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The effects of dipyridamole on the myocardial vasodilator actions of noradrenaline, isoprenaline and adenosine

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Summary

1. In anaesthetized cats, intravenous adenosine infusions decreased resistance to blood flow in the myocardial vascular bed. This effect of adenosine was augmented during the 60 min following the intravenous injection of dipyridamole (1 mg/kg).

2. In different experiments, intravenous infusions of noradrenaline caused either a slight increase or a slight decrease in myocardial vascular resistance. The dilator component of the action of noradrenaline was not augmented by dipyridamole.

3. Isoprenaline infusions decreased the resistance of the myocardial bed. This effect was unaltered after dipyridamole.

4. These results do not support the hypothesis that part of the effect of catecholamines on myocardial vascular resistance involves the release of adenosine from hypoxic myocardial cells.

Introduction

The blood flow through the myocardial circulation is highly dependent upon the rate of oxygen utilization by the heart. The balance between oxygen requirements and availability is continuously maintained under widely varying conditions, including reactive hyperaemia (Olsson & Gregg, 1965), systemic hypoxia (Eckenhoff, Hafkenschiel, Landmesser & Harmel, 1947), autoregulation (Fischbach, Burnett & Scher, 1959), increased cardiac output (Braunwald, Sarnoff, Case, Stainsby & Welch, 1958) and cardiac nerve stimulation (Shipley & Gregg, 1945). A coupling mechanism that would maintain this equilibrium has been suggested in the following terms: ' reductions in myocardial oxygen tension induced by reduction in coronary blood flow, by hypoxaemia or by increased myocardial metabolic activity lead to a breakdown of adenine nucleotides to adenosine, which diffuses out of the cells to produce dilation of the arterioles ' (Berne, 1964).

Isoprenaline increases myocardial blood flow and this effect is, in part, secondary to the increase in work and in oxygen demand (Parratt & Wadsworth, 1970a). It was considered possible that catecholamines, by increasing the work of the heart, cause a release of adenosine, and that this contributes to the vasodilatation produced by these amines.

It should be possible to test this hypothesis by the use of drugs, such as dipyridamole, which augment the actions of adenosine (Bretschneider, Frank, Bernard, Kochsiek & Scheler, 1959). Thus, if adenosine is released in physiologically significant amounts during catecholamine infusions, then dipyridamole would be expected

to augment the dilator actions of these infusions. In this paper, experiments are described which seek to determine whether the 'adenosine hypothesis' has any relevance to the myocardial vasodilator effects of catecholamines.

Methods

Experiments were performed in 17 cats of either sex, which were anaesthetized with pentobarbitone. The myocardial thermal conductivity increment was used as an index of blood flow and was measured by means of a heated thermocouple implanted into the left ventricular wall. Details of the experimental technique involved and applications of this method for the measurement of blood flow have been previously described (McInnes & Parratt, 1969 ; Grayson, Coulson & Winchester, 1971). Myocardial vascular resistance was calculated as the myocardial thermal conductivity increment divided by the diastolic aortic pressure.

The animals were artificially ventilated with room air from a Palmer pump (tidal volume = 20 ml/kg). Aortic blood pressure was measured with a capacitance transducer (Elema-Schönander EMT 35) by way of a catheter introduced through the left femoral artery. Aortic blood pressure, aortic dp/dt and the electrocardiogram (lead II) were recorded on an ink-jet recorder (Elema- Schönander Mingograf 81), and heart rate was measured from the E.C.G. Drugs were administered via an external jugular vein.

A 5 min infusion of adenosine, noradrenaline or isoprenaline was given as a control. This infusion was repeated 5 min after the intravenous injection of dipyridamole (1 mg/kg) or of another coronary vasodilator drug, hexobendine (Raberger & Kraupp, 1971). Recovery from the effects of dipyridamole was monitored by repetition of the infusion every 15 min thereafter. Since potentiation of adenosine infusions by dipyridamole did not last for longer than one hour it was possible to study the effects of several administrations of dipyridamole in any one animal.

The drugs used were: adenosine (Sigma), (±)-noradrenaline HCl (Sigma), (−)-isoprenaline bitartrate (Wyeth), dipyridamole (Boehringer Ingelheim), hexobendine (Beecham), adenosine 5′-triphosphate, disodium salt (Sigma).

