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DISTRIBUTION AND TYPES OF ADRENOCEPTORS IN THE GUINEA-PIG ILEUM: THE ACTION OF α- AND β-ADRENOCEPTOR AGONISTS

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1 Segments of guinea-pig ileum and the myenteric plexus-longitudinal smooth muscle preparation were used for a study of the actions of adrenaline, noradrenaline, isoprenaline, ephedrine and phenylephrine on the responses of coaxially stimulated ileum at different distances from the ileocaecal valve.

2 The responses of the ileum to electrical stimulation were suppressed by adrenaline, noradrenaline and ephedrine, while phenylephrine and isoprenaline inhibited them only partially.

3 The twitch inhibition elicited by these adrenoceptor agonists was the same at all distances from the ileocaecal valve. There was no significant difference between their cumulative and non-cumulative concentration-response curves.

4 Smooth muscle relaxation was induced only by isoprenaline and contraction only by phenylephrine at all distances from the ileocaecal junction. Adrenaline and noradrenaline evoked smooth muscle contraction in the terminal (0 to 20 cm), a concentration-dependent, biphasic response in the intermediate part (21 to 50 cm) and a relaxation in the proximal ileum (> 50 cm from the ilecocaecal valve). Ephedrine did not change significantly the smooth muscle tension in the terminal and the intermediate segments and induced smooth muscle relaxation in the proximal ones.

5 Ouabain and a potassium-free solution did not appear to influence the prejunctional action of noradrenaline nor the amplitude of smooth muscle relaxation in the proximal ileum, whereas the concentration-contractor response curves were significantly depressed and shifted to the right by ouabain and in a potassium-free solution.

6 The brief initial (phasic) contraction induced by acetylcholine was not influenced during the sustained increase or decrease in tension induced by catecholamines. On the contrary, the stimulatory catecholamine actions disappeared or were changed to smooth muscle relaxation by acetylcholine pretreatment. Potassium chloride pretreatment did not change the character of the adrenoceptor agonist action of the agonists studied.

7 Since there is a similar prejunctional action at all distances from the ileocaecal valve and a different postjunctional effect of the adrenoceptor agonists at different distances from the ileocaecal junction, it could be suggested that in the guinea-pig ileum there are at least two α-adrenoceptors (inhibitory prejunctional-α_2, stimulatory postjunctional-α_1), an inhibitory postjunctional β-adrenoceptor and an as yet uncharacterized inhibitory postjunctional receptor.

8 Based on the specific postjunctional action of phenylephrine and the prejunctional action of ephedrine in the guinea-pig ileum, these drugs could be used with success as 'specific' α_1- and α_2-adrenoceptor stimulants.

Introduction

The inhibitory action of catecholamines in the small intestine of several mammalian species is mediated by α- and β-adrenoceptors (Ahlquist & Levy, 1959; Furchgott, 1960; Andersson & Mohme-Lundholm, 1969; Kosterlitz, Lydon & Watt, 1970; Bowman & Hall, 1970). McDougal & West (1952; 1954), Kosterlitz *et al.* (1970) and Paton & Vizi (1969) were of the opinion that adrenaline and noradrenaline act upon the intramural neuronal elements, whereas the action of isoprenaline was ascribed to direct smooth muscle effects (McDougal & West, 1952; Kosterlitz & Watt, 1965; Kosterlitz *et al.*, 1970). However, there is evidence that in the terminal ileum the mechanisms controlling the contraction-relaxation cycle are different from those operating elsewhere in the gastrointestinal tract (Kažič, 1975; Bauer, 1976). A postjunctional α-adrenoceptor agonist action of catecholamines was found in the small intestine of rabbit and in the longi-

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tudinal smooth muscle of taenia coli (Bülbring & Tomita, 1969a, b; Bauer & Zakhari, 1977) similar to those on other types of smooth muscle, e.g. uterus (Willems, Bernard, Delaunois & de Schaepdryver, 1965), blood vessels (Somlyó & Somlyó, 1968). The biochemical and biophysical events associated with the activation of α- and β-adrenoceptors differ in many respects (Burnstock, 1958; Brody & Diamond, 1967; Bülbring & Tomita, 1969a, b; Andersson & Mohme-Lundholm, 1970; Andersson, 1972; Bauer, 1976; Bauer & Zakhari, 1977). The decreased acetylcholine release from the myenteric plexus by adrenaline and noradrenaline (Paton & Vizi, 1969; Kosterlitz *et al.*, 1970; Wikberg, 1977) and the rich adrenergic innervation of the intramural ganglia in the myenteric plexus (Norberg, 1964; Gabella, 1972) and minimal adrenergic innervation in the longitudinal muscle layer of the small intestine of several mammalian species (including guinea-pig) led Norberg (1964) and Costa & Gabella (1971) to assume that α-adrenoceptor stimulation in the intestine affected intramural cholinergic neurones or nerve endings. Lee (1970), basing his experiments on α-adrenoceptor and muscarinic receptor antagonists, suggested the possible role of both pre- and postjunctional α-adrenoceptor sites in the action of catecholamines. Pre- and postjunctional α-adrenoceptors have been described in preparations of rabbit jejunun (Wikberg, 1977) and guinea-pig ileum (Anderson & Lees, 1976; Drew, 1977; 1978). Moreover, Innes & Kohli (1969) reported sympathomimetically-induced contraction of the upper, i.e. nonterminal, portion of the guinea-pig ileum.

