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Contractile Responses of Chicken Rectum to Stimulation of Remak's Nerve or Purinoceptors: Effect of Suramin

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Abstract—Stimulation of Remak's nerve produced a rapid contraction of the rectal muscle which was resistant to blockade by hyoscine, followed by a slow contractile response which was cholinergic. With hyoscine (1 μM) in the Tyrode solution, the non-cholinergic contractile response to nerve stimulation was compared with the responses to adenosine triphosphate (ATP) and α,β-methylene adenosine triphosphate (α,β-Me ATP). In the presence of suramin (300 μM), the contractile response to ATP was increased by about 100%, whereas that to nerve stimulation was inhibited by approximately 17%. Suramin (60 μM) also discriminated between the contractile responses to ATP and nerve stimulation, only the former being potentiated by the drug. It seems probable that suramin potentiated the action of ATP in the rectum through inhibition of ectonucleotidase activity. If so, the potentiation should not extend to α,β-Me ATP, as this analogue of ATP is resistant to inactivation by the enzyme. Suramin inhibited the contractile response to α,β-Me ATP. Thus the neurotransmitter which mediated the hyoscine-resistant contractile response to stimulation of Remak's nerve was dissimilar to ATP, in that it was not potentiated by suramin and presumably was not inactivated by ectonucleotidase activity.

The contractile response of the chicken rectum to stimulation of Remak's nerve is not completely abolished by hyoscine or atropine, and when the nerve is stimulated for 5 s only, the response is usually unaffected by these drugs. It was concluded that stimulation of Remak's nerve liberates two motor transmitters—an unidentified substance and acetylcholine (Bartlet & Hassan 1971; Bartlet 1974).

It has been suggested that adenosine triphosphate (ATP) may be the neurotransmitter responsible for atropine-resistant contractile responses in many tissues (Burnstock 1972). The time course of the contractile responses of chicken rectum to ATP and electrical field stimulation in the presence of atropine are very similar. Moreover, following desensitization of purinoceptors with α,β-methylene adenosine triphosphate (α,β-Me ATP) (10 μM), contractile responses to ATP or nerve stimulation in the presence of atropine were reported to be abolished, whereas the response to carbachol was unaffected (Meldrum & Burnstock 1985). There is evidence which suggests, however, that desensitization of the chicken rectum with α,β-Me ATP is not specific for purinoceptors. Komori et al (1988) found that prolonged exposure of the chicken rectum to α,β-Me ATP (0·2 or 4 μM) inhibited the contractile responses to ATP, neurotensin and K^+. They suggested that the effect of α,β-Me ATP was due mainly to a change in the electrical properties of the membrane of the smooth muscle cells. It was concluded that α,β-Me ATP is an unsatisfactory substance for investigating whether or not ATP is the neurotransmitter mediating non-cholinergic motor transmission in the Remak nerve/chicken rectum preparation.

The experiments presented here were undertaken to characterize the effect of suramin in the Remak nerve/chicken rectum preparation. Previous studies with this drug have shown that it is an antagonist at P_2 purinoceptors (Dunn & Blakely 1988). Furthermore, Hourani & Chown (1989) found that suramin (10 mM) inhibited the degradation of ATP incubated with strips of guinea-pig urinary bladder, and attributed this to inhibition of ectonucleotidase activity. Although suramin has these potentially opposing effects on the action of ATP, it should not discriminate between the hyoscine-resistant contractile responses to nerve stimulation and ATP if the former is purinergic.

