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EVIDENCE IN SUPPORT OF THE P_1/P_2 PURINOCEPTOR HYPOTHESIS IN THE GUINEA-PIG TAENIA COLI

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1 The relaxations induced by adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP) and adenosine on the carbachol-contracted taenia coli of the guinea-pig have been studied. ATP and ADP produce similar responses which differ in nature and time course from those of AMP and adenosine.

2 Theophylline, at concentrations (25–200 μM) lower than those which produce significant phosphodiesterase inhibition, blocks the effects of AMP and adenosine but fails to antagonize the responses elicited by ATP and ADP. The antagonism of adenosine by theophylline appears to be competitive.

3 Apamin (1–100 nM) blocks the inhibitory effects of ATP and ADP but fails to antagonize the responses to AMP and adenosine. The antagonism by apamin is non-competitive.

4 The results indicate that ATP and adenosine relax the taenia coli by activating different receptors and are consistent with the P_1, P_2 purinoceptor hypothesis.

Introduction

Based primarily on the relative potencies of agonists and selectivity of antagonists on a wide variety of tissues, Burnstock (1976; 1978) has proposed two types of 'purinergic' receptors and named them P_1 and P_2-purinoceptors. The P_1-receptor was characterized by its susceptibility to blockade by the methylxanthines (theophylline and caffeine) and agonist potency ranking of adenosine = adenosine 5′-monophosphate (AMP) > adenosine 5′-diphosphate (ADP) > adenosine 5′-triphosphate (ATP). The P_2-receptor was not susceptible to blockade by the methylxanthines and the agonist potency ranking was in reverse, ATP being the most potent. 2,2′-Pyridylisatogen tosylate, although not a specific ATP antagonist, has been shown to block the effects of ATP and ADP in the guinea-pig taenia coli without having significant effects upon the relaxations to AMP or adenosine (Spedding & Weetman, 1976). Furthermore, when the taenia coli was desensitized to adenosine, the relaxations to ATP were still apparent and were in fact, increased by this procedure. On this basis these authors also suggested that there may be separate receptors for adenosine and ATP in this preparation.

Changes in cyclic AMP levels were believed to be associated with activation of the P_1-receptor. Londos & Wolff (1977), in a study of the effects of adenosine and its analogues on adenylate cyclases from several tissues, identified two adenosine reactive sites which they termed 'R' and 'P' sites. Occupation of the extracellular 'R' site led to activation of the cyclase system with a subsequent increase in cyclic AMP levels. This 'R' site was competitively antagonized by theophylline and thus may be comparable to the P_1-receptor of Burnstock's classification. The intracellular 'P' site, which requires much higher concentrations of adenosine for activation, mediates inhibition of the cyclase system and is not antagonized by theophylline. Since this inhibitory site has been shown to bind adenosine in the range of 50–100 μM (Braun & Levitzki, 1979) and the intracellular adenosine concentration *in vivo* is usually below 1 μM (Schrader, Berne & Rubio, 1972) it may very well be that the intracellular 'P' site for adenosine never becomes operational *in vivo*.

The recent finding of Vladimirova and colleagues (Vladimirova & Shuba, 1978; Baidan, Vladimirova, Miroshnikov & Taran, 1978) that low concentrations of apamin (a polypeptide found in bee venom) abolished the hyperpolarizing action of externally applied ATP on intestinal smooth muscle, guinea-pig stomach and taenia coli, led them to suggest that it might be a specific ATP receptor antagonist. We have, therefore, used this compound in conjunction with theophylline in an attempt to characterize the purinoceptors in the guinea-pig taenia coli.

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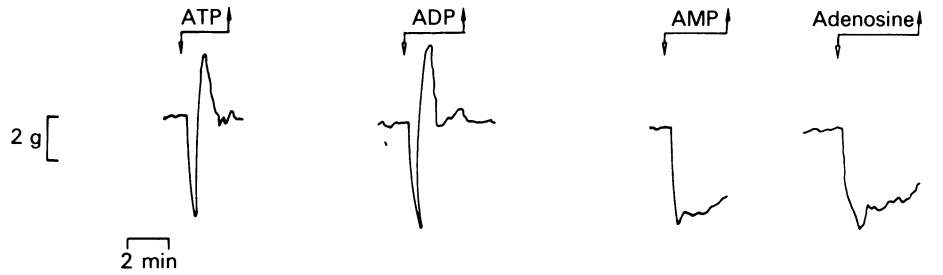

Figure 1 Relaxation of a carbachol-contracted guinea-pig taenia coli preparation to ATP (3 μM); ADP (1 μM); AMP (100 μM) and adenosine (100 μM); horizontal brackets indicate the period of exposure to the agonist.