The aim of the present study was to provide additional information about the distribution of the intestinal adrenoceptors and to find out whether there are differences between α- and β-adrenoceptor actions on smooth muscle as have been described for the taenia coli (Bülbring & Tomita, 1969b; Bauer & Zakhari, 1977) in different parts of the ileum and on the prejunctional nerve fibres of the myenteric plexus. Preliminary reports of some of the results were made at the International Symposium on the Physiology and Pharmacology of smooth muscle (Bauer, 1976) and to the Czechoslovak Pharmacological Society (Bauer, 1978).

Methods

Experiments were performed on the isolated ileum of the guinea-pig. The preparations used were ileal segments up to 70 cm from the ileocaecal valve. The action of catecholamines was analysed upon the whole ileal preparation or upon a longitudinal muscle-myenteric plexus preparation of guinea-pig ileum.

After exsanguination of animals, pieces of ileum 3 to 4 cm long were quickly dissected and inserted into an organ bath filled with a modified Krebs solution of the following composition (mM): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, $NaHCO_3$ 15.4, $MgCl_2$ 1.2, NaH_2PO_4 1.2 and glucose 11.5. The solution was aerated with a mixture 95% O_2 and 5% CO_2. The whole ileum was stimulated transmurally (Paton, 1955) with supramaximal pulses of 0.3 ms duration at a frequency of 0.07 Hz. The method of dissection of the preparation of myenteric plexus and longitudinal smooth muscle was similar to the modification described by Paton & Vizi (1969). A segment of ileum 3 to 5 cm long was gently slid along a glass rod of 6 mm diameter and arranged so that the mesenteric attachment was in a straight line. With a wisp of cotton wool soaked in a saline solution the longitudinal muscle layer was separated from the circular muscle along the mesenteric attachment by firm strokes in a lateral direction and then proximo-distally along the whole circumference of the ileal segment. The adherence of a functional myenteric plexus to the longitudinal muscle was checked by electrical field stimulation of the intramural myenteric plexus with similar rectangular pulses to those used for transmural stimulation, one electrode being at the top and the other at the bottom of the organ bath. When the action of a drug on the responses to electrical stimulation was investigated, the stimuli were applied continuously throughout the experiments. Isometric smooth muscle contractions (twitches) were recorded with a strain gauge transducer. The experiments were performed at 37°C under an initial tension of 1.5 g.

The following drugs were used: acetylcholine hydrochloride (VEB Berlin Chemie), adrenaline hydrochloride (Spofa), atropine sulphate (Spofa), ephedrine hydrochloride (Spofa, Merck), G-strophantin (Spofa), hexamethonium dibromide (VEB Rodleben), isoprenaline hydrochloride (Spofa), noradrenaline hydrogentartrate (Spofa), phenylephrine hydrochloride (Koch Light). Fresh stock solutions were prepared in distilled water. The agonists were in contact with the preparation for 45 to 120 s, a time ensuring a maximal effect. All concentrations are expressed as mol (base)/l (M).

Results are expressed as arithmetic means with $\pm$ s.e. mean. Differences were tested by Student's t test for paired observations. The concentrations of agonists producing 50% of maximum contraction or relaxation (EC_{50} values) were calculated by linear regression (Delaunois, 1973).

Results

The responses of the whole ileum and of the preparations of the myenteric plexus and longitudinal

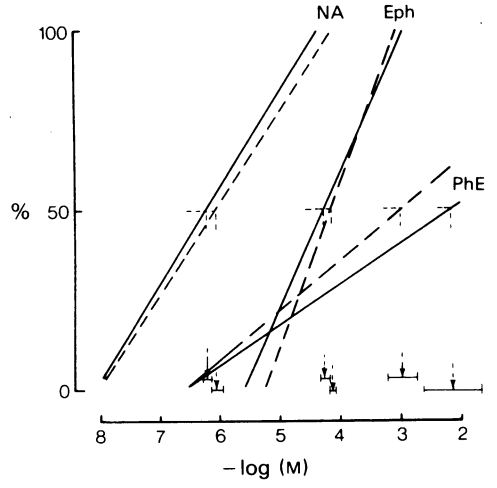

Figure 1 Regression lines for the inhibitory action of cumulatively-applied noradrenaline (NA), ephedrine (Eph) and phenylephrine (PhE) on the smooth muscle twitches evoked by electrical transmural stimulation in the terminal (full line) and proximal (broken line) ileum. Note the similarity of the slopes for noradrenaline and ephedrine as opposed to those for phenylephrine. The EC_{50} values are given with their s.e. mean.

