Br. J. Pharmac. (1980), **71**, 245–252

THE EFFECTS OF CALCIUM CONCENTRATION ON THE INHIBITION OF CHOLINERGIC NEUROTRANSMISSION IN THE MYENTERIC PLEXUS OF GUINEA-PIG ILEUM BY ADENINE NUCLEOTIDES

E.B. DOWDLE & R. MASKE[1]

Department of Clinical Science and Immunology, University of Cape Town, Medical School, Observatory 7925, Cape, South Africa

1 Adenosine and the adenine nucleotides AMP, ADP, ATP, cyclic AMP, NAD, NADP and NADH produced a dose-related inhibition of the contractile response of guinea-pig ileum longitudinal muscle-myenteric plexus strips to low frequencies (< 1 Hz) of electrical field stimulation.

2 These compounds inhibited hexamethonium-sensitive contractions induced by nicotine but did not alter the responses to exogenous acetylcholine, and the acetylcholine output from the myenteric plexus was inhibited by the adenyl compounds. These findings indicate that adenine derivatives act at a presynaptic site on postganglionic cholinergic neurones.

3 The degree of inhibition produced by adenine compounds was inversely related to the calcium concentration of the bath fluid over a range of calcium concentrations (1 to 5 mM) that had no effect on the responses of the muscle to exogenous acetylcholine.

4 The inhibition produced by adenine derivatives was antagonized by theophylline and augmented by dipyridamole. Both of these interactions were sensitive to, and synergistic with, alterations of the concentration of calcium in the bath fluid.

5 The results suggest that adenine compounds inhibit acetylcholine release from the myenteric plexus by diminishing the availability of intracellular calcium ions required for neurotransmitter release.

Introduction

Adenosine and adenine nucleotides, in addition to serving as biosynthetic precursors of nucleic acids and cofactors in chemical energy transfer, have reversible inhibitory effects upon contractile responses of isolated intestinal preparations that suggest that this class of compounds may function *in vivo* as regulators of neuromuscular activity in the gut.

Various mechanisms have been proposed to account for the pharmacological effects of adenine compounds. These include suggestions that adenosine, adenosine 5′-pyrophosphate (ADP) and adenosine 5′-triphosphate (ATP) function as neurotransmitters released by inhibitory 'purinergic' nerve endings (Burnstock, Campbell, Satchell & Smythe, 1970; Burnstock, 1972; Satchell & Burnstock, 1975; Okwuasaba, Hamilton & Cook, 1977); that their action is mediated by alterations in intracellular levels of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (Sattin & Rall, 1970; Clark, Gross, Su & Perkins, 1974); or that they affect the passage of sodium (Imai & Takeda, 1967), potassium (de Gubareff & Sleator, 1965) or calcium (Herlihy, Bockman, Berne & Rubio, 1976; Ribeiro, Sá-Almeida & Namorado, 1979) across cell membranes.

Since calcium ions are critically and ubiquitously involved in the function of excitable cells, we have studied the effects of adenosine and adenine derivatives on contractile responses of the guinea-pig ileum longitudinal muscle-myenteric plexus preparation at different calcium concentrations. Our results have shown that adenosine-containing compounds act at a presynaptic site on postganglionic cholinergic neurones in the myenteric plexus and that this action may be mediated by modulation of intra-axonal calcium concentration.

Methods

Preparation of longitudinal muscle-myenteric plexus strips

Guinea-pigs weighing 300 to 600 g were killed by cervical dislocation and exsanguination. Strips of

[1] Present address: Department of Physiology, John Curtin School for Medical Research, Australian National University, Canberra ACT 2601, Australia.

