Br. J. Pharmac. (1979), **67**, 301–308

THEOPHYLLINE ANTAGONIZES SOME EFFECTS OF PURINES IN THE INTESTINE BUT NOT THOSE OF INTRAMURAL INHIBITORY NERVE STIMULATION

R.C. SMALL & A.H. WESTON

Department of Pharmacology, Materia Medica and Therapeutics, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT

1 Hyoscine- and guanethidine-treated preparations of longitudinal muscle of rabbit duodenum, guinea-pig taenia caeci and fundic strip relaxed when exposed to noradrenaline, adenosine, adenosine triphosphate (ATP) or to field stimulation of their intramural nerves.

2 In guinea-pig taenia caeci and fundus, theophylline 100 μmol/l had no effect on responses to noradrenaline, adenosine, ATP and intramural nerve stimulation.

3 In rabbit duodenum, theophylline 100 μmol/l antagonized some responses to adenosine but had no effect on responses to noradrenaline, ATP and intramural nerve stimulation.

4 Theophylline 1 mmol/l itself relaxed the intestinal tissues and in the fundic strip and taenia caeci, these relaxant effects were associated with abolition of spike activity and cellular hyperpolarization. In the taenia caeci, the amplitude of inhibitory post-junctional potentials was reduced.

5 Theophylline 1 mmol/l antagonized the twitch suppression produced by adenosine and ATP in the transmurally-stimulated guinea-pig ileum but not that evoked by noradrenaline.

6 It is concluded that theophylline can selectively antagonize some actions of purines in the intestine but that it does not specifically antagonize the effects of intramural inhibitory nerve stimulation.

Introduction

The evidence that intestinal smooth muscle is innervated by inhibitory fibres which are not adrenergic has recently been reviewed by Small & Weston (1979a). The possibility that such neurones might utilize adenosine triphosphate (ATP) or a related nucleotide as a transmitter was suggested by Burnstock, Campbell, Satchell & Smythe (1970). However, subsequent attempts to assess the validity of this 'purinergic' hypothesis have been frustrated by the absence of a selective antagonist of purine action on smooth muscle. Such an agent would be a powerful analytical tool, provided that it could selectively antagonize the actions of exogenous purines on smooth muscle receiving non-adrenergic inhibitory innervation, without itself influencing smooth muscle tone.

Ally & Nakatsu (1976) showed that theophylline could antagonize the relaxant effects of adenosine in rabbit duodenum at concentrations which altered neither the tone of the tissue nor its sensitivity to adrenaline. This observation prompted the use of theophylline as an investigative tool in the guinea-pig fundic strip experiments of Okwuasaba, Hamilton & Cook (1977). These workers claimed that theophylline could, without lowering the tone of the fundus, virtually abolish the effects of stimulating its intramural inhibitory neurones. They further claimed that theophylline antagonized the relaxant effects of ATP and adenosine but did not antagonize relaxations evoked by noradrenaline or stimulation of extrinsic sympathetic nerves. Okwuasaba *et al.* (1977) thus concluded that purinergic inhibitory neuroeffector transmission occurred in the fundic strip.

The object of the present study was to investigate whether theophylline would provide similar evidence for purinergic transmission in other preparations of rabbit and guinea-pig intestine where non-adrenergic inhibitory innervation had already been demonstrated. Some of the results of these experiments have been communicated to the British Pharmacological Society (Small & Weston, 1979b).

Methods

Adult rabbits and guinea-pigs of either sex were killed by stunning and bleeding. Tissues isolated from these animals were incubated in Krebs solution gassed with 95% O_2 and 5% CO_2 and maintained at 37.5°C.

0007-1188/79/100301-08$01.00

Experiments with rabbit duodenum, guinea-pig taenia caeci and guinea-pig fundic strip

Strips of longitudinal muscle from rabbit duodenum were prepared and set up for isometric recording of mechanical activity as described by Weston (1973). Guinea-pig fundic strips were prepared as described by Okwuasaba *et al.* (1977). The fundic strips and segments of guinea-pig taenia caeci (2 to 3 cm long) were set up for isotonic recording of mechanical activity under a load of 1 g.

