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Inhibition of rabbit intestine mediated by α- and β-adrenoceptors

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Summary

1. The effects of some α- and β-adrenoceptor agonists and antagonists were studied on isolated segments of rabbit intestine in an attempt to characterize the two types of inhibitory response produced by sympathomimetic amines.

2. Phenylephrine, an α-adrenoceptor agonist, produced an inhibition of rapid onset, from which recovery occurred despite the continued presence of the drug. On washout there was an overshoot in contraction height. Isoprenaline, a β-adrenoceptor agonist, produced an inhibition of slow onset which was maintained throughout the presence of the drug and there was no overshoot on washout.

3. Adrenaline resembled phenylephrine more closely than it resembled isoprenaline, in that it showed more affinity for α-adrenoceptors, whereas noradrenaline, and the transmitter released on periarterial nerve stimulation, behaved more like isoprenaline, although both types of receptor were affected.

4. Adenosine-5′-triphosphate produced an inhibition resembling that produced by an α-adrenoceptor agonist, whereas the dibutyryl analogue of cyclic adenosine 3′,5′-monophosphate (cyclic 3′,5′-AMP) produced an inhibition resembling that produced by a β-adrenoceptor agonist.

5. In critical concentrations theophylline augmented and imidazole inhibited β-adrenoceptor mediated responses, as well as responses to dibutyryl cyclic AMP. However, additional actions of theophylline and imidazole were also demonstrated.

6. Responses mediated by α-adrenoceptors, but not those mediated by β-adrenoceptors, were blocked by membrane stabilizers, quinidine being the most potent of those studied.

7. The results are discussed in relation to the possible mechanisms of action of α- and β-adrenoceptor agonists.

Introduction

Activation of either α- or β-adrenoceptors results in inhibition of intestinal movements or tone in the rabbit (Furchgott, 1960), the guinea-pig (Brody & Diamond, 1967), the dog (Levy & Ahlquist, 1967) and man (Bucknell & Whitney, 1964). In the experiments described in this paper, an attempt has been made to characterize these two types of inhibition more fully in the isolated intestine of the rabbit.

Methods

Isolated segments of small intestine (duodenum, jejunum or ileum) from Dutch strain rabbits of either sex were suspended in Krebs solution (NaCl 118 mM, KCl 4·7 mM, $CaCl_2$ 2·5 mM, $MgCl_2$ 1·2 mM, $NaHCO_3$ 25 mM, NaH_2PO_4 1·1 mM, glucose 5·6 mM) at 37° C and gassed with 5% carbon dioxide in oxygen. Hyoscine hydrobromide (1 μg/ml) was always added to the reservoir of Krebs solution. Pendular movements were recorded on a kymograph with an isotonic frontal writing lever which was loaded with 3 g and which magnified the contractions 8 times, or with an isometric microdynamometer exerting a 3 g load (Ugo Basile Instruments). The large load was applied to prevent development of a high level of background tone in the tissue (Lum, Kermani & Heilman, 1966). Under these conditions, drugs caused an inhibition of pendular movements with little or no effect on the baseline. The periarterial adrenergic nerves were stimulated with rectangular pulses of 0·5 ms duration and of strength greater than that necessary to give a maximal response at the frequency used. Four of the rabbits had been pretreated with reserpine (1 mg/kg intraperitoneally 48 and 96 hr before the experiment). No differences were observed between the responses of the different parts of the intestine, or between isotonic and isometric recording; therefore no distinction is made in the following description.

The drugs used were (−)-adrenaline, (−)-phenylephrine hydrochloride, (−)-noradrenaline hydrochloride, (±)-metanephrine hydrochloride, (±)-normetanephrine hydrochloride, adenosine, sodium adenosine-5′-monophosphate (AMP), sodium adenosine-5′-diphosphate (ADP), sodium adenosine-5′-triphosphate (ATP), creatine phosphate (CP), ribose-5′-phosphate, guanine, guanosine, guanosine triphosphate, inosine, inosine triphosphate, uracil, uridine, uridine triphosphate, hypoxanthine, imidazole (Sigma); (−)-isoprenaline bitartrate (Wyeth); theophylline, quinidine base, reserpine, hyoscine hydrobromide (British Drug Houses), cyclic adenosine 3′,5′-monophosphate (cyclic AMP), dibutyryl cyclic adenosine 3′,5′-monophosphate sodium (dibutyryl cyclic AMP) (Boehringer-Ingelheim); tetrodotoxin (Sankyo); guanethidine sulphate, phentolamine mesylate (Ciba); propranolol hydrochloride, procaine hydrochloride (Imperial Chemical Industries); MJ1999 hydrochloride (Mead Johnson); methysergide (Sandoz); mepyramine maleate (May & Baker); lignocaine hydrochloride, cocaine hydrochloride (Macfarlane Smith). Concentrations given in the text are the final bath concentrations. Where concentrations are given by weight, they refer to the base unless otherwise stated. Stock solutions were prepared in distilled water or in dilute HCl and were diluted with 0·9% NaCl solution before use. Where necessary drug solutions were adjusted to pH 7 before addition to the organ bath.

Results

Sympathomimetic amines

Figure 1a illustrates equivalent submaximal responses of the intestine to (−)-phenylephrine, (−)-adrenaline, (−)-noradrenaline and (−)-isoprenaline, left in contact with the tissue for 1 min, and to periarterial nerve stimulation (15 Hz for 30 s). Inhibition produced by phenylephrine (0·6 μM, 0·1 μg/ml) was abrupt in onset but was not maintained at full intensity. Instead the pendular movements

always increased in amplitude towards the control level despite the continued presence of the drug, and in thirty-two out of forty experiments there was a temporary increase in the amplitude of contractions over the control level on washing the tissue (Fig. 1a). Responses to larger or smaller doses of phenylephrine differed quantitatively but not qualitatively. In contrast, an equivalent degree of inhibition produced by isoprenaline (0·047 μM, 10 ng/ml) was relatively slow to reach its maximum and was then maintained throughout the presence of the drug (Fig. 1a). The time from addition of isoprenaline (0·047 μM) to the maximum depression was measured in ten experiments and was found to be 35 ± 5 s. On washing out the isoprenaline, the pendular movements returned slowly to the control level but did not exceed it (Fig. 1a). The shapes of the responses to adrenaline and noradrenaline fell between those to phenylephrine and isoprenaline. The response to adrenaline shown in Fig. 1a resembles that to phenylephrine, whereas that to noradrenaline is similar to the isoprenaline response except that it is more rapid in onset. In other experiments the responses to adrenaline and noradrenaline were more intermediate in character. In the experiment illustrated by Fig. 1b, concentrations of the amines necessary to give equal degrees of inhibition were again selected, but this time left in contact with the tissue for 5 min. In the case of phenylephrine, the amplitudes of the contractions recovered to the control level within 2 min, where they were maintained until the drug was washed out, at which point a brief overshoot occurred. As usual, the response to isoprenaline was maintained at full intensity. In this experiment the intermediate characters of the responses to adrenaline and noradrenaline are more obvious, but, in general, adrenaline behaved more like phenyl-

