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POTENTIATION OF ADENOSINE AND THE ADENINE NUCLEOTIDES BY DIPYRIDAMOLE

BY

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The pharmacological actions of adenosine and related compounds were first described by Drury & Szent-Györgyi (1929). The early work has been reviewed by Drury (1936). In general, adenosine relaxes smooth muscle, and causes a fall in blood pressure and dilatation of the coronary arteries. The guinea-pig uterus, however, contracts in response to adenosine (Bennet & Drury, 1931 ; Deuticke, 1932). Adenosine produces bradycardia in cats or rabbits, but in guinea-pigs and rats it produces a transient period of heart block, usually associated with prolongation of the P-R interval and bradycardia. The pharmacological actions of adenylic acid, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are similar to those of adenosine. The actions of adenosine and the adenine nucleotides are transient, probably because of their rapid deamination in blood and tissues (Conway & Cooke, 1939) to the corresponding inosine derivatives, which are pharmacologically inactive.

Dipyridamole (2,6-bis(diethylamino)-4,8-dipiperidinopyrimido (5,4-d) pyrimidine) was introduced recently as a coronary dilator for the treatment of angina (Charlier, 1961 ; Winbury, 1964). Dipyridamole has been shown to affect the metabolism of adenosine and the adenine nucleotides in cardiac muscle, and produces an increased content of adenosine or ATP, particularly under conditions of hypoxia (Hockerts & Bögelmann, 1959 ; Siess, 1962 ; Gerlach & Deuticke, 1963). Dipyridamole also inhibits the uptake of adenosine into red blood cells, thus delaying its deamination (Koss, Beisenherz & Maerkisch, 1962 ; Bunag, Douglas, Imai & Berne, 1964). This action of dipyridamole probably contributes to its potentiation of adenosine in inhibiting platelet agglutination (Emmons, Harrison, Honour & Mitchell, 1965*a*, *b*).

It seemed possible that dipyridamole might potentiate the pharmacological actions of adenosine and the adenine nucleotides. This paper describes experiments that demonstrate such a potentiation, and also attempts to explain its mechanism. A preliminary account of the prolongation of the action of adenosine on the guinea-pig heart *in situ* is in the press.

METHODS

Guinea-pigs of either sex weighing 300 to 800 g were anaesthetized with urethane (1.5 g/kg I.P.) and intra-atrial injections of adenosine, adenine nucleotides or acetylcholine were given by the

modification of the method of Drury, Lutwak-Mann & Solandt (1938) described by Rand, Stafford & Thorp (1955*a*). Responses were measured from the electrocardiogram. A similar technique was used for rats (320 to 480 g). Hearts isolated from guinea-pigs or kittens (0.6 to 1 kg) were perfused by retrograde flow through the aorta into the coronary vessels, as in Langendorff's method. The perfusion fluid was McEwen's (1956) solution, warmed to $33 \pm 0.5°$ C, and was delivered at a constant flow rate with a roller pump (Watson Marlow type MHRE). Perfusion pressure was measured with a Condon manometer, and contractions of the heart were recorded with a spring lever; heart rate was recorded with a Thorp impulse counter. Rabbit isolated duodenum or ileum was suspended in McEwen's solution at 37 to 38° C, and the contractions recorded with an isotonic lever. Guinea-pig isolated uterus was set up as described in the 1932 *British Pharmacopoeia.*

Adenosine, 5-adenylic acid, ADP and ATP were obtained from Koch-Light Laboratories Ltd., and the dipyridamole (Persantin) was a gift from Boehringer Ingelheim.

RESULTS

Guinea-pig heart in situ

Potentiation of adenosine. When adenosine (25 μg) is injected into the left atrium of a guinea-pig it produces a heart block for 2 to 5 sec. This transient heart block in response to adenosine is clearly defined, easy to measure, and reproducible for several hours. Figure 1 is a graph showing the effect of intravenous injections of various doses of dipyridamole on the duration of the heart block produced by injections of adenosine given at 3-min intervals. The potentiation of adenosine by single injections of dipyridamole is transient, suggesting that the dipyridamole is rapidly inactivated. A similar, transient potentiation of adenosine was seen in seven other experiments in guinea-pigs after giving single intravenous injections of dipyridamole (50 to 800 μg/kg).