0007-1188/80/130245-08 $01.00

tissue approximately 5 cm in length and comprising longitudinal muscle with attached myenteric plexus were prepared according to the method of Paton & Vizi (1969) from segments of ileum approximately 20 cm proximal to the ileo-caecal junction. The strips were suspended, at a resting tension of 0.3 g, in an organ bath (7.5 ml) containing Krebs solution maintained at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. A period of 90 min was allowed for equilibration before experiments were started. During this period, strips were washed every 10 min. Changes of the bath fluid were made by overflow. The composition of the Krebs solution was (mM): NaCl 118, KCl 4.7, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, choline chloride 0.03, $MgCl_2$ 1.2 and $CaCl_2$ 2.5. Solutions containing different calcium concentrations were prepared in Krebs solution and the effects of adenine derivatives were studied after a 30 min equilibration period with each calcium concentration.

Contractile responses were recorded isometrically with a force-displacement transducer and a single pen recorder (Philips PM 8202).

Electrical stimulation

Field stimuli were applied to the strip through immersed platinum electrodes situated at the top and the bottom of the organ bath (Paton, 1963; Paton & Vizi, 1969). Square-wave, biphasic pulses of 2 ms duration and supramaximal voltage (45 V; 10 V/cm) were delivered at low frequencies (0.1 to 0.2 Hz) by means of a pulse generator.

Acetylcholine assay

Acetylcholine was assayed by a bracketing technique as described by Paton & Zar (1968) and Paton & Vizi (1969). A 10 cm 'donor' segment of longitudinal muscle-myenteric plexus was suspended in 3 ml Krebs solution containing 3 μM physostigmine sulphate. Samples of the bath fluid were collected into glass beakers after periods of 10 min and were assayed in duplicate within 10 min of collection.

The acetylcholine released into the bath fluid over timed periods, under conditions of rest or electrical field stimulation at 0.2 Hz, was assayed with a second strip of longitudinal muscle-myenteric plexus. This 'assay' strip was suspended in 4.0 ml Krebs solution containing 7.7 nM physostigmine sulphate and 13 μM morphine sulphate. The acetylcholine content of each sample was estimated by interpolation from dose-response curves constructed with freshly prepared acetylcholine standards. Both samples and standards were added to the assay bath in volumes of 0.5 ml. When donor strips were exposed to drugs, standard curves were constructed with acetylcholine solutions containing the relevant drug at concentrations equivalent to the final concentrations in the sample assay bath. Acetylcholine released by the donor strips is expressed as pmol g^{-1} min^{-1}.

Addition of drugs

Drug solutions were added to the bath in volumes of 5 to 75 μl to give the desired final concentrations. To quantitate the effects of drugs, both 'single dose' and 'cumulative dose' methods were used to construct dose-response curves (Kosterlitz & Watt, 1968). In each case the drug remained in the bath until it had produced a maximal effect. The rest intervals between drug additions varied from 5 to 10 min. In evaluating the effects of adenyl derivatives on nicotine- and acetylcholine-induced contractions, the adenyl compounds were added to the bath 30 to 60 s before the spasmogens.

The effect of any particular adenine compound was expressed as a percentage inhibition of the twitch height. The maximum inhibition observed with 100 μM adenosine was taken as 100% and lesser degrees of inhibition were expressed as a percentage of this maximum. Since the inhibition produced by adenine compounds was usually followed by some degree of spontaneous recovery, the degree of inhibition produced by any given concentration of compound was read as the maximal inhibition for that concentration. The ID_{50} values (concentrations producing 50% inhibition of the twitch response) were determined by interpolation from linear dose-response plots of the logit transform of the effect as a function of the logarithm of the concentration. The logit transform of the % inhibition was calculated from the formula

$$\text{Logit (\% inhibition)} = \text{Log}_e\left[\frac{\text{\% inhibition}}{100 - \text{\% inhibition}}\right]$$

ED_{50} values for acetylcholine excitation were obtained by interpolation from log-logit transform plots of cumulative dose-effect curves.