Responses of the fundic strip and taenia caeci to relaxant agonists approached equilibrium after approximately 45 s. The effects of noradrenaline, ATP and adenosine on these tissues were investigated by constructing cumulative concentration-effect curves, ten fold increments in agonist concentration being made at 45 s intervals. In the rabbit duodenum, concentration-effect curves for noradrenaline, ATP and adenosine were constructed in an acute manner. Each drug concentration was applied to the tissue for 30 s and then washed from the bath. Field stimulation of the intramural inhibitory nerves of the three tissues was carried out at supramaximal voltage with ring electrodes. Pulses of 0.5 ms width were delivered in trains of 10 s duration. Frequency-response curves were constructed using two fold frequency increments.

In test tissues, the effects of noradrenaline, ATP, adenosine and field stimulation were investigated in the absence and presence (30 min preincubation) of theophylline. Control tissues were not exposed to theophylline and were always run concurrently with test preparations.

Experiments with transmurally stimulated guinea-pig ileum

Segments of ileum, 1 to 2 cm long, were set up for transmural stimulation (Paton, 1955) and isometric recording of mechanical activity under a resting tension of 1 g. Transmural stimulation was carried out at supramaximal voltage with pulses of 0.5 ms duration and frequency 0.2 Hz. The effects of noradrenaline, ATP and adenosine on the twitches evoked by transmural stimulation were studied by the construction of cumulative concentration-effect curves, ten fold increments in agonist concentration being made at 15 s intervals. In test tissues, agonist action was studied both in the absence and presence (30 min preincubation) of theophylline. Control tissues were not exposed to theophylline and were always run concurrently with test preparations.

Electrophysiological studies

Simultaneous intracellular recordings of membrane potential changes and mechanical activity were made by the method of Small & Weston (1977; 1979c). Mechanical activity was recorded either isometrically (resting tension 1 g) or isotonically (load 1 g). Field stimulation of the taenia caeci was carried out with platinum ring electrodes delivering 0.5 ms pulses of supramaximal voltage every 25 s. The recording microelectrode was situated approximately 1 cm from the stimulating cathode towards the colonic end of the tissue.

Intracellular recordings of electrical activity were only evaluated provided that they contained no sudden changes in spike amplitude or resting potential indicative of temporary displacement of the microelectrode from the cell of interest. The apparent resting membrane potential was taken as the potential change occurring on deliberate withdrawal of the microelectrode from the cell (see Figure 5) following a stable recording of at least 2 min duration.

The effects of theophylline on spontaneous electrical activity and inhibitory post-junctional potentials (i.p.j.ps) were studied by impalement of six cells before theophylline treatment. Theophylline was added to the bathing medium during the course of a seventh impalement so that the onset of the electrical changes evoked by the drug could be studied. Following a 30 min preincubation with theophylline, a further six cells were impaled.

Drugs and solutions

The Krebs solution had the following composition (mmol/l): Na^+ 143.5, K^+ 5.94, Ca^{2+} 2.55, Mg^{2+} 1.19, Cl^- 125, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, glucose 11.1. Except in the experiments with transmurally-stimulated ileum, the Krebs solution contained hyoscine 0.03 mmol/l and guanethidine 0.01 mmol/l. Drugs used were adenosine (Koch Light), the disodium salt of ATP (Sigma), guanethidine sulphate (Ciba), hyoscine hydrobromide (Sigma), (−)-noradrenaline bitartrate (Koch Light) and theophylline (Sigma).

Dilutions of noradrenaline were prepared from a stock solution in 0.1 N HCl on the day of use. Each noradrenaline dilution contained ascorbic acid 0.2 mg/ml as an antoxidant. Dilutions of adenosine and ATP were prepared by dissolving (or suspending) the solid in 0.9% w/v NaCl solution (saline) immediately before use.

