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The mechanism of adenosine release from hypoxic rat liver cells

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1 Uptake of [^{14}C]-adenosine into freshly dispersed rat hepatocytes was inhibited 44% by dipyridamole (50 μM) and 60% by nitrobenzylthioinosine (NBTI, 20 μM). The results are consistent with the known ability of these drugs to inhibit adenosine transport in other cell types.

2 The nucleotide analogue, α,β-methylene adenosine diphosphate (AOPCP, 50 μM), inhibited by 84% the degradation of exogenous 5′ AMP that occurred rapidly when this substrate alone was presented to isolated hepatocytes. This confirms the ecto-5′-nucleotidase inhibitory properties of this analogue in isolated hepatocytes.

3 During hypoxic incubation, isolated hepatocytes released adenosine, which accumulated in the extracellular volume. Dipyridamole and NBTI each markedly attenuated this extracellular adenosine accumulation. In contrast, AOPCP had no inhibitory effect on net hypoxic adenosine release.

4 It is concluded that hypoxic rat hepatocytes produce adenosine intracellularly and that this adenosine is released via facilitated diffusion to the extracellular space, based on the inhibition observed with the transport inhibitors. The plasma membrane enzyme ecto-5′-nucleotidase does not appear to participate in hypoxic adenosine release from these cells as indicated by the lack of effect of the nucleotidase inhibitor, AOPCP.

Introduction

Adenosine appears to act as a multi-purpose intercellular messenger in mammalian tissues. It has been proposed as a mediator of local vasoregulation and as a modulator of cardiac mechanical and electrical function, peripheral and central nervous system synaptic transmission, and platelet function (Berne *et al.*, 1983). These actions are, for the most part, mediated via adenosine receptors on the external surface of the target cells (Schrader *et al.*, 1977; Bruns *et al.*, 1980; Berne *et al.*, 1983). The general paradigm, then, is that adenosine is released from one or more cell types into the interstitial space, whence it can act on the target cell(s).

Two mechanisms to explain adenosine release from cells have been proposed. One possible mechanism is that adenosine is made at the surface of cells by 5′-nucleotidase bound to the plasma membrane (Frick & Lowenstein, 1978; Berne, 1980). The other possibility is that adenosine is made inside the cell and leaves via facilitated transport (Schütz *et al.*, 1981). We wished to test these hypotheses using an isolated cell model to avoid any confusion arising from the complex tissue geometry and multiple barriers to adenosine diffusion that are found in an intact tissue with a microcirculation. Consequently, we performed experiments with freshly isolated, dispersed rat liver cells. Our results favour the intracellular production of adenosine.

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Methods

Liver cell suspensions

Suspensions of liver cells were prepared according to the method of Seglen (1976). Rats weighing 250–400 g were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, or to effect i.p.). Heparin (1000 u kg^{-1}) was administered into the inferior vena cava. The portal vein was exposed and cannulated and the liver was perfused with a Ca^{2+}-free, non-recirculating buffer at 50 ml min^{-1} for 10 min. Next, the liver was perfused for 10 min with recirculated buffer containing collagenase (0.5 mg ml^{-1}, Sigma Type IV). The liver was carefully transferred to chilled buffer in which the cells were dispersed by gentle scraping with a plastic fork. The suspension was filtered through

nylon mesh (250 μm) and incubated under oxygen for 30 min. The suspension was filtered once more (88 μm nylon mesh) and centrifuged gently (3 *g*). The supernatant containing blood cells, vascular cells and damaged cells (Seglen, 1976) was discarded and the sedimented cells were resuspended in buffer. Four more such washing cycles yielded a final cell suspension composed of hepatocytes (90–100% of the total number of cells) that were 64–91% viable (average = 83 ± 1% for 19 preparations) as judged by their ability to exclude Trypan blue. Total protein yield averaged 1080 ± 82 mg protein per liver.

