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A comparison of the excitatory and inhibitory effects of non-adrenergic, non-cholinergic nerve stimulation and exogenously applied ATP on a variety of smooth muscle preparations from different vertebrate species

G. BURNSTOCK, D. G. SATCHELL AND ANNE SMYTHE

Department of Zoology, University of Melbourne, Parkville, Victoria-3052, Australia

Summary

1. The responses to non-adrenergic, non-cholinergic nerve stimulation have been compared with those to exogenously applied ATP on seventeen different tissues from a number of vertebrate classes.

2. Stimulation of all the mammalian gut preparations studied (with the exception of the guinea-pig ileum) after blockade of the effects of adrenergic and cholinergic nerve stimulation by guanethidine (3·5 μM) and hyoscine (1·3 μM) caused inhibition ; exogenously applied ATP mimicked this inhibitory response.

3. Stimulation of the guinea-pig ileum in the presence of hyoscine and guanethidine, usually caused a diphasic response, relaxation followed by contraction ; exogenously applied ATP mimicked this response, in contrast to acetylcholine and noradrenaline which caused excitation and relaxation respectively.

4. Stimulation of preparations of lower vertebrate gut and guinea-pig bladder in the presence of hyoscine and guanethidine caused contraction ; exogenously applied ATP mimicked this contractile response.

5. In each preparation the time course of the response to ATP was similar or identical to the response to non-adrenergic, non-cholinergic nerve stimulation.

6. The results are consistent with the hypothesis that a purine nucleotide may be the transmitter substance released from non-adrenergic, non-cholinergic nerves supplying smooth muscle preparations from a number of vertebrate classes.

Introduction

Evidence has been presented that a purine nucleotide, probably ATP, is the transmitter released from non-adrenergic, non-cholinergic inhibitory nerves to the gastrointestinal tract (Burnstock, Campbell, Satchell & Smythe, 1970 ; Satchell & Burnstock, 1971 ; Su, Bevan & Burnstock, 1971 ; Burnstock, 1972 ; Satchell, Lynch, Bourke & Burnstock, 1972). Consequently these nerves have been tentatively termed 'purinergic' (Burnstock, 1971). In addition to purinergic neurones in the wall of the gut, fibres which are neither adrenergic nor cholinergic have been shown to supply a variety of organs (Burnstock, 1969, 1972 ; Campbell, 1970). Some of these are excitatory, as for example those to the urinary bladder (Henderson & Roepke, 1934 ; Chesher, 1967 ; Ambache & Zar, 1970 ; Dumsday,

1971 ; Burnstock, Dumsday & Smythe, 1972) and to segments of the gut in lower vertebrates (Everett, 1968 ; Carter, 1969 ; Bartlet & Hassan, 1971 ; Sneddon, Smythe, Satchell & Burnstock, to be published). Others are inhibitory, for example those to the amphibian and reptile lung (Wood & Burnstock, 1967 ; Campbell, 1971 ; Robinson, McLean & Burnstock, 1971 ; Berger, 1972 ; Schnizer, Hoang & Brecht, 1968) and parts of the vascular system (Hughes & Vane, 1967, 1970). It is not yet known whether any, some, or all of these nerves are purinergic or whether they release yet further neurotransmitters, although some evidence has been presented that those supplying the toad lung (Robinson *et al.*, 1971), guinea-pig bladder (Dumsday, 1971 ; Burnstock *et al.*, 1972), toad intestine (Sneddon *et al.*, 1972) and rabbit portal vein (Hughes & Vane, 1967) may release ATP.

If an adenine nucleotide is indeed a transmitter in a variety of smooth muscle preparations and species, causing contraction in some instances and inhibition in others it would be expected that responses to ATP should match the responses to non-adrenergic, non-cholinergic nerve stimulation. In order to test this hypothesis, the effects of non-adrenergic, non-cholinergic nerve stimulation and those of exogenously applied ATP have been compared in seventeen different tissues from four classes of vertebrates.

