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Cardiovascular actions of substituted adenosine analogues

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Summary

1. The effects of adenosine on systemic blood pressure, coronary blood flow and cardiac contractility and rate were studied in anaesthetized open-thorax dogs.

2. The potency and duration of action of substituted adenosine analogues were compared with that of adenosine.

3. With the exception of 2-chloroadenosine, in which coronary dilator activity was enhanced, substitution in the 2-position of the adenosine molecule reduced activity.

4. In all cases 2-substitution prolonged the duration of the coronary dilator activity of adenosine.

5. N^6-Methylation of adenosine and 2-chloroadenosine reduced their coronary dilator activities, but had no effect on the duration of the response.

6. Comparison of the coronary dilator potencies and hypotensive activities of 2-ethylaminoadenosine and 2-methoxyadenosine indicates that these analogues have some specificity for the coronary bed.

Introduction

The coronary dilator activity of adenosine is well documented (Drury & Szent-Gyorgi, 1929; Winbury, Papierski, Hemmer & Hambourger, 1953; Green & Stoner, 1950). This activity is usually accompanied by bradycardia, cardiac depression and hypotension (Drury & Szent-Gyorgi, 1929). All of these effects are of short duration, however, because of the rapid uptake of adenosine into red blood cells and tissues (Pfleger, Seifen & Schoendorf, 1969), and its conversion to inosine by adenosine deaminase (Baer, Drummond & Duncan, 1966; Rockwell & Maguire, 1966).

Substitution of the adenosine molecule by certain groups in the 2-position and N^6-alkylation produces compounds which inhibit adenosine deaminase (Rockwell & Maguire, 1966). Such chemical modifications have been found to reduce the hypotensive activity of adenosine, with the exception of 2-chloroadenosine, in which hypotensive activity is considerably enhanced (Thorp & Cobbin, 1959). Some of the substituted analogues, however, although reduced in potency compared with adenosine, induce a much longer lasting hypotension (Clarke, Davoll, Philips & Brown, 1952; Michal, 1970).

The results of preliminary experiments with 2-chloroadenosine indicated that some chemical modifications of the adenosine molecule might increase the relative

degree of dilation in the coronary system compared with that in the peripheral vessels and might also reduce its cardiac depressant activity. Such compounds would therefore have potential as specific coronary vasodilators.

To evaluate this possibility, the effects of a number of adenosine analogues on coronary blood flow, systemic blood pressure and cardiac rate and contractility were measured.

Method

The adenosine analogues used in the study were synthesized in this Department (Gough & Maguire, 1967 ; Maguire, Nobbs, Einstein & Middleton, 1970), and were analytically pure.

Mongrel dogs of both sexes weighing between 17 and 30 kg were anaesthetized with sodium pentobarbitone (30 mg/kg i.v.), and subsequent doses administered as required. Respiration was assisted by a Starling Ideal Pump, through a cuffed endotracheal tube. Systemic blood pressure (1 mmHg$\equiv$1·333 mbar) was recorded from the femoral artery via a Statham P23AC pressure transducer. The thorax was opened by a mid-sternal incision and the pericardium was slit to expose the left descending branch of the coronary artery. One centimetre of the artery was cleared for the placement of an electromagnetic flow probe. Contractions of the ventricular muscle were recorded by means of a Walton-Brodie strain gauge sutured to an area supplied by the left descending coronary artery. The Lead II ECG was continuously monitored on an oscilloscope, and the QRS complex used to trigger a cardiotachometer. Measurements were recorded on a Grass Model 7 polygraph.

No drugs were administered for 45 min after the completion of surgery, to allow the animals to reach a steady state. Injections were made into the left atrium via an indwelling polythene cannula. This procedure allowed for adequate mixing, and passage of adenosine through the coronary circulation before the lungs, where extensive uptake is known to occur (Pfleger, *et al.*, 1969). Control responses to 1·5 ml of normal saline were recorded, and all injection volumes were kept below this.

A dose-response relationship was obtained for adenosine at the start of each experiment because the analogues were known to have adenosine deaminase inhibitor activity. The animals then received three to five of the analogues in random order. Dose ranges of the analogues were selected to produce increases in coronary flow similar in magnitude to those elicited by 1–10 μg/kg of adenosine.