Reagents

All the chemicals used to prepare the buffers were of analytical grade. Stock solutions of adenyl compounds were prepared in buffered saline and stored at −20°C. The following drugs and compounds were used: adenosine, AMP, ADP, ATP, cyclic AMP, adenine, 2'-deoxyadenosine, theophylline, guanosine and cyclic GMP (Schwarz/Mann, New York); inosine, hypoxanthine, GTP, 6-chloropurine ribonucleoside, physostigmine (eserine) sulphate, NAD, NADP, NADH and hexamethonium bromide (Sigma Chemical Company); *o*-acetylcholine chloride, nicotine hydrogen tartrate and D-ribose (BDH Ltd.); morphine sulphate (Petersen Ltd.); dipyridamole (Boehringer

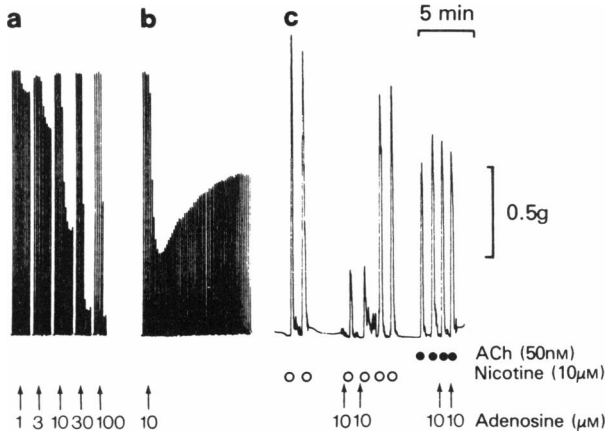

Figure 1 The inhibitory effect of adenosine on the contractile responses of the longitudinal muscle-myenteric plexus strip obtained from guinea-pig ileum. Tracing (a) shows the twitch response elicited by 0.1 Hz field stimulation and the dose-related inhibition produced by adenosine at the concentrations indicated by the arrows. Tracing (b) shows the spontaneous recovery following inhibition produced by 10 μM adenosine; adenosine remained in the bath during recording of the tracing. Tracing (c) shows the contractions induced by 10 μM nicotine (O) and 50 nM acetylcholine (ACh, ●) before and after exposure to 10 μM adenosine for 1 min (indicated by the arrows). The preparation was washed between additions.

Ingelheim (Pty) Ltd); and A23187 (Eli Lilly and Co., Indianapolis, U.S.A.). Coformycin was obtained from Prof. H. Umezawa, Chemistry Research Foundation, Tokyo. Drug concentrations refer to the final bath concentration in terms of molarity of the salt.

Results

Effects of adenine compounds on the twitch response

Adenosine produced a dose-dependent inhibition of the electrically evoked twitch response at 0.1 Hz with a threshold concentration of 0.1 μM and a maximum inhibitory concentration of 100 μM. The effect was rapid in onset (usually within 6 s) and reached a maximum 30 to 60 s after addition of adenosine (Figure 1a). If adenosine was left in contact with the preparation, some degree of spontaneous recovery was observed (Figure 1b). The rate of recovery followed first-order kinetics at all concentrations investigated, and could be prevented by pretreatment of the preparation with 3 μM coformycin, a potent inhibitor of adenosine deaminase (Henderson, Brox, Zombor, Hunting & Lomax, 1977). The inhibitory effect of adenosine was readily and rapidly (within 0.5 to 2 min) reversible by washing the preparation with adenosine-free buffer.

Table 1. Inhibitory effects of adenine compounds on the field stimulated guinea-pig ileum preparation

Compound	ID_{50} (μM)*
Adenosine	3.9 ± 0.4 (50)
AMP	4.4 ± 0.5 (8)
ADP	4.5 ± 1.0 (8)
ATP	5.8 ± 0.9 (10)
Cyclic AMP	4.6 ± 0.5 (5)
NAD	4.2 ± 0.4 (3)
NADP	3.7 ± 0.6 (3)
NADH	4.3 ± 0.5 (3)

* ID_{50} values were calculated from cumulative dose-response curves and are expressed as the mean ± s.e. mean in each case. The number of experiments is indicated in parentheses.

