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Adenosine and dipyridamole: actions and interactions on the contractile response of guinea-pig ileum to high frequency electrical field stimulation

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1 The action of adenosine on the myenteric plexus-longitudinal muscle strips from guinea-pig ileum to high frequency electrical field stimulation (10 Hz) was investigated.

2 Electrically induced contractions were reduced markedly by tetrodotoxin (0.2 μM) and atropine (1 μM), and partially by noradrenaline (3 μM) and morphine (3 μM).

3 Adenosine, adenosine 5′-monophosphate (AMP) and adenosine triphosphate (ATP) produced a concentration-dependent inhibition of the high frequency contractions over the range of 0.1–100 μM, the most potent being adenosine.

4 The concentration-response curve for adenosine was significantly shifted to the left by dipyridamole (10 nM), while dipyridamole at higher concentrations (30 nM–10 μM), depressed the contraction markedly by itself.

5 Dipyridamole decreased [^{3}H]-adenosine uptake into strips of ileum in a concentration-dependent manner. There was a significant correlationship between the reduction of adenosine uptake and the inhibition of the contraction induced by dipyridamole ($r = 0.970$).

6 In strips desensitized to adenosine or treated with adenosine deaminase, the inhibitory effect of dipyridamole was significantly reduced.

7 The present investigation revealed that adenosine depressed responses of guinea-pig ileum to high frequency electrical stimulation and suggested that the inhibitory effect of dipyridamole may be closely associated with the behaviour of endogenous adenosine or related compounds.

Introduction

Adenosine and adenine nucleotides have been shown to exert a prejunctional inhibitory action in many tissues (Ginsborg & Hirst, 1972; McIlwain, 1974; Hedqvist & Fredholm, 1976; Kuroda, 1978; Reese & Cooper, 1982). In guinea-pig ileum, these compounds inhibit the twitch response to low frequency electrical stimulation by a reduction of acetylcholine release from intramural cholinergic nerves (Sawynok & Jhamandas, 1976; Vizi & Knoll, 1976). Further, it has been shown that the most potent inhibitor among them was adenosine and the inhibitory effect of adenosine was competitively antagonized by theophylline and potentiated by dipyridamole (Hayashi *et al.*, 1977; Moody & Burnstock, 1982).

Previously we examined the effect of adenosine on the electrically (10–30 Hz) induced contractions of guinea-pig isolated ileal longitudinal muscle strip and showed that adenosine also depressed the high frequency contractions. Furthermore, we found that a progressive decline of amplitude of the contraction developed (tachyphylaxis) when the same electrical stimulation was applied to the strip at 1 min intervals, and suggested that the release of endogenous adenosine-related compounds may be involved in the development of tachyphylaxis (Hayashi *et al.*, 1978b).

Burnstock (1972) proposed that a purine compound, probably ATP, is the transmitter released from non-adrenergic, non-cholinergic inhibitory nerves present in the myenteric plexus. Vizi & Knoll (1976) and Okwuasaba *et al.* (1977) also raised the possibility that ATP or related compounds might act as endogenous inhibitory substances on the myenteric neurones in the guinea-pig ileum. Further, in the other tissues, evidence has accumulated in favour of the assumption that they may function as inhibitory modulators or transmitters (McIlwain, 1974; Su, 1975; Muramatsu, *et al.*, 1980).

The aim of the present investigation was to study the

effect of adenosine on the high frequency contractile response of guinea-pig ileum longitudinal muscle.

Methods

Male guinea-pigs weighing 300 to 500 g were killed by a blow on the head and ileal longitudinal muscle strips with the attached myenteric plexus were prepared according to the method of Paton & Vizi (1969). The strip was set up in an organ bath (5 ml capacity) containing Tyrode solution at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. Tyrode solution had the following composition (mM): NaCl 136.7, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.5.

