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THE EFFECTS OF DIPYRIDAMOLE ON THE GUINEA-PIG ILEAL LONGITUDINAL MUSCLE-MYENTERIC PLEXUS PREPARATION

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1 The longitudinal muscle-myenteric plexus strip prepared from guinea-pig ileum has been used to study the actions of dipyridamole and its interactions with adenine derivatives.

2 Dipyridamole augmented the inhibitory effects of adenine derivatives on the twitch response induced by 0.1 Hz field stimulation of the preparation. This synergistic effect was apparent with relatively low concentrations of dipyridamole (10 to 100 nM) and after short pretreatment times (1 to 2 min) that did not inhibit the twitch response on their own. Appropriate studies suggested that dipyridamole-adenosine synergism followed a pattern of facilitative agonist competition.

3 Dipyridamole did not inhibit either uptake of [^{3}H]-adenosine by the preparation or adenosine deaminase activity under the same conditions that it exhibited synergism with adenosine.

4 Higher concentrations of dipyridamole inhibited the twitch response, mainly by decreasing acetylcholine release but partly by a direct action on smooth muscle. The direct action of dipyridamole on muscle was not synergistic with adenosine.

5 Fluorescence microscopy showed preferential binding of dipyridamole to the myenteric plexus.

Introduction

The drug dipyridamole is known to potentiate the ability of adenosine and adenine nucleotides to cause heart block (Stafford, 1966; Kolassa, Pfleger & Träm, 1971), coronary vasodilatation (Stafford, 1966; Parratt & Wadsworth, 1972), relaxation of guinea-pig taenia coli (Satchell & Burnstock, 1975) and tracheal muscle (Coleman, 1976), and inhibition of ileal contractile responses to electrical stimulation (Hayashi, Mori, Yamada & Kunitomo, 1978; Moritoki, Kanbe, Maruoka, Ohara & Ishida, 1978). Although well documented, the synergism between dipyridamole and adenine compounds is not fully understood, nor is it known whether the neural and muscular elements in any given responsive tissue are equally susceptible to the primary and to the adenosine-potentiating effects of dipyridamole.

The study described in this paper was designed to clarify some of these uncertainties using the intact guinea-pig ileum longitudinal muscle-myenteric plexus strip as a convenient experimental preparation that comprises both nervous and muscular tissues whose separate responses to drugs can be detected and quantitated. Our results have shown that dipyridamole acts upon both the post-ganglionic cholinergic neurone and the muscle cell. The former action is synergistic with adenosine in a manner that suggests facilitative agonist competition. Dipyridamole, at concentrations that were synergistic with adenosine, did not appear to inhibit adenosine uptake or adenosine deaminase.

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Methods

Organ-bath studies

The methods described by Paton & Zar (1968) and Paton & Vizi (1969) were used for the preparation of longitudinal muscle-myenteric plexus strips from guinea-pig ileum and for assay of acetylcholine output. The strips were suspended in organ baths containing 7.5 ml Krebs solution supplemented with 0.03 mM choline chloride (Gintzler & Musacchio, 1975) maintained at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. Contractile responses were recorded isometrically with a force-displacement transducer and a single pen recorder. Strips were stimulated electrically by means of two platinum electrodes placed at the top and the bottom of the organ bath (field stimulation) as described by Paton & Vizi

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(1969). Square-wave biphasic pulses of 2 ms duration and supramaximal voltage (45 V) were delivered at frequencies of 0.1 or 0.2 Hz by means of a pulse generator. Effects of adenosine and dipyridamole were expressed as the percentage twitch inhibition related to drug-free controls. Acetylcholine output was expressed as pmol g^{-1} min^{-1}. Stock solutions of adenine compounds were prepared in buffered saline and stored at −20°C. Dipyridamole was dissolved in distilled water.

