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The possible role of adenosine in the coronary dilator action of some pyrimidopyrimidines and pteridines

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Summary

1. The abilities of some pyrimidopyrimidines (including dipyridamole) and pteridine derivatives to potentiate heart block produced by adenosine in the guinea-pig and to produce coronary dilatation in kitten hearts were investigated.

2. The results demonstrated a correlation between adenosine potentiating and coronary dilating activities of the compounds studied, and are therefore compatible with the concept that compounds of the dipyridamole type owe their coronary dilator activity to potentiation of endogenous adenosine.

Introduction

Dipyridamole (2,6 - bis(diethanolamino) - 4,8 - dipiperidino - pyrimido(5,4 - d)pyrimidine) is a coronary dilator drug introduced for the treatment of angina (Charlier, 1961 ; Winbury, 1964). This compound has been shown to potentiate adenosine in its actions on several tissues (Scholtholt, Bussman & Lochner, 1965 ; Stafford, 1966) and it has been suggested that some of its effects, including dilatation of the coronary vessels, may arise indirectly through potentiation of endogenous adenosine (West, Bellet, Manzoli & Müller, 1962 ; Gerlach & Deuticke, 1963 ; Stafford, 1966).

In the experiments described in this paper the coronary dilator and adenosine potentiating activities of several pyrimidopyrimidines (including dipyridamole) and pteridines were studied in an attempt to determine whether any correlation was evident between the two effects.

Methods

Adenosine potentiation

Adenosine produces a transient period of heart block in the guinea-pig, and this response was chosen as suitable for studying the degree of adenosine potentiation by pyrimidopyrimidines and pteridines because dipyridamole by itself does not produce it (Stafford, 1966).

Guinea-pigs of either sex, weighing 450–750 g, were anaesthetized with urethane (1·5 g/kg intraperitoneally) and placed on artificial respiration. Adenosine was injected in a volume of 10 μl every 2 min through a polythene cannula (1 mm

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external diameter) tied into the left atrium (Rand, Stafford & Thorp, 1955). The pyrimidopyrimidine and pteridine derivatives were injected into the jugular vein. Duration of heart block—that is, the interval during which conduction block was evident—was measured from the electrocardiogram.

Experiments with adenosine and dipyridamole were also carried out on isolated atria from guinea-pigs (300–360 g). The atria were suspended in McEwen's (1956) solution at 35° ±0·5° C gassed with 95% oxygen and 5% carbon dioxide. The spontaneous contractions were recorded isometrically with a Statham universal transducing cell (Type UC2) and a Beckman Dynograph pen recorder.

Coronary dilatation

It is difficult to study coronary dilator activity in isolated guinea-pig hearts because the vessels lack sufficient tone. Isolated kitten hearts, on the other hand, maintain a high level of tone in their coronary arteries and were chosen as a suitable preparation to study coronary dilator activity. Kitten hearts do not respond to adenosine by an obvious and precisely defined heart block, so that it was not possible to study the two parameters in the same species.

Hearts isolated from kittens (0·5–1·2 kg) were perfused by retrograde flow through the aorta into the coronary vessels. The perfusion fluid, McEwen's (1956) solution warmed to 35 ±0·5° C, was delivered by a Watson Marlow (MHRE) flow inducer. Perfusion pressure was measured in mmHg (1 mmHg≡1·333 mbar) with a Condon manometer, and contractions of the heart were recorded with a spring loaded lever. The pyrimidopyrimidine or pteridine derivatives were added to the reservoir of perfusion fluid and perfusion was continued until the maximum dilator effect for the concentrations used was produced. In order to avoid the complication of cumulative effects of the drugs, each heart was subjected to only one application of one drug.

Drugs used

Adenosine (Koch-Light Laboratories Ltd.).

Pyrimidopyrimidines: Dipyridamole, 2,6-bis(diethanolamino)-4,8-diperidino-pyrimido (5,4-d) pyrimidine [RA 8]; 2,6-bis(diethanolamino)-4,8-di(3′-hydroxy-piperidino)-pyrimido (5,4-d) pyrimidine [RA 159]; 2,4,6,8-tetra(diethanolamino)-pyrimido (5,4-d) pyrimidine [RA 137].

Pteridines: 4-ethylethanolamino-2,7-dimorpholino-6-phenylpteridine [RE 61]; 4-diisopropanolamino-2,7-dimorpholino-6-phenylpteridine [RE 102]; 4-ethanol-isopropanolamino-2,7-di(2′-methyl-morpholino)-6-phenylpteridine [RE 244]; 4-methylethanolamino-2,7-dimorpholino-6-phenylpteridine [RE 57].