Results

Adenosine

Intravenous adenosine infusions (0·5 (mg/kg)/min) produced a fall in aortic blood pressure, in heart rate and in calculated myocardial vascular resistance. When administered in smaller doses (0·25 and 0·1 (mg/kg)/min) these effects were usually slight or absent (Fig. 1).

All of these effects of adenosine were augmented when the infusion was given after the intravenous administration of dipyridamole. This is clearly demonstrated in Fig. 1 which shows records from an experiment in which 0·1 (mg/kg)/min of adenosine was administered before and 6 min after dipyridamole (1 mg/kg). This dose of adenosine had no detectable effect before, but after dipyridamole it had effects on blood flow and on vascular resistance greater than that produced by the previous administration of 0·5 (mg/kg)/minute. Infusions of 0·1 or 0·25 (mg/kg)/min were administered to six cats. The mean effects of control infusions were

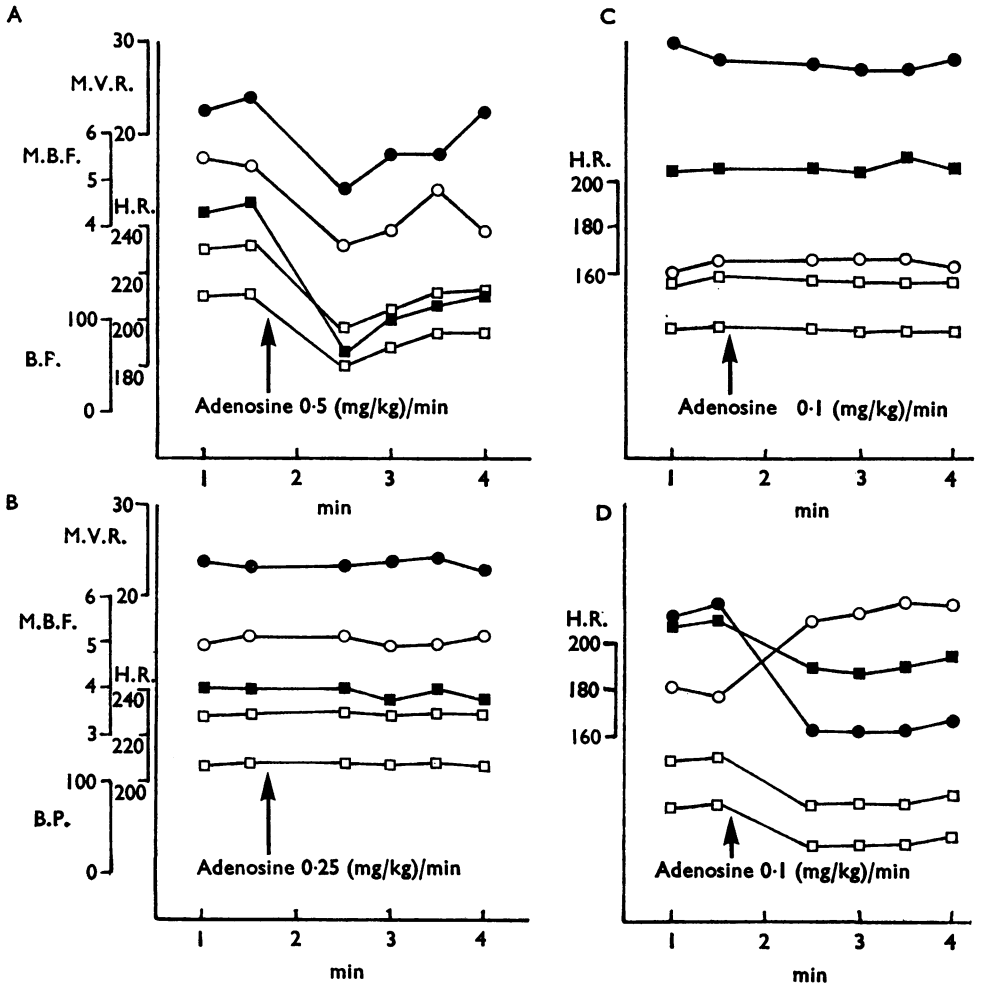

FIG. 1. The effect of adenosine infusions on myocardial blood flow (○) (measured as the myocardial thermal conductivity increment, cal/cm/s/°C), myocardial vascular resistance (●) (arbitrary units), heart rate (■) (beats/min) and on systolic and diastolic aortic blood pressure (□) (mmHg (1 mmHg≡1·333 mbar)). Three infusions of adenosine (A, B and C) were administered to this anaesthetized cat; only the first 2 min of each (commencing at the arrow) is shown. Six min after the intravenous injection of dipyridamole (1 mg/kg), the lowest dose of adenosine was infused again (D).

negligible; myocardial vascular resistance fell by $9 \pm 10\%$ and myocardial blood flow increased by $2 \pm 5\%$. However, 6 min after dipyridamole, repetition of the infusions produced a marked reduction in resistance (of $62 \pm 6\%$) while myocardial blood flow increased by $61 \pm 23\%$.

