Br. J. Pharmac. (1970), **39**, 398–413.

The effects of adrenaline, noradrenaline and isoprenaline on inhibitory α- and β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum

H. W. KOSTERLITZ, R. J. LYDON AND A. J. WATT*

Pharmacology Section, Department of Therapeutics and Pharmacology, University of Aberdeen

Summary

1. Two preparations, a segment of the ileum and the myenteric plexus-longitudinal muscle preparation, have been used for an analysis of the inhibitory effects of adrenaline, noradrenaline and isoprenaline on the contractor responses of the longitudinal muscle to acetylcholine or to electrical, coaxial or field, stimulation.

2. Since the inhibitory effects of adrenaline, noradrenaline and isoprenaline on the acetylcholine-induced contractions were not affected by phenoxybenzamine but were antagonized by propranolol, it is concluded that β-adrenoceptors are present on the muscle cells.

3. The responses to electrical stimulation were suppressed by adrenaline or noradrenaline but only partly inhibited by isoprenaline. Propranolol antagonized the effect of isoprenaline and, to some extent, that of noradrenaline, but scarcely affected the action of adrenaline. Phenoxybenzamine, on the other hand, antagonized most of the effect of adrenaline and, to some extent, that of noradrenaline ; it usually potentiated the effect of isoprenaline.

4. The output of acetylcholine evoked by electrical stimulation was diminished by adrenaline or noradrenaline but was not affected by isoprenaline. The depressant effect on acetylcholine release was antagonized by phenoxybenzamine but not affected by propranolol ; therefore these effects of adrenaline and noradrenaline are mediated by α-adrenoceptors.

5. It may be assumed that α-adrenoceptors *in situ* are stimulated mainly by circulating adrenaline and possibly noradrenaline and thus cause a prejunctional inhibition at the nerve-smooth muscle junction.

Introduction

It is a well established observation that inhibition in the small intestine is mediated by α- and β-adrenoceptors (Ahlquist & Levy, 1959 ; Furchgott, 1960). In the guinea-pig ileum it would seem that the actions of adrenaline and noradrenaline are mainly on neuronal elements (McDougal & West, 1952, 1954 ;

* Present address: Department of Physiology, University of Manchester.

Kosterlitz & Robinson, 1957), whereas the effect of isoprenaline is mainly on the muscle (McDougal & West, 1952). There is evidence, however, that in the guinea-pig taenia coli and the rabbit small intestine, α-adrenoceptors are also situated on the smooth muscle cells (Jenkinson & Morton, 1967 ; Bowman & Hall, 1970).

These findings have been further analysed by examining the effects of phenoxybenzamine and propranolol on the inhibitory actions of catecholamines on acetylcholine release and on the responses of the longitudinal muscle of the guinea-pig ileum to electrical stimulation. Preliminary reports of some of the results have been made to the Pharmacological and Physiological Societies (Kosterlitz & Watt, 1965 ; Kosterlitz & Lydon, 1968) and to the International Symposium on Gastro-Intestinal Motility in September, 1967 (Kosterlitz & Cowie, 1968).

Methods

Experimental procedures

All experiments were performed on the guinea-pig isolated ileum ; the terminal portion was used 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). The depressant action of catecholamines was tested on the contractions of the longitudinal muscle of a segment of ileum or on the contractions of the myenteric plexus-longitudinal muscle preparation. Excitatory effects of catecholamines were seen only rarely and, when they occurred, the preparations were discarded.

The method of dissection of the myenteric plexus-longitudinal muscle preparation was a modification of that reported by Ambache (1954) and Rang (1964) ; recently, Paton & Vizi (1969) described a similar modification. A segment of ileum, 5–10 cm long, was gently slid on to a glass rod of 5–6 mm diameter and arranged so that the mesenteric attachment was in a straight line. With a wisp of cotton wool soaked in Krebs solution the longitudinal muscle was separated from the circular muscle along the mesenteric attachment by stroking firmly into the attachment along the whole segment of ileum. This gave an edge of longitudinal muscle bearing the mesenteric attachment. The other free edge of the longitudinal muscle, still attached to the circular muscle, was then stroked away with cotton wool along the whole length of the segment and the whole circumference of the ileum. The strip consisted of all the longitudinal muscle of the segment ; the mesenteric attachment was seen as whitish material and was easily removed by taking one end in fine forceps and pulling it off the muscle. The myenteric plexus adhered firmly to the longitudinal muscle.

The bath fluid was a low Mg^{2+} Krebs solution to which hexamethonium bromide (70 μM) and mepyramine maleate (0·125 μM) were added (Gyang & Kosterlitz, 1966) ; it was bubbled with 95% oxygen and 5% carbon dioxide. Its composition was as follows (mM): NaCl 118, KCl 4·75, $CaCl_2$ 2·54, KH_2PO_4 1·19, $MgSO_4$ 0·12, $NaHCO_3$ 25, glucose 11. The volume of bath fluid was 40 ml when contractions of the segment of ileum were recorded, and 4 ml in all other experiments. The temperature was 36° C.

The stimuli were 1·5 times maximal rectangular pulses of 0·5 ms duration, at a frequency of 6 or 20/min ; the whole segment of ileum was stimulated coaxially (Paton, 1955), and the plexus-longitudinal muscle preparation by field stimulation

by means of two platinum electrodes, one at the top and one at the bottom of the organ bath. When the effects of drugs on the responses to electrical stimulation were investigated, stimuli were applied continuously throughout the experiment. The twitch-like contractions of the longitudinal muscle were recorded by an auxotonic lever (Paton, 1957) writing on a smoked drum ; the initial tension was 1 g for the whole ileum and 0·3 g for the plexus-longitudinal muscle preparation.

In experiments in which the spontaneous and evoked outputs of acetylcholine were to be assayed, physostigmine was added only after the effects of catecholamines on the contractile responses had been recorded. For the determination of acetylcholine output, the ileum segment or the plexus-longitudinal muscle preparation was suspended in 4 ml of Krebs solution containing physostigmine sulphate (7·7 μM) ; to ensure inactivation of cholinesterase, the preparation was incubated for 1 h before the beginning of the experiment. The collection periods varied (2–6 min), depending on the amount of acetylcholine released. At the end of a collection period, samples of fluid from the donor bath were withdrawn into a glass syringe and transferred to a small beaker and two aliquots of the sample assayed within 15 min.

Assay of acetylcholine

Acetylcholine was assayed by a bracketing technique on a segment of guinea-pig ileum suspended in 4 ml Krebs solution containing mepyramine (0·125 μM), morphine (1·3 μM) and hexamethonium (70 μM) but not physostigmine. The solution was gassed with 95% oxygen and 5% carbon dioxide. The aboral end of the ileum remained open and was tied over a piece of glass tubing mounted in the base of the organ bath and connected to a reservoir containing Krebs solution. The level of this solution was kept 0·5 cm below the level of the fluid in the organ bath to exert gentle suction on the gut contents including shed mucosa. The contractions of the gut were recorded with a tension of 0·5 g with an isotonic lever writing on smoked paper.