The pyrimidopyrimidines and pteridines were a gift from Boehringer Ingelheim Pty. Ltd.

Results

Adenosine potentiation

Heart block

A dose of adenosine (0·08–0·12 μmol) that initially caused a heart block of 3–6 s duration was chosen. This dose was repeated at 2 min intervals for the duration of the experiment (2–3 h). When injected at 20–40 min intervals, all the pyrimido-

pyrimidine and pteridine derivatives prolonged the period of heart block produced by adenosine as described for dipyridamole by Stafford (1966). In each of the first eight experiments several of the potentiating compounds were injected in succession in order to obtain a rough estimate of their relative potency. In each of a further twenty experiments, the activities of submaximal doses of one or other of the compounds were compared with those of dipyridamole using a bracketing procedure. The concentrations of dipyridamole used ranged from 0·08 to 0·30 μmol/kg in different experiments. Figure 1 shows the results of one of these experiments in which the relative activity of RA 159 was studied. Approximate estimates of relative potency were obtained and the results from all twenty experiments are shown in Table 1.

Isolated atria

In three experiments adenosine (4–40 μM) caused marked slowing of the rate of beating and diminution of contractile force of isolated guinea-pig atria. These effects of adenosine were powerfully augmented by dipyridamole (0·4–1·2 μM)

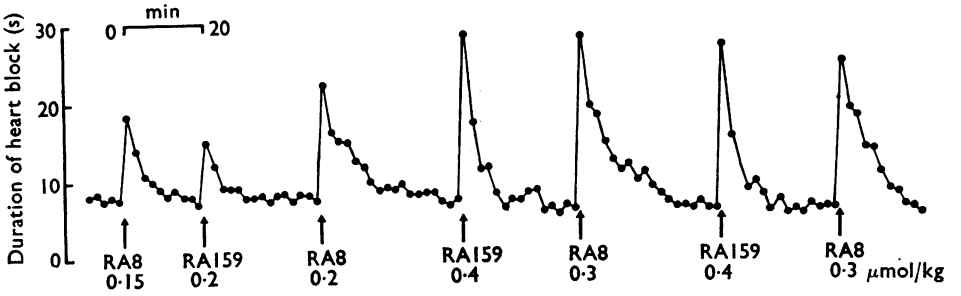


FIG. 1. Each point represents the duration of heart block produced by 0·08 μmol adenosine, injected every 2 min into the left atrium of a guinea-pig heart *in situ*. RA 8 (dipyridamole) and RA 159 were injected intravenously and they prolonged the period of heartblock produced by adenosine. In this experiment RA 159 was 0·6–0·8 times as potent as RA 8.

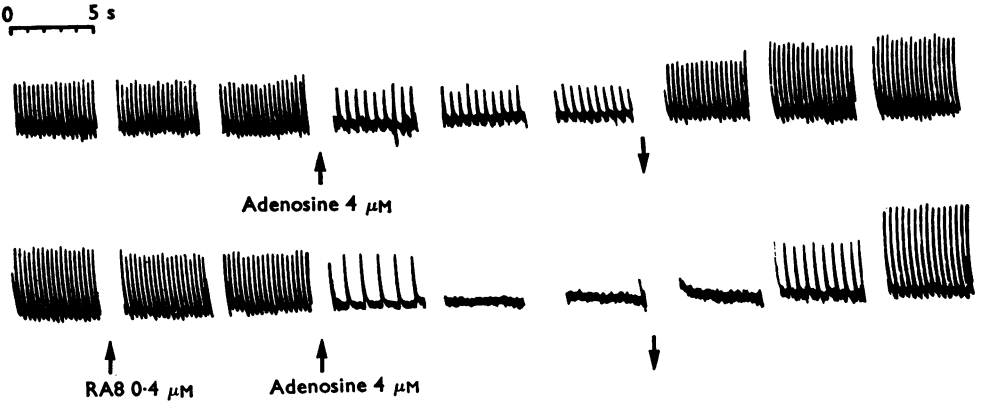


FIG. 2. Isometric contractions of spontaneously beating isolated atria from guinea-pigs. Recordings were made for 5 s in every min. RA 8 (dipyridamole, 0·4 μM) augmented the diminution of rate and contractile force in response to adenosine (4 μM).