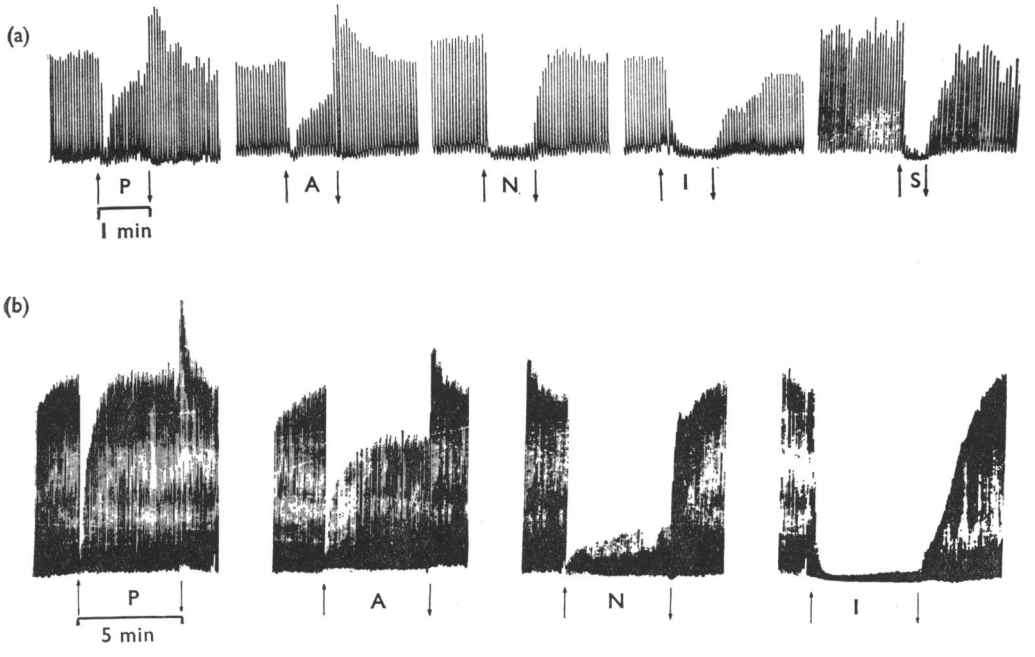

FIG. 1. Responses to sympathomimetic amines and to periarterial nerve stimulation. (a) and (b) are from different experiments. At P, phenylephrine (0·598 μM, 0·1 μg/ml); at A, adrenaline (0·055 μM, 10 ng/ml), at N, noradrenaline (0·118 μM, 20 ng/ml); at I, isoprenaline (0·047 μM, 10 ng/ml) and at S, periarterial nerve stimulation (15 Hz for 30 s). Drugs were added at the first arrow and washed out at the inverted arrow. In (a) drugs were left in contact with the tissue for 1 min. In (b) drugs were left in contact with the tissue for 5 min.

ephrine than like isoprenaline, whereas noradrenaline resembled isoprenaline more closely. Periarterial nerve stimulation always produced a response most closely resembling that to noradrenaline in the same experiment (Fig. 1a).

The responses to the sympathomimetic amines or to periarterial nerve stimulation were not changed by the previous addition of the MAO inhibitor, iproniazid (30 μg/ml), nor of the COMT inhibitor pyrogallol (40 μg/ml). Nor were the effects of the added amines changed by the previous addition of cocaine (0·5 μg/ml), and the effect of periarterial nerve stimulation was only very slightly augmented. Other workers have also commented on the negligible ability of cocaine to augment responses to nerve stimulation in the rabbit intestine (Roszkowski & Koelle, 1960 ; Stafford, 1963). Metanephrine and normetanephrine were completely without effect in concentrations exceeding the effective concentrations of the sympathomimetic amines, although in very large amounts (20 μg/ml) they produced effects similar to those produced by much smaller doses of phenylephrine. These results indicate that the different rates of recovery from the inhibitory effects of the amines were probably not related to metabolism or uptake.

Responses to the sympathomimetic amines were unaffected by guanethidine (2 μg/ml) or by tetrodotoxin (0·1 μg/ml), although both of these substances abolished

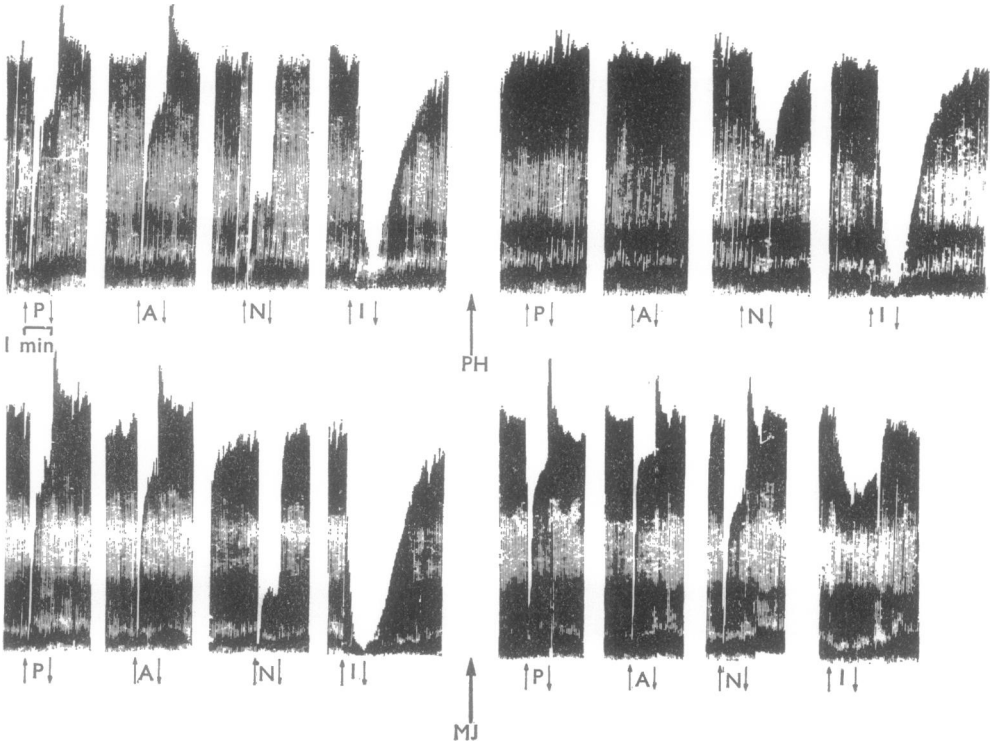

FIG. 2. Effects of adrenoceptor blocking drugs. At P, phenylephrine (0·598 μM); at A, adrenaline (0·055 μM); at N, noradrenaline (0·118 μM) and at I, isoprenaline (0·047 μM). Control responses are shown on the left. Responses on the right of the upper row were recorded in the presence of phentolamine 0·2 μg/ml (PH). Responses on the right of the lower row recorded in the presence of MJ1999 20 μg/ml (MJ). Phentolamine blocked responses to phenylephrine and adrenaline and reduced that to noradrenaline. MJ1999 reduced the response to isoprenaline (a larger dose completely blocked it) and converted the noradrenaline response to one resembling that to phenylephrine.

the effects of periarterial nerve stimulation. In intestine taken from rabbits pretreated with reserpine, responses to periarterial nerve stimulation were absent, but responses to the sympathomimetic amines remained of the same form as in intestine from control animals. These results show that all components of the responses to the amines were independent of nervous mechanisms.