In some of the later experiments the plexus-longitudinal muscle preparation was used for the assay. The bath fluid was Krebs solution with an increased Mg^{2+} content (3 mM instead of 1·2 mM) and contained mepyramine (0·125 μM), morphine (1·3 μM) and hexamethonium (70 μM). The contractions were recorded by means of an isotonic transducer (Sanborn) and a pen oscillograph.

The solution in the assay bath was renewed by overflow. The standard acetylcholine solutions were made up from the stock solution (5·5 mM) by diluting with either distilled H_2O or a salt solution containing 136 mM NaCl, 2·5 mM $CaCl_2$ and 4·8 mM KCl, adjusted to pH 4 by the addition of N HCl. When the whole ileum was used as assay preparation, propranolol was added to the bath fluid in a concentration (0·85 μM) that blocked the inhibitor effects of catecholamines without depressing the response to acetylcholine. In later experiments, in which the plexus-longitudinal muscle preparation was used, drugs were not added to the standard solution of acetylcholine since it was found that the final concentrations of catecholamines, propranolol or phenoxybenzamine did not affect the responses of the longitudinal muscle to acetylcholine.

The results were expressed as ng/min for the whole ileum or (ng/min)/g tissue for the plexus-longitudinal muscle preparation.

Drugs

Drugs used were: acetylcholine chloride, carbamylcholine chloride, choline chloride, physostigmine sulphate, (−)-adrenaline (British Drug Houses), (−)-noradrenaline bitartrate, (±)- isoprenaline hydrochloride (Winthrop Laboratories), hexamethonium bromide, mepyramine maleate (May & Baker), morphine hydrochloride, phenoxybenzamine hydrochloride (Smith, Kline & French) and propranolol hydrochloride (Imperial Chemical Industries). Stock solutions were prepared in distilled water, except for adrenaline, which was made up in 0·01 N HCl, and acetylcholine, which was made up in 5% (w/v) NaH_2PO_4 solution. Dilutions of the catecholamines were made with a solution of 0·2 mg ascorbic acid/ml H_2O; this concentration of ascorbic acid did not affect the responses to acetylcholine. The concentrations were expressed as μM of base.

The contact times chosen were such as to ensure maximum effects of the drugs. Catecholamines were added 30 s before addition of acetylcholine which, in turn, remained in contact for another 30 s; thus the catecholamines were in the bath for 60 s. When the effect of catecholamines on the responses to electrical stimulation was investigated, they were left in the bath until their effect was seen to be maximal. Propranolol was added at least 10 min before its antagonist action was tested. Phenoxybenzamine in a concentration of 3 μM was added for 20 min and then washed out; in a concentration of 0·015 μM it was added at least 120 min before its antagonist action was tested.

Results

There were no significant differences between the results obtained on a segment of ileum or on the plexus-longitudinal muscle preparation, so the results are given for only one of the two preparations.

Choline requirements of the plexus-longitudinal muscle preparation

It should be noted that whereas the responses of the longitudinal muscle to electrical stimulation remained constant for several hours when a segment of ileum was used (Gyang & Kosterlitz, 1966), the responses of the plexus-longitudinal muscle preparation diminished after 3–4 h; at the same time the inhibitory effect of adrenaline increased two to threefold over a period of 3 h (Fig. 1). If choline was then added to the bath fluid, the height of the electrically induced twitch was restored to its original value (Fig. 2) and the dose-response curve for adrenaline moved in the direction of its original position (Fig. 1). In preliminary experiments it was found that choline in a concentration of 20 μM prevented the described increase of the inhibitory effect of adrenaline and therefore the Krebs solution always contained this concentration of choline when the plexus-longitudinal muscle preparation was used.

Depressant effects of catecholamines on the responses of the longitudinal muscle to electrical stimulation

The depressant effects of adrenaline, noradrenaline and isoprenaline are shown in Fig. 3. The results with adrenaline and noradrenaline confirm and extend earlier observations (Schaumann, 1958; Härtfelder, Kuschinsky & Mosler, 1958). The

threshold concentrations of the three compounds were similar (5–10 nM or 1–2 ng/ml). Maximal effects were obtained with concentrations of 0·5–1 μM. It must be emphasized, however, that the effect of isoprenaline was fundamentally different from that of either of the other two catecholamines. In sufficiently high concentrations, adrenaline and noradrenaline caused complete inhibition of the twitch but the maximum inhibition produced by isoprenaline was usually not more than 20 to 40% and only occasionally 60% (Figs. 3–5).

High concentrations of adrenaline and noradrenaline led to tachyphylaxis, particularly when the intervals between additions to the bath were short. In order to minimize tachyphylaxis, concentrations resulting in more than 90% inhibition were avoided, the exposure time was not more than 90 s and the intervals between additions were 5 min or longer.

Effects of blockade of β-adrenoceptors by propranolol

In preliminary experiments, it was found that a concentration of 0·425 μM (0·11 μg/ml) of propranolol was sufficient to produce the maximum antagonistic effect for the concentrations of catecholamines used (Fig. 4); in all later experiments concentrations of 0·85 or 1 μM were used.

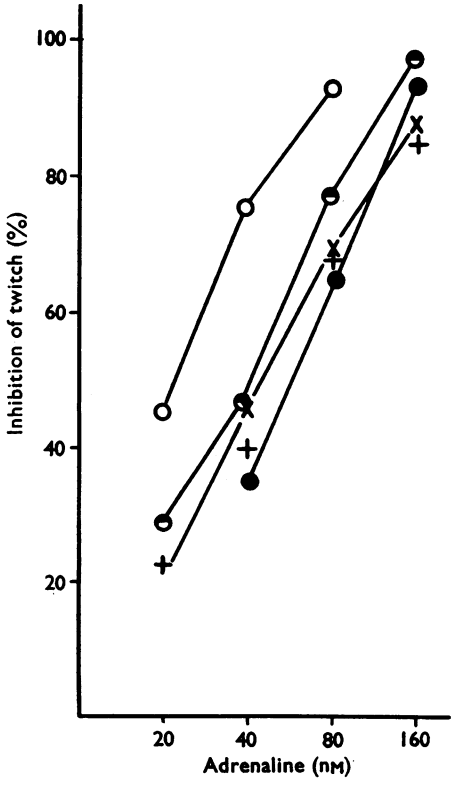

FIG. 1. Effect of choline on the depressant action of adrenaline on the responses of the plexus-longitudinal muscle preparation to electrical stimulation. Intervals between additions of adrenaline, 7 min. Time after setting-up of preparation in choline-free Krebs solution: ●, 90 min; ⊖, 140 min; ○, 280 min. Choline (7 μM) added at 320 min; ×, 350 min, +, 370 min.

