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Nerve-mediated inhibition of mechanical activity in rabbit duodenum and the effects of desensitization to adenosine and several of its derivatives

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Summary

1. Inhibition of mechanical activity in longitudinal muscle strips of rabbit duodenum was induced by perivascular and intramural nerve stimulation.

2. The effects of perivascular stimulation were abolished by phentolamine + propranolol, guanethidine, reserpine and by tetrodotoxin. The effects of intramural stimulation were abolished only by tetrodotoxin.

3. Noradrenaline, phenylephrine, isoprenaline, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine each produced an inhibition of mechanical activity. The relative potencies of these agonists were noradrenaline>isoprenaline>phenylephrine>ATP>ADP>adenosine>AMP.

4. Exposure of tissues to high concentrations of either ATP or adenosine desensitized the tissue to further exposure to ATP, ADP, AMP and adenosine, whilst the inhibitory effects of noradrenaline, phenylephrine and isoprenaline and of perivascular and intramural stimulation were unaffected. The effects of desensitization were reversible.

5. It was concluded that the effects of intramural stimulation were mediated neither by noradrenaline nor by adenosine or its derivatives.

Introduction

There has been much speculation about the existence in intestinal smooth muscle of a population of intramural inhibitory neurones whose effects are mediated by an unidentified transmitter substance. Weisenthal, Hug, Weisbrodt & Bass (1971) commented that much of the discussion about the intramural inhibitory pathway was based on the assumption that such a pathway existed rather than on the quality of the evidence in favour of its existence. The whole subject, including the possibility that adenosine triphosphate (ATP) is the transmitter involved, has been reviewed by Burnstock (1972). It is apparent that there have been no detailed attempts to test the hypothesis of Paton & Vane (1963) that intramural and extramural stimulation of the same pathway could itself give rise to the differences about which there is speculation. If the differences are real, the absence of a specific antagonist to the action of ATP is an important limiting factor in assessing its transmitter role. In view of this, it is surprising that the ability to desensitize a tissue to the inhibitory effects of ATP (Holman & Hughes, 1965) has not been further investigated.

In the present investigation, the inhibition produced in rabbit duodenum by intramural stimulation and by stimulation of the perivascular nerves was subjected to a detailed pharmacological analysis. In addition, the tissues were desensitized to the effects of several adenosine derivatives to assess their possible intramural transmitter role.

Some of the results have been presented to the British Pharmacological Society (Weston, 1971a).

Methods

Two cm lengths of the longitudinal muscle layer were removed from rabbit duodenum as previously described (Weston, 1971b) except that the attached mesentery was removed with the muscle strip by altering the position of the incisions. Histological examination showed that the strips contained Auerbach's plexus. The lower part of the strip was pulled through one pair of ring electrodes and the mesentery through a similar pair.

All experiments were conducted at 37° C in Krebs solution containing atropine, 10 μM, to eliminate cholinergic responses. Tissues which failed to show regular mechanical activity were discarded. Tension changes were recorded isometrically (Ether UF1) on a potentiometric recorder (Rikadenki). Rectangular pulses of 0·5 ms duration and 30 V strength were delivered at various frequencies (1–64 Hz) for periods of 10 s every 4 min from a Grass stimulator.

Successive doses of noradrenaline were administered at 4 min intervals as were phenylephrine, ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine. A 5 min interval was allowed between responses to isoprenaline. Two intermediate changes of Krebs solution were made in each drug cycle. Inhibition of mechanical activity was measured as described by Kim, Schulman & Levine (1968).

Desensitization of the tissue to adenosine derivatives was achieved by exposure to Krebs solution containing either ATP 100 μM or adenosine 100 μM. When phentolamine + propranolol and guanethidine were used as antagonists and when ATP and adenosine were used as desensitizing agents, these drugs were added to the Krebs solution and a 30 min equilibration period was allowed before the experiment was continued. The concentrations of phentolamine (pA_{100} against phenylephrine) and propranolol (pA_{100} against isoprenaline) used were derived from a previous study (Weston, 1971b).

In experiments involving reserpine treatment, rabbits were injected with reserpine 2 mg/kg intraperitoneally on each of two days prior to the experiment. The catecholamine content of a portion of duodenum was assayed spectrophotofluorimetrically (Bertler, Carlsson & Rosengren, 1958).

