Effect of 5′-deoxy-5′-isobutylthioadenosine on formation and release of adenosine from neonatal and adult rat ventricular myocytes

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1. Studies in rat polymorphonuclear leucocytes have suggested that 5′-deoxy-5′-isobutylthioadenosine (IBTA), an inhibitor of the IMP-selective cytosolic 5′-nucleotidase, may be used to test its role in adenosine formation in intact cells. We investigated adenosine formation in neonatal and adult rat cardiomyocytes. 2. 2-Deoxyglucose (30 mM) with oligomycin (2 μg/ml) induced a 90–100% fall in ATP concentration in 10 min in neonatal and 60 min in adult heart cells. Adenosine accumulation was substantially increased, accounting for 13% of the fall in ATP concentration in neonatal cells and 56% in adult cells. 3. Anti-(rat liver ecto-5′-nucleotidase) serum did not inhibit adenosine accumulation. Furthermore, dipyridamole (10 μM), a nucleoside-transport blocker, inhibited by 80% the appearance of the newly formed adenosine in the medium, showing that adenosine is produced intracellularly by both adult and neonatal-rat myocytes in response to inhibition of oxidative metabolism. 4. IBTA (3 mM) inhibited by 80% the appearance of adenosine in the medium, but did not inhibit total adenosine accumulation by neonatal-rat myocytes and only modestly inhibited total adenosine accumulation by adult myocytes. 5. IBTA, like dipyridamole, inhibited incorporation of extracellular adenosine (10 μM) into neonatal and adult ventricular myocyte nucleotides by 60–70%. Transport of IBTA (100 μM) into the cells did not appear to be inhibited by dipyridamole (30 μM). 6. We conclude that IBTA acted primarily to inhibit adenosine release from myocytes. The small effect on adenosine formation rates implies that the IMP-selective cytosolic 5′-nucleotidase plays a minor role in this tissue.

INTRODUCTION

Evidence has accumulated for two distinct pathways of adenosine formation from extracellular and intracellular nucleotides (Newby et al., 1990). In the heart, there is long-standing evidence, which has been confirmed recently, for the release of nucleotides from ischaemic or hypoxic preparations (Paddle and Burnstock, 1974; Clemens and Forrester, 1981; Schrader et al., 1982; Van Belle et al., 1987; Imai et al., 1989; Borst and Schrader, 1990). Adenosine may then be formed extracellularly via ecto-nucleotidases (Pearson, 1985). The quantitative importance of this mechanism of adenosine formation in the heart is still controversial, however. In other studies, for example, no ATP release was detected from hypoxic rat or guinea-pig myocardium, but an active intracellular pathway of adenosine formation was identified (Frick and Lowenstein, 1976; Schütz et al., 1981; Schrader et al., 1981; Deussen et al., 1988). During ATP catabolism, intracellular adenosine is produced from AMP via cytosolic 5′-nucleotidases (EC 3.1.3.5), of which there are at least two forms, an AMP-selective form (Truong et al., 1988; Newby, 1988) and an IMP-selective form (Van den Berghe et al., 1977; Itoh, 1981a,b; Itoh and Oka, 1985; Itoh et al., 1986). This pathway links adenosine formation to the balance between energy supply and demand, and thus to the formation and degradation of cytosolic AMP derived from cytosolic ATP (Newby, 1984; Newby et al., 1990). Adenosine must then leave the cell through the nucleoside transporter to act on extracellular receptors. Borst and Schrader (1990) reported that, although ischaemia stimulated the release of both adenine nucleotides and adenosine in the isolated guinea-pig heart, only the release of adenosine was greatly increased during hypoxic perfusion.

The use of isolated cardiac myocytes has also produced evidence for both pathways of adenosine formation, but has not established their relative importance. Release of ATP during hypoxia (Forrester and Williams, 1977; Williams and Forrester, 1983) and production of adenosine via ecto-5′-nucleotidase during metabolic poisoning (Bukoski and Sparks, 1986) have been shown for adult rat ventricular myocytes. There is, however, much less evidence for the extracellular pathway of adenosine formation in neonatal-rat or embryonic-chick heart cells, where use of inhibitors of ecto-5′-nucleotidase and nucleoside transport have indicated that adenosine is produced largely, if not exclusively, intracellularly during either hypoxia or metabolic poisoning (Meghji et al., 1985, 1988).