The coronary dilator potency of each analogue with respect to adenosine was calculated from the dose-response lines for each dog. A mean value and standard error was obtained from five to six animals. The hypotensive potency of each analogue with respect to adenosine was calculated from the dose-response lines, but here the value was computed from the combined data obtained from all the animals receiving each compound.

Results

Adenosine

A typical response to the range of doses of adenosine used is shown in Fig. 1. Femoral blood pressure was lowered by doses greater than 1 μg/kg. In many

animals this fall in blood pressure resulted in compensatory increases in cardiac contractile force and rate. In approximately half the animals, however, contractile force and/or rate were initially depressed. The effect of adenosine on coronary blood flow and systemic blood pressure was dose dependent and was of 30–60 s duration.

Adenosine analogues

The results obtained for the adenosine analogues are incorporated in Table 1.

2-Alkylthioadenosines

2-Methyl-, -ethyl-, -*n*-propyl- and isopropyl-thioadenosines were examined. The effects of 2-methylthioadenosine were tested on only two dogs, because of its low

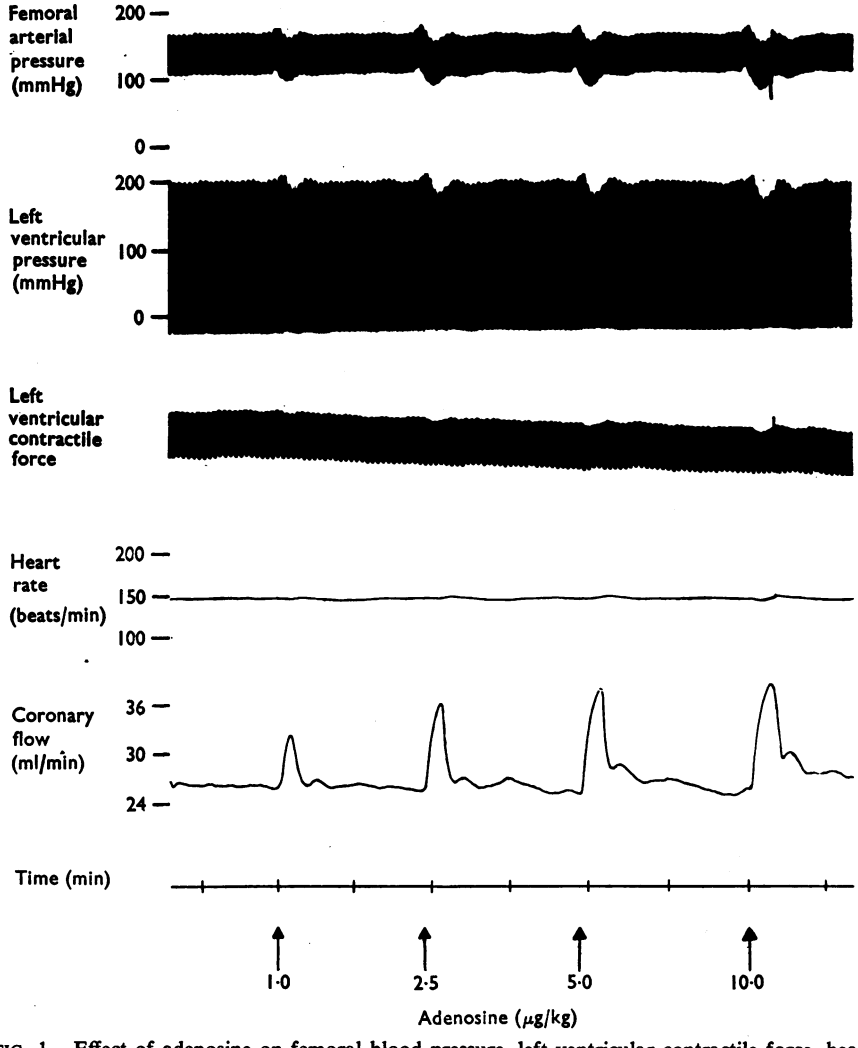

FIG. 1. Effect of adenosine on femoral blood pressure, left ventricular contractile force, heart rate and coronary blood flow of anaesthetized open-thorax dogs.

solubility. With larger animals it proved impossible to dissolve sufficient of the compound in normal saline to enable injection volumes to be kept below 1·5 ml. (Injection of up to 1·5 ml of normal saline had no effect in any of the animals, but larger volumes produced coronary dilation in some animals which could not be distinguished from the drug effect.)

