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DIRECT AND INDIRECT EFFECTS OF ADENOSINE 5′-TRIPHOSPHATE ON GUINEA-PIG ILEUM

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1 The inhibitory effects of adenosine 5′-triphosphate (ATP) were compared on the responses to electrical stimulation, and to direct and indirect stimulation by drugs of the longitudinal smooth muscle of guinea-pig ileum before and after blocking nervous activity.

2 While the major inhibitory effect of ATP was an indirect one on the intramural excitatory nerves, there was also a small direct effect on the muscle.

3 ATP also had direct and indirect excitatory effects. The direct effect particularly was only seen with high concentrations of ATP, but the appearance of these excitatory effects may be affected by the inhibitory actions.

Introduction

Several studies over the past few years have obtained evidence for the existence of a nonadrenergic inhibitory mechanism in guinea-pig ileum (Holman & Hughes, 1965; Kosterlitz, 1967; Hirst & McKirdy, 1974; Van Nueten, Fontain, Helsen & Jansson, 1977).

Among the most prominent candidates for the role of a nonadrenergic inhibitory transmitter in the past few years have been the purine nucleotides. Burnstock (1972, 1979) has reviewed the evidence that a purine nucleotide may play such a role at various sites in the alimentary canal, including the ileum, as well as in various other tissues and organs.

If a purine nucleotide such as adenosine 5′-triphosphate (ATP) was to act as an inhibitory transmitter, it should have a direct inhibitory effect on the muscle involved. In looking for a direct effect of a purine nucleotide on the longitudinal muscle of guinea-pig ileum, different workers have obtained different results. While Sawynok & Jhamandas (1976), Cook, Hamilton & Okwuasaba (1979) and Gustafsson, Hedqvist, Fredholm & Lundgren (1978) found that purine nucleotides did not depress the direct response of guinea-pig ileum to stimulants such as acetylcholine, Sehorn & Borowitz (1971) and McDougal & Borowitz (1972) reported that adenosine, had a small direct inhibitory effect on the smooth muscle, as well as a more prominent indirect inhibitory effect, exerted on the excitatory intramural nerves.

In the experiments described here the effects of ATP in producing direct and indirect inhibition of guinea-pig ileum are compared.

Methods

All experiments were carried out on segments of guinea-pig ileum. The segments were taken from the terminal end after discarding the last 10 cm (Munro, 1953). The preparation was set up so that it could be coaxially stimulated (Paton, 1955). The stimuli used were square wave pulses of 0.5 ms duration, at a frequency of 0.1 Hz and just submaximal voltage.

The bath fluid was a Krebs solution of the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11. The bath temperature was 37°C.

The longitudinal movements of the preparation were recorded auxotonically by an isometric transducer connected to the preparation by a light coil spring with a compliance of 0.75 cm/g. The resting tension was 1 g. The inhibitory effect of ATP on the response to coaxial stimulation was expressed as a percentage inhibition of the control response before the addition of ATP. The inhibitory effect of ATP on the responses to the spasmogens was studied by using a dose of the spasmogen which gave a contraction approximately equal in size to the coaxial twitch and measuring the percentage inhibition produced when ATP was added 15 s before the spasmogen.

Statistical methods

All results are expressed as the mean ± s.e.mean. In order to normalize the ratios, a log transformation was performed. The s.e. on either side of the mean is therefore different. Significance of difference in the experimental results was calculated using a paired *t* test. *P* values of less than 0.05 were considered

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significant. When an EC_{50} value for ATP is calculated this is defined as the concentration of ATP producing a 50% inhibition of the test response.

Drugs

The drugs used were acetylcholine chloride (BDH), adenosine 5'-triphosphate (sodium salt) (BDH), histamine acid phosphate (BDH), 5-hydroxytryptamine creatinine sulphate (BDH), hyoscine hydrobromide (BDH), nicotine hydrogen tartrate (BDH) and tetrodotoxin (Sankyo). These drugs were dissolved in distilled water. I am grateful to Dr J. Pike (Upjohn) for a gift of prostaglandin E_2 (PgE_2). Stock solutions of PGE_2 were prepared in absolute ethanol (1 mg/ml) and dilutions were made with distilled water.