Drugs and solutions

The Krebs solution used had the following composition (mM): Na^+ 143·0, K^+ 5·9, Ca^{++} 2·5, Mg^{++} 1·2, Cl^- 125·0, HCO_3^- 25·0, SO_4^{--} 1·2, $H_2PO_4^-$ 1·2, dextrose 11·1. The pH of this solution was 7·4 while bubbling with 5% CO_2 in O_2.

Drugs used were adenosine, adenosine monophosphate, adenosine 5′ diphosphate, adenosine 5′ triphosphate (Boehringer); (±)-isoprenaline bitartrate (Ward-Blenkinsop); (−)-noradrenaline bitartrate (Ward-Blenkinsop); phentolamine hydrochloride

(Ciba); (−)-phenylephrine hydrochloride (Boots); (±)-propranolol hydrochloride (I.C.I.); reserpine (B.D.H.); tetrodotoxin (Koch-Light).

Results

Analysis of the effects of perivascular and intramural stimulation

Some of the results are summarized in Figure 1. Both perivascular and intra-

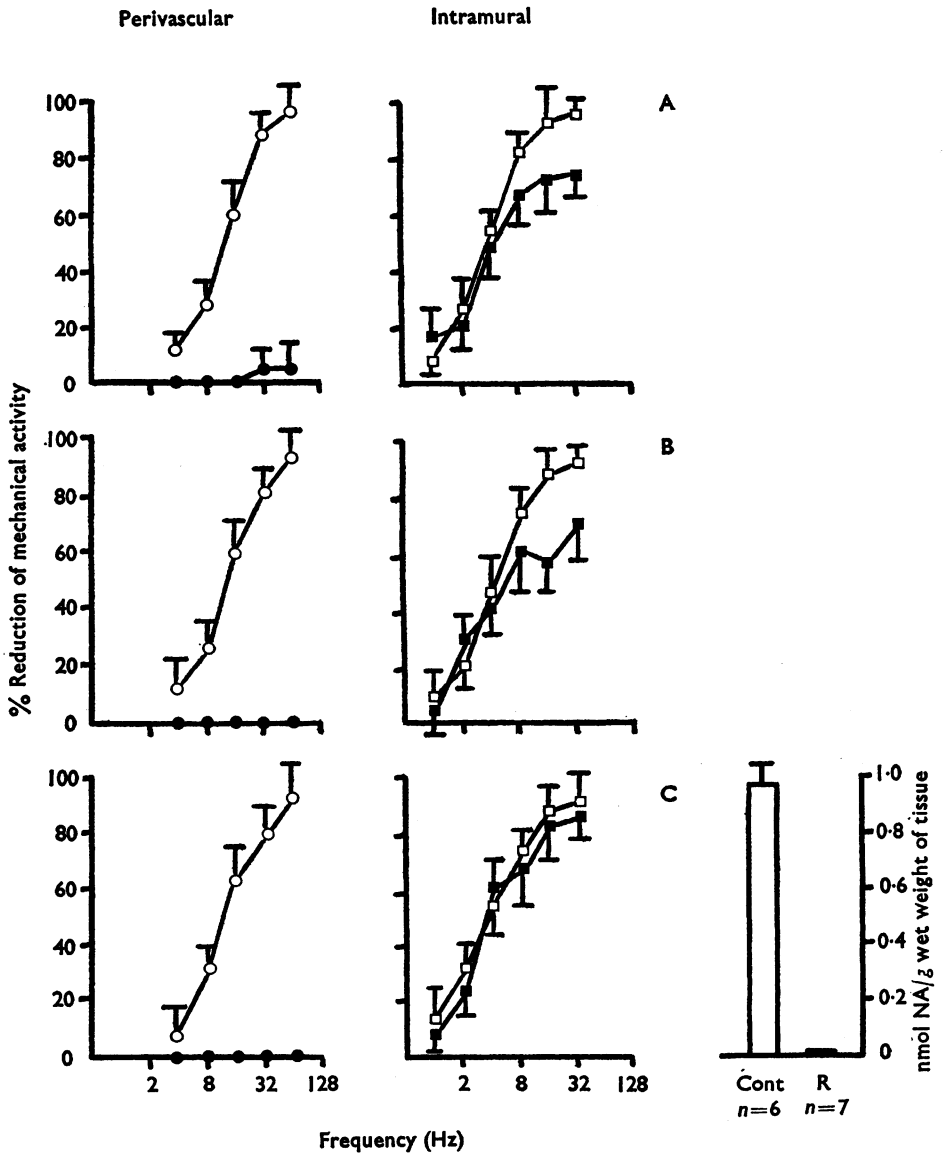

FIG. 1. Effect of drugs on the frequency-response curves to perivascular and intramural stimulation. A, Effect of phentolamine (2·5 μM)+propranolol (4·75 μM). B, Effect of guanethidine (10 μM). C, Effect of reserpine (2 mg/kg i.p.). Responses to perivascular stimulation before (○) and after (●) drug exposure. Responses to intramural stimulation before (□) and after (■) drug exposure. Vertical columns show tissue noradrenaline (NA) content before (Cont) and after (R) reserpine treatment. Unless stated, each point is the mean of 8 experiments ($n=8$); vertical bar indicates S.E.M.

mural stimulation produced a frequency-dependent reduction of mechanical activity. The threshold stimulation frequency was lower for intramural than for perivascular stimulation. After intramural stimulation, the control pattern of mechanical activity rapidly returned and this was followed by an increase in the amplitude of spontaneous contractions which persisted for about 10 seconds. The amplitude of the after-contraction (10–40% of control levels) was greater as the stimulation frequency increased. The inhibition produced by perivascular stimulation persisted for several seconds after stimulation and after-excitation was rarely seen.