FIG. 2. Effect of addition of choline (7 μM) on the height of the twitch of the plexus-longitudinal muscle preparation. Same experiment as in Fig. 1. Choline was added at arrow.

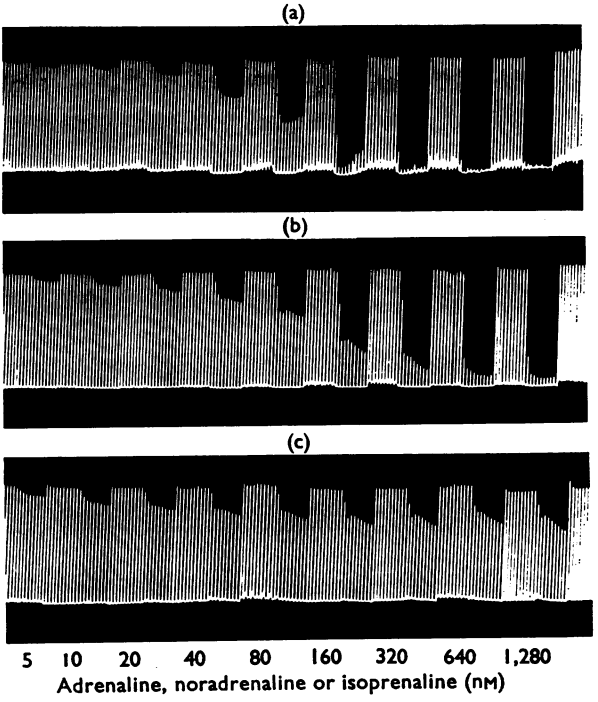

FIG. 3. Depressant effects of adrenaline, noradrenaline and isoprenaline on the responses of the ileum to electrical stimulation (6/min). The numbers refer to the concentrations (nM) of adrenaline (a), noradrenaline (b) and isoprenaline (c). Intervals between additions of drugs, 10 min.

The depressant effect of adrenaline was antagonized only very little, that of noradrenaline somewhat more and that of isoprenaline very considerably but not completely (Figs. 4 and 5). In experiments on segments of the ileum the mean dose-ratios and S.E.M. after exposure to 0·85 μM propranolol for at least 10 min were as follows: adrenaline $1{\cdot}3 \pm 0{\cdot}1$ (twelve experiments), noradrenaline $3{\cdot}2 \pm 0{\cdot}6$ (eleven experiments) and isoprenaline $7{\cdot}8 \pm 0{\cdot}7$ (ten experiments). Without antagonist, the inhibitory effects of catecholamines increased slightly during the course of the experiment.

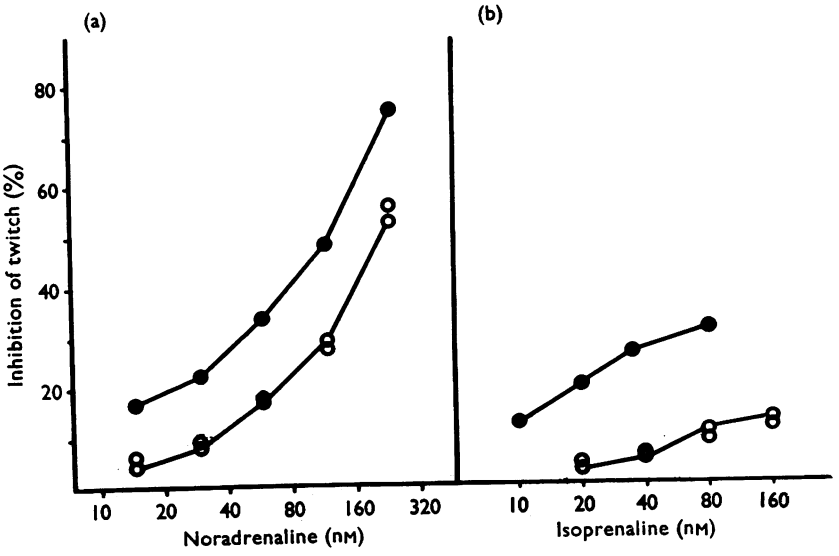

FIG. 4. Effects of propranolol on the depression by catecholamines of the responses of the ileum to electrical stimulation. Coaxial stimulation, 6/min, (●) before and (○) after addition of 0·425 μM propranolol; unconnected (○), in the presence of 0·85 μM propranolol.

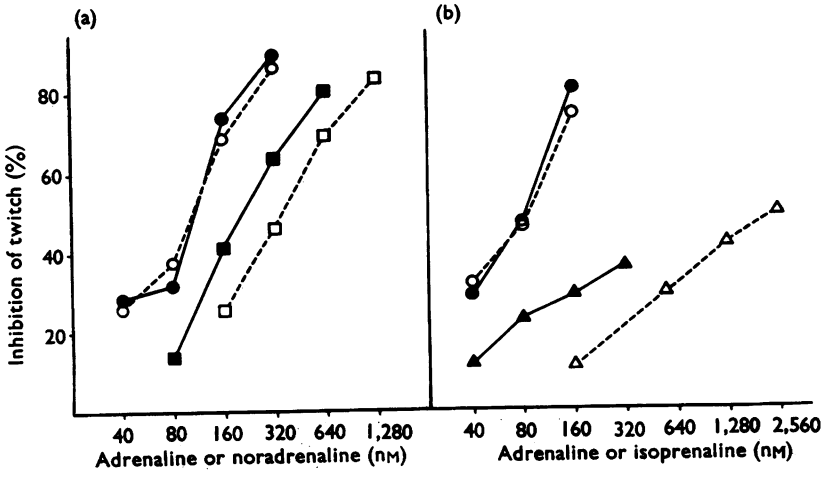

FIG. 5. Effects of propranolol on the depression by catecholamines of the responses of two plexus-longitudinal muscle preparations to electrical stimulation. Adrenaline before (●) and after (○) addition of propranolol (1 μM); noradrenaline, before (■) and after (□) addition of propranolol; isoprenaline, before (▲) and after (△) addition of propranolol. Adrenaline and noradrenaline (a) or adrenaline and isoprenaline (b) were added alternately; the first dose was given 50 min after propranolol.

Effects of blockade of α-adrenoceptors by phenoxybenzamine

Phenoxybenzamine has an anti-acetylcholine action which depresses the responses of the longitudinal muscle to electrical stimulation, (Boyd, Burnstock, Campbell, Jowett, O'Shea & Wood, 1963 ; Benfey & Grillo, 1963). While this effect was found to be present when the concentration of phenoxybenzamine was higher than 0·07 μM (22 ng/ml), concentrations as low as 0·015 μM antagonized the depressant action of adrenaline, provided the ileum was exposed to the drug for not less than 2 h (Fig. 7a). With higher concentrations (3 μM), a much shorter exposure (20 min) was needed but the contractor response to electrical stimulation was abolished also ; this latter effect was lessened by saturation of the acetylcholine receptors with acetylcholine during exposure to phenoxybenzamine. Thus, the antagonism by phenoxybenzamine of the inhibitory action of adrenaline on the responses to electrical stimulation could be demonstrated (Fig. 6).

