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ACTIONS OF PUTATIVE TRANSMITTERS IN THE CHICKEN VAGUS NERVE/OESOPHAGUS AND REMAK NERVE/RECTUM PREPARATIONS

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1 Histamine (0.02-0.1 μg/ml) contracted the chicken rectum preparation. This effect was antagonized by mepyramine (0.01 μg/ml) but not by hyoscine (0.02 μg/ml).

2 5-Hydroxytryptamine (0.05-0.25 μg/ml) relaxed the rectum preparation and at higher concentration produced a biphasic response. These responses were not antagonized by methysergide (0.01 μg/ml), and the relaxation was not antagonized by tetrodotoxin (0.1 μg/ml) or a combination of propranolol (0.05 μg/ml) and phentolamine (0.1 μg/ml).

3 Neither mepyramine (0.1 μg/ml) nor methysergide (0.01 μg/ml) antagonized the contractions produced by nerve stimulation in vagus nerve/oesophagus and Remak nerve/rectum preparations.

4 5-Hydroxytryptamine (2 μg/ml) in the presence of methysergide (0.01 μg/ml), inhibited the contractions produced by nerve stimulation in Remak nerve/rectum and vagus nerve/oesophagus preparations.

5 Adenosine, adenosine 5′-phosphate (AMP), adenosine 5′-diphosphate (ADP) and adenosine 5′-triphosphate (ATP), in order of decreasing potency, produced slow contractions in most oesophagus preparations. The action of ATP in this preparation was antagonized by tetrodotoxin (0.1 μg/ml), hyoscine (0.1 μg/ml) and strychnine (5 μg/ml).

6 Desensitization of the vagus nerve/oesophagus preparation to ATP did not produce any antagonism of the contractions to nerve stimulation.

7 Adenosine and AMP produced relaxations and ADP and ATP contractions in the rectum preparation. ATP was about 100 times as potent as ADP in producing fast contractions which were not antagonized by tetrodotoxin, hyoscine or strychnine.

8 Desensitization of the Remak nerve/rectum preparation to ATP resulted in the contractions to nerve stimulation and acetylcholine being inhibited to the same extent.

9 Prostaglandin E_2 produced slow contractions in the oesophagus and rectum preparations which were not antagonized by tetrodotoxin (0.1 μg/ml). Polyphloretin phosphate (10 μg/ml) antagonized spontaneous movements and responses to prostaglandin E_2 in the rectum but not the oesophagus.

10 Neither polyphloretin phosphate (60 μg/ml) nor indomethacin (20-100 μg/ml) antagonized the contractions produced by nerve stimulation in vagus nerve/oesophagus (with hyoscine in the bathing solution) and Remak nerve/rectum preparations.

11 These experiments seem to exclude histamine, 5-hydroxytryptamine, adenosine and its nucleotides and prostaglandin E_2 as possible motor transmitters in synapses and neuromuscular junctions in the chicken vagus nerve/oesophagus and Remak nerve/rectum preparations.

Introduction

The longitudinal contractions in preparations of chicken rectum produced by stimulation of Remak's nerve are abolished by nicotine and tubocurarine, but similar contractions in the oesophagus are affected very little by these drugs. Thus ganglionic transmission in the parasympathetic nerves to the oesophagus may not be wholly cholinergic. In preparations of chicken oesophagus the contractions produced by stimulation of the vagus and descending oesophageal nerves are only partly antagonized by hyoscine or atropine, and in the chicken rectum preparation the contractions to nerve stimulation are almost wholly unaffected by these drugs

(Hassan, 1969; Bartlet & Hassan, 1971; Bartlet, 1972, 1973). The ineffectiveness of atropine and hyoscine in producing a blockade of neuromuscular transmission in these preparations suggests that the postganglionic motor nerves may not be wholly cholinergic.

In the present experiments several putative transmitters have been tested on the chicken vagus nerve/oesophagus and Remak nerve/rectum preparations. Some of these substances produced longitudinal contractions which were analysed pharmacologically to find out how closely they resembled the motor responses produced by nerve stimulation in the presence of antagonists of acetylcholine.

Methods

Vagus nerve/oesophagus and Remak nerve/rectum preparations

Vagus nerve/oesophagus and Remak nerve/rectum preparations were made from chicks aged 1-13 days as described by Bartlet & Hassan (1968a, 1971). The preparations were mounted in a 25 or 50 ml organ bath filled with Krebs solution gassed with 5% CO_2 in O_2 and maintained at 35° C. The nerves were stimulated through platinum electrodes for periods of 5 s with square wave pulses (1 ms, 30 Hz, 2-10 V) which were monitored. In those experiments in which the preparations were stimulated only indirectly the interval between trains of stimuli was 5 min, other preparations were exposed to agonists for periods of 30 s-2 min with an interval of 10 min between tests. The responses were recorded on smoked paper with an isotonic lever, load 1.5 g, which magnified the responses 10 or 20 times.