It has been shown that 5′-deoxy-5′-isobutylthioadenosine (IBTA) non-competitively inhibits the partially purified IMP-selective cytosolic 5′-nucleotidases from rat liver, polymorphonuclear leucocytes and heart (Skladanowski et al., 1989), but was much less potent against the AMP-selective cytosolic 5′-nucleotidase from pigeon or rat heart (Newby, 1988; Skladanowski and Newby, 1990). Furthermore, IBTA was shown to inhibit adenosine production in intact polymorphonuclear leucocytes during 2-deoxyglucose-induced ATP catabolism, thereby implying the involvement of the IMP-selective 5′-nucleotidase (Skladanowski et al., 1989).

In the present study we examined the effects of IBTA on adenosine formation in both neonatal and adult rat ventricular myocytes. Initial experiments showed that IBTA acted as a

Abbreviations used: IBTA, 5′-deoxy-5′-isobutylthioadenosine; 2′-deoxycoformycin, *R*-3-(deoxy-β-D-pentofuranosyl)-3,6,7,8-tetrahydro[4,5-*d*]-diazapin-8-ol; 5-iodotubercidin, 4-amino-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine.

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nucleoside-transport inhibitor. We therefore compared its action with that of a known transport inhibitor, dipyridamole.

MATERIALS AND METHODS

Materials

AnalaR water used for isolation of adult ventricular myocytes was obtained from BDH. Collagenase was obtained from Lorne Laboratories U.K. (Worthington collagenase, type CLS2). Rabbit anti-(rat liver 5′-nucleotidase) serum was a gift from Dr. J. P. Luzio, Department of Clinical Biochemistry, University of Cambridge, Cambridge, U.K. Plastic Petri dishes (Falcon) were obtained from Becton Dickinson (Lincoln Park, NJ, U.S.A.). All other materials were obtained from sources described elsewhere (Gordon et al., 1986; Skladanowski et al., 1989).

Preparation of neonatal rat myocytes

Neonatal rat myocytes were prepared in 6- or 24-well plates from 4–5-days-old Sprague–Dawley rats as described previously (Newby et al., 1983).

Preparation of adult rat myocytes

Adult rat myocytes were prepared in Petri dishes (35 mm diameter) from female Sprague–Dawley rats (200–300 g) as described previously (Meghji et al., 1992).

Cell numbers

The number of neonatal cells was estimated by measurement of DNA as described by Kissane and Robins (1958). Data were expressed per 10^7 cells. The number of attached adult cells and the fraction of rod-shaped cells were determined by counting several random fields per dish under a light microscope. Attached cells consisted of $91.1 \pm 0.5\%$ ($n = 16$) rod-shaped cells. The estimated volume of adult rat myocytes is 25 times that of neonatal rat myocytes (Katzberg et al., 1977). The observed cellular ATP content of adult rat myocytes was also higher than that of neonatal rat myocytes. Data were expressed per 10^5 rod-shaped cells, thus matching initial ATP levels with neonatal rat myocytes and allowing direct comparison between the two cell types.

Adenosine formation

Incubations of neonatal myocytes were performed in duplicate at 37 °C in the medium described previously (Meghji et al., 1985). Adult myocytes were incubated in a slightly different buffer (125 mM NaCl, 2.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 10 mM Hepes, 1 mM $CaCl_2$ and 0.4 % BSA; pH 7.4, equilibrated with air) as described by Piper et al. (1982). Cells were pre-incubated for at least 60 min with inhibitors of adenosine deaminase (2′-deoxycoformycin; 30 μM) and adenosine kinase (5-iodotubercidin; 10 μM) to minimize further metabolism of adenosine (Newby, 1980; Newby et al., 1983), with in addition, IBTA (3 mM), dipyridamole (10 μM) or rabbit anti-(rat liver 5′-nucleotidase) serum (1:100) as indicated. After preincubation, cells were rinsed three times with Krebs buffer. ATP catabolism was induced by placing the cells in 1 ml of Krebs buffer containing 30 mM 2-deoxyglucose, 2 μg/ml oligomycin and in addition IBTA (3 mM) or dipyridamole (10 μM) as indicated, on a gently shaking water bath (30 strokes/min). Incubations were terminated 2–10 min later (neonatal cells) or 10–60 min later (adult cells) by removal of 0.9 ml of supernatant medium, of which 0.12 ml was reserved for assaying lactate dehydrogenase. Trichloroacetic acid (87 μl of 50 %, w/v) was added to the remaining 0.78 ml of medium, and 1 ml of 5 % (w/v) to the cells, and samples were then deproteinized and neutralized as described previously (Meghji et al., 1988).