Adrenoceptor blocking drugs

Figure 2 illustrates the effects of phentolamine and MJ1999 on the responses to the amines. In the concentrations used responses to phenylephrine were abolished by the α-adrenoceptor antagonist, phentolamine (0·1–1·0 μg/ml), but were hardly, if at all, changed by the β-adrenoceptor antagonists, MJ1999 (10–50 μg/ml) or propranolol (1 μg/ml). Responses to isoprenaline were reduced or abolished by MJ1999 (10–50 μg/ml) or propranolol (0·1–1 μg/ml) but were unaltered by phentolamine (0·1–2 μg/ml).

Responses to adrenaline (0·055 μM, 10 ng/ml) were usually (Fig. 2) completely blocked by phentolamine (0·2 μg/ml) but were little affected by MJ1999 or propranolol. However, in larger concentrations in the presence of phentolamine, adrenaline (1·0 μM, 0·2 μg/ml) produced an inhibition of slower onset which persisted throughout the presence of the drug and which was not followed by an overshoot on washout; that is, after phentolamine, the response to a larger concentration of adrenaline resembled that to isoprenaline, and this was confirmed by the fact that it was then blocked by MJ1999 or propranolol.

Responses to noradrenaline (0·118 μM, 20 ng/ml) or to periarterial nerve stimulation were reduced in size but never abolished in the presence of either phentolamine or MJ1999. Both blocking agents were necessary to abolish responses to noradrenaline or periarterial nerve stimulation. In the presence of MJ1999 only, the reduced responses to noradrenaline (Fig. 2) or periarterial nerve stimulation resembled in shape those to phenylephrine, whereas in the presence of phentolamine alone they resembled those to isoprenaline (Fig. 2).

Mepyramine, methysergide and hyoscine

The responses to the amines and to periarterial nerve stimulation were unaltered by mepyramine (1 μg/ml) or methysergide (1 μg/ml). These concentrations were ten times greater than those necessary to block responses to histamine or 5-hydroxytryptamine respectively. All experiments were carried out in the presence of hyoscine hydrobromide (1 μg/ml), which was added to the reservoir of physiological salt solution. This concentration of hyoscine was ten times greater than that necessary to block the responses of the preparation to acetylcholine. Innes & Kohli (1969) found that the stimulant action of some sympathomimetic amines on guinea-pig gut was blocked by 5-HT antagonists. The present experiments on rabbit gut, however, showed that no part of the responses to the amines or to periarterial nerve stimulation, including the secondary increase in amplitude of contractions that occurred, particularly with phenylephrine or adrenaline, could be attributed to the release of histamine, 5-hydroxytryptamine or acetylcholine.

Nucleotides, nucleosides and bases

Cyclic AMP in concentrations of 0·1–1·0 mM (0·035–0·35 mg/ml) produced a response resembling that to phenylephrine in about 90% of preparations. That is,

the onset of inhibition was rapid, partial recovery occurred during the continued presence of the compound and overshoot occurred on wash out (see first responses of Figs. 5a and 9b). In the remaining preparations, however, the response to cyclic AMP more closely resembled that to noradrenaline or isoprenaline ; the inhibition was maintained throughout the presence of the compound and no overshoot occurred on washout (see first response of Fig. 5b). The dibutyryl analogue of cyclic AMP always produced a slowly developing inhibition of pendular movement. The onset of the effect occurred 90–120 s after addition of the compound ; it continued to develop after washing the tissue and was slow to recover (Figs. 5c and 7c). The concentrations of dibutyryl cyclic AMP required were about 10 times greater than those of cyclic AMP.

The effects of adenine, adenosine and the adenine nucleotides (AMP, ADP and ATP) were tested. These compounds are known to inhibit the rabbit intestine (Drury, 1936). On a molar basis, ATP was 4 to 5 times more potent than ADP in depressing the amplitude of contractions. Concentrations of 2·0 μM (1·1 μg/ml) of ATP produced a response equivalent to that produced by 0·6 μM (0·1 μg/ml) of phenylephrine. AMP was about 80 times and adenosine about 150 times less potent than ATP. Adenine was without effect. The responses to adenosine and the nucleotides resembled that to phenylephrine in that onset was rapid, recovery began during their presence and overshoot occurred on washout. This is evident in the control responses to ATP in Fig. 8.

The effects of ribose-5-phosphate, guanine, guanosine, guanosine triphosphate, uracil, uridine, uridine triphosphate, hypoxanthine, inosine, inosine triphosphate and creatine phosphate were also tested, but found to be either very weak in their activity compared with adenosine and its derivatives, or inactive.

The effects of cyclic AMP, dibutyrylcyclic AMP, adenosine, AMP, ADP and ATP were unaltered in the presence of phentolamine (1 μg/ml), MJ1999 (50 μg/ml) or propranolol (1 μg/ml). These results with cyclic AMP and adrenoceptor blocking drugs confirm those of Kim, Shulman & Levine (1968).

Theophylline and imidazole

Theophylline (0·1–1 mM) itself produced a decrease in the frequency of spontaneous pendular movements (Figs 3d, 4 and 8b) and with high concentrations both frequency and amplitude were depressed (Fig. 4).