Drugs used

A bathing solution of the composition described by Krebs & Henseleit (1932) was made with ANALAR salts and de-ionized water. The drugs used were acetylcholine chloride (B.D.H.), adenosine (Sigma), adenosine 5′-diphosphate sodium salt (ADP) (Sigma), adenosine 5′-monophosphate sodium salt (AMP) (Sigma), adenosine 5′-triphosphate disodium salt (ATP) (Sigma), histamine acid phosphate (B.D.H.), 5-hydroxytryptamine creatinine sulphate (B.D.H.), (−)-hyoscine hydrobromide (B.D.H.), indomethacin (Merck, Sharp & Dohme), mepyramine maleate (May & Baker), methysergide bimaleate (Sandoz), (−)-noradrenaline bitartrate (Koch-Light), phentolamine mesylate (Ciba), polyphloretin phosphate-standard 4 (Leo 101K) (AB Leo), (±)-propranolol hydrochloride (I.C.I.), prostaglandin E_2 (Upjohn), strychnine hydrochloride (B.D.H.) and tetrodotoxin (Sigma). Prostaglandin E_2 (5 mg) was dissolved in 95% v/v ethanol (0.5 ml) and the solution diluted with 0.02% w/v Na_2CO_3 in 0.9% w/v aqueous NaCl (4.5 ml). Polyphloretin phosphate (1 g) was dissolved in 0.1 M NaOH (46 ml). The nucleotides of adenosine (200 mg) were dissolved in 0.9% w/v aqueous NaCl (10 ml) and the pH adjusted to 6.8. These solutions were stored frozen. Other drug solutions were freshly prepared. Adenosine was dissolved in 0.1 M HCl and the pH of the solution adjusted to 5.5. Indomethacin was dissolved in a few drops of 95% v/v ethanol and the solution diluted with Krebs solution. Solutions of the remaining drugs were made in 0.9% w/v NaCl solution (saline). The drug concentrations in the text, table and figures are concentrations of the above compounds in the organ bath.

Results

Responses to nerve stimulation in the presence of hyoscine

Submaximal responses of the vagus nerve/oesophagus preparation to nerve stimulation were only partly antagonized on exposure to hyoscine (Fig. 1) at a concentration which antagonized acetylcholine more than 250 times. No further antagonism of the residual contractions to nerve stimulation were demonstrable when the concentration of hyoscine was increased 10 or 20 fold. In some of the present experiments the actions of putative transmitters in the oesophagus have been compared to the response to nerve stimulation in the presence of hyoscine 10 μg/ml, a concentration that seemed to antagonize fully the responses to stimulation of the cholinergic fibres. In experiments with the Remak nerve/rectum preparation hyoscine (5-100 μg/ml) did not antagonize significantly the contractions produced by nerve stimulation (Fig. 2), thus in this preparation the responses to putative transmitters were compared with the contractions to nerve stimulation in the absence of hyoscine.

Actions of histamine and 5-hydroxytryptamine in the Remak nerve/rectum preparation

The actions of histamine and 5-hydroxytryptamine in the oesophagus preparation have been described by Bartlet & Hassan (1968b). In the rectum preparation histamine (0.02-0.1 μg/ml) produced contractions which were antagonized by mepyramine (0.01 μg/ml) with a dose-ratio of

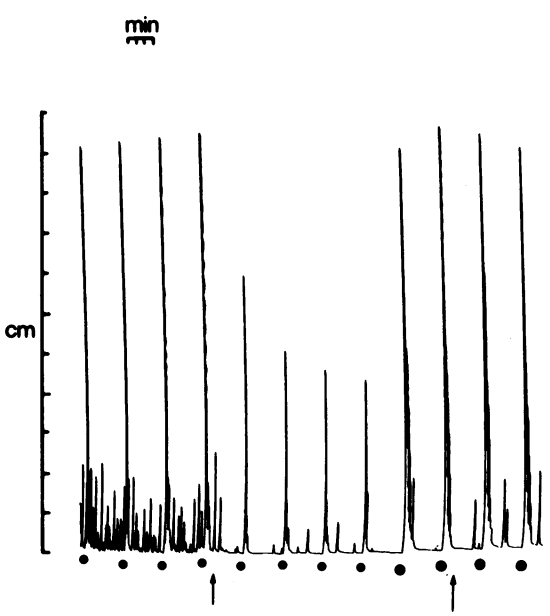


Fig. 1 Chicken vagus nerve/oesophagus preparation. The dots mark 5 s periods of nerve stimulation (1 ms, 30 Hz) at intervals of 5 min, the stimulus strength being 2 V at the smaller dots and 5 V at the larger dots. At the first arrow hyoscine (5 μg/ml) was added to the bathing solution and from the second arrow hyoscine (50 μg/ml) was present. Hyoscine (5 μg/ml) partly antagonized the contractions, and the residual responses were not further antagonized when the concentration of hyoscine was increased ten-fold. Magnification x 10.

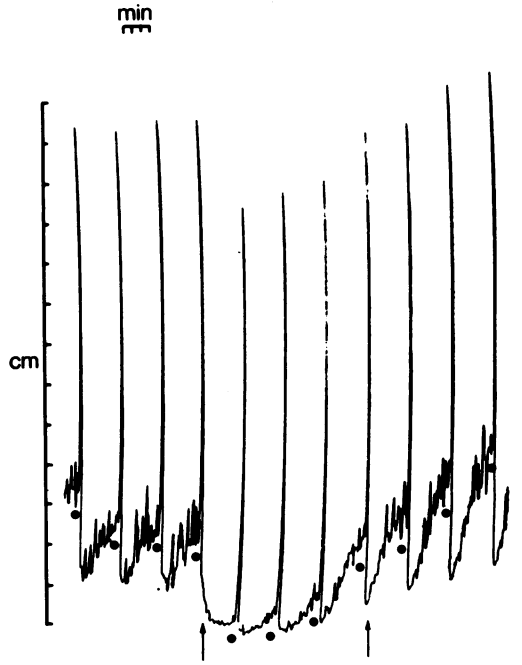


Fig. 2 Chicken Remak nerve/rectum preparation. The dots mark 5 s periods of nerve stimulation (1 ms, 30 Hz, 5 V) at 5 min intervals. At the first arrow hyoscine (10 μg/ml) was added to the bathing solution and from the second arrow hyoscine (100 μg/ml) was present. Hyoscine did not antagonize the contractions significantly. Magnification x 10.

