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STUDIES ON THE CORONARY DILATOR ACTIONS OF SOME ADENOSINE ANALOGUES

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1 The cardiovascular actions of 23 adenosine analogues have been examined in anaesthetized open thorax dogs; the analogues were substituted in the 2-position of the purine ring, or in the exocyclic amino group, or were modified in the imidazole or sugar rings.

2 The effects of these compounds on coronary blood flow, peripheral blood pressure, and heart rate were compared with those of adenosine.

3 9-β-D-Arabinofuranosyladenine had no cardiovascular action; the other analogues on intra-atrial administration caused an immediate increase in coronary blood flow, the magnitude and duration of which varied with the structures of the analogues.

4 2-Fluoro-, 2-bromo-, 2-isobutylthio-, 2-ethylamino-, and 5′-deoxy-5′-chloro- adenosines had coronary dilator potencies equal to or greater than that of adenosine. No relationship was found between the dilator potency of the adenosine analogues and their duration of coronary dilator action.

5 The coronary dilator action of adenosine was potentiated by inosine, 9-β-D-arabinofuranosyladenine, tubercidin, N^6-methyladenosine and 2-trifluoromethyl-N^6-methyladenosine.

6 There was no correlation between the substrate specificities of the shorter-acting analogues for adenosine deaminase or adenosine kinase and their duration of coronary dilator action.

7 It is proposed that in the anaesthetized dog, uptake into tissues is a more important mode of removal of adenosine and adenosine analogues from the vascular system than inactivation by adenosine deaminase, that the duration of coronary dilator action of the analogues is related primarily to their specificity for the carrier which mediates adenosine uptake, and that the adenosine carrier is not associated with kinase action.

Introduction

Several classes of substituted analogues of adenosine have been found to mimic the cardiovascular actions of adenosine. The hypotensive properties of certain 2-substituted derivatives of adenosine were first reported by Clarke, Davoll, Philips & Brown (1952), and we recently investigated the effects of a more extensive series of 2-substituted adenosines on coronary blood flow and systemic blood pressure in the anaesthetized dog (Angus, Cobbin, Einstein & Maguire, 1971). N^6-Aralkylated adenosine analogues have been shown to elicit peripheral vasodilation (Dietmann, Birkenheier & Schaumann, 1970) and to increase coronary blood flow (Jahn, 1969; Juhran, Voss, Dietmann & Schaumann, 1971), and the hypotensive effects of 5′-modified derivatives of adenosine were described by Jahn (1965). Many of these analogues induce a long lasting vasodilatation, unlike that caused by adenosine, which is transient, but only 2-chloro-, 2-hydroxy- and 2-methyl- adenosines (Clarke *et al.*, 1952; Thorp & Cobbin, 1959) have been found to be more potent than adenosine itself.

In order to investigate further the coronary dilator activities of adenosine analogues, we have examined the cardiovascular effects of 23 structurally diverse adenosine analogues in anaesthetized open thorax dogs. The modified analogues included 2-substituted and N^6-substituted derivatives of adenosine, and nucleosides similar to adenosine but having altered sugar moieties or modified imidazole ring systems. The effects of these compounds on coronary blood flow, femoral arterial blood pressure and heart rate were compared with those of adenosine. In

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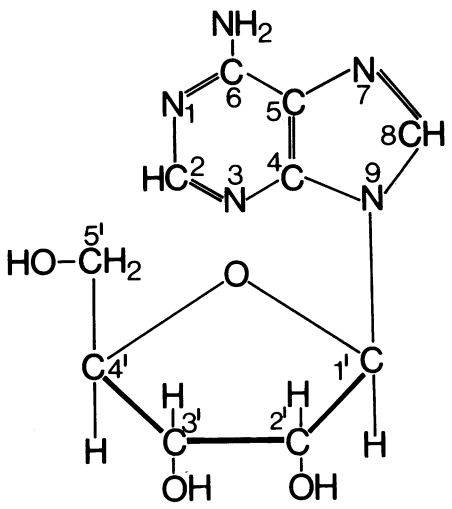

Fig. 1 Structure of adenosine, showing the numbering of the ring systems.

addition, the potentiation of the coronary dilator action of adenosine by several of the analogues and by inosine was investigated.