Methods

The following gut segments were suspended in organ baths as Magnus preparations: ileum and colon of the guinea-pig ; duodenum, ileum and rectum of the rat ; duodenum, ileum, colon and rectum of the mouse ; duodenum and ileum of the toad, *Bufo marinus* ; ileum of the lizard, *Tiliqua rugosa* ; large intestine of the fish, *Carassius auratus*. Other preparations dissected as strips and mounted in organ baths were: the guinea-pig stomach and the taenia coli preparation ; guinea-pig bladder ; human colon. Mammalian preparations were suspended in a modified Krebs solution (Bülbring, 1953) at 35° C. All other preparations were mounted in McKenzie's solution (McKenzie, 1953) at room temperature ; the composition of this solution was (mM) 115 NaCl, 3·2 KCl, 20 $NaHCO_3$, 3·0 NaH_2PO_4, 1·2 $MgSO_4$, 16·5 glucose and 1·3 $CaCl_2$. Nutrient media were bubbled with a mixture of 95% O_2 and 5% CO_2. Muscle activity was recorded with an isotonic lever (magnification 7·5 : 1) writing on a smoked drum. Rectangular pulses from Grass S5 stimulators were used for nerve stimulation, either transmurally or via perivascular nerves by means of platinum ring electrodes shielded in plastic.

Nutrient media contained hyoscine (1·3 μM) and guanethidine (3·5 μM) except where stated. The drugs were added at least 30 min prior to stimulation and abolished responses to cholinergic and adrenergic stimulation. Experiments were carried out on at least five preparations of each tissue cited.

Drugs. These were adenosine-5′-triphosphate disodium salt (ATP) hyoscine hydrobromide, guanethidine sulphate, acetylcholine chloride and (−)-noradrenaline bitartrate. All drugs were dissolved in water and concentrations are expressed in terms of the forms given above.

Results

Responses of different preparations

Guinea-pig gastrointestinal tract and bladder. In experiments on three out of the four preparations of different regions of the guinea-pig gastrointestinal tract,

namely stomach, taenia coli and colon, transmural stimulation or exogenously applied ATP in the presence of hyoscine and guanethidine consistently caused relaxation. In each case the shape of the response to ATP (1–5 μM) closely resembled the shape of the response to transmural stimulation (Fig. 1). In the fourth preparation, the guinea-pig ileum, transmural stimulation caused a diphasic response in the form of a small relaxation followed by a large contraction and this response was again matched by responses to ATP (Fig. 1c). The tone of the preparation affected the relative amplitudes of the relaxation and contraction phases of the responses to both nerve stimulation and ATP. A diphasic response was not seen during application of either acetylcholine or noradrenaline (hyoscine omitted) which caused contraction and relaxation respectively, regardless of the tone of the preparations (Fig. 1c). Stimulation of strips of the guinea-pig bladder in the presence of hyoscine and guanethidine caused contraction. Again the response was closely matched by ATP (Fig. 1e). Both responses were slower and longer lasting than the inhibitory responses of the gut to non-adrenergic inhibitory nerve stimulation and ATP.

Human colon. Biopsy specimens of human colon were small thick sections of gut with a low ratio of surface area to volume and therefore a decreased sensitivity