7.5 ± 0.8 (three experiments). Hyoscine (0.02 μg/ml) did not antagonize the actions of histamine in the rectum preparation although at one twentieth of this concentration it antagonized acetylcholine 20 times (two experiments). Thus histamine did not stimulate cholinergic nerves in the chicken rectum as it did in preparations of oesophagus (Bartlet, 1968; Bartlet & Hassan, 1968b).

5-Hydroxytryptamine (0.05-0.25 μg/ml) produced a relaxation in the rectum which was not antagonized by a combination of propranolol (0.05 μg/ml) and phentolamine (0.1 μg/ml) (Figure 3). Thus the relaxation did not seem to be the outcome of a release of catecholamines. As the concentration of 5-hydroxytryptamine was increased the response became biphasic, a relaxation followed by a contraction, and eventually changed to a contraction only (Figure 4). The relaxations produced by 5-hydroxytryptamine were not antagonized by methysergide (0.01 μg/ml) (three experiments) or tetrodotoxin (0.1 μg/ml) (three experiments) or by a combination of these drugs (three experiments) (Figure 3). The contractions produced by 5-hydroxytryptamine at higher concentrations were not antagonized by methysergide (0.01 μg/ml) (two experiments), although 5-hydroxytryptamine acting in the oesophagus was antagonized more than 800 times by methysergide (0.001 μg/ml) (Bartlet & Hassan, 1968b). Thus methysergide seemed to have relatively little affinity for the 5-hydroxytryptamine receptors in the plain muscle of chicken rectum.

As methysergide did not antagonize 5-hydroxytryptamine acting in the Remak nerve/rectum preparation an attempt was made to desensitize the tissue specifically to this substance (Gaddum, 1953). When 5-hydroxytryptamine (2 μg/ml) was added to the bathing solution the Remak nerve/rectum preparation became unresponsive to further doses of this substance, and the contractions to nerve stimulation, but not acetylcholine were partly antagonized (four experiments) (Figure 5). Three concentrations of

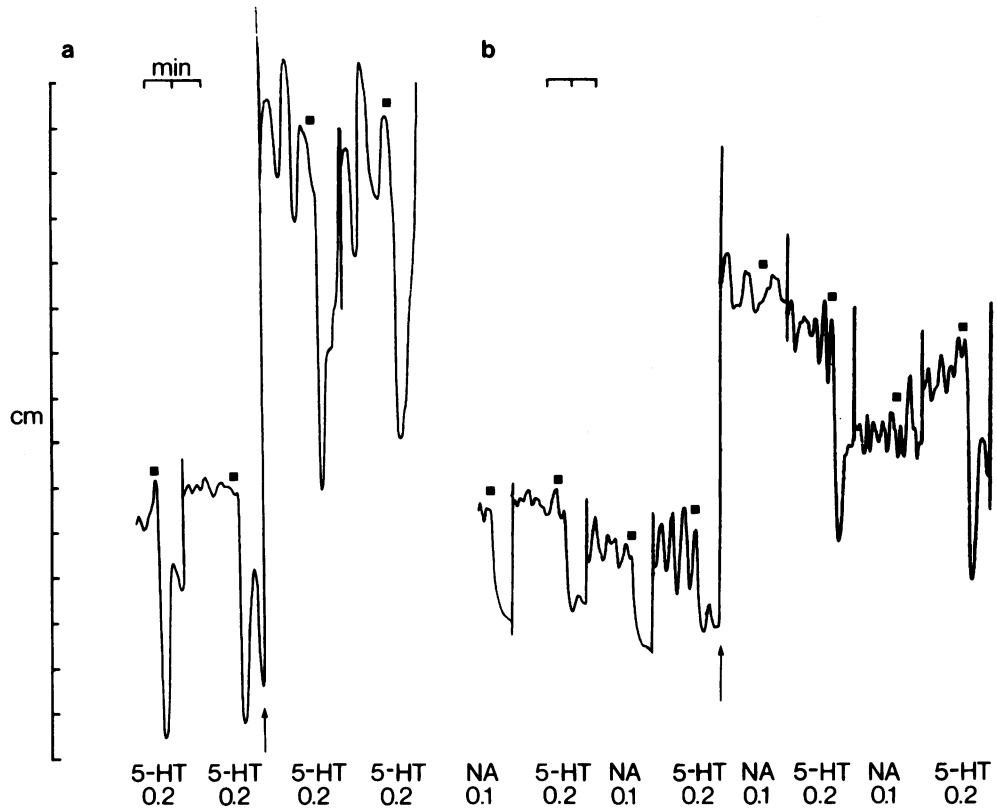
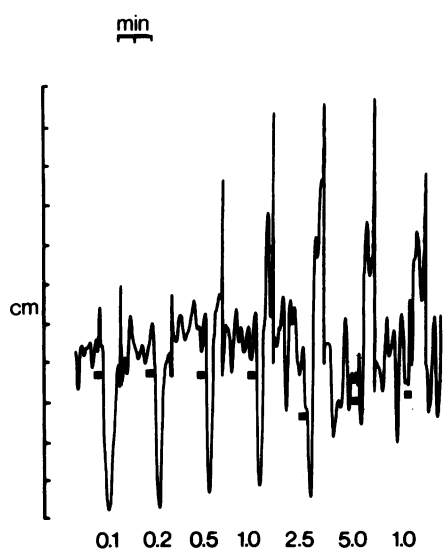