Adenosine analogues

The numbering of the purine and sugar rings of adenosine is shown in Figure 1. In carbocyclic adenosine (9-[β-DL-2α, 3α-dihydroxy-4-β(hydroxymethyl)cyclopentyl] adenine) a methylene group (CH_2), replaces the ether oxygen of the ribofuranose ring; in tubercidin (4-amino-7-β-D-ribofuranosyl-7*H*-[pyrrolo-2,3-(+)-pyrimidine]) the nitrogen of position 7 of adenosine is replaced by a methine group (CH); in formycin (7-amino-3-β-D-ribofuranosyl-[pyrazolo-4,3-(+)-pyrimidine]) C_8 and N_9 of adenosine are transposed. N^6-(Δ^2-isopentenyl) adenosine is referred to by the abbreviated name, N^6-isopentenyladenosine. 2-Hydroxyadenosine and 3′-deoxyadenosine are also known by their non-generic names (crotonoside and cordycepin, respectively) but the systematic names are used here.

The following nucleosides were gifts from the investigators indicated: 2-fluoro- and 2-bromoadenosines, Dr J.A. Montgomery, and carbocyclic adenosine, Dr Y.F. Shealy, both of the Southern Research Institute; formycin, Dr E. Balis, N^6-hydroxyadenosine and 2-hydroxyadenosine, Dr A. Giner-Sorolla, and 3′-deoxyadenosine, Dr G.B. Brown, all of the Sloan-Kettering Institute for Cancer Research; 8-azaadenosine and 9-β-D-arabinofuranosyladenine, Dr R.K. Robins, ICN Nucleic Acid Research Institute; N^6-isopentenyladenosine, Dr M.P. Rathbone, McMaster University.

2-Trifluoromethyladenosine and 2-trifluoromethyl-N^6-methyladenosine were synthesized as reported (Gough & Maguire, 1965; Gough & Maguire, 1967). 2-Methoxy-, 2-ethoxy-, 2-methylamino-, 2-ethylamino-, 2-*n*-butylthio-, 2-*sec*-butylthio- and 2-isobutylthio- adenosines (Maguire, Nobbs & Einstein, unpublished), and N^6-methyladenosine were synthesized by Mr D.M. Nobbs. 5′-Deoxy-5′-chloroadenosine was prepared by Mr J.C. Middleton (Middleton and Maguire, unpublished). Adenosine was a Fluka product, tubercidin was obtained from Calbiochem and inosine from Sigma.

N^6-Isopentenyladenosine was purified by silica gel chromatography in chloroform : methanol, 75 : 25, and was free of adenosine. All nucleosides were checked for purity by paper chromatography in several solvent systems, and only chromatographically pure samples were used.

Methods

Mongrel dogs of either sex, weighing between 13 and 30 kg, were anaesthetized with sodium pentobarbitone (30 mg/kg i.v.) and supplementary doses were administered as required. The animals were intubated with a cuffed endotracheal tube and respiration was maintained with a Bird Mark 8 respirator. The right femoral artery was cannulated and systemic blood pressure measured with a Statham P23A pressure transducer.

A thoracotomy was performed through the fourth left interspace and the pericardium slit to expose the descending and circumflex branches of the left coronary artery. One cm of one of the arteries was cleared to place a cuff-type electromagnetic flow probe (Goodman, 1969a & b). The QRS complex of the Lead II ECG was used to trigger a Grass cardiotachometer and all measurements were recorded on a Grass Model 7 polygraph. Cannulae were inserted into the left atrial appendage and the femoral vein for the administration of drugs. Drugs were administered in solution in normal saline (0.9% w/v NaCl solution) into the left atrium, unless they were not very soluble and the volume required exceeded 2 ml; these were injected intravenously. No drugs were administered until 30 min had elapsed from the completion of surgery, in order to allow the animals to reach a steady state; a log dose-response relationship for adenosine was then obtained. A range of intra-atrial injections of adenosine was administered and the effects on coronary flow and