When dipyridamole was administered in a dose of 1 mg/kg intravenously its potentiating effects could be detected for about one hour. In Fig. 2 are plotted the mean results from six experiments in which adenosine infusions (0·1 or 0·25 (mg/kg)/min) were repeated at 15 min intervals after dipyridamole.

The direct effects of dipyridamole were similar to, but much more prolonged than, those of adenosine. There was a fall in aortic pressure and a slight rise in myocardial blood flow. Calculated myocardial vascular resistance therefore

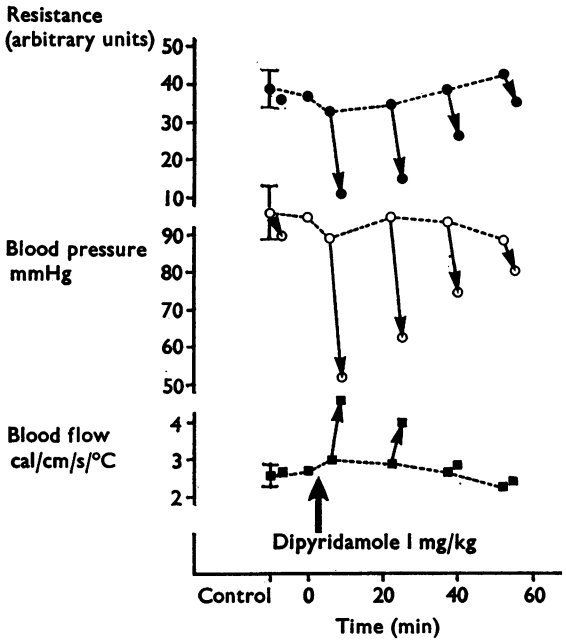

FIG. 2. The effect of adenosine infusions before and at various times after dipyridamole in the anaesthetized cat. Each point is the mean of six observations. Each arrow shows the mean effect of an infusion of adenosine on the myocardial thermal conductivity increment, an index of blood flow (■), the myocardial vascular resistance (●) and the diastolic aortic blood pressure (○). Standard error bars are included for the first measurement. The control adenosine infusions had negligible haemodynamic effects. However, after dipyridamole, adenosine caused a marked increase in blood flow, despite a fall in pressure.

decreased. These effects were marked 6 and 22 min after injection, but had almost disappeared after 36 min (see Table 1).

In three cats, hexobendine (0·02–0·1 mg/kg) was administered. As with dipyridamole, this substance markedly potentiated the effects of infusions of adenosine. It was, however, about 10 times more potent.

Adenosine triphosphate (0·1–0·5 (mg/kg)/min) produced effects similar to those of adenosine. The effects of adenosine triphosphate were potentiated both after dipyridamole and after hexobendine.

TABLE 1. *The direct effects of dipyridamole in the anaesthetized cat*

	Control	After dipyridamole			
		6 min	22 min	36 min	*n*
Diastolic blood pressure	94 ±5	83 ±5	88 ±5	89 ±5	27
Myocardial blood flow	2·4±0·2	2·6±0·2	2·6±0·2	2·4±0·2	22
Myocardial vascular resistance	41 ±3	33 ±3	36 ±3	39 ±4	22
Heart rate	193 ±7	191 ±7	191 ±7	192 ±7	20

Changes in diastolic blood pressure (mmHg), myocardial blood flow as thermal conductivity increment (cal/cm/sec/°C), myocardial vascular resistance (arbitrary units) and in heart rate (beats/min) were measured at various times after the intravenous injection of dipyridamole (1 mg/kg). Each result is the mean (± standard error) of the number of observations (*n*) indicated.