The nucleotides AMP, ADP, ATP, cyclic AMP, NAD, NADP and NADH produced inhibitory effects that were, in all qualitative respects, similar to those produced by adenosine.

The ID_{50} values for adenosine and the adenine nucleotides tested are given in Table 1. Although, in terms of the molar concentration required to produce 50% inhibition, adenosine was the most potent compound with activity diminishing as the number of phosphate groups increased, the ID_{50} values observed for the various compounds were not significantly different. At concentrations less than 100 μM, adenine, ribose, inosine, hypoxanthine, 6-chloropurine ribonucleoside, 2'-deoxyadenosine, guanosine, cyclic GMP and GTP had no inhibitory effect, indicating a structural requirement for both adenine and ribose for inhibitory activity.

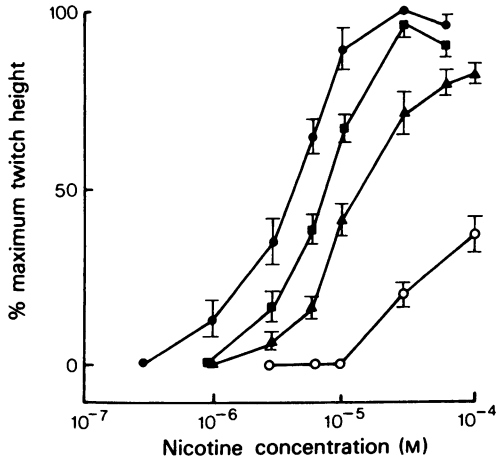

Figure 2 Effects of adenosine and hexamethonium on the dose-response curves for nicotine. Contractile responses are expressed as percentages of the maximum twitch height elicited by 30 μM nicotine. Contractions were elicited before (●) and after exposure to adenosine 1 μM (■) and 10 μM (▲) for 1 min. Inhibition produced by 100 μM hexamethonium (○) was measured after exposure to the drug for 2 min. Each point represents the mean of 4 experiments; vertical lines show s.e. means.

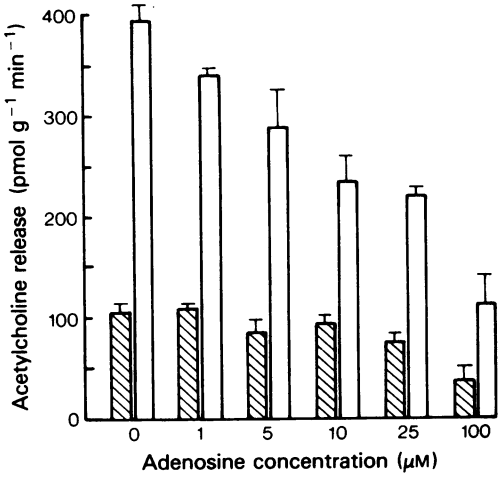

Figure 3 Effect of adenosine on acetylcholine release from the longitudinal muscle-myenteric plexus preparation. Acetylcholine output was measured in pmol g^{-1} min^{-1} at rest (hatched bars) and during field stimulation at 0.2 Hz (open bars). Samples were collected over a 10 min period and the mean values for 3 experiments are shown in each case; vertical lines show s.e. means.

Site of action of adenine compounds

To determine the site of action of adenine compounds, the inhibitory effects of adenosine and adenine nucleotides were studied on contractions induced by nicotine and acetylcholine. Hexamethonium-sensitive concentrations of nicotine (less than 10 μM) elicit contractile responses by stimulation of the ganglion cells while acetylcholine, in the presence of hexamethonium, stimulates the smooth muscle directly (Day & Vane, 1963; Paton & Zar, 1968). Adenine compounds inhibited the contractions produced by nicotine (Figures 1c and 2) whereas no significant inhibition of the acetylcholine-induced contractions was produced by adenosine or adenine nucleotides at concentrations up to 100 μM (Figure 1c). Hexamethonium, at a concentration of 100 μM that abolished nicotine-induced contractions, had no effect on adenosine-induced inhibition of 0.1 Hz electrically evoked twitch response. The site of action of adenine compounds could therefore be localised to the post-ganglionic cholinergic nerves.