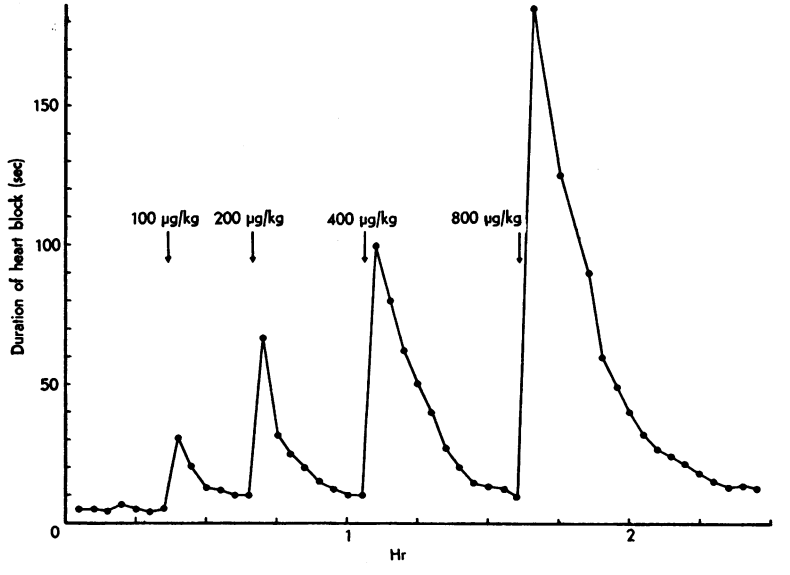

Fig. 1. The duration of each period of heart block produced by the injection of adenosine (25 μg) into the left atrium of a guinea-pig is plotted against the time of the injection. Single intravenous injections of dipyridamole, in the doses indicated above the arrows, produced a transient potentiation of the responses to adenosine.

During a continuous injection of dipyridamole the responses of the guinea-pig heart to intra-atrial injections of adenosine were greatly prolonged. Infusions of 40 to 150 μg/kg/min produced a very high degree of potentiation of adenosine, although there was some variation between animals in the degree of potentiation produced by any particular rate of infusion. This was probably due to differences in the rate of inactivation of dipyridamole by different guinea-pigs. Figure 2 shows the greatly

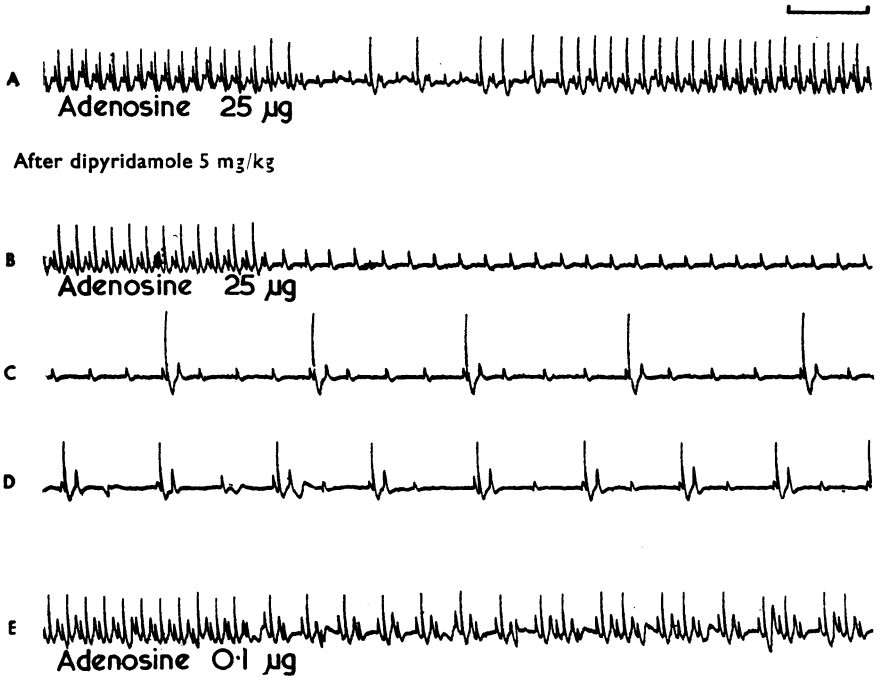

Fig. 2. Guinea-pig ECG. Panel A shows the control response to adenosine (25 μg) injected into the left atrium. Panels B, C and D were recorded at 0, 2 and 4 min after the injection of adenosine (25 μg) when the guinea-pig had been given an infusion of dipyridamole (100 μg/kg/min) for 50 min; the degree and duration of the heart block were greatly increased. Panel E shows the response to the threshold dose of adenosine (0.1 μg) when the guinea-pig had received the infusion of dipyridamole for 60 min. This response consists of a long period of low grade heart block (2:1), which differs from the control response.

