The control of adenosine concentration in polymorphonuclear leucocytes, cultured heart cells and isolated perfused heart from the rat

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1. Rat polymorphonuclear leucocytes or neonatal-rat heart cells in culture were treated with 2'-deoxycoformycin and 5-iodotubercidin at concentrations that inhibited adenosine deaminase (EC 3.5.4.4) and adenosine kinase (EC 2.7.1.20) inside the intact cells, and the rate of adenosine accumulation was determined. 2. The basal rate of adenosine formation was 2% (polymorphonuclear leucocytes) or 9% (heart cells) of the maximal activity of adenosine kinase also measured in intact cells. 3. Greatly increased rates of adenosine formation were observed during adenine nucleotide catabolism. This condition also led to a decrease in adenosine kinase activity. 4. When isolated rat hearts were perfused with 5-iodotubercidin alone at a concentration which inhibited adenosine kinase, no increase in tissue or perfusate adenosine or inosine concentration was observed. However, perfusion with hypoxic buffer or infusion of adenosine into the coronary circulation at a rate (20 nmol/min) equivalent to 40% of the activity of adenosine kinase caused large increases in effluent perfusate adenosine and inosine concentrations. 5. These data argue unanimously against the existence of a substrate cycle controlling adenosine concentration. 6. They suggest instead that an increase in the rate of adenosine formation is the principal cause of elevations in adenosine concentration during ATP catabolism.

Adenosine functions as a 'local hormone' which communicates the energy status of a cell to its neighbours within a tissue (Berne, 1964; Arch & Newsholme, 1978a). Many animal tissues contain enzymes capable of both synthesizing and degrading adenosine (Murray, 1971; Arch & Newsholme, 1978b). Thus the concentration of the nucleoside could be elevated either by stimulating adenosine formation or by inhibiting its degradation. A provocative hypothesis put forward by Arch & Newsholme (1978a,b) further suggested that a 'substrate cycle' existed between AMP and adenosine, the purpose of which was to amplify the

increase in adenosine concentration resulting from increased AMP hydrolysis (see Scheme 1). Such an amplification can occur, provided that in the basal state (upper values in Scheme 1) the rate of adenosine formation approximates to the maximal rate of adenosine kinase. Little adenosine is thus available for deamination, and the steady-state concentration remains low. An increase in 5'-nucleotidase activity (see the lower values in Scheme 1) cannot be matched by the adenosine kinase, since it is saturated, and hence disproportionately more adenosine becomes available to the deaminase. Since this enzyme has a relatively high K_m (see Arch & Newsholme, 1978a,b), it can only increase its activity after a proportional rise in adenosine concentration. Thus when the new steady state is reached, a 2-fold increase in 5'-nucleotidase activity, for example (Scheme 1), could result in an 11-fold increase in adenosine concentration.

In this study we have quantified the effect of stimulating ATP breakdown on the rate of adenosine formation determined in the absence of its further metabolism (Newby & Holmquist, 1981) and

Abbreviations used: 2'-deoxycoformycin, R-3-(2-deoxy- β -D-erythro-pentofuranosyl)-3,6,7,8-tetrahydro[4,5-d][1,3]diazapin-8-ol; 5-iodotubercidin, 4-amino-5-iodo-7-(β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine; h.p.l.c., high-pressure liquid chromatography.

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Scheme 1. A substrate cycle to control adenosine concentration

The numbers represent hypothetical values for the relative fluxes (in arbitrary units) through the individual steps in the basal state (upper values) and during ATP catabolism (lower values).

its effect on adenosine kinase activity. We have also tested the hypothesis that the basal rate of adenosine formation approximates to the maximal activity of the kinase.

Materials and methods

D-[U-¹⁴C]Sorbitol (9 GBq/mmol) was obtained from Amersham International, Amersham, Bucks., U.K. The sources of other materials have been given previously (Newby, 1980, 1981).