Results

Experiments with taenia caeci

Segments of guinea-pig taenia caeci, set up as described in Methods, developed a high, stable level of

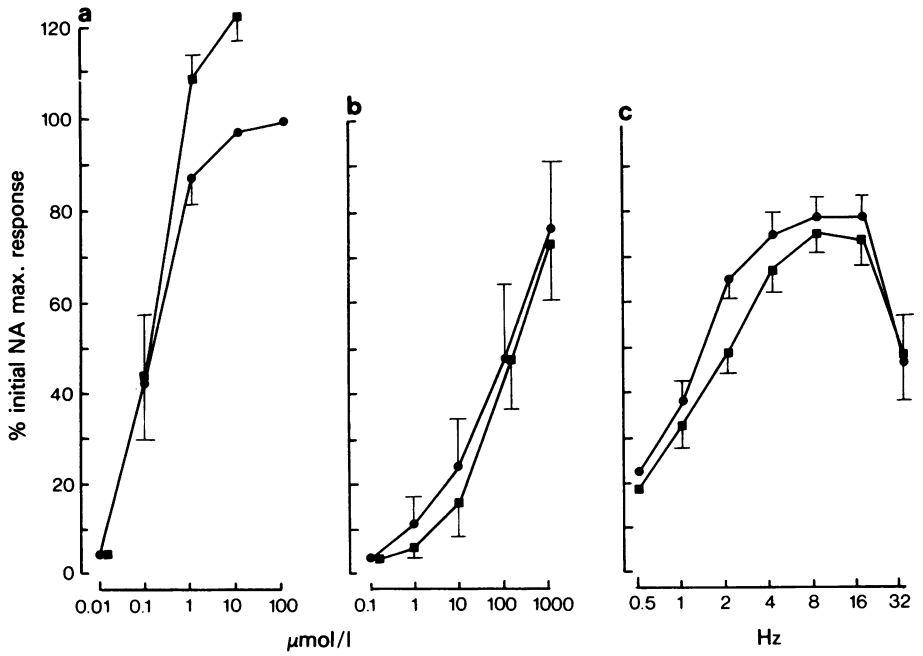

Figure 1 Inhibition of mechanical activity in guinea-pig taenia caeci produced by (a) noradrenaline, (b) adenosine and (c) electrical field stimulation. (●) Initial responses; (■) responses after 30 min preincubation with and in the presence of theophylline 100 μmol/l. Responses are expressed as % of the initial noradrenaline (NA) maximum response and are means of six experiments; vertical lines show s.e. mean.

tone within 15 min, allowing the relaxant effects of drugs and nerve stimulation to be studied easily. Theophylline 100 μmol/l caused little or no change in the resting tone of test tissues and relaxant responses of these tissues to noradrenaline, adenosine or field stimulation were little, if at all affected after exposure to theophylline (Figure 1). Any changes which did occur were also seen in concurrent control tissues and were therefore attributable to the passage of time rather than to the action of theophylline. A parallel series of experiments showed that the relaxant effects of ATP (100 nmol/l to 1 mmol/l) were unaffected by theophylline (100 μmol/l).

Experiments with rabbit duodenum

The strips of longitudinal muscle from rabbit duodenum exhibited spontaneous phasic mechanical changes which were reduced in amplitude by noradrenaline, adenosine and field stimulation. Exposure of the test tissues to theophylline (100 μmol/l) did not modify their spontaneous activity but the relaxant effects of some concentrations of adenosine were significantly reduced (Figure 2). Responses of these tissues to noradrenaline or field stimulation were unchanged by the presence of theophylline. Concurrent control experiments detected no change in the responses of the muscle strips to noradrenaline, adenosine and field stimulation attributable to the passage of time. The reduction in the effects of adenosine seen in the test tissues therefore represented a selective antagonism by theophylline. A parallel series of experiments showed that the relaxant effects of ATP (1 μmol/l to 10 mmol/l) were unaffected by theophylline (100 μmol/l).