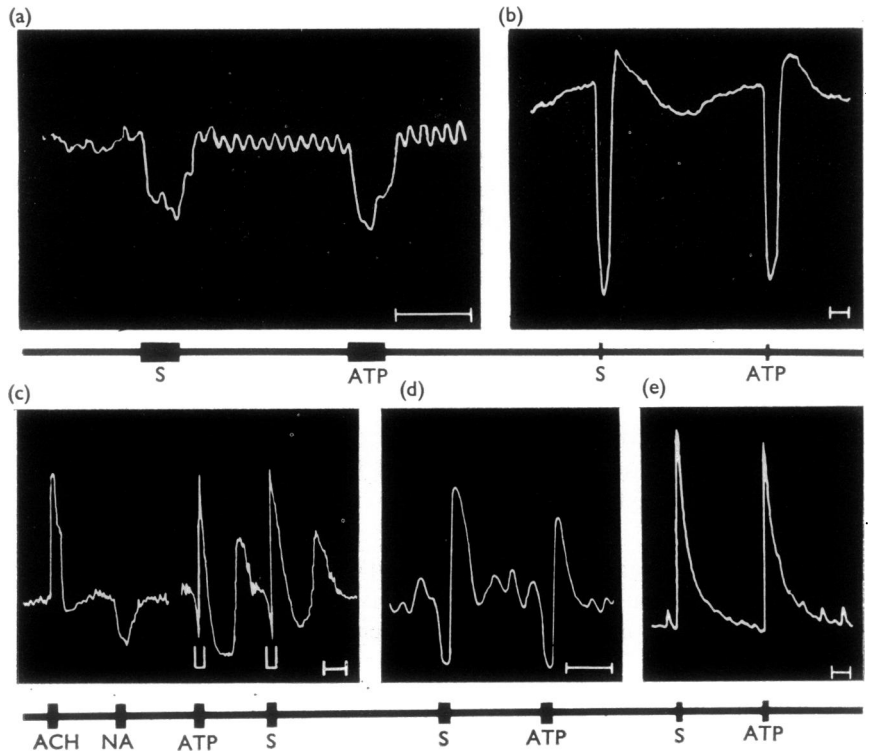

FIG. 1. Responses of various guinea-pig smooth muscle preparations to transmural stimulation and ATP. Hyoscine (1·3 μM) and guanethidine (3·5 μM) were present except where stated. (a) Guinea-pig stomach preparations consisting of strips (4×40 mm) cut as a spiral around the mid portion of the stomach; transmural stimulation (S, 5 Hz for 30 s), ATP (5 μM for 30 s); (b) guinea-pig taenia coli, transmural stimulation (S, 5 Hz for 15 s), ATP (1 μM for 15 s); (c) guinea-pig ileum, acetylcholine (ACH, 0·006 μM for 30 s, hyoscine omitted), noradrenaline (NA, 0·17 μM for 30 s, hyoscine omitted), ATP (5 μM for 30 s), transmural stimulation (S, 5 Hz for 30 s); (d) guinea-pig colon, transmural stimulation (S, 5 Hz for 15 s), ATP (5 μM for 15 s); (e) guinea-pig bladder, transmural stimulation (S, 5 Hz for 20 s), ATP (5 μM for 20 s). Time marker, 1 min.

to applied drugs. Transmural stimulation in the presence of hyoscine and guanethidine or the application of high concentrations of ATP (400 μM) caused relaxation (Fig. 2a).

Rat intestine. In preparations of rat duodenum (Fig. 2b) and ileum (Fig. 2c), transmural stimulation and ATP (10–50 μM) caused similar relaxations. In rat rectum, transmural stimulation consistently failed to cause a marked relaxation; but there was a reduction in the size of the spontaneous activity together with a large rebound contraction following cessation of the stimulus (Fig. 2d). Similarly, ATP (200 μM) was found to reduce the size of spontaneous activity in this preparation and a large rebound contraction was observed following washout.

Mouse intestine. In preparations of mouse duodenum and ileum, transmural stimulation caused a relaxation. Relaxations were also observed in response to ATP (20–40 μM), and in each preparation the response to ATP resembled the response to transmural stimulation (Fig. 3a, b). In mouse colon, responses to transmural stimulation were larger than in the above preparations and were