other parameters were recorded. Coronary dilator responses to adenosine and the adenosine analogues occurred approximately 15-20 s after administration, and were measured as the peak increases in coronary blood flow, and expressed as a percentage of the control values (regarded as 100%). Each animal received three to five analogues in random order; the dose ranges which were used produced increases in coronary blood flow similar to those elicited by 3.7-37.4 nmol/kg of adenosine. Sufficient time was allowed between injections for all measurements to return to steady state levels. Adenosine and each of the analogues in the chosen dose ranges produced coronary dilator responses which were linearly related to the logarithm of the dose, and all the log dose-response lines were parallel. Potencies of the analogues relative to adenosine were calculated from these lines, and a mean ± standard error was obtained for each drug from the results in five to six animals.

The duration of coronary dilator action of adenosine and the adenosine analogues was measured as the time interval from the initial increase in coronary blood flow until the return to the pre-injection rate of flow. An estimate of the duration of coronary dilator action of each analogue relative to that of adenosine was obtained by comparing the duration of action of equiactive doses of adenosine and the analogues, usually at the 19 nmol/kg level of adenosine. With most of the analogues studied the duration relative to adenosine varied from animal to animal, and is therefore expressed as a range. The influence of inosine, and several adenosine analogues, on the coronary dilator response to adenosine was examined. Control responses to the intravenous administration of 37.4-374 nmol/kg of adenosine were recorded and compared with those obtained after the simultaneous intravenous administration of adenosine and inosine, or the adenosine analogue, in at least three dogs.

Results

With the exception of 9-β-D-arabinofuranosyladenine, all the adenosine analogues produced coronary dilation. The arabino epimer in doses up to 3.7 μmol/kg did not affect coronary blood flow. The coronary dilator potencies of the other adenosine analogues relative to that of adenosine, and the duration of action of doses of each compound compared to that of an equipotent dose of adenosine, are summarized in Table 1. There was great variation in both potency and duration of dilator action amongst the analogues. The most potent coronary dilators were 2-fluoro-, 2-bromo-, 2-isobutylthio- and 5′-deoxy-5′-chloroadenosines, which were more potent than adenosine. 2-Ethylaminoadenosine was equipotent with adenosine, and 2-hydroxyadenosine was almost as potent as the parent compound.

All the other analogues were less potent than adenosine. The three analogues with modifications in the imidazole ring, i.e. tubercidin, formycin and 8-azaadenosine, were very weakly active, as was 2-trifluoromethyl-N^6-methyladenosine. Substitution of the exocyclic amino group of adenosine with either a hydroxy or an isopentenyl radical, resulted in a reduction of dilator potency to 6% of that of adenosine, similar to the reduction in potency which we reported for N^6-methyladenosine (Angus *et al.*, 1971).

The duration of the coronary dilator responses elicited by the analogues varied from transient to prolonged, as indicated in Table 1. Figure 2 shows typical responses of adenosine and two of the potent coronary dilators; 2-fluoroadenosine was relatively short-acting and 2-isobutylthioadenosine was long-acting. Figure 3 compares typical responses of adenosine and three weak coronary dilators. Formycin induced a transient dilation like that of adenosine, N^6-isopentenyladenosine was short-acting, and 2-benzylthioadenosine was long-acting. These illustrations and the results summarized in Table 1 show that there is no correlation between coronary dilator potency and duration of coronary dilator action. At the dose levels used in this study the fall in systemic blood pressure was not usually greater than 25 mmHg and was often too small to be measured accurately. The hypotensive effect was of the same duration as the coronary dilator response.