The effect of theophylline on responses to phenylephrine, adrenaline, or ATP depended on the concentration of theophylline added. In the presence of 0·1–0·4 mM (0·02–0·08 mg/ml) of theophylline, the extent of the inhibition produced by phenylephrine (Fig. 3a), adrenaline (Fig. 3b) or ATP (Fig. 8b) was unchanged, but it was then maintained in the presence of the drug and there was no overshoot on washout, even when this had been formerly present. In contrast, higher concentrations of theophylline (0·4–1 mM, 0·08–0·2 mg/ml) reduced or abolished the inhibitory responses produced by phenylephrine (Fig. 4a), adrenaline or ATP (Fig. 8b). In preparations in which the response to cyclic AMP resembled that to phenylephrine, it behaved like phenylephrine in its interaction with theophylline. Thus, with low concentrations of theophylline (0·1–0·4 mM), the inhibitory component of the cyclic AMP response was often prolonged, whereas with high concentrations of theophylline (0·4–1·0 mM), as illustrated in Fig. 5a, the response to cyclic AMP

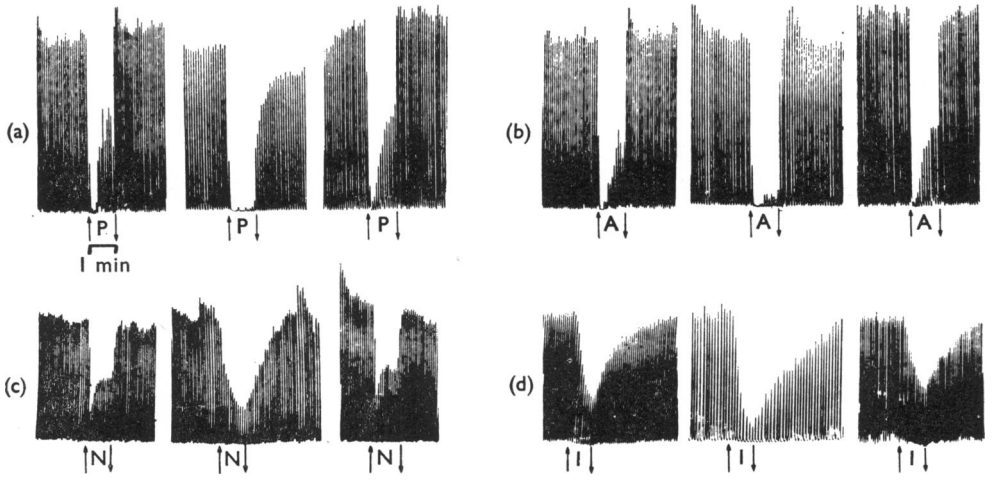

FIG. 3. Effects of theophylline (0·4 mM) on responses to sympathomimetic amines. At P, phenylephrine (0·598 μM); at A, adrenaline (0·165 μM); at N, noradrenaline (0·118 μM); at I, isoprenaline (0·038 μM). The records are from four different experiments. The middle response of each group of three was recorded in the presence of theophylline 0·4 mM. Theophylline prevented the onset of recovery during the presence of phenylephrine, adrenaline and noradrenaline, changed the shape of the noradrenaline response to one resembling that to isoprenaline, and augmented the isoprenaline response. Theophylline itself slowed the frequency of pendular movements and this is particularly evident in the middle response of (d).

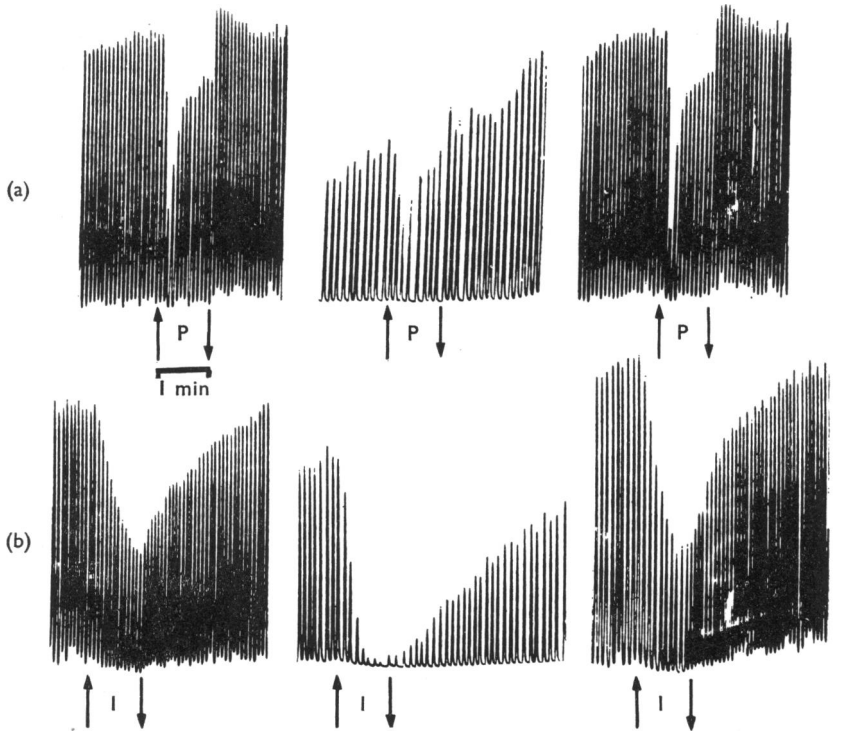

FIG. 4. Effects of a higher concentration of theophylline (0·8 mM) on responses to phenylephrine (0·359 μM at P) and isoprenaline (0·028 μM at I). The middle response in each row was recorded in the presence of theophylline. This large concentration of theophylline reduced the response to phenylephrine but still augmented that to isoprenaline (compare Fig. 3). Theophylline itself depressed and slowed the pendular movements.

was antagonized. Concentrations of theophylline near the middle of the total range (0·1–1·0 mM) modified responses to phenylephrine, adrenaline, ATP or cyclic AMP in different ways in different experiments. Thus, the same intermediate concentration of theophylline (for example 0·4 mM) might prolong an inhibitory response in one preparation, but antagonize it in another.

Responses to isoprenaline (Figs. 3d and 4b), noradrenaline (Fig. 3c), periarterial nerve stimulation, dibutyryl cyclic AMP (Fig. 5c), and to cyclic AMP when it resembled that to noradrenaline (Fig. 5b) were never antagonized but were slightly augmented by all concentrations of theophylline (0·1–1 mM). In the presence of theophylline, responses to noradrenaline (Fig. 3c), to periarterial nerve stimulation or to cyclic AMP (Fig. 5b) then resembled those to isoprenaline even more closely.

In the presence of imidazole (10–50 mM, 0·65–3·25 mg/ml), inhibition produced by phenylephrine was blocked (Fig. 6a) and inhibitory responses to adrenaline (Fig.