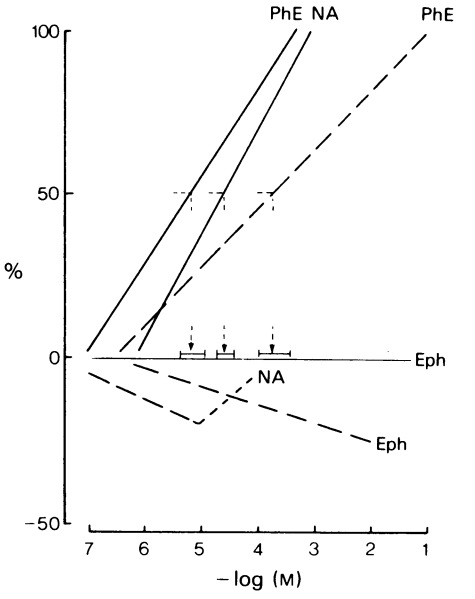

Figure 2 As in Figure 1 for smooth muscle contraction or relaxation. The inhibitory responses are expressed as the percentage of maximal stimulatory effect. Note the activity of phenylephrine (PhE) on both ileal parts similar to that of noradrenaline (NA) in the terminal ileum and the relaxant effect of noradrenaline and ephedrine (Eph) in the proximal ileum.

smooth muscle to catecholamines and other stimulants used were not significantly different. Therefore, the results obtained in the whole ileum and the longitudinal muscle preparation were considered together unless stated otherwise. In preliminary experiments, there was no difference between the action of catecholamines in preparations treated with hexamethonium (10^{-5} M) and untreated preparations; therefore, the study was carried out in preparations without hexamethonium pretreatment.

The effect of cumulatively-applied adrenoceptor agonists on the twitch response and basal tension

Concentration-dependent effect of cumulatively applied noradrenaline (10^{-8} to 10^{-4} M) on the smooth muscle twitches induced by supramaximal electrical stimulation of the intramural 'postganglionic' nerve fibres showed a similar inhibition at both terminal (0 to 3 cm) and proximal (>50 cm from the ileocaecal valve) parts of ileum. However, there were pronounced differences in the changes in smooth muscle tension induced by noradrenaline at different distances from the ileocaecal junction. While in the terminal ileum noradrenaline evoked only contractions, at distances greater than 50 cm from the ileocaecal junction it induced only relaxations (Figures 1 and 2).

The inhibition of twitch induced by cumulatively-applied adrenaline was similar to that of noradrenaline. Adrenaline induced contractions of the terminal ileum and relaxations in proximal segments (an insignificant contraction occurred at the highest concentration (10^{-4} M) used). The EC_{50} values of adrenaline-induced contraction and twitch inhibition were similar to those of noradrenaline (Table 1).

The inhibitory action of cumulatively-applied isoprenaline (10^{-8} to 10^{-4} M) was studied on twitches evoked by transmural stimulation at a distance of 0 to 3 cm and >50 cm. After the highest concentration used, the inhibition was $43.5 \pm 5.82\%$ and $47.2 \pm 6.12\%$, respectively. The relaxation was also similar at all distances along the ileum and reached $62.4 \pm 7.5\%$ of the initial tension at distances greater than 50 cm and $59.6 \pm 6.4\%$ in the 3 cm nearest to the ileocaecal valve.

Ephedrine (10^{-6} to 10^{-3} M) inhibited the ileal responses to transmural stimulation in the terminal and the proximal ileum (Figure 1). The EC_{50} values for the inhibition of the twitch were similar in both parts of the ileum (Table 1). The slope of the concentration-response curves was parallel to and not significantly different from those of noradrenaline and adrenaline. The smooth muscle tone of the terminal ileum was not significantly influenced by ephedrine; only a small

contraction was occasionally observed at a higher ephedrine concentration. In the proximal part of the ileum, more than 70% of preparations were relaxed by ephedrine (Figure 2).

Phenylephrine in concentrations of 10^{-7} to 10^{-3} M inhibited to the same extent the responses of both the terminal and the proximal ileum to transmural stimulation but the inhibition hardly reached 50% with the highest phenylephrine concentration used. At the same time, phenylephrine evoked contractions of terminal and proximal ileal segments, the amplitude of contractions being relatively greater in the terminal part (Figure 1 and 2). The concentration-response curves of phenylephrine for inhibition of the twitch had a smaller maximum and their slopes were significantly flatter than those of adrenaline, noradrenaline and ephedrine. The concentration-response curves for the contractions of the terminal and the proximal ileum had the same slope as those of adrenaline and noradrenaline in the terminal part of the ileum. The EC_{50} values for twitch inhibition and muscle contraction in the terminal and proximal parts of the ileum are given in Table 1.