TABLE 1. *Chemical formulae and relative adenosine-potentiating and coronary dilating activities of the compounds studied. (Dipyridamole=1 for both activities)*

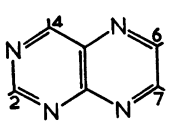
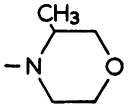
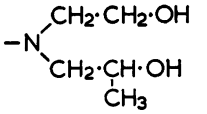
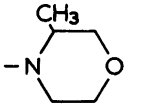
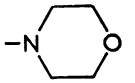
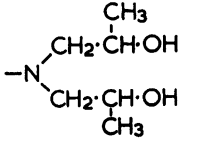
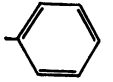
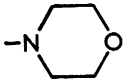
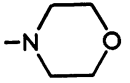
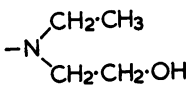
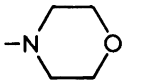
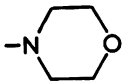
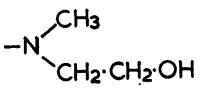
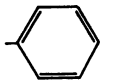
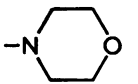
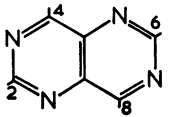
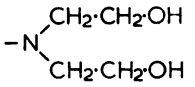
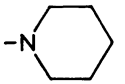
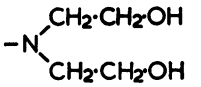
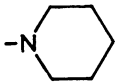
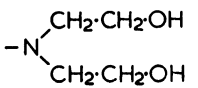
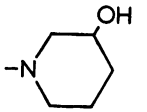
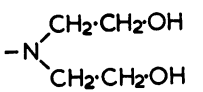
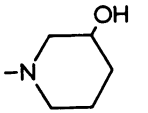
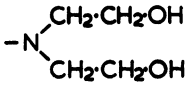
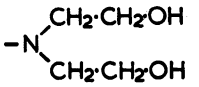
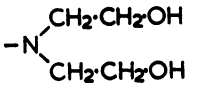
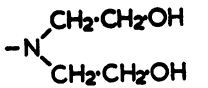
Pteridines	Substitute in position 2	4	6	7	Adenosine potentiation RA 8=1	Coronary dilatation RA 8=1
						
RE 244					4·0–5·0, 4·0–6·0, 6·0–8·0 **5·5**	**18**
RE 102					3·0–4·5, 4·0–5·0, 2·8–3·5 **3·6**	**2·5**
RE 61					2·0–3·0, 2·2–2·8, 1·8–2·2 **2·3**	**7·2**
RE 57					1·0–1·1, 1·0–1·1, 1·4–1·6, 0·8–0·9 **1·1**	**1·5**

TABLE 1—*continued*

Pyrimidopyrimidines	Substitute in position 2	4	6	8	Adenosine potentiation RA 8=1	Coronary dilatation RA 8=1
						
RA 8					**1·0**	**1·0**
RA 159					0·8–1·0, 0·5–0·8, 0·6–0·8, 0·7–1·0 **0·78**	**0·3**
RA 137					0·3–0·5, 0·4–0·5, 0·3–0·5 **0·42**	**0·1**

Adenosine potentiating activity was determined by bracketing the responses to various doses of a compound around those to dipyridamole. Thus each experiment yielded an activity range (ordinary type). The numbers in bold type are the mean values obtained from different experiments. For coronary dilatation, relative potency was estimated at concentrations required to produce a 20% fall in perfusion pressure.