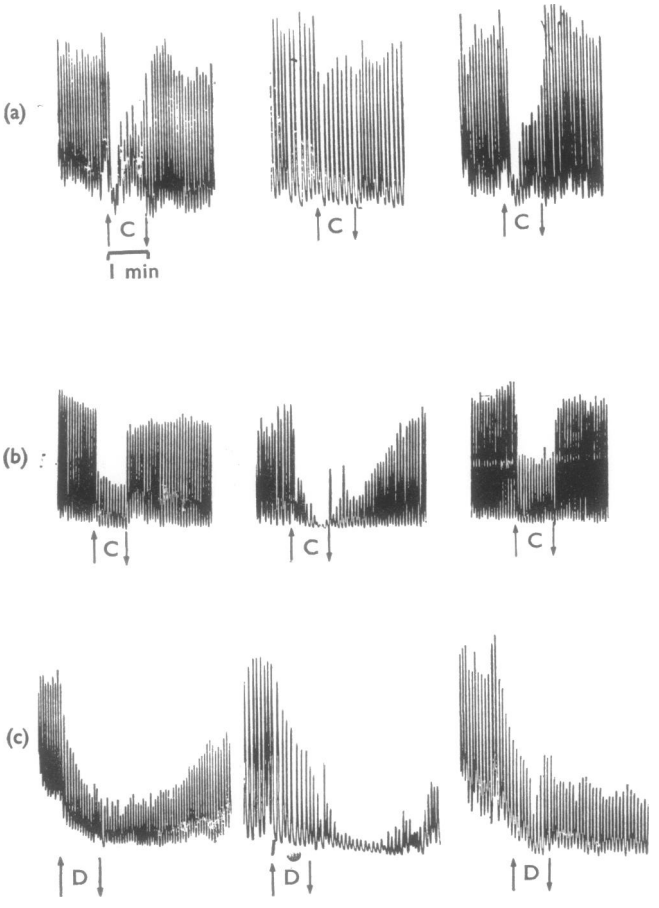

FIG. 5. Effects of theophylline (0·4 mM) on responses to cyclic AMP and dibutyryl cyclic AMP. Responses are from three different experiments. The middle response in each row was recorded in the presence of 0·4 mM theophylline. (a) At C, 0·5 mM cyclic AMP. The control response to cyclic AMP resembled in shape that to phenylephrine and it was antagonized by theophylline. (b) At C, 1·0 mM cyclic AMP. The control response to cyclic AMP resembled that to noradrenaline or isoprenaline and was augmented by theophylline. (c) At D, 8 mM dibutyryl cyclic AMP. The inhibition was slow to develop and persisted after washing out the nucleotide. Theophylline augmented the response.

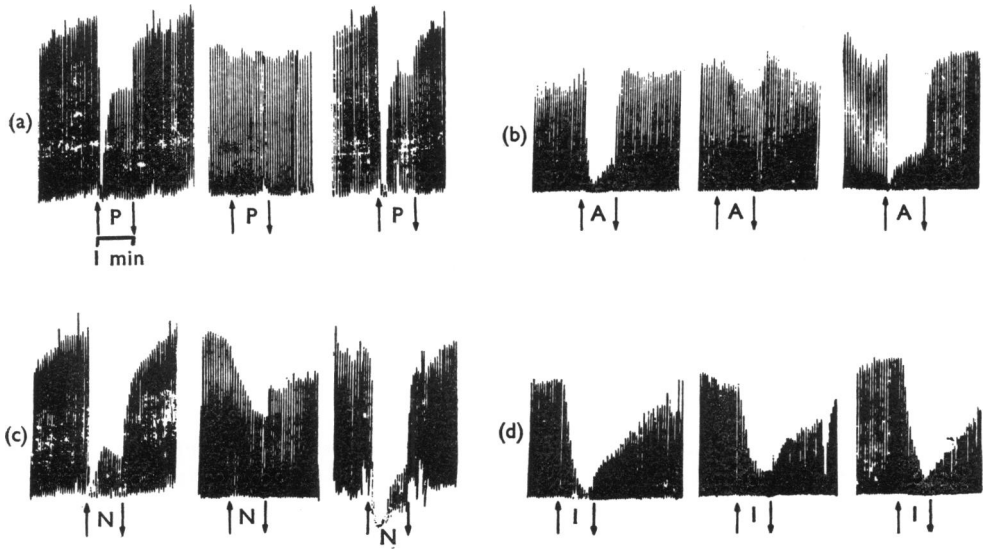

FIG. 6. Effects of imidazole on responses to sympathomimetic amines. Responses are from four different experiments. At P, phenylephrine (1·794 μM); at A, adrenaline (0·218 μM); at N, noradrenaline (0·474 μM); at I, isoprenaline (0·190 μM). The middle response of each group of three was recorded in the presence of 0·06 M imidazole. Imidazole blocked the response to phenylephrine, markedly reduced that to adrenaline, and reduced, to a lesser extent, that to noradrenaline. The response to isoprenaline was little affected.

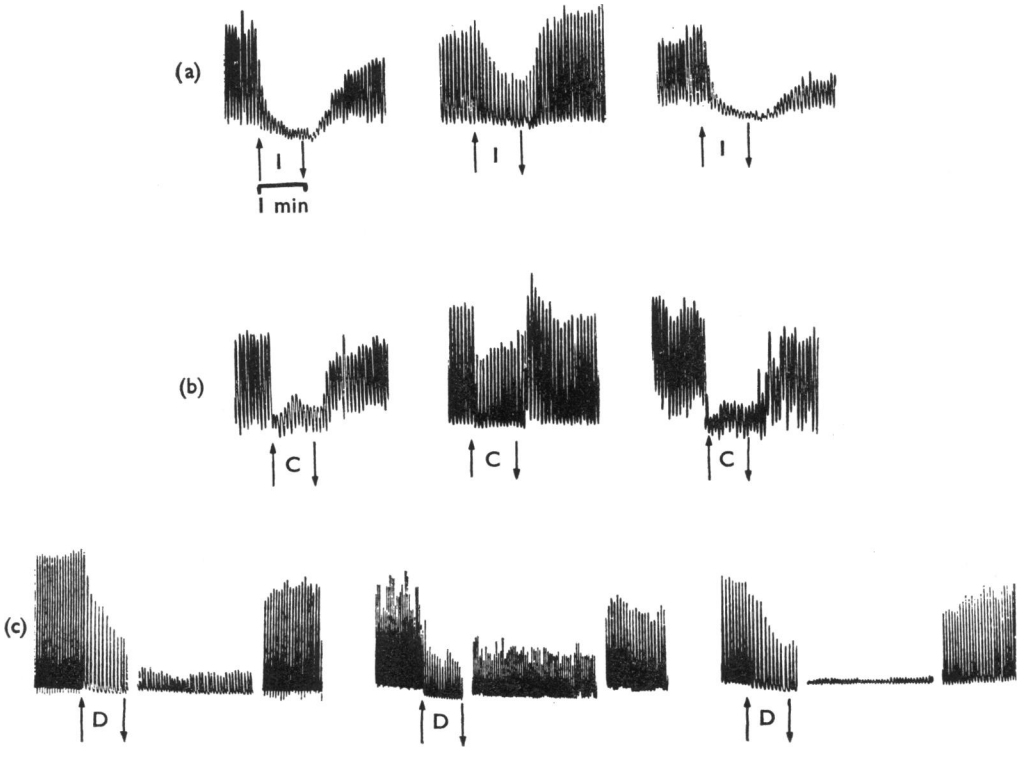

FIG. 7. Effects of a higher concentration of imidazole (0·08 M) on responses to isoprenaline (0·047 μM), cyclic AMP (0·5 mM at C), and dibutyryl cyclic AMP (7 mM at D). Responses are from three different experiments. The middle response in each row was recorded in the presence of imidazole. The two gaps in each of the responses to dibutyryl cyclic AMP correspond to 3·5 min and 6·25 min respectively. This large concentration of imidazole reduced the responses to isoprenaline, to cyclic AMP (which in this experiment, as was usual, resembled that to isoprenaline) and to dibutyryl cyclic AMP.