increased duration of the response to adenosine (25 μg) 50 min after starting an infusion of dipyridamole (100 μg/kg/min). Panel A shows the initial response to 25 μg of adenosine and panels B, C and D were recorded at 0, 2 and 4 min after the injection of the same dose of adenosine into the guinea-pig receiving an infusion of dipyridamole (total dose 5 μg/kg), which itself had no marked effect on the control ECG. The duration of the response to adenosine was increased from 3.5 sec to 8 min. In order to determine the degree of sensitization to adenosine produced by dipyridamole, two cannulae were tied into the left atrium; each contained adenosine, one at a concentration of 2 mg/ml. and the other of 10 μg/ml. In this way, two doses of adenosine could be injected in approximately the same volume (0.005 to 0.02 ml.). After the response to

25 μg of adenosine had been greatly increased by dipyridamole, injections of much lower doses of adenosine (0.05 to 0.2 μg) were given, in an attempt to match the control response. Panel E in Fig. 2 illustrates why this was not possible. A threshold dose of adenosine, in a guinea-pig infused with dipyridamole, produced a prolonged period of low grade heart block, whereas a threshold dose of adenosine in an untreated guinea-pig produced a short period of higher grade heart block. Because the responses were not the same, it was difficult to estimate the degree of potentiation of adenosine by dipyridamole. However, the threshold dose of adenosine was decreased by 0.004, 0.006 and 0.005 in the three experiments, indicating an increase in sensitivity of the order of 200-fold.

Potentiation of the adenine nucleotides. The actions of adenylic acid, ADP and ATP on the guinea-pig electrocardiogram were identical with those of adenosine, and the same molar doses were required to produce responses of the same duration (Rand, Stafford & Thorp, 1955*b*). Figure 3 illustrates the results from one of two experiments

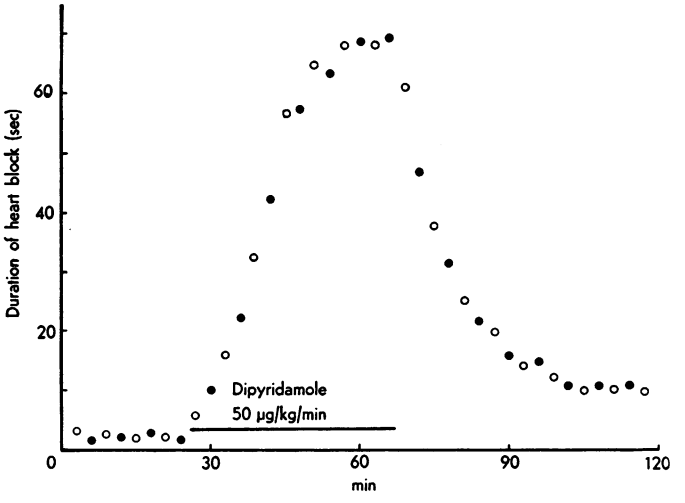

Fig. 3. The duration of responses to alternate injections of equimolar doses of adenosine (closed circles) and adenylic acid (open circles) into the left atrium of a guinea-pig is plotted against time of injection. During the infusion of dipyridamole (50 μg/kg/min), responses to both adenosine and adenylic acid were potentiated equally. After the infusion of dipyridamole was stopped, the responses to adenosine and adenylic acid declined at the same rate.

in which adenosine and adenylic acid were injected alternately into the left atrium of a guinea-pig; the durations of the responses of heart block were plotted graphically. During the infusion of dipyridamole (50 μg/kg/min), the responses to both adenosine and adenylic acid were potentiated to the same extent. After the infusion of dipyridamole was stopped, the responses to adenosine and adenylic acid both declined at the same rate. In two similar experiments in which ADP was used instead of adenylic acid, and in three in which ATP was used, the effects were potentiated by dipyridamole to the same extent as were those of adenosine.

