerated Tyrode solution maintained at 37°C and having the following composition (mM): NaCl 138, KCl 5.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 1.5, NaH_2PO_4 0.36 and glucose 5.0. It also contained cocaine (1.0 μM), corticosterone (10.0 μM) and propranolol (1.0 μM). Drugs were diluted in saline (0.9% w/v NaCl solution) from their stock solutions and were added to the bath fluid in volumes of 0.1 ml. Maximal transmural stimulation was by square pulses (30 V, 1 ms, 0.125 Hz) delivered from an S8 Grass Stimulator via a pair of coaxial platinum electrodes.

Interaction of noradrenaline with phentolamine, yohimbine, prazosin and phenoxybenzamine in the non-stimulated ileum

Graded doses of noradrenaline were added cumulatively to the bath fluid containing the suspended ileum until maximal relaxation was attained. This procedure was repeated in the presence of phentolamine (0.71 μM), prazosin (1.8 μM), yohimbine (1.3 μM) or phenoxybenzamine (29 nM). In these experiments, the agonist was added for a period sufficient to ensure that the response to the administered concentration of the drug reached its maximum. A contact time of 1 min was found to be adequate for noradrenaline. The antagonist drugs were added to the bath fluid reservoir to obtain the desired concentrations. There was a contact time of 20 min for antagonists after which they were washed out and the bath filled with antagonist-free Tyrode solution before starting experimental tests. By use of a similar experimental procedure, the effects of isoprenaline, methoxamine and phenylephrine in this tissue were studied. In experiments with isoprenaline, propranolol was not added to the bathing fluid.

In a series of experiments, the noradrenaline concentration-response curves were determined before and after exposing the tissue to either a combination of prazosin and phenoxybenzamine or a combination of yohimbine and phenoxybenzamine or a combination of phentolamine and phenoxybenzamine. In these experiments, phenoxybenzamine was added 10 min after prazosin, yohimbine or phentolamine and the two were washed out after a further 20 min before repeating the noradrenaline dose-response curve.

Receptor protection studies

The selective receptor protection technique (Furchgott, 1954; Innes, 1962) was used to determine whether there was any difference between receptor sites for phentolamine and prazosin on the guinea-pig ileum.

Preliminary experiments to establish dosages

Noradrenaline was added cumulatively to the bath fluid containing the suspended ileum until maximum relaxation was obtained. From this series of six experiments, a submaximal dose of noradrenaline (24 nM) producing 50–70% maximal relaxation was found. The effect of different concentrations of α-adrenoceptor antagonists on this submaximal dose was tested and it was found that 0.29 μM phenoxybenzamine, 3.6 μM phentolamine, 3.9 μM yohimbine or 3.7 μM prazosin regularly abolished the responses to the chosen submaximal dose of noradrenaline.

In another series of experiments the effect of a very high concentration of noradrenaline (50 μM), on the response to a subsequent submaximal concentration (24 nM), was studied. After determining the response to the submaximal concentration of noradrenaline three times, the high concentration of noradrenaline was added and allowed to act for 25 min; it was then washed out. Two additional washes were carried out at intervals of 10 min after which the response to the submaximal concentration was determined at 5 min intervals. In this series of experiments, it was found that the high concentration of noradrenaline produced a relaxation which was sustained throughout the period of contact of noradrenaline with the tissue. Up to 10 min after exposure to the high concentration of noradrenaline had ceased, the response to the submaximal concentration was depressed but at 15 min, this response had reached a mean of 95% (range 85–110%, $n = 6$) of the value before exposure to the high concentration. It subsequently remained steady so that the means ± s.e.mean of the responses in six experiments at 15, 20, 25 and 30 min after washing out the high concentration of noradrenaline were 95.0 ± 4.0, 97.7 ± 3.1, 102.3 ± 1.7 and 105.8 ± 2.8% respectively. In the control tissues which were not treated with high concentrations of noradrenaline, the response to 24 nM noradrenaline was practically unchanged throughout the experiment (Figure 3). In view of these results, in subse-

quent experiments to determine the effect of the high dose of noradrenaline on the blockade of the α-receptor by the antagonists, the tissue was rechallenged with the low dose of noradrenaline 20 min after washing out the high dose of noradrenaline and the antagonists.