Confirmation that the adenine compounds acted on the cholinergic nerves was obtained from the experimental results summarized in Figure 3 from which it can be seen that adenosine, at concentrations of 1 to 100 μM, reduced the resting output of acetylcholine to a small extent and markedly inhibited acetylcholine release evoked by 0.2 Hz stimulation in a concentration-dependent fashion. Similar reductions of stimulated acetylcholine release were observed with AMP, ADP and ATP.

Effects of calcium on adenine inhibition

The inhibitory effects of adenosine on the 0.1 Hz twitch response were studied in the presence of calcium concentrations varying from 0.5 mM to 10 mM. As can be seen from the results summarized in Figure 4, adenosine dose-response curves were shifted to the right as the calcium concentrations increased. In other words, the magnitude of the effect of any given concentration of adenosine was inversely related to the calcium concentration. Similar results were obtained with AMP, ADP and ATP.

In Figure 5, the ID_{50} values for adenosine inhibition of the 0.1 Hz twitch response and the ED_{50} values for acetylcholine-induced contractions are plotted as functions of the calcium concentration at which the respective dose-effect curves were constructed. As is evident from these experiments, the sensitivity of the smooth muscle to the direct action of acetylcholine was unaffected by changes in the calcium concentration over the range 1 to 5 mM. However, over this same range the ID_{50} for adenosine was directly related to calcium concentration. We conclude, therefore, that adenosine-calcium interactions

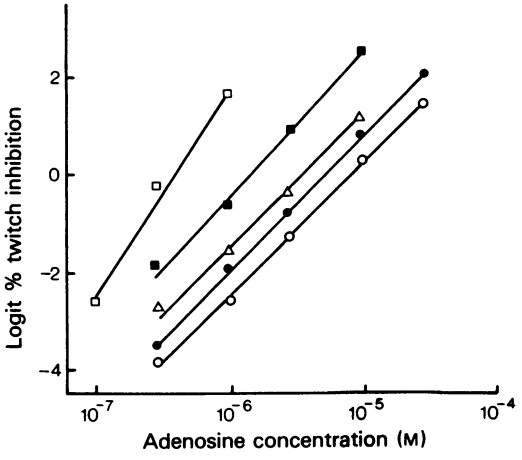

Figure 4 Effect of the calcium chloride concentration on the inhibitory action of adenosine. Strips of longitudinal muscle-myenteric plexus were equilibrated for 30 min at each calcium concentration before addition of adenosine. Calcium chloride concentrations (mM): 0.5 (□); 1.25 (■); 2.5 (△); 5.0 (●); 10.0 (○). The inhibitory effect of adenosine is plotted as the logit transform of the inhibition expressed as a percentage of the maximal inhibition produced by 100 μM adenosine. Each point represents the mean for 3 experiments and the lines were fitted by least squares regression.

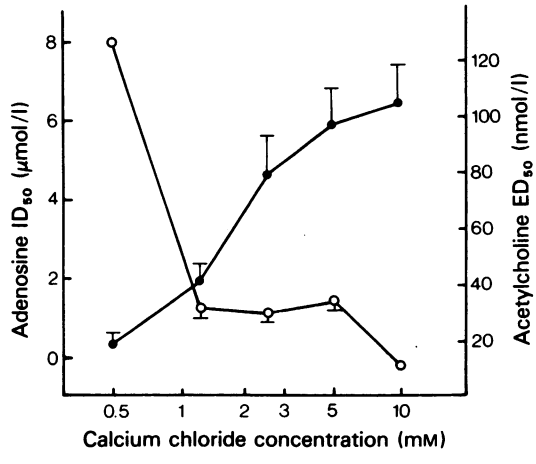

Figure 5 The effect of calcium chloride concentration on the ID_{50} for adenosine inhibition of the 0.1 Hz twitch response of the guinea-pig ileum preparation (left hand scale; ●) and the ED_{50} for acetylcholine contractions (right hand scale; ○). The ID_{50}s for adenosine inhibition and the ED_{50}s for acetylcholine contractions were estimated from cumulative dose-effect curves. Each point represents the mean for 3 experiments and vertical lines indicate s.e. means.