Fig. 3 Chick rectum, two preparations. Numbers refer to concentrations (μg/ml) of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) present in the organ bath for 1 min in every 10 minutes. In (a), the arrow marks the addition of tetrodotoxin (0.1 μg/ml) and methysergide (0.01 μg/ml) to the bathing solution, and in (b), phentolamine (0.1 μg/ml) and propranolol (0.05 μg/ml). Both combinations of antagonists raised the tone of the preparations and failed to block the responses to 5-hydroxytryptamine. Magnification x 10.



5-hydroxytryptamine were tested in random order in four Remak nerve/rectum preparations responding to nerve stimulation only. After 15 min exposure to 5-hydroxytryptamine, at concentrations of 0.2, 2.0 and 20 μg/ml, the contractions produced by stimulation of Remak's nerve were antagonized by 9.2 ± 4.7, 21.8 ± 1.5 and 46.4 ± 4.4%, respectively. The addition of 5-hydroxytryptamine (2 μg/ml) to the bathing solution also partly antagonized the responses of the oesophagus to nerve stimulation in the presence of hyoscine (10 μg/ml) and methysergide (0.01 μg/ml; two experiments).

Fig. 4 Chick rectum preparation. Numbers refer to concentrations of 5-hydroxytryptamine (5-HT) (μg/ml) which were present in the organ bath for 1 min in every 10 minutes. 5-Hydroxytryptamine in low concentration relaxed the preparation; as the concentration of the drug was increased a motor response was produced. Magnification x 10.

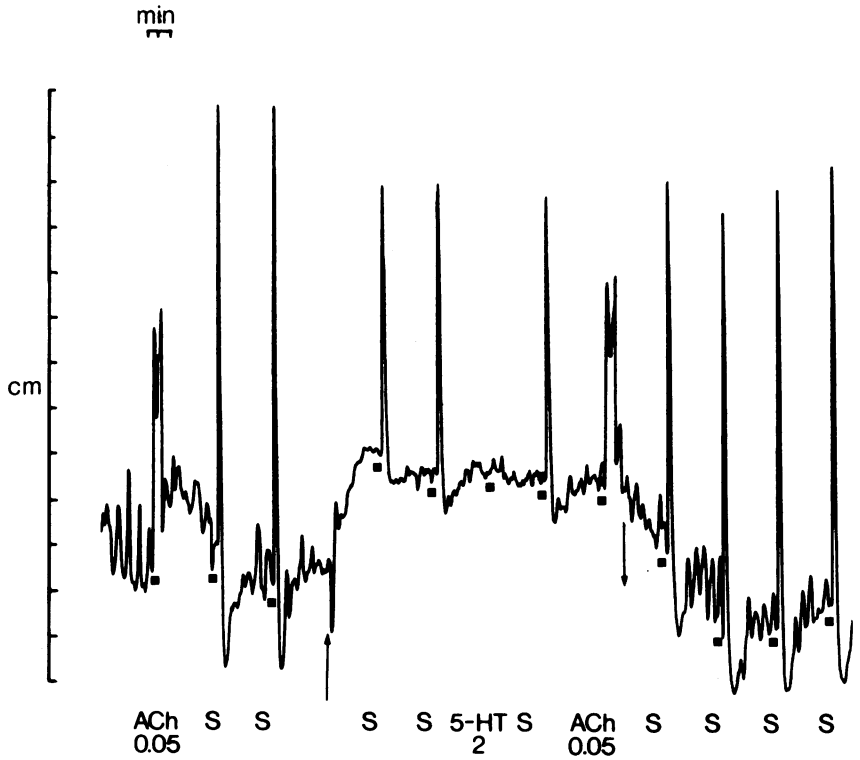


Fig. 5 Chicken Remak nerve/rectum preparation. The dots mark 5 s periods of nerve stimulation (S) (1 ms, 30 Hz, 5 V) or exposure of the preparation to acetylcholine (ACh) or 5-hydroxytryptamine (5-HT) for 1 min, with a 10 min interval between tests. Numbers refer to concentrations of agonists (μg/ml). Between the arrows 5-hydroxytryptamine (2 μg/ml) was present in the bathing solution. 5-Hydroxytryptamine desensitized the preparation to its own action, antagonized the response to nerve stimulation and did not affect that to acetylcholine. Magnification x 10.

Mepyramine (0.1 μg/ml) and methysergide (0.01 μg/ml) did not antagonize the contractions produced by nerve stimulation in the vagus nerve/oesophagus preparation (with hyoscine in the bathing solution) and Remak nerve/rectum preparation (two experiments in each instance).