Noradrenaline

Noradrenaline infusions increased aortic pressure and myocardial blood flow, while heart rate was reduced. The rise in blood flow was secondary to the rise in

pressure, and calculated myocardial vascular resistance was, in most experiments, unaltered. Sometimes, however, noradrenaline had a slight vasodilator effect, while in other experiments a mild vasoconstrictor action was noted in the myocardial bed. These results are similar to those obtained by Parratt & Wadsworth (1970b). The effects of an infusion of noradrenaline in one experiment are illustrated in Figure 3.

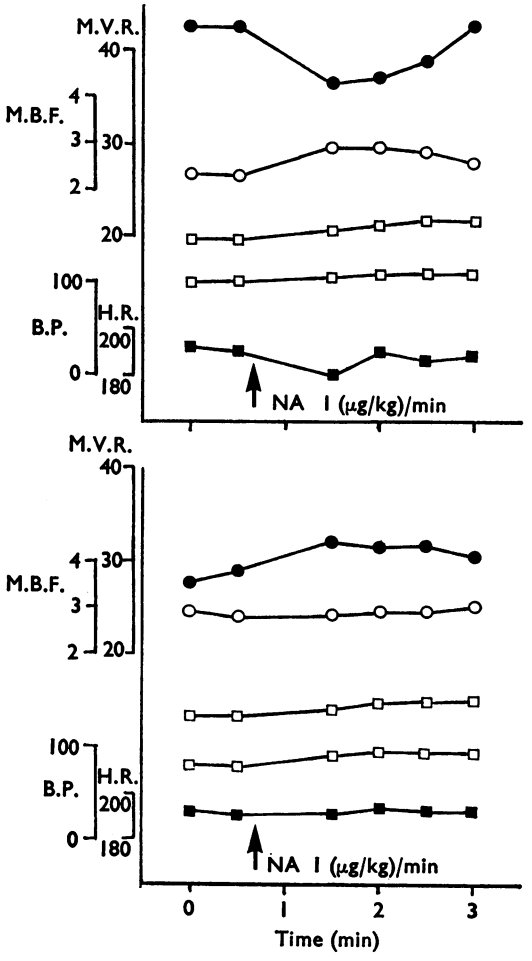

FIG. 3. The effects of noradrenaline (NA) infusions on myocardial blood flow (○) (measured as the myocardial thermal conductivity increment, cal/cm/s/°C), myocardial vascular resistance (●) (arbitrary units), heart rate (■) (beats/min) and on aortic blood pressure (□) (mmHg). In this cat an infusion was administered before (upper panel) and after (lower panel) dipyridamole (1 mg/kg). The control infusion produced mild myocardial vasodilatation and an increase in blood pressure. Seven minutes after dipyridamole, the basal level of resistance was considerably less, and noradrenaline now produced slight vasoconstriction. Only the first 2 min of each infusion (commencing at the arrow) are shown.

The mean results from seven experiments in which noradrenaline infusions (0·5 (μg/kg)/min) were administered are plotted in Figure 4. The mean change in myocardial vascular resistance in the control infusions was an increase of $4 \pm 7\%$. When noradrenaline was administered 6 min after dipyridamole, it still increased resistance, in this case by $24 \pm 9\%$. This increased constrictor effect is probably a reflection of the fall in base-line resistance following the injection of dipyridamole. There was

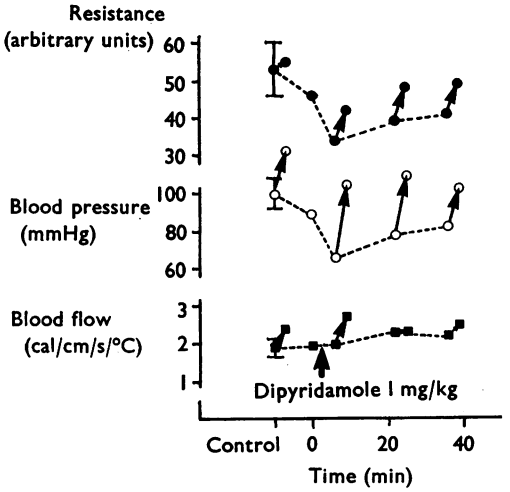

FIG. 4. The effects of noradrenaline infusions (0·5 (μg/kg)/min) before, and at various times after, dipyridamole. Each point is the mean of seven observations. Each arrow indicates the mean effect of noradrenaline on myocardial thermal conductivity increment, a measure of blood flow (■), myocardial vascular resistance (●) and on the aortic blood pressure (○). Standard error bars are included for the first measurement. Control infusions increased the blood pressure, while changes in blood flow and resistance were slight. There was no evidence that dipyridamole augmented the vasodilator component of the action of noradrenaline.

no evidence in any experiment that dipyridamole augmented the dilator component of the action of noradrenaline.