Experiments with guinea-pig fundus

When fundic strips were prepared for isotonic recording as described in Methods, only about 25% of preparations generated sufficient resting tone to enable relaxant responses to be examined. Theophylline (100 μmol/l) caused little or no change in the resting tone of test tissues. Relaxant responses of these tissues to noradrenaline, adenosine and field stimulation were not reduced after exposure to theophylline (Figure 3), an increase apparently occurring in responses to some concentrations of noradrenaline. A similar change in the noradrenaline concentration-effect relationship was seen in concurrent control tissues and could

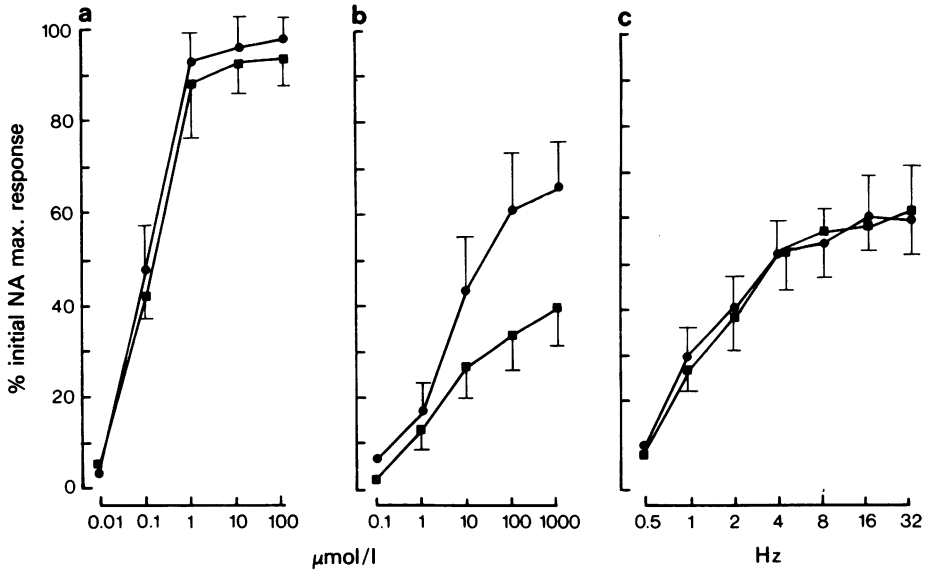

Figure 2 Inhibition of mechanical activity in rabbit duodenum longitudinal muscle produced by (a) noradrenaline, (b) adenosine and (c) electrical field stimulation. (●) Initial responses; (■) responses after 30 min preincubation with and in the presence of theophylline 100 μmol/l. Responses are expressed as % of the initial noradrenaline (NA) maximum response and are means of six experiments; vertical lines show s.e. mean.