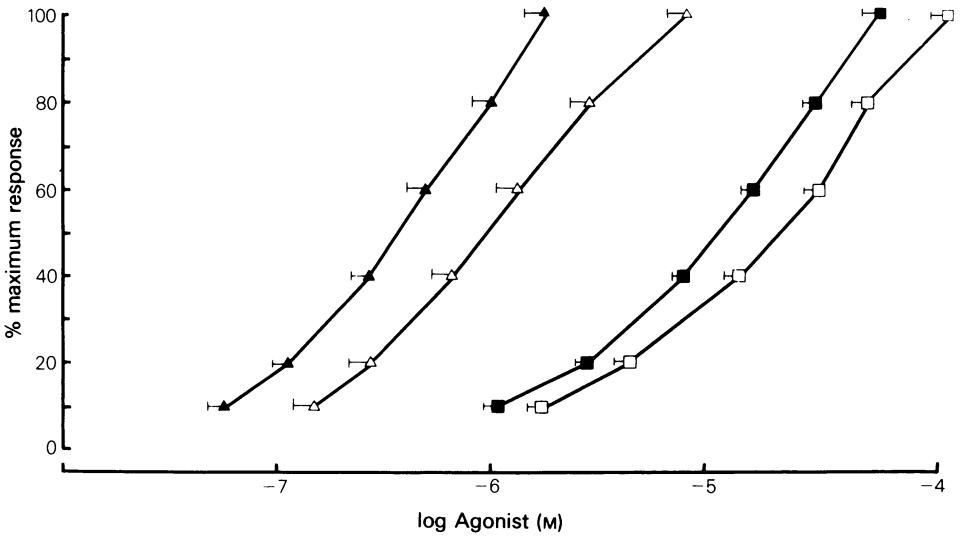

Figure 2 Log dose-response curves for the relaxation of the isolated carbachol-contracted guinea-pig taenia coli by ADP (▲), ATP (△), adenosine (■) and AMP (□). Each point is the mean of at least 10 values obtained from at least 5 experiments; horizontal lines show s.e.mean.

Methods

Guinea-pigs of either sex (300–400 g) were killed by a blow on the head and were then exsanguinated and the abdomen opened. Longitudinal strips (2–3 cm) of the taenia with the underlying Auerbach's plexus were dissected free and suspended in a 10 ml organ bath containing modified Krebs solution at 37°C (Bülbring, 1953) of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.4, $NaHCO_3$ 16.3, $MgSO_4$ 2.5 and glucose 7.84; when this solution had been saturated with a gas mixture of 95% O_2 and 5% CO_2, 2.5 mM $CaCl_2$ was added.

The muscles were initially stretched to a tension of 0.25 g and contractions recorded by a Grass FT10 isometric transducer and displayed on a Grass polygraph. Relaxations to adenosine and its derivatives were demonstrated as a reduction in the submaximal contraction induced by carbachol (25 nM). The carbachol was added and once the contraction had reached a plateau, one concentration of the purine compound was added and the maximum relaxation obtained with the agonist measured. The bath was then washed with fresh Krebs solution and 5 min later the carbachol was re-added and the next concentration of agonist tested. Responses were calculated as the percentage reduction in the carbachol contraction. The response of the tissue to at least seven concentrations of agonist, applied in the sequence 1, 3, 10, 30, 100, 300 and 1000 molar concentration units was determined and log dose-response curves were constructed for the inhibitory agonists by calculating the mean ± s.e.mean of the effective