The same concentration-dependent effects of noradrenaline and adrenaline on the smooth muscle twitches as for terminal and proximal parts could be elicited also in intermediate (18 to 21, 35 to 38 cm) parts (Table 1). However, there were pronounced differences in changes of smooth muscle tension induced by noradrenaline and adrenaline in this region. They induced smooth muscle contraction or a biphasic effect (smooth muscle relaxation at low and contraction at high concentrations). The EC_{50} values for the smooth muscle contraction in the terminal and intermediate ileal parts were significantly different (Table 1).

The effect of single supramaximal concentrations of adrenoceptor agonists at all points along the ileum

Noradrenaline (10^{-5} M) evoked contractions in the part of the ileum nearest to the ileocaecal valve; the amplitude of this contraction reached that evoked by transmural stimulation. At a distance of 9 to 12 cm, the fast and brief initial contraction (phasic response) was only one third and at a distance of 18 to 21 cm only one eighth of that evoked by transmural stimulation. The sustained increase in tension (tonic response) induced by noradrenaline was about one half of the phasic responses at all distances from the ileocaecal junction. In contrast at 27 to 30 cm, the ileum was slightly relaxed and at >50 cm the muscle tone was markedly decreased by noradrenaline. The muscle twitches were depressed by 80 to 100% and the depression was independent of the distance from the ileocaecal junction. The action of isoprenaline (10^{-5} M), unlike that of noradrenaline, was not dependent on the distance of the ileal segment from the ileocaecal valve (Figure 3). In one fifth of the preparations, isoprenaline caused a biphasic response with a small and transient smooth muscle contraction before the smooth muscle relaxation appeared. The action of adrenaline (10^{-5} M) was similar to that of noradrenaline except that the muscle contraction was

Table 1 EC_{50} values of cumulatively-applied adrenoceptor agonists

Agonist	*Distance from the ileocaecal valve* (cm)	*EC_{50} ± s.e. mean (μM)* *Twitch inhibition*		*Muscle contraction*	
Noradrenaline	0–3	0.62	(0.53–0.73)	7.32	(5.24–10.47)*
	18–21	0.87	(0.74–1.03)	$10^2 \times 7.5$	(1.49–37.63)*†
	35–38	0.86	(0.72–1.05)	NC	
	>50	0.58	(0.48–0.67)	NC	
Adrenaline	0–3	0.62	(0.49–0.78)	2.56	(1.91–3.4)*
	18–21	0.61	(0.47–0.78)	NC	
	35–38	0.53	(0.42–0.69)	NC	
	>50	0.51	(0.36–0.72)	NC	
Phenylephrine	0–3	$10^4 \times 1.52$	(0.44–5.43)	5.23	(3.02–9.13)*
	>50	$10^3 \times 1.12$	(0.63–1.98)	$10^2 \times 1.86$	(0.88–3.91)†*
Ephedrine	0–3	57.2	(49.7–65.9)	NC	
	>50	85.5	(78.7–92.8)	NC	

Isoprenaline reduced the basal tension at all distances and the EC_{50} value for its twitch inhibition could not be calculated exactly.

* Significantly different from the twitch inhibitory value at $P < 0.05$.

† Significantly different from the value at distance of 0–3 cm at $P < 0.05$.

NC = EC_{50} value could not be calculated.

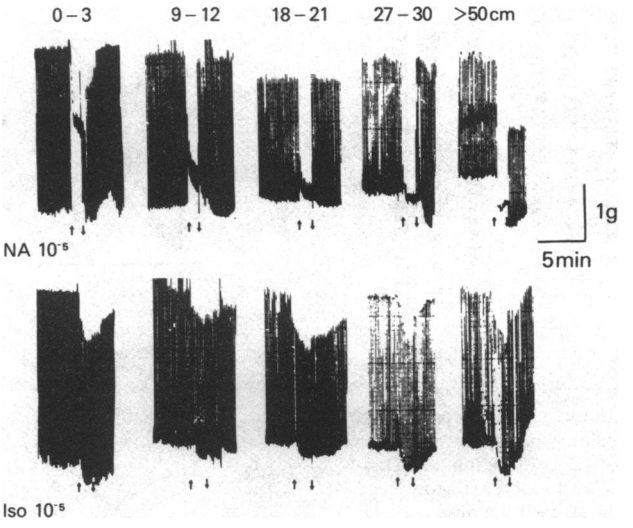

Figure 3 Effect of noradrenaline (NA) and isoprenaline (Iso) on the smooth muscle basal tension and the responses of the guinea-pig ileum to transmural stimulation at different distances from the ileocaecal valve. Note a similar effect of isoprenaline at every distance and large differences between noradrenaline effects in the terminal and the proximal ileum. Arrows indicate start and finish of exposure to agent.

changed to a relaxation at distances closer (15 to 20 cm) to the ileocaecal valve than it was in the case of noradrenaline (Figure 4).

Non-cumulative addition of noradrenaline gave the same results for twitch inhibition and basal tension changes as cumulative addition. The EC_{50} values and the slope of the concentration-response curves were not significantly different in these two types of noradrenaline administration.