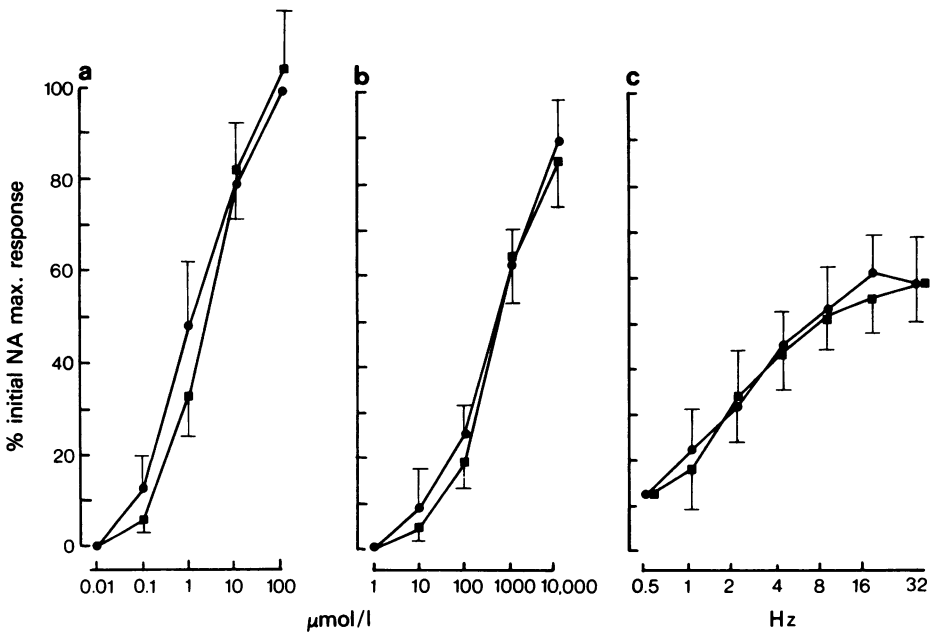

Figure 3 Inhibition of mechanical activity in guinea-pig fundic strips produced by (a) noradrenaline, (b) adenosine and (c) electrical field stimulation. (●) Initial responses; (■) responses after 30 min preincubation with and in the presence of theophylline 100 μmol/l. Responses are expressed as % of the initial noradrenaline (NA) maximum response and are means of six experiments; vertical lines show s.e. mean.

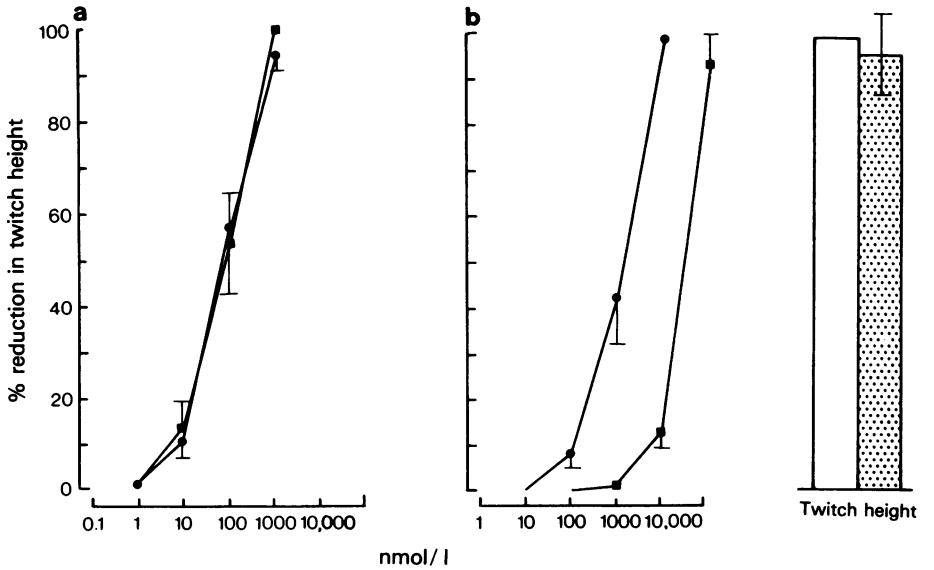

Figure 4 Inhibition of twitches of transmurally stimulated guinea-pig ileum produced by (a) noradrenaline and (b) adenosine. (●) Initial responses; (■) responses after 30 min preincubation with and in the presence of theophylline 1 mmol/l. The columns show twitch height initially (open column) and after exposure to theophylline 1 mmol/l (stippled column). The initial twitch height is expressed arbitrarily as 100%. All responses shown are means of six experiments; vertical lines show s.e. mean.

therefore be attributed to the passage of time rather than to an action of theophylline. The sensitivity of control tissues to adenosine and field stimulation remained constant. A parallel series of experiments showed that the relaxant effects of ATP (1 μmol/l to 10 mmol/l) were unaffected by theophylline (100 μmol/l).

Experiments with guinea-pig ileum

Transmural stimulation of segments of ileum with single pulses produced characteristic twitches which were reduced in amplitude by noradrenaline and adenosine (Figure 4). Theophylline (1 mmol/l) itself had no significant effect on the amplitude of the twitches or on the ability of noradrenaline to suppress them. However, in the presence of theophylline, the concentration-effect curve for adenosine was moved to the right (Figure 4). Concurrent control experiments detected no changes in either the shapes or positions of the concentration-effect curves of noradrenaline or adenosine. The reduced sensitivity of test tissues to adenosine therefore represented a selective antagonism by theophylline.