Rat polymorphonuclear leucocytes

Cell preparation and incubation (Newby, 1980), sonication, measurement of adenosine deaminase in the sonicated preparation (Newby, 1980) and the incorporation of [³H]adenosine into nucleotides (Newby, 1981) have all been previously described. Adenosine kinase in the sonicated preparation was measured under conditions found to give maximal activity by Miller *et al.* (1979a). Assay cocktail consisted of (final concns.) 25 mM-sodium phosphate (pH 6.8), 1 mM-ATP, 0.2 mM-MgSO₄, 5 mM-phosphocreatine, 1 μM-erythro-9-(2-hydroxynon-3-yl)-adenine (to inhibit adenosine deaminase; Carson *et al.*, 1977), 0.5 mg of creatine kinase/ml, 5 μM-adenosine and 730 kBq of [2-³H]adenosine/ml. [³H]AMP formation was measured by the filter-disc method (De Jong & Kalman, 1973) as described previously (Newby, 1981).

Neonatal-rat heart cells

Beating cells were prepared from the ventricular portions of hearts from 4–5-day-old rats by the method of Harary & Farley (1963). The cells were 60–70% myocytes as judged by beating and by selective staining (Blondel *et al.*, 1971) and were less than 2% broken as judged by measuring lactate dehydrogenase release into the medium.

Cells (0.5×10^6 – 1.0×10^6 in 1 ml) were pre-incubated for 1 h with the addition of 10 μM-2'-deoxycoformycin to inhibit adenosine deaminase. They were then washed four times with 1 ml of

Krebs–Ringer solution (Cohen, 1957) containing 13 mM-NaHCO₃ and 13 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/NaOH, pH 7.4. Cells were then further incubated in 1 ml of the same buffer supplemented with 1 μM-iodotubercidin. Supernatant buffer (0.9 ml) and the remaining cells were extracted with trichloroacetic acid (final concn. 5%, w/v), and metabolites were measured by h.p.l.c. (Newby, 1980). Cell number was measured by the assay method for DNA of Kissane & Robins (1958).

For the measurement of incorporation of [³H]-adenosine into nucleotides, 0.25×10^6 – 0.5×10^6 cells were incubated for 10 min at 37°C in 0.22 ml of Krebs–Ringer buffer containing 10 μM-adenosine, 0.1 mM-hypoxanthine and 15–19 kBq of [2-³H]-adenosine. Incubations were terminated and nucleotide formation was quantified as described by Newby (1981). For measurement of adenosine kinase activity, cells were lysed in 0.2 ml of 10 mM-Tes (2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)-amino]ethanesulphonic acid)/NaOH, pH 7.0, containing 0.1% Triton X-100, and assayed as for polymorphonuclear leucocytes. Alternatively, adenosine deaminase was measured by adding 20 μl of 0.55 mM-adenosine, 1.1 mM-inosine and 15–18 kBq of [2-³H]adenosine. The mixture was incubated at 37°C for 10 min and then a 70 μl sample was removed and boiled for 3 min. Inosine production was assessed by t.l.c. (Shimizu *et al.*, 1970; Newby, 1981). Lactate dehydrogenase in supernatants and lysates was measured as described by Luzio *et al.* (1976).

Isolated perfused rat hearts

Hearts were obtained from male Wistar rats (250–350 g), paced at 5 beats/s and perfused at a constant flow of 20 ml/min by the Langendorff (1895) method by using apparatus and procedures detailed previously (Illingworth *et al.*, 1975; Illingworth & Mullins, 1976). The perfusing buffer was Krebs–Ringer bicarbonate (1.3 mM-Ca²⁺; Cohen, 1957) supplemented with 1 mM-sodium pyruvate and gassed with O₂/CO₂ (19:1).

Effluent perfusates (approx. 5 ml) were collected into tubes containing 5 μl of 0.1 μM-2'-deoxycoformycin and kept frozen at –20°C. Thawed samples were filtered (Newby, 1980) and 0.2 ml portions subjected to h.p.l.c. Hearts were freeze-clamped, the frozen ventricular tissue was separated, pulverized and extracted with aq. 5% (w/v) HClO₄/20% (v/v) methanol (Williamson & Corkey, 1969). Extracts were neutralized (Khym, 1975) and filtered (Newby, 1980), and 10 μl samples taken for the analysis of nucleotides or 30 μl samples for measurement of adenosine by h.p.l.c.