Receptor protection by noradrenaline against phenoxybenzamine, phentolamine, yohimbine and prazosin

Two segments of the terminal ileum were set up at the same time, one serving as the test tissue and the other as control. Initial relaxation of both the test and the control tissues to a submaximal concentration of noradrenaline (24 nM) was obtained and the tissue washed. A high concentration of noradrenaline (50 μM) was then added to the bath fluid of both tissues. Five min later, and with the high concentration of noradrenaline still in the bath, an α-adrenoceptor blocker, i.e. phenoxybenzamine (0.29 μM), phentolamine (3.6 μM), prazosin (3.7 μM) or yohimbine (3.9 μM), was added to the test tissue while 0.1 ml bath fluid without any antagonist dissolved was added to the control tissue; 20 min later, the bath fluid was replenished and the tissue allowed to recover. Response to the submaximal concentration of noradrenaline was again determined 20, 25 and 30 min after the washout. A similar series of experiments was performed in which exposure to the antagonists was not preceded by a high dose of noradrenaline.

Effect of exogenous noradrenaline on electrically evoked twitches

Twitches of the guinea-pig ileum suspended in a 50 ml organ bath were obtained by maximal transmural stimulation as described by Paton (1957). After the amplitude of the twitches had become steady, noradrenaline was added cumulatively to the bath fluid. This procedure was repeated in the presence of various concentrations of prazosin, phentolamine, yohimbine and phenoxybenzamine. The percentage inhibition of the evoked twitches was plotted logarithmically against the molar concentration of antagonist. From the graphs, the concentrations of noradrenaline and antagonists which were used in the acetylcholine release experiments were chosen. A similar experimental procedure was followed to test the effect of other agonists: isoprenaline, phenylephrine, methoxamine and dopamine, on the evoked twitches.

Acetylcholine release studies

For the determination of acetylcholine output, a modification of the method of Paton (1957) was used. Two segments of the terminal ileum were used, one serving as the donor tissue and the other as the assay tissue.

The donor tissue was mounted in a 10 ml bath containing aerated Tyrode solution at 37°C. Physostigmine (6.2 μM) was added to prevent hydrolysis of the released acetylcholine. The tissue was transmurally stimulated as described earlier.

α-Adrenoceptor blockers were left in the bath for 20 min and then washed out before their effect was tested. Propranolol and practolol were added for 10 min and remained in contact with the tissue during the test period. Five minute collection periods were used.

The preparation of the assay tissue was similar to that of the donor tissue but to the bath fluid were added morphine (30 μM) to reduce endogenous release of acetylcholine and mepyramine (0.5 μM) to prevent interaction with histamine receptors. Acetylcholine released from the donor tissue was assayed by allowing the fluid bathing the donor tissue to run directly on to the assay tissue and the acetylcholine concentration found by interpolation on a standard acetylcholine dose-resonse curve obtained by duplicate determination of responses of the same assay tissue to standard concentrations of acetylcholine before and after contact with the donor fluid. In experiments involving exposure of the donor tissue to other drugs, the standard curve for acetylcholine was also obtained in the presence of the same concentrations of the drugs present in the donor fluid. Acetylcholine was identified by (a) specific antagonism by hyoscine and (b) destruction by increase of pH over 11. At the end of each experiment the tissue was blotted dry on a filter paper and weighed. The output of acetylcholine was expressed as pmol $min^{-1} g^{-1}$ wet tissue.

Results are given in the text and Table as means ± s.e. mean. The significance of differences between sets of data was assessed by Student's *t* test and probability levels less than 0.05 were taken to indicate significant differences between group means.

Drugs used were: (−)-noradrenaline bitartrate (Sigma), phentolamine mesylate (CIBA), cocaine hydrochloride (B.P.), corticosterone (Sigma), phenoxybenzamine hydrochloride (S.K.&F.), yohimbine hydrochloride (Sigma), (±)-propranolol (ICI), prazosin hydrochloride (Pfizer), hyoscine hydrochloride (BDH), isoprenaline sulphate (BDH), acetylcholine chloride (BDH), phenylephrine bitartrate (Sigma).

Results

Effect of noradrenaline in the unstimulated ileum

Noradrenaline (6.0 nM – 96 nM) produced a dose-dependent relaxation of the ileum which was antagonized by phentolamine, yohimbine, prazosin and phenoxybenzamine.