Actions of adenosine, AMP, ADP and ATP in the vagus nerve/oesophagus preparation

Adenosine and its nucleotides produced similar contractions in the oesophagus. The contractions commenced after a delay of 10-30 s and developed fully in 1-2 min; after washing out the drugs the preparation required about 5 min for relaxation to the control tone. The potency of ATP was compared to that of adenosine, AMP and ADP, three preparations being used for each comparison. It was found that 1 μg ATP was equivalent to 0.49 ± 0.11 μg adenosine, 0.64 ± 0.07 μg AMP and 0.80 ± 0.11 μg ADP. A few preparations failed to respond to adenosine and its nucleotides although they exhibited a normal sensitivity to acetylcholine and 5-hydroxytryptamine.

The influence of some drugs on the actions of ATP, histamine, 5-hydroxytryptamine and acetylcholine in the oesophagus is summarized in Table 1. The action of ATP was abolished on exposure of the oesophagus to tetrodotoxin (0.1 μg/ml); washing the preparation producing a rapid recovery of the response (Figure 6). ATP acting in the oesophagus was antagonized by hyoscine (0.1 μg/ml) and strychnine (5 μg/ml). Histamine, which stimulates neural structures in the chicken oesophagus preparation (Bartlet & Hassan, 1968b), was also antagonized by tetrodotoxin, hyoscine and strychnine.

The effect of desensitization of the preparation to ATP on its responses to nerve stimulation was examined in the absence and presence of hyoscine. Exposure to ATP (500 μg/ml) selectively desensitized the preparation to the action of this

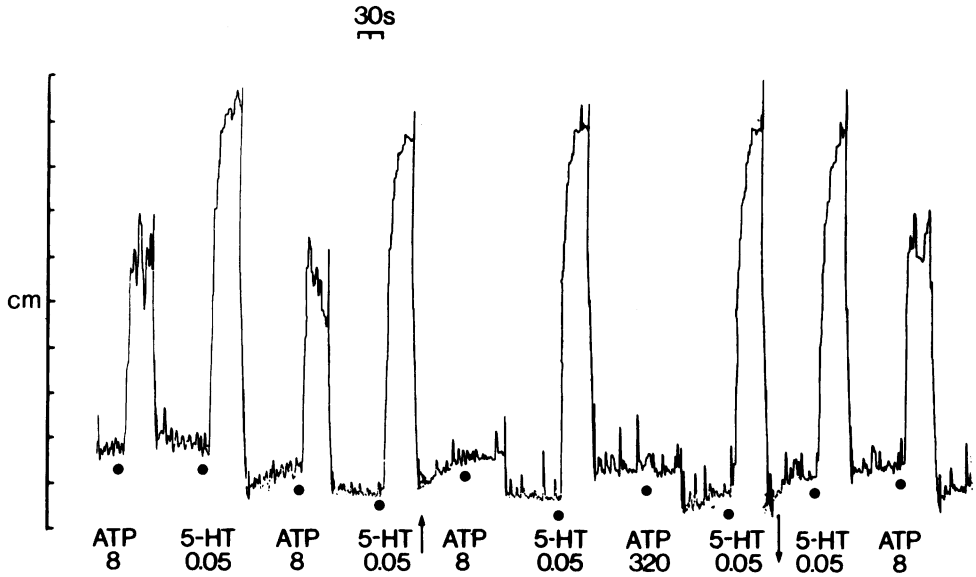


Fig. 6 Chicken post-crop oesophagus preparation. Numbers refer to concentrations (μg/ml) of adenosine triphosphate (ATP) and 5-hydroxytryptamine (5-HT) which were present in the organ bath for 1.5 min at intervals of 10 minutes. Between the arrows tetrodotoxin (0.1 μg/ml) was present in the bathing solution. The response to ATP was abolished by tetrodotoxin and recovered on washing. Magnification x 10.

substance, the responses to nerve stimulation being potentiated (two experiments) or unaffected (two experiments) and those to acetylcholine little affected (Figure 7). ATP has little action in the oesophagus in the presence of hyoscine, nevertheless four preparations contracting to nerve stimulation and 5-hydroxytryptamine in the presence of hyoscine (10 μg/ml) were exposed to ATP (1,500 μg/ml). The ATP antagonized 5-hydroxytryptamine in three of the preparations but did not affect the responses to nerve stimulation.

Actions of adenosine, AMP, ADP and ATP in the Remak nerve/rectum preparation

Adenosine and AMP produced relaxations and ADP and ATP contractions in the rectum preparation (Figure 8). ATP and ADP produced fast contractions which commenced within 2 s of the addition of the drug to the bathing solution and reached a maximum in 10-20 s; on further exposure the preparation relaxed partly and then sometimes began to contract slowly. ATP (1 μg) was found to be equipotent with 83 ± 24 μg ADP

Table 1 Antagonism of adenosine 5'-triphosphate (ATP), histamine, 5-hydroxytryptamine and acetylcholine in the chick oesophagus preparation

		Dose-ratio*			
Antagonist	*Concentration*	*ATP*	*Histamine*	*5-Hydroxytryptamine*	*Acetylcholine*
Tetrodotoxin	0.1 μg/ml	>20 (4)	15.6 ± 4.7 (3)	no antagonism (4)	no antagonism (3)
Hyoscine	0.1 μg/ml	3, 6	5 (2)	no antagonism (2)	>500, >150
Strychnine	5.0 μg/ml	4.5 ± 0.5(3)	1.8 ± 0.1 (3)	potentiation (3)	no antagonism (4)

Dose-ratios were measured when the antagonism became steady and are given as means and s.e. mean except where only one or two observations were made. The number of observations is given in parentheses.
* Gaddum, Hameed, Hathway & Stephens, 1955.