The depressant effect of noradrenaline was not always antagonized but was sometimes potentiated (Fig. 8a) ; if noradrenaline was antagonized, it was less so than adrenaline (Fig. 6). The inhibitory effect of isoprenaline was never antagonized but usually potentiated (Fig. 9a), as indicated by a dose-ratio of less than unity. Calculation of the dose-ratios after exposure to 0·015 μM phenoxybenzamine for at least 2 h of a segment of ileum gave the following means and S.E.M.: adrenaline $3{\cdot}6 \pm 0{\cdot}3$ (seventeen experiments), noradrenaline $2{\cdot}0 \pm 0{\cdot}5$ (thirteen experiments) and isoprenaline $0{\cdot}39 \pm 0{\cdot}025$ (six experiments).

Combined effects of phenoxybenzamine and propranolol

When the preparation was treated first with phenoxybenzamine and then with propranolol, the inhibitory actions of the three catecholamines were reduced more

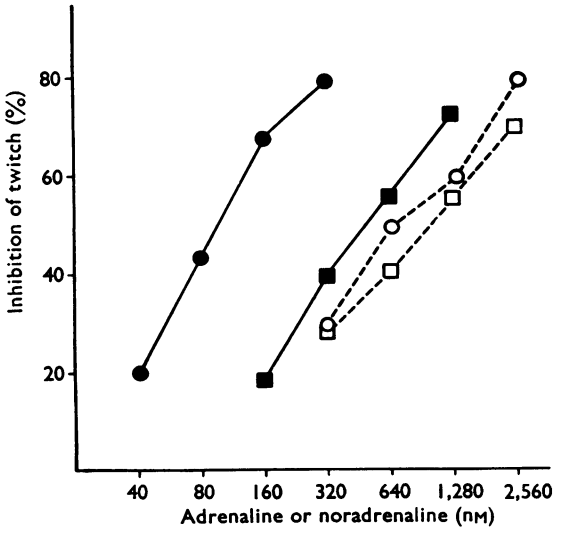

FIG. 6. Effects of phenoxybenzamine on the depression by catecholamines of the responses of the plexus-longitudinal muscle preparation to electrical stimulation. Exposure to phenoxybenzamine (3 μM) for 20 min in the presence of acetylcholine (150 μM), added before phenoxybenzamine to protect the acetylcholine receptors ; this procedure resulted in a reduction of the twitch height by 50%. Adrenaline, before (●) and after (○) exposure to phenoxybenzamine, noradrenaline before (■) and after (□) exposure to phenoxybenzamine.

than by phenoxybenzamine alone, propranolol being more effective with noradrenaline and isoprenaline than with adrenaline (Figs. 7a, 8a, 9a). When propranolol was added before phenoxybenzamine, the final results were similar.

Comparison of the inhibitory effects of adrenaline and noradrenaline on the contractor responses to electrical stimulation or to acetylcholine

The effects of catecholamines on the contractor responses to maximal electrical stimulation were compared with their effects on contractor responses of equal size

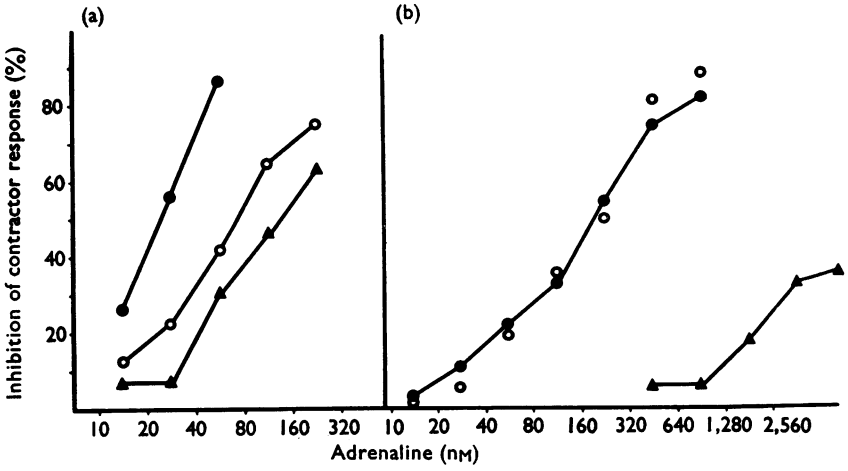

FIG. 7. Inhibitory effect of adrenaline on the responses of the ileum to electrical stimulation (a) or to an equiactive concentration of acetylcholine (b) and the antagonist actions of phenoxybenzamine (0·022 μM) and propranolol (0·85 μM). Adrenaline alone (●); after 150 (a) or 180 (b) min exposure to phenoxybenzamine (○); after 220 (a) or 250 (b) min exposure to phenoxybenzamine and 10 (a) or 40 (b) min exposure to propranolol (▲).

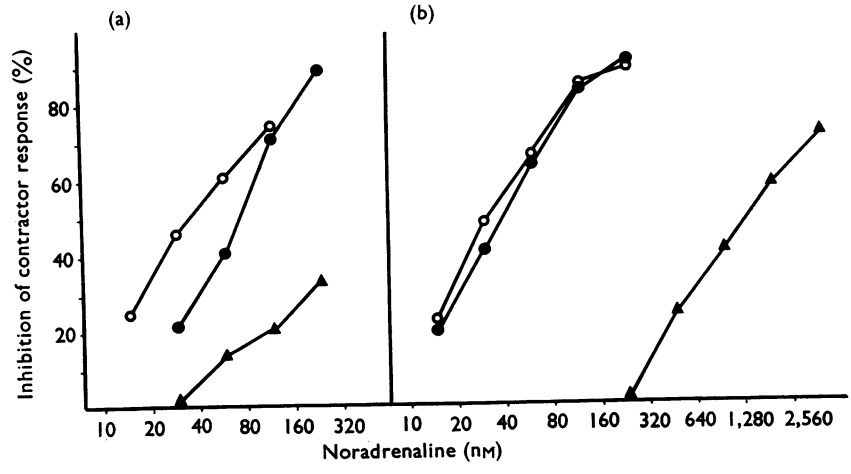

FIG. 8. Inhibitory effect of noradrenaline on the responses of the ileum to electrical stimulation (a) or to an equiactive concentration of acetylcholine (b) and the antagonist actions of phenoxybenzamine (0·022 μM) and propranolol (0·85 μM). Noradrenaline alone (●); after 135 (a) or 160 (b) min exposure to phenoxybenzamine (○); after 200 (a) or 230 (b) min exposure to phenoxybenzamine and 10 (a) and 40 (b) min exposure to propranolol (▲). Note that in this experiment phenoxybenzamine potentiated the inhibitory effect of noradrenaline on the responses to electrical stimulation.

caused by submaximal concentrations of acetylcholine. It was found that adrenaline reduced the responses to electrical stimulation more readily than the responses to acetylcholine (Fig. 7). On the other hand, noradrenaline, which in this preparation has a greater effect on β-adrenoceptors and a smaller effect on α-adrenoceptors than adrenaline, was as effective in inhibiting the responses to acetylcholine as those to electrical stimulation (Fig. 8). Isoprenaline, which depressed the responses to electrical stimulation only partly, could abolish the responses to acetylcholine (Fig. 9). The number of experiments was insufficient to determine the relative potencies, but the results agree with the findings of Wilson (1964), namely, that isoprenaline had the highest activity and adrenaline the lowest.