Materials and Methods

Remak's nerve/chicken rectum preparation

These were made from J-line chickens, 3–14 days old (Institute of Animal Physiology & Genetics Research, Roslin, Midlothian, UK), which were killed by dislocation of the neck, and bled. The Remak's nerve/rectum preparation was made as described by Bartlet & Hassan (1971), and mounted in an organ-bath filled with Tyrode solution (29°C) gassed with 5% CO_2–95% O_2. For recording the contractile responses of the longitudinal muscle, the rectum was attached to an isotonic transducer (load, 600 mg) and a potentiometric recorder. Remak's nerve was pulled through platinum ring electrodes for stimulation with trains of 0·5 ms square wave pulses for 5 s, at 20 Hz and 10 V. In the experiments investigating the hyoscine-sensitive component of the response to nerve stimulation, the duration of the trains of pulses was increased to 60 s. The responses of the rectum to nerve stimulation were abolished by tetrodotoxin (1 μM). In some experiments the smooth muscle was stimulated directly by exposure to ATP or α,β-Me ATP for 30 s, or neurotensin for 45 s. The interval between successive trains of nerve stimulations or exposures to agonists was 8 min. Before starting an experiment, an equilibrium period of 48 min was allowed during which time the preparation was washed with Tyrode solution every 8 min.

The composition of the Tyrode solution (mM) was as follows: NaCl 136·9, KCl 2·7, NaH_2PO_4 0·4, $CaCl_2$ 1·8, $MgCl_2$ 2·5, $NaHCO_3$ 11·9 and glucose 5·6. It was made with Analar salts and deionized water. Responses to catecholamines released by electrical stimulation of Remak's nerve

were excluded by inclusion of propranolol (0·17 μM) and phentolamine (0·27 μM) in the Tyrode solution (Bartlet & Hassan 1970). Hyoscine (1 μM) was present in the Tyrode solution, except in experiments to demonstrate the hyoscine-sensitive component in the contractile response to nerve stimulation.

Dose ratios

Dose ratios (Gaddum et al 1955) were estimated for ATP and α,β-Me ATP acting on the chicken rectum in the presence of suramin. Each preparation was exposed to ATP and α,β-Me ATP for 30 s at intervals of 8 min. After obtaining three reproducible control responses to each agonist, suramin was added to the organ-bath to produce a final drug concentration of 60 or 300 μM in the Tyrode solution. The control concentrations of ATP and α,β-Me ATP were then reduced or increased, respectively, to obtain contractile responses in the presence of suramin which matched the controls. The responses obtained after 30 min exposure to suramin were used for estimating the dose ratios. It was impracticable to expose the preparation to suramin for a longer period, because of development of spontaneous activity.

Drugs

The drugs used and their sources were: adenosine triphosphate (Sigma, UK), α,β-methylene adenosine-5-triphosphate (Sigma), hyoscine hydrobromide (BDH, UK), neurotensin (Sigma), nicotine acid tartrate (Sigma), phentolamine mesylate (Ciba, UK), propranolol hydrochloride (ICI, UK), suramin (Bayer, UK), tetrodotoxin (Sigma), and tubocurarine chloride (Sigma). Suramin was dissolved in 0·9% w/v aqueous NaCl (saline) at the time of use. The other drugs were dissolved in distilled water, stored at −20°C and diluted with saline just before use.

Table 1. Effect of suramin on responses of chicken rectum to ATP and α,β-Me ATP.

	Dose ratios in the presence of suramin		*P* Comparison of suramin at 60 and 300 μM
	60 μM	300 μM	
α,β-Me ATP	1·31 ± 0·04 (3)	8·75 ± 1·14 (6)	< 0·01
ATP	0·14 ± 0·01 (3)	0·18 ± 0·04 (6)	> 0·6

Values are the mean ± s.e.m. of the dose ratios obtained from the number of experiments shown in parentheses.

Statistical analysis

The values in the text and Table 1 are expressed as the mean ± s.e.m. The significance of a difference between means was estimated by Student's *t*-test and probability (*P*) values are given. A value of $P < 0.05$ was regarded as significant.