Adenosine release

Suspensions were prepared in pairs from a single liver with each member of the pair having the same cell concentration. The suspending physiological salt solution (Seglen, 1976) was equilibrated with 95% O_2 + 5% CO_2 and had a pH of 7.4 maintained by $NaHCO_3$ and 10 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethane-sulphonic acid). Glucose (10 mM) was also present. One of the paired suspensions contained either 50 μM AOPCP (α,β-methylene adenosine diphosphate, Sigma), 50 μM dipyridamole (Sigma) or 5–20 μM 6-(4-nitrobenzyl)-thioinosine (NBTI, Calbiochem-Behring). The other member of the pair served as a control. In the dipyridamole series both control and drug-containing suspensions contained 1% ethanol, which was used as a solvent for the drug. During these experiments, the suspensions were maintained at 37°C and aerated continuously. After a 15 min equilibration, baseline samples were obtained. Then both suspensions were made hypoxic by substituting 95% N_2 + 5% CO_2 as the aerating gas. Further samples were obtained after 10 and 20 min of hypoxia. At these times, a 1 ml sample of the suspension was layered onto 0.3 ml of oil (4 parts silicone oil, Dow Corning No. 550, plus one part light mineral oil) in a 1.5 ml microcentrifuge tube. This was quickly capped and centrifuged for 10 s. An aliquot of the top layer, composed solely of extracellular fluid (Wohlhueter *et al.*, 1978), was transferred to cold perchloric acid + methanol and processed for h.p.l.c. analysis as described below. When adenosine was added to a pooled volume of extracellular fluid obtained in this fashion, its concentration (5 μM) was stable for 2 min. The elapsed time between the introduction of sample into the centrifuge tube and the transfer of an aliquot of the top layer to acid was less than 1 min.

Samples of extracellular fluid (obtained as described above) were acidified with cold (− 15°C) perchloric acid (2N) in methanol (3:1). The acid was neutralized with KOH, the $KClO_4$ precipitate removed by centrifugation (1,000 *g*) and the supernatant concentrated by freeze-drying and resuspension in distilled water. Adenosine content was measured by reversed-phase high pressure liquid chromatography (h.p.l.c., Hartwick & Brown, 1977; Belloni *et al.*, 1984) using a Beckman-Altex Ultrasphere-5μ-C_{18} stainless steel column (25 cm × 4.6 mm) preceded by a Spherisorb-5μ-C_{18} guard column (3 cm × 4.6 mm). The mobile phase was 10% methanol in 4 mM KH_2PO_4 (pH 3.6) applied at a flow rate of 1.4 ml min^{-1}. Adenosine levels were quantified by comparing peak heights to those of separate injections of adenosine standards. Peak identity was verified by using adenosine deaminase (Boehringer Mannhein) to eliminate the putative adenosine peak. Protein concentrations of cell suspensions were determined by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Adenosine uptake

Adenosine uptake studies were conducted at 23°C. Individual 1 ml aliquots of liver cell suspension were mixed with 1 ml of starter solution containing [8-^{14}C]-adenosine and [6,6′(n)-^{3}H]-sucrose (Amersham, final concentrations of 2 μM and 1 nM, respectively). The starter solution also contained dipyridamole, nitrobenzylthioinosine or the appropriate vehicle. After a prescribed time, a 1 ml aliquot of the incubation mix was centrifuged through a layer of oil as already described. An aliquot of the extracellular fluid (top layer) was obtained for subsequent analysis of ^{14}C and ^{3}H content. Another aliquot was processed for h.p.l.c. determination of adenosine content and specific radioactivity. The remainder of the top and oil layers were discarded and the tube was washed twice with water. The cell pellet was dissolved overnight in

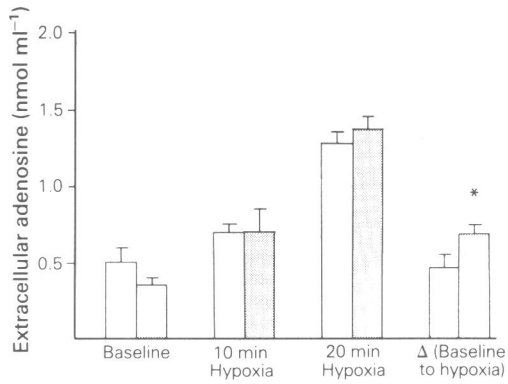

Figure 1 Baseline and hypoxic levels of extracellular adenosine in suspensions of isolated liver cells of rat. One of each suspension pair (stippled column) contained 50 μM α,β-methylene adenosine diphosphate (AOPCP); open columns = controls. The last pair of columns represent the difference between the baseline and the average (10 min and 20 min points) hypoxic adenosine levels. * signifies $P < 0.05$. $n = 4$.