Further evidence for the view that the actions of catecholamines on the acetylcholine-induced contractions were different from those evoked by electrical stimulation, was obtained from an analysis of the effects of drugs blocking α-adrenoceptors and β-adrenoceptors. The inhibitory effects on the acetylcholine-induced contractions were never affected by phenoxybenzamine but readily antagonized by propranolol (Figs. 7–9).

Effects of catecholamines on acetylcholine release

The results obtained so far indicate that the receptors on the smooth muscle cells are β-adrenoceptors and are activated by isoprenaline, noradrenaline and adrenaline. In addition, there are α-adrenoceptors which are of importance for the depression of the electrically evoked twitch and are activated by adrenaline and, to a lesser extent, by noradrenaline. If this interpretation is correct, these inhibitory effects of adrenaline and noradrenaline are likely to be prejunctional and caused by a depression of the release of the transmitter, acetylcholine.

Our results confirm and extend the findings of Watt (1966), Vizi (1968) and Paton & Vizi (1969). The depressant effects of adrenaline and noradrenaline on the spontaneous and the evoked outputs of acetylcholine are shown in Figs. 10–13. The

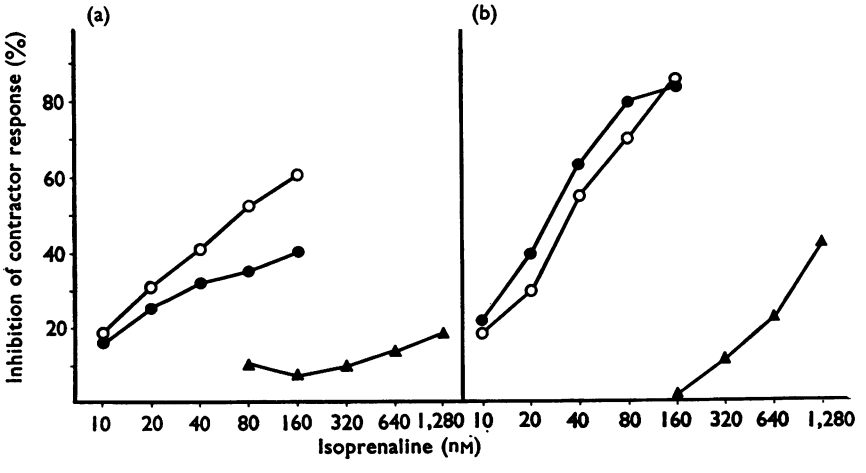

FIG. 9. Inhibitory effect of isoprenaline on the responses of the ileum to electrical stimulation (a) or to an equiactive concentration of acetylcholine (b) and the antagonist actions of phenoxybenzamine (0·022 μM) and propranolol (0·85 μM). Isoprenaline alone (●); after 120 (a) or 150 (b) min exposure to phenoxybenzamine (○); after 190 (a) or 220 (b) min exposure to phenoxybenzamine and 10 (a) or 40 (b) min exposure to propranolol (▲).

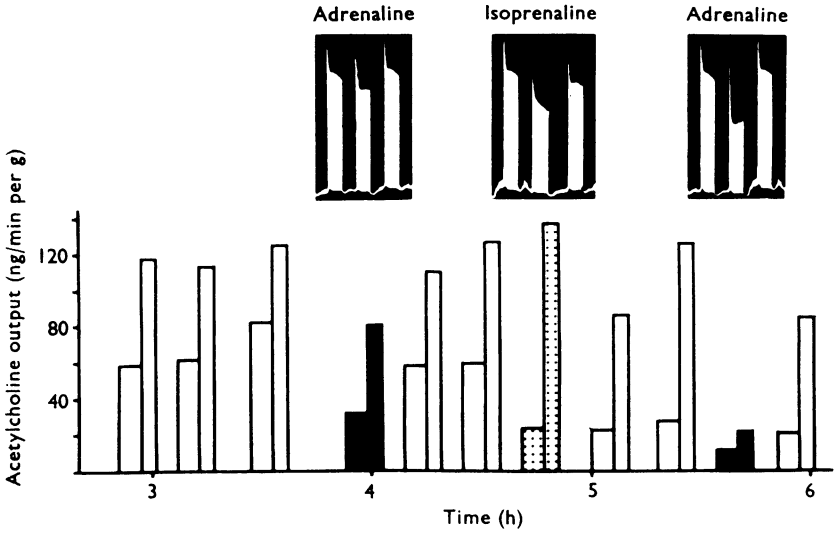

FIG. 10. Comparison of the effects of adrenaline and isoprenaline on the responses of the myenteric plexus-longitudinal muscle preparation to electrical stimulation and on the release of acetylcholine. Abscissa, time (h) since setting up the preparation; ordinate, acetylcholine output (ng/min per g tissue). The first of each pair of columns shows the spontaneous output and the second the output evoked by electrical stimulation (20/min). Clear columns, controls; black columns, during exposure to adrenaline (62·5 nM at 4 h and 125 nM at 5 h 40 min); shaded columns, exposure to isoprenaline (1 μM). The inserts show the effects of 2 min periods of stimulation before the addition of physostigmine to the bath; adrenaline or isoprenaline was present during the second period of stimulation in each set of three periods.