A parallel series of experiments showed that theophylline (1 mmol/l) antagonized the twitch suppression evoked by ATP (10 nmol/l to 1 mmol/l) to an extent similar to that observed for adenosine.

Electrophysiological experiments with taenia caeci and fundic strips

The spontaneous electrical activity and the electrical changes (i.p.j.ps) associated with non-adrenergic inhibitory transmission in the taenia caeci are both well documented. Accordingly, this tissue was selected for the electrophysiological studies of the effects of theophylline.

Many cells of the taenia caeci exhibited stable resting membrane potentials. Spike activity in such cells was continuous (though of variable frequency) and i.p.j.p. amplitude was constant. Other cells exhibited slow oscillations of resting membrane potential, spike activity occurring only during the depolarizing phase of the cycle. The amplitude of successive i.p.j.ps in cells of this type was not constant, being maximal during the depolarizing phase of the cycle and minimal when the cell was quiescent and relatively hyperpolarized.

When the taenia was exposed to theophylline (1 mmol/l), several minutes elapsed before any change was apparent in the electrical activity of the impaled cell. Generally, spike discharge became more periodic, the quiescent phases gradually lengthening so that in many cells spike activity ceased altogether (Figure 5). In other cells, spike discharge was restricted to rebound bursts of activity on recovery from each i.p.j.p.

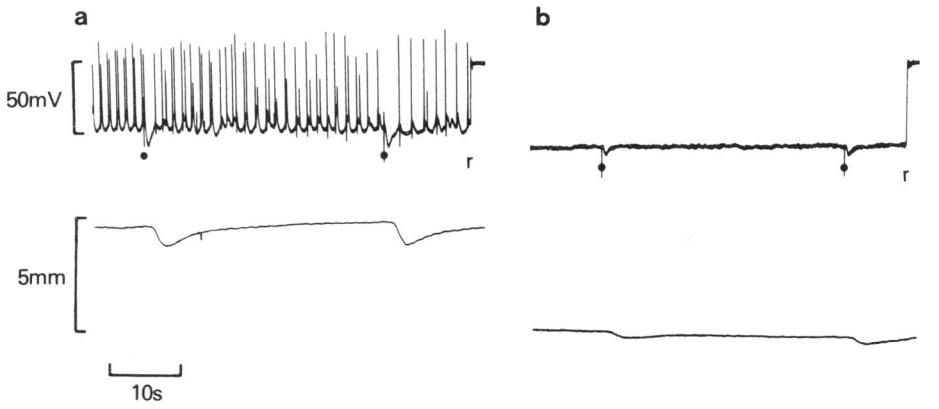

Figure 5 Effect of theophylline 1 mmol/l on electrical (upper trace) and isotonic mechanical activity (lower trace) in guinea-pig taenia caeci. (a) Control record showing the i.p.j.ps evoked by single pulses of supramaximal voltage and 0.5 ms duration (at dots) and associated relaxations. At r, the microelectrode was deliberately withdrawn from the cell to give a reading of apparent resting membrane potential. (b) Record obtained from the same tissue (but from a different cell) in the presence of theophylline 1 mmol/l. Cessation of spike discharges, reduction in i.p.j.p. amplitude and a fall in mechanical tone are clearly visible.

In all tissues, the changes in spike activity were accompanied by a gradually developing, weak hyperpolarization of the cell and overall relaxation. I.p.j.ps were reduced in amplitude at this stage but were not abolished (Figure 5, Table 1). Analysis of the results (analysis of variance and linear regression) revealed that values of the apparent resting membrane potential and of i.p.j.p. amplitude did not alter with time during both the control and test periods of the experiment. Values of these parameters obtained before and after exposure to theophylline 1 mmol/l were therefore compared using a two-tailed, unpaired t test (Table 1). The probability that the elevated apparent membrane potential or the reduced i.p.j.p. amplitude seen after theophylline occurred by chance was less than 0.05 in each case. Preliminary experiments with the fundic strip also showed that the relaxant effects of theophylline 1 μmol/l were associated with abolition of spontaneous spike activity and a gradually developing, weak hyperpolarization of the impaled cell.