Potentiation of acetylcholine. Intra-atrial injections of acetylcholine produced responses similar to those produced by adenosine, but acetylcholine was three to five times more potent than adenosine on a molar basis. Alternate injections of adenosine and acetylcholine were given into the left atria of six guinea-pigs in doses which produced a 3 to 6 sec heart block. In three of the guinea-pigs, dipyridamole was infused at the rate of 40 μg/kg/min, and in the other three, at 100 μg/kg/min. At both rates of infusion, the potentiation of adenosine was considerable, but the responses to acetylcholine were only slightly increased. After the infusion of dipyridamole at 40 μg/kg/min for 60 min, the heart block produced by adenosine lasted 60 to 85 sec, but that produced by acetylcholine only 12 to 15 sec. After dipyridamole (100 μg/kg/min for 60 min) the heart block produced by adenosine lasted 5 to 8 min, but that produced by acetylcholine only 20 to 45 sec.

Guinea-pig isolated heart

Injections of acetylcholine (1 to 5 μg) or adenosine (5 to 20 μg) into the perfusion fluid of guinea-pig isolated hearts produced heart block. Dipyridamole potentiated the effects of adenosine and acetylcholine on the isolated heart, but those of adenosine were potentiated much more than those of acetylcholine (Fig. 4). The lowest dose of

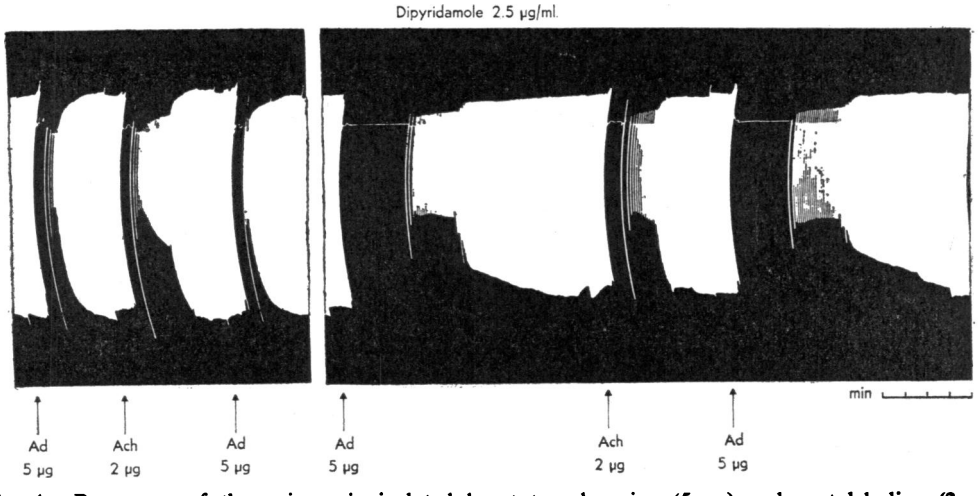

Fig. 4. Responses of the guinea-pig isolated heart to adenosine (5 μg) and acetylcholine (2 μg) before and after addition of dipyridamole (2.5 μg/ml.) to the perfusion fluid. Dipyridamole potentiated adenosine much more than acetylcholine.

dipyridamole that increased the response to adenosine in these experiments was 0.025 to 0.05 μg/ml.; maximum potentiation of adenosine was produced by 1 to 2.5 μg/ml. of dipyridamole. Attempts to measure the degree of potentiation of adenosine by dipyridamole in the isolated heart were difficult for the same reason as with the guinea-pig heart *in situ*—that is, threshold doses of adenosine in the hearts treated with dipyridamole produced a long period of bradycardia or low grade heart block which was different from the response to threshold doses of adenosine in the untreated heart, i.e., a higher grade of heart block.

The actions of 8-azaguanine and papaverine

In vitro, 8-azaguanine is an inhibitor of intestinal adenosine deaminase of comparable potency to dipyridamole. In order to see whether potentiation of adenosine by dipyridamole was related to inhibition of adenosine deaminase, the effect of 8-azaguanine on the heart block produced by adenosine was measured in three experiments on the guinea-pig heart *in situ*, and in three experiments on the isolated heart. Perfusion of isolated hearts with 8-azaguanine in concentrations up to 100 μg/ml. did not alter the response to adenosine. On the guinea-pig heart *in situ*, 8-azaguanine (10 to 30 mg/kg) produced only a very slight and transient potentiation of adenosine.

Papaverine prevents uptake of adenosine into red blood cells, but is less potent than dipyridamole (Koss *et al.*, 1962). In two experiments, papaverine, in the maximum tolerated dose (10 mg/kg), produced only a slight increase in the response of the guinea-pig heart *in situ* to adenosine.

