Adenosine formation and release from neonatal-rat heart cells in culture

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1. The incorporation of [^3H]adenosine ($10\ \mu\text{M}$) into neonatal-rat heart cell nucleotides was inhibited in a concentration-dependent manner, such that 50% inhibition was obtained with $0.75\ \mu\text{M}$ -dipyridamole, $0.26\ \mu\text{M}$ -hexobendine or $0.22\ \mu\text{M}$ -dilazep. 2. Adenosine formation was accelerated 2.5-fold to $2.1 \pm 0.3\ \text{nmol}/10^7$ cells in 10 min when cells were incubated with a combination of $30\ \text{mM}$ -2-deoxyglucose and $2\ \mu\text{g}$ of oligomycin/ml. 3. Of the newly formed adenosine, $6 \pm 2\%$ was in the cells. 4. Dipyridamole, hexobendine or dilazep ($10\ \mu\text{M}$) increased the amount of adenosine in the cells and decreased that in the medium such that 45–50% of the newly formed adenosine was in the cells. 5. Antibodies which inhibited ecto-5'-nucleotidase by $98.7 \pm 0.3\%$ did not alter the rate of adenosine formation or its distribution between cells and medium. 6. We conclude that adenosine was formed in the cytoplasm during catabolism of cellular ATP and was released via the dipyridamole-sensitive symmetric nucleoside transporter.

Adenosine increases coronary blood flow (Drury & Szent-Györgyi, 1929; Berne, 1963), antagonizes the positive inotropic effect of catecholamines both pre- (Hedqvist & Fredholm, 1979; Lokhandwala, 1979) and post-synaptically (Schrader *et al.*, 1977; Dobson, 1978, 1983; Endoh & Yamashita, 1980) and decreases heart rate (James, 1965). These actions tend to increase substrate and oxygen availability and to decrease myocardial energy consumption. It has been suggested that adenosine functions in heart and other tissues as a 'retaliatory metabolite' to match energy demands to the availability of cellular ATP (Newby, 1984). This demands a direct biochemical link between myocardial ATP breakdown and release of adenosine so that it may act locally on smooth-muscle cells, neighbouring myocytes and nerve terminals. These studies were designed to test the three hypotheses that have been advanced to explain adenosine release from the heart: (1) adenosine is formed in the cytoplasm and then transported out (Schütz *et al.*, 1981); (2) AMP or other nucleotides are released into the extracellular space and then degraded by ecto-nucleotidases (Paddle & Burnstock, 1974; Forrester & Williams, 1977; Pearson *et al.*, 1980); (3) adenosine is formed at the cytoplasmic face of the ecto-5'-nucleotidase and is then translocated by the enzyme to the

external face (Newby, 1975; Arch & Newsholme, 1978; Frick & Lowenstein, 1978).

In studying adenosine formation and release from heart tissues, cultured cells offer advantages of intactness and ease of experimental replication. Their compartmental simplicity allows agents such as antibodies to penetrate to all available sites of action. Moreover, released nucleotides and adenosine cannot be trapped in an interstitial compartment (Degenring *et al.*, 1976; Knabb *et al.*, 1984). The role of the well-characterized symmetric nucleoside transporter (Plagemann & Wohlheuter, 1980; Paterson *et al.*, 1981) was tested by using the selective transport inhibitors dipyridamole, hexobendine and dilazep. The participation of ecto-5'-nucleotidase (EC 3.1.3.5) was assessed by using inhibitory antibodies.

Materials and methods

Dipyridamole {2,2',2'',2'''-(4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine-2,6-diyl)dinitrilo}tetraethanol was obtained from Boehringer Ingelheim, Bracknell, Berks, U.K. Dilazep {1,4-bis-[3-(3,4,5-trimethoxybenzoyloxy)propyl]perhydro-1,4-diazepine} and hexobendine {*NN'*-dimethyl-*NN'*-bis-[3-(3',4',5'-trimethoxybenzoyloxy)propyl]ethylene-diamine} were generously given by Asta-Werke, Bielefeld, Germany, and Chemie Linzag, Linz, Austria, respectively. Scintillant (cocktail T) was

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obtained from BDH, Poole, Dorset, U.K. Antisera were raised in sheep to rat liver 5'-nucleotidase immunoaffinity-purified to apparent homogeneity as described by Bailyes *et al.* (1982). The sources of other materials have been given previously (Newby, 1981).