The negative chronotropic effect of adenosine is known to be associated with doses of adenosine larger than those required to cause coronary vasodilation (Schöndorf, Rummel & Pfleger, 1969). No negative chronotropic effect was observed at the dose levels used for the determination of coronary dilator potencies, but in most animals a transient increase in rate was apparent after administration of the analogues, probably a manifestation of a reflex response to the hypotension induced by the compounds.

Potentiation of the coronary dilator action of adenosine

Inosine (administered intravenously at 3.7 μmol/kg) had no effect on coronary blood flow, but inosine (0.93-3.7 μmol/kg) injected intravenously together with adenosine briefly potentiated both the magnitude and the duration of the adenosine response (Figure 4). N^6-Methyladenosine (35.5-355 nmol/kg) caused only small

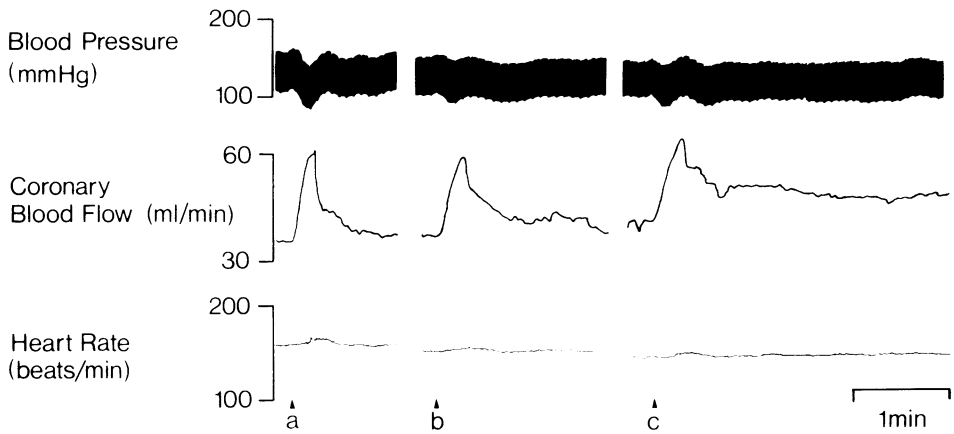

Fig. 2 Effects of intraatrially administered nucleosides on femoral arterial blood pressure, coronary blood flow and heart rate in the anaesthetized open-thorax dog; (a) adenosine (18.5 nmol/kg); (b) 2-fluoroadenosine (1.75 nmol/kg); (c) 2-isobutylthioadenosine (7.05 nmol/kg).

Table 1 Coronary vasodilator properties of adenosine analogues

Compound	*Molar coronary dilator potency (± s.e.)*	*Relative duration of coronary dilator activity**
Adenosine	1	1
2-Fluoroadenosine	5.3 ± 0.6	4
2-Bromoadenosine	2.5 (1.8-3.2)†	5-8
2-Trifluoromethyladenosine	0.05 ± 0.008	10-15
2-Hydroxyadenosine	0.82 ± 0.1	2-4
2-Methoxyadenosine	0.31 ± 0.04	10-15
2-Ethoxyadenosine	0.42 ± 0.07	10-15
2-Aminoadenosine	0.14 ± 0.01	2
2-Methylaminoadenosine	0.07 ± 0.01	7-10
2-Ethylaminoadenosine	1.10 ± 0.15	5-10
2-*n*-Butylthioadenosine	0.27 ± 0.03	5-8
2-Isobutylthioadenosine	2.30 ± 0.05	8
2-*sec*-Butylthioadenosine	0.43 ± 0.015	5-8
2-Benzylthioadenosine	0.09 ± 0.009	5-6
N^6-Isopentenyladenosine	0.06 ± 0.008	2-3
N^6-Hydroxyadenosine	0.06 ± 0.008	1-2
2-Trifluoromethyl-N^6-methyladenosine	0.001	10-15
Tubercidin	0.005	1
Formycin	0.005 ± 0.004	1
8-Azaadenosine	0.003	1
9-β-D-Arabinofuranosyladenine	inactive	
3'-Deoxyadenosine	0.06	2-4
Carbocyclic adenosine	0.23 (0.20-0.27)†	2
5'-Deoxy-5'-chloroadenosine	1.9 ± 0.22	1-2

* Duration is expressed relative to that of an equipotent dose of adenosine; the increase in coronary flow elicited by 18-37 nmol per kg of adenosine lasted for 0.5-1.0 minute.
† Range where less than five estimations were made.