Accumulation of [^3H]-adenosine

Accumulation of [^{3}H]-adenosine was determined according to a modification of the methods of Satchell, Lynch, Bourke & Burnstock (1972) and Kalsner (1975). Strips of longitudinal muscle-myenteric plexus (approximately 10 cm in length) were collected and equilibrated at 37°C for 5 min in Krebs solution gassed with 95% O_2 and 5% CO_2. They were incubated under the same conditions with dipyridamole (10 nM to 1 μM) after which [^{3}H]-adenosine (3 μM; specific activity 3.3 mCi/μmol) was added, using two experimental protocols: (1) dipyridamole pretreatment for 2 min followed by incubation with [^{3}H]-adenosine for 2 min and (2) dipyridamole pretreatment for 15 min followed by incubation with [^{3}H]-adenosine for 10 min. All solutions contained [^{14}C]-inulin (0.1 μCi/ml) to correct for ^{3}H activity in extracellular fluid. After incubation, the strips were blotted gently on a tissue, weighed and transferred to counting vials containing 2 ml Soluene-350. The vials were kept in a 50°C waterbath for 2 h to speed solubilization. Dimilune 10 ml was added to each vial and the samples were assayed for ^{14}C and ^{3}H by dual-channel counting in a Packard Tricarb liquid scintillation spectrometer (Model 3380). Uptake of adenosine was expressed as nmol adenosine/g of tissue after correction for extracellular ^{3}H activity calculated from the [^{14}C]-inulin space. This was estimated to be 0.5 ± 0.02 ml/g (mean ± s.e. mean for 30 determinations). Inhibition of incorporation of label by dipyridamole was expressed as a percentage of control uptake in the absence of dipyridamole.

Adenosine deaminase assay

The effect of dipyridamole was investigated using adenosine deaminase obtained from two sources: (1) type I adenosine deaminase (E.C. No. 3.5.44) purified from calf intestinal mucosa (Sigma) and (2) a crude homogenate of guinea-pig ileum longitudinal muscle-myenteric plexus. The activity of adenosine deaminase was determined by the method of Kalckar (1947) in which the rate of deamination of adenosine to inosine is followed spectrophotometrically by recording the decrease in optical density at 265 nm ($-\Delta A_{265}$).

In the case of the pure enzyme, the effects of dipyridamole were studied by pretreatment of the enzyme solution with the compound (10 nM to 30 μM) for 2 or 15 min before assay. In the case of the longitudinal muscle-myenteric plexus enzyme, the tissue was incubated in Krebs solution containing dipyridamole (100 nM and 10 μM) for periods of 2 or 15 min. At the end of the incubation period, the strips were blotted dry, weighed and pulverized after freezing in liquid nitrogen. The pulverized tissue was homogenized in 1 ml 0.05 M phosphate buffer, pH 7.5, with a Dounce homogenizer, followed by centrifugation at 7000 *g* for 10 min. The supernatant fluid was tested for adenosine deaminase activity by the assay procedure described above. The protein content of each supernatant fluid was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

Fluorescent-staining technique

Strips of longitudinal muscle-myenteric plexus were incubated in Krebs solution containing dipyridamole 100 μM for 10 min in a 37°C waterbath. The preparations were washed three times with dipyridamole-free Krebs solution and were mounted on clean glass slides. The slides were examined in a Zeiss fluorescent microscope equipped with 380 nm (maximum) activating filters and 470 nm barrier filters.

Materials and drugs

All standard chemicals and laboratory reagents used were obtained from recognized drug houses and chemical firms. The following drugs and compounds were used: Adenosine, adenosine 5'-phosphate (AMP), adenosine 5'-pyrophosphate (ADP), adenosine 5'-triphosphate (ATP) and cyclic adenosine 3',5'-monophosphate (cyclic AMP) (Schwarz/Mann, New York); adenosine deaminase and physostigmine (eserine) sulphate (Sigma Chemical Company); acetylcholine chloride (BDH Ltd.); morphine sulphate (Petersen Ltd.); dipyridamole (Boehringer Ingelheim (Pty) Ltd.). [2-^{3}H]-adenosine (23 Ci/mmol) and inulin-[^{14}C] carboxylic acid (13.25 mCi/mmol) were obtained from the Radiochemical Centre, Amersham. Soluene-350 and Dimilune were obtained from the Packard Instrument Company. Coformycin was obtained from Prof. H. Umezawa, Chemistry Research Foundation, Tokyo, Japan.

Results

Synergistic effects of dipyridamole on adenyl inhibition of guinea-pig ileum

Exposure of the preparation to concentrations of dipyridamole and adenosine inhibited the contractile

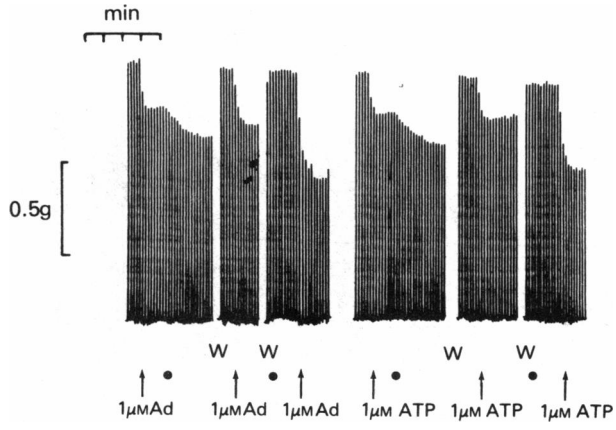


Figure 1 Effect of sequential order of addition of dipyridamole and adenosine or ATP on the contractile responses of longitudinal muscle-myenteric plexus strips to 0.1 Hz field stimulation. Addition of drugs is shown as follows: 1 μM adenosine (Ad); 1 μM ATP (ATP); 60 nM dipyridamole (at ●). The preparations were washed at W.