The action of adrenoceptor agonists and the activation of muscarinic receptors

To analyse whether or not the action of noradrenaline in different ileal segments was the result of a different sensitivity of the ileum to agonists, the action of noradrenaline was compared with that of exogenous and endogenous acetylcholine (transmural stimulation). The responses elicited by endogenous acetylcholine did not differ significantly at various distances from the ileocaecal junction, being 2.15 ± 0.24 and 2.31 ± 0.17 g in proximal and terminal segments, respectively, and were taken as 100%. The actions of noradrenaline (10^{-5} M) and of exogenous acetylcholine (5×10^{-6} M) were expressed as percentages of the responses evoked by transmural stimulation. The acetylcholine-induced phasic and tonic contractions were not significantly changed by increasing the distance from the ileocaecal junction. In contrast, the postjunctional action of noradrenaline was markedly decreased and changed in its character as mentioned above.

When the effects of catecholamines on acetylcholine action were studied, acetylcholine was applied either during the tonic phase of the noradrenaline-induced contraction in the terminal ileum or during the noradrenaline-induced relaxation in the intermediate and proximal parts or during the decreased basal tension induced by isoprenaline at all distances from the ileo-

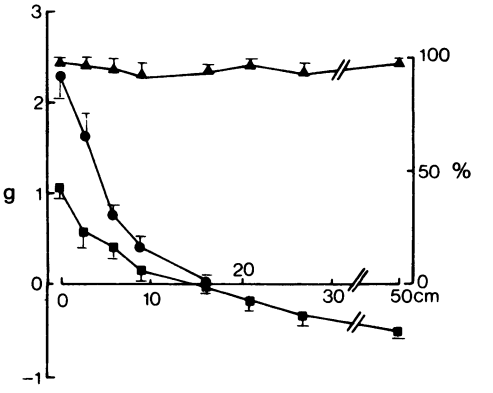

Figure 4 Effects of adrenaline (10^{-5} M) on the smooth muscle initial brief contraction (phasic; ●) and sustained increase in tension (tonic contraction; ■) and twitches (▲) evoked by transmural stimulation at different distances from the ileocaecal valve. Note the difference between the effect of adrenaline on the smooth muscle tension and on the twitches. Each point represents the mean of at least six trials; vertical lines show s.e. mean.

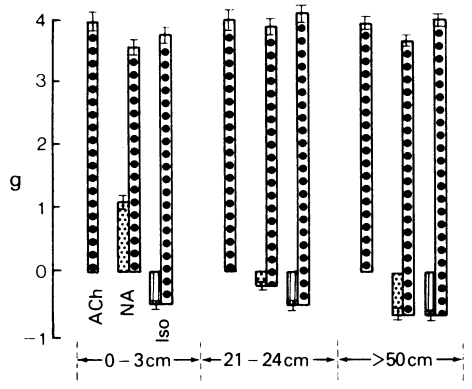

Figure 5 Acetylcholine-induced phasic contraction (5×10^{-6} M, filled circles) before and during the tonic phase of noradrenaline induced contraction or relaxation (10^{-5} M, stippled bars) and isoprenaline-induced relaxations (10^{-5} M, vertical stripes) at different distances from the ileocaecal valve. Note the same maximal response evoked by acetylcholine under all conditions. Each column represents the mean of at least six determinations; vertical lines show s.e. mean.

caecal valve. Isoprenaline and noradrenaline pretreatment did not influence significantly the maximal value of the phasic acetylcholine contraction. However, the changes in the smooth muscle responses caused by acetylcholine were significantly smaller when acetylcholine was applied at the time of an increased

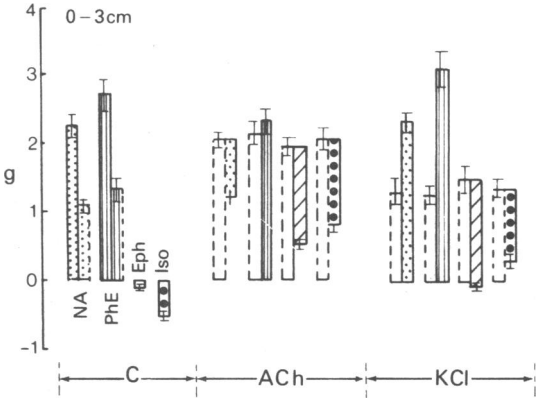

Figure 6 The action of noradrenaline (10^{-5} M, stippled bars), phenylephrine (10^{-4} M, vertical stripes), ephedrine (5×10^{-4} M, hatched) and isoprenaline (10^{-5} M, filled circles) before (C) and during the acetylcholine (ACh 10^{-5} M) or KCl (20 mM) tonic contractions in the terminal ileum (open columns with broken lines). Note the difference between noradrenaline action during the acetylcholine or the KCl contracture. Each column represents the mean of at least six determinations; vertical lines show s.e. mean.