observed at calcium concentrations between 1 and 5 mM were mediated by effects upon the myenteric plexus and not by direct effects upon the smooth muscle component of the preparation.

Synergistic effects of theophylline and calcium concentration

Methylxanthine derivatives have been shown to antagonize the inhibitory effects of adenine compounds on ileal preparations (Ally & Nakatsu, 1976; Sawynok & Jhamandas, 1976; Vizi & Knoll, 1976) and theophylline is known to have effects on the intracellular calcium concentration (Moritoki, Morita & Kanbe, 1976; Kažić, 1977). It was therefore of interest to look for interactions between adenosine, calcium ions and theophylline by performing a series of experiments in which the ID_{50} values for adenosine were determined in the presence or absence of 100 μM theophylline and/or 5 mM $CaCl_2$. As shown in Table 2, the ID_{50}s for adenosine were increased by 10.4 μM in the presence of 100 μM theophylline and by 0.9 μM in the presence of 5 mM $CaCl_2$. When 100 μM theophylline and 5 mM $CaCl_2$ were present in combination, the ID_{50} was increased by 27.3 μM. Calcium and theophylline therefore acted synergistically, and not additively, to reverse the inhibitory effect of adenosine.

The synergistic effects of dipyridamole

Since dipyridamole has been shown to act synergistically with adenosine and adenine nucleotides to inhibit contractile responses of isolated intestinal preparations (Satchell & Burnstock, 1975; Okwuasaba *et*

Table 2 Effects of theophylline and calcium concentration on adenosine inhibition of 0.1 Hz twitch response

Treatment	*Calcium concentration* 2.5 mM	5.0 mM
Control	4.2 ± 0.2	5.1 ± 0.3
Theophylline (100 μM)	14.6 ± 0.5	31.5 ± 1.0

Table entries give the mean ID_{50} values (μM) ±s.e. mean for 3 experiments calculated from cumulative dose-response curves in the presence of theophylline and calcium concentration shown. The pretreatment time with theophylline was 2 min.
Two-way analysis of variance yielded f values as follows: calcium effect: 240 ($P < 0.001$); theophylline effect: 1026 ($P < 0.001$); calcium × theophylline interaction: 194 ($P < 0.001$).

al., 1977; Hayashi, Mori, Yamada & Kunitomo, 1978), we felt it appropriate to study the effects of varying calcium concentrations on the magnitude of this synergistic effect. Experiments were accordingly performed in which the inhibitory effects of 1 μM adenosine on the 0.1 Hz twitch response were measured in the presence of normal (2.5 mM) or reduced (2.0 mM) calcium concentrations with or without the addition of 10 nM dipyridamole. These concentrations were chosen since neither the slight reduction in calcium concentration nor 10 nM dipyridamole alone had any detectable effect on the amplitude of the 0.1 Hz twitch response in the absence of adenosine.

As can be seen from the data presented in Table 3 a reduction of the calcium concentration augmented the inhibitory effect of adenosine by 1.9% and dipyridamole increased the inhibitory effects of adenosine by 8.5%. However, the combined effect of reduction in calcium concentration and 10 nM dipyridamole was to increase adenosine inhibition by 19.2%. Calcium reduction and dipyridamole therefore acted synergistically and not additively.