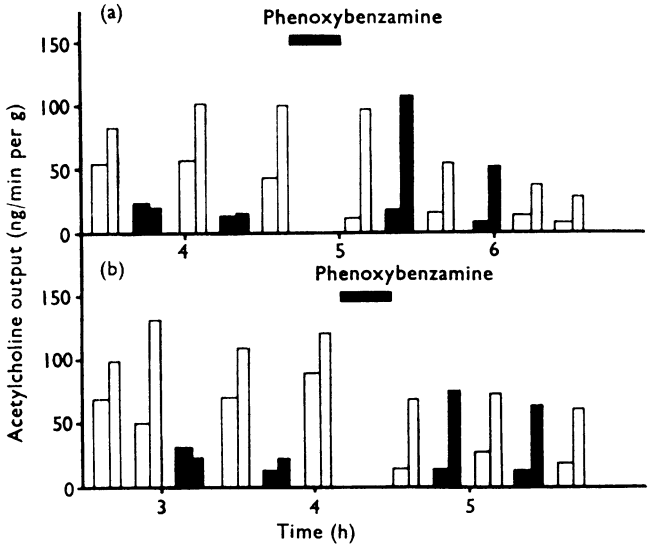

FIG. 11. Effects of adrenaline (a) and noradrenaline (b) on the release of acetylcholine from the myenteric plexus-longitudinal muscle preparation. Abscissa, time (h) since setting up preparation; ordinate, output of acetylcholine (ng/min per g tissue). The first of each pair of columns shows the spontaneous output and the second the output evoked by electrical stimulation (20/min). Clear columns, controls; black columns, during exposure to (a) adrenaline (0·64 μM) or (b) noradrenaline (3·2 μM). Horizontal bars, exposure to phenoxybenzamine (3 μM).

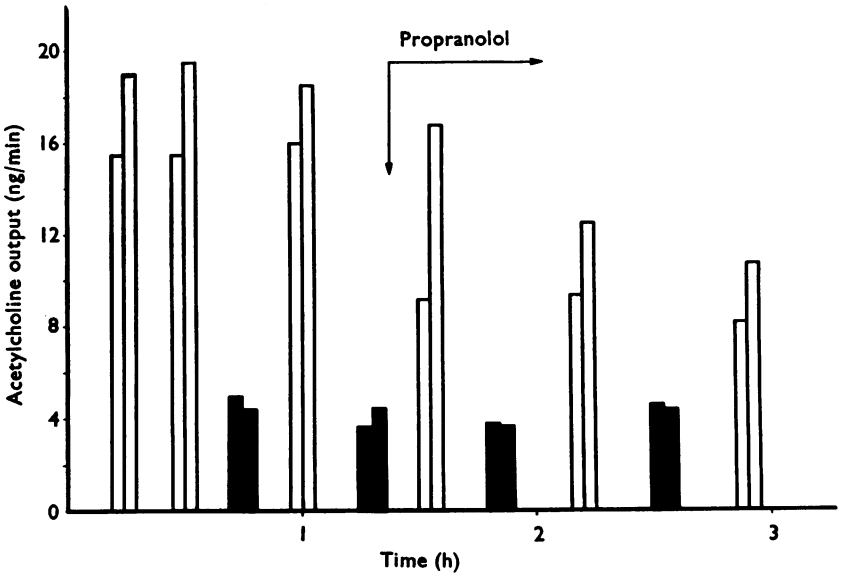

FIG. 12. Absence of an effect of propranolol on the depression by adrenaline of the release of acetylcholine from a segment of ileum. Abscissa, time (h) since the beginning of the acetylcholine assays; ordinate, acetylcholine output (ng/min). The first of each pair of columns shows the spontaneous output and the second the output evoked by electrical stimulation (20/min). Clear columns, controls; black columns, during exposure to adrenaline (0·28 μM). From the arrow until the end of the experiment propranolol (0·85 μM) was present.

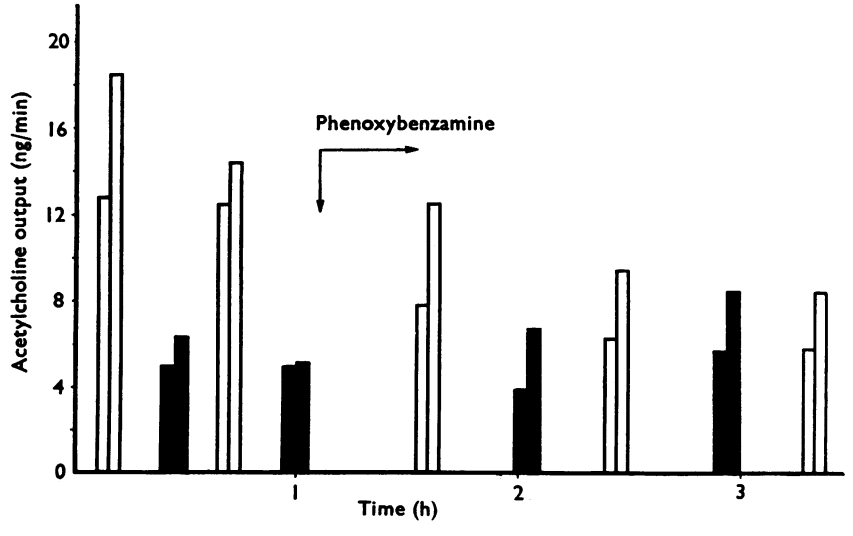

FIG. 13. Effect of phenoxybenzamine on the depression by adrenaline of the release of acetylcholine from a segment of ileum. Abscissa, time (h) since beginning of the acetylcholine assays; ordinate, acetylcholine output (ng/min). The first of each pair of columns shows the spontaneous output and the second the output evoked by electrical stimulation (20/min). Clear columns, controls; black columns, during exposure to adrenaline (0·28 μM). From the arrow until the end of the experiment phenoxybenzamine (0·015 μM) was present.

concentrations of adrenaline used varied between 60 and 640 nM (12–130 ng/ml); with the lowest concentration the inhibition was small (Fig. 10) and with the highest concentration it was almost complete (Fig. 11). Noradrenaline in a concentration of 3·2 μM (0·6 μg/ml) caused almost maximal inhibition (Fig. 11).

On the other hand, 1 μM isoprenaline, which depressed the twitch by about 30%, did not inhibit the evoked acetylcholine output. This absence of any effect was in contrast to the effect of 62·5 nM adrenaline which, although causing a smaller depression of the twitch, depressed acetylcholine release by almost 25% (Fig. 10). Isoprenaline did not usually affect the spontaneous release of acetylcholine; the apparent effect in Fig. 10 was probably due to a decline in the output with time since there was no recovery after washing out of isoprenaline. Further evidence that β-adrenoceptors are not involved in the depression of acetylcholine release by catecholamines was obtained from the observation that propranolol did not antagonize the depressant effect of adrenaline on either the spontaneous or the evoked release of acetylcholine (Fig. 12).

The experiments with phenoxybenzamine were sometimes complicated by the fact that this and other α-adrenoceptor blocking drugs tended to diminish both spontaneous and evoked acetylcholine release. There was, however, no doubt that, in intestinal segments and in the plexus-longitudinal muscle preparation, phenoxybenzamine antagonized the depressant actions of both adrenaline and noradrenaline. This effect was obtained whether a high concentration of phenoxybenzamine (3 μM) was used for 20 min (Fig. 11) or a low concentration (0·015 μM) for 2 h (Fig. 13).

These observations therefore confirm the view formulated on the basis of observations of the actions of the catecholamines and their antagonists on the mechanical responses, namely, that the α-adrenoceptors are involved in the modulation of transmitter release.