Results

Effects of ATP on the responses to coaxial stimulation, acetylcholine and nicotine

ATP in concentrations above about 0.1 μM inhibited the response to coaxial stimulation (Figure 1). The EC_{50} was 3.0 μM. Complete inhibition of the coaxial twitch was usually produced with a concentration of 12.8 μM. Figure 1 also illustrates the rebound contractions produced by ATP. Although these reached their maximum immediately after washout, they began before the bath was washed out.

ATP also inhibited the responses to acetylcholine and to nicotine. The response to acetylcholine was only affected by concentrations of ATP greater than 3.2 μM and the maximum of the response to acetylcholine produced was about 25% (Figure 2). Concentrations of ATP greater than 51.2 μM were not used in these experiments since, as will be described later, such concentrations of ATP themselves produce a contraction. The inhibitory effect of ATP on the response to coaxial stimulation was significantly greater ($P<0.05$) than on the responses to acetylcholine or nicotine with all the concentrations of ATP used in Figure 2.

Although the lower concentrations of ATP used did not produce significantly different inhibitions of the responses to acetylcholine and nicotine, the response to nicotine was inhibited to a significantly greater extent by 25.6 μM ATP ($P<0.5$) and 51.2 μM ATP ($P<0.01$). The higher concentration of ATP almost abolished the response to nicotine.

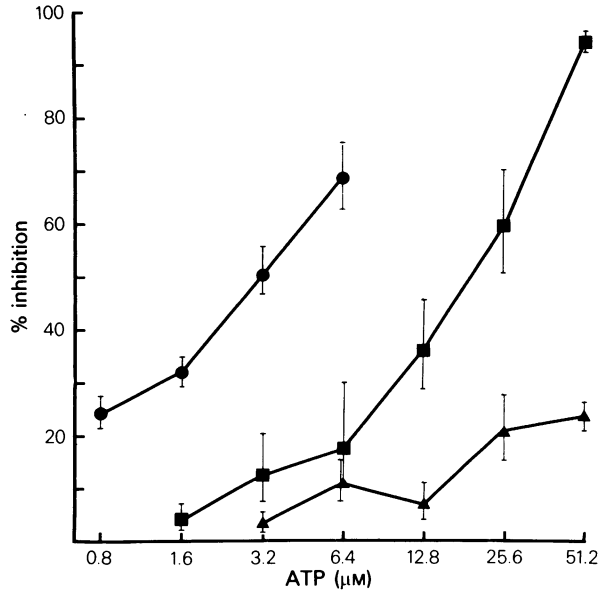

Figure 2 Percentage inhibition produced by ATP of responses to coaxial stimulation (●), nicotine (■) and acetylcholine (▲). Each point represents the mean of 8 experiments. Vertical bars show s.e.mean.

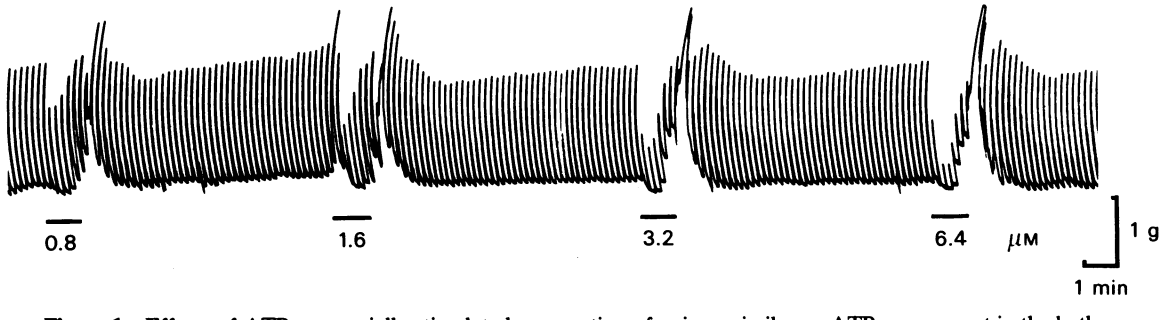

Figure 1 Effects of ATP on coaxially stimulated preparation of guinea-pig ileum. ATP was present in the bath during the periods indicated by the horizontal bars below the trace. The figures below the bar refer to the concentration of ATP (μM). Scales 1 g tension and 1 min.