Isoprenaline

Isoprenaline infusions (0·25 (μg/kg)/min) increased heart rate, pulse pressure and myocardial blood flow; diastolic blood pressure and calculated myocardial vascular resistance were reduced (see Fig. 5).

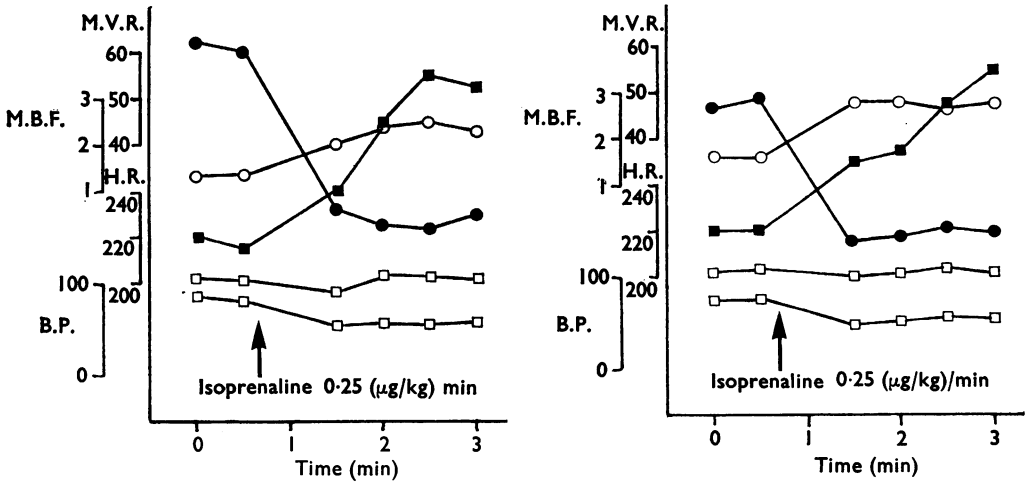

FIG. 5. The effects of isoprenaline infusions on myocardial blood flow (○) (measured as myocardial thermal conductivity increment, cal/cm/s/°C), myocardial vascular resistance (●) (arbitrary units), heart rate (■) (beats/min) and aortic blood pressure (□) (mmHg). In this cat an infusion was administered before (left panel) and again 5 min after the intravenous injection of dipyridamole (1 mg/kg). The effects of isoprenaline were not altered by dipyridamole. Only the first 2 min of each infusion (commencing at the arrow) are shown.

The mean results from seven experiments, in which isoprenaline infusions were administered at intervals after dipyridamole, are shown in Figure 6. The effects of isoprenaline were uninfluenced by the prior administration of dipyridamole. Thus, before dipyridamole, isoprenaline decreased myocardial vascular resistance by $41 \pm 7\%$; after dipyridamole resistance fell by $40 \pm 8\%$. Isoprenaline increased myocardial blood flow by $62 \pm 16\%$ before dipyridamole, and by $57 \pm 13\%$ after. There was thus no evidence that dipyridamole caused any potentiation of the dilator effects of isoprenaline in the myocardial vasculature.

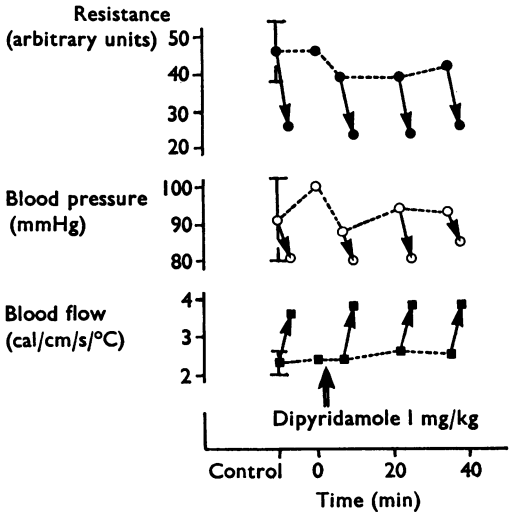

FIG. 6. The effects of isoprenaline infusions (0·25 (μg/kg)/min) before, and at various times after, dipyridamole. Each point is the mean of 7 observations. Each arrow indicates the mean effect on myocardial thermal conductivity increment, an index of blood flow (■), myocardial vascular resistance (●) and the aortic blood pressure (○). Standard error bars are included for the first measurement. Control infusions increased blood flow and decreased resistance. These effects were not noticeably affected by dipyridamole.