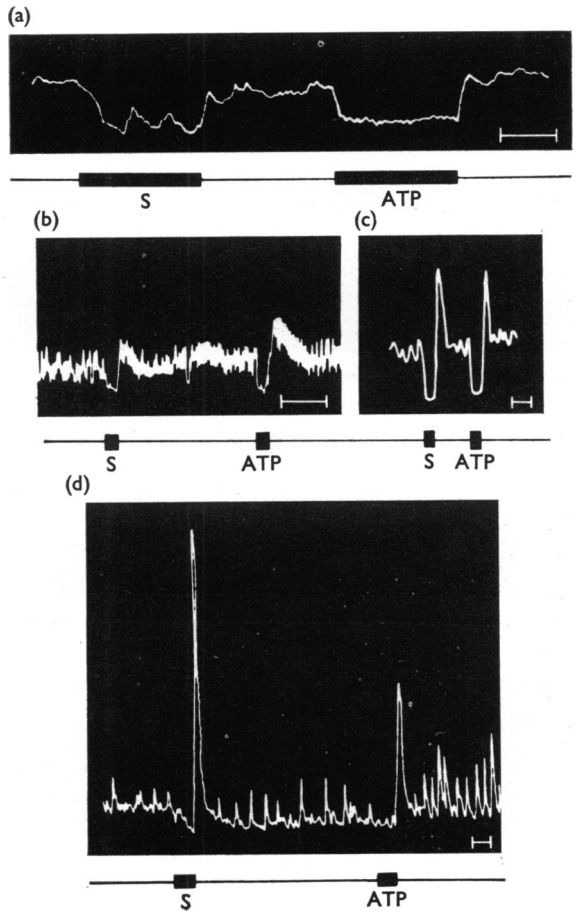

FIG. 2. Responses of rat and human intestinal preparations to transmural stimulation and ATP in the presence of hyoscine (1·3 μM) and guanethidine (3·5 μM). (a) Biopsy specimens of human colon cut as 10×5×4 mm strips; transmural stimulation (S, 5 Hz for 2 min), ATP (400 μM for 2 min); (b) rat duodenum, transmural stimulation (S, 5 Hz for 20 s), ATP (10 μM for 20 s); (c) rat ileum, transmural stimulation (S, 5 Hz for 30 s), ATP (50 μM for 30 s); (d) rat rectum, transmural stimulation (S, 3 Hz for 1 min), ATP (200 μM for 1 min). Time marker, 1 min.

similar in shape to responses to ATP (Fig. 3c). The mouse rectum has a high degree of spontaneous activity. Both transmural stimulation and ATP abolished the spontaneous activity and caused a small relaxation in this preparation. The relaxation was followed by a marked rebound contraction after cessation of stimulation or washout of the ATP (Fig. 3d).

Gut from lower vertebrates. In contrast to the responses of most preparations of mammalian gut, with the exception of the guinea-pig ileum, transmural stimulation of toad duodenum and ileum (Fig. 4a, b) lizard ileum (Fig. 4c) and goldfish intestine (Fig. 4d) caused contraction in the presence of hyoscine and guanethidine. In each case the response to ATP (10–25 μM) resembled the response to transmural stimulation.

Comparison of the time course of the responses to perivascular sympathetic nerve stimulation and noradrenaline with that of the responses to non-adrenergic inhibitory nerve stimulation and ATP

If ATP is the transmitter substance released by non-adrenergic inhibitory nerve fibres in mammalian gut, then it is possible that responses to stimulation of these nerves and to ATP might exhibit distinct characteristics which are different from the inhibitory responses to sympathetic nerve stimulation and noradrenaline. It