1% SDS, then mixed with scintillation mixture (Liquiscint, National Diagnostics) and analyzed for ^{3}H and ^{14}C content using a liquid scintillation counter (Nuclear Chicago Mark I) and correcting for quenching through use of an external radium standard and a channels-ratio calculation. The ^{3}H content of the pellet and the $^{14}C/^{3}H$ ratio in the extracellular fluid were used to correct the ^{14}C content of the cell pellet for contamination by extracellular fluid. This contamination was usually negligible.

Activity of ecto-5'-nucleotidase

A 1 ml aliquot of cell suspension was added to 1 ml of a starter solution containing [U-^{14}C]-AMP (Amersham) (final extracellular concentration: 5 μM) with or without AOPCP (50 μM). An extracellular aliquot and the washed cell pellet were obtained as described above after a prescribed incubation time. The pellet was analyzed for ^{14}C content. The extracellular fluid sample was fractionated with a polyethyleneimine cellulose (Sigma) anion exchange column (Magnusson *et al.*, 1976). The AMP, ADP, ATP and adenosine + inosine + hypoxanthine fractions were analyzed separately for ^{14}C content using the scintillation counting techniques described above.

Statistical analysis

Student's *t* test for paired data (drug vs. control) was used to analyze the adenosine release data. Student's *t* test for unpaired data was used to analyze the adenosine uptake and AMP degradation data. The critical value of *t* was adjusted to compensate for the number of simultaneous multiple comparisons within each experiment by the method of Bonferroni (Wallenstein *et al.*, 1980). Data are presented as the mean value ± one standard error of the mean (s.e.mean). The value of *n* is either the number of experiments or the number of samples as indicated.

Results

As seen in Figures 1–3, extracellular adenosine concentration rose during 20 min of hypoxia in control suspensions. We have previously shown that there is no progressive net adenosine accumulation in these suspensions when oxygenation is maintained (Belloni *et al.*, 1984).

Extracellular adenosine levels were not affected by the presence of 50 μM AOPCP (Figure 1). If anything, there was a tendency (not statistically significant) for baseline levels to be slightly less in the presence of the drug and the average hypoxic increment in adenosine concentration (average of 10 and 20 min hypoxic valves minus baseline value) was slightly greater in the presence of AOPCP. Extracellular adenosine levels expressed per mg cellular protein are given in Table 1.

[U-^{14}C]-AMP (5 μM) was metabolized rapidly by dispersed liver cells at 23°C (Table 2). The amount of ^{14}C recovered in the extracellular AMP fraction declined with incubation time. This ^{14}C decrement was

Table 2 Degradation of 5'-AMP by isolated liver cells of rat in suspension

Incubation time (sm)	*Control*	*AOPCP* (50 μM)
9–15	63 ± 3 (10)	99 ± 3 (8)*
25	31 ± 3 (4)	81 ± 2 (4)*
45–62	19 ± 4 (5)	82 ± 3 (5)*
120	23 ± 3 (6)	78 ± 5 (6)*

Values are means ± s.e. with number of trials indicated in parentheses. Data are expressed as percentage of exogenous [U-^{14}C]-AMP originally present (4.9 ± 0.3 nmol ml^{-1}) that remains after the incubation time indicated. *indicates $P<0.001$ comparing drug treatment to appropriately timed controls. Trials were conducted at 23°C.

Table 1 Extracellular adenosine levels before and during hypoxia.