Effects of the calcium ionophore, A23187

Adenosine inhibitory dose-response curves were constructed in the presence or absence of 0.1 μM A23187. The results are presented in Figure 6 from which it can be seen that this concentration of the calcium ionophore increased the ID_{50} for adenosine from 4.3 ± 0.4 μM to 7.8 ± 0.9 μM (mean ±s.e. mean for three experiments).

Discussion

The results of the experiments we describe in this paper have shown that adenosine and adenine nucleotides inhibit the contractile responses of the guinea-pig ileum longitudinal muscle-myenteric plexus preparation to low frequency electrical field stimulation. Since these compounds inhibited both the electrically stimulated release of acetylcholine and nicotine-induced contractions but had no effect upon the amplitude of acetylcholine-evoked contractions it may be concluded that they act upon postganglionic cholinergic nerves in the myenteric plexus and not directly upon smooth muscle. These results are in keeping with the findings of other workers who have studied the guinea-pig ileum (Takagi & Takayanagi, 1972; Sawynok & Jhamandas, 1976; Vizi & Knoll, 1976; Hayashi *et al*, 1978).

The lack of a direct action on smooth muscle, considered in conjunction with the observations (a) that adenosine and its nucleotides were essentially equipotent in their inhibitory activity and (b) that the pharmacologically active derivatives of adenosine were not restricted to the adenosine-AMP-ADP-ATP series but included NAD, NADH and NADP, indicate that the concept of purinergic neuromuscular transmission as originally formulated (see review by Burnstock, 1972) does not apply to the guinea-pig ileum.

Considerable support exists for the view that adenosine and adenine nucleotides play a regulatory role in neurological and other tissues (Arch & Newsholme, 1978), but relatively little is known of the manner in which the action of adenosine is mediated. There is evidence that adenosine stimulates adenylate cyclase

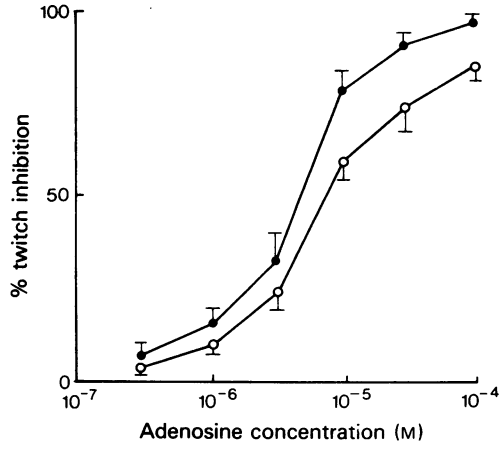

Figure 6 Effect of calcium ionophore, A23187, on the inhibitory action of adenosine. Adenosine inhibition of the 0.1 Hz twitch response was recorded before (●) and after (○) exposure to 0.1 μM A23187 for 5 min. Each point represents the mean for 3 experiments and vertical lines indicate s.e. means.

Table 3 Effects of dipyridamole and calcium concentration on 1 μM adenosine inhibition of 0.1 Hz twitch response

Treatment	*Calcium concentration* 2.5 *mM*	2.0 *mM*
Control	10.9 ± 0.9	12.8 ± 1.2
Dipyridamole (10 nM)	18.4 ± 1.8	30.1 ± 1.3

Each result is the mean value ± s.e. mean (3 experiments) of the % inhibition produced by 1 μM adenosine in the presence of dipyridamole and calcium concentrations shown. Dipyridamole pretreatment time was 2 min.

Two-way analysis of variance gave f values as follows: calcium effect: 25.9 ($P < 0.001$); dipyridamole effect 87.3 ($P < 0.001$); calcium × dipyridamole interaction: 13.7 ($P < 0.01$).

leading to elevated cyclic AMP levels in brain tissue slices (Sattin & Rall, 1970; Schultz & Daly, 1973) and nerve cells in tissue culture (Clark *et al.*, 1974; Blume & Foster, 1975). Since an increase in cyclic AMP is associated with acetylcholine release (Goldberg & Singer, 1969; Wilson, 1974), it seems unlikely, on circumstantial grounds, that this mechanism operates in the adenine-induced inhibition of acetylcholine release that we have documented.