Electrical stimulation was carried out by the method essentially similar to that described by Paton & Vizi (1969). The strip was stimulated by means of two electrodes, one at the top and the other at the bottom of the organ bath. Rectangular pulses (0.1–30 Hz, 0.4 ms) were delivered from a stimulator (Nihon Kohden, SEN–3201). The muscle strip was adjusted to an initial resting tension of approximately 0.5 g and allowed to stabilize for 60 min with frequent washes. The response was recorded by means of an isometric force-displacement transducer (Nihon Kohden, TB–612T) on a recorder (Nihon Kohden, RJG–3024).

Tissues were desensitized to adenosine by exposure to Tyrode solution containing 50 μM adenosine for 45–60 min.

Measurement of uptake of [^{3}H]-adenosine

Strips of ileum were prepared as described above and preincubated individually at 37°C for 60 min in test tubes containing 10 ml Tyrode solution and gassed with 95% O_2 and 5% CO_2 throughout the experiment. The strips were then incubated for 10 min with [2,8-^{3}H]-adenosine (2 μCi ml^{-1}, 1 μM). Dipyridamole, when used, was added 30 min before incubation with the tritiated adenosine. After the incubation, strips were blotted gently, weighed, chopped and placed in scintillation vials containing 1 ml of solubilizer (Protosol). The vials were then maintained overnight in a water bath at 50°C to speed solubilization. Five ml of scintillation solution (10 g of DPO, 50 mg of POPOP and 100 g of naphthalene per litre of dioxane) was added to each vial. The radioactivity of the samples were counted in a Aloka scintillator and expressed as d.p.m. per g wet weight tissue.

Data analysis

Inhibitory responses of adenosine, dipyridamole and other drugs were calculated as percentages of control contraction. Values of $-\log EC_{50}$ (concentration producing 50% inhibition) were calculated from the concentration-inhibition curves. Mean values of all data are shown with their standard errors and were compared by Student's t test for unpaired data. Differences with P values less than 0.05 were considered significant. Regression lines were calculated using the least-squares method.

Drugs

Drugs used were: adenosine (Kohjin), adenosine deaminase (Sigma), atropine sulphate (Merk), dipyridamole (Takeda), hexamethonium bromide (Merk), morphine hydrochloride (Takeda), noradrenaline bitartrate (Daiichi), protosol (New England Nuclear), tetrodotoxin (Sankyo). [2,8-^{3}H]-adenosine was obtained from New England Nuclear, Boston, U.S.A.. Drugs were dissolved in distilled water and enzyme was added directly to the bath. The concentrations of drugs are expressed as μM of the final concentrations.

Results

Characteristics of the contractile response to high frequency stimulation

Electrical stimulation at frequencies of 0.1 to 30 Hz induced a monophasic contraction to each stimulation, the height of which was dependent on the frequency. The effects of 10 μM adenosine on these responses is shown in Table 1. Adenosine depressed the contractions at all frequencies of stimulation, but was less effective against the contraction at higher frequency than that at 0.1 Hz. In subsequent experiments we used 10 Hz as high frequency stimulation.

Figure 1 shows the effects of several blocking agents on the high frequency (10 Hz) contractile response.

Table 1 Inhibitory effect of adenosine (10 μM) on the electrically induced contractile response of guinea-pig isolated ileal longitudinal muscles

Frequency (Hz)	*% inhibition**	P-*value*†	n
0.1	78 ± 1.7	—	57
1	63 ± 4.0	<0.001	14
10	58 ± 2.6	<0.001	43
30	54 ± 4.2	<0.001	13

*Inhibition produced by adenosine at a given frequency is expressed as a percentage of the control response at that frequency.
†Significantly different from 0.1 Hz.