Incorporation of [³H]adenosine into cellular nucleotides was measured by infusing at 1 ml/min

perfusion buffer supplemented with 21–2100 μM -adenosine, 1 mM-hypoxanthine and 10 kBq of [^3H]adenosine/ml. After 10 min infusion was stopped, but perfusion continued for a further 5 min. The heart was then freeze-clamped, extracted with HClO_4 and the radioactivity in the neutralized extract determined. Over 95% of the radioactive material bound to AG1-X2 quaternary-amine anion exchanger (Bio-Rad Laboratories, Watford, Herts, U.K.), showing it to be present as nucleotides (Newby, 1981).

The distribution of 5-iodotubercidin (1 μM final concn.) or 5'-amino-5'-deoxyadenosine (10 μM final concn.) into cardiac tissue was assessed by infusing the drugs simultaneously with D-[U- ^{14}C]sorbitol (final concn. 100–150 Bq/ml). After 10 min of infusion, hearts were freeze-clamped and neutralized acid extracts produced. The radioactivity in the acid extract and in the influent perfusate was determined and used to calculate the extracellular water space (Morgan *et al.*, 1959). The concentration of 5-iodotubercidin in the neutralized acid extract was determined by loading 1 ml on to a 0.5 ml bed of AG1-X2 quaternary-amine anion exchanger (Cl^- form) followed by 1 ml of water. The drug was eluted with 2 ml of aq. 20% (v/v) acetonitrile. The eluate was freeze-dried, redissolved in 0.3 ml of water and 0.2 ml was subjected to h.p.l.c. (see below). 5'-Amino-5'-deoxyadenosine was determined by loading 1 ml of neutralized extract on to a 0.5 ml bed of AG 50W-X8 sulphanilic acid anion exchanger (Na^+ form) (Bio-Rad Laboratories) followed by 7 ml of water. The drug was eluted with 6 ml of 10 mM- NaOH . The eluate was immediately neutralized with 120 μl of 0.5 M- H_3PO_4 , and 0.2 ml samples were subjected to h.p.l.c. Over 95% of either drug added to a neutralized acid extract was recovered after these procedures. Wet weight and dry weight of the frozen tissue were determined as described previously (Illingworth *et al.*, 1975). Total tissue water was estimated as the difference between these values.

H.p.l.c.

Nucleosides were determined as described previously (Newby & Holmquist, 1981). ATP, ADP, AMP, IMP and hypoxanthine were determined with the buffer system described previously (Newby & Holmquist, 1981), but chromatography was performed at 12°C. This greatly improved the resolution of IMP from ATP. 5'-Amino-5'-deoxyadenosine was measured by its A_{254} during reversed-phase chromatography on a Waters Associates (Hartford, Northwich, Cheshire, U.K.) $\mu\text{Bondapak C}_{18}$ column eluted with 50 mM-Tris/phosphate (pH 7.5)/10% (v/v) methanol. 5-Iodotubercidin was measured by its A_{280} during chromatography on the same column with 50 mM- $(\text{NH}_4)_2\text{HPO}_4$ (pH 7.0)/15% (v/v) acetonitrile.

Results and discussion

Studies with intact polymorphonuclear leucocytes and cultured neonatal heart cells

The maximal activity of adenosine kinase was 155 pmol/min per 10^7 polymorphonuclear leucocytes and 460 pmol/min per 10^7 neonatal heart cells (Table 1). It was important to decide whether these values, determined in homogenates, accurately measured the activity of the enzyme inside the intact cells. This was assessed by measuring the rate of incorporation of [^3H]adenosine into cellular nucleotides. The concentration-dependence of this process (results not shown) indicated that the high-affinity component (see Arch & Newsholme, 1978a) was saturated at 10 μM -adenosine. At this concentration, the rate of incorporation was 182 pmol/min per 10^7 polymorphonuclear leucocytes and 510 pmol/min per 10^7 neonatal heart cells (Table 1). In both cell types, the incorporation of [^3H]adenosine into nucleotides could be inhibited by inhibitors of adenosine kinase. The maximum inhibition obtained was 54% with polymorphonuclear leucocytes (Newby, 1981) and 99% with neonatal heart cells (results not shown). Thus we concluded that the