response to 0.1 Hz field stimulation to an extent that was greater than the sum of the inhibitions observed when either of these two compounds was added alone at the same concentrations (Figure 1). The action of dipyridamole was, therefore, synergistic with adenosine and not additive. This synergistic effect was evident after relatively short preincubation periods (1 to 2 min) with low concentrations of dipyridamole (10 to 100 nM) that, when present alone for the same period of time, did not inhibit contractile responses to field stimulation or to exogenously applied acetylcholine. Synergism with AMP, ADP, ATP and cyclic AMP was kinetically similar to that observed with adenosine. The dipyridamole synergistic effect was dose-related as indicated by a shift to the left of the adenosine and ATP dose-response curves (Figure 2).

While most experiments were performed with a pretreatment time of 2 min, synergism with adenosine was evident after preincubation with dipyridamole for as short a time as 30 s. Synergistic augmentation of adenosine inhibition increased progressively, but at a decreasing rate, over a 20 min period following addition of dipyridamole to the organ bath. This effect was almost maximal, for a given concentration of dipyridamole, within 10 min.

Subthreshold concentrations of dipyridamole (i.e. concentrations that produced no effect on their own) produced a slowly developing inhibition when added after a small dose of adenosine or nucleotide. Figure 1 shows that 1 μM adenosine produced an inhibition of 18%, and 60 nM dipyridamole had no effect when added alone. When 60 nM dipyridamole was added after 1 μM adenosine, an additional inhibition of 13% was observed. Thus a small dose of adenosine facilitated the effect of dipyridamole in producing twitch inhibition. ATP facilitated the inhibitory effect of dipyridamole to a similar extent. However, it is worth noting that the degree of synergistic inhibition observed with given concentrations of dipyridamole and adenosine depended on the sequential order in which these two compounds were added. Adenosine followed by dipyridamole inhibited less than when the compounds were added in the reverse order (Figure 1).

Once exposed to dipyridamole, the longitudinal muscle-myenteric plexus preparation remained sensitized to the inhibitory action of adenosine despite repeated washings with dipyridamole-free buffer, indicating that dipyridamole was not readily dislodged from binding sites responsible for the synergistic effect. This post-washing 'residual augmentation' could be eliminated by adding dipyridamole in the presence of 100 μM adenosine (Figure 3). The high concentration of adenosine, therefore, effectively competed with dipyridamole for binding to its receptors. Interaction between dipyridamole and adenosine at the same receptor site was further suggested by the observation that the residual synergistic effect of dipyridamole could be washed out more rapidly if the washing solution contained 100 μM adenosine (Figure 4), indicating that adenosine displaced dipyridamole from the receptor.

Effects of dipyridamole on the contractile responses of guinea-pig ileum

Dipyridamole alone produced a dose-dependent inhibition of the 0.1 Hz twitch response over a concentration range of 0.1 to 100 μM (Figure 5) with a con-