Lactate dehydrogenase activity was determined by the method of Keiding et al. (1974), and cell intactness was assessed by comparison with lactate dehydrogenase activity in cells lysed with 10 mM Tes/NaOH (pH 7) containing 0.1 % Triton X-100.

Nucleotide and nucleoside analysis

The concentrations of metabolites were measured by h.p.l.c. as previously described (Gordon et al., 1986). When calculating values for metabolites in the cells, correction was made for the 100 μl of medium extracted with the cells.

Incorporation of adenosine into cellular nucleotides

Cells were preincubated with dipyridamole (10 μM) or IBTA (1 μM–3 mM) for 30 min. Cells were washed three times and then incubated in duplicate at 37 °C on a shaking water bath in 0.6 ml of Krebs buffer in Petri dishes (adult cells) or in 0.2 ml in 24-well plates (neonatal cells). The reaction was initiated by adding 20 μl containing 100 μM adenosine and 20 kBq of [^{3}H]adenosine (neonatal myocytes) or 60 μl containing 110 μM adenosine and 60 kBq of [^{3}H]adenosine (adult myocytes). After 10 min the reaction was terminated by addition of 220 μl (neonatal cells) or 660 μl (adult cells) of unlabelled carrier (1 mM each of adenosine, inosine, hypoxanthine and AMP in 1 % SDS). A 20 μl sample of cell lysate was spotted on to silica-gel-coated plastic plates and chromatographed in the solvent system of Shimizu et al. (1970).

Some experiments were done with suspensions of adult rat ventricular myocytes rather than adherent cells. Cells were preincubated alone or together with dipyridamole (30 μM) for 30 min in a 6-well plate. Incubations were then initiated by addition of IBTA (100 μM) alone or together with ^3H_2O (0.5 MBq), [^{14}C]mannitol (0.05 MBq) or dipyridamole (30 μM). At 5 min the entire content of each well (1 ml) was collected and placed in an Eppendorf tube containing 0.8 ml of bromododecane oil, beneath which was 0.2 ml of 5 % trichloroacetic acid, which was then centrifuged for 1 min at 10000 rev./min in a Wifug microfuge. The cells sedimented into the trichloroacetic acid solution at the bottom of the tube, whereas the supernatant remained above the oil layer. Medium (700 μl) was removed and added to 70 μl of 50 % trichloroacetic acid. The remainder of the supernatant on top of the oil and the oil in the Eppendorf tube were discarded. The medium and cell extracts were then neutralized as described previously (Meghji et al., 1988). IBTA was quantified by h.p.l.c. Samples (50 μl) were added to scintillation fluid and counted for ^{3}H or ^{14}C radioactivity.

Inhibition of ecto-5′-nucleotidase activity

Adult ventricular myocytes were preincubated with anti-(rat liver 5′-nucleotidase) serum (1:100) as indicated for at least 30 min. Cells were washed three times and then incubated at 37 °C in 1 ml of Krebs buffer on a shaking water bath. The reaction (in duplicate) was started by addition of 100 μl containing 2 mM AMP, [^{3}H]AMP (1.85 KBq), 15 mM adenosine and 103 mM sodium β-glycerophosphate. The reaction was terminated at 5 min and ecto-5′-nucleotidase activity was measured as described previously (Meghji et al., 1985).

RESULTS

Effects of IBTA and dipyridamole on adenosine formation by neonatal rat myocytes

Cells remained 99.4±0.3% intact in the absence and 99.7±0.1% in the presence of 2-deoxyglucose (30 mM) and oligomycin (2 μg/ml) at 10 min. In the additional presence of IBTA or dipyridamole, cells were 99.6±0.2% and 99.5±0.1% intact respectively ($n = 3$ in each case). Cell breakage did not exceed 5% in any of these experiments. IBTA is structurally similar to *S*-adenosyl-L-homocysteine. In separate experiments (results not shown) we established that it was not metabolized by purified *S*-adenosyl-L-homocysteine hydrolase to produce adenosine.

The changes in concentrations of nucleotides and nucleosides in response to metabolic poisoning for 2, 5 and 10 min are shown in Figures 1 and 2. 2-Deoxyglucose and oligomycin produced a 90% fall in ATP concentration in the cells in 10 min (Figure 1a). The rapid initial fall in ATP concentration (33±2%/min) was accelerated (43±4%/min) by IBTA, but was unaltered (29±4%/min) by dipyridamole.