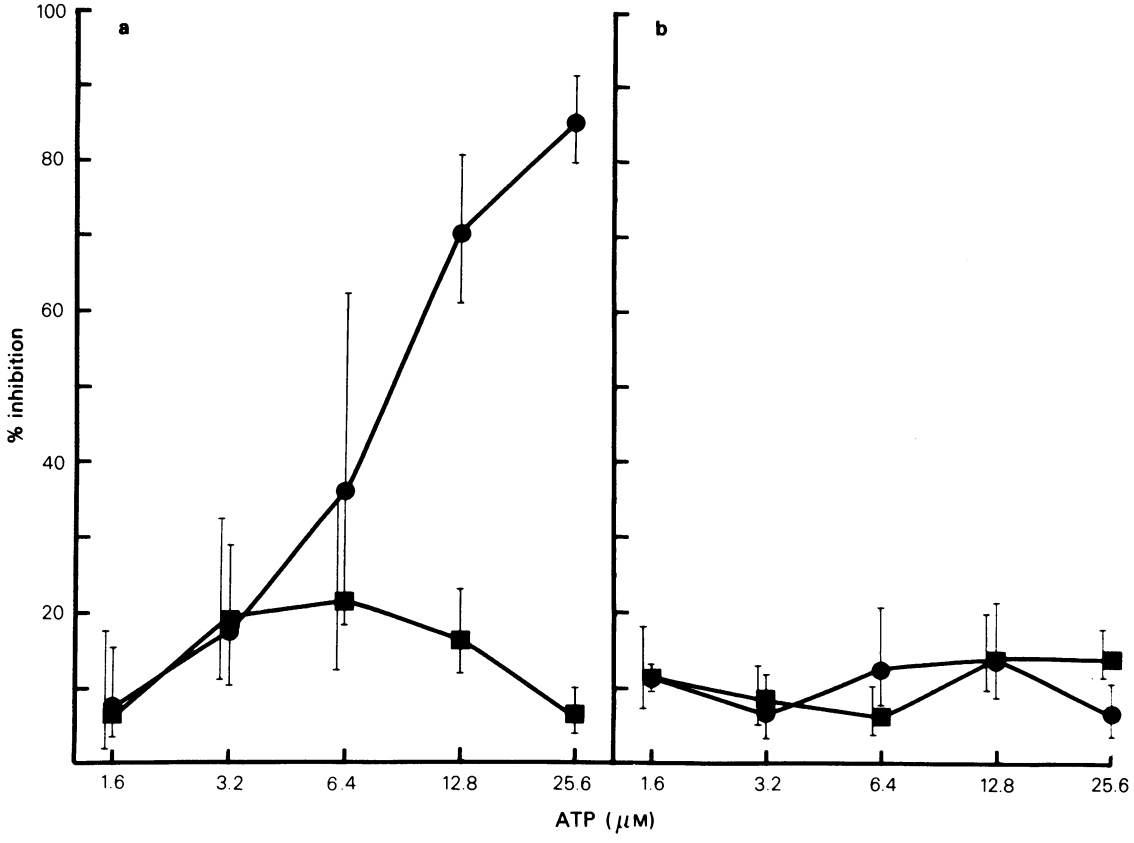

Figure 3 Percentage inhibition produced by ATP of the responses to (a) 5-hydroxytryptamine (5-HT) and (b) acetylcholine. In both panels the control responses to ATP are shown by (●) and the responses to ATP in the presence of tetrodotoxin (69 nM) by (■). Each point is the mean value; vertical lines show s.e.mean ($n = 7$).