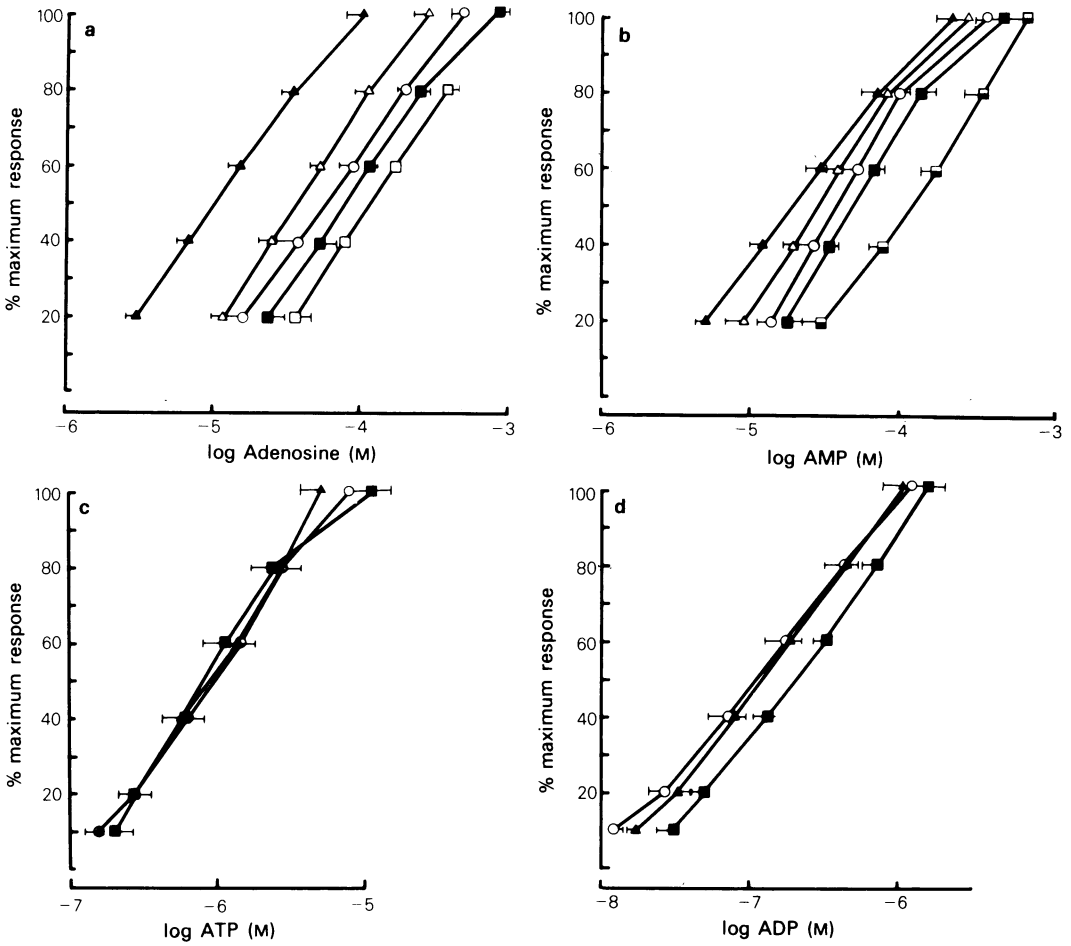

Figure 3 The effect of theophylline on the log dose-response curves to adenosine and the adenine nucleotides in the isolated carbachol-contracted guinea-pig taenia coli. (a) Log dose-response curves to adenosine in the absence (▲) and in the presence of 25 μM (△), 50 μM (○), 75 μM (■) and 100 μM (□) theophylline. Each point is the mean of at least 12 values obtained from at least 6 animals. (b) Log dose-response curves to AMP in the absence (▲) and in the presence of 25 μM (△), 50 μM (○), 75 μM (■) and 200 μM (□) theophylline. Each point is the mean of at least 10 values obtained from at least 5 animals. (c) Log dose-response curves to ATP in the absence (▲) and in the presence of 50 μM (○) and 100 μM (■) theophylline. Each point is the mean of at least 8 values obtained from at least 5 animals. (d) Log dose-response curves to ADP in the absence (▲) and in the presence of 50 μM (○) and 100 μM (■) theophylline. Each point is the mean of at least 10 values obtained from 5 preparations. In all cases horizontal lines show s.e.mean.

concentrations required to give a certain percentage of maximum response. The maximum response for each inhibitory agonist was equivalent to a 100% reduction in the carbachol contraction. This method of combining log dose-response curves avoids biasing the curves towards a lower slope (Waud, 1975).