Discussion

The results obtained in this investigation confirm the findings of Ahlquist & Levy (1959) and Furchgott (1960) that, in the small intestine, there are α and β-adrenoceptors, both of which have inhibitory effects on the contractions of the longitudinal smooth muscle.

The acetylcholine-induced contraction of the longitudinal muscle is depressed by adrenaline, noradrenaline and isoprenaline and these inhibitory effects are not affected by phenoxybenzamine but are antagonized by propranolol. Since in these experiments the bath fluid contained hexamethonium and therefore acetylcholine acted directly on the smooth muscle, it may be concluded that β-adrenoceptors, but no α-adrenoceptors, are present on the cells of the longitudinal muscle of the guinea-pig ileum. There is evidence, however, that in the guinea-pig taenia coli and the rabbit small intestine, the smooth cells also have α-adrenoceptors (Jenkinson & Morton, 1967; Bowman & Hall, 1970).

The inhibition of the responses of the longitudinal muscle to electrical stimulation is more complex in that α-adrenoceptors also are involved. Since activation of the α-adrenoceptors results in a decrease in acetylcholine release and Paton & Zar (1968) have shown that this release originates from nervous tissue only, it is likely that these adrenoceptors are situated on neuronal elements. This view is supported by the findings of McDougal & West (1952) and Lum, Kermani & Heilman (1966)

that α-adrenoceptors are less resistant than β-adrenoceptors to damage by storage of the intestine at 6°–8° C for 24–72 h. There is no evidence that β-adrenoceptors play a part in the depression of acetylcholine release by adrenaline or noradrenaline because, first, isoprenaline does not affect acetylcholine output and, second, the β-adrenoceptor blocking drug, propranolol, does not antagonize the depressant effect of adrenaline on acetylcholine release.

The depression of responses of the longitudinal muscle by isoprenaline is mediated solely by β-adrenoceptors and the depression by adrenaline almost wholly by α-adrenoceptors ; the inhibitory effect of noradrenaline is caused by activation of both receptors, the β-adrenoceptors on the muscle being more involved than the neuronal α-adrenoceptors. Although adrenaline can act on both receptors, the inhibition of the electrically evoked contraction is mediated almost exclusively by α-adrenoceptors because the sensitivity of the α-adrenoceptors for adrenaline is much greater than that of the β-adrenoceptors. Similar findings have been obtained in the guinea-pig colon by Beani, Bianchi & Crema (1969).

An interesting observation is the enhancement by phenoxybenzamine of the inhibitory action of isoprenaline on the responses to electrical stimulation. This effect does not seem to be due to a facilitation of the activation of the β-adrenoceptors by isoprenaline, since there is no potentiation of the inhibition by isoprenaline of the acetylcholine-induced contraction. Since phenoxybenzamine depresses acetylcholine output, the enhancement of the effect of isoprenaline may be due to a reduction in the safety factor of transmission. Sometimes a similar potentiation has been observed with noradrenaline, which activates β-adrenoceptors rather than α-adrenoceptors, but never with adrenaline, the inhibitory action of which is antagonized by phenoxybenzamine. Earlier observations with Dibenamine (Furchgott, 1960) and phenoxybenzamine (Stafford, 1963) could possibly be interpreted in the same way. A similar potentiation of the inhibitory effect of adrenaline is observed with the myenteric plexus-longitudinal muscle preparation when the responses to electrical stimulation are reduced by choline deficiency of the bath fluid. Another possibility is the prevention by phenoxybenzamine of the uptake of isoprenaline by storage sites ; it is unlikely that α-adrenoceptors take up isoprenaline since there is no interaction between adrenaline and isoprenaline (Watt, 1966). At present, it is impossible to give an explanation for the observation that isoprenaline can inhibit completely the acetylcholine-induced contraction but depresses the response to electrical stimulation usually by 20–40% and only occasionally by 60%.

The site of the depressant action of adrenaline and noradrenaline on the output of acetylcholine is not finally established. Since the effect of adrenaline is much greater at low than at high frequencies of stimulation (Cowie, Kosterlitz & Watt, 1968 ; Kosterlitz & Cowie, 1968 ; Vizi, 1968 ; Paton & Vizi, 1969), it is likely that diminished release rather than inhibition of synthesis is the cause of the decreased output of acetylcholine ; adrenaline or noradrenaline do not affect the acetylcholine content of the intestine (Schaumann, 1958). The absence of an inhibitory action of isoprenaline, the lack of an antagonistic effect of propranolol and the antagonistic effect of phenoxybenzamine support the view that the depressant action of adrenaline on acetylcholine release is mediated by α-adrenoceptors. Watt (1966), Vizi (1968) and Paton & Vizi (1969) arrived at a similar conclusion.

From the experiments on the release of acetylcholine it is impossible to decide whether the action of adrenaline is on nerve cells, on axons, on nerve terminals in

contact with nerve cells or on nerve terminals innervating smooth muscle cells. Histochemical evidence has shown that, whereas the longitudinal muscle layer of the guinea-pig ileum has only a few adrenergic fibres, the intramural ganglion cells are surrounded by a network of adrenergic fibres (Norberg, 1964 ; Hamberger, Norberg & Ungerstedt, 1965 ; Jacobowitz, 1965). These histochemical findings suggest that the action of the adrenergic transmitter may be mainly on the intramural nerve cells. However, the analysis of the effects of exogenous adrenaline on the responses of the longitudinal muscle to electrical stimulation indicates that the site of action is more likely to be at the nerve terminals innervating the muscle cells, since the effect is seen when the experiments are carried out in the presence of hexamethonium and the stimuli are supramaximal—that is, they stimulate all parts of the neurone innervating the muscle.

Two factors are of importance for the assessment of the physiological significance of the inhibitory action of adrenaline, namely, the lack of adrenergic innervation of the longitudinal muscle and the great sensitivity of the neuronal α-adrenoceptors to adrenaline. This combination would suggest that *in vivo* the α-adrenoceptors may be activated by circulating adrenaline secreted by the adrenal medulla. There is evidence in favour of this view ; Kock (1959) has shown that, in the cat, the reflex inhibition of the intestine elicited by stimulation of somatic nerves depends on the presence of the adrenal medulla.

The findings that the α-adrenoceptors are more sensitive to adrenaline than to noradrenaline and that the order of sensitivity of the β-adrenoceptors is isoprenaline $>$noradrenaline$>$adrenaline agree with the observations of MacDougal & West (1952) and Härtfelder *et al.* (1958) for α-adrenoceptors and of Wilson (1964) for β-adrenoceptors. Furchgott (1960) found a similar order of potencies for the rabbit duodenum.

This investigation was supported in part by U.S. Public Health Service Grant NB 03026, National Institute of Neurological Diseases and Stroke. We thank Mr. S. Bourner for skilful technical assistance. Gifts of hexamethonium bromide (May & Baker Ltd.) and propranolol hydrochloride (Imperial Chemical Industries Ltd.) are gratefully acknowledged.