	Extracellular adenosine pmol (mg cell protein)$^{-1}$			
	Baseline	*10 min hypoxia*	*20 min hypoxia*	*Average hypoxia*
Control ($n=4$)	51 ± 6	79 ± 26	133 ± 25	106 ± 25
AOPCP	42 ± 12	84 ± 29	164 ± 49	124 ± 38
Control ($n=6$)	38 ± 8	104 ± 24	170 ± 44	137 ± 33
NBTI	68 ± 33	87 ± 30	115 ± 40***	101 ± 35**
Control ($n=5$)	51 ± 13	304 ± 62	385 ± 99	345 ± 78
Dipyridamole	59 ± 19	191 ± 34**	178 ± 63†	184 ± 45*

AOPCP = α,β-methylene adenosine diphosphate; NBTI = nitrobenzylthioinosine.
* $P<0.025$; ** $P<0.01$; *** $P<0.001$; † $P<0.07$ for comparison to paired control.

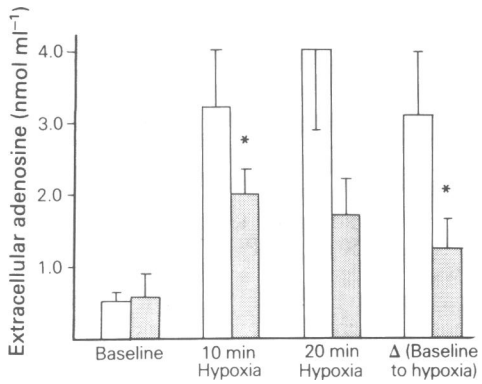

Figure 2 Effect of dipyridamole (50 μM) (stippled columns) on extracellular adenosine levels. Format identical to that of Figure 1. $n = 5$.

Table 3 Uptake of adenosine by isolated liver cells of rat in suspension

	Adenosine pmol (mg cell protein)$^{-1}$		
	15 s	30 s	60 s
Control (8)	59 ± 4	106 ± 9	183 ± 20
NBTI, 20 μM (8)	24 ± 4*	46 ± 7*	66 ± 12*
Control (16)	33 ± 3	67 ± 4	108 ± 10
Dipyridamole, 50 μM (16)	19 ± 2	27 ± 3*	45 ± 4*

Values are means ± s.e. Values in parentheses are numbers of samples. * $P < 0.001$ comparing drug treatment to its timed control. Tests were conducted at 23°C. NBTI = nitrobenzylthioinosine.

accounted for by increased ^{14}C recovery in the cellular fraction and in the earliest extracellular fraction eluted from the PEIC columns (adenosine + inosine + hypoxanthine). At any incubation time tested, more ^{14}C remained as extracellular AMP in those suspensions containing 50 μM AOPCP than in control suspensions. Estimates of the initial reaction velocities based on the AMP contents of the samples representing the shortest incubation times (9–15 s) and those of the starter solutions themselves (treated exactly as cell samples except for the absence of cells) indicated that the AMP breakdown rate was inhibited by 84% with this concentration of AOPCP.

The experiments measuring AMP degradation were performed at 23°C (room temperature) because of the technical difficulty involved in performing the entire protocol at 37°C. When we performed experiments in which all solutions and cell mixtures were kept at 37°C as much as possible, with only the centrifugation through oil performed at room temperature, we found greater control rates of degradation but similar drug inhibition (data not shown).

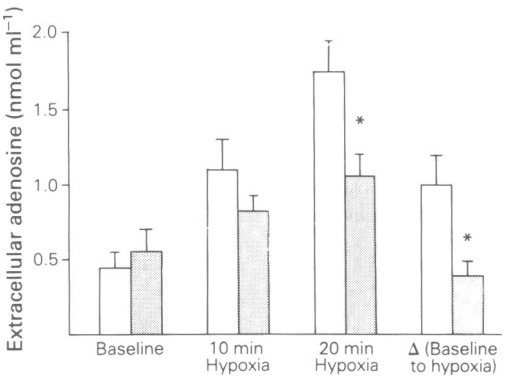

Figure 3 Effect of nitrobenzylthioinosine (NBTI) (stippled columns) on extracellular adenosine levels. NBTI concentration was 5 μM in two experiments and 20 μM in four experiments. Each experiment showed the same trend. Format identical to that of the other figures. $n = 6$.