The following drugs were used: adenosine (Sigma), adenosine 5′-monophosphate (AMP: Sigma), adenosine 5′-diphosphate (ADP: Sigma), adenosine 5′-triphosphate (ATP: Sigma), apamin (Dr B. Banks, Department of Physiology, UCL), carbamylcholine chloride (carbachol: Sigma), theophylline (Sigma). Concentrations refer to final molar concentrations in the organ bath unless otherwise stated.

Results

Responses of the guinea-pig taenia coli to adenosine and related nucleotides

ATP (0.1–10 μM), ADP 0.01–1 μM), AMP (1–100 μM) and adenosine (1–100 μM) all produced

concentration-dependent relaxations of the carbachol-contracted guinea-pig taenia coli. However, differences in the onset and time to peak relaxation were apparent. Both ATP and ADP elicited a rapid relaxation of the smooth muscle which faded rapidly and was often followed by a contraction. In contrast, adenosine and AMP elicited slower relaxations of the taenia coli which, once developed, faded slowly during maintained contact with the tissue but which were rapidly reversed on washing. The relaxations to adenosine and AMP were not followed by a contraction. A comparison of the nature of the relaxations to ATP, ADP, AMP and adenosine is shown in Figure 1.

ADP was the most potent of the adenine compounds tested and was found to be three times more potent than ATP, and approximately one hundred times more potent than AMP and adenosine (Figure 2).

Effects of theophylline

Theophylline in concentrations of up to 100 μM did not alter the sensitivity of the taenia coli strips to carbachol and on addition of theophylline to the bathing fluid, no alteration in basal tone was noted.

The effects of theophylline on the log dose-response curves to adenosine and the adenine nucleotides in the carbachol-contracted guinea-pig taenia coli are shown in Figure 3 (a–d). Theophylline (25–200 μM) following a 20 min incubation period, produced a progressive parallel shift to the right of the adenosine and AMP log dose-response curves, though to different degrees, without diminishing the maximal responses. This antagonism was found to be surmountable on addition of greater concentrations of the agonists. Higher concentrations of theophylline were not employed, as it is known to inhibit phosphodiesterase (Amer & Kreighbaum, 1975) from a number of tissues and in the millimolar range, to affect Ca^{2+} fluxes (Isaacson & Sandow, 1967), although neither of these effects have been demonstrated in the guinea-pig taenia coli.

The data in Figure 3 were used to determine the apparent pA_2 values for theophylline according to the method of Arunlakshana & Schild (1959). Figure 4 shows the relevant Schild plot for adenosine and AMP. It can be seen that the apparent pA_2 values for theophylline with adenosine and AMP as inhibitory agonists were $4.98 \pm .32$ and $4.27 \pm .30$ respectively, these values not being significantly different. Determination of the slopes of the plot yielded values of 0.96 ± 0.09 for adenosine and 1.21 ± 0.09 for AMP. The value for adenosine was not significantly different from unity and therefore, is compatible with an antagonism of a competitive nature.

The relaxations induced by ATP and ADP were not antagonized by theophylline (50–100 μM) and therefore, there was no significant shift to the right in the log dose-response curves in the presence of theophylline.

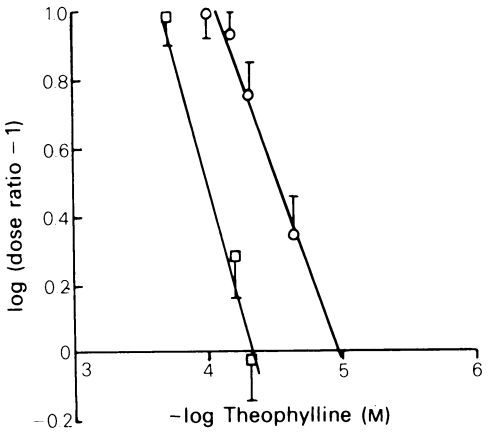

Figure 4 A Schild plot constructed from the results shown in Figure 3 (a and b) of the antagonism of adenosine (○) and AMP (□) by theophylline. The dose-ratios were calculated at the ED_{50} level, after a 20 min exposure to theophylline. Vertical lines show s.e.mean.