REFERENCES

AHLQUIST, R. P. & LEVY, B. (1959). Adrenergic receptive mechanism of canine ileum. *J. Pharmac. exp. Ther.*, **127**, 146–149.

AMBACHE, N. (1954). Separation of the longitudinal muscle of the rabbit's ileum as a broad sheet. *J. Physiol., Lond.*, **125**, 53–55*P*.

BEANI, L., BIANCHI, C. & CREMA, A. (1969). The effect of catecholamines and sympathetic stimulation on the release of acetylcholine from the guinea-pig colon. *Br. J. Pharmac.*, **36**, 1–17.

BENFEY, B. G. & GRILLO, S. A. (1963). Antagonism of acetylcholine by adrenaline antagonists. *Br. J. Pharmac. Chemother.*, **20**, 528–533.

BOWMAN, W. C. & HALL, M. T. (1970). Inhibition of rabbit intestine mediated by α- and β-adrenoceptive receptors. *Br. J. Pharmac.*, **38**, 399–415.

BOYD, H., BURNSTOCK, G., CAMPBELL, G., JOWETT, A., O'SHEA, J. & WOOD, M. (1963). The cholinergic blocking action of adrenergic blocking agents in the pharmacological analysis of autonomic innervation. *Br. J. Pharmac. Chemother.*, **20**, 418–435.

COWIE, A. L., KOSTERLITZ, H. W. & WATT, A. J. (1968). Mode of action of morphine-like drugs on autonomic neuro-effectors. *Nature, Lond.*, **220**, 1040–1042.

FURCHGOTT, R. F. (1960). Receptors for sympathetic amines. In *Adrenergic Mechanisms, Ciba Foundation Symposium*, ed. Vane, J. R., Wolstenholme, G. E. W. & O'Connor, C. M. London: J. & A. Churchill.

GYANG, E. A. & KOSTERLITZ, H. W. (1966). Agonist and antagonist actions of morphine-like drugs on the guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.*, **27**, 514–527.

HÄRTFELDER, G., KUSCHINSKY, G. & MOSLER, K. H. (1958). Der Antagonismus verschiedener Sympatholytica gegenüber dem inhibitorischen Adrenaline- oder Noradrenalineffekt am elektrisch gereizten Meerschweinchenileum. *Arch. exp. Path. Pharmak.*, **234**, 91–101.

HAMBERGER, B., NORBERG, K. A. & UNGERSTEDT, U. (1965). Adrenergic synaptic terminals in autonomic ganglia. *Acta physiol. scand.*, **64**, 285–286.

JACOBOWITZ, D. (1965). Histochemical studies of the autonomic innervation of the gut. *J. Pharmac. exp. Ther.*, **149**, 358–364.

JENKINSON, D. H. & MORTON, I. K. M. (1967). Adrenergic blocking drugs as tools in the study of the action of catecholamines on the smooth muscle membrane. *Ann. N.Y. Acad. Sci.*, **139**, 762–771.

KOCK, N. G. (1959). An experimental analysis of mechanisms engaged in reflex inhibition of intestinal motility. *Acta physiol. scand.*, suppl., **164**, 1–54.

KOSTERLITZ, H. W. & COWIE, A. L. (1968). Some aspects of transmission at the nerve-smooth muscle junction in the longitudinal muscle of the guinea-pig ileum. *Am. J. dig. Dis.*, N.S., **13**, 415–417.

KOSTERLITZ, H. W. & LYDON, R. J. (1968). The actions of choline, adrenaline and phenoxybenzamine on the innervated longitudinal muscle strip of the guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **32**, 422*P*.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1957). Inhibition of the peristaltic reflex of the isolated guinea-pig ileum. *J. Physiol., Lond.*, **136**, 249–262.

KOSTERLITZ, H. W. & WATT, A. J. (1965). Adrenergic receptors in the guinea-pig ileum. *J. Physiol., Lond.*, **177**, 11–12*P*.

LUM, B. K. B., KERMANI, M. H. & HEILMAN, R. D. (1966). Intestinal relaxation produced by sympathomimetic amines in the isolated rabbit jejunum; selective inhibition by adrenergic blocking agents and by cold storage. *J. Pharmac. exp. Ther.*, **154**, 463–471.

MCDOUGAL, M. D. & WEST, G. B. (1952). The action of isoprenaline on intestinal muscle. *Archs int. Pharmacodyn. Thér.*, **90**, 86–92.

MCDOUGAL, M. D. & WEST, G. B. (1954). The inhibition of the peristaltic reflex by sympathomimetic amines. *Br. J. Pharmac. Chemother.*, **9**, 131–137.

MUNRO, A. F. (1953). Effect of autonomic drugs on the responses of isolated preparations from the guinea-pig intestine to electrical stimulation. *J. Physiol., Lond.*, **120**, 41–52.

NORBERG, K. A. (1964). Adrenergic innervation of the intestinal wall studied by fluorescence microscopy. *Int. J. Neuropharmac.*, **3**, 379–382.

PATON, W. D. M. (1955). The response of the guinea-pig to electrical stimulation by coaxial electrodes. *J. Physiol., Lond.*, **127**, 40–41*P*.

PATON, W. D. M. (1957). A pendulum auxotonic lever. *J. Physiol. Lond.*, **137**, 35–36*P*.

PATON, W. D. M. & VIZI, E. S. (1969). The inhibitory action of noradrenaline and adrenaline on acetylcholine by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmac.*, **35**, 10–28.

PATON, W. D. M. & ZAR, M. A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strip. *J. Physiol., Lond.*, **194**, 13–33.

RANG, H. P. (1964). Stimulant actions of volatile anaesthetics on smooth muscle. *Br. J. Pharmac. Chemother.*, **22**, 356–365.

SCHAUMANN, W. (1958). Zusammenhänge zwischen der Wirkung der Analgetica und Sympathicomimetica auf den Meerschweinchen-Dünndarm. *Arch. exp. Path. Pharmak.*, **233**, 112–124.

STAFFORD, A. (1963). Potentiation of some catechol amines by phenoxybenzamine, guanethidine and cocaine. *Br. J. Pharmac. Chemother.*, **21**, 361–367.

VIZI, E. S. (1968). The inhibitory action of noradrenaline and adrenaline on release of acetylcholine from guinea-pig ileum longitudinal strips. *Arch. exp. Path. Pharmak.*, **259**, 199–200.

WATT, A. J. (1966). Inhibitory mechanisms in guinea-pig isolated ileum. Ph.D. thesis, Univ. Aberdeen.

WILSON, A. B. (1964). Beta sympathetic inhibitory receptors in the small intestine of the guinea-pig. *J. Pharm. Pharmac.*, **16**, 834–835.

(*Received January* 23, 1970)