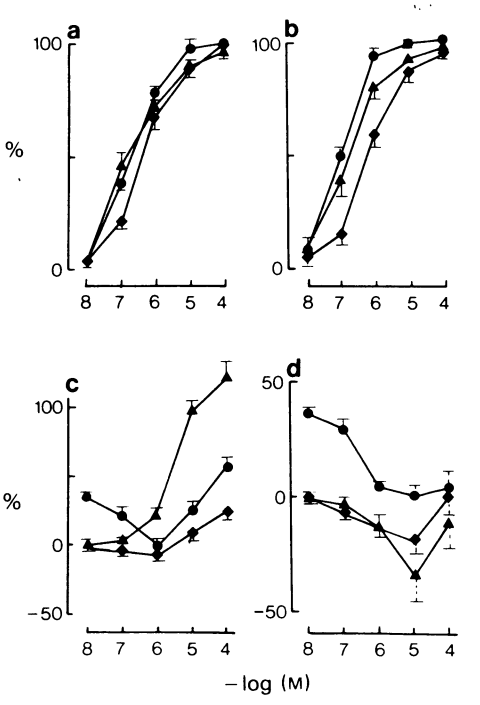

Figure 7 Effect of noradrenaline on the terminal (a, c) and the proximal (b, d) ileum on the electrically-evoked twitches (a, b) and smooth muscle tension (c, d) before (▲) and after ouabain pretreatment (●, 2×10^{-6} M) or in potassium-free solution (◆) for 10 min. Note the lack of effect of ouabain and potassium-free conditions on the twitch inhibition and their pronounced effect on the smooth muscle contraction. Each point represents the mean of at least six determinations; vertical lines show s.e. mean.

smooth muscle tone and they were higher when acetylcholine was applied at a decreased smooth muscle tone caused by catecholamines (Figure 5).

In all ileal segments noradrenaline (10^{-5} M) applied during the tonic phase of acetylcholine (10^{-5} M) contraction evoked a simple relaxation, the amplitude of which was several times larger than that of the relaxation elicited by noradrenaline in the proximal ileum. Similarly, the amplitude of isoprenaline-induced (10^{-5} M) relaxation was also increased. Ephedrine, which did not influence the tension of the terminal but relaxed the proximal ileum, evoked the largest smooth muscle relaxation compared to that of noradrenaline and isoprenaline during the acetylcholine-induced increase of the basal tone. Phenylephrine, which contracted the smooth muscle of both terminal and proximal ileum, did not change significantly the tonic phase of acetylcholine action (Figure 6).

Atropine (5×10^{-6} M) decreased the smooth muscle tone and completely blocked the responses to

transmural stimulation under the standard conditions used. Pretreatment with atropine increased the amplitude of the smooth muscle contractions elicited by noradrenaline (10^{-5} M); even at distances up to 50 cm, no significant smooth muscle relaxation was induced by noradrenaline. In the presence of atropine the smooth muscle relaxation elicited by isoprenaline (10^{-5} M) was not significantly decreased.

The stimulatory action of adrenaline and noradrenaline on the smooth muscle of the terminal ileum and their inhibitory effects on the proximal ileum were not changed when they were applied during contractions induced by potassium chloride (20 mM). Similarly, the contraction evoked by phenylephrine remained unchanged in both the terminal and the proximal ileum. Ephedrine here again significantly relaxed the tonic phase of potassium-induced contractions and the action of isoprenaline remained unchanged; however, the amplitude of the relaxation was greater during potassium tonic contractions (Figure 6).

The action of noradrenaline and sodium pump activity

Potassium-free solution or ouabain (2×10^{-6} M) evoked smooth muscle contractions. The smooth muscle tension in the potassium-free solution returned to the initial value in up to 3 min, while in ouabain-treated preparations it remained increased by more than 30% for up to 5 to 10 min. Pretreatment by ouabain or a potassium-free solution in the terminal and the proximal parts of ileum for 3 to 10 min did not change significantly the inhibitory action of cumulatively-applied noradrenaline on the electrically-evoked smooth muscle twitches but it depressed significantly the contraction of the terminal ileum induced by noradrenaline. The smooth muscle relaxation caused by noradrenaline in the proximal parts of the ileum was not changed by pretreatment with a potassium-free solution and was increased in amplitude in ouabain-pretreated and contracted preparations (Figure 7).

Discussion

Muscle contraction as a result of the activation of neuronal elements of the intestine may be counteracted by inhibitory mechanisms located in the intramural myenteric plexus and in the smooth muscle. When the smooth muscle is contracted by direct muscle cell activation without neuronal participation, the smooth muscle could be relaxed only by the direct effect on smooth muscle cells. Since the smooth muscle twitches evoked by short (0.3 ms) electrical rectangular pulses were abolished by atropine in our experiments, as described also by Paton (1955), and were not changed by the action of hexamethonium in concentrations large enough to block the transmission in intramural cholinergic ganglia, the twitches may be assumed to be the result of activation of intramural cholinergic 'postganglionic' nerve fibres.