The effects of perivascular stimulation were greatly reduced by phentolamine (2·5 μM) + propranolol (4·75 μM), guanethidine (10 μM) and by pretreatment of the animals with reserpine, whereas the effects of intramural stimulation were little affected. Tetrodotoxin (313 nM) abolished the effects of both perivascular and intramural stimulation (9 experiments). These antagonists produced no significant shift in the log concentration-effect curve to ATP (0·16–40 μM) used as control.

Relative potencies of inhibitory agonists

The relative potencies of the agonists used are illustrated in Figure 2. ATP was the most potent of the adenosine derivatives. Phenylephrine and the adenosine derivatives each produced an inhibition of mechanical activity which was rapid in onset and which declined in the presence of the drug. On washout, there was an increase in the amplitude of mechanical activity, which was independent of the degree to which mechanical activity had previously been inhibited. The after-contraction following intramural stimulation in the range 4–32 Hz was greater than that following washout of phenylephrine and the adenosine derivatives (8 experiments, $P \leqslant 0{\cdot}025$). The inhibition produced by noradrenaline and by isoprenaline

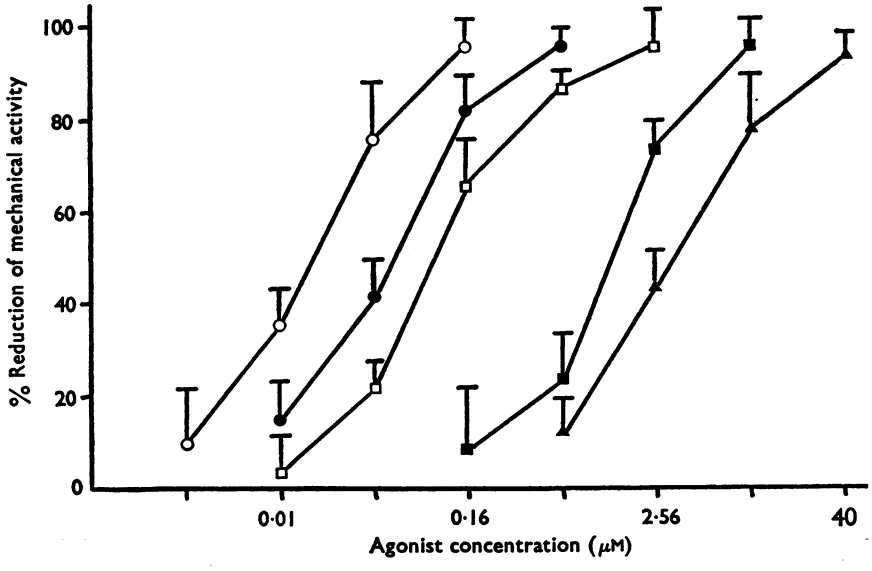

FIG. 2. Concentration-effect curves for the inhibitory agonists. Responses to noradrenaline (○), isoprenaline (●), phenylephrine (□), ATP (■), AMP (▲). The curves for ADP and adenosine are located between those for ATP and AMP and have been omitted for clarity. Each point is the mean of 8 experiments; vertical bar indicates S.E.M.