Figure 1 shows the cumulative dose-response curves for noradrenaline alone, and in the presence of phentolamine (0.71 μM), prazosin (1.8 μM), yohimbine (1.3 μM) and phenoxybenzamine (29 nM). At these concentrations, phentolamine, yohimbine and prazosin caused approximately equal displacement to the right of the noradrenaline concentration-response curve without affecting the maximum response, while phenoxybenzamine shifted the curve to the right and reduced the maximum response to about 25% of the control value. In experiments in which the tissue was treated with phenoxybenzamine

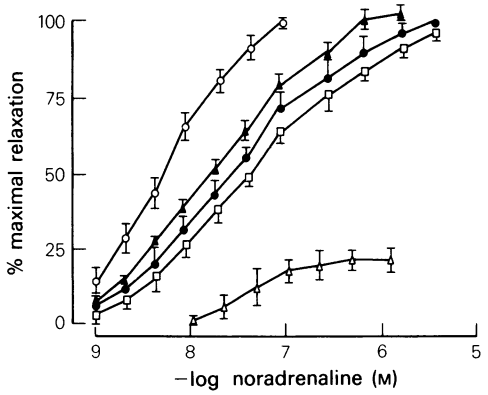

Figure 1 Guinea-pig isolated ileum: concentration-response curves for noradrenaline in the absence of α-adrenoceptor antagonists. (○) No antagonist; (▲) yohimbine (1.3 μM); (●) prazosin (1.8 μM); (□) phentolamine (0.71 μM); (△) phenoxybenzamine (29 nM). Each point represents the mean of six experiments and vertical bars show s.e.mean.

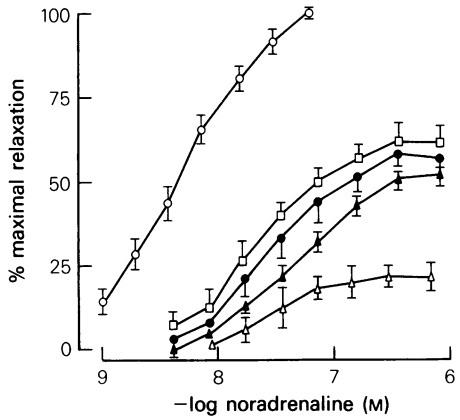

Figure 2 Guinea-pig isolated ileum: concentration-response curves for noradrenaline in the absence and presence of α-adrenoceptor antagonists. (○) No antagonists; (△) phenoxybenzamine (29 nM); (●) phenoxybenzamine (29 nM) plus prazosin (1.8 μM); (□) phenoxybenzamine (29 nM) plus phentolamine (0.71 μM), (▲) phenoxybenzamine (29 nM) plus yohimbine (1.3 μM).

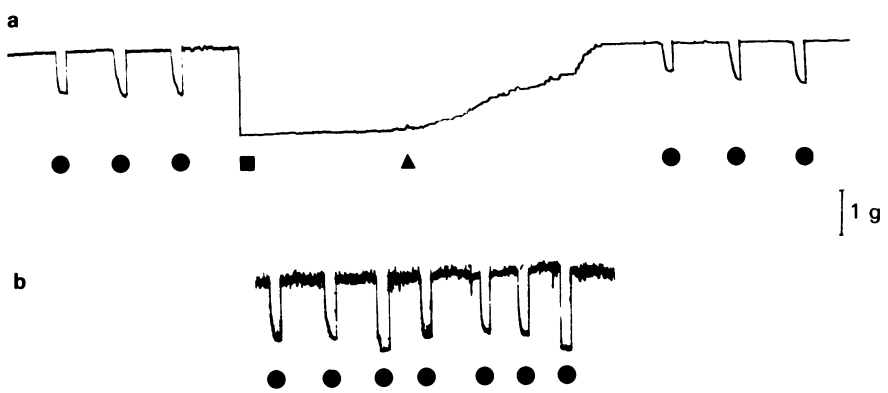

Figure 3 Guinea-pig isolated ileum: (a) effect of high concentration of noradrenaline (50 μM) added at (■) on response to subsequent sub-maximal dose of noradrenaline (24 nM) added at (●); (▲) indicates washout. (b) Response to sub-maximal concentration of noradrenaline (24 nM) added at (●) in tissue not treated with the high concentration of noradrenaline.