Neonatal-rat heart cells

Cell cultures, consisting of 60–70% myocytes, were prepared from the ventricles of 4–5-day-old rats by the method of Harary & Farley (1963) as described previously (Newby *et al.*, 1983) either in 6-well plates (surface area 960 mm²/well) or in 24-well plates (surface area 200 mm²/well).

Incorporation of adenosine into nucleotides

Incorporation of [³H]adenosine (10 μM) into nucleotides was measured in the absence and presence of adenosine-transport inhibitors by the method described previously (Newby *et al.*, 1983). Cells ($3.98 \times 10^5 \pm 0.64 \times 10^5$ per well in 24-well plates) were preincubated at 37°C with the nucleoside-transport inhibitors dipyrindamole, hexobendine or dilazep (10 nM–0.1 mM) for at least 30 min before their effects were investigated.

Adenosine formation

Adenosine formation was measured as described previously (Newby *et al.*, 1983) in the presence of 10 μM-2'-deoxycoformycin, which inhibited adenosine deaminase (EC 3.5.4.4) by $99 \pm 1\%$, 1 μM-5-iodotubercidin, which inhibited adenosine kinase (EC 2.7.1.20) by $95 \pm 2\%$, and 10 μM-allopurinol, which inhibited xanthine oxidase (EC 1.2.3.2) by $98 \pm 2\%$. These inhibitory potencies were determined on the enzymes inside intact cells, as detailed previously (Newby, 1980, 1981). Incubations were stopped by removing 0.9 ml of the supernatant buffer from the wells and adding this to 0.1 ml of 50% (w/v) trichloroacetic acid. After aspirating as much as possible of the remaining buffer, the cells were extracted with 1 ml of 5% trichloroacetic acid. Nucleoside and nucleotide concentrations were measured by h.p.l.c. as described previously (Newby & Holmquist, 1981; Newby *et al.*, 1983).

Inhibition of ecto-5'-nucleotidase and measurement of enzyme activity

Cells ($2.62 \times 10^6 \pm 0.14 \times 10^6$ in 6-well plates) were washed with 4×1 ml of modified Krebs–Ringer bicarbonate buffer (Cohen, 1957) containing 13 mM-NaHCO₃ and 13 mM-Hepes/NaOH, pH 7.4. The cells were then incubated at 37°C in 1 ml of the same buffer, to which 10 μl of sheep anti-(rat liver ecto-5'-nucleotidase) serum was added in some cases, for at least 30 min. ATP catabolism was then induced and metabolites were

measured as described above. Alternatively, ecto-5'-nucleotidase activity was measured by a slight modification of the method of Newby *et al.* (1975). The medium was replaced with 0.5 ml of fresh buffer, and the reaction was initiated by the addition of 50 μl containing 15 mM-adenosine, 103 mM-sodium β-glycerophosphate, 2 mM-AMP and [³H]AMP (0.9 kBq). The mixture was incubated at 37°C for 5 min. The reaction was terminated by removing 0.5 ml of the medium and adding it to 0.3 ml of 0.15 M-ZnSO₄ (Avruch & Wallach, 1971). AMP was precipitated by the addition of 0.2 ml of 0.15 M-Ba(OH)₂. Supernatants (0.5 ml) were added to 5 ml of scintillant and the radioactivity was measured.

Homogenates were prepared by transferring the cells and buffer to conical plastic test tubes and then sonicating for 5 s with a MSE sonicator (model no. P.G. 100).

Cell numbers

Cell numbers were estimated by the measurement of DNA by the assay method of Kissane & Robins (1958).

Cell intactness

The intactness of cells incubated in buffer with or without nucleoside-transport inhibitors or anti-(rat liver ecto-5'-nucleotidase) serum was judged by measurement of cytosolic lactate dehydrogenase (EC 1.1.1.27) in aspirated supernatants as described by Keiding *et al.* (1974). Values were compared with the activity present in cells lysed with 10 mM-Tes/NaOH (pH 7.0)/0.1% Triton X-100.

Extracellular water space

The cross-contamination of the cell fraction with supernatant medium was assessed by incubating cells ($2.36 \times 10^6 \pm 0.40 \times 10^6$ in 6-well plates) with 1 ml of buffer containing 50 kBq of inulin[¹⁴C]-carboxylic acid/ml for 2 or 5 min at 37°C. After removal of as much as possible of the supernatant medium, 1 ml of buffer supplemented with 0.1% Triton X-100 was added and the lysate transferred to a scintillation vial. The well was washed with a further 1 ml of Triton-containing buffer, and the washing was also added to the same scintillation vial. Scintillant (18 ml) was added and radioactivity determined.