Discussion

The report by Okwuasaba *et al.* (1977) of highly selective antagonism by theophylline of the effects of intramural nerve stimulation and of ATP in guinea-pig fundus was the stimulus for the experiments described in this paper. Initially, the guinea-pig taenia caeci and the longitudinal muscle of rabbit duodenum were chosen for further exploration of the effects of theophylline since the inhibitory actions of purines and non-adrenergic neurones are well established in these tissues (Burnstock, 1972; Weston, 1973).

In the guinea-pig taenia caeci, theophylline failed to antagonize the effects of adenosine, ATP and intramural nerve stimulation. The failure of theophylline to antagonize ATP and nerve stimulation in this tissue has recently been confirmed by Hooper, Spedding, Sweetman & Weetman (1979). In rabbit duodenum, theophylline produced some antagonism of adenosine, thus confirming the findings of Ally & Nakatsu (1976). However, theophylline was without effect on duodenal responses to ATP or intramural nerve stimulation.

In order to investigate whether the reported inhibition of intramural inhibitory nerve activity by theophylline in guinea-pig fundus was restricted to this

Table 1 The effects of theophylline (1 mmol/l) on apparent resting membrane potential (r.m.p.) and inhibitory post-junctional potential (i.p.j.p.) amplitude in guinea-pig taenia caeci

	r.m.p. (*mV*)	*i.p.j.p.* (*mV*)
Control	49.2 ± 0.7 (36)	10.1 ± 0.7 (36)
Theophylline 1 mmol/l	54.6 ± 0.7 (30)	6.8 ± 0.5 (34)
P (2-tailed *t* test)	0	0.0004

Mean values ± s.e. mean are given, numbers of observations in parentheses.

tissue, an attempt was made to repeat the observations of Okwuasaba *et al.* (1977). However, theophylline was found to have no effect on responses to adenosine, ATP and intramural nerve stimulation in this tissue, results which are in agreement with those of an independent study (Baer & Frew, 1979).

In the present experiments, a concentration of 100 μmol/l theophylline was first used. Since this concentration had little or no effect, experiments were carried out with 1 mmol/l theophylline in an attempt to elicit inhibition of purine action and intramural nerve stimulation. However, in all tissues, this ten fold increase in theophylline concentration produced a marked relaxation which rendered the evaluation of subsequent inhibitory responses very difficult.

In a further attempt to determine whether theophylline 1 mmol/l could antagonize the effects of intramural nerve stimulation, electrophysiological experiments were carried out on the guinea-pig taenia caeci. It was reasoned that, if theophylline 1 mmol/l produced its relaxant effects by uncoupling electrical activity from mechanical activity, measurement of the i.p.j.p. following intramural nerve stimulation might provide evidence of specific antagonism by theophylline of the intramural inhibitory transmission process. These electrophysiological experiments showed that theophylline (1 mmol/l) was able to reduce significantly the size of the i.p.j.p. However, this reduction was accompanied by significant cellular hyperpolarization, an event which itself would be expected to reduce the size of the i.p.j.p. In view of the failure of theophylline 100 μmol/l to antagonize the relaxation mediated by intramural nerves, it is concluded that the reduction in i.p.j.p. amplitude evoked by theophylline results from general tissue hyperpolarization and not from specific antagonism of inhibitory neuroeffector transmission.