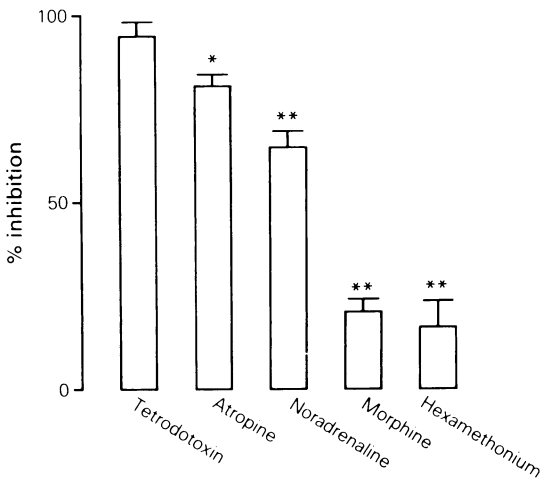

Figure 1 Effects of several blocking agents on the contractile response of ileal longitudinal muscle to high frequency stimulation (10 Hz). Inhibition produced by following antagonists is expressed as a percentage of the control response: tetrodotoxin, 0.2 μM ($n = 7$); atropine, 1 μM ($n = 17$); noradrenaline, 3 μM ($n = 6$); morphine, 3 μM ($n = 5$), hexamethonium, 100 μM ($n = 4$). An asterisk indicates a significant difference from tetrodotoxin (*: $P < 0.05$, **: $P < 0.001$).

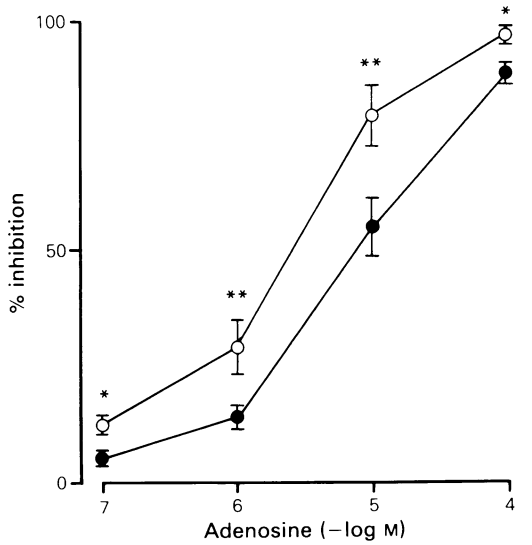

Figure 2 Concentration-response curve for adenosine in the ileal longitudinal muscle. Ordinate scale; percentage inhibition of the contractile response produced by adenosine. Abscissa scale; concentration (− log M) of adenosine. (●) Control, $n = 14$; (○) in the presence of dipyridamole (0.01 μM), $n = 6$. An asterisk indicates a significant difference of values between inhibition in the absence and presence of dipyridamole (*: $P < 0.01$; **: $P < 0.001$).

Tetrodotoxin (0.2 μM) and atropine (1 μM) depressed the response maximally by 94 ± 3.8% ($n = 7$), and 81 ± 2.9% ($n = 17$), respectively, while noradrenaline (3 μM) depressed it by 65 ± 4.8% ($n = 6$). Morphine (3 μM) and hexamethonium (100 μM) depressed contractions by 21 ± 4.2% ($n = 5$) and 17 ± 4.5% ($n = 4$), respectively.

Effect of adenosine on the high frequency response

Adenosine, AMP and ATP produced a concentration-dependent depression of the high frequency contractile response; (for $-\log EC_{50}$ values see Table 2.) Adenosine was the most potent among them and the inhibition at 100 μM was 88 ± 2.3% ($n = 14$) (Figure 2). Adenine and inosine produced no inhibition at concentrations less than 1 mM. In the presence of 10 nM dipyridamole, the concentration-response curve for adenosine was shifted to the left and the $-\log EC_{50}$ was significantly increased (Figure 2, Table 2). Furthermore, the inhibitory effect of adenosine was antagonized by theophylline (100 μM) (Table 2). Table 3 shows the effect of adenosine in the ileal longitudinal muscle strips pretreated with

Table 2 Inhibitory effects of adenosine and related compounds on the contractile response of ileal longitudinal muscle induced by high frequency stimulation (10 Hz)

Compounds	$-\log EC_{50}$*	P-*value*†	n
ATP	4.73 ± 0.165	<0.05	8
AMP	4.82 ± 0.113	<0.05	11
Adenosine	5.18 ± 0.097	—	18
Inosine	No inhibition	—	5
Adenine	No inhibition	—	5
Adenosine + theophylline	4.48 ± 0.065	<0.001	6
Adenosine + dipyridamole	5.67 ± 0.108	<0.02	5

* E_{50}; concentration (M) producing 50% inhibition.
† Significantly different from adenosine. Theophylline: 100 μM, dipyridamole: 0.01 μM.