Rat heart in situ

Intra-atrial injections of adenosine in the rat (5 to 20 μg) produced heart block, as in the guinea-pig. However, in the rat, adenosine was not potentiated by dipyridamole. In three rats, doses of dipyridamole up to 10 mg/kg were injected intravenously, but the response to adenosine was never increased. Doses of dipyridamole as high as 20 mg/kg produced transient ventricular arrhythmias, and during and shortly after these the response to adenosine was abolished or reduced.

Coronary flow

The coronary dilator action of adenosine was measured in hearts isolated from six kittens. During the first 15 to 30 min of perfusion of the heart at a flow rate of 15 ml./min, the perfusion pressure gradually increased to 120 to 130 mm Hg, and then remained steady at this level. Injections of adenosine (1 to 5 μg) into the perfusion fluid caused a fall in perfusion pressure of 20 to 60 mm Hg, indicating dilatation of the coronary vessels. ATP produced a similar fall in perfusion pressure, but was about three times more potent than adenosine on a molar basis. These doses of adenosine and ATP were too low to produce any change in heart rate. There was considerable tachyphylaxis to the dilator action of adenosine and ATP; even so potentiation of both adenosine and ATP by dipyridamole could be clearly demonstrated. The concentration of dipyridamole in the perfusion fluid that was needed to potentiate adenosine and ATP (0.1 to 0.5 μg/ml.) approached that which itself produced coronary dilatation (0.5 to 1 μg/ml.). Figure 5 illustrates the increased dilatation of coronary vessels produced by adenosine in the presence of dipyridamole (1 μg/ml.).

Rabbit isolated intestine

Adenosine (0.05 to 5 μg/ml.) produced relaxation of isolated rabbit intestine, and reduction of the spontaneous contractions. This action of adenosine was increased in the presence of dipyridamole (5 to 10 ng/ml.), but the maximum potentiation of adenosine observed was only 2.5-fold in twelve experiments, and was not increased by increasing the concentration of dipyridamole. Higher concentrations of dipyridamole (>2 μg/ml.) reduced or blocked the response to adenosine.

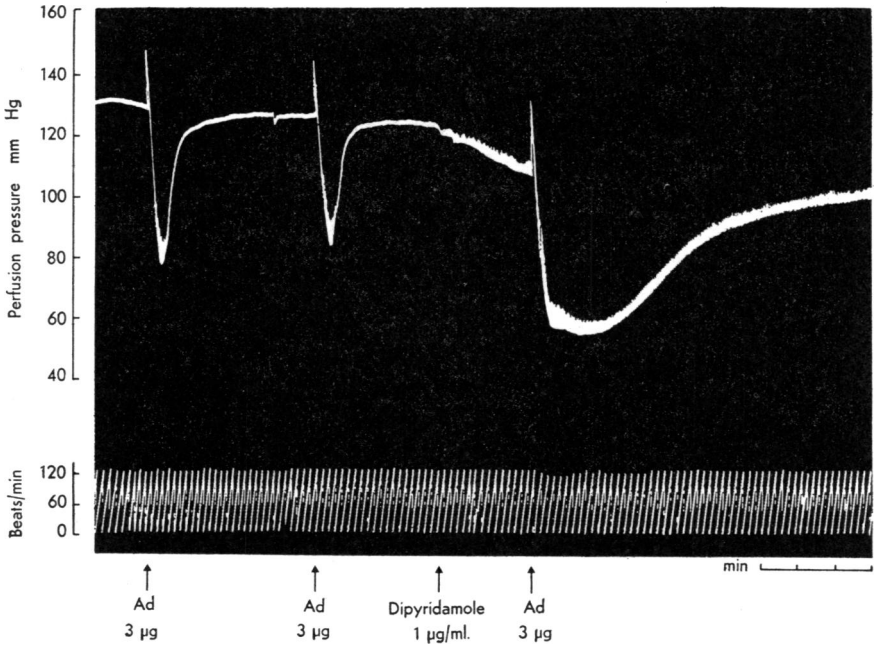

Fig. 5. Injections of adenosine (3 μg) into the fluid perfusing a kitten isolated heart caused a fall in perfusion pressure indicating coronary dilatation. Heart rate was not affected. After the addition of dipyridamole (1 μg/ml.) to the perfusion fluid the action of adenosine was increased and greatly prolonged.