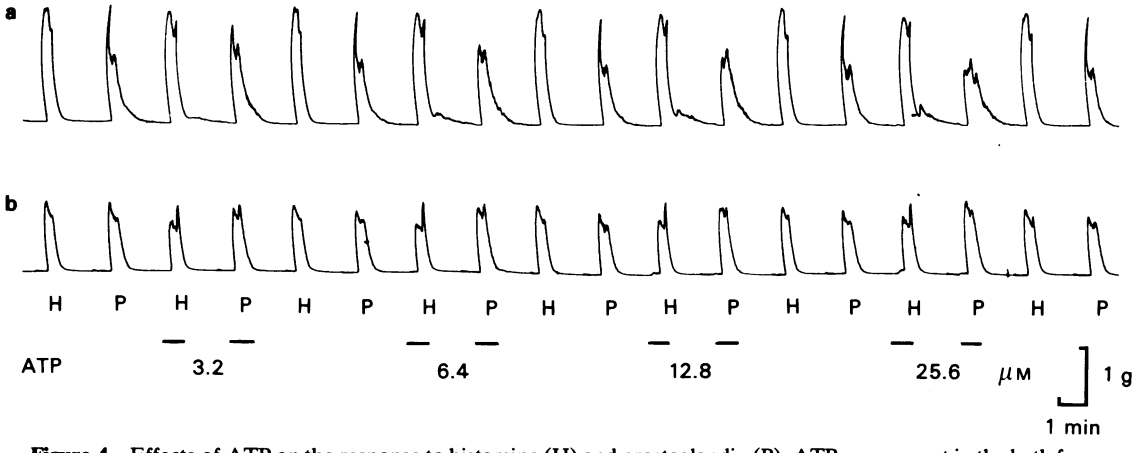

Figure 4 Effects of ATP on the response to histamine (H) and prostaglandin (P). ATP was present in the bath for the periods indicated by the horizontal bars at the bottom of the figure, which refer to both upper and lower traces. (a) Control trace; (b) trace obtained from a preparation in the presence of hyoscine 60 nM. Numbers below the bars refer to concentration of ATP (μM). Scales 1 g tension and 1 min.

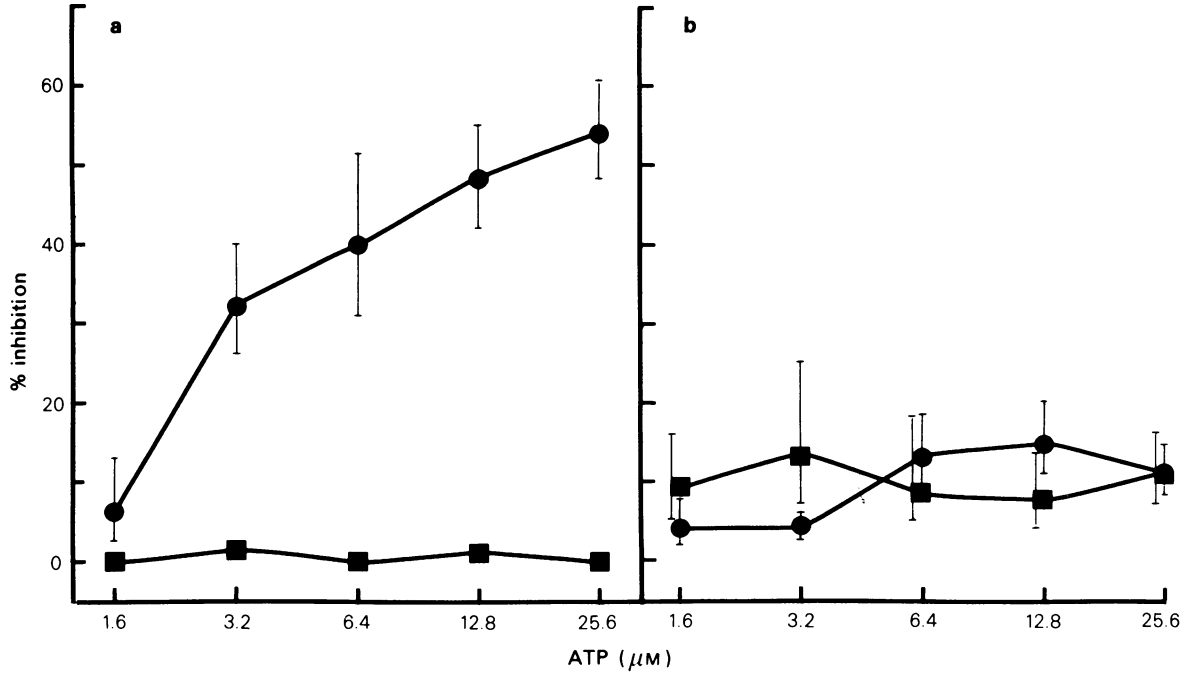

Figure 5 Percentage inhibition produced by ATP of the responses to (a) prostaglandin E_2 and (b) histamine. In both panels the control responses to ATP are shown by (●) and the responses to ATP in the presence of hyoscine (60 nM) by (■); each point is the mean value and vertical lines show s.e.mean ($n = 5$).