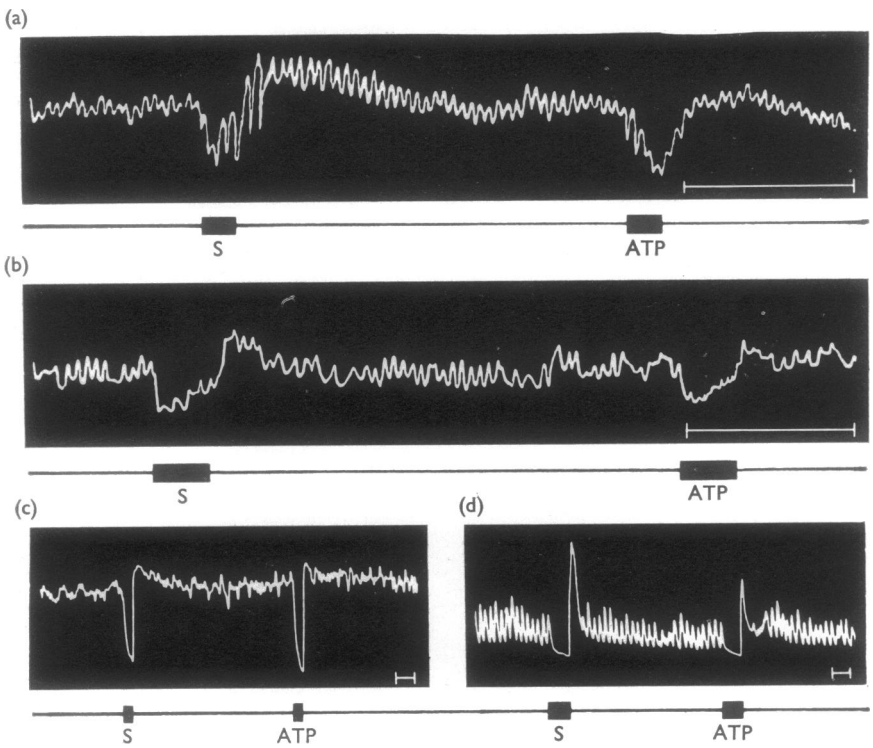

FIG. 3. Responses of segments of the gut of mouse to transmural stimulation and ATP. Hyoscine (1·3 μM) and guanethidine (3·5 μM) were present throughout. (a) Mouse duodenum; transmural stimulation (S, 5 Hz for 1 min), ATP (40 μM for 1 min); (b) mouse ileum; transmural stimulation (S, 5 Hz for 20 s), ATP (20 μM for 20 s); (c) mouse colon, transmural stimulation (S, 5 Hz for 30 s), ATP (40 μM for 30 s); (d) mouse rectum, transmural stimulation (S, 5 Hz for 1 min), ATP (40 μM for 1 min). Time marker (a) 5 min; (b), (c) and (d) 1 min.

has been found that the responses of the guinea-pig taenia coli to perivascular sympathetic nerve stimulation or noradrenaline (0·17 μM) were slow in onset, taking approximately 30 s to reach maximum, whereas the responses to non-adrenergic inhibitory nerve stimulation or ATP (25 μM) were rapid in onset and took approximately 15 s to reach maximum (Fig. 5).

Discussion

The possibility that ATP is a transmitter substance in a variety of vertebrate tissues causing contraction in some instances and relaxation in others has been investigated. The effects of non-adrenergic, non-cholinergic nerve stimulation and ATP have been compared on sixteen gastrointestinal preparations from four vertebrate classes and one bladder preparation. In each instance it has been observed that the response to ATP mimicked the response to non-adrenergic, non-cholinergic nerve stimulation. Thus, if the nerve-mediated response was a relaxation, then ATP caused relaxation; if the nerve-mediated response was a contraction, then ATP caused a contraction; finally, if the nerve-mediated response was diphasic, then ATP caused a diphasic response.