6b) were much reduced. Those to noradrenaline (Fig. 6c) and periarterial nerve stimulation were also reduced, but to a lesser extent than were those to adrenaline. Responses to isoprenaline (Fig. 6d), ATP (Fig. 8a), cyclic AMP or dibutyryl cyclic AMP were barely altered. Higher concentrations of imidazole (80 mM, 5·2 mg/ml) reduced but did not abolish responses to isoprenaline (Fig. 7a), noradrenaline, periarterial nerve stimulation, ATP (Fig. 8a), cyclic AMP (Fig. 7b) and dibutyryl cyclic AMP (Fig. 7c). With rabbit intestine we were therefore unable to confirm the observations of Bueding, Bülbring, Gercken, Hawkins & Kuriyama (1967) with the taenia coli of the guinea-pig that imidazole in a concentration of 50 mM blocks the response to ATP.

When first applied, imidazole (50 mM) itself produced a large contraction which waned almost to the control level within about 5 min without washing it from the tissue. At the same time imidazole produced a maintained increase in the frequency of the spontaneous contractions when the frequency had been low to start with. Higher concentrations of imidazole (80 mM and above) produced a similar effect when first applied, but the contractions often waned to become constant at a level below that of the control amplitude.

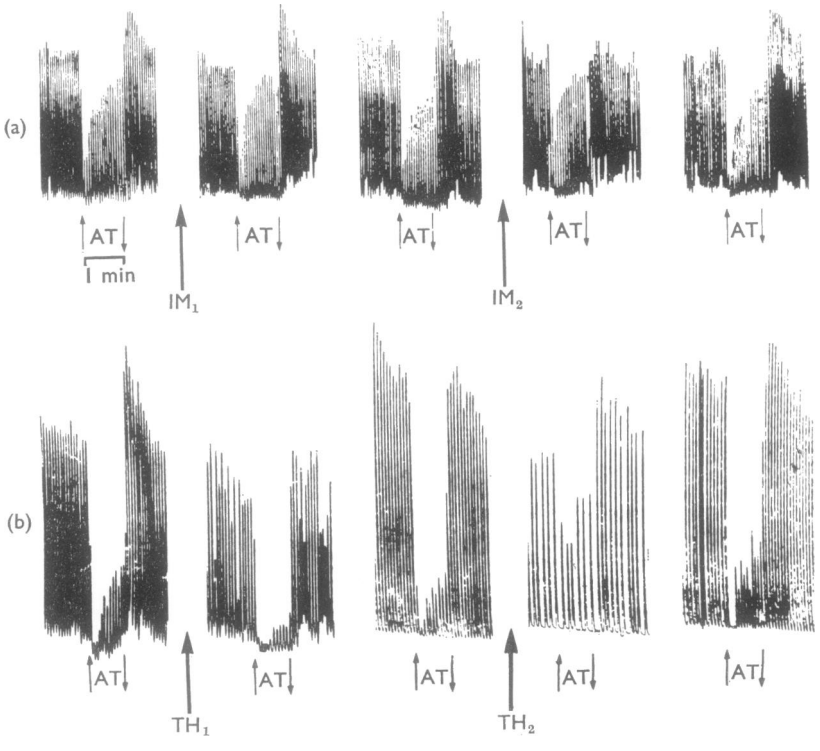

FIG. 8. Effects of imidazole and theophylline on responses to ATP. Responses are from two different experiments. (a) At AT, 0·05 mM ATP ; at IM_1, 0·05 M and at IM_2, 0·075 M imidazole. The smaller concentration of imidazole was without effect on the response to ATP, whereas the larger concentration reduced it. (b) At AT, 0·1 mM ATP ; at TH_1, 0·4 mM and at TH_2, 0·6 mM theophylline. The smaller concentrations of theophylline reduced the degree of recovery occurring during the presence of ATP, whereas the larger concentration of theophylline antagonized the inhibitory effect of ATP.

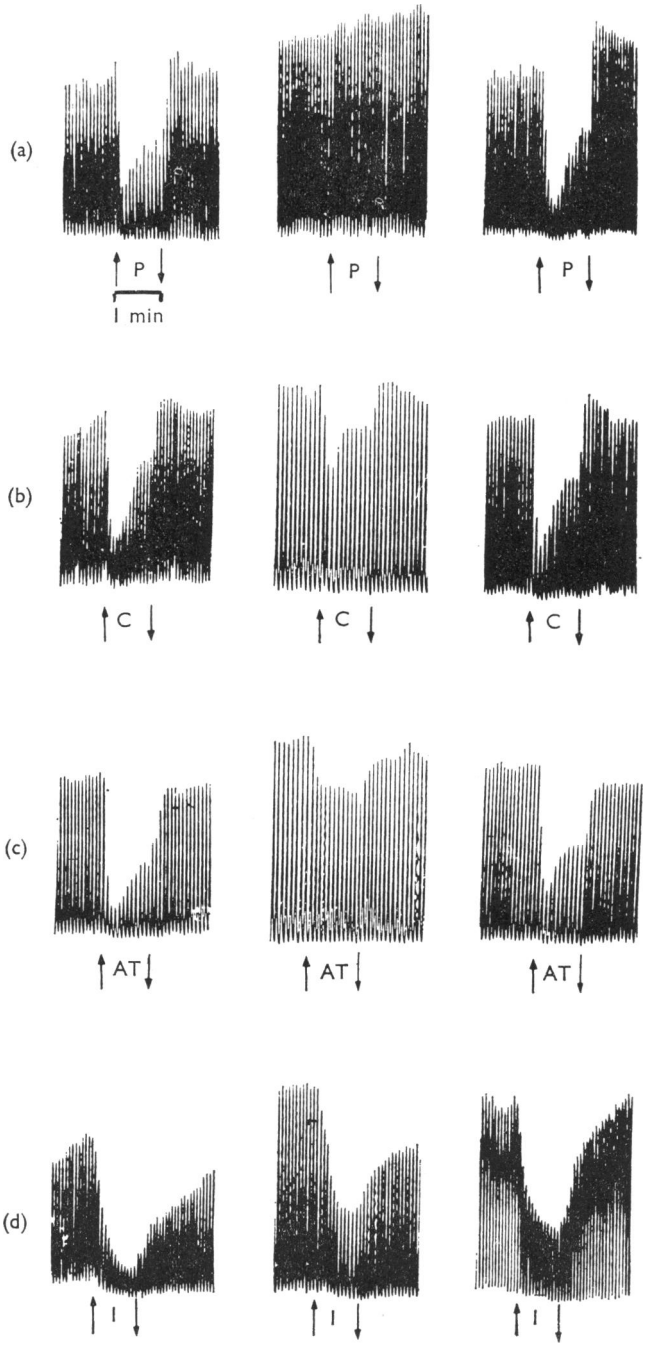

FIG. 9. Effects of quinidine on responses to phenylephrine (0·598 μM at P), cyclic AMP (0·5 mM at C), ATP (0·003 mM at AT) and isoprenaline (0·024 μM at I). Responses are from four different experiments. In each row, the middle response was recorded in the presence of quinidine, 5 μg/ml in (a) and 15 μg/ml in (b), (c) and (d). Quinidine blocked the response to phenylephrine and markedly reduced those to cyclic AMP (when, as in this experiment, it resembled in shape that to phenylephrine) and to ATP. Slightly larger doses of quinidine completely blocked these responses to cyclic AMP and ATP. Quinidine had little effect on the response to isoprenaline. The reduction shown in (d) was the greatest recorded and the response differed little from the second control response obtained after removal of the quinidine.