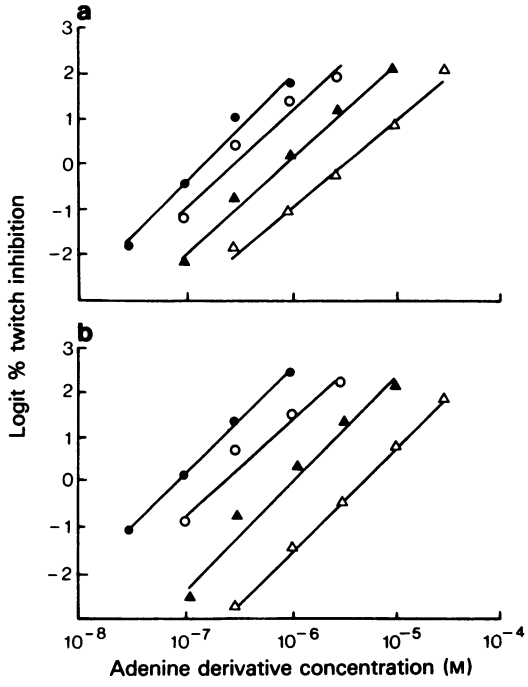


Figure 2 Dipyridamole augmentation of the inhibitory effects of (a) adenosine and (b) ATP on the 0.1 Hz twitch response of the longitudinal muscle-myenteric plexus preparation. Inhibition by adenosine or ATP was recorded in the absence of dipyridamole (△) and following 2 min exposure to dipyridamole at 10 nM (▲), 30 nM (○) or 100 nM (●). Least squares regression lines were fitted to each set of experimental points representing the relationship between the log of adenosine or ATP concentration and the logit transform of the % inhibition observed. The logit transformation was performed as described elsewhere (Dowdle & Maske, 1980).

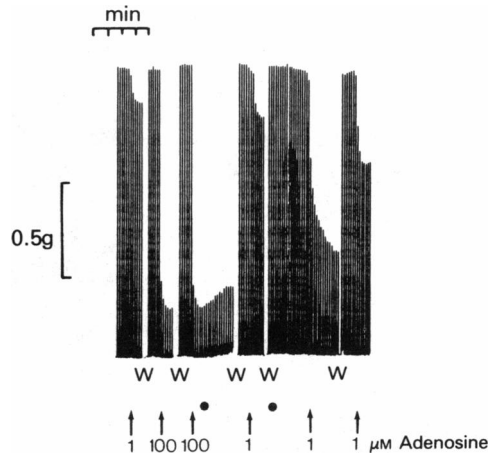


Figure 3 Effect of 100 μM adenosine on residual dipyridamole-adenosine synergism. Each record shows the responses of a longitudinal muscle-myenteric plexus strip to 0.1 Hz field stimulation. Arrows indicate addition of adenosine at the concentrations shown; 60 nM dipyridamole addition is shown by the closed circles (●). The strip was washed at W.

centration producing 50% inhibition (ID_{50}) of 1.5 ± 0.4 μM (mean ± s.e. mean for 3 experiments). This is close to the ID_{50} for adenosine of 3.9 ± 0.4 μM reported by Dowdle & Maske (1980). In comparison to adenosine-induced inhibition of the twitch response (which reached a maximum 30 to 60 s after addition of adenosine), dipyridamole-induced inhibition developed slowly, reaching a maximum after only 5 to 15 min, depending on the concentration of dipyridamole added. Recovery from dipyridamole inhibition was slow (2 to 4 h) and was usually incomplete despite frequent washes. High concentrations of dipyridamole stained the preparation with the yellow colour of the drug and it was difficult to wash this colour off the tissue.

Dipyridamole reduced the contractile responses to exogenously applied acetylcholine in a concentration-dependent and time-related fashion. Relatively low concentrations of dipyridamole (100 nM) had no effect on acetylcholine-induced contractions after 2 min but produced 5% inhibition after 15 min. Higher doses of dipyridamole (10 to 100 μM) inhibited the acetylcholine twitches after pretreatment for 2 min (Figure 6). The further addition of 10 μM adenosine did not augment the dipyridamole effect (Figure 6). Dipyridamole, therefore, had a direct effect on the smooth muscle cells that was not synergistic with adenosine.

Dipyridamole also inhibited evoked acetylcholine release in a dose-dependent manner (Figure 7). Samples for acetylcholine estimations were collected after stimulation at 0.2 Hz for 10 min. In order to allow for the direct inhibitory effect on the muscle, the appropriate final concentrations of dipyridamole were included in the acetylcholine standards used to estimate the acetylcholine content of the samples collected. After exposure to high concentrations of dipyridamole, inhibition of electrically evoked acetylcholine release reversed very slowly.

These experiments indicate that, at low concentrations (10 nM to 1 μM), dipyridamole inhibited the twitch response mainly by an action on cholinergic nerves. When compared with adenosine derivatives, dipyridamole showed reaction kinetics that were slow