Table 1 Receptor protection experiments

Procedure	*Response to noradrenaline* (g) Before procedure	After procedure
1 High dose of noradrenaline only	0.98 ± 0.04	0.96 ± 0.03
2 High dose of noradrenaline followed by:		
(a) phentolamine	0.85 ± 0.02	0.82 ± 0.03
(b) prazosin	0.91 ± 0.04	0.87 ± 0.04
(c) phenoxybenzamine	0.76 ± 0.10	0.71 ± 0.06
3 Saline only in place of high dose of noradrenaline	1.04 ± 0.03	1.05 ± 0.04
4 Saline followed by:		
(a) phentolamine	0.84 ± 0.07	No response
(b) prazosin	0.84 ± 0.06	No response
(c) phenoxybenzamine	0.84 ± 0.12	No response

Tension developed by a strip of guinea-pig ileum to noradrenaline (24 nM) before and after a series of experimental procedures. The first of two responses before the procedure and the third response after the procedure were used in computing the table. Each procedure was repeated in 6 separate experiments and the values represent the mean ± s.e.mean of the 6 experiments.

(29 nM) during incubation with phentolamine (0.71 μM) or prazosin (1.8 μM) or yohimbine (1.3 μM), phenoxybenzamine reduced the maximum response to about 60% (Figure 2). Isoprenaline (1.7 nM–112 nM), phenylephrine (7.0 nM), dopamine (10 nM–250 nM) and methoxamine (4.0 nM–128 nM) also relaxed the ileum.

Receptor protection studies

In six experiments the tissues were treated with phenoxybenzamine, prazosin or phentolamine during incubation with a high concentration of noradrenaline. The adrenoceptors were protected from the blocking effect of the antagonists as revealed by the retention of the response of the tissue to noradrenaline after this treatment (Table 1 and Figure 4). In tissues where exposure to the antagonists was not preceded by a high dose of the agonist, subsequent addition of a submaximal dose of noradrenaline produced no effect (Table 1, Figures 3 and 4).

Effect of exogenous noradrenaline on electrically evoked twitches.

Noradrenaline (1.0 nM–32 nM) inhibited the transmurally evoked twitches. The block by noradrenaline was antagonized by phentolamine (0.026 μM–2.6 μM) and yohimbine (0.051 μM–0.51 μM) but not by prazosin (0.053 μM–5.3 μM) or phenoxybenzamine (4.2 μM–42 μM) (Figure 5). Although phentolamine and yohimbine antagonized the effect of noradrenaline, it was not possible, by increasing the concentration of these antagonists, to abolish completely the inhibitory effect of noradrenaline even on addition of a much higher concentration of propranolol. Similarly, phenylephrine (0.1 μM–5 μM) methoxamine (0.047 μM–4.7 μM), isoprenaline (0.069 μM–6.8 μM) and dopamine (0.05 μM–5 μM) inhibited the electrically evoked twitches. The mean maximum inhibition by 5 μM phenylephrine was 75.0 ± 4.7%.

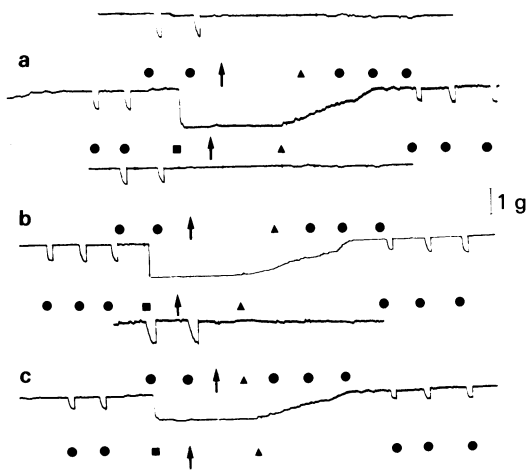

Figure 4 Guinea-pig isolated ileum: protection by a high concentration of noradrenaline against (a) prazosin block (b) phentolamine block and (c) phenoxybenzamine block. At (●) noradrenaline (24 nM) was added to the bath fluid; at (■) noradrenaline (50 μM) was added followed at ↑ by prazosin (3.7 μM) or phentolamine (3.6 μM)or phenoxybenzamine (0.29 μM) and washout at (▲). The upper tracings represent the control tissues without the high concentration of noradrenaline.