Membrane-stabilizing drugs

Quinidine (5 μg/ml), procaine (100 μg/ml) and lignocaine (200 μg/ml) completely blocked responses to phenylephrine (0·6 μM). Fig. 9a illustrates this effect of quinidine. Cocaine was ineffective in concentrations up to 200 μg/ml and further increase in the concentration of cocaine abolished the spontaneous pendular movements of the intestine. The three effective antagonists also reduced or blocked responses to ATP (Fig. 9c) and to cyclic AMP (Fig. 9b) when, as was usual, it resembled phenylephrine in its action. The ability of quinidine to antagonize similar responses to adenosine has been described before (Arulappu, 1967). The rank order of potency in blocking the effects of the two nucleotides was the same as that for phenylephrine, but in the same preparation higher concentrations of quinidine than those necessary to block responses to phenylephrine were required. Thus, quinidine 15–20 μg/ml was required to block a response to ATP (2·0 μM) or to cyclic AMP (0·5 mM) equivalent in size to that produced by phenylephrine. In the same preparation, quinidine 5 μg/ml was sufficient to abolish the phenylephrine response.

In doses below those producing abolition of pendular movements, quinidine, procaine, lignocaine and cocaine had little or no effect on responses to isoprenaline (Fig. 9d) or to dibutyryl cyclic AMP.

All four drugs blocked responses of the intestine to periarterial nerve stimulation. The effective concentrations were quinidine 20–30 μg/ml, cocaine 75 μg/ml, lignocaine 100 μg/ml and procaine 200 μg/ml. Thus, the rank order of potency of the drugs in this test differed from that found when they were tested against phenylephrine, ATP or cyclic AMP, and, although quinidine was again the most potent, considerably higher concentrations were required to abolish the effects of periarterial nerve stimulation.

Discussion

The results demonstrate a difference in the forms of the inhibitory responses of the rabbit intestine to phenylephrine on the one hand and to isoprenaline on the other. The inhibitory response to phenylephrine was rapid in onset, recovery began during the presence of the drug and overshoot in contraction height usually occurred when the drug was removed from the organ bath. In contrast, the isoprenaline response was relatively slow in onset, was maintained throughout the presence of the drug and was not followed by overshoot on washout. Phenylephrine is known to act mainly on α-adrenoceptors, whereas isoprenaline acts mainly on β-adrenoceptors (Levy & Ahlquist, 1967), and the use of the appropriate antagonists confirmed that, in the concentrations used, these amines acted specifically on one type of receptor or the other. The results therefore suggest that the two forms of response are characteristic of α- and β-adrenoceptor mediated responses respectively. Van Rossum & Mujić (1965) characterized the two types of response on the basis of their different rates of onset.

Adrenaline and noradrenaline are known to act on both types of receptor in rabbit intestine (Furchgott, 1960), and this was confirmed in the present experiments both by the shapes of the responses and by the use of selective antagonists. The rabbit intestine seems to be unusual, however, in that noradrenaline showed more selectivity for β-adrenoceptors than did adrenaline, which, in the concentrations used, acted mainly on α-adrenoceptors. According to Lawson & Mackenna (1969),

the transmitter released on stimulation of the sympathetic nerves excites only β-adrenoceptors. This was not found to be so in the present experiments. Stimulation of the periarterial nerves produced responses resembling those to noradrenaline; they were mainly due to β-adrenoceptor stimulation but α-adrenoceptors were also involved.

The return of the spontaneous pendular movements towards or to the control amplitude during the continued presence of an α-adrenoceptor agonist did not seem to be due to metabolism or uptake of the amine. It is possible that the waning α-adrenoceptor mediated response is due to desensitization of the α-adrenoceptors, as suggested for adrenaline by Finkleman (1930), and that the overshoot on washout is a consequence of over compensation by the ion pump and a rebound depolarization of the membrane following the α-adrenoceptor mediated primary hyperpolarization. Bennett (1966) explained the secondary stimulation of the guinea-pig taenia coli following intramural inhibitory nerve stimulation in this way. However, an alternative explanation is that α-adrenoceptor stimulation evokes two opposing responses—a primary inhibitory response which partially masks a secondary, more slowly developing but more persistent stimulant action. The brief overshoot in contraction height which usually followed washout would then be attributable to unmasking of the remnant of this secondary stimulant action when the drug was removed from the organ bath. If this is so, the secondary stimulant effect could not be due to release of 5-hydroxytryptamine, histamine or acetylcholine or to some mechanism involving intramural nervous structures, because it was not reduced by methysergide, mepyramine or hyoscine in concentrations greatly in excess of those necessary to block the relevant agonist. Nor was it modified by concentrations of tetrodotoxin great enough to abolish the effects of nerve stimulation. So far, it has not proved possible to separate the two components of the α-adrenoceptor response to phenylephrine. Drugs that modified the one component also modified the other in a similar way, so that it appears that the secondary "stimulant" effect may be a consequence of some change arising from the primary inhibition. However, stimulation of pendular movement is the main response evoked by the indirectly acting amines, tyramine and amphetamine (Van Rossum & Mujić, 1965; and confirmed under the present experimental conditions) and it is possible that this effect is related to the secondary effect of α-adrenoceptor stimulants.

Inhibition of tone in the guinea-pig intestine by α-adrenoceptor agonists can be the result of an action on intramural cholinergic nerves with a consequent reduction in spontaneous acetylcholine output (McDougal & West, 1954; Kosterlitz & Watt, 1965; Paton & Vizi, 1969). There is, however, no evidence that pendular movements in the rabbit intestine involve a cholinergic mechanism, and, in any case, hyoscine was always present in the bathing fluid, and the responses were not changed by tetrodotoxin. An action involving neural elements can therefore be ruled out, and it is concluded that both the α- and the β-adrenoceptors involved were present in the smooth muscle cells.