was slower in onset, was maintained in the presence of the drug and no increase in mechanical activity above control levels occurred after washout.

Desensitization by ATP and adenosine

When the tissue was exposed to ATP 100 μM, smooth muscle activity was completely inhibited at first but returned to about 90% of the control value in about 15 minutes. Under these conditions, the sensitivity of the tissue to noradrenaline, phenylephrine, isoprenaline, perivascular and intramural stimulation was unaffected whilst the inhibitory effects of ATP (0·16–40 μM) were greatly reduced (Figure 3).

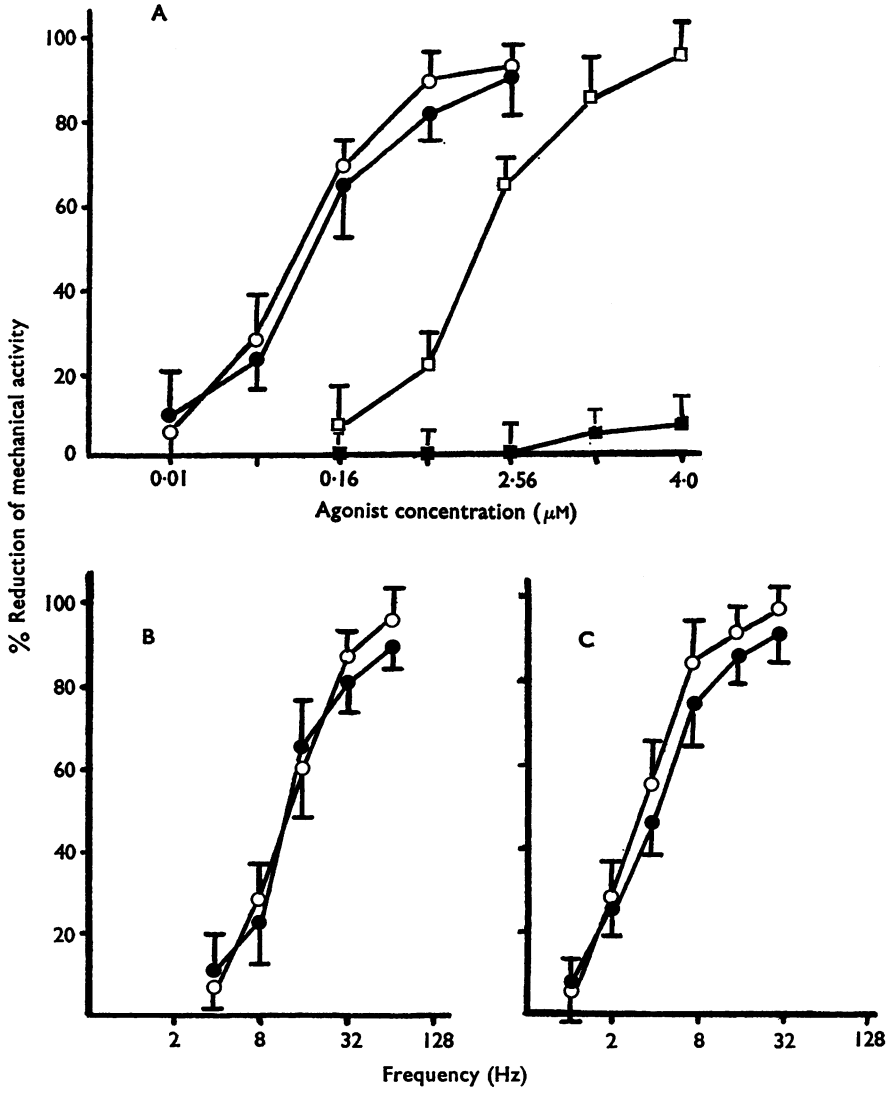

FIG. 3. Effects of desensitization with ATP 100 μM. A, Responses to phenylephrine before (○) and after (●) desensitization; responses to ATP before (□) and after (■) desensitization. B, Responses to perivascular stimulation and C, responses to intramural stimulation before (○) and after (●) desensitization. Each point is the mean of 8 experiments; vertical bar indicates S.E.M.