Sawynok & Jhamandas (1976) showed that adenosine could suppress the twitches of transmurally-stimulated guinea-pig ileum by preventing acetylcholine release and that this action could be antagonized by theophylline. This effect of theophylline has been confirmed in the present experiments. Paton (1979) found that purines could inhibit neural noradrenaline release and that this action too could be antagonized by theophylline. It seems clear, therefore, that theophylline can selectively antagonize the pre-junctional actions of purines in inhibiting transmitter release. In the intestinal tissues investigated in the present study, theophylline reduced the effects of adenosine only in rabbit duodenum and guinea-pig ileum. Theophylline does not antagonize the effects of intramural inhibitory nerve activity in rabbit or guinea-pig intestine and the original observations of Okwuasaba *et al.* (1977) appear to have been artefacts since Baer & Frew (1979) and Cook & Hamilton (personal communication) have been unable to reproduce them.

We conclude that theophylline is of little value as a tool in testing the purinergic nerve hypothesis.

This work was supported by grants for apparatus from the Friends of the Duchess of York Hospital for Babies, the Medical Research Council, the Royal Society and the Smith, Kline and French Foundation. We gratefully acknowledge the expert technical assistance of Miss J. Bamber and Miss A.J. Whitehead.

References

ALLY, A.I. & NAKATSU, K. (1976). Adenosine inhibition of isolated rabbit ileum and antagonism by theophylline. *J. Pharmac. exp. Ther.*, **19**, 208–215.

BAER, H.P. & FREW, R. (1979). Relaxation of guinea-pig fundic strip by adenosine, adenosine triphosphate and electrical stimulation: lack of antagonism by theophylline or ATP treatment. *Br. J. Pharmac.*, **67**, 293–299.

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.*, **24**, 509–581.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D.G. & SMYTHE, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmac.*, **40**, 668–688.

HOOPER, M., SPEDDING, M., SWEETMAN, A.J. & WEETMAN, D.F. (1979). The effects of 2-2′-pyridylisatogen on responses to adenosine 5′-triphosphate in isolated smooth muscle preparations. In *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides.* ed. Baer, H.P. & Drummond, G. New York: Raven Press (in press).

OKWUASABA, F.K., HAMILTON, J.T. & COOK M.A. (1977). Relaxations of guinea-pig fundic strip by adenosine, adenine nucleotides and electrical stimulation; antagonism by theophylline and desensitisation to adenosine and its derivatives. *Eur. J. Pharmac.*, **46**, 181–198.

PATON, D.M. (1979). The effects of adenosine and adenine nucleotides on adrenergic transmission. In *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides*, ed. Baer, H.P. & Drummond, G. New York: Raven Press (in press).

PATON, W.D.M. (1955). The responses of the guinea-pig ileum to electrical stimulation by co-axial electrodes. *J. Physiol.*, **127**, 40–41P.

SAWYNOK, J. & JHAMANDAS, K.H. (1976). Inhibition of acetylcholine release from cholinergic nerves by adenosine, adenine nucleotides and morphine; antagonism by theophylline. *J. Pharmac. exp. Ther.*, **197**, 379–390.

SMALL, R.C. & WESTON, A.H. (1977). Simultaneous recording of electrical and mechanical activity from intestinal and vascular smooth muscle. *Br. J. Pharmac.*, **61**, 491–492P.

SMALL, R.C. & WESTON, A.H. (1979a). Intramural inhibition in the rabbit and guinea-pig intestine. In *Physio-*

logical and regulatory Functions of Adenosine and Adenine Nucleotides, ed. Baer, H.P. & Drummond, G. New York: Raven Press (in press).

SMALL, R.C. & WESTON, A.H. (1979b). Theophylline is not an antagonist of intramural nerve activity in rabbit and guinea-pig intestine. *Br. J. Pharmac.*, **66**, 84P.

SMALL, R.C. & WESTON, A.H. (1979c). Simultaneous long-term recording of the mechanical and intracellular electrical activity of smooth muscles. *J. Pharmac. Meth.*, (in press).

WESTON, A.H. (1973). Nerve-mediated inhibition of mechanical activity in rabbit duodenum and the effects of desensitization to adenosine and several of its derivatives. *Br. J. Pharmac.* **48**, 302–308.

(Received February 5, 1979.)