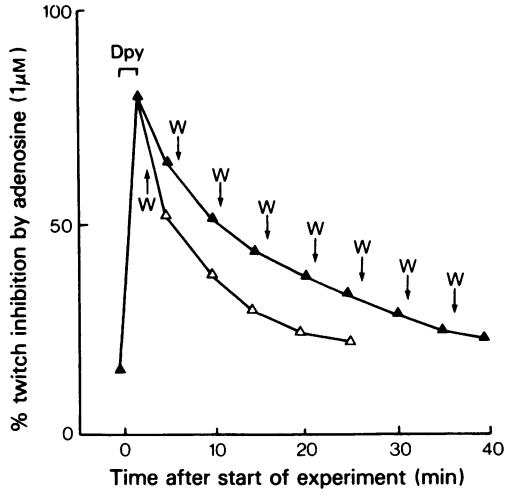


Figure 4 Displacement of residual dipyridamole synergistic effect by adenosine. The graph shows the % inhibition, of the 0.1 Hz twitch response, produced by 1 μM adenosine before exposure to dipyridamole (15%), after exposure to 60 nM dipyridamole (Dpy) for 2 min (80%) and at regular intervals thereafter following washes (W) with buffer (▲) or buffer containing 100 μM adenosine (△). Each wash was with 6 consecutive 10 ml volumes of warmed wash solution over a period of 1 to 2 min.

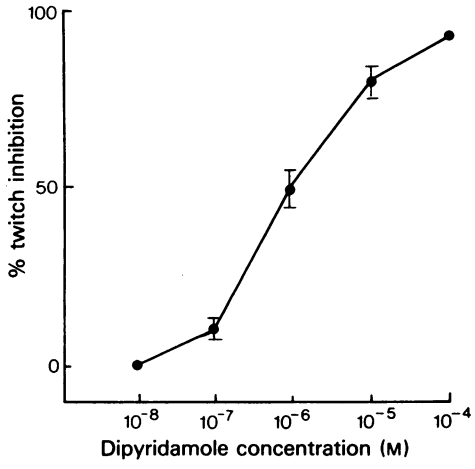


Figure 5 Inhibition by dipyridamole of the 0.1 Hz twitch response of the longitudinal muscle-myenteric plexus strip. The tissue was exposed to dipyridamole until a maximal effect was observed. Each point represents the mean for at least 3 experiments; vertical lines show s.e. mean.

in onset and reversal. At higher concentrations (10 to 100 μM), the direct action on the smooth muscle became apparent.

Effects of dipyridamole on [^{3}H]-adenosine accumulation by guinea-pig ileum strips

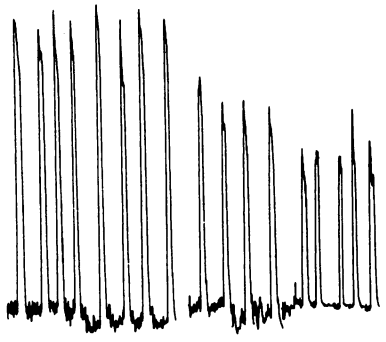
As is evident from the results summarized in Table 1, no inhibition of [^{3}H]-adenosine accumulation by longitudinal muscle-myenteric plexus strips was observed with concentrations of dipyridamole (10 nM and 100 nM) and incubation times (2 min) that were effectively synergistic with adenosine in the bath experiments. In fact, within these dose-time exposures dipyridamole appeared to increase rather than inhibit adenosine uptake. These concentrations of dipyridamole did inhibit adenosine accumulation after 15 min. A higher concentration of dipyridamole (1 μM) inhibited adenosine uptake to the same extent after 2 min and 15 min incubation.

Effects of dipyridamole on adenosine deaminase activity

No inhibition of calf intestinal adenosine deaminase (E.C. No. 3.5.44) activity was demonstrated following pretreatment of the enzyme with dipyridamole (10 nM to 30 μM). With 10 μM adenosine solution as substrate, it was still not possible to show inhibition of adenosine deaminase with concentrations of dipyridamole up to 30 μM. However, when as a 'positive control', the enzyme was preincubated for 2 min with coformycin, a structural analogue of inosine that inhibits adenosine deaminase (Henderson, Brox, Zombor, Hunting & Lomax, 1977), its activity was inhibited in a dose-related manner. The results of a second experiment in which dipyridamole was incubated with ileal strips are given in Table 2. As can be seen from these results, dipyridamole had only a slight effect upon the activity of adenosine deaminase extracted from guinea-pig ileal strips.

Preferential binding of dipyridamole to the neural elements of the myenteric plexus

Dipyridamole is a fluorescent compound. This fact was exploited for the tissue localization of dipyridamole by incubating longitudinal muscle-myenteric plexus strips in Krebs solution containing 100 μM dipyridamole for 10 min. When the preparation was washed and examined in a fluorescent microscope equipped with 380 nm (max) activating filters and 470 nm barrier filters, the appearance presented in Figure 8 was observed. Although background, dipyridamole fluorescent-staining of muscle fibres could be detected, there was a clearly visible concentration of the drug over the neural elements of the myenteric plexus. No fluorescence was obtained in control strips incubated in the absence of dipyridamole.