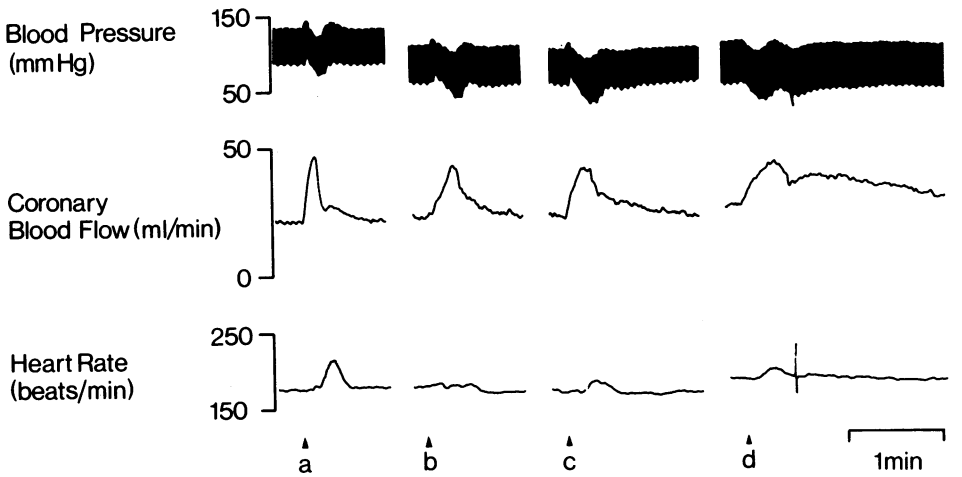

Fig. 3 Effects of intraatrially administered nucleosides on femoral arterial blood pressure, coronary blood flow and heart rate in the anaesthetized open-thorax dog; (a) adenosine (18.5 nmol/kg); (b) formycin (280 nmol/kg); (c) N^6-isopentenyladenosine (298 nmol/kg); (d) 2-benzylthioadenosine (193 nmol/kg).

increases in coronary blood flow, but when administered together with adenosine, caused a transient potentiation of the dilator action of adenosine. Similarly, tubercidin (185-370 nmol/kg) had little effect on coronary flow, but briefly potentiated the action of simultaneous doses of adenosine, as did 9-β-D-arabinofuranosyladenine (185-925 nmol/kg), which had no intrinsic dilator properties. In contrast 2-trifluoromethyl-N^6-methyladenosine (0.29-2.5 μmol/kg), which alone induced little increase in coronary flow, caused a prolonged potentiation of the dilator response of simultaneous doses of adenosine; at high doses of the analogue, potentiation of the adenosine response could be detected up to 10 min after administration.

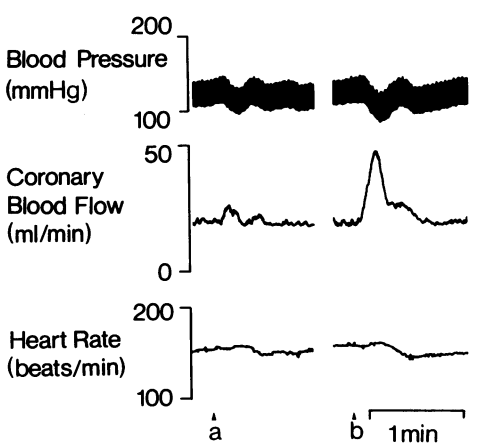

Fig. 4 Potentiation by inosine of the effects of intravenously administered adenosine on femoral arterial blood pressure, coronary blood flow and heart rate in the anaesthetized open-thorax dog; (a) adenosine (93.5 nmol/kg); (b) adenosine (93.5 nmol/kg) plus inosine (3.7 μmol/kg).