Substitution with an alkylthio group in the 2-position of the adenosine molecule resulted in a reduction of its coronary dilator activity. As the length of the alkyl chain was increased, however, the activity was increased towards that of the parent compound, but branching of the propyl chain reduced activity (Table 1).

The duration of dilator activity was increased by 2-alkylthio substitution. Elevation of coronary blood flow was maintained for 5–7 min after injection of the 2-alkylthioadenosines, which was 10–15 times as long lasting as the effect of adenosine.

Femoral blood pressure was reduced by the 2-alkylthioadenosines. This hypotensive activity was of shorter duration than the coronary dilator activity, and was only evident for 1–3 min after injection of the compounds. Dose-response lines were calculated for this effect; and found to be statistically parallel to that for adenosine ($P=0·05$). Thus the systemic depressor potencies of the 2-alkylthioadenosines could be computed (Table 1). 2-Ethylthioadenosine was less potent as a hypotensive agent than as a coronary dilator. 2-Isopropylthioadenosine was not significantly more effective as a coronary than as a systemic dilator, while 2-*n*-propylthioadenosine appeared more active in the peripheral vessels.

Cardiac rate and contractility were not significantly affected by any of the 2-alkylthioadenosines.

N^6-Methylated adenosines

Coronary dilator activity was markedly reduced by the N^6-methylation of adenosine and 2-chloroadenosine. N^6,N^6-Dimethylation of adenosine abolished activity altogether, and no response could be elicited with doses of up to 0·5 mg/kg (Table 1).

N^6-Methyladenosine was effective for only 30–60 s, and was thus not noticeably different from adenosine. 2-Chloro-N^6-methyladenosine, however, had a duration of action 10 times that of adenosine.

TABLE 1. *Hypotensive and coronary dilator potencies of a series of adenosine analogues*

Drug	Coronary dilator potency ± S.D.	Duration of coronary dilator activity	Hypotensive potency (95% confidence limits)	Ratio of coronary dilator potency to hypotensive potency
Adenosine	1	1	1	1
2-Alkylthioadenosines				
2-Methylthioadenosine	0·03	10–15		
2-Ethylthioadenosine	0·12 ± 0·02	10–15	0·05 (0·02–0·09)	2·3
2-*n*-Propylthioadenosine	0·48 ± 0·14	10–15	0·76 (1·5 –0·38)	0·6
2-Isopropylthioadenosine	0·37 ± 0·06	8–12	0·35 (0·77–0·16)	1·1
N^6-Methylated adenosines				
N^6-Methyladenosine	0·06 ± 0·01	1	0·06 (0·11–0·03)	1
N^6,N^6-Dimethyladenosine	< 0·02			
2-Chloro-N^6-methyladenosine	0·04 ± 0·01	10	0·05 (0·07–0·03)	0·9
Miscellaneous 2-substituted adenosines				
2-Chloroadenosine	6·6 ± 1·6	8–12	2·4 (3·3 –1·7)	2·8
2-Ethylaminoadenosine	0·91 ± 0·26	10–12	0·24 (0·34–0·17)	3·8
2-Methoxyadenosine	0·28 ± 0·08	20–25	0·09 (0·14–0·05)	3·2

The N^6-methyl derivatives of adenosine and 2-chloroadenosine lowered systemic blood pressure. The relative potencies of these compounds as hypotensive agents were approximately equal to their potencies as coronary dilators (Table 1). The duration of the hypotensive effect of N^6-methyladenosine was not significantly different from that of adenosine. After the administration of 2-chloro-N^6-methyladenosine blood pressure returned to normal within 1–2 minutes. The effects of the N^6-methylated compounds on heart rate and contractile force were qualitatively similar to those of adenosine.