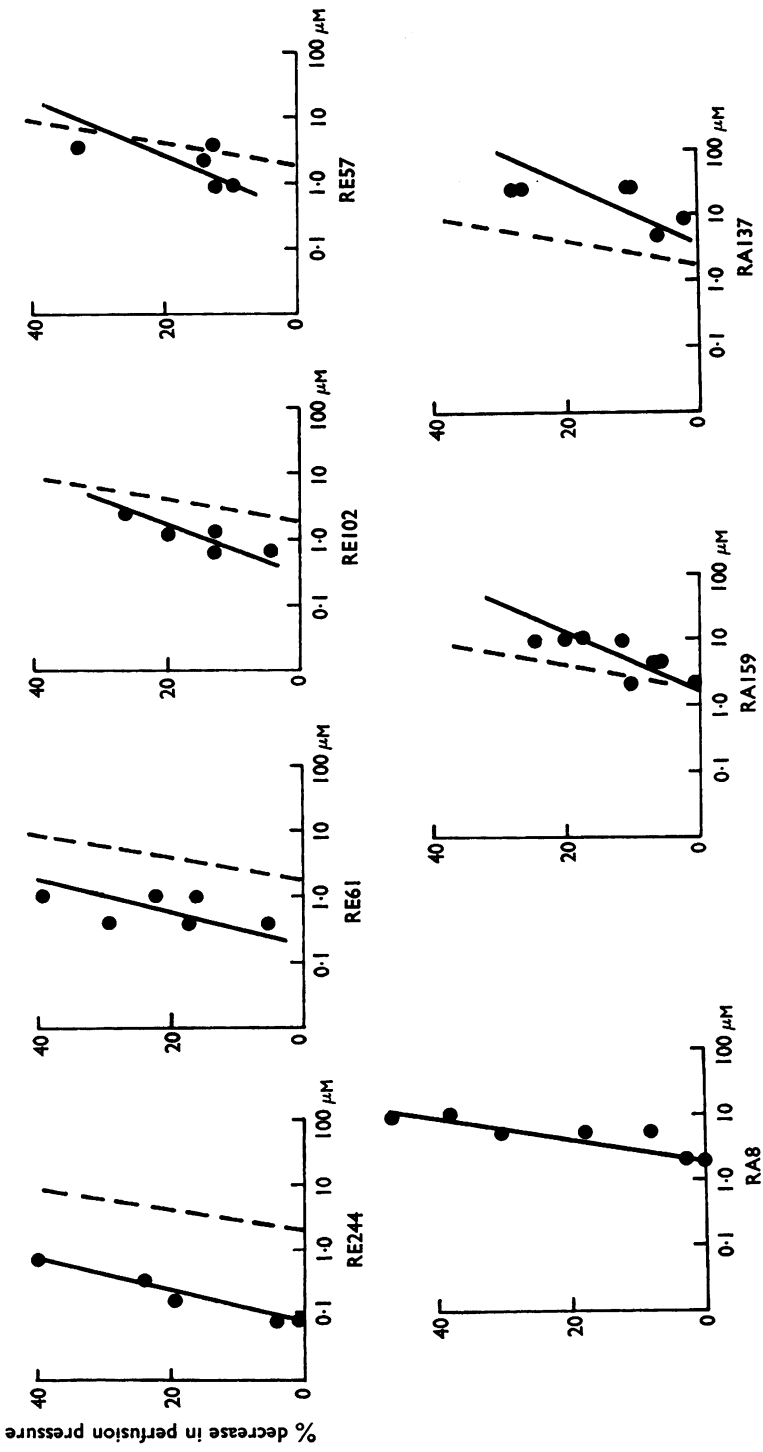


FIG. 3. Percentage fall in perfusion pressure plotted against log concentration of the compounds introduced into the perfusate of kitten isolated hearts. Each point is the result of one experiment. The regression lines were plotted by the least-squares method and that of dipyridamole (RA 8) is included for comparison in each graph as an interrupted line. Deviation from parallelism of each regression line with that of RA 8 is not significant ($P>0{\cdot}05$).

(Fig. 2). The concentrations of dipyridamole used had no apparent effect on atrial contractions in the absence of adenosine. The effects of other pyrimidopyrimidines and pteridines were not studied on this preparation.

Coronary dilatation

Flow rate was adjusted to 10–12 ml/min. At this rate, perfusion pressure slowly rose to 90–120 mmHg and remained constant within this range until a pyrimidopyrimidine or pteridine derivative was introduced into the perfusion fluid. Effective concentrations of these compounds caused coronary dilatation which was reflected by a fall in perfusion pressure. The coronary dilator activity of the compounds was expressed as percentage reduction in pressure and was determined at two or three concentrations for each of the seven compounds, enabling log concentration-response lines to be drawn (Fig. 3). In Fig. 3 the regression line for dipyridamole is included for comparison in each graph. The approximate relative potency of each compound calculated from the concentrations necessary to produce a 20% decrease in perfusion pressure is given in Table 1. Coronary dilator activity was marked in the absence of change in amplitude and rate of contractions, except at higher concentrations of pyrimidopyrimidines and pteridines when slight increases in amplitude were observed. The absence of any change in contractility over most of the dose range used avoids the complications to which such changes give rise when studying coronary dilator activity (Charlier, 1961).