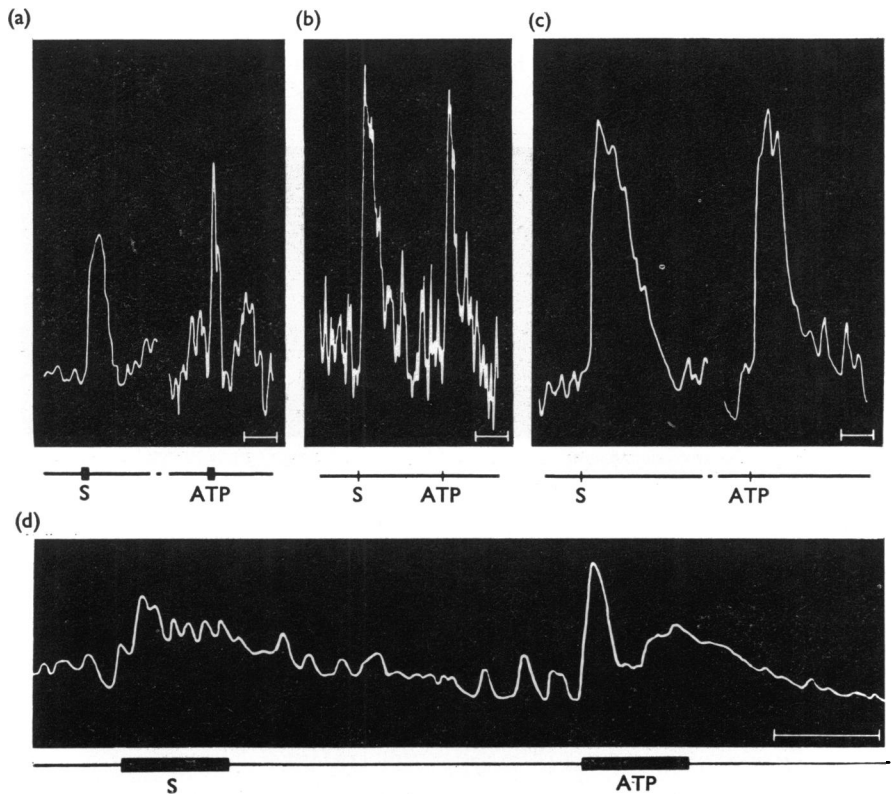

FIG. 4. Responses of gut segments from lower vertebrates to transmural stimulation or ATP. Hyoscine (1·3 μM) and guanethidine (3·5 μM) were present throughout. (a) Lizard ileum; transmural stimulation (S, 10 Hz for 1 min), ATP (10 μM for 1 min); (b) toad duodenum, transmural stimulation (S, 5 Hz for 15 s), ATP (10 μM for 15 s); (c) toad ileum, transmural stimulation (S, 5 Hz for 15 s), ATP (25 μM for 15 s); (d) goldfish large intestine, transmural stimulation (S, 10 Hz for 1 min), ATP (12 μM for 1 min). Time markers, (a), (b) and (c), 5 min, (d) 1 min.

Stimulation of non-adrenergic, non-cholinergic nerves and application of ATP caused similar and distinctive types of responses, i.e. in most cases the rates of onset and decline of the two responses were similar or identical. For example, both ATP and non-adrenergic, non-cholinergic nerve stimulation produced a response of the guinea-pig taenia coli which was rapid in onset, whereas perivascular sympathetic nerve stimulation and noradrenaline produced a response which was less rapid in onset and took twice as long to reach maximum. It should be noted too, that Ambache & Zar (1970) drew particular attention to the fact that in the mammalian bladder the time-course of contraction produced by ATP mimicked that of non-cholinergic nerve stimulation more closely than any other excitatory substance tested. Further, the diphasic response to ATP and non-adrenergic, non-cholinergic nerve stimulation in the guinea-pig ileum was not matched by either noradrenaline or acetylcholine which caused only relaxation and contraction respectively.

The responses to non-adrenergic, non-cholinergic nerve stimulation and ATP have been reported to be variable in the toad stomach, occurring as relaxations in some instances and contractions in others; occasionally, the response to ATP was a contraction while the response to transmural stimulation was a relaxation or *vice versa* (Smythe, 1971). The toad stomach is supplied by intramural non-adrenergic inhibitory neurones and also by postganglionic non-cholinergic excitatory nerves (Campbell, 1971). Thus, the variation in response may be explicable