Discussion

Earlier studies have shown that a number of 2-substituted, N^6-alkylated and 5′-modified derivatives of adenosine have coronary dilator effects (Jahn, 1965; Jahn, 1969; Angus *et al.*, 1971; Juhran *et al.*, 1971). Our present investigations have revealed that different types of adenosine analogues (in particular those with modified sugar and imidazole ring systems) also have coronary dilator activity, although in some instances this activity is weak. Only one of the four sugar-modified adenosine analogues examined, 9-β-D-arabinofuranosyladenine, was without coronary dilator action. Three 2-substituted adenosine analogues, 2-fluoro-, 2-bromo-, and 2-isobutylthio-adenosines, which have not been screened previously for coronary dilator effects, were found to be more potent coronary dilators than adenosine. 2-Fluoroadenosine was the most potent of these, but was still less potent than 2-chloroadenosine, which was found to be seven times as potent as adenosine (Angus *et al.*, 1971).

Jahn (1965) reported that 5′-deoxy-5′-chloro-

adenosine increased blood flow in the left coronary artery of dogs on infusion into this artery, but not after intravenous injection. We found that this analogue was more than twice as potent as adenosine, and that the dilator response it induced was almost as transient as that of adenosine, indicating that like adenosine it is rapidly rendered inactive in the cardiovascular system. The short duration of action of 5′-deoxy-5′-chloroadenosine could explain why the analogue was ineffective after intravenous administration; this route would require high dose levels to elicit coronary vasodilation.

The results in Table 1 show that there is a wide range of coronary dilator potencies in the analogues studied, together with considerable diversity in the duration of activity. However, there is no correlation between these two properties, even in closely related congeners, e.g. 2-hydroxy- and 2-methoxy- adenosines. Many of the analogues examined in this study are characterized by the prolonged coronary dilator responses which they elicit, but others (listed in Table 2) are relatively short-acting, with a maximum duration of dilator action up to four times that of adenosine; of these, N^6-methyladenosine, formycin, tubercidin, and 8-azaadenosine are as transient in their effects as adenosine itself. Studies in several species have indicated that the brevity of the adenosine response is due to its rapid removal from the vascular system by deamination by plasma adenosine deaminase, and by uptake into the cellular components of blood (van Belle, 1969) and into tissues (Pfleger, Seifen & Schöndorf, 1969; Miyazaki, Hattari & Nakamura, 1970; Kolassa, Pfleger & Träm, 1971; Afonso & O'Brien, 1971), where it is metabolized by phosphorylation by adenosine kinase and ATP, or by deamination by intracellular adenosine deaminase (Lerner & Rubinstein, 1970; Liu & Feinberg, 1971; Maguire, Lukas & Rettie, 1972). It is probable that considerable species differences exist in the relative importance of the two routes of inactivation, i.e. plasma deamination and tissue uptake, and in the relative importance of the various uptake compartments. The diversity in duration of coronary dilator actions of the different analogues in the anaesthetized dog appears to be structure-dependent, and indicates some specificity for the different routes of inactivation.

Van Belle (1969) showed that the level of plasma adenosine deaminase in the dog is low, and that the main route of inactivation of adenosine in canine whole blood *in vitro* is via uptake into platelets. Even so, *in vivo*, some deamination of

Table 2 Comparison of the substrate specificities of adenosine analogues for adenosine deaminase and adenosine kinase with their duration of coronary dilator action.