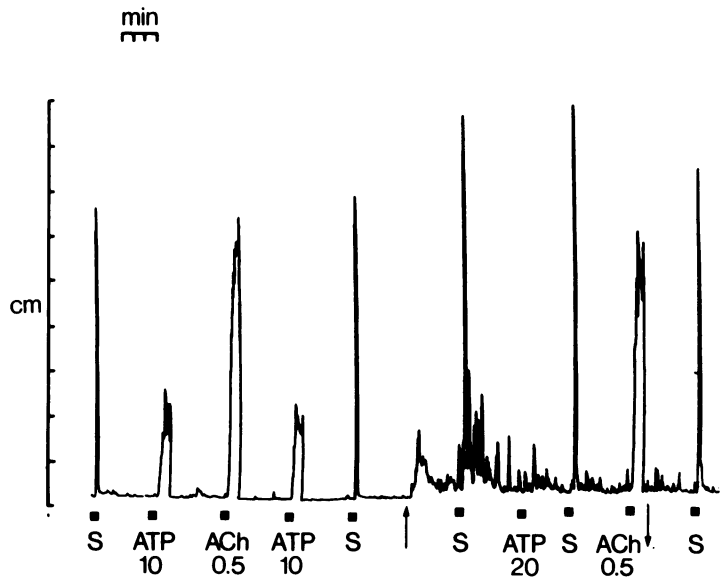


Fig. 7 Chicken vagus nerve/oesophagus preparation. The dots mark exposure of the preparation to adenosine triphosphate (ATP) or acetylcholine (ACh) for 1 min or nerve stimulation (S) (1 ms, 30 Hz, 3 V) for 5 seconds. Numbers refer to concentrations of agonists present in the organ bath (μg/ml). Between the arrows ATP, 500 μg/ml, was present in the bathing solution. ATP desensitized the preparation to its own action, potentiated the response to nerve stimulation and did not affect that to acetylcholine. Magnification x 10.

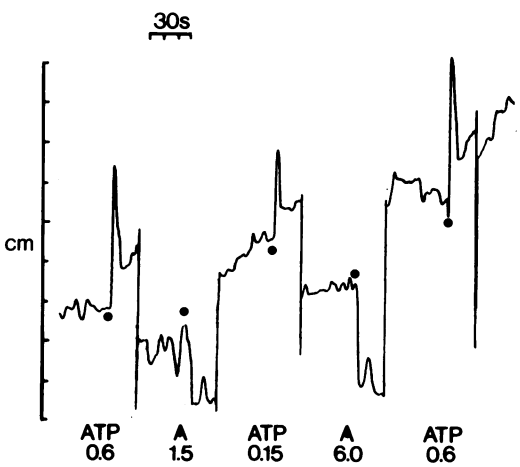


Fig. 8 Chick rectum preparation. Numbers refer to concentration (μg/ml) of adenosine (A) and adenosine triphosphate (ATP) present in the organ bath for 1 min in every 10 minutes. ATP produced a contraction and adenosine relaxed the preparation. Magnification x 10.

(three experiments) in the production of fast contractions in the rectum preparation. The relaxations produced by adenosine and AMP continued until the drugs were removed from the bathing solution.

The action of ATP in the rectum preparation was not antagonized by hyoscine (0.1 μg/ml) or strychnine (5 μg/ml; three experiments in each instance). Tetrodotoxin (0.1 μg/ml), which produced a large increase in the tone in the rectum, did not affect the action of ATP markedly.

ATP (1,000 μg/ml) was added to the solution bathing five Remak nerve/rectum preparations contracting to nerve stimulation, acetylcholine and ATP. In the presence of ATP at this high concentration the responses to acetylcholine and nerve stimulation were antagonized to about the same extent and that to the initial stimulant dose of ATP abolished (Figure 9). The effect of exposure to ATP was reversed rapidly when the drug was removed from the bathing solution.

Actions of prostaglandin E_2 in vagus nerve/oesophagus and Remak nerve/rectum preparations

Prostaglandin E_2 (0.2-1.0 μg/ml) produced slow contractions in preparations of oesophagus. Most preparations were exposed to prostaglandin E_2 for