Discussion

All the pyrimidopyrimidine and pteridine derivatives investigated potentiated adenosine in its action on the guinea-pig heart *in situ*, and were active as coronary

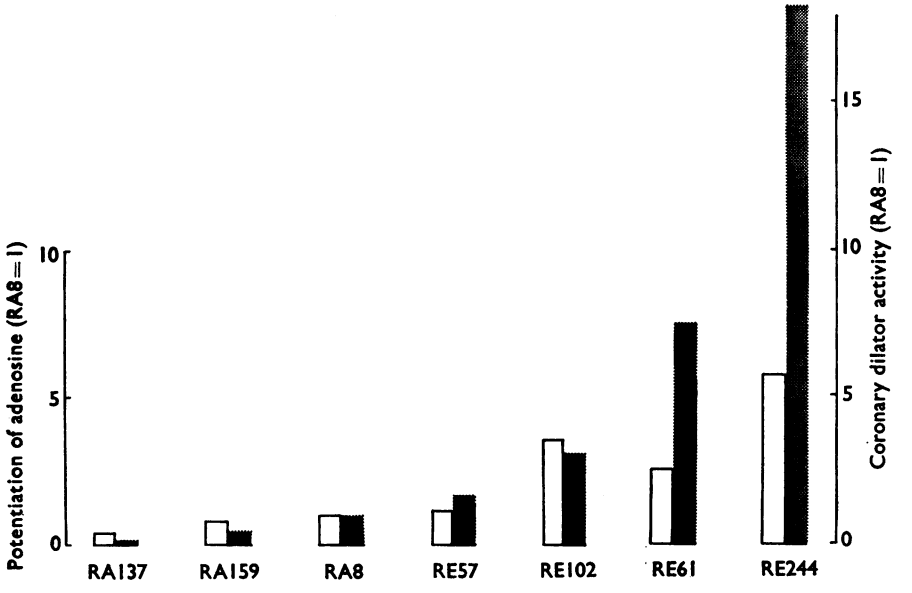


FIG. 4. Relative potencies (dipyridamole = 1) of pyrimidopyrimidines and pteridines in potentiating adenosine in the guinea-pig heart *in situ* (open columns) and in dilating the coronary vessels of perfused isolated kitten hearts (shaded columns). The values are those shown in Table 1.

dilators in isolated kitten hearts. That there is a reasonable correlation between the rank orders of potency in producing these effects is illustrated in Fig. 4. A perfect correlation would not be expected in view of the different species used to measure the two effects, and the fact that the calculated log concentration-response lines for the different agents were not parallel, even though not differing significantly from parallelism, so that the level of response at which they were compared was necessarily arbitrary. A correlation might of course be demonstrable if the coronary dilatation produced by the compounds, whatever their mechanism of action, resulted in a correspondingly greater proportion of the injected adenosine reaching the site of the action responsible for producing heart block. Indeed a non-specific potentiating action of this type produced by dipyridamole was demonstrated in the cat nictitating membrane (Bowman & Stafford, 1968). However, it is unlikely that coronary dilatation is responsible for potentiation of adenosine-induced heart block in the guinea-pig, because the potentiation is selective for adenyl compounds (Stafford, 1966). Furthermore, in isolated atria, changes in vascular tone are unlikely to affect the access of drugs to receptors, yet dipyridamole powerfully augmented the reduction in rate and force of contractions produced by adenosine. The degree of correlation between coronary dilator activity and the ability to potentiate adenosine of the various compounds used in the present experiments is thus compatible with the concept that the group of compounds studied produces coronary dilatation by potentiating endogenous adenosine or adenyl nucleotides in their action on coronary vessels.

The compounds may potentiate adenosine by suppressing its deamination, either directly by blocking adenosine deaminase, or indirectly by interfering with its uptake into intracellular sites of deamination. However, the pyrimidopyrimidine and pteridine derivatives were effective in potentiating adenosine in doses of 0·01–0·1 μmol/kg. These are equivalent to concentrations much lower than those found by Deuticke & Gerlach (1966) to inhibit adenosine deaminase (around 0·1 mM). The concentrations used in the present experiments are consistent with those required to exert an adenosine sparing action in membrane-intact erythrocytes (Koss, Beisenherz & Maerkisch, 1962) and cardiac cells (Kübler & Bretschneider, 1964), an action not shown in broken cells. It thus seems likely that by blocking the re-uptake of endogenous adenosine into myocardial cells, or into erythrocytes, pyrimidopyrimidine and pteridine derivatives may make more adenosine available to act on receptors, and thereby produce coronary dilatation.

It is of interest that the order of potency of the compounds studied in the present experiments appears to be related to their probable oil/water partition coefficients, as forecast from their chemical structures, the more lipid soluble compounds being the more potent. Potency, within this group, may therefore be dependent on the ability of the compounds to penetrate to and combine with some lipid component of the cell membrane.

I thank Dr. Anne Stafford for her kind help and advice throughout this work, and Mr. A. Kerr for carrying out some of the experiments. I am grateful to Dr. K. Higgins, of Boehringer Ingelheim Pty. Ltd., for supplying the compounds and for his interest in this work.

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