Analogue	*Duration of coronary dilator activity*	*Relative substrate specificity**			
		Adenosine deaminase	*Source, reference*	*Adenosine kinase*	*Source, reference*
Adenosine	1	+++	placenta *a*	+++	liver *j*
N^6-Methyladenosine	1	+	intestine *b*	+++	liver *j*
Tubercidin	1	–	intestine *c*	+++	liver *j*
Formycin	1	+	intestine *d*	++	liver *j*
8-Azaadenosine	1	+++	intestine *e*	+++	tumor *k*
N^6-Hydroxyadenosine	1-2	++	placenta *a*	+	tumor *k*
5′-Deoxy-5′-chloroadenosine	1-2	+	intestine *f*	–	
Carbocyclic adenosine	2	+	intestine *g*	+	tumor *k*
2-Aminoadenosine	2	++	placenta *a*	+	liver *j*
N^6-Isopentenyladenosine	2-3	+	intestine *h*	+++	sarcoma *l*
3′-Deoxyadenosine	2-4	++	intestine *i*	+	liver *j*
2-Hydroxyadenosine	2-4	+	intestine *h*		
2-Fluoroadenosine	4	+	placenta *a*	++	liver *j*

* The order of substrate specificity of each analogue is expressed as +++, if equal to that of adenosine, or ++, if better than one-tenth of that of adenosine, or +, if less than one-tenth of that of adenosine, and has been estimated from K_m, V_{max} or rate values reported in the references listed for bovine placental or calf intestinal mucosa adenosine deaminase, and rabbit liver, human tumor or mouse sarcoma 180 adenosine kinase.

a, Maguire & Sim, 1971; *b,* Chassey & Suhadolnik, 1967; *c,* Bloch, Leonard & Nichol, 1967; *d,* Sheen, Martin & Parks, 1970; *e,* Simon, Bauer, Tolman & Robins, 1970; *f,* Maguire, 1973; *g,* Bennett, Allan & Hill, 1968; *h,* Maguire, unpublished results; *i,* Corey & Suhadolnik, 1965; *j,* Lindberg, Klenow & Hansen, 1967; *k,* Schnebli, Hill & Bennett, 1967; *l,* Divekar & Hakala, 1971.

adenosine, and those analogues which are good substrates of adenosine deaminase, may be expected to occur as a result of the action of the plasma enzyme.

With one exception, tubercidin, the short-acting dilators are all substrates of adenosine deaminase; their relative substrate specificities for the enzyme are compared in Table 2. 8-Azaadenosine is an excellent substrate, and 3′-deoxyadenosine, N^6-hydroxyl- and 2-amino- adenosines are moderately good substrates; the inactivation of these compounds in the cardiovascular system may be due at least in part to the action of plasma adenosine deaminase. When N^6-hydroxyadenosine was administered i.v. in dogs, it disappeared rapidly from the blood and a concomitant increase in plasma allantoin levels occurred (Philips, personal communication), suggesting that there was some conversion of the analogue to inosine by plasma adenosine deaminase. The transient dilators, formycin and N^6-methyladenosine, are such poor substrates of adenosine deaminase that it is unlikely that they are inactivated to any extent by the enzyme in plasma. Tritiated N^6-methyladenosine has been shown to be taken up by the myocardium on intracoronary infusion in the anaesthetized dog (Olsson, unpublished results), and although there is no similar evidence for the uptake of formycin from the canine vascular system, it seems probable that the rapid tissue uptake of both these nucleosides is the reason for the brevity of their dilator effects, and that they have a high affinity for the carrier mediating adenosine uptake. Studies in which tritiated tubercidin was administered i.v. in dogs showed that the label quickly disappeared from the serum and was found mainly in the lungs, muscle and liver (Smith, Gray, Carlson & Hanze, 1967). It seems probable, therefore, that the transient nature of the coronary dilator response of tubercidin in the anaesthetized dog is due to its rapid uptake by tissues similar to the uptake of adenosine itself. It is likely also that those less transient coronary dilators which are poor substrates of adenosine deaminase (Table 2) are also inactivated by tissue uptake.