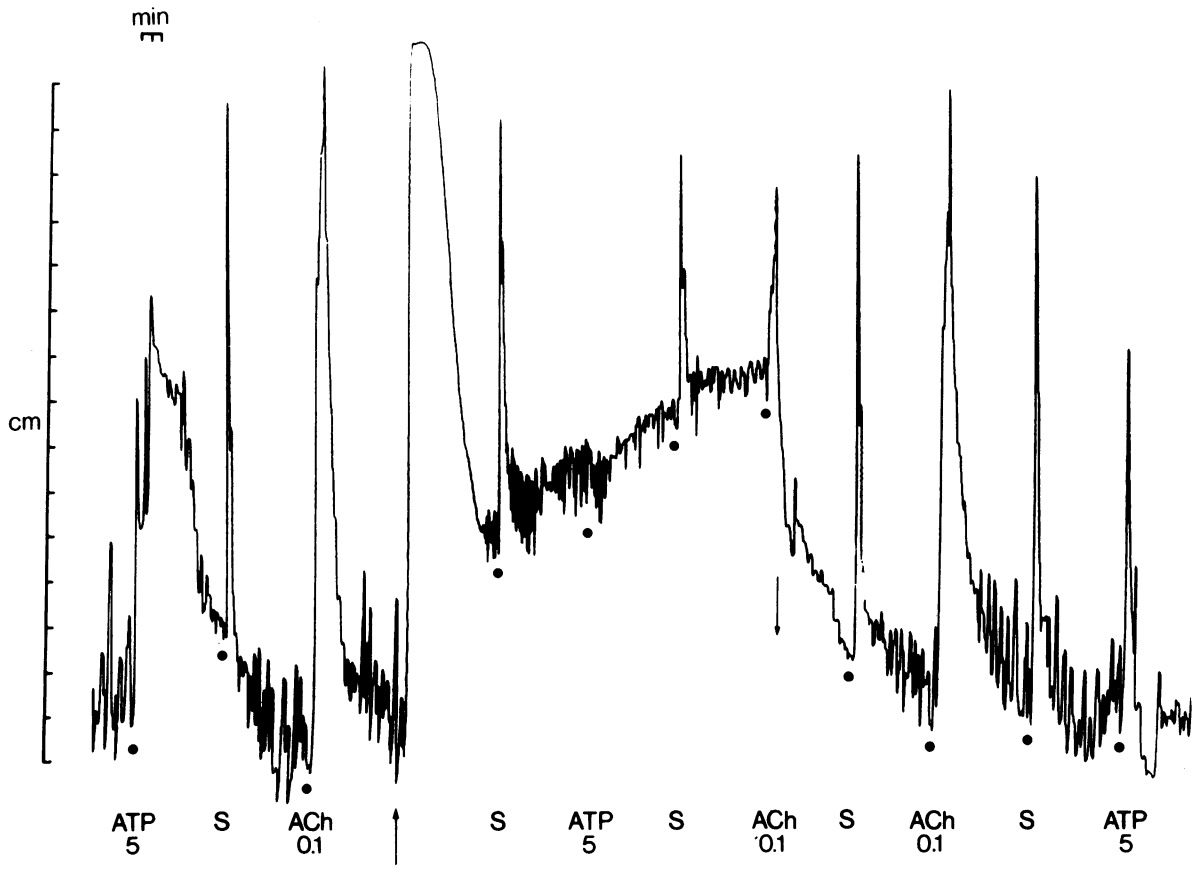


Fig. 9 Chicken Remak nerve/rectum preparation. The dots mark 5 s periods of nerve stimulation (S) (1 ms, 30 Hz, 5 V) or exposure of the preparation to adenosine triphosphate (ATP) or acetylcholine (ACh) for 1 min, with a 10 min interval between tests. The preparation contracted spontaneously after washing out the first dose of ATP. Numbers refer to concentrations of agonists present in the organ bath (μg/ml). Between the arrows ATP (1,000 μg/ml) was present in the bathing solution. ATP desensitized the preparation to its own action and antagonized the responses to ACh and nerve stimulation to about the same extent. Magnification x 10.

2 min and required 5 min for relaxation to the control tone after washing out the drug. The action of prostaglandin E_2 in the oesophagus was not antagonized by tetrodotoxin (0.1 μg/ml; two experiments) or polyphloretin phosphate (30-60 μg/ml; four experiments), and polyphloretin phosphate did not affect the spontaneous movements in this preparation. Exposure of vagus nerve/oesophagus preparations to polyphloretin phosphate (60 μg/ml; two experiments) or indomethacin (20 μg/ml; three experiments) for an hour did not antagonize the contractions produced by nerve stimulation in the presence of hyoscine (10 μg/ml).

The rectum preparation was more sensitive to prostaglandin E_2 than the oesophagus and responded to the drug at concentrations of 0.002-0.05 μg/ml, the time course of the contractions being similar to those in the oesophagus preparation (Figure 10). Tetrodotoxin (0.1 μg/ml) did not antagonize the action of prostaglandin E_2 on the rectum preparation (three experiments). Polyphloretin phosphate (10 μg/ml) inhibited the spontaneous movements in the rectum preparation and antagonized the actions of both prostaglandin E_2 and acetylcholine (six experiments; Figure 10). The antagonism of prostaglandin E_2 was insurmountable, the slow contraction becoming rapid and poorly sustained when the concentration of the agonist was increased in the presence of the antagonist. In most preparations the control responses to prostaglandin E_2 were not fully antagonized by polyphloretin phosphate (10 μg/ml), and it seemed