Effects of apamin

Apamin, at concentrations of 0.5–20 nM, increased the spontaneous activity of the taenia coli strip and occasionally caused a slight increase in the tone of the preparation. At these concentrations, following a 20 min exposure period, apamin produced a concentration-dependent blockade of the inhibitory responses to ATP and ADP in the carbachol-contracted guinea-pig taenia coli whilst the contractile component of the response (Figure 5) was still apparent. Figure 6 (a, b) shows the non-competitive nature of the antagonism of the inhibitory response as indicated by the non-parallel shift of the log dose-response curves in the presence of apamin. At these and higher apamin concentrations (10–50 nM) a slight increase in the tone of the preparation was usually observed, which was compensated for by reducing the amount of carbachol used to contract the tissue. The maximum inhibitory responses induced by both ATP and ADP, in the presence of apamin (10–50 nM) were reduced, and frequently a pure excitatory response was observed.

The relaxations induced by AMP and adenosine were not antagonized by apamin (1–10 nM) (Figure 7a, b) and in each of four other such experiments, 500 nM apamin did not abolish or significantly reduce the adenosine inhibition. Apamin has previously been found to block the relaxations induced by α-

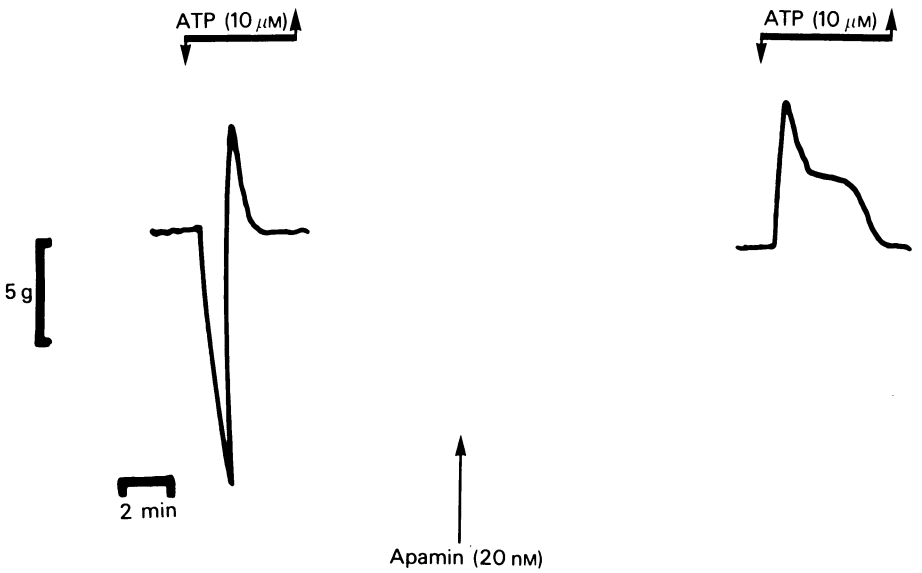

Figure 5 Response of a carbachol-contracted guinea-pig taenia coli preparation to ATP (10 μM) before (left) and after (right) apamin (20 nM) treatment. Horizontal brackets indicate the period of exposure to the agonist.

adrenoceptor agonists on the guinea-pig taenia coli, while leaving the responses to β-adrenoceptor agonists unaltered (Banks, Brown, Burgess, Burnstock, Claret, Cocks & Jenkinson, 1979).

Discussion

The results of the present study have demonstrated the inhibitory actions of adenosine and related nucleotides on the carbachol-contracted guinea-pig taenia coli, and are consistent with previous reports (Burnstock, Campbell, Satchell & Smythe, 1970; Satchell & Maguire, 1975; Spedding & Weetman, 1976; Maas & Den Hertog, 1980). The most potent inhibitory compound tested was ADP, the order of potency being ADP > ATP > adenosine = AMP. However, the accurate measurement of potency ratios requires the concentration of the agonist in the region of the receptors to be in diffusion-equilibrium with that in the external solution at the time a response is measured. Thus, the rate of removal of the agonist from the receptor region due to enzymatic action, transport into cells, and binding should be negligible compared with the rate due to diffusion back to the outside solution. Since quantitative information on the relative uptake into cells and enzymatic inactivation of these various purine agonists in the taenia coli is not available at present, the above potency ratio cannot be taken as definitive. Indeed there is some evidence to suggest that ADP is metabolized more slowly than ATP in certain tissues which may explain why ADP appears to be three times more potent than ATP in the taenia coli while in other tissues they have been found to be equipotent (Burnstock, Dumsday & Smythe, 1972; Sjöberg & Wahlstrom, 1975; Okwuasaba, Hamilton & Cook, 1977).