Table 1. Summary of estimates of the activity of adenosine kinase and the basal and stimulated rates of adenosine production

Measurements were performed in triplicate or quadruplicate by procedures described in the Materials and methods section. ATP catabolism was induced at 37°C in polymorphonuclear leucocytes with 5 mM-2-deoxyglucose and adenosine formation was measured over the first 2 min, or in neonatal heart cells with 30 mM-2-deoxyglucose and 2 μg of oligomycin/ml and adenosine formation measured over the first 5 min. Values are means $\pm$ S.E.M. for the numbers of separate experiments shown in parentheses.

Process	Reaction velocity (pmol/min per 10^7 cells)	
	Polymorpho- nuclear leucocytes	Neonatal heart cells
Homogenate adenosine kinase	155 $\pm$ 20* (5)	460 $\pm$ 60 (7)
Incorporation of 10 μM -adenosine into nucleotides	182 $\pm$ 13* (12)	510 $\pm$ 90 (14)
Incorporation of 10 μM -adenosine inhibited by 10 μM -5-iodotubercidin	98 (54%)*	505 (99%)
Adenosine accumulation in the basal state	1.9 $\pm$ 0.3 (8)	47 $\pm$ 6 (14)
Adenosine accumulation during ATP catabolism	141 $\pm$ 18 (8)	330 $\pm$ 60 (5)

* Values taken from Newby (1981).

Fig. 1. Time course of adenosine accumulation in polymorphonuclear leucocytes and neonatal-rat heart cells

(a) Polymorphonuclear leucocytes were incubated in triplicate at 37°C for the times indicated with 30 μM-2'-deoxycoformycin together with 1 μM-5-iodotubercidin (○), or with 50 μM-5'-amino-5'-deoxyadenosine (●), or without either inhibitor (■). Values are means ± S.E.M. for one of eight similar experiments. (b) Neonatal-rat heart cells, either 1.27×10^6 in 2 ml (●) or 2.56×10^6 in 2 ml (○), were preincubated in duplicate with 10 μM-2'-deoxycoformycin for 1 h at 37°C, washed three times, and then incubated in buffer supplemented with 1 μM-5-iodotubercidin. Adenosine concentrations in the cells (■) or in the supernatant (●, ○) were then determined as described in the Materials and methods section. Values are means with ranges for two examples of 14 similar experiments.

activity of adenosine kinase inside the intact cells was 98 pmol/min per 10^7 polymorphonuclear leucocytes and 505 pmol/min per 10^7 neonatal heart cells (Table 1). These activities were close to the values for the homogenate.

To determine the basal rate of adenosine forma-

tion, cells were first preincubated with 2'-deoxycoformycin to inhibit adenosine deaminase (Newby, 1980, 1981). Incubation was then continued in the presence of 5'-iodotubercidin or 5'-amino-5'-deoxyadenosine to inhibit adenosine kinase. Cells so treated experienced approximately linear increases in adenosine concentration (Fig. 1). The rate of adenosine formation was 1.9 pmol/min per 10^7 polymorphonuclear leucocytes (Table 1), which was approx. 2% of the activity of adenosine kinase. Adenosine formation in cultured neonatal heart cells proceeded at a rate of 47 pmol/min per 10^7 cells (Table 1), which was approx. 9% of the activity of adenosine kinase.

The rate of adenosine formation could be dramatically increased during ATP catabolism. Challenging polymorphonuclear leucocytes with 2-deoxyglucose (Newby & Holmquist, 1981) led to a 70-fold activation, to 140 pmol/min per 10^7 cells (Table 1). Adenosine formation in neonatal heart cells was increased 7-fold in the presence of 2-deoxyglucose and oligomycin (Seraydarian *et al.*, 1969), to 330 ± 60 pmol/min per 10^7 cells (Table 1).