Exposure to ATP 100 μM also desensitized the tissue to the effects of ADP, AMP and adenosine (each 0·16–40 μM). Identical experiments performed in the presence of adenosine 100 μM showed that this drug desensitized the tissue to subsequent exposure to ATP, ADP and AMP whereas responses to phenylephrine, isoprenaline, perivascular and intramural stimulation were unaffected.

After washing with Krebs solution for 45 min, the effects of desensitization were abolished and control responses to adenosine and its derivatives were obtained.

Discussion

The results show that the decrease in mechanical activity following perivascular stimulation was mediated by noradrenaline. Inhibition following intramural stimulation occurred at lower pulse frequencies than that following perivascular stimulation. Similar findings have been reported for other tissues (Burnstock, Campbell & Rand, 1966). Intramural inhibition was also largely unaffected by phentolamine + propranolol, guanethidine and reserpine pretreatment. Consideration of these results individually is insufficient to discount noradrenaline as the mediator of intramural inhibition. Collectively, however, they indicate that another transmitter is involved and discount the suggestion that intramural stimulation excites adrenergic neurones at a point distal to the site of action of the blocking drugs (Paton & Vane, 1963).

Burnstock (1972) has stressed the relevance of the after-contraction so often seen following intramural stimulation. In the present experiments, the after-contraction occurred after intramural stimulation and also after washout of phenylephrine, ATP, ADP, AMP and adenosine. It seems possible that this phenomenon is the inevitable consequence of rapid primary inhibition (Bowman & Hall, 1970) since it did not occur on washout of noradrenaline and isoprenaline or after perivascular stimulation. The greater magnitude of the after-contraction following intramural stimulation in the duodenum could be due to additional endogenous release of acetylcholine against which the concentration of atropine in the tissue bath was not completely effective.

Exposure to high concentrations of ATP desensitized the tissue to the effects of ATP, ADP, AMP and adenosine whereas the responses to perivascular and intramural stimulation, noradrenaline, phenylephrine and isoprenaline were unaffected. This specific desensitization indicates that a derivative of adenosine is not responsible for mediating the effects of intramural stimulation and is consistent with the low potency of ATP relative to that of noradrenaline. It is possible that it is more difficult to desensitize a tissue to the effects of an endogenously-released transmitter than to the effects of that transmitter when exogenously applied. However, in guinea-pig ileum, desensitization to the effects of exogenous acetylcholine is also effective in preventing the cholinergic excitatory component of the peristaltic reflex (Schaumann, Jochum & Schmidt, 1953 ; Weston, 1973).

Burnstock, Campbell, Satchell & Smythe (1970) reported that desensitization to the effects of exogenous ATP reduced or abolished the inhibitory effects of intramural stimulation, which contrasts with observations made in the present study. It is impossible to assess the extent of the discrepancy because of the absence of detailed quantitative results in the report of Burnstock *et al.* (1970). Campbell (1970) has pointed out that, if the intramural inhibitory transmitter is a nucleotide, the ubiquitous nature of these substances will render proof of their transmitter role

very difficult and the above differences emphasize the necessity for a quantitative approach to this problem.

Adenosine itself was as effective as ATP in desensitizing the tissue to the effects of adenosine derivatives. This suggests that the adenosine nucleus is the active inhibitory and desensitizing moiety and is consistent with the similarity in the potencies of ATP, ADP, AMP and adenosine. A similar suggestion concerning the adenosine moiety has been made by Kim *et al.* (1968).

The function of intramural inhibitory nerves in intestinal tissues has been the subject of speculation (Campbell, 1970), one suggestion being that inhibitory responses are mediated by antidromic stimulation of sensory neurones in enteric plexuses. An alternative hypothesis is that the neurones form the inhibitory pathway of a peristaltic reflex and this suggestion is currently being investigated.

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