Results

Effect of acetylcholine antagonists

When Remak's nerve was stimulated for 1 min with 0·5 ms pulses at 20 Hz, a biphasic contractile response was obtained (Fig. 1). A rapid contraction was produced within 10 s, and

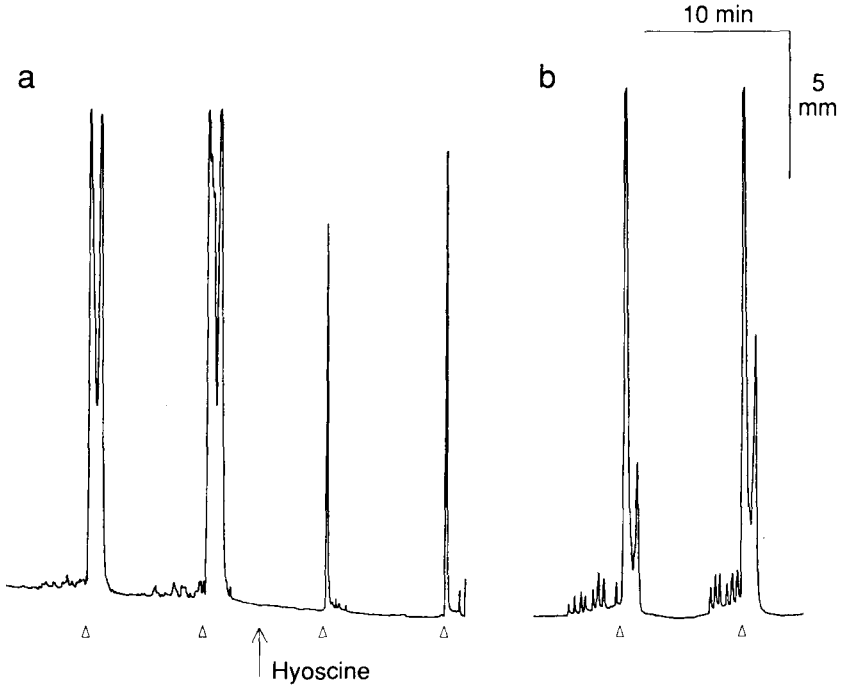

FIG. 1. Effect of hyoscine on the response of a chicken rectum preparation to stimulation of Remak's nerve. At △, the nerve was stimulated with 0·5 ms pulses at 20 Hz for 1 min, the interval between periods of stimulation being 8 min. The bath fluid was changed after each period of stimulation. In a, the arrow marks the addition of hyoscine (1 μM) to the bath fluid. Between a and b, hyoscine was removed from the organ bath and 48 min elapsed. In the presence of hyoscine, the fast contractile response to nerve stimulation was little affected, whereas the slow contractile response was abolished. The slow contractile response began to recover when hyoscine was removed from the bath fluid. The Tyrode solution contained propranolol (0·17 μM) and phentolamine (0·27 μM) throughout the experiment. Calibrations for time and mm of contraction in the rectal muscle.