The rate of incorporation of 10 μM-adenosine into nucleotides was diminished during ATP catabolism. In polymorphonuclear leucocytes a 13–27% inhibition and in heart cells a 61–84% inhibition (ranges from three experiments) occurred when ATP concentration was decreased by 40%.

Further inhibition occurred at lower ATP concentrations (results not shown). These inhibitions could not be explained by isotopic dilution, since the concentration of endogenous adenosine produced was less than 0.1 μM.

Studies with the isolated perfused rat heart

It was not possible to observe accumulation of adenosine in either polymorphonuclear leucocytes or neonatal heart cells in culture without the use of combinations of several inhibitors. Although the inhibitors used did not affect 5'-nucleotides assayed *in vitro* (Newby, 1980, 1981; Worku & Newby, 1983), inhibitors of adenosine deaminase in particular influence many other aspects of nucleoside metabolism (Henderson *et al.*, 1977; Rogler-Brown & Parks, 1980; Plagemann & Wohlheuter, 1981; Newby, 1981). With the isolated perfused rat heart it was possible to show net formation of adenosine without using inhibiting drugs. The strategy was therefore firstly to determine whether inhibition of adenosine kinase alone could result in increases in tissue and perfusate nucleoside concentrations similar to those seen during ATP catabolism caused by hypoxic perfusion. Secondly, if one makes the assumption that, in the normoxic state, the rate of adenosine formation approximates to the activity of adenosine kinase, then inhibition of the enzyme should lead to net formation of adenosine at this rate.

Table 2. *Heart tissue nucleotide, adenosine and inosine concentrations*

Hearts were perfused for 20–30 min with normoxic buffer. They were then infused for 10 min with normoxic buffer with or without 5-iodotubercidin or 5'-amino-5'-deoxyadenosine. Alternatively they were switched over to perfusion with hypoxic buffer for 10 min. Nucleotide and nucleoside concentrations were determined as described in the Material and methods section. Values are the means $\pm$ S.E.M. for the numbers of hearts shown in parentheses. Abbreviation: N.D., not determined.

Perfusion buffer	Infusion	Metabolite concn. (μ mol/g dry wt.)					
		ATP	ADP	AMP	IMP	Inosine	Adenosine
Krebs–Ringer bi-carbonate equilibrated with O ₂ /CO ₂ (19:1) (normoxic buffer)	None	18.0 $\pm$ 0.3 (8)	3.0 $\pm$ 0.3 (8)	0.42 $\pm$ 0.05 (8)	0.059 $\pm$ 0.009 (7)	0.32 $\pm$ 0.02 (5)	0.047 $\pm$ 0.009 (8)
	5-Iodotubercidin (1 μ M)	18.9 $\pm$ 0.5 (7)	3.1 $\pm$ 0.3 (7)	0.33 $\pm$ 0.03 (7)	0.056 $\pm$ 0.007 (6)	0.31 $\pm$ 0.01 (3)	0.043 $\pm$ 0.007 (7)
	5'-Amino-5'-deoxyadenosine (10 μ M)	16.8 $\pm$ 0.6 (5)	2.1 $\pm$ 0.1 (5)	0.34 $\pm$ 0.01 (5)	0.028 $\pm$ 0.003 (5)	0.21 $\pm$ 0.01 (5)	0.034 $\pm$ 0.006 (5)
Krebs–Ringer bi-carbonate equilibrated air/CO ₂ (19:1) (hypoxic buffer)	None	11.7 $\pm$ 0.8 (6)	4.7 $\pm$ 0.3 (6)	2.3 $\pm$ 0.2 (6)	1.4 $\pm$ 0.3 (6)	N.D.	0.18 $\pm$ 0.04 (6)

To simulate the effect of such a net rate of adenosine formation on effluent perfusate nucleoside concentrations, we infused adenosine into the coronary circulation at an equivalent rate.