The relaxations induced by ATP and ADP differed qualitatively from those elicited by AMP and adenosine, the former giving rapid relaxations of the smooth muscle, often followed by a contraction, while those elicited by AMP and adenosine were much slower in onset and were not followed by a contraction. This observation by itself can only be taken as suggestive of a differing underlying mechanism for the two responses but further evidence using selective antagonists is required to strengthen the case.

The relaxations induced by adenosine and AMP were selectively antagonized by theophylline. The nature of this antagonism appears to be competitive, the evidence to support this assumption being: (1) the slopes of the log dose-response curves in the presence of theophylline were not significantly different from the controls, suggesting a parallel shift which is indicative of competitive antagonism; (2) the slope of the Schild plots yielded values of 0.96 and 1.21 for adenosine and AMP respectively, the value for adenosine being not significantly different from the theoretical value of unity for a competitive antagonist. The slope of the Schild plot for AMP is significantly greater than unity, and although theories have been proposed to explain log-log plots

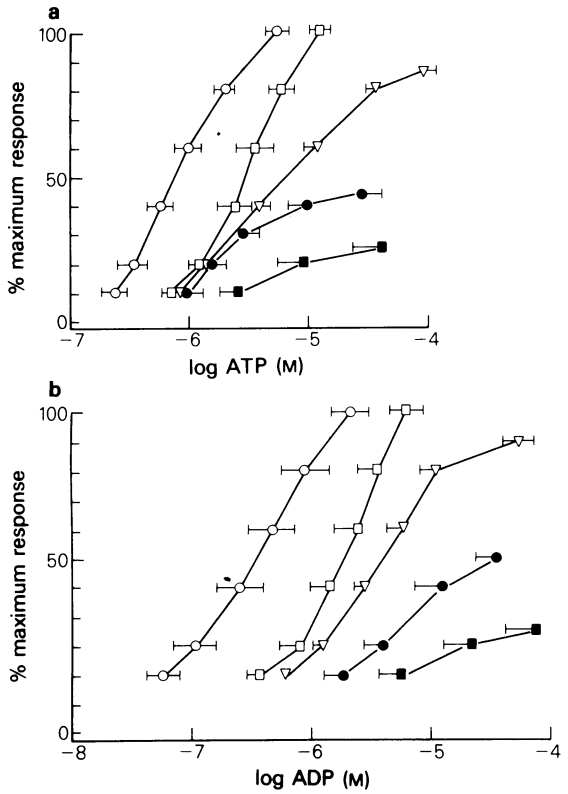

Figure 6 Log dose-response curves for the relaxations of the isolated carbachol-contracted guinea-pig taenia coli by ATP (a) and ADP (b) in the absence (○) and presence of apamin, 0.5 nM (□), 5 nM (▽), 10 nM (●) and 20 nM (■). Each point is the mean of at least 8 values obtained from at least 5 preparations; horizontal lines show s.e.mean.

with slopes differing from 1.0 (Wenke, Lincova, Cepelik, Cernohorsky & Hynie, 1967), it is more probable that this results from unsatisfactory experimental conditions. For example, the concentration range over which theophylline can be tested is limited by its known phosphodiesterase inhibiting properties (Amer & Kreighbaum, 1975); thus, the Schild plot cannot be constructed over as wide a range of antagonist concentrations as is desirable. The apparent pA_2 values for adenosine and AMP as calculated from the Schild plots are $4.98 \pm .32$ and $4.27 \pm .30$ respectively. pA_2 values differing by more than 0.5 are sometimes taken as evidence of a difference between receptor sites. However, as theophylline was quite a weak antagonist at the purinoceptors and since the pA_2 values were not significantly different, there is not sufficient evidence at present to suggest that a difference exists.