In the presence of 2′-deoxyglucose and oligomycin the concentration of ADP transiently increased and then fell to below control levels (Figure 1b). The initial increase in ADP concentration was abolished by IBTA, but was not affected by dipyridamole.

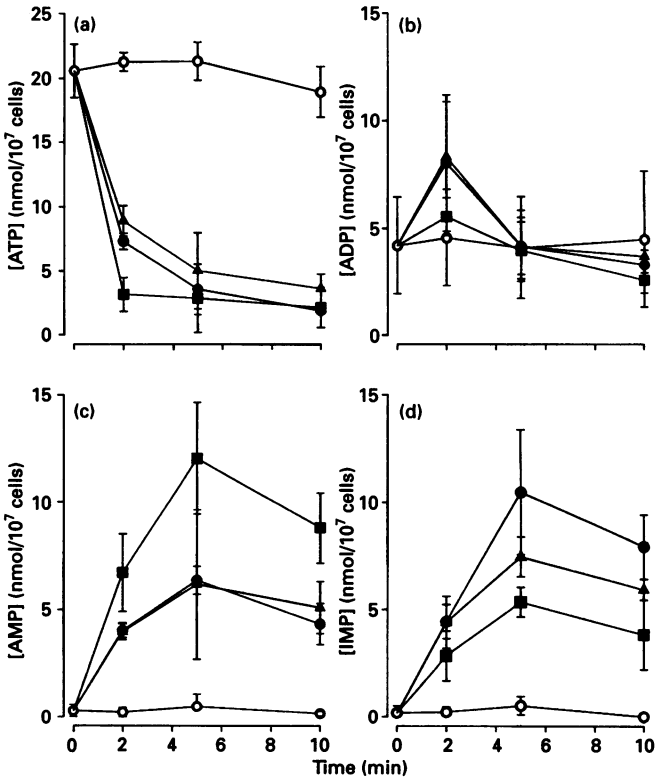

Figure 1 Nucleotide concentrations in 2-deoxyglucose- and oligomycin-treated neonatal rat ventricular myocytes

Myocytes [(2.48±0.43)×10⁶ cells/well] were incubated at 37 °C in Krebs bicarbonate/Hepes solution alone (○) or to which was added 30 mM 2-deoxyglucose and 2 μg/ml oligomycin (●), or the same drugs plus either 3 mM IBTA (■) or 10 μM dipyridamole (▲). In each experiment metabolites were measured in duplicate at zero time and at one of the indicated times because of the limited cell numbers available in each preparation. Experiments were repeated so that three measurements in duplicate were obtained for each time point; means±S.D. are shown.