The adenosine kinase activity in a homogenate of rat heart was 60 $\pm$ 5 nmol/min per g wet wt. (n = 5). The rate of incorporation of [³H]adenosine into cellular nucleotides was maximal at 30 μ M and then declined, possibly owing to high-substrate inhibition of the adenosine kinase (Miller *et al.*, 1979a). The maximal rate of adenosine incorporation (33 nmol/min per g wet wt.) was approx. 50% of the maximal activity of adenosine kinase and was equivalent to approx. 50 nmol/min per 1.5 g heart.

The incorporation of 10 μ M-adenosine into heart nucleotides was inhibited by 90% by 1 μ M-5-iodotubercidin, a similar potency to that seen with adenosine kinase in homogenates (Newby, 1981). The drug penetrated throughout the heart, occupying 143 $\pm$ 6% (n = 9) of the total water space. Its accumulation within the tissue may be explained by its lipid-solubility (A. C. Newby, unpublished work). 5'-Amino-5'-deoxyadenosine inhibited the incorporation of adenosine into heart nucleosides only 54% at 10 μ M, although it produced 50% inhibition of the homogenate enzyme at 50 nM (Miller *et al.*, 1979b; Newby, 1981). This may be explained by poor penetration of the inhibitor into the tissue, since it occupied only 68 $\pm$ 1% (n = 5) of the total water space, which was close to the extracellular D-sorbitol space (66 $\pm$ 2%; n = 17).

Inhibition of adenosine kinase had no effect on tissue nucleotide, adenosine or inosine concentrations (Table 2). Neither was there any significant increase in effluent perfusate adenosine or inosine

concentrations (Table 3). Large changes in these parameters were produced on perfusion with hypoxic buffer (Tables 2 and 3). This demonstrated that inhibition of adenosine kinase, without a simultaneous increase in the rate of adenosine formation, could not account for the increase in nucleoside concentrations seen on hypoxic perfusion.

The failure to observe an increase in steady-state concentrations of nucleosides or in the rate of nucleoside release on inhibition of adenosine kinase suggested that the basal rate of adenosine synthesis was much less than the maximal activity of adenosine kinase. However, a positive control was needed to demonstrate that a net rate of adenosine synthesis similar in magnitude to the activity of the kinase would lead to a measurable change in perfusate nucleoside concentrations. To simulate such a net rate of adenosine formation, adenosine was infused into the coronary circulation at 20 nmol/min (final concn. 1 μ M). This was calculated to be equivalent to 40% of the maximal rate of incorporation of adenosine into nucleotides and 20% of the maximal activity of adenosine kinase. Increases in effluent perfusate adenosine and inosine concentrations were readily observed (Table 3).

Hypoxic perfusion of isolated rat hearts for 10 min diminished ATP concentration to 65% of its control value (Table 2). This did not result in a significant decrease in the rate of incorporation of 10 μ M-adenosine into heart nucleotides.

Effect of adenosine kinase inhibitors on coronary vasodilation

Perfused hearts display an easily quantified physiological response, coronary vasodilation. This

Table 3. *Increments in adenosine and inosine concentration in heart perfusates*

Hearts were perfused at 20 ml/min with normoxic buffer for 20–30 min. Then either normoxic buffer or the same buffer supplemented with adenosine or 5-iodotubercidin was infused. Alternatively hearts were switched over to perfusion with hypoxic buffer. Samples of effluent perfusate were taken for 15 s immediately before infusion or hypoxic perfusion was begun and for 15 s after the treatments had been effected for 5 min and 10 min respectively. The absolute concentrations of nucleosides in normoxic-effluent perfusates were for adenosine 65 ± 9 nM ($n = 17$) and for inosine 78 ± 10 nM ($n = 17$). To assess the effect of the various treatments, the concentrations of nucleosides in each perfusate were compared with the values obtained before commencement of that treatment. Each heart thus served as its own control. Values shown are the differences in concentration before and after treatment began. All values are the means $\pm$ S.E.M. for the numbers of observations shown in parentheses.