Statistical methods

Means $\pm$ S.E.M. are given for the numbers of observations in parentheses. Differences between groups were tested for significance by Student's paired *t* test. A probability level of $P < 0.05$ was considered significant.

Results

Adenosine incorporation into cellular nucleotides

The rate of incorporation of 10 μM-adenosine into nucleotides was 448 ± 55 pmol/min per 10⁷ cells (n = 15). All three transport inhibitors tested

inhibited adenosine incorporation in a concentration-dependent manner (Fig. 1). Incorporation of adenosine (10 μM) into nucleotides was inhibited by 50% in the presence of 0.75 μM-dipyridamole, 0.26 μM-hexobendine or 0.22 μM-dilazep. Cell intactness was 99.6 ± 0.2% in control cells, 99.1 ± 0.5% (n = 6) in the presence of 100 μM-dipyridamole, 98.0 ± 1.0% (n = 5) in the presence of 100 μM-hexobendine and 98.5 ± 0.8% (n = 4) in the presence of 100 μM-dilazep.

ATP breakdown and adenosine formation

Incubations were routinely separated into medium and a cell-containing fraction with a small remnant of surrounding medium (see below). Nucleotides were found exclusively in cell-containing fractions (results not shown), whereas adenosine and hypoxanthine were found in both medium and cell-containing fractions (Table 1).

When cells were incubated with 2'-deoxycoformycin and 5-iodotubercidin, there was no change in cellular ATP concentration (Table 1). Adenosine accumulated linearly (cf. Newby *et al.*, 1983) at a rate of 0.84 ± 0.10 nmol/10⁷ cells in 10 min (Table 1). Of the newly formed adenosine, 6.7 ± 2.3% was present in the cell-containing fraction.

Addition of 2-deoxyglucose (30 mM) and oligomycin (2 μg/ml) induced a 56 ± 11% fall in the ATP concentration in the neonatal heart cells in 10 min (Table 1). This was accompanied by an increase in hypoxanthine accumulation, from an undetectable value to 4.5 ± 0.8 nmol/10⁷ cells in 10 min (Table 1). The rate of adenosine formation increased 1.9–3.3-

Fig. 1. Inhibition of incorporation of adenosine into cellular nucleotides

Cells (3.98 × 10⁵ ± 0.64 × 10⁵) were preincubated for at least 30 min with different concentrations of dipyridamole (●), hexobendine (■) or dilazep (▲). Incorporation of [³H]adenosine (10 μM) into cellular nucleotides was measured in triplicate as described in the Materials and methods section. Each point is the mean ± S.E.M. from at least three different experiments. Values for the rate of incorporation of adenosine are expressed as a percentage of that obtained in the absence of inhibitor.

Table 1. Effect of adenosine transport inhibitors on the concentrations of nucleotides and nucleosides in cells and medium during ATP catabolism

Cells (2.43 × 10⁶ ± 0.18 × 10⁶) were incubated at 37°C for 0 or 10 min in Krebs–Ringer bicarbonate/Hepes solution to which was added 30 mM-2-deoxyglucose and 2 μg of oligomycin/ml, and 10 μM-dipyridamole, 10 μM-hexobendine or 10 μM-dilazep as indicated. 2'-Deoxycoformycin (10 μM), 5-iodotubercidin (1 μM) and allopurinol (10 μM) were present in all cases. Metabolites were measured in duplicate. The values given are means ± S.E.M. for six different preparations.