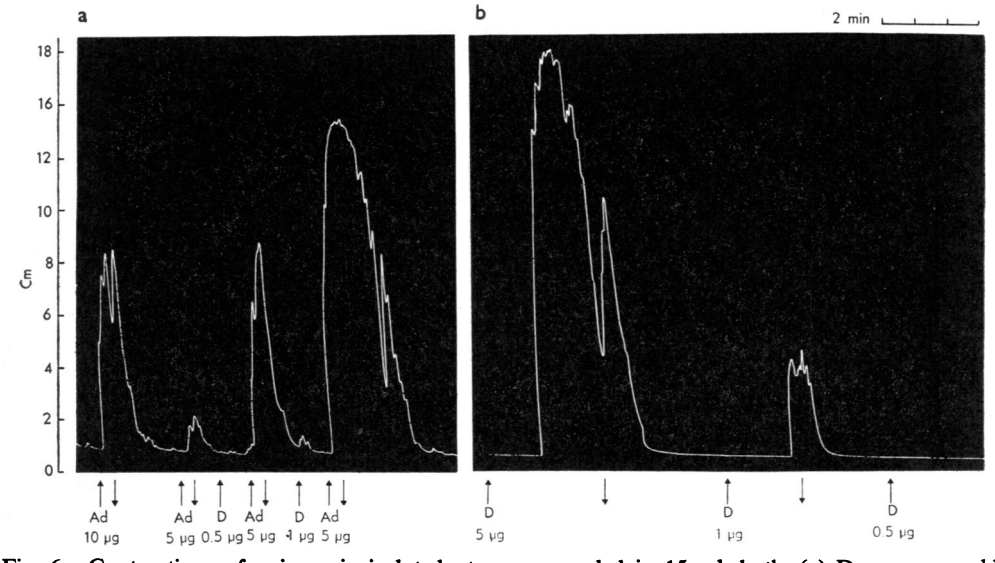

Fig. 6. Contractions of guinea-pig isolated uterus suspended in 15 ml. bath. (a) Drugs were added at the upward pointing arrows and the bath was washed at the downward pointing arrows. In the presence of dipyridamole (D) (0.5 or 1 μg) the response to 5 μg of adenosine (Ad) was greatly increased. (b) Contractions of the guinea-pig isolated uterus occurred 3 to 4 min after the addition to the bath of dipyridamole (5 or 1 μg).

Guinea-pig isolated uterus

The guinea-pig uterus contracted in response to adenosine (0.1 to 1 μg/ml.). In eight experiments, the response to threshold concentrations of adenosine was greatly increased by dipyridamole (0.01 to 0.075 μg/ml.). One experiment is illustrated in Fig. 6*a*. Concentrations of dipyridamole higher than 0.1 μg/ml. produced contractions of the guinea-pig uterus. This was seen in five experiments in which concentrations of dipyridamole ranging from 0.1 to 2 μg/ml. produced either a slowly developing spasm of the uterus, or a sudden contraction after a latent period of 3 to 5 min. Contractions of the guinea-pig uterus produced by dipyridamole are shown in Fig. 6*b*. Acetylcholine (0.01 to 0.04 μg/ml.) also produced a contraction of the guinea-pig uterus. In two experiments, dipyridamole (0.04 μg/ml.) slightly increased the response to acetylcholine, but higher concentrations of dipyridamole (0.075 to 0.1 μg/ml.) reduced or abolished the response to acetylcholine.

DISCUSSION

Dipyridamole potentiates the pharmacological actions of adenosine and the adenine nucleotides as was predicted from its actions on their metabolism. Potentiation of the effects of adenosine is most dramatic on the guinea-pig heart *in situ*, but also occurs in isolated hearts of guinea-pigs and kittens, and on the isolated intestine and uterus. The action of adenosine on the rat heart, however, is not increased by dipyridamole. On the guinea-pig heart *in situ*, adenylic acid, ADP and ATP are potentiated to the same extent as adenosine, so any explanation of the mechanism of potentiation of adenosine by dipyridamole must also hold for the adenine nucleotides as well. However, acetylcholine produces an identical response which is hardly potentiated by dipyridamole. In this respect, the action of dipyridamole is more specific than that of the cardiac glycosides, which potentiate adenosine, the adenine nucleotides and acetylcholine to exactly the same extent (Rand, Stafford & Thorp, 1955*b*; Rand & Stafford, 1957).

On the basis of the known actions of dipyridamole, there are two possible explanations for its ability to potentiate adenosine: it could inhibit the deamination of adenosine, or it could inhibit the uptake of adenosine into red blood cells, thus delaying its deamination.