Dipyridamole (50 μM, Figure 2) and nitrobenzylthioinosine (NBTI) (5–20 μM, Figure 3) significantly and substantially attenuated the rise in extracellular adenosine concentration during hypoxia. Baseline extracellular adenosine levels were not significantly affected by either drug. Adenosine levels in these experiments are also expressed in terms of pmol extracellular adenosine per mg cell protein in Table 1.

Both dipyridamole (50 μM) and NBTI (20 μM) significantly and substantially attenuated the incorporation of ^{14}C into liver cells when cells were incubated with [^{14}C]-adenosine (2 μM) (Table 3). Based on net uptakes during the shortest incubation times (15 s), adenosine uptake was attenuated by 44% in the presence of dipyridamole and by 60% in the presence of NBTI. Net uptake during longer incubations (30 or 60 s) was similarly attenuated by each drug.

Discussion

The major finding of this study is that adenosine release from hypoxic rat liver cells was attenuated by inhibitors of facilitated adenosine transport, but not by an inhibitor of ecto-5'-nucleotidase. An ancillary finding of this study is that adenosine uptake by rat isolated liver cells was sensitive to dipyridamole and NBTI. Also, it appeared that the membrane-bound 5'-nucleotidase of hepatocytes is capable of degrading extracellular AMP and is, hence, an ecto-enzyme.

We have used extracellular adenosine concentration

changes as an index of adenosine release. In reality, this variable represents the net adenosine release, which, in turn, reflects the difference between unidirectional adenosine efflux on the one hand, and adenosine uptake and extracellular degradation on the other. Its use as an index of adenosine release seems to be justifiable. Adenosine uptake does not appear to involve the membrane-bound 5'-nucleotidase; human lymphocytes with a genetic lack of this enzyme take up adenosine as avidly as do normal lymphocytes (Fleit *et al.*, 1975). Thus, the unaltered net adenosine release we observed in the presence of the nucleotidase inhibitor probably reflects an unchanged unidirectional adenosine release. The transport blockers did, of course, inhibit adenosine uptake, but this would have caused an enhanced net adenosine release if the unidirectional release remained normal; thus, the decreased net release we observed in the presence of these drugs must reflect a decreased unidirectional adenosine release.

Our experiments measuring breakdown of exogenous AMP suggest that 50 μM AOPCP can inhibit an ecto-5'-nucleotidase in rat liver cells. This adenine nucleotide analogue has such an action on skeletal and cardiac muscle (Frick & Lowenstein, 1976; Schütz *et al.*, 1981). We have assumed that 5'-nucleotidase is the enzyme responsible for the observed AMP breakdown, based on its proven presence in hepatocyte membrane (Drummond & Yamamoto, 1971; Frick & Lowenstein, 1976) but we have provided no independent data to confirm this. Our estimates of the rate of this reaction probably underestimate the true initial rate since the substrate level fell noticeably during even the initial 15 s. If anything, however, this caused us to underestimate the inhibitory effect of AOPCP.

The uptake experiments confirm that dipyridamole and NBTI can inhibit adenosine uptake by rat liver cells, as they inhibit adenosine uptake and transport in many other cell types (Paterson *et al.*, 1983). Uptake of purine ribonucleosides by mammalian cells reflects both trans-membrane transport and intracellular metabolism. As a consequence, special experimental design features, including the use of very short incubation times ($\sim$ 1 s), must be used in order to characterize precisely any adenosine transport system in itself (Plagemann & Wohlhueter, 1983). Our data, obtained using relatively longer incubation times, probably underestimate the adenosine transport rate of rat hepatocytes but, nonetheless, reveal a substantial adenosine transport capacity. Using a liver cell diameter of 18 μm and a protein content of 1 mg protein per 7×10^5 cells (our unpublished observations), the 15 s uptake data indicate an uptake rate of 5 pmol (μl cell H_2O)$^{-1}$s^{-1}. If we consider our use of 23°C instead of 37°C as the experimental temperature and our use of 2 μM adenosine compared to the transport K_m of 50–150 μM typical of mammalian cells, the adenosine transport capacity of rat hepatocytes seems comparable to that of other mammalian cells (Plagemann & Wohlhueter, 1983). Adenosine release was not completely blocked by dipyridamole and NBTI in our experiments. We presume this reflects the competitive nature of the adenosine transport system inhibition by the agents used (Paterson *et al.*, 1983) and also the existence of passive diffusion of adenosine through the hepatocyte plasma membrane.