Acetylcholine release

In the absence of electrical stimulation there was a spontaneous release of acetylcholine at an average rate of 400.6 ± 72.4 (s.e.mean) pmol g^{-1} min^{-1} ($n = 48$). The level of spontaneous release varied substantially between tissues but in the same tissue, the variation was much less. Nevertheless, control spontaneous release was measured repeatedly in the course of a single experiment in which effects of drugs were measured. All the values for control spontaneous release have been pooled in analysing the results. Noradrenaline (59.0 nM–5.9 μM) and phenylephrine (15.0 μM) produced a reduction in the spontaneous output of acetylcholine. By contrast, methoxamine (0.48 μM–24 μM), isoprenaline (0.36 μM–3.6 μM) and dopamine (1.0 μM–5.0 μM) had no effect on the resting release of acetylcholine. The effect of these agonists on the tone of the ileum had earlier been determined in a separate series of experiments. At all doses used, the agonists caused relaxation of the ileum. This result, therefore, showed that whereas noradrenaline, isoprenaline, phenylephrine and dopamine would relax the ileum, only noradrenaline and the high concentration of phenylephrine would produce a decrease in the resting output of acetylcholine. There is, therefore, a dissociation between the effects of phenylephrine, isoprenaline, methoxamine and dopamine on acetylcholine output and relaxation of the ileum. Even in the case of noradrenaline, 0.059 μM produced about 70% relaxation while 0.59 and 5.9 μM produced maximal relaxation of the ileum; yet the reduction in the resting output of acetylcholine by these three concentrations of noradrenaline were 23.4, 44.7 and 49.4% respectively (Figure 6). A similar dissociation of the effect of morphine on acetylcholine output and contraction of the guinea-pig ileum has been observed by Kosterlitz & Waterfield (1975).

The output of acetylcholine during periods of transmural electrical stimulation (1366.4 ± 89.4 pmol g^{-1} min^{-1}) was greater than the resting output and was similarly diminished by noradrenaline and a high concentration of phenylephrine (15.0 μM) but not by isoprenaline, methoxamine and dopamine (Figure 6).

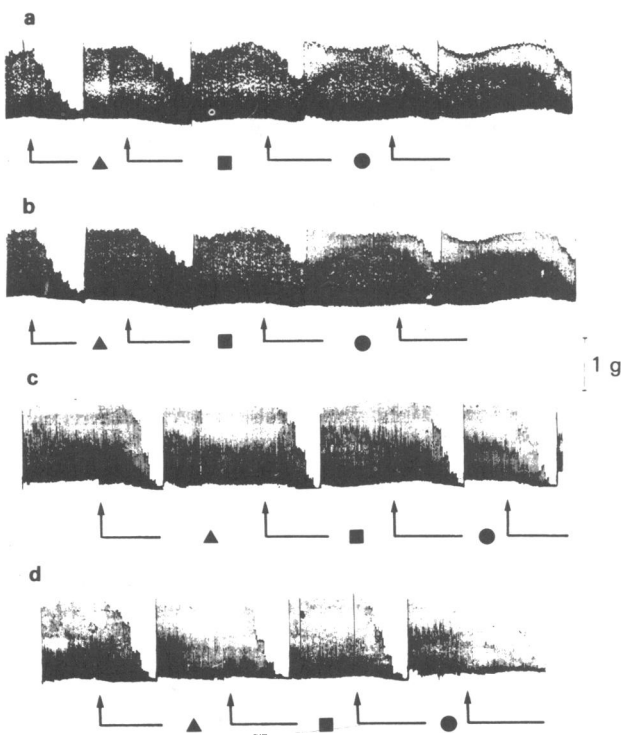

Figure 5 Guinea-pig isolate ileum: inhibition of transmurally evoked twitches by exogenous cumulative doses of noradrenaline (1–32 nM) added at ⌊___ in the presence of (a) phentolamine (0.026, 0.26 and 2.6 μM); (b) yohimbine (0.051, 0.251 and 0.51 μM); (c) prazosin (0.053, 0.53 and 5.3 μM) and (d) phenoxybenzamine (4.2, 21 and 42 nM). (▲) indicates first concentration of antagonists, (■) indicates the 2nd concentration of antagonist and (●) indicates the 3rd concentration of antagonist.