Miscellaneous 2-substituted adenosines

2-Chloroadenosine, 2-ethylaminoadenosine, 2-methoxyadenosine. 2-Chloroadenosine was the only analogue significantly more potent as a coronary dilator than adenosine. Substitution of an ethylamino group in the 2-position of the adenosine molecule did not significantly affect its coronary dilator activity, whereas substitution with a methoxy group markedly reduced activity (Table 1).

The duration of the coronary dilator activity of adenosine was significantly increased by these substitutions (Table 1); the increases in coronary blood flow persisted for 3–10 min, depending on the doses injected. When equipotent doses were compared, the activity of 2-chloroadenosine and 2-ethylaminoadenosine was evident for approximately 10 times as long as for adenosine. 2-Methoxyadenosine was even longer lasting, its duration of action being 20 times that of adenosine.

Although all the compounds had some hypotensive activity (Table 1), this was less marked and of shorter duration than the coronary dilator effect. In most cases the blood pressure returned to normal within 1–2 min after injection of the analogues. Cardiac contractility and rate were not affected by these analogues.

Discussion

It has been postulated that adenosine is a mediator in the adjustment of coronary blood flow which occurs in myocardial ischaemia (Rubio, Berne & Katori, 1969). Although some doubt still remains concerning its role as a natural autoregulatory hormone (Juhran & Dietman, 1970), the effects of exogenously administered adenosine on coronary blood flow are fully documented (Rowe, Afonso, Gurtner, Chelius, Lowe, Castillo & Crumpton, 1962; Wolf & Berne, 1956; Raberger, Kraupp, Stühlinger, Nell & Chirikdjian, 1970). The increase in coronary blood flow which follows a single injection of adenosine is of brief duration since adenosine is rapidly removed from the circulation by uptake into tissues (Pfleger *et al.*, 1969) and deamination (Baer *et al.*, 1966).

In the normal heart, adenosine released by the myocardium into the interstitial fluid is capable of dilating the coronary arterioles (Rubio & Berne, 1969). The concentration of adenosine in the interstitial fluid depends on its rate of release, uptake by myocardial cells and erythrocytes, and enzymic inactivation (Rubio *et al.*, 1969). The coronary dilation caused by the adenosine analogues could result from their own intrinsic activity, inhibition of adenosine deaminase, inhibition of adenosine uptake into the red cells or tissues or any combination of these effects.

All the analogues are competitive inhibitors of adenosine deaminase (Rockwell & Maguire, 1966; Sim & Maguire, unpublished). Their relative potencies as inhibitors, derived from their affinity constants for the active site of the enzyme, are

shown in Table 2. With the exception of the alkylthio group, there appears to be no direct correlation between the coronary dilator potencies and the adenosine deaminase inhibitor activities for these derivatives, indicating that enzymic inhibition is probably not the sole mode of action of the analogues, although it could be a contributing factor.

The adenosine analogues may exert their action by inhibiting the uptake of adenosine into red blood cells. This can be assessed by comparison with the effects of dipyridamole, a known coronary vasodilator and inhibitor of adenosine deaminase.

It has been postulated that an important component of the coronary vasodilator effect of dipyridamole is an increase in the local concentration of adenosine at the vascular receptors of the heart (Kraupp, 1969). These local increases could be due to both the inhibition of the uptake of adenosine by red blood cells (Koss, Beisenherz & Maerkisch, 1962; Gerlach, Deuticke & Koss, 1965) and myocardial cells (Kolassa, Pfleger & Rummel, 1970) and/or the inhibition of adenosine deaminase (Bunag, Douglas, Imai & Berne, 1964). However, the 'adenosine-sparing' effect is more likely to be due to a reduction in the permeability of the red cell membrane to adenosine, and hence a decrease in its rate of uptake (Bunag, Douglas, Imai & Berne, 1964; Stafford, 1966). A comparison of the coronary dilator activities of dipyridamole and the adenosine analogues suggested that they had different modes of action. Dipyridamole reached peak effect 2–10 min after injection, whereas the adenosine analogues were almost instantly effective.