Discussion

The effects of catecholamines on the myocardial vascular bed involve both direct and indirect actions. The direct effects are exerted via the adrenoceptors of arteriolar smooth muscle. Noradrenaline interacts with α-receptors (causing constriction), while isoprenaline interacts with β_2-adrenoceptors, causing dilatation (Parratt, 1965 ; Gaal, Kattus, Kolin & Ross, 1966). Both amines interact with β_1-adrenoceptors in the myocardium, causing an increase in cardiac work and in oxygen demand, and these changes underlay the indirect effect (Hashimoto, Shigei, Imai, Saito, Yago, Uei & Clark, 1960).

Thus β_2-adrenoceptor antagonists reduce the myocardial vasodilator action of isoprenaline by inhibiting the direct effect ; β_1 antagonists can also reduce isoprenaline dilatation, but in this case by inhibiting the indirect effect (Parratt & Wadsworth, 1970a). The constrictor actions of noradrenaline become apparent after β-receptor blockade, which removes the indirect dilator component of action of the amine (Parratt & Wadsworth, 1970b). These direct and indirect effects have also been clearly distinguished in isolated hearts (Broadley, 1970).

The indirect vasodilator effect may be mediated by the release of a vasoactive metabolite. In the case of the dilatation that accompanies systemic hypoxia and

coronary ischaemia there is evidence that adenosine may be the mediator (Berne, Rubio, Dobson & Curnish, 1971; Rubio, Berne & Katori, 1969). Adenosine, hypoxanthine and inosine are released from the isolated perfused cat heart by adrenaline in the presence of the deaminase inhibitor, 8-azaguanine (Katori & Berne, 1966). If adenosine were also being released from the myocardium in significant quantities during catecholamine infusions *in vivo* then it would be expected that, after dipyridamole, the dilator effect of isoprenaline would be potentiated and the dilator component of the action of noradrenaline would become more evident.

In the experiments described in this paper dipyridamole (1 mg/kg) potentiated the effects (including myocardial vasodilatation) of adenosine. This action of dipyridamole lasted for about one hour. However, no potentiation of the dilator effects of isoprenaline was observed and the constrictor effect of noradrenaline was not diminished by dipyridamole. It is therefore concluded that noradrenaline and isoprenaline, in the doses used in this study, do not release physiologically significant quantities of adenosine from the heart.

Presently available evidence suggests that dipyridamole potentiates the actions of adenosine by two different mechanisms. It prevents the uptake of adenosine into erythrocytes (Pfleger, 1969), and this prolongs its life in the blood stream. It also prevents adenosine uptake into cardiac tissue (Kubler, Spieckermann & Bretschneider, 1970), and this action accounts for the potentiation of the cardiac effects of adenosine even in blood-free systems (Stafford, 1966). It might, therefore, be expected that dipyridamole would more readily potentiate the effects of exogenous adenosine than the effects of adenosine released from the myocardium. Intravenously administered adenosine would be susceptible to both inactivation mechanisms but locally released adenosine would only be available to the second. Dipyridamole has been found to produce a less than 2 fold potentiation of adenosine administered via a branch of the left coronary artery, while in the same experiments it produced a 52 fold potentiation of intravenously administered adenosine (Scholtholt, Bussmann & Lochner, 1965). Dipyridamole was found to prolong the reactive hyperaemia following coronary artery occlusion (Parratt & Wadsworth, 1972). This is taken as evidence that dipyridamole is able to potentiate 'physiologically' released adenosine and, therefore, that dipyridamole would potentiate the dilator effects of isoprenaline if adenosine were being released by this amine.

Hashimoto & Sano (1968) have found that the vasodilator effects of noradrenaline in the isolated perfused dog heart are augmented after dipyridamole. Since in their experiments the acceleration of the heart rate induced by noradrenaline was also greater after dipyridamole, it is probable that the potentiation was not the result of an adenosine-sparing action. A non-selective potentiating effect of dipyridamole has also been observed in the cat nictitating membrane, where vasodilatation allowed greater access of agonist drugs to their site of action (Bowman & Stafford, 1968).

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