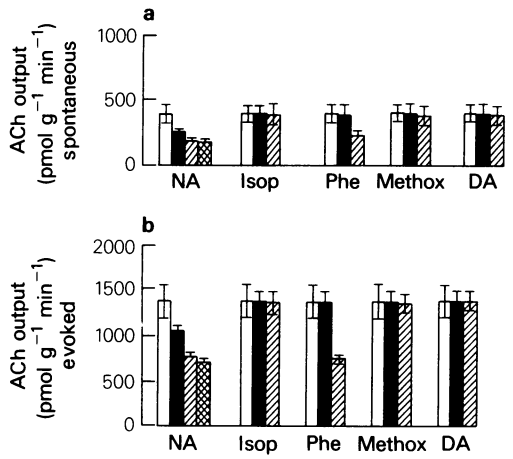

Figure 6 Guinea-pig isolated ileum: spontaneous (a) and evoked (b) release of acetylcholine (open columns) and the effect upon it of noradrenaline (NA: solid columns 0.059 μM; hatched columns 0.59 μM; cross-hatched columns 5.9 μM); isoprenaline (Isop: solid columns 0.36 μM; hatched columns 3.6 μM); phenylephrine (Phe: solid columns 0.5 μM; hatched columns 1.5 μM); methoxamine (Methox: solid columns 0.48 μM; hatched columns 24 μM); dopamine (DA: solid columns 1.0 μM; hatched columns 5.0 μM). The control release was estimated from 48 samples. Each drug was tested in 4–5 experiments. The columns show mean values and vertical lines indicate s.e.mean.

Phentolamine (0.13 and 2.6 μM) and yohimbine (0.051 and 0.51 μM) had no effect on the resting output of acetylcholine and no statistically significant increase in the stimulation output (Figure 7). Further increase in the concentration of the two antagonists did not produce a further increase in acetylcholine output in response to transmural stimulation. Prazosin (0.53 and 5.3 μM) and phenoxybenzamine (4.2 and 42 nM) had no effect on the resting nor on the stimulation output of acetylcholine (Figure 8).

The inhibitory effect of noradrenaline (0.59 μM) on acetylcholine release was antagonized by phentolamine (0.13 and 2.6 μM) and yohimbine (0.051 and 0.51 μM) such that, when noradrenaline diminished resting output to about 46% of the control value, in the presence of phentolamine (2.6 μM) or yohimbine (0.5 μM) the resting output was reduced by only 4% and 2%, respectively. This dose of noradrenaline alone produced 100% relaxation of the ileum but 75% and 85% relaxation in the presence of phentolamine (0.71 μM) and yohimbine (1.3 μM), respectively. Unlike phentolamine and yohimbine, prazosin (0.53–5.3 μM) and phenoxybenzamine (4.2–42 nM) did not antagonize the inhibitory effect of noradrenaline on the resting and the electrically evoked acetylcholine output. At all the concentrations used, none of the four antagonists had an effect on the tone of the ileum.

Propranolol and practolol had no effect of their own on the spontaneous and evoked release of acetylcholine nor did they antagonize the effect of added noradrenaline.

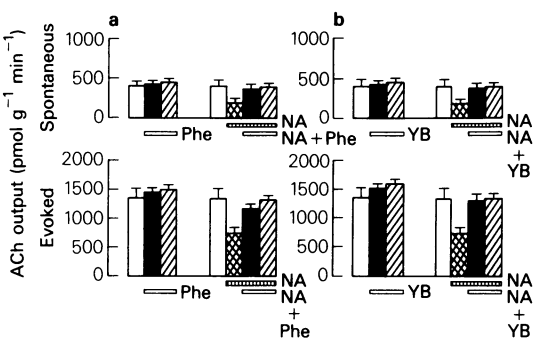

Figure 7 Reversal of the inhibitory effect of noradrenaline on spontaneous and evoked release of acetylcholine by (a) phentolamine and (b) yohimbine. Open columns, control output; the concentrations of phentolamine used were 0.13 (solid columns) and 2.6 (hatched columns) μM, while the concentrations of yohimbine were 0.051 (solid columns) and 0.51 (hatched columns) μM. The concentration of noradrenaline used was 0.59 μM. NA = Noradrenaline; YB = yohimbine; Phe = phentolamine.