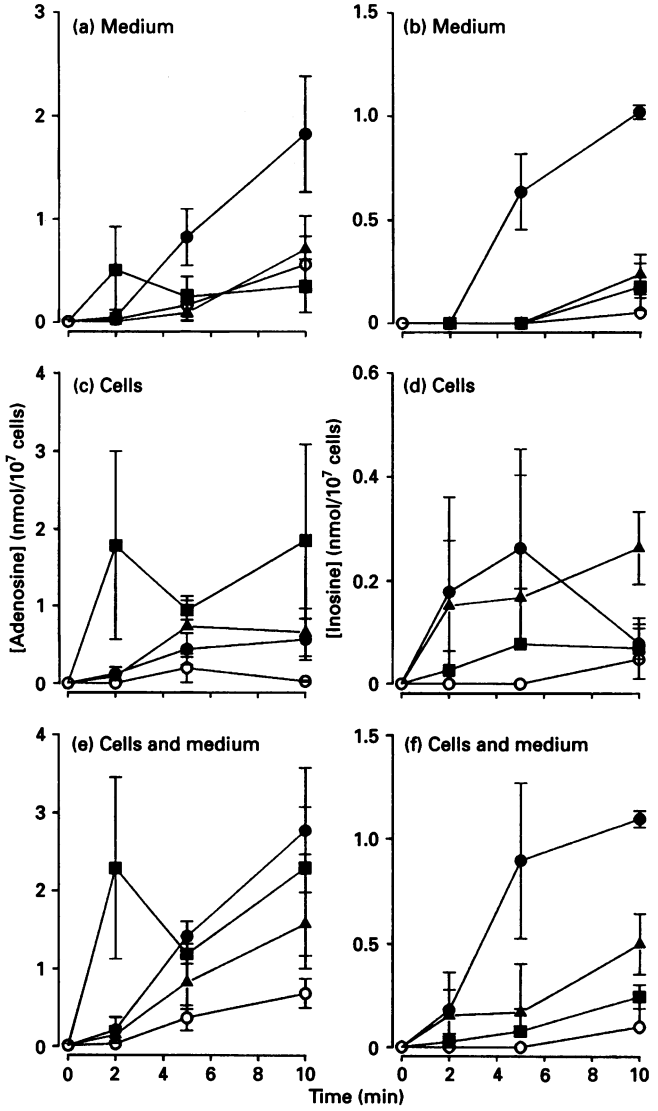

Figure 2 Nucleoside concentrations in 2-deoxyglucose- and oligomycin-treated neonatal rat ventricular myocytes

Myocytes [(2.48±0.43)×10⁶ cells/well] were incubated and metabolites measured as described in Figure 1.

The rate of AMP production over the first 2 min in presence of 2-deoxyglucose and oligomycin alone (1.9±0.1 nmol/min per 10^7 cells; Figure 1c) was increased by IBTA (3.2±1.0 nmol/min per 10^7 cells), but not by dipyridamole (1.9±0.1 nmol/min per 10^7 cells). IBTA led to a more sustained increase in AMP concentrations (Figure 1c). IMP production followed a similar time course to AMP production, but in this case addition of either IBTA or dipyridamole slightly inhibited the increase (Figure 1d).

Incubation with 2-deoxyglucose and oligomycin promoted both adenosine and inosine production (Figure 2), confirming and extending our previous result (Meghji et al., 1985). IBTA inhibited release of both adenosine and inosine into the medium and promoted the increase in adenosine concentration in the cell fraction (2 min and 10 min). Intracellular inosine levels tended to rise more slowly in the presence of IBTA. The data suggested that IBTA inhibited inosine production, but inhibited adenosine release rather than production. The effects of IBTA were therefore

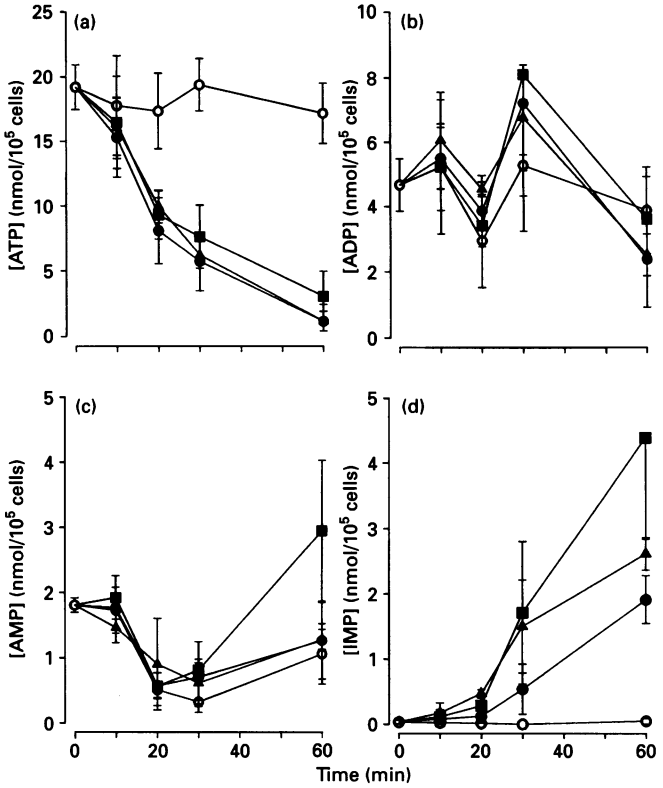

Figure 3 Nucleotide concentrations in 2-deoxyglucose- and oligomycin-treated adult rat ventricular myocytes

Myocytes [(7.57±1.18) × 10^4 cells/well] were incubated at 37 °C in Krebs Hepes solution alone (○) or to which was added 30 mM 2-deoxyglucose and 2 μg/ml oligomycin (●), or the same drugs plus either 3 mM IBTA (■) or 10 μM dipyridamole (▲). In each experiment metabolites were measured in duplicate at zero time and at one of the indicated times because of the limited cell numbers available in each preparation. Experiments were repeated so that three measurements in duplicate were obtained for each time point; means±S.D. are shown.