The relaxations induced by ATP and ADP were not antagonized by theophylline in concentrations up to 100 μM which, taken in conjunction with the observation that theophylline appears to act as a competitive antagonist of adenosine and possibly AMP in the taenia coli, suggests that the purines ATP and ADP act through a different receptor complex to produce their characteristic responses.

Apamin antagonized the inhibitory responses to ATP and ADP in the taenia coli muscle (see also Vladimirova & Shuba, 1978; Baidan *et al.*, 1978). However, the mode of action of this polypeptide, as indicated by the log dose-response curves, does not appear to be that of a competitive receptor antagonist, since the shift in the curves, although parallel at low concentrations, became non-parallel at the higher apamin concentrations and the maximum responses to ATP and ADP were depressed. In contrast, the relaxations induced by AMP and adenosine were not antagonized by apamin and no shift in the log dose-response curves was observed. Indeed, further work carried out on this neurotoxin has led to the suggestion that apamin is not a receptor blocking drug but that it abolishes the responses to ATP and ADP by inhibiting the increase in potassium permeability that these agents initiate (Banks *et al.*, 1979).

It has been postulated that adenosine and AMP act in many tissues through activation of the adenylate cyclase system (see review by Fain & Malbon, 1979); if this were the case in the taenia coli preparation, then apamin would not be expected to antagonize their inhibitory responses but confirmation of this view will have to await experimentation.

The results presented in this study therefore, are in agreement with the hypothesis (Burnstock, 1978) that there are two distinct purine receptors, one of which, P_1, is activated by adenosine and AMP and is competitively blocked by theophylline. Activation of this receptor leads to a slow relaxation which is not followed by a contraction. In many tissues the adenosine receptor has been linked with the adenylate cyclase system but as yet, this has not been studied in the taenia coli. However, unlike ATP and ADP, the inhibitory responses elicited by adenosine and AMP were not antagonized by apamin.

The P_2 purinoceptor, in contrast, is activated by ATP and ADP, and activation of this receptor elicits a rapid relaxation which fades during maintained contact with the tissue and is followed by a contraction. Theophylline does not antagonize this receptor site in the concentrations tested, and as yet, there is no known competitive P_2 antagonist. However, in systems where P_2 activation leads to a specific increase in K^+ permeability, apamin may be used to block the response.

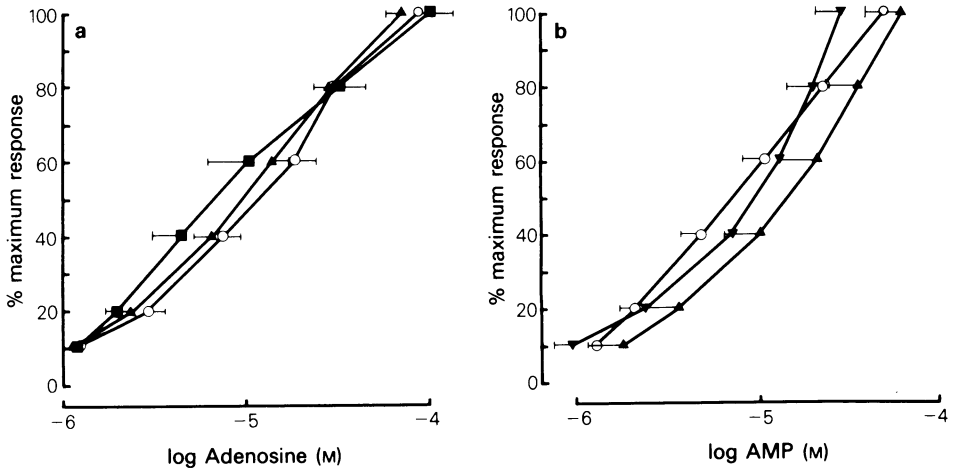

Figure 7 Log dose-response curves for the relaxations of the isolated carbachol-contracted guinea-pig taenia coli by adenosine (a) and AMP (b) in the absence (▲) and presence of apamin 5 nM (○), 10 nM ( ) and 500 nM (■). Each point is the mean of at least 8 values obtained from at least 5 preparations; horizontal lines show s.e.mean.