Time ...		0min	10min				
Metabolites (nmol/10 ⁷ cells)		Control	Control	Deoxyglucose + oligomycin	Deoxyglucose + oligomycin + dipyridamole	Deoxyglucose + oligomycin + hexobendine	Deoxyglucose + oligomycin + dilazep
Cells	ATP	22.64 ± 3.42	24.26 ± 2.90	10.59 ± 2.91	10.73 ± 2.40	6.28 ± 1.04	6.36 ± 0.87
	ADP	7.38 ± 1.41	5.85 ± 1.33	5.21 ± 0.84	5.17 ± 0.85	4.35 ± 0.24	4.74 ± 0.33
	AMP	0.86 ± 0.08	0.73 ± 0.26	2.65 ± 0.45	2.21 ± 1.08	1.94 ± 0.72	2.27 ± 0.78
	IMP	0.64 ± 0.25	0.96 ± 0.52	3.80 ± 2.66	3.64 ± 2.30	3.97 ± 2.41	4.21 ± 2.74
	Adenosine	0.11 ± 0.02	0.20 ± 0.02	0.31 ± 0.04	0.70 ± 0.10	0.66 ± 0.04	0.78 ± 0.05
	Hypoxanthine	2.30 ± 0.33	2.09 ± 0.42	2.07 ± 0.27	1.99 ± 0.33	1.75 ± 0.31	1.86 ± 0.36
Medium	Adenosine	0.08 ± 0.02	0.87 ± 0.10	2.03 ± 0.26	0.70 ± 0.11	0.67 ± 0.09	0.71 ± 0.11
	Hypoxanthine	0.13 ± 0.04	0.36 ± 0.06	5.09 ± 0.84	9.34 ± 0.95	9.35 ± 0.75	8.67 ± 0.73
	Total nucleotide and nucleoside concn.	34.15 ± 3.11	35.31 ± 3.95	31.75 ± 4.06	34.47 ± 3.48	28.97 ± 3.46	29.61 ± 3.58

fold, to 2.1 ± 0.3 nmol/ 10^7 cells in 10 min (Table 1). Approx 39% of the decrease in ATP was accounted for by hypoxanthine, 18% by adenosine, 15% by AMP and 26% by IMP (Table 1). Of the newly formed adenosine, $6.0 \pm 1.8\%$ was present in the cell-containing fraction (Table 1).

Fig. 2. Influence of nucleoside-transport inhibitors on ATP breakdown

Cells were incubated at 37°C for 0 (a) or 10 min (b–f) as described in the legend to Table 1. Abbreviations: DOG, 2-deoxyglucose; Oligo, oligomycin; Dipyrid., dipyridamole; Hexo., hexobendine. The cellular ATP concentrations in control cells at 10 min were compared with values obtained under the other conditions: ns, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 3. Influence of adenosine-transport inhibitors on hypoxanthine formation and release

Cells were incubated at 37°C for 0 (a) and 10 min (b–f) as described in the legend to Table 1. For abbreviations see Fig. 2 legend. Values in the supernatant were compared with those for the supernatant from control cells at 10 min (b) (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) or with the values for the supernatant from cells inhibited for 10 min in the presence of 2-deoxyglucose and oligomycin (c) (ns = not significant, † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$).

Incubation with 2-deoxyglucose and oligomycin in the additional presence of hexobendine or dilazep ($10\mu\text{M}$), but not dipyridamole ($10\mu\text{M}$), resulted in a further fall in cellular ATP concentration but this was not statistically significant (Fig. 2). Transport inhibitors increased the rate of hypoxanthine formation by 1.7–1.9-fold during ATP catabolism (Table 1, Fig. 3) and decreased the rate of accumulation of adenosine by 40–50% (Table 1, Fig. 4). This may be explained by residual activity of adenosine deaminase on the nucleoside trapped within the cells (see below). Hypoxanthine release was not inhibited by dipyridamole, hexobendine or dilazep (Fig. 3), consistent with its mediation by a separate transporter (Plagemann & Wohlhueter, 1980). Adenosine release was de-

Fig. 4. Influence of nucleoside-transport inhibitors on adenosine formation and release

Cells were incubated at 37°C for 0 (a) and 10 min (b–f) as described in the legend to Table 1. For abbreviations, see Fig. 2 legend. Values in the supernatants (a) and in the cells (b) were compared with those for control cells at 10 min (b) (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) or with the values for cell inhibited for 10 min in the presence of 2-deoxyglucose and oligomycin (c) (ns = not significant, † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$).

creased by $66 \pm 3\%$ by $10 \mu\text{M}$ -dipyridamole, by $66 \pm 3\%$ by $10 \mu\text{M}$ -hexobendine and by $63 \pm 6\%$ by $10 \mu\text{M}$ -dilazep (Fig. 4a). In each case inhibition of adenosine release was accompanied by an accumulation of adenosine in the cell-containing fraction (Fig. 4b). Dipyridamole caused retention of $47.4 \pm 6.7\%$, hexobendine $47.7 \pm 2.5\%$, and dilazep $51.5 \pm 3.0\%$ of the newly formed adenosine. Cells remained $99.6 \pm 0.2\%$ intact in the presence of dipyridamole, hexobendine and dilazep ($10 \mu\text{M}$).