In the smooth muscle cells of the guinea-pig taenia coli, α-adrenoceptors are believed to be located on the cell membrane, whereas β-adrenoceptors may be located intracellularly (Brody & Diamond, 1967) or may lead to a chain of intracellular events responsible for the mechanical response. Inhibition evoked by α-adrenoceptors is the result of hyperpolarization of the cell membranes consequent

upon a primary increase in K^+ permeability, whereas hyperpolarization evoked by β-adrenoceptor activation (if it occurs, and there is controversy in the literature over this point) may be secondary to some intracellular action of the amines (Jenkinson & Morton, 1967). The relatively rapid onset of the α-adrenoceptor mediated inhibition of the rabbit intestine, together with the observation that α-adrenoceptor responses were antagonized by membrane stabilizers such as quinidine and procaine, is compatible with a membrane location of α-adrenoceptors in this tissue and an action primarily involving a change in membrane permeability. On the other hand, β-adrenoceptor mediated responses were resistant to the action of membrane stabilizers, suggesting that their effects are not dependent on a change in membrane potential.

In addition to blocking α-adrenoceptor mediated responses, membrane stabilizers blocked the similar responses produced by ATP and cyclic AMP. This suggests that the response to ATP and the more usual phenylephine-like response to cyclic AMP are exerted on the cell membrane, and raises the question whether α-adrenoceptor mediated responses involve membrane ATP or some other nucleotide as an intermediary. Bueding *et al.* (1967) found adrenaline, in guinea-pig taenia coli, to cause an increase in ATP and CP levels coincident with the inhibitory effect on activity.

The rank order of potency of the membrane stabilizers in blocking the smooth muscle responses differed from that in blocking the effects of periarterial nerve stimulation. The latter is probably mainly a reflection of the action of the drugs on adrenergic neurones, because the muscle response to the chemical transmitter (presumably noradrenaline) is mainly β-adrenoceptor mediated and therefore largely resistant to these drugs. It was of interest that quinidine, although the most potent of the membrane stabilizers in both tests, was considerably more potent in blocking smooth muscle responses than in depressing nerve conduction. Quinidine was somewhat more potent against phenylephrine than against ATP. Quinidine has been reported to possess some α-adrenoceptor blocking activity (Hiatt, 1950), and this may account for its greater antagonism towards phenylephrine.

There is a considerable volume of circumstantial evidence that some β-adrenoceptor mediated mechanical effects, particularly those in the heart, are secondary to activation of membrane adenyl cyclase and the production of increased intracellular levels of cyclic AMP (Sutherland & Robison, 1966 ; Robison, Butcher & Sutherland, 1967), and Bueding & Bülbring (1964, 1967) and Bueding, Butcher, Hawkins, Timms & Sutherland (1966) have suggested that the relaxing effect on guinea-pig taenia coli may also be related to increases in the tissue concentration of this nucleotide. In only about 10% of the present experiments, cyclic AMP itself produced an inhibitory response of the rabbit intestine resembling, in shape, that produced by β-adrenoceptor agonists. This low percentage may have been due to the poor ability of the cyclic nucleotide to penetrate the cell membranes. Its dibutyryl analogue always produced a response resembling that to β-adrenoceptor agonists, and according to Posternak, Sutherland & Henion (1962) and Butcher, Ho, Meng & Sutherland (1965) this compound penetrates cell membranes more readily and is more resistant to breakdown by phosphodiesterase. The slow onset of inhibition produced by dibutyryl cyclic AMP and the persistence of its effect are compatible with an intracellular site of action. The effective concentrations of dibutyryl cyclic AMP were high, possibly because, in order to be effective, it has first to be converted to the

parent nucleotide (Henion, Sutherland & Posternak, 1967 ; Sutherland, Robison & Butcher, 1968) and the rabbit intestine may be deficient in the necessary enzymes.

The effects of theophylline and imidazole were tested because, in broken cell preparations, these substances, in concentrations similar to those used in the present experiments, inhibit and activate respectively the phosphodiesterase responsible for destroying cyclic AMP (Butcher & Sutherland, 1962). Theophylline itself inhibited the rabbit intestine and, in all effective concentrations, it potentiated isoprenaline, noradrenaline and periarterial nerve stimulation, and inhibited the secondary " stimulant " effect of α-adrenoceptor agonists. It also augmented isoprenaline-like responses produced by dibutyryl cyclic AMP and those occasionally produced by cyclic AMP. Imidazole stimulated the rabbit intestine and, in high concentrations, reduced the β-adrenoceptor mediated responses to isoprenaline, noradrenaline and periarterial nerve stimulation, and the similar responses produced by the cyclic nucleotides. These effects with theophylline and imidazole may be explained on a basis of their interactions with phosphodiesterase, and they add support to the possibility that β-adrenoceptor mediated effects involve the production of increased levels of cyclic AMP. These results should be treated with some reservation, however, because the actions of theophylline, and especially of imidazole, were not specific. Higher concentrations of theophylline blocked responses to phenylephrine, adrenaline, ATP and to cyclic AMP when, as was usual, it resembled phenylephrine in its action. Relatively low concentrations of imidazole also blocked responses to phenylephrine and adrenaline, and in unpublished experiments the same concentrations of imidazole were found to block acetylcholine. It thus appears that, in addition to any actions they may have on phosphodiesterase, in organized tissue theophylline and imidazole exert a wide ranging depressant action on the cell membrane. The related xanthine, caffeine, has also been shown to block certain responses to adenosine and the adenine nucleotides (Nichols & Walaszek, 1963 ; De Gubareff & Sleator, 1965).

Jenkinson & Morton (1967) showed that isoprenaline inhibits calcium ion-induced contractions of the guinea-pig taenia coli depolarized by K_2SO_4 solution. α-Adrenoceptor stimulants were much less effective in this respect. In skeletal muscle homogenate, Rabinowitz, Desalles, Meisler & Lorand (1965) demonstrated a parallelism between the distribution of adenyl cyclase, the enzyme responsible for cyclic AMP production, and the particles which show relaxing activity. It may therefore be that intracellular cyclic AMP is involved in the active removal of calcium ions from the vicinity of the contractile elements, and that inhibition of spontaneous pendular movements of rabbit intestine by β-adrenoceptor stimulants is the result of increased levels of cyclic AMP facilitating the binding of intracellular calcium ions so that fewer cross linkages between actin and myosin are formed. It may also be that a secondary effect of α-adrenoceptor stimulants is to reduce the intracellular levels of cyclic AMP as suggested for some tissues by Robison *et al.* (1967). Such an effect might then account for the secondary stimulant effect of α-adrenoceptor agonists on pendular movements. Smooth muscle has a relatively poorly developed sarcoplasmic reticulum, and so membrane bound calcium may be a source of these ions for the contractile process. The postulated inability of the membrane to rebind these ions in the absence of adequate levels of cyclic AMP may account for its instability and for the " rebound " depolarization recorded by Bennett (1966) in the taenia coli of the guinea-pig. It is also tempting to speculate that rhythmic

changes in the levels of the cyclic nucleotide may play a role in initiating or controlling spontaneous pendular movements.

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