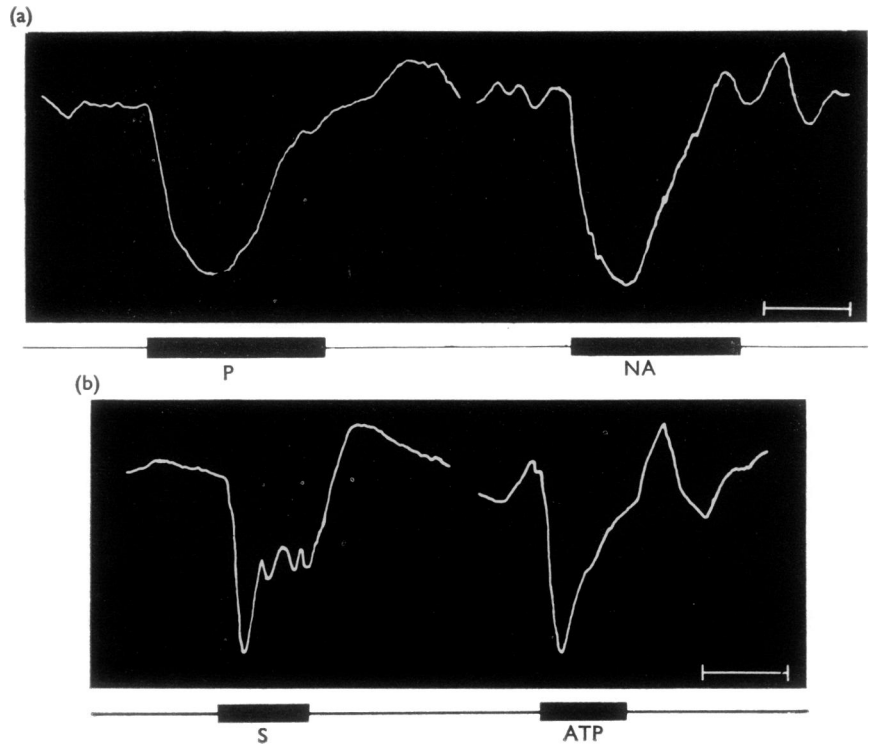

FIG. 5. Responses of the guinea-pig taenia coli to (a) perivascular sympathetic nerve stimulation (P, 30 Hz for 2 min) and noradrenaline (NA, 0·17 μM for 2 min); (b) transmural stimulation (S, 5 Hz for 1 min) and ATP (25 μM for 1 min). Hyoscine (1·3 μM) and guanethidine (3·5 μM) were present throughout. Time marker, 1 min.

in terms of the disposition of receptors which mediate excitatory or inhibitory responses, the geometry of electrode placement and the location or direction of dissection of the strip preparation. It is possible that the diphasic response of the guinea-pig ileum to non-adrenergic, non-cholinergic nerve stimulation could be explained in a similar way. Non-adrenergic inhibitory innervation of ileum is well established (Holman & Hughes, 1965; Kuriyama, Osa & Toida, 1967; Kosterlitz & Lydon, 1969; Furness, 1969), and atropine-resistant contraction of the longitudinal muscle of guinea-pig intestine has also been described (Munro, 1953; Ambache & Freeman, 1968; Kottegoda, 1968; Kosterlitz & Watt, 1963; Goldenberg, 1969; Furness, 1971; Ambache, Verney & Zar, 1970). It is known that the contractions are not mediated by well-known pharmacologically active substances such as histamine, 5-hydroxytryptamine, prostaglandin or catecholamines (Ambache *et al.*, 1970). If both non-adrenergic inhibitory and non-cholinergic excitatory responses of the guinea-pig ileum are mediated by adenine nucleotides, then the diphasic response could be due to a combination of the characteristically rapid onset and short duration of the inhibitory response followed by the slow and longer-lasting excitatory response to these substances.

While the findings that responses to ATP match the variety of responses to non-adrenergic, non-cholinergic nerve stimulation does not necessarily imply a causal relationship, it would be surprising if this close correlation observed in the seventeen preparations examined was only a coincidence. Thus the present experiments appear to add support to the hypothesis that ATP is a transmitter substance in vertebrate gastrointestinal tract and bladder.

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