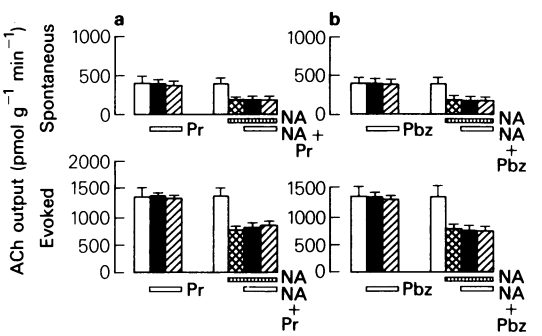

Figure 8 Lack of effect of prazosin (a) and phenoxybenzamine (b) on the inhibition of the spontaneous and evoked release of acetylcholine by noradrenaline. Open columns, control output; the concentrations of prazosin used were 0.53 (solid columns) and 5.3 (hatched columns) μM, while the concentrations of phenoxybenzamine were 4.2 (solid columns) and 42 (hatched columns) nM. The concentration of noradrenaline used was 0.59 μM. NA = noradrenaline; Pr = prazosin; Pbz = phenoxybenzamine.

Discussion

The results of the receptor protection experiments show that high concentrations of noradrenaline protect α-adrenoceptors from blockade by phenoxybenzamine, yohimbine, prazosin and phentolamine. The results also show that prazosin, yohimbine and phentolamine partially protect noradrenaline from blockade by phenoxybenzamine and that phentolamine is about two and a half times as potent as prazosin in this respect. These results, therefore, suggest that the receptor sites at which prazosin acts are the same as those on which noradrenaline, phentolamine and phenoxybenzamine act.

The observation that there is a spontaneous release of acetylcholine raises the possibility that the state of tone of the ileum at rest may be due, at least in part, to spontaneous activity in the cholinergic fibres innervating the tissue. The reduction of this spontaneous release by noradrenaline may be responsible, wholly or partially, for the relaxation of the ileum which the drug produces. Direct action on smooth muscle is not excluded by this study. Phentolamine and yohimbine prevent the inhibition of acetylcholine release and reverse the relaxation induced by noradrenaline. Phenoxybenzamine and prazosin also reverse the relaxation produced by noradrenaline without preventing the inhibition of acetylcholine release. These results suggest that exogenous noradrenaline has at least two possible components to its effect on the resting ileum, one presynaptic and the other postsynaptic, and only the postsynaptic component is affected by prazosin and phenoxybenzamine. Studies by other workers (e.g. Kosterlitz, Lydon & Watt, 1970) have shown that higher concentrations of phenoxybenzamine or longer periods of incubation are needed to demonstrate its presynaptic effect in this tissue. Since the concentrations and contact time for phenoxybenzamine used in the receptor protection experiments are similar to those used in the acetylcholine release studies, then the identical receptors on which phenoxybenzamine, prazosin and phentolamine are presumed to act in the receptor protection experiments could only be the postsynaptic α-adrenoceptor.

The results also show that acetylcholine release in the stimulated ileum is greater than in the resting tissue and that the evoked release is inhibited by noradrenaline. This inhibitory effect of noradrenaline is antagonized by phentolamine and yohimbine but not by prazosin or phenoxybenzamine. In parallel with this, the reduction of the evoked twitches by noradrenaline is antagonized by phentolamine and yohimbine but not by prazosin or phenoxybenzamine. These results show that the presynaptic effect of noradrenaline on the cholinergic nerves is not affected by preferentially postsynaptic blockers like prazosin and phenoxybenzamine. The inability of phenoxybenzamine to block responses to noradrenaline in the transmurally stimulated gut has also been reported by Wikberg (1978).

The lack of effect of isoprenaline and dopamine on the spontaneous release of acetylcholine and the failure of propranolol and practolol to antagonize the noradrenaline-induced reduction in spontaneous and evoked release show that α-adrenoceptors and receptors for dopamine take no part in the regulation of acetylcholine release at the cholinergic nerve endings in the guinea-pig ileum.

In conclusion, these studies confirm that the relaxation of the unstimulated ileum by noradrenaline is due mainly to action on postsynaptic α-adrenoceptors although a presynaptic component cannot be excluded. The inhibition of the evoked twitches and reduction in acetylcholine release are due to action on presynaptic α-adrenoceptors. Prazosin reacts with the same subgroup of postsynaptic α-receptors as noradrenaline, phentolamine and phenoxybenzamine. In the guinea-pig ileum, prazosin has no effect on the α-adrenoceptors situated on the cholinergic nerve endings.

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