Effect of ATP on the responses to 5-hydroxytryptamine and acetylcholine before and after the addition of tetrodotoxin

Figure 3 shows the inhibitory effect of ATP on the responses to 5-hydroxytryptamine (5-HT) and acetylcholine. In the control period, before the addition of tetrodotoxin (TTX), ATP had a significantly greater inhibitory effect on the response to 5-HT when used in concentrations of 12.8 μM and 25.6 μM ($P<0.002$). When the experiment was repeated in the presence of 67 nM TTX, a concentration which, in each experiment, completely blocked the response to coaxial stimulation, the effect of ATP on the TTX-resistant responses to 5-HT was much reduced and there was no significant difference in the inhibition by ATP of the responses to 5-HT and acetylcholine.

Effect of ATP on the responses to prostaglandin E_2 and histamine before and after the addition of hyoscine

Figures 4 and 5 illustrate the effect of ATP on the responses to prostaglandin E_2 (PGE_2). ATP produced an inhibition of the initial spike component of the PGE_2-induced contraction. This component of the response to PGE_2 was also abolished by hyoscine (60 nM) and ATP had no effect on the hyoscine-resistant component of the PGE_2 response. This difference in the effect of ATP was significant ($P<0.005$) with each concentration tested. ATP also produced a small inhibition of the response to histamine, the maximum inhibition seen being 28%. The inhibitory effect of ATP on the response to histamine was not significantly different in the hyoscine-treated preparation. The inhibitory effect of ATP before the addition of hyoscine was significantly greater ($P<0.05$) on the response to PGE_2 than on the response to histamine. In the presence of hyoscine the inhibitory effects of ATP on the responses to histamine was significantly greater ($P<0.01$) than on the response to PGE_2.

Responses to ATP alone and the effect of hyoscine

Figure 6 illustrates the responses of the unstimulated ileum to a range of concentrations of ATP. In concentrations from 1–50 μM the direct response was never very large. With concentrations of ATP greater than 50 μM the direct response was a contraction. A maximum response was produced with about 1.5 mM ATP and this response was at least 90% of the maximum contraction of the preparation which could be produced with acetylcholine. With concentrations of ATP above 1 μM a rebound contraction was seen

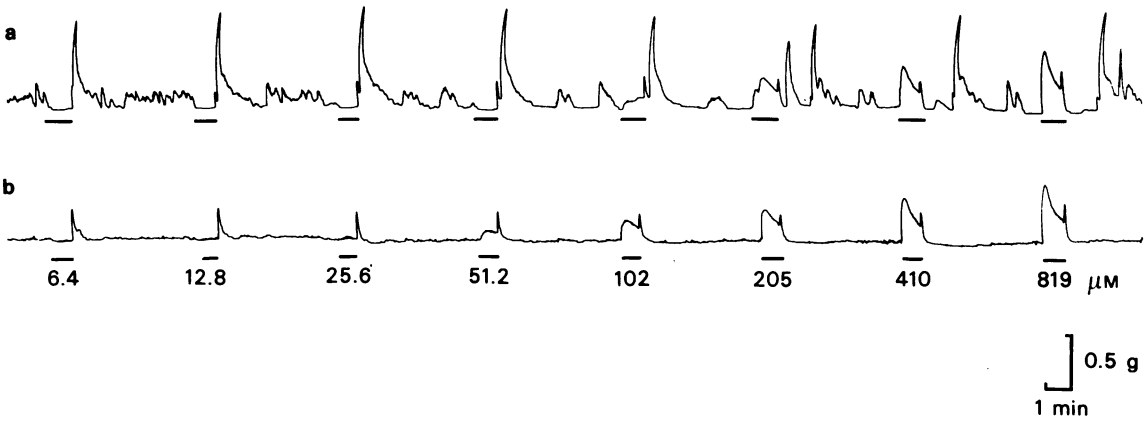

Figure 6 Effects of ATP on unstimulated preparation. ATP was present in organ bath for periods indicated by the horizontal bars at the foot of each figure, which refer to both traces. The concentration of ATP (μM) is shown by the number below each bar. (a) Control responses to ATP. (b) Responses to ATP in a preparation treated with hyoscine (60 nM). Scales 1 g tension and 1 min.

after washing out the organ bath. With the largest concentrations of ATP the rebound contraction could be delayed for several minutes.