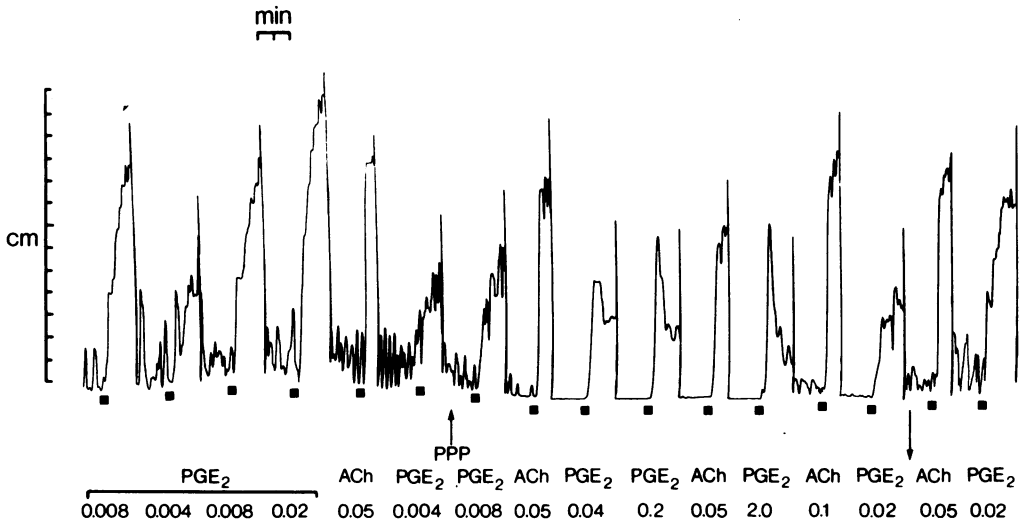


Fig. 10 Chick rectum preparation. Numbers refer to organ bath concentrations (μg/ml) of prostaglandin E_2 (PGE_2) and acetylcholine (ACh). The preparation was exposed to prostaglandin E_2 for 2 min or acetylcholine for 1 min at intervals of 10 minutes. Between the arrows polyphloretin phosphate (PPP, 10μg/ml) was present in the bathing solution. Polyphloretin phosphate antagonized effects of both agonists, the antagonism of prostaglandin E_2 being insurmountable. Magnification x 10.

improbable that a strong and specific antagonism would be obtained with this antagonist at a lower concentration. The antagonism of acetylcholine was weak; the dose-ratio was 2.5 ± 0.3 (6). Exposure of Remak nerve/rectum preparations to polyphloretin phosphate (60 μg/ml; two experiments) or indomethacin (100 μg/ml; three experiments) for an hour did not produce any antagonism of the contractions to nerve stimulation.

Discussion

Although mepyramine antagonized the effects of histamine on plain muscle and neural structures in the oesophagus preparation (Bartlet & Hassan, 1968b) and plain muscle in the rectum, it did not affect the contractions produced by nerve stimulation. Thus it seems improbable that histamine is a transmitter in synapses or neuromuscular junctions in these preparations.

In the chicken oesophagus preparation 5-hydroxytryptamine stimulates plain muscle and this action is antagonized by methysergide (Bartlet & Hassan, 1968b). Since methysergide did not antagonize the hyoscine-resistant responses of the oesophagus to nerve stimulation however, 5-hydroxytryptamine is unlikely to be a transmitter in this preparation. Desensitization of the Remak nerve/rectum preparation to 5-hydroxytryptamine partly antagonized the contractions to nerve stimulation. If the antagonism of the response to nerve stimulation was the outcome of desensitization of 5-hydroxytryptamine receptors in the plain muscle, one might postulate that 5-hydroxytryptamine was the transmitter at neuromuscular junctions in the rectum preparation. The antagonism of the response to nerve stimulation seems, however, to be an outcome of an action of 5-hydroxytryptamine in the neural structures, as this substance similarly antagonized contractions to nerve stimulation in the chicken vagus nerve/oesophagus preparation (with hyoscine and methysergide in the bathing solution), in which preparation 5-hydroxytryptamine has no demonstrable methysergide-resistant action in the muscle. A further possibility was that 5-hydroxytryptamine might be a transmitter at synapses, but this is also unlikely since a tetrodotoxin-sensitive response to this substance could not be demonstrated in either preparation.

In the experiments with adenosine and its nucleotides only ATP and ADP produced contractions in both the oesophagus and rectum preparations. In the rectum preparation ATP was about 100 times as potent as ADP in the production of fast contractions which were not antagonized by tetrodotoxin, hyoscine or strychnine, indicating that these nucleotides stimulated the plain muscle directly. In the

oesophagus preparation ATP was less potent than adenosine, AMP and ADP in the production of slow contractions which were antagonized by tetrodotoxin, hyoscine and strychnine. These observations suggest that the plain muscle in the oesophagus, unlike that in the rectum, was insensitive to adenosine and its nucleotides, and that the oesophagus preparation responded to these substances when they had diffused through the outer tissue and reached neural structures. The relative potencies of these substances in the oesophagus approximates to their adenosine content, and may be an outcome of hydrolysis of the nucleotides in diffusion to their receptors.

According to Burnstock (1972) ATP is a transmitter in many peripheral nerves. In the present experiments however, desensitization of the tissues to ATP failed to produce a selective inhibition of the responses of the preparations to nerve stimulation. This observation seems to exclude the possibility that ATP might be a transmitter in the chicken vagus nerve/oesophagus and Remak nerve/rectum preparations.

Prostaglandin E_2 produced slow contractions in the oesophagus and rectum which were not antagonized by tetrodotoxin, indicating that the muscle was directly stimulated by the drug. It was interesting to note that polyphloretin phosphate antagonized both the spontaneous movements and the action of prostaglandin E_2 in the rectum preparation but antagonized neither in the oesophagus preparation. This observation supports the hypothesis that a prostaglandin regulates spontaneous motor activity in certain organs (Bennett & Posner, 1971; Ferreira, Herman & Vane, 1972; Davison, Ramwell & Willis, 1972). Prostaglandin synthesis and release is blocked by indomethacin (Vane, 1971; Smith & Willis, 1971; Ferreira, Moncada & Vane, 1971). Exposure of vagus nerve/oesophagus and Remak nerve/rectum preparations to indomethacin or polyphloretin phosphate, however, did not antagonize the contractions produced by nerve stimulation. Thus it seems improbable that a prostaglandin is a transmitter in these preparations.

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