Considering the similarities of the structures of the analogues to adenosine, it is likely that they would have at least some intrinsic adenosine-like activity, and thus it is reasonable to suggest that the coronary dilation seen in these experiments might be explicable in these terms.

The duration and magnitude of the coronary dilation and the hypotensive activity of adenosine were significantly affected by modification of the parent molecule. All

TABLE 2. *Comparison of the coronary dilator potencies of a series of adenosine analogues with their relative affinities for the active site of adenosine deaminase*

Drug	Relative affinity	Coronary dilator potency
Adenosine	1	1
2-Chloroadenosine	0·7	6·6
2-Methoxyadenosine	0·8	0·28
2-Methylthioadenosine	1·2	0·03
2-Ethylaminoadenosine	1·4	0·91
2-Ethylthioadenosine	1·8	0·12
2-Isopropylthioadenosine	2·5	0·37
2-*n*-Propylthioadenosine	3·4	0·48
N^6-Methyladenosine	6·7	0·06
2-Chloro-N^6-methyladenosine	10·8	0·04

The affinities are obtained from the values of K_m or K_i and are expressed relative to the affinity of adenosine $\left(\frac{1}{3{\cdot}97\times10^{-5}}\right)$.

TABLE 3. *Comparison of the duration of coronary dilator activities of a series of adenosine analogues with their relative affinities for the active site of adenosine deaminase*

Drug	Relative affinity	Duration of coronary dilator activity
Adenosine	1	1
2-Chloroadenosine	0·7	10
N^6-Methyladenosine	6·7	1
2-Chloro-N^6-methyladenosine	10·8	10

The affinities are expressed relative to that of adenosine as in Table 2.

substitutions in the 2-position increased the duration of the dilator activity to at least 10 times that of adenosine. The most outstanding in this group was 2-methoxyadenosine, the dilator activity of which persisted for approximately 20 times that of adenosine.

The prolongation of dilator activity is unlikely to be due to inhibition of adenosine deaminase. Table 3 shows the duration of action of 2-chloroadenosine, N^6-methyladenosine and 2-chloro-N^6-methyladenosine, and their relative affinity constants for the active site of the enzyme. The N^6-methylated derivatives have a higher affinity constant, and hence are more potent inhibitors, although their coronary dilator activity is not significantly longer lasting than that of adenosine.

The only modification to the structure of adenosine which resulted in an increase in coronary dilator potency with respect to the parent compound was 2-chloro substitution, an observation which has been previously reported (Thorp & Cobbin, 1959). 2-Ethylamino substitution did not significantly decrease dilator activity. The remaining analogues were all less potent as coronary dilators than adenosine. With the alkylthio substituted compounds, as the length of the alkyl chain was increased, the potency approached that of adenosine ; branching of the chain, however, reduced activity.

An unsubstituted 6-amino group appears to be essential for high dilator activity, illustrated by the marked loss of activity caused by the N^6-methylation of 2-chloroadenosine, and the complete lack of activity of N^6,N^6-dimethyladenosine.

Evidence of cardiac depression due to adenosine was not seen in all the animals. Approximately half the animals showed signs of cardiac depression in response to 10 μg/kg of adenosine and in these animals it was observed that increasing the dose of adenosine above this concentration did not elicit any greater increases in coronary flow. This observation is in accord with the report of Schondorf, Rummel & Pfleger (1969), showing that the doses required to produce cardiac depression were 100 times those producing increases in coronary flow.

In comparing the relative potencies of the adenosine analogues as coronary dilators and hypotensive agents, the difference in the duration of these two effects must be taken into account. In all cases where coronary dilator activity was significantly more persistent than that of adenosine, it was observed that the hypotensive activity was of relatively short duration. This could have been due to reflex mechanisms, activated by the fall in systemic blood pressure, exerting their effect to restore blood pressure to normal levels.

Table 1 shows the relative potencies of the analogues as coronary dilators and hypotensive agents with respect to adenosine. The ratio of the two potencies gives an indication of the specificity of each analogue as a coronary dilator. It appears, from these results, that 2-ethylaminoadenosine, 2-methoxyadenosine, 2-chloroadenosine and 2-ethylthioadenosine have some specific coronary dilator activity.

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