All the adrenoceptor agonists studied inhibited the smooth muscle twitches induced by transmural stimulation. Their concentration-response curves had the same slope, irrespective of the distance of the ileal segment from the ileocaecal valve. Noradrenaline, adrenaline and ephedrine had about the same activity but isoprenaline and phenylephrine were less than half as potent. The depression of electrically-evoked responses by the agonists used in this study confirms the findings of Kosterlitz & Watt (1965), Kosterlitz *et al.* (1970), Anderson & Lees (1976) and Wikberg (1977). Since the activation of α-receptors by noradrenaline and adrenaline results in a decreased release of acetylcholine (Paton & Zar, 1968; Paton & Vizi, 1969; Kosterlitz *et al.*, 1970), it is likely that the described inhibitory action of noradrenaline, adrenaline, ephedrine, phenylephrine and isoprenaline (in part) could be the result of their action on prejunctional adrenoceptors. Inhibition of the twitch is probably due to decreased acetylcholine release rather than decreased acetylcholine synthesis since adrenaline and noradrenaline do not change the acetylcholine content of the intestine (Schaumann, 1958) and adrenaline has less effect at high frequencies of stimulation than at low frequencies (Paton & Vizi, 1969; Vizi, 1974).

In spite of the histological evidence that only the intramural ganglionic neurones are highly adrenergically innervated (Norberg, 1964; Hamberger, Norberg & Ungerstedt, 1965; Gabella, 1972) the adrenergic twitch inhibition was independent of the presence of ganglionic transmission involving acetylcholine. It therefore seems reasonable that the adrenergic inhibitory action is predominantly directed towards the 'postganglionic' or final motor cholinergic nerve terminals and not the intramural ganglionic cells, a conclusion which is in good agreement with the suggestions of Bowman & Hall (1970) and Lee (1970).

Noradrenaline and isoprenaline had little or no influence on the acetylcholine phasic contractions in a concentration that inhibited the responses to electrical stimulation; these observations are in good agreement with the proposed prejunctional origin of the inhibition caused by adrenoceptor agonists. The great sensitivity of the neuronal α-adrenoceptors to adrenaline, noradrenaline and ephedrine gives further evidence of the possible significance of prejunctional adrenoceptors under physiological conditions. In these experiments there was, contrary to the results of McDougal & West (1952), Härtfelder, Kushinsky & Mosler (1958) and Kosterlitz *et al.* (1970), no pronounced difference between the action of cumulatively

applied adrenaline and noradrenaline. The EC_{50} values of adrenaline were only slightly higher than those of noradrenaline, while ephedrine had a 90 to 100 times lower potency than noradrenaline and adrenaline. The activity of these three agonists and the slopes of their concentration-response curves were the same at every distance studied from the ileocaecal junction, indicating that same receptor was probably involved in their action. The effect of phenylephrine and isoprenaline was significantly smaller, perhaps as a result of a lower affinity for or efficacy at prejunctional adrenoceptors. The very low potency of phenylephrine in inhibiting the twitches is consistent with its ineffectiveness in reducing acetylcholine release (Paton & Vizi, 1969). Contrary to the results of Wikberg (1978), phenylephrine in our experiments inhibited the twitches not only in proximal but also in the terminal part of ileum and, as in the case of the other agonists, there was no difference between the sensitivity of prejunctional α-adrenoceptors to agonists whatever their distance from the ileocaecal junction.

There was a large difference between agonists with respect to their ability to stimulate adrenoceptors on the smooth muscle. Although the β-adrenoceptor agonist, isoprenaline, relaxed the ileal smooth muscle independently of the distance from the ileocaecal valve, the action of α-adrenoceptor agonists differed at various distances from the ileocaecal junction. Since Munro's (1953) observation of the excitatory adrenotropic action near the ileocaecal valve, the first 10 cm of ileum has usually been discarded when the action of drugs on ileal preparations has been studied. Our experiments have shown that noradrenaline and adrenaline have stimulatory action at 0 to 20 cm from the ileocaecal valve; furthermore relaxations appeared, which had the same amplitude in segments at distances more than 50 cm from the ileocaecal junction. While the excitatory action of phenylephrine was present at all distances from the ileocaecal valve, demonstrating the presence of stimulatory α-adrenoceptors in the proximal ileum, the potency of phenylephrine decreased with increasing distance from the ileocaecal junction. Based on the EC_{50} values, the order of potency of α-adrenoceptor agonists was adrenaline > phenylephrine > noradrenaline. The activity of phenylephrine did not differ significantly from that of adrenaline and noradrenaline in the first 3 cm from the ileocaecal valve, but, at a distance of more than 50 cm, was significantly smaller. Innes & Kohli (1969) were of the opinion that the stimulatory action of some adrenoceptor agonists is the result of 5-hydroxytryptamine receptor activation at larger distances from the ileocaecal junction. Since the slopes of the concentration-response curves for noradrenaline and adrenaline in the first 3 cm and that of phenylephrine at all points along the ileum were parallel, it is probable that they act upon the same receptor site at all distances from the ileocaecal junction. The postjunctional action of ephedrine was negligible in the terminal ileum, a finding which is in good agreement with the results of Minker, Koltai & Jánossy (1977), and appeared as a relaxant effect in proximal segments, similar to noradrenaline and adrenaline. Moreover, the action of adrenaline and noradrenaline in the intermediate parts of the ileum was biphasic; at low concentrations there was a relaxation, whereas at higher concentrations contractions were evoked. This means that the relaxation could be overcome by the activation of stimulatory adrenoceptors in the terminal parts; the opposite situation appeared in the proximal parts.