References

AMER, M.S. & KREIGHBAUM, W.E. (1975). Cyclic nucleotide phosphodiesterases: properties, activators, inhibitors, structure-activity relationships, and possible role in drug development. *J. Pharm. Sci.*, **64**, 1–37.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BAIDAN, L.V., VLADIMIROVA, I.A., MIROSHNIKOV, A.I. & TARAN, G.A. (1978). Action of apamin on synaptic transmission in various types of synapses. *Doklady Akademi Nauk SSSR*, **241**, 1224–1227.

BANKS, B.E.C., BROWN, C., BURGESS, G.M., BURNSTOCK, G., CLARET, M., COCKS, T. & JENKINSON, D.H. (1979). Apamin blocks certain neurotransmitter-induced increases in potassium permeability. *Nature, Lond.*, **282**, 415–417.

BRAUN, S. & LEVITZKI, A. (1979). The attenuation of epinephrine-dependent adenylate cyclase by adenosine and the characteristics of the adenosine stimulatory and inhibitory sites. *Mol. Pharmac.*, **16**, 737–748.

BÜLBRING, E. (1953). Measurements of oxygen consumption in smooth muscle. *J. Physiol.*, **122**, 111–134.

BURNSTOCK, G. (1976). Purinergic receptors. *J. theor. Biol.*, **62**, 491–503.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*. ed. Straub, R.W. & Bolis, L. pp 107–118. New York: Raven Press.

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.

BURNSTOCK, G., DUMSDAY, B.H. & SMYTHE, A. (1972). Atropine resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing a purine nucleotide. *Br. J. Pharmac.*, **44**, 451–461.

FAIN, J.N. & MALBON, C.C. (1979). Regulation of adenylate cyclase by adenosine. *Mol. cell. Biochem.*, **25**, 143–169.

ISAACSON, A. & SANDOW, A. (1967). Quinine and caffeine effects on ^{45}Ca movements in frog sartorius muscle. *J. gen. Physiol.*, **50**, 2109–2128.

LONDOS, C. & WOLFF, J. (1977). Two distinct adenosine-sensitive sites on adenylate cyclase. *Proc. natn. Acad. Sci. U.S.A.*, **74**, 5482–5486.

MAAS, A.J.J. & DEN HERTOG, A. (1980). The effect of the phenyl phosphorate N-0164 on prostaglandin action and on post-inhibitory excitation in the taenia of guinea-pig caecum. *Eur. J. Pharmac.*, **62**, 157–166.

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 desensitization to adenosine and its derivatives. *Eur. J. Pharmac.*, **46**, 181–198.

SATCHELL, D.G. & MAGUIRE, M.H. (1975). Inhibitory effects of adenine nucleotide analogs on the isolated guinea-pig taenia coli. *J. Pharmac. exp. Ther.*, **195**, 540–548.

SCHRADER, J., BERNE, R.M. & RUBIO, R. (1972). Uptake and metabolism of adenosine by human erythrocyte ghosts. *Am. J. Physiol.*, **223**, 159–166.

SJÖBERG, F. & WAHLSTROM, B.A. (1975). The effect of ATP and related compounds on spontaneous mechanical activity in the rat portal vein. *Acta. physiol. scand.*, **94**, 46–53.

SPEDDING, M. & WEETMAN, D.F. (1976). Identification of

separate receptors for adenosine and adenosine 5'-triphosphate in causing relaxations of the isolated taenia of the guinea-pig caecum. *Br. J. Pharmac.*, **57,** 305–310.

VLADIMIROVA, I.A. & SHUBA, M.F. (1978). Strychnine, hydrastine and apamin effect on synaptic transmission in smooth muscle cells. *Neurophysiology USSR*, **10,** 295–299.

WAUD, D.R. (1975). Analysis of dose response curves. In *Methods of Pharmacology*, Vol. 3. *Smooth Muscle*, ed. Daniel, E.E. & Paton, D.M. pp 471–506. New York and London: Raven Press.

WENKE, M., LINCOVA, D., CEPELIK, J., CERNOHORSKÝ, M. & HYNIE, S. (1967). Some aspects of the action of beta adrenergic blocking drugs on adrenergic lipid mobilization. *Ann. N.Y. Acad. Sci.*, **139,** 860–878.

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