Dipyridamole μM	0	0.1	0	0.1	10	10	100	100
Adenosine μM	0	0	10	10	0	10	0	10

Figure 6 Recordings of contractile responses of a longitudinal muscle-myenteric plexus preparation to 75 nM acetylcholine after treatment with dipyridamole or adenosine at the indicated concentrations. In all cases acetylcholine was added 2 min after dipyridamole or 30 s after adenosine. The strip was washed between additions.

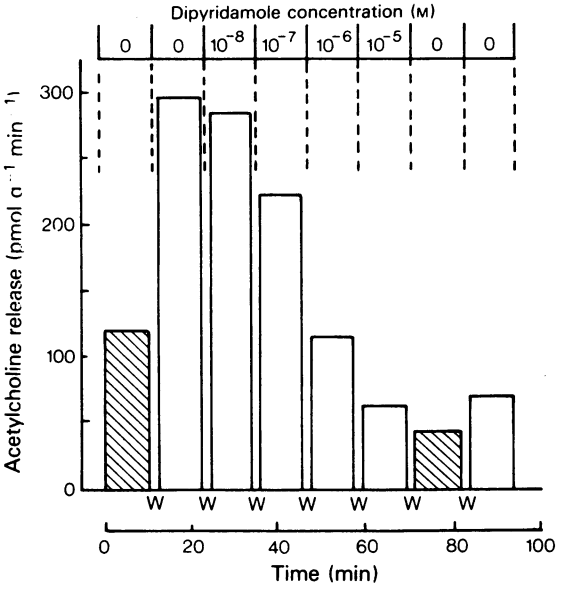


Figure 7 Dose-dependent inhibition by dipyridamole of acetylcholine release from a strip of longitudinal muscle-myenteric plexus treated with physostigmine. Samples were assayed for acetylcholine content after 10 min periods of rest (hatched bars) or 0.2 Hz field stimulation (open bars). Dipyridamole was added to the bath at the beginning of each 10 min period at the final concentrations given above. The preparation was washed during the intervals indicated by W. The abscissa scale shows the time course of the experiment.

Discussion

The results we describe in this paper have confirmed the observations of others (Okwuasaba, Hamilton & Cook, 1977; Hayashi *et al.*, 1978; Moritoki *et al.*, 1978) that dipyridamole interacts synergistically with adenosine and adenine nucleotides to inhibit the contractile responses of the guinea-pig ileum. In attempting to explain this synergism, most workers have referred to two alleged actions of dipyridamole, its ability to interfere with intracellular accumulation of adenosine and its inhibitory effect upon the enzyme, adenosine deaminase. A critical review of the subject reveals that efforts to accommodate these two actions of dipyridamole into a unifying, general concept of its pharmacological action have not been entirely successful.

In guinea-pig heart (Kolassa *et al.*, 1971; Hopkins, 1973), ileum (Moritoki *et al.*, 1978) and taenia coli preparations (Satchell *et al.*, 1972; Satchell & Burnstock, 1975), dipyridamole-induced inhibition of adenosine uptake (as measured by radio-assay of tissue preparations after exposure to radio-labelled adenosine) correlated with the synergism observed between

Table 1 Effect of dipyridamole on [^{3}H]-adenosine accumulation by strips of guinea-pig ileum

Dipyridamole pretreatment	*Adenosine uptake (nmol/g)**		*% control*
Control	0.7 ± 0.03	(in 2 min)	—
10^{-8} M (2 min)	0.8 ± 0.18	(in 2 min)	118
10^{-7} M (2 min)	0.8 ± 0.12	(in 2 min)	114
10^{-6} M (2 min)	0.3 ± 0.04	(in 2 min)	41
Control	3.4 ± 0.30	(in 10 min)	—
10^{-8} M (15 min)	2.8 ± 0.39	(in 10 min)	83
10^{-7} M (15 min)	2.1 ± 0.03	(in 10 min)	61
10^{-6} M (15 min)	1.3 ± 0.27	(in 10 min)	39

* The results represent the mean ± s.e. mean for 3 experiments in each case.