Extracellular water space

To assess contamination of the cell fraction with medium, the extracellular water space was determined with inulin [^{14}C]carboxylic acid. A value of $53 \pm 5 \mu\text{l/ml}$ was obtained from three separate experiments. This value can be used to correct the adenosine concentration in the cells. For instance, after 10 min incubation with 2-deoxyglucose and oligomycin, the adenosine concentration in the cells should be corrected from 311 ± 36 to $203 \pm 36 \text{ pmol}/10^7$ cells. In the additional presence of dipyridamole, the corrected adenosine concentration increased to $667 \pm 100 \text{ pmol}/10^7$ cells, in the

presence of hexobendine to $623 \pm 36 \text{ pmol}/10^7$ cells and in the presence of dilazep to $747 \pm 42 \text{ pmol}/10^7$ cells.

Inhibition of ecto-5'-nucleotidase

AMP added to intact heart cells was dephosphorylated at a rate of $47 \pm 11 \text{ nmol/min per } 10^7$ cells ($n = 10$). Sonicated cells exhibited $190 \pm 40\%$ of the intact-cell activity, demonstrating an internal pool of enzyme, as in other cells (Stanley *et al.*, 1980). Incubation with anti-(rat liver ecto-5'-nucleotidase) serum decreased the cell-surface enzyme activity to $1.3 \pm 0.3\%$ ($n = 10$), which increased to $1.9 \pm 0.7\%$ after five washings (Fig. 5). Sonication of the washed cells revealed an activity equivalent to $8 \pm 4\%$ of the cell-surface activity (Fig. 5), showing that antibody inhibited both pools of enzyme. Inhibition of the internal pool on

Fig. 5. Effect of anti-(rat liver ecto-5'-nucleotidase) serum on 5'-nucleotidase activity in intact and homogenized neonatal rat myocytes

Intact cells ($2.49 \times 10^6 \pm 0.11 \times 10^6$) were preincubated in duplicate for 30 min at 37°C with anti-(rat liver ecto-5'-nucleotidase) serum at a 1:100 final dilution (b, c, e). Two sets of duplicates (c, e) were washed with $5 \times 1 \text{ ml}$ of buffer. Inhibited or control cells were sonicated (d-f). One set of duplicates (f) containing control sonicated cells was resuspended with $500 \mu\text{l}$ of the fifth wash from duplicate (c). 5'-Nucleotidase activity was then determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for the number of experiments shown.

Fig. 6. Influence of anti-(rat liver ecto-5'-nucleotidase) serum on adenosine formation and release

Cells ($2.62 \times 10^6 \pm 0.14 \times 10^6$) were preincubated for 30 min at 37°C with anti-(rat liver ecto-5'-nucleotidase) serum at 1:100 final dilution (d-f) and then incubated at 37°C for 0 (a,d) or 10 min (b, c, e, f) in Krebs-Ringer bicarbonate/Hepes solution supplemented with 30 mM-2-deoxyglucose (DOG) and $2 \mu\text{g}$ of oligomycin/ml (Oligo) (c, f). Adenosine concentrations were measured in supernatants (a) and cells (b) in duplicates. Values given are means $\pm$ S.E.M. from four separate experiments. There was no significant difference between values obtained in the presence and absence of anti-(rat liver ecto-5'-nucleotidase) serum.

sonication by excess antibody was ruled out, since the fifth wash from inhibited cells failed to inhibit fresh cells (Fig. 5f).

Inhibition of ecto-5'-nucleotidase had no effect on the rate of formation or the distribution of adenosine between medium and cells (Fig. 6). Neither did it affect the concentrations in the cells or medium of any of the other metabolites measured (results not shown). Cells remained $99.2 \pm 0.2\%$ ($n = 3$) intact.