Table 3 Inhibitory effect of adenosine on the contractile response of ileal longitudinal muscle induced by high frequency stimulation (10 Hz) in the presence of atropine

Compounds	n	*% inhibition**	P-*value*
Adenosine (100 μM)	14	88 ± 2.3	
Atropine (1 μM)	17	81 ± 3.0	<0.001
Atropine (1 μM) + adenosine (100 μM)	9	95 ± 2.0	<0.05
Tetrodotoxin (0.2 μM)	7	94 ± 3.8	NS

*Inhibition produced by each agents is expressed as a percentage of the control response.
NS:not significant.

atropine (1 μM). The atropine-resistant contraction was 19% of the control; adenosine also reduced this contraction significantly. The extent of inhibition induced by atropine and adenosine together was almost equal to that of tetrodotoxin (0.2 μM).

Effect of dipyridamole on the high frequency response

Dipyridamole (10 nM), which potentiated the inhibitory effect of adenosine, slightly depressed the high frequency contractile response. A higher concentration of dipyridamole caused greater inhibition: at 10 μM it was 45 ± 1.7% ($n = 14$) (Figure 3). The inhibitory effect of dipyridamole was antagonized by theophylline (100 μM).

Table 3 shows the effect of dipyridamole on [^{3}H]-adenosine uptake into the ileal longitudinal muscle strips. The ileal strips rapidly accumulated tritium and this accumulation was strikingly prevented by dipyridamole in a concentration-dependent manner. There was a significant positive correlation ($r = 0.970$, $P < 0.01$) between the inhibitory effect of dipyridamole on the contractile response and [^{3}H]-

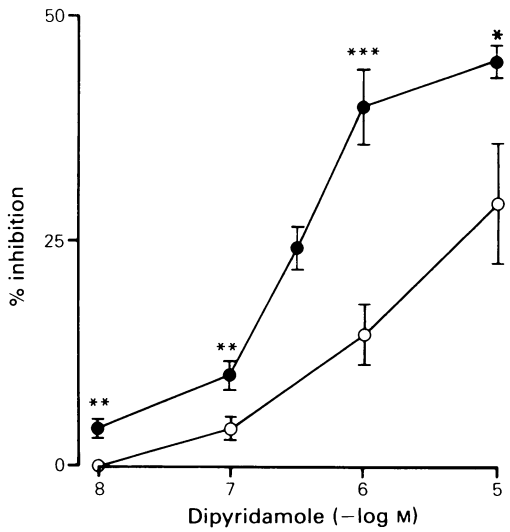

Figure 3 Inhibitory effect of dipyridamole on the contractile response to high frequency stimulation (10 Hz). Ordinate scale; percentage inhibition of the response produced by dipyridamole. Abscissa scale; concentration (− log M) of dipyridamole. (●) Control, $n = 14$; (○) in the presence of theophylline (100 μM), $n = 9$. An asterisk indicates a significant difference of values between inhibition in the absence and presence of theophylline (*: $P < 0.05$; **: $P = 0.01$; ***: $P = 0.001$).

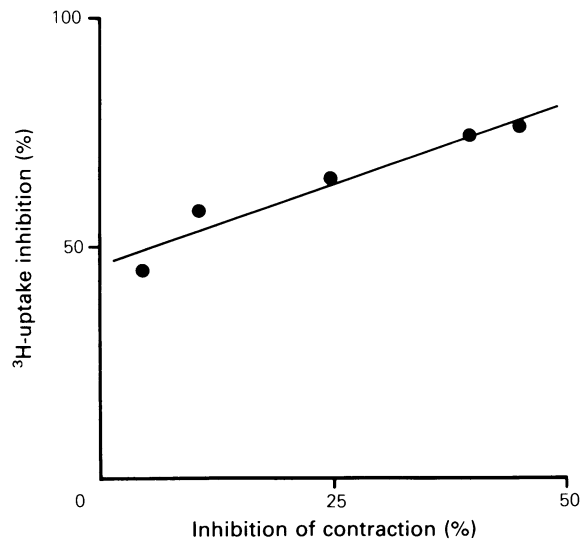

Figure 4 Relationship between percentage inhibition of the contractile response and percentage inhibition of [^{3}H]-adenosine uptake for 5 different concentrations of dipyridamole. The correlation coefficient is 0.970 ($P < 0.01$).