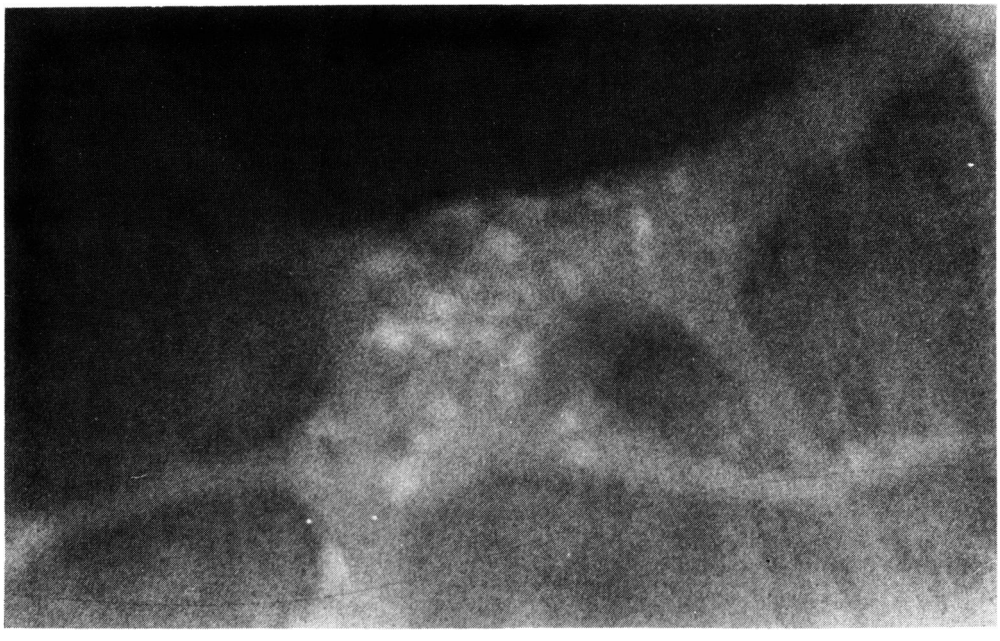


Figure 8 Dipyridamole-fluorescent staining of the guinea-pig ileum preparation. The photograph, taken with 380 nm (max.) activating filters and 470 nm barrier filters, shows preferential staining of the neural elements of the myenteric plexus. A ganglion containing intensely fluorescent cells is shown (X1200).

these compounds and the authors were generally ready to conclude that this was a quantitatively sufficient explanation for the phenomenon. This led these workers to suggest that dipyridamole acted by prolonging the action of adenosine at extracellular receptor sites. On the other hand, Kalsner (1975) found that dipyridamole inhibited the uptake of [^{3}H]-adenosine by bovine coronary artery strips but that this effect was quantitatively insufficient to account for the extent to which dipyridamole sensitized the preparation for adenosine-induced relaxation. There is, moreover, reason to doubt the specificity of dipyridamole on cellular transport mechanisms since, in addition to inhibiting the uptake of adenosine, dipyridamole also inhibits the transport of other nucleosides, nucleic acid bases and monosaccharides into Novikoff hepatoma cells (Plagemann & Richey, 1974).

In our experiments with the intact electrically stimulated longitudinal muscle-myenteric plexus preparation, when adenosine was added to the bath after 2 min of preincubation with 10 nM dipyridamole, the synergistic effect was evident within 60 s of adding the nucleoside. When these conditions were simulated in the uptake experiments, no consistent effect of dipyridamole on the incorporation of radioactivity into the tissue could be detected. However, it is possible that these low doses of dipyridamole did inhibit adenosine uptake into postganglionic cholinergic nerves, but, by virtue of the small fraction of the total tissue mass that these structures represent, the magnitude of this effect on total tissue uptake could not be discerned with certainty. When large concentrations of dipyridamole were present for longer preincubation periods, diminished incorporation of radioactivity into the tissue was observed. This may have represented inhibition of binding of adenosine to cell surface receptors or to interference with a transmembrane transport mechanism. These considerations emphasize the fact that the longitudinal muscle-myenteric plexus preparation is a complex system of nervous tissue and smooth muscle cells so that experimental protocols in which these strips are incubated in the presence of radioactive adenosine, removed, blotted and assayed for total content of radioactivity are unlikely to yield definitive results from which conclusions can be drawn regarding the precise mode of action of dipyridamole.