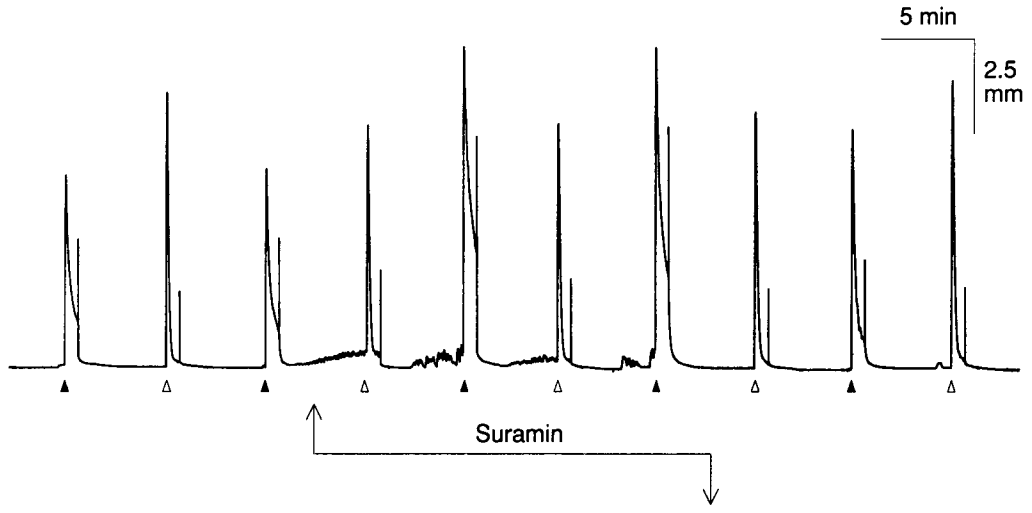

FIG. 2. Effect of suramin on the responses of a chicken rectum preparation to ATP and nerve stimulation in the presence of hyoscine. At △ Remak's nerve was stimulated with 0·5 ms pulses at 20 Hz for 5 s. At ▲ the preparation was exposed to ATP (16·5 μM) for 30 s. The interval between stimuli was 8 min, and the bath fluid was changed after each period of stimulation. The Tyrode solution contained hyoscine (1 μM), propranolol (0·17 μM) and phentolamine (0·27 μM) throughout the experiment. Suramin (300 μM) was present in the bathing solution between the arrows. It potentiated the response to ATP and slightly inhibited that to nerve stimulation. Calibration for time and mm of contraction of the rectal muscle.

before the preparation had relaxed completely a slow contractile response ensued and continued to develop until cessation of the stimuli. When hyoscine (1 μM) was added to the bathing solution, the height of the rapid contractile response was inhibited transiently and then recovered to the control height in the presence of the antagonist. The slow contractile response was abolished by hyoscine, and only recovered slowly after the antagonist was removed from the bath fluid. In two other preparations, the response to nerve stimulation and the effect of hyoscine were similar to that shown in Fig. 1.

The contractile response to stimulation of Remak's nerve was abolished by tubocurarine (20 μM) or nicotine (20 μM), and recovered slowly when the ganglion blocking agent was removed from the bath fluid.

Effect of suramin

Fig. 2 shows the effect of suramin on contractile responses of the rectum to ATP (16·5 μM) or stimulation of Remak's nerve in the presence of hyoscine (1 μM). Suramin (300 μM) potentiated the response to ATP and inhibited that to nerve stimulation. The effect of suramin was established rapidly, and the responses to ATP and nerve stimulation were reproducible in the presence of the drug. When suramin was removed from the bath fluid, the effect of the drug was reversed. In three other experiments, suramin discriminated between the contractile responses to ATP and Remak's nerve stimulation in the presence of hyoscine, as illustrated in the experimental record shown in Fig. 2. After 24 or 32 min exposure to suramin (300 μM), the contractile responses to ATP and nerve stimulation were 195·5 ± 26·3% (four experiments) and 83·5 ± 3·5% (four experiments), respectively, of the corresponding control responses.

The potentiation of ATP by suramin may have been an outcome of inhibition of ectonucleotidase activity in the preparation (Hourani & Chown 1989). This possibility was investigated by comparing the effect of suramin on responses to ATP (25 μM) and α,β-Me ATP (0·25 μM), which is a methylene isostere of ATP that is resistant to inactivation by dephosphorylation (Welford et al 1987).

Dose ratios for ATP and α,β-Me ATP acting on the rectum in the presence of suramin are shown in Table 1. Suramin antagonized the contractile response to α,β-Me ATP. The dose ratio for α,β-Me ATP increased 6·7-fold as the concentration of suramin was raised from 60 to 300 μM ($P < 0·01$). In contrast, suramin potentiated the response to ATP about 6-fold. The magnitude of this effect was similar in the presence of suramin at 60 or 300 μM ($P > 0·6$).

The possibility that suramin, at concentrations less than 300 μM, might potentiate the hyoscine-resistant response of the rectum to nerve stimulation, was investigated in preparations responding to stimulation of Remak's nerve or neurotensin (2·5 nM). Suramin (100 μM) did not affect the responses of the preparation (two experiments). In three experiments, suramin (60 μM) did not affect the contractile responses to nerve stimulation or neurotensin. In two other experiments, however, suramin (60 μM) produced an increase in the tone and spontaneous contractile activity in the preparation, together with potentiation of the contractile responses to both neurotensin and nerve stimulation.