The first explanation is unlikely for several reasons. When tested on adenosine deaminase from intestinal mucosa (Bunag *et al.*, 1964), high concentrations of dipyridamole were needed to produce inhibition ($1–4 \times 10^{-4}$M), whereas potentiation of the response of the isolated guinea-pig heart to adenosine is produced by a much lower concentration of dipyridamole (5×10^{-8}M). In addition, 8-azaguanine, which also inhibits adenosine deaminase from intestinal mucosa, does not appreciably alter the response of the heart to adenosine. Finally, it would be difficult to account for the identical potentiation of adenosine, adenylic acid, ADP and ATP on this basis, because in the heart the adenine nucleotides must first be broken down to adenosine before deamination (Angkapindu, Stafford & Thorp, 1959; Berne, 1964) and it is unlikely that this breakdown would occur sufficiently rapidly. Nevertheless, it would be of interest to test dipyridamole as an inhibitor of adenosine deaminase from cardiac muscle of various species, and these investigations are now in progress.

The second explanation has the one attractive feature that it is possible to explain the inability of dipyridamole to potentiate adenosine in the rat. Haemolysis of rat blood, which allows the adenosine easily to come in contact with the red cell deaminase, does not greatly increase the rate of inactivation of adenosine, whereas in guinea-pig blood haemolysis greatly accelerates the rate of deamination (Koss *et al.*, 1962). Thus, if dipyridamole prevented uptake of adenosine into red cells, it would delay inactivation of adenosine in guinea-pig blood, but not appreciably alter the rate of inactivation in rat blood. However, if dipyridamole potentiates adenosine by blocking its uptake into red blood cells, it is difficult to account for the potentiation of adenosine by dipyridamole on the isolated heart and other isolated organs.

Another possible explanation is that, as well as inhibiting uptake of adenosine into red cells, dipyridamole also inhibits uptake of adenosine into cardiac and smooth muscle tissue. In this way, in the presence of dipyridamole, more adenosine would be available to act on receptors. Thus the potentiation of adenosine by dipyridamole might be analogous to the potentiation of noradrenaline by cocaine. Adenosine is known to be taken up by the isolated, perfused cat heart (Jacob & Berne, 1960); when the heart was perfused with 200 ml. of Tyrode solution containing 0.6–1.3 μM of C^{14} labelled adenosine, about half of the adenosine was taken up into the heart, where it could be recovered from an acid-soluble supernatant of the heart homogenate, most of it incorporated into AMP, ADP and ATP. The location of the sites of uptake and incorporation is not known but it could be in the granules of the sympathetic nerve endings, which are known to contain ATP as well as noradrenaline.

It is of some interest that dipyridamole itself will produce similar effects to those of adenosine, but its onset of action is slow. Both adenosine and dipyridamole relax most smooth muscle, but contract the guinea-pig uterus. The contractions produced by dipyridamole, however, develop only after several minutes contact. It is possible that dipyridamole causes the accumulation of adenosine or adenine nucleotides, either by inhibiting their degradation, or by preventing their uptake. This action might be analogous to the sympathomimetic action of cocaine. This raises the possibility that all of the pharmacological actions of dipyridamole depend upon its ability to potentiate adenosine. Perhaps the therapeutically desirable action of dipyridamole in relieving angina may result from the accumulation of adenosine or adenine nucleotides in the heart, where they act to dilate the coronary vessels.

SUMMARY

1. Dipyridamole (0.1–1 mg/kg) greatly increased the heart block produced by intra-atrial injections of adenosine, adenylic acid, ADP and ATP in the guinea-pig, but only very slightly increased the response to acetylcholine. Potentiation of adenosine by dipyridamole was also seen on the guinea-pig isolated heart.

2. Dipyridamole increased the dilatation of the coronary vessels produced by adenosine and ATP in the kitten isolated heart, and increased the relaxation of the rabbit intestine produced by adenosine.

3. Dipyridamole (0.01–0.075 μg/ml.) increased the contractions of the guinea-pig isolated uterus produced by adenosine, and in higher concentrations (0.1–2 μg/ml.) itself produced contraction of the uterus.

4. Possible mechanisms for the potentiation of adenosine by dipyridamole are discussed, and it is suggested that all of the pharmacological actions of dipyridamole may result from its ability to allow the accumulation of adenosine or adenine nucleotides in tissues.

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