Evidence to support the theory that dipyridamole potentiates adenosine action by inhibition of adenosine deaminase activity is also unsatisfactory. Although studies have shown that dipyridamole is a competitive inhibitor of adenosine deaminase purified from calf intestinal mucosa (Deuticke & Gerlach, 1966), most workers do not consider that this is an important aspect of dipyridamole's action (Stafford, 1966; Hopkins & Goldie, 1971). The concentrations of dipyridamole required to inhibit adenosine deaminase are much higher than the concentrations required to augment the effects of adenosine or to inhibit its uptake. Furthermore, because adenosine deaminase is an intracellular enzyme (Pull & McIlwain, 1972), transport of adenosine into the cell is required before deamination takes place. Finally, dipyridamole acts synergistically with adenine nucleotides and adenosine to the same extent. Since the nucleotides must first be broken down to adenosine and taken up into the cell before deamination can take place (Burnstock, 1972), it seems unlikely that inhibition of adenosine deaminase by dipyridamole plays a significant role in synergism with nucleotides. When these arguments are considered in conjunction with our inability to show that dipyridamole inhibited adenosine deaminase appreciably, one is led to conclude that inhibition of adenosine deaminase activity by dipyridamole does not play a role in the synergistic effect that it exerts on adenine inhibition of the twitch response of the guinea-pig ileum.

Our studies of the synergism between dipyridamole and adenine compounds suggest a mechanism based upon the concept of facilitation between competitive agonists (Ariëns, Simonis & van Rossum, 1964). This phenomenon has been described in biological systems that exhibit all-or-none responses. An all-or-none response is produced by an effector unit when the stimulus reaches a critical trigger value. Since most responsive biological preparations comprise a large number of individual effector units with varying trigger values, graded dose-response curves may be observed despite the quantal, all-or-none responses of the constituent effector units. The drug concentration at which the composite preparation as a whole starts to respond in a graded way is referred to as the threshold value. For cooperation between two competitive agonists (A and B), each with a certain threshold value, addition of a subthreshold dose of A results in a stimulus insufficient to produce a response. Addition of small doses of B, in the presence of subthreshold doses of A, are sufficient to bring the

Table 2 Effect of dipyridamole on adenosine deaminase extracted from longitudinal muscle-myenteric plexus

Dipyridamole treatment	*Adenosine deaminase activity* ($-\Delta A_{265}$/*min per μg Pr*)*
Control	19.1 ± 0.5
10^{-7} M (2 min)	17.3 ± 0.6
10^{-7} M (15 min)	19.8 ± 1.0
10^{-5} M (2 min)	17.5 ± 0.3
10^{-5} M (15 min)	17.8 ± 0.1

* Results are the mean values ± s.e. mean for 3 experiments.

stimulus to the critical value to induce an effect. Without previous addition of A, higher doses of B are necessary to produce the same effect. There is thus facilitation of the effect of B by A, and the reverse holds true. The interaction between adenosine and dipyridamole follows closely this pattern of facilitation (Figure 1), suggesting that the two compounds may act at the same presynaptic receptor site. This suggestion is supported by the following experimental results that were obtained. (1) 'Protection' of the receptor from dipyridamole by large concentrations of adenosine (100 μM) prevented the residual synergistic effect that normally followed a wash procedure (Figure 3). (2) Washing the preparation, after exposure to dipyridamole, with buffer containing 100 μM adenosine removed the residual synergistic effect more rapidly than did washing with adenosine-free buffer (Figure 4), indicating that adenosine displaced receptor-bound dipyridamole by a mass-action effect. (3) The kinetics of dipyridamole synergism with adenosine and with adenine nucleotides were quantitatively similar without evidence of a delay in the case of the nucleotides, indicating that no prior conversion to adenosine was required. One may conclude, therefore, that dipyridamole interacts at the presynaptic receptor site with an adenosine receptor. This conclusion would be implicit in a model proposing facilitation by competitive agonists at a single class of receptor sites.

For the most part, published accounts of the pharmacology of dipyridamole have considered the drug in association with adenosine. There have been relatively few reports dealing with the action of dipyridamole acting alone. Our studies have shown that dipyridamole at low concentrations inhibited contractile responses mainly by an action on cholinergic nerves and, to a lesser extent and at higher concentrations, by direct action on the longitudinal muscle. The concentration of dipyridamole required to inhibit the twitch response by 50% was similar to that found for adenosine and both inhibited acetylcholine release. Dipyridamole differed from adenosine in that the rates at which inhibition developed and recovery took place after washing were considerably slower in the case of dipyridamole. Furthermore dipyridamole had a direct action on muscle that was not shown by, nor was synergistic with, adenosine.

We suggest, therefore, that dipyridamole inhibits acetylcholine release by an action on the same receptor site that binds adenosine. This receptor site is situated on the cholinergic nerves in the myenteric plexus, and appears to be the same site involved in dipyridamole-adenosine interactions. Dipyridamole inhibition differs from adenosine inhibition in its slow 'on and off' kinetic rates. Preferential binding of dipyridamole to the myenteric plexus (Figure 8) is consistent with these conclusions.

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