adenosine uptake in the ileal strips. The inhibitory effect of dipyridamole on the contractile response was examined further, using ileal longitudinal preparations treated with adenosine deaminase or desensitized by adenosine (Table 4). Adenosine deaminase (0.5 unit ml^{-1}) caused a slight increase of the contractile response to high frequency stimulation. In the presence of adenosine deaminase, the inhibitory effect of dipyridamole was significantly reduced (Table 5); the effects of the enzyme were abolished by boiling it.

The exposure of ileum strips to 50 μM adenosine caused a small relaxation and little depression of the response to high frequency stimulation, which recovered within 45–60 min. Under these conditions, a second dose of adenosine (10 μM) failed to depress the electrically-evoked contractions appreciably. The inhibitory effect of dipyridamole on the high frequency contraction was also reduced significantly in these adenosine-desensitized preparations (Table 5). The inhibitory effect of noradrenaline (3 μM) was little affected in adenosine-desensitized preparations.

Table 4 Effect of dipyridamole on [^{3}H]-adenosine uptake into ileal longitudinal muscle strips

Dipyridamole (μM)	^{3}H-uptake (d.p.m. × 10 per g)	P-*value**	% inhibition *of uptake*†
0	*5.47* ± 0.812	—	0
0.01	3.00 ± 0.612	<0.01	45
0.1	2.30 ± 0.099	<0.01	58
0.3	1.92 ± 0.167	<0.01	65
1	1.41 ± 0.113	<0.01	74
10	1.31 ± 0.166	<0.01	76

:Means are presented with their standard errors (n = 5).
*Comparison are with corresponding normal group without dipyridamole treatment.
†Inhibition was expressed as a percentage of the ^{3}H-uptake without dipyridamole.
Strips were exposed to [^{3}H]-adenosine in the presence or absence of dipyridamole and the accumulation of radioactivity after a 10 min incubation period was measured.

Table 5 Influence of adenosine deaminase and desensitization of adenosine on the inhibitory effect of dipyridamole (0.3 μM)

Pretreatment	*Contraction to 10 Hz**	*% inhibition by dipyridamole*†	n
Normal	1.00	24 ± 2.6	14
Adenosine deaminase	108 ± 0.05	4 ± 2.4‡	7
Desensitization Adenosine (50 μM)	1.12 ± 0.10	10 ± 2.00‡	4

*Contractile response was expressed as a ratio of normal response.
†Inhibition was expressed as a percentage of the each contractile response without dipyridamole.
‡Significantly different from normal ($P < 0.01$).

Discussion

Electrically (10 Hz)-induced contraction of guinea-pig ileal longitudinal muscle was subdivided into several components by the use of appropriate blocking agents. Most of the contractile response was atropine-sensitive and therefore this component represents the response of smooth muscle to acetylcholine released from electrically stimulated myenteric plexus. The small residual part of the response was shown to be due to two components, i.e. one was atropine-resistant and tetrodotoxin-sensitive, whilst the other was both atropine and tetrodotoxin-resistant. The former component may be similar to the contraction due to excitation of non-cholinergic neurones as previously reported by Ambache & Freeman (1969), whereas the latter may be the response of the longitudinal muscle to direct electrical stimulation.

Noradrenaline and morphine, in concentrations which abolished the twitch response to low frequency stimulation (Hayashi *et al.*, 1978a), reduced only a part of the contraction to high frequency stimulation. These data are consistent with the view that two mechanisms constitute the cholinergic component; one is important at low frequency of stimulation and is sensitive to morphine and adrenaline, whereas the other is effective at higher frequencies of stimulation and is resistant to these drugs (Paton, 1963; Takagi & Takayanagi, 1966).