Perfusion buffer	Infusion	Time (min)	Change in nucleoside concn. (nM)	
			Inosine	Adenosine
Krebs–Ringer bicarbonate equilibrated with O ₂ /CO ₂ (19:1) (normoxic buffer)	Buffer	5	22 $\pm$ 20 (3)	7 $\pm$ 7 (3)
	Buffer	10	9 $\pm$ 2 (3)	26 $\pm$ 30 (3)
	5-Iodotubercidin (1 μ M)	5	15 $\pm$ 13 (5)	14 $\pm$ 13 (5)
	5-Iodotubercidin (1 μ M)	10	0 $\pm$ 5 (5)	2 $\pm$ 14 (5)
	Adenosine (1 μ M)	5	270 $\pm$ 20 (6)	350 $\pm$ 80 (6)
	Adenosine (1 μ M)	10	250 $\pm$ 40 (6)	340 $\pm$ 100 (6)
Krebs–Ringer bicarbonate equilibrated with air/CO ₂ (19:1) (hypoxic buffer)	None	5	1200 $\pm$ 90 (8)	990 $\pm$ 130 (8)
	None	10	1060 $\pm$ 100 (8)	660 $\pm$ 140 (6)

is at least in part mediated by an increase in adenosine concentration (Berne, 1980). The aortic pressure of 56 normoxically perfused hearts was 104 ± 19 (mean $\pm$ S.D.) mmHg. This group excluded those that did not dilate (as judged by a minimum 25% fall in aortic pressure) on infusion of 1 μ M-adenosine. The dilation observed on adenosine infusion in this group averaged $34 \pm 7\%$ ($n = 56$). Hearts infused with 1 μ M-5-iodotubercidin dilated $34 \pm 7\%$ ($n = 7$), which was 87% of the dilation observed with adenosine in the same hearts. Hearts infused with 10 μ M-5'-amino-5'-deoxyadenosine dilated $26 \pm 6\%$ ($n = 5$), which was 77% of the dilation observed with adenosine in the same hearts. Thus both kinase inhibitors caused vasodilation to approximately the same extent as adenosine.

In view of the different chemical structures of 5'-amino-5'-deoxyadenosine and 5-iodotubercidin, it is unlikely that both agents could be directly vasoactive by binding to either an A₁ or an A₂ adenosine receptor (Wolff *et al.*, 1981). Vasodilation can be elicited by inhibitors of adenosine transport. However, neither kinase inhibitor interferes with this process at the concentrations used (Newby, 1981). Alternatively, these agents may have caused a sufficient rise in adenosine concentration in the region of the coronary resistance vessels. Since these only represent a small proportion of the total tissue mass and are distal to the majority of the coronary circulation, no overall change in adenosine concentration might have been observed.

Control of adenosine concentration

The ability to measure the rate of adenosine

formation in the absence of its further metabolism (Newby & Holmquist, 1981) has allowed us to quantify precisely and unequivocally the variation in this process in response to ATP catabolism. Large increases were observed in both polymorphonuclear leucocytes and cultured neonatal heart cells. Acceleration of adenosine formation was also implicated as the determinant of elevated adenosine concentration in the hypoxically perfused heart. Adenosine production may be regulated by the cytosolic 5'-nucleotidase (Worku & Newby, 1983).

Results from all three experimental models demonstrate that the basal rate of adenosine formation is a small fraction of the maximal activity of adenosine kinase. Thus inhibiting adenosine kinase alone without simultaneously stimulating adenosine formation would be an ineffective means, physiologically, of elevating adenosine concentration. However, it also implies that the presence of adenosine kinase would tend to diminish the rise in adenosine concentration consequent on an acceleration of adenosine formation. It was therefore noteworthy that ATP catabolism resulted in an inhibition of adenosine kinase in the isolated-cell preparations (cf. Lomax & Henderson, 1973) as well as an activation of nucleoside formation.

The concept of 'substrate cycling' (Newsholme & Start, 1973; Newsholme & Crabtree, 1976) does not simply imply formation of adenosine from AMP and rephosphorylation occurring simultaneously. To achieve a more sensitive regulation of adenosine concentration through this mechanism, the basal rate of adenosine production must be close to the maximal activity of adenosine kinase (see the introduction). Thus the participation of adenosine

kinase in a substrate cycle is ruled out by the present work, at least for the models studied.

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