Our study evaluates two hypotheses that have been proposed to explain the release of adenosine from mammalian cells. The first hypothesis holds that adenosine is produced by membrane-bound 5'-nucleotidase from intracellular AMP (Frick & Lowenstein, 1978; Berne, 1980) with hydrolysis and translocation across the plasma membrane being somehow linked (Frick & Lowenstein, 1978). The second hypothesis holds that adenosine is produced intracellularly, rather than at the cell surface, and its efflux from the cell is via passive and facilitated diffusion (Schütz *et al.*, 1981).

In the present study, hypoxic adenosine release continued unabated despite substantial inhibition of the plasma membrane 5'nucleotidase by AOPCP. This agrees with results from studies using perfused hearts (Frick & Lowenstein, 1976; Schütz *et al.*, 1981) and also with a study by Newby & Holmquist (1981), in which rat polymorphonuclear leukocytes, challenged by 2'-deoxyglucose, were found to produce adenosine at equal rates in the presence and absence of an antibody against the membrane-bound 5'-nucleotidase. All of these studies speak against the required participation of plasma membrane 5'-nucleotidase in the adenosine production of mammalian cells in response to hypoxia or 2-deoxyglucose.

We found clear and substantial inhibition of net adenosine release from hypoxic liver cells in the presence of dipyridamole or NBTI. This result agrees with that of Schütz *et al.* (1981), who found a decreased release of adenosine from perfused, hypoxic hearts in the presence of NBTI, and it extends their findings to another type of mammalian cell. What is more important, our use of isolated cells ensures that the blocking drugs are acting on the parenchymal cells themselves. The finding of Schütz *et al.* (1981) could have resulted from an inhibition of trans-capillary adenosine movement. Such a possibility was suggested by Knabb *et al.* (1984) who found increased adenosine levels in a pericardial suffusate, but not in coronary venous blood, in the presence of dipyridamole. There were, of course, no capillary barriers in the isolated cell suspensions used in the present study and so our finding confirms a direct action of the transport blocking drugs on efflux from the parenchymal cells.

It has been suggested that the liver in rats and rabbits is a major exporter of adenosine for use in other tissues, such as intestinal mucosa and eryth-

rocytes, that have poor capability for *de novo* purine synthesis (Pritchard *et al.*, 1975). The present results confirm the capacity of rat liver cells to produce and release adenosine and to enhance adenosine release during periods of hypoxia. This hypoxic response might reflect simply the deleterious effects of inadequate oxygen supply on adenine nucleotide maintenance in hepatocytes, as in other tissues. From another perspective. however, adenosine export from the liver during hypoxia might be important to the dependent peripheral tissues for the replenishment of their cellular nucleotide levels upon regaining a normoxic state.

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References

BELLONI, F.L., RUBIO, R. & BERNE, R.M. (1984). Intracellular adenosine in isolated rat liver cells. *Pflügers Arch.*, **400**, 106–108.

BERNE, R.M. (1980). The role of adenosine in the regulation of coronary blood flow. *Circulation Res.*, **47**, 807–813.

BERNE, R.M., RALL, T.W. & RUBIO, R. (ed.) (1983). *Regulatory Function of Adenosine*. The Hague: Martinus Nijhoff.

BRUNS, R.F., DALY, J.W. & SNYDER, S.H. (1980). Adenosine receptors in brain membranes: binding of N^6-cyclohexyl [^{3}H] adenosine and 1,3-diethyl-8-[^{3}H] phenylxanthine. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 5547–5551.