The results of the experiments in which we studied the interaction between calcium concentration and adenosine effects suggest that adenosine derivatives may act by limiting the availability of intracellular calcium required for the coupling of excitation and neurotransmitter release. In support of this suggestion are our findings of synergistic relationships between: (a) calcium deprivation and adenosine inhibition of the twitch response (Figure 4); (b) calcium excess and the antagonistic effect of theophylline (Table 2); and (c) low calcium concentrations and potentiation of adenosine inhibition by dipyridamole (Table 3). Furthermore, the concentration of adenosine required to produce 50% inhibition of the twitch response was increased by increasing the calcium concentration in the organ bath (Figure 5) and by the action of the calcium ionophore, A23187 (Figure 6).

Circumstantial support for this suggestion is available from established relationships between calcium ions and acetylcholine release. The electrophysiological studies of Katz & Miledi (1967a, b) on the giant squid synapse have shown that there is a depolarization-dependent increase in calcium permeability at the presynaptic terminal that is essential for neurotransmitter release. Miledi (1973) has obtained direct evidence for this and has shown that the crucial factor determining the rate of release of acetylcholine is the intracellular level of ionized calcium. A reduction in calcium entry during depolarization reduces the output of transmitter (Baker, 1972).

Relatively few studies have considered the action of adenosine in terms of its possible effects upon intracellular calcium levels. De Gubareff & Sleator (1965) suggested that adenosine acted on heart muscle by blocking the normal action of calcium on excitation-contraction coupling and a decrease in calcium influx was proposed as a mechanism for the adenosine-induced relaxation of guinea-pig taenia coli (Axelsson & Holmberg, 1969) and hog vascular smooth muscle (Herlihy *et al.*, 1976). Vizi & Knoll (1976) considered the possibility that adenine nucleotides inhibited the release of acetylcholine from guinea-pig ileum by preventing the entry of calcium ions into the nerve terminals. They noted, however, that the inhibitory action of the adenine nucleotides was not prevented by a calcium excess (5 mM calcium chloride) and suggested that adenine compounds inhibited acetylcholine release by stimulating membrane ATPase activity. Recently, Ribeiro *et al.* (1979) have shown that adenosine (0.1 to 2 mM) and ATP (0.2 mM) decreased the uptake of ^{45}Ca by potassium-depolarized synaptosomes prepared from rat cerebral cortex. Although these concentrations are high, the results support the view that adenine inhibition is mediated by a decrease in calcium entry into nerve endings.

The results we have obtained with theophylline confirm the well documented observation that this compound antagonizes the inhibitory effects of adenine derivatives on ileal preparations. There is good evidence to indicate that methylxanthines increase intracellular ionized calcium pools by releasing bound calcium (Hoffmann, 1969) and by increasing membrane permeability to calcium (Moritoki *et al.*, 1976; Kažić, 1977). Taken in conjunction with our observations, these findings suggest that the antagonistic effects of theophylline and adenosine on acetylcholine release may be mediated by the opposite effects that they exert on intracellular calcium ion concentrations in postganglionic cholinergic nerves.

We propose, therefore, that modulation, by adenine derivatives, of cholinergic neurotransmission in the myenteric plexus is mediated by effects upon intracellular calcium economy. Although tentative and yet to be examined by more definitive micro-electrode studies, this proposed mechanism is consistent with our experimental observations and with the widening concept of calcium as an important 'second messenger' in cellular regulatory processes.

We are grateful to the South African Medical Research Council and the University of Cape Town Staff Research Fund for financial support; to Professor H. Umezawa, Tokyo for coformycin; to Boehringer Ingelheim (Pty) Ltd. for dipyridamole; and to Eli Lilly and Co. for A23187.

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(Received December 21, 1979.)