In four preliminary experiments, it was shown that prolonged exposure of preparations to neurotensin (1·25 μM) did not antagonize the contractile response to nerve stimulation in the presence of hyoscine, although contractile responses to neurotensin (2·5 nM or 1·25 μM) were abolished. Thus the non-cholinergic motor transmitter does not combine with neurotensin receptors in chicken rectal muscle.

Discussion

Effect of acetylcholine antagonists

The contractile response of the rectal muscle to stimulation

of Remak's nerve was abolished by tubocurarine or nicotine, demonstrating that it was produced by stimulation of cholinergic preganglionic fibres in the nerve. Remak's nerve stimulation produced a rapid contractile response which was resistant to blockade by hyoscine, followed by a slower contractile response which was abolished by the drug. Thus, although the postganglionic motor fibres in Remak's nerve are not wholly cholinergic it would be incorrect to describe them as non-cholinergic.

The biphasic nature of the contractile response of the chicken rectum to stimulation of Remak's nerve is similar to the contractile responses of rat or guinea-pig urinary bladder to intramural nerve stimulation (Brading & Williams 1990). In each instance, a rapid contractile response which is non-cholinergic is followed by a cholinergic contractile response. The significance of this is unclear, although it has been suggested that co-transmission mediated by two transmitters released from the same neuron may occur (Campbell 1987).

Effect of suramin

Suramin (10 mM) inhibits the inactivation of ATP by strips of guinea-pig urinary bladder (Hourani & Chown 1989). This was attributed to inhibition of ectonucleotidase activity, and it was predicted that this would potentiate the pharmacological actions of ATP. In further experiments, however, the limited availability of suramin only allowed the drug to be tested at one hundredth of the concentration which inhibited inactivation of ATP. This was insufficient to potentiate the contractile response of urinary bladder to ATP, and so a pharmacological demonstration of inhibition of ectonucleotidase activity was not obtained (Hourani & Chown 1989).

In the present experiments, however, the contractile response of chicken rectum to ATP was potentiated strongly by suramin (300 μM), which suggests that the drug inhibited ectonucleotidase activity in the preparation. This interpretation was supported by the observation that suramin did not potentiate the contractile response to α,β-Me ATP, an analogue of ATP which is resistant to inactivation by ectonucleotidase activity (Welford et al 1987). Thus the present experiments provide pharmacological support for the view that suramin is an inhibitor of ectonucleotidase activity (Hourani & Chown 1989).

Suramin was an effective antagonist of α,β-Me ATP in the rectum. Thus the drug must have had a dual effect on the contractile response to ATP, potentiating and antagonizing it by inhibition of ectonucleotidase activity and P_2 purinoceptors, respectively. Suramin, at concentrations of 60 or 300 μM, potentiated the response to ATP to the same extent. Thus, increases in the potentiation and antagonism of ATP produced by the increase in suramin concentration, seemed to counteract each other.

Suramin (300 μM) discriminated between the contractile responses of the rectum to ATP and stimulation of Remak's nerve in the presence of hyoscine, the latter being antagonized by the drug. Thus the neurotransmitter mediating the hyoscine-resistant contractile response to nerve stimulation was unlike ATP, in that it was insensitive to potentiation by suramin and most probably not inactivated by ectonucleotidase activity. It could be reasoned, however, that the effect of suramin (300 μM) was difficult to interpret because of its dual effect on the response to ATP. Suramin (60 μM) produced a minimal effect on purinoceptors (dose ratio for α,β-Me ATP, 1·3), however, and potentiated ATP sevenfold. In spite of this, suramin (60 μM) did not selectively potentiate the contractile response of the rectum to stimulation of Remak's nerve in the presence of hyoscine. These observations seem inconsistent with the notion that Remak's nerve is purinergic. The putative role of ATP as a neurotransmitter mediating atropine-resistant contractions in chicken rectum has also been brought into question by Komori & Ohashi (1988). They noted that in chicken rectal muscle exposed to atropine, excitatory junction potentials produced by stimulation of the intramural nerves were normal, at a time when purinoceptors were desensitized to ATP.

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