Tubercidin and N^6-methyladenosine potentiated the coronary dilator response of adenosine, as did inosine and 2-trifluoromethyl-N^6-methyladenosine at higher dose levels. Inosine is readily taken up by guinea-pig and rat isolated perfused hearts, and by the canine heart *in situ*, and both inosine and N^6-methyladenosine inhibited the uptake of adenosine by these preparations (Kolassa, Pfleger & Rummel, 1970; Maguire & Lukas, unpublished; Olsson, personal communication; Olsson, Snow & Gentry, 1972). Inosine, N^6-methyladenosine and 2-trifluoromethyl-N^6-methyladenosine are inhibitors of adenosine deaminase (Rockwell & Maguire, 1966). The trifluoromethyl-N^6-methyl derivative is a particularly potent inhibitor with a K_i of 2 μM; at the dose levels at which this analogue potentiated the response of adenosine, its concentration in blood (3-24 μM), would have certainly inhibited the action of plasma adenosine deaminase. Unlike inosine or N^6-methyladenosine, 2-trifluoromethyl-N^6-methyladenosine appears to be cleared only very slowly from the vascular system; its longlasting potentiating effect is probably due in part to its inhibiting action on plasma adenosine deaminase. It is conceivable that some of the brief potentiating action of inosine and N^6-methyladenosine may be due to their inhibition of plasma adenosine deaminase. However, as tubercidin, which does not inhibit adenosine deaminase (Corey & Suhadolnik, 1965), potentiated the action of adenosine with similar brevity, it seems probable that interference with the tissue uptake of adenosine is the basis of the potentiating action of tubercidin, inosine, N^6-methyladenosine and 9-β-D-arabinofuranosyladenine, on the adenosine coronary dilator response. These four nucleosides and formycin are all reported to inhibit the transport of adenosine into rabbit polymorphonuclear leucocytes by binding to the adenosine carrier (Taube & Berlin, 1972).

The present studies of the duration of coronary dilator actions of adenosine analogues in anaesthetized dogs, and of the potentiation of the coronary dilator effect of adenosine by certain nucleosides, suggest that the adenosine carrier responsible for adenosine uptake from the canine cardiovascular system will accept analogues in which a hydroxyl replaces the 6-amino group, in which the imidazole ring is modified, and in which the sugar is altered as in 9-β-D-arabinofuranosyladenine. Adenosine analogues with substituents in position 2 larger than fluorine, as in the long-acting dilators, are probably not acceptable.

It has been suggested that carrier-mediated nucleoside transport is related to kinase action (Scholtissek, 1968). Three of the four transient coronary dilators are excellent substrates for adenosine kinase, while the fourth, formycin, is a moderately good substrate (Table 2); however, 5′-deoxy-5′-chloroadenosine, which is only slightly longer-acting, lacks a 5′-hydroxyl and cannot be phosphorylated.

Thus, while the duration of the coronary dilator responses of the analogues listed in Table 2 can be correlated with their apparent specificities for the adenosine receptor mediating uptake, there is no correlation between duration and substrate specificity for adenosine kinase. In addition, inosine, which appears to be transported via the

adenosine carrier (see above) is not a substrate for the adenosine kinase (Schnebli, Hill & Bennett, 1967). These findings indicate that in the anaesthetized dog there is no direct involvement of adenosine kinase in the transport of adenosine and its analogues from the vascular system into tissues.

All the analogues with long-acting coronary dilator properties are inhibitors of adenosine deaminase (Rockwell & Maguire, 1966; Maguire & Sim, 1971; Maguire, 1973). This common property, however, does not appear to be related to their duration of action, which must be due rather to a slow uptake into tissues or slow renal clearance. Few studies have been reported on the excretion of nucleosides which are not substrates of adenosine deaminase, and yet unknown metabolic pathways may be involved, as indicated by the results of the metabolism in man of a very poor substrate, N^6-isopentenyladenosine (Chheda & Mittelman, 1972).

Our studies have shown that a number of adenosine analogues with diverse structural modifications have coronary dilator activity, and that the structural modifications which result in increased dilator potency, i.e. enhanced activity at a putative vascular smooth muscle receptor, are different from the structural modifications which result in rapid clearance of the analogues from the vascular system. The evidence presented suggests that the analogues which are rapidly inactivated are those with affinity for the carrier mediating adenosine uptake into tissues.

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