When the preparation was treated with 60 nM hyoscine (Figure 6b), the direct contractile response to ATP was not affected except that it became apparent at a lower concentration of ATP, but the rebound contraction was abolished. The same effect was produced by tetrodotoxin (69 nM).

Discussion

While some workers have found that ATP and other purines have no direct inhibitory effect on the longitudinal smooth muscle of guinea-pig ileum (Sawynok & Jhamandas 1976; Gustafsson *et al.*, 1978; Cook *et al.*, 1979) the results described in this paper support the earlier findings of Sehorn & Borowitz (1970) and McDougal & Borowitz (1972) with adenosine, that a small direct inhibitory action is present as well as the more prominent indirect inhibitory action. This direct effect of ATP, as seen by the inhibition of the responses to directly acting muscle stimulants such as acetylcholine and histamine, is present in the same concentration range, 1–25 μM, as the indirect inhibitory effect.

Since ATP is metabolized to adenosine in the gut (Burnstock 1972, 1979), it is possible that the formation of adenosine might occur during the experiments and that the direct inhibitory effect is a response to adenosine, the purine used by Sehorn & Borowitz (1970) and McDougal & Borowitz (1972). However Sawynok & Jhamandas (1976) found both ATP and adenosine to be without effect on the response of the longitudinal muscle of the ileum to acetylcholine and Gustafsson *et al.* (1978) also found that adenosine did not inhibit the response to acetylcholine.

The results obtained in the experiments in which the inhibitory effect of ATP was compared on the responses to 5-HT and acetylcholine confirm that while the most prominant effect of ATP is on the response to 5-HT mediated by the M receptors (Gaddum & Picarelli, 1957), there is a small inhibitory effect on the direct response of the muscle to 5-HT (Figure 3). This direct effect of 5-HT, mediated by the D receptors, and obtained in a preparation treated with TTX to block nervous activity so that 5-HT could not act via the M receptors, was inhibited by ATP to approximately the same extent as the response to acetylcholine in the absence or presence of TTX.

The experiments with PGE_2 again confirmed that ATP had a strong inhibitory effect on the indirect component of the response to PGE_2. However, the direct component of the response to PGE_2, obtained in the presence of hyoscine, was not inhibited at all by ATP, although in the same experiments ATP inhibited the responses to histamine in the absence or presence of hyoscine.

This suggests that the direct excitatory effect of PGE_2 on smooth muscle has a different mechanism from that of histamine. Coceani & Wolfe (1966) and Paton & Daniel (1967) have previously found evidence for a difference in the mechanisms by which the direct contractile actions of PGE and acetylcholine on smooth muscle are produced. It is not possible from the present experiments to draw any definite conclusions about the different modes of action.

Thus while ATP does have a direct inhibitory effect on the longitudinal muscle of guinea-pig ileum,

as would be required if it were to act as an inhibitory transmitter, its prominent inhibitory effect is on the cholinergic excitatory innervation.

In these experiments ATP was also found to have both direct and indirect excitatory actions, confirming the findings of Burnstock, Campbell, Satchell & Smythe (1970). The indirect action was seen as a rebound contraction on washing out the preparation (Burnstock *et al.*, 1970), with increasing concentrations of ATP, the interval between washing out the preparations and the appearance of the rebound contraction increased. The direct excitatory effect of ATP was only obtained with a range of concentrations higher than those needed to elicit the inhibitory effects. The maximum contraction produced by ATP acting directly on the muscle was always less than the maximum contraction which could be elicited by acetylcholine or histamine.

In conclusion, application of ATP to guinea-pig ileum preparations produces both direct and indirect inhibition of the smooth muscle. An indirect rebound excitation and, with higher concentrations, a direct excitation, are also seen.

It is possible that the metabolic conversion of ATP to adenosine might account for some of the differences in the type of inhibition observed by different workers. Further experiments are required to resolve this point.

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