DRUMMOND, G.I. & YAMAMOTO, M. (1971). Nucleotide phosphomonoesterases. In *The Enzymes*, Vol. IV, 3rd edition, ed. Boyer, P.D. pp. 337–354. New York: Academic Press.

FLEIT, H., CONKLYN, M., STEBBINS, R.D. & SILBER, R. (1975). Function of 5'-nucleotidase in the uptake of adenosine from AMP by human lymphocytes. *J. biol. Chem.*, **250**, 8889–8892.

FRICK, G.P. & LOWENSTEIN, J.M. (1976). Studies of 5'-nucleotidase in the perfused rat heart including measurements of the enzyme in perfused skeletal muscle and liver. *J. biol. Chem.*, **251**, 6372–6378.

FRICK, G.P. & LOWENSTEIN, J.M. (1978). Vectorial production of adenosine by 5'-nucleotidase in the perfused rat heart. *J. biol. Chem.*, **253**, 1240–1244,

HARTWICK, R.A. & BROWN, P.R. (1977). Selective analysis for adenosine using reversed-phase high pressure liquid chromatography. *J. Chromatog.*, **143**, 383–389.

KNABB, R.M., GIDDAY, J.M., ELY, S.W., RUBIO, R. & BERNE, R.M. (1984). Effects of dipryidamole on myocardial adenosine and active hyperemia. *Am. J. Physiol.*, **247** (Heart Circ. Physiol. 16), H804–H810.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

MAGNUSSON, R.P., PORTIS, A.R. & McCARTY, R.E. (1976). Quantitative analytical separation of adenine nucleotides by column chromatography on polyethyleneimine-coated cellulose. *Anal. Biochem.*, **72**, 653–657.

NEWBY, A.C. & HOLMQUIST, C.A. (1981). Adenosine production inside rat polymorphonuclear leucocytes. *Biochem. J.*, **200**, 399–403.

PATERSON, A.R.P., JAKOBS, E.S., HARLEY, E.R., FU, N.-W., ROBIUS, M.J. & CASS, C.E. (1983). Inhibition of nucleoside transport. In *Regulatory Function of Adenosine*, ed. Berne, R.M., Rall, T.W. & Rubio, R. pp. 203–220. The Hague: Martinus Nijhoff.

PLAGEMANN, P.G.W. & WOHLHUETER, R.M. (1983). Nucleoside transport in mammalian cells and interaction with intracellular metabolism. In *Regulatory Function of Adenosine*. ed. Berne, R.M., Rall, T.W. & Rubio, R., pp. 179–201. The Hague: Martinus Nijhoff.

PRITCHARD,, J.B., O'CONNOR, B., OLIVER, J.M. & BERLIN, R.D. (1975). Uptake and supply of purine compounds by the liver. *Am. J. Physiol.*, **229**, 967–972.

SCHRADER, J., NEES, S. & GERLACH, E. (1977). Evidence for a cell surface adenosine receptor on the coronary myocytes and atrial muscle cells. *Pflügers Arch.*, **369**, 251–257.

SCHÜTZ, W., SCHRADER, J. & GERLACH, E. (1981). Different sites of adenosine formation in the heart. *Am. J. Physiol.*, **240** (Heart Circ. Physiol. 9), H963–H970.

SEGLEN, P.O. (1976). Preparation of isolated rat liver cells. In *Methods in Cell Biology*. ed. Prescott, D.M. Vol. 13 pp. 29–83. New York: Academic Press.

WALLENSTEIN, S., ZUCKER, C.L. & FLEISS, J.L. (1980). Some statistical methods useful in circulation research. *Circulation Res.*, **47**, 1–9.

WOHLHUETER, R.M., MARZ, R., GRAFF, J.C. & PLAGEMANN, P.G.W. (1978). A rapid-mixing technique to measure transport in suspended animal cells: Applications to nucleoside transport in Novikoff rat hepatoma cells. In *Methods in Cell Biology*. ed. Prescott, D.M. Vol. 20, pp. 211–236, New York: Academic Press.

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