Discussion

Cellular ATP concentration was decreased by approx. 60% on incubation for 10 min with 2-deoxyglucose and oligomycin, in agreement with previous work (Seraydarian *et al.*, 1969). Adenosine formation was only modestly accelerated (cf. Mustafa *et al.*, 1975; Newby *et al.*, 1983) because of the high basal rate of adenosine formation. This may result from the abnormally low value (0.85) of energy charge ($[ATP] + [\frac{1}{2}ADP]/[ATP] + [ADP] + [AMP]$) in cultured heart cells compared with the value in perfused rat hearts (0.91; Newby *et al.*, 1983). Adenosine accumulation accounted for 18% of the ATP degraded, whereas IMP and hypoxanthine accounted for 65%. This indicates a more rapid flux through AMP deaminase (Lomax & Henderson, 1973; Van den Berghe *et al.*, 1977) than through 5'-nucleotidase (Worku & Newby, 1983).

Dipyridamole, hexobendine and dilazep, selective inhibitors of nucleoside transport (Turnheim *et al.*, 1978; Plagemann & Wohlheuter, 1980; Paterson *et al.*, 1983), inhibited incorporation of adenosine into the nucleotide pool of neonatal rat heart cells by approx. 80% at $10 \mu M$. Our results are consistent with other studies showing that the nucleoside transporter in the rat is less sensitive than that of other species (Kolassa *et al.*, 1971; Kolassa & Pfleger, 1975; Verma & Marangos, 1984; Williams *et al.*, 1984). Nucleoside-transport inhibitors ($10 \mu M$) increased the cellular adenosine concentration by 3–4-fold and decreased adenosine efflux by 63–66%. Higher concentrations produced non-specific effects on nucleotide degradation (results not shown). Assuming a value of 5 mM for ATP concentration gives an estimate for intracellular adenosine concentration (corrected for extracellular water) in the cells incubated with deoxyglucose, oligomycin and nucleoside-transport inhibitors of 0.13–0.15 mM. This compares with a value of 0.16–0.17 μM for extracellular adenosine concentration and demonstrates that a large concentration gradient was maintained by the transport inhibitors. Since inhibition of the nucleoside transporter is, at least in part, competitive (Turnheim *et al.*, 1978; Plagemann & Wohlheuter, 1980),

incomplete prevention of adenosine efflux may be explained by the high intracellular concentration. We cannot rule out that a proportion of the adenosine was released by another mechanism.

Mustafa *et al.* (1975) and Mustafa (1979) found that dipyridamole, dilazep, hexobendine, lidoflazine and papaverine did not inhibit net release of adenosine from embryonic-chick heart cells prelabelled with [3H]adenosine. This disagreement might arise from uncertainties in the specific radioactivity of labelled pools or from further metabolism and reincorporation of adenosine. Alternatively, in contrast with our results, chick heart cells may have released nucleotides which were subsequently degraded by ecto-5'-nucleotidase (Paddle & Burnstock, 1974; Forrester & Williams, 1977; Pearson & Gordon, 1979; Lollar & Owen, 1981; Forrester, 1981). A third possibility remains that, in some species, nucleoside-transport inhibitors act asymmetrically.

Some studies (Frick & Lowenstein, 1978; Dornand *et al.*, 1979) have suggested that AMP can be vectorially transported into cells via the ecto-5'-nucleotidase, implying that the enzyme may function physiologically in the reverse direction (Newby, 1975; Arch & Newsholme, 1978). However, the failure of inhibition of ecto-5'-nucleotidase to alter the distribution of newly formed adenosine, which has not previously been documented, discounts a role for the enzyme in vectorial export of adenosine.

The present results support the conclusions drawn from studies of perfused hearts (Frick & Lowenstein, 1976; Schütz *et al.*, 1981) and from isolated polymorphonuclear leucocytes (Newby, 1980; Newby & Holmquist, 1981) that adenosine formation takes place in the cytoplasm and that the nucleoside is only subsequently exported. Furthermore, they identify the symmetric nucleoside permease as the mechanism of adenosine efflux. Dipyridamole and other nucleoside-transport inhibitors (nitrobenzylthioinosine, papaverine and lidoflazine) have been shown to decrease nucleoside efflux from perfused hearts (Kübler *et al.*, 1970; Kukovetz & Pösch, 1971; Degenring *et al.*, 1976; Schütz *et al.*, 1981). The vasodilator effects of nucleoside-transport inhibitors seem paradoxical if they inhibit both adenosine release and adenosine uptake. It is possible, but unlikely, that such a group of structurally unrelated compounds could have another common mechanism of action. Alternatively it is necessary to explain how inhibition of influx and efflux of nucleoside can lead to an increase in interstitial-fluid adenosine concentration.

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