Such contractile responses to high frequency stimulation, were markedly inhibited by adenosine in a concentration-dependent manner. The $-\log EC_{50}$ was 5.18 and at concentration of 100 μM, adenosine depressed the response by approximately 90%. However, the inhibitory effect of adenosine was somewhat less on the response to stimulation at

0.1 Hz. Our previous experiments indicated that adenosine abolished the twitch response at 30 μM, the pD_2 value being 5.83 (Hayashi *et al.*, 1978a). There are two possible explanations why the high frequency contractile response was inhibited less effectively by adenosine than the response to low frequency stimulation. (1) The high frequency response involves components other than the cholinergic one, and such components may be resistant to the inhibitory action of adenosine, (2) The adenosine-resistant response may also represent a cholinergic component which is only effective at high frequencies of stimulation. Maske *et al.* (1980) proposed that such high frequency stimulation activated an additional population of cholinergic neurones in the myenteric plexus which were not depolarized by low frequency stimuli, and were relatively resistant to the effects of adenosine. However, it is now unlikely that the first explanation is correct because the non-cholinergic contraction was reduced by adenosine. Since the inhibitory effect of adenosine was antagonized by theophylline, it is possible that adenosine acts on prejunctional P_1-purinoceptors, similar to those described by Burnstock (1978).

Dipyridamole has been shown to potentiate the coronary vasodilator and negative chronotropic effects of adenosine on the heart (Stafford, 1966; McInnes & Parratt, 1969; Kolassa *et al.*, 1970; 1971; Kalsner, 1975), as well as the inhibitory effects of adenosine and adenine nucleotides in the intestine (Satchell *et al.*, 1972; Satchell & Burnstock, 1975) and trachea (Coleman, 1976). Previously, we found that dipyridamole prevented adenosine uptake into the ileal longitudinal muscle strip and suggested that the potentiation of the action of adenosine by dipyridamole appears to be exerted mainly through the blockade of adenosine uptake (Hayashi *et al.*, 1978a). Such a potentiating effect of dipyridamole was also demonstrated for the high frequency contractile response in the present experiments. Furthermore, dipyridamole depressed the response to high frequency stimulation by itself, in a concentration-dependent manner. This inhibitory effect was significantly correlated with the inhibition of adenosine uptake into the preparation induced by dipyridamole. In the preparations desensitized with exogenous adenosine, the inhibitory effect of dipyridamole was clearly reduced.

Satchell (1981) found that the enzyme, nucleotide pyrophosphatase, reduced inhibitory responses to stimulation of non-adrenergic nerves, and suggested that ATP is the transmitter released by these nerves. When we examined the effect of adenosine deaminase on the dipyridamole action, dipyridamole failed to depress the high frequency response. It is well known that adenosine deaminase converts adenosine to inosine. From these results, it is suggested that dipyridamole may increase the amounts of endogenous adenosine-related compounds in the synaptic cleft by the blocking of adenosine uptake into the tissue and the accumulating compounds may depress the contractile response. We have already showed that high frequency electrical stimulation increased [^{3}H]-purine compounds output from [^{3}H]-adenosine preloaded ileal longitudinal muscle strips in the presence of dipyridamole (Hayashi *et al.*, 1978b). Possibly, in the absence of dipyridamole, the high frequency response may not be affected by such endogenous purine compounds so much, as the result of the predominant uptake of adenosine, because both the adenosine desensitization and adenosine deaminase scarcely affected the high frequency contractile response. Previously, we found a progressive decline of the contraction (tachyphylaxis) when ileal longitudinal muscles of guinea-pig were stimulated electrically with 10 or 30 Hz for 10 s at 15 min intervals and suggested that the development of tachyphylaxis may be closely associated with the release of endogenous adenosine derivatives (mostly adenosine) (Hayashi *et al.*, 1978b). Thus, under these circumstances, repetitive stimulation may exceed the capacity of the adenosine uptake system.

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