The proposed (Vizi, 1974) relationship between acetylcholine release and sodium pump activity is questionable as far as there was a pronounced difference between the time course of the contractions of the guinea-pig ileum evoked by ouabain- and potassium-free solution. The results described in the present paper, contrary to those of Paton, Vizi & Zar (1971) and Vizi (1974), have shown that the action of agonists on the presynaptic α-adrenoceptors measured as the twitch inhibition is probably not related to changed sodium pump activity. Although the stimulatory postjunctional actions of α-adrenoceptor agonists were inhibited, the relaxation was not significantly influenced by the decreased sodium pump activity. These results may indicate only a loose association between adrenergic smooth muscle stimulation and sodium pump activity. On the other hand, there could not have been any relationship between their smooth muscle inhibitory action and sodium pump activity, as has been demonstrated for taenia coli (Bauer, 1976), where noradrenaline and adrenaline effects on the membrane potential, muscle tension and membrane resistance were not prevented by ouabain or potassium-free solution pretreatment for 30 min, a time long enough to block pump activity (Casteels, Droogmans & Hendrickx, 1971; Bauer, 1978).

The action of endogenous and exogenous acetylcholine, potassium chloride and isoprenaline remained unchanged in various parts of the ileum. The different effects of α-adrenoceptor agonists in the terminal (0 to 20 cm), intermediate (21 to 50 cm) and proximal (> 50 cm) parts of the ileum could, therefore, hardly have been due to a different sensitivity of the postjunctional membrane to stimulants and relaxants but could be explained either by different α-adrenoceptor subtypes in the terminal and the proximal parts of the ileal smooth muscle or by different mechanisms connected with the activation of same postsynaptic α-adrenoceptor types. Another explanation could be a change in balance between the contractile and relaxant effects of the agonists.

The changed direction of the noradrenaline and adrenaline action during the increased basal tone induced by acetylcholine and the same acetylcholine action before and during the noradrenaline-induced tonic contraction provides evidence of a possible common link between excitation and contraction which is activated by acetylcholine and noradrenaline. The stimulatory (α_1) postjunctional adrenoceptor site could be the allosteric part of the acetylcholine receptor, different from the postsynaptic receptors mediating ileal smooth muscle relaxation. The receptor mediating this effect is, as yet, uncharacterized. Since the mechanism of potassium-induced contraction is different from that of acetylcholine and noradrenaline, potassium did not affect the noradrenaline and phenylephrine contraction.

Some differences between the postjunctional and prejunctional action of adrenoceptor agonists were described by Kosterlitz *et al.* (1970), Drew (1977) and Wikberg (1977). Our results concerning the postjunctional inhibitory action of isoprenaline are in good agreement with the results of McDougal & West (1952), Kosterlitz *et al.* (1970) and others, and this action is probably related to an increased adenylate cyclase activity and an increased cyclic adenosine 3'5'-monophosphate concentration (Bär, 1974; Andersson, 1972). The occasional occurrence of an action of high concentrations of isoprenaline may be similar to its proposed stimulatory action on the lamina muscularis mucosae of canine ileum (Tansy, Martin, Landin & Kendall, 1979). It was suggested that the effect of α-adrenoceptor agonists was only prejunctional (Paton & Vizi, 1969; Kosterlitz *et al.*, 1970). The differences found between the inhibitory prejunctional (α_2) and stimulatory postjunctional (α_1) action of agonists described in the present results were similar to those found in other smooth muscle preparations (e.g. gallblader, *vas deferens*, Lee & Fujiwara, 1977; Langer, 1977).

These results indicate the possibility of differentiating between several adrenoceptor subtypes in the guinea-pig ileal preparation. There seems to be inhibitory pre-junctional (α_2) and stimulatory postjunctional (α_1) with inhibitory postjunctional (β) receptors and an inhibitory postjunctional receptor as yet uncharacterised.

It has been suggested that ephedrine has both α- and β-adrenoceptor agonist properties (Innes & Nickerson, 1970). The large difference between the ephedrine and isoprenaline action found in the proximal and terminal ileum indicated that, in the guinea-pig ileum, ephedrine is without a postjunctional β-adrenoceptor stimulant action.

It has been shown that ephedrine could be a suitable α_2-agonist with small postjunctional inhibitory activity, while phenylephrine is predominantly an α_1-agonist. Noradrenaline and adrenaline then represent drugs with a combined α_1, α_2 and β-adrenoceptor stimulatory properties and they have a higher potency at α_2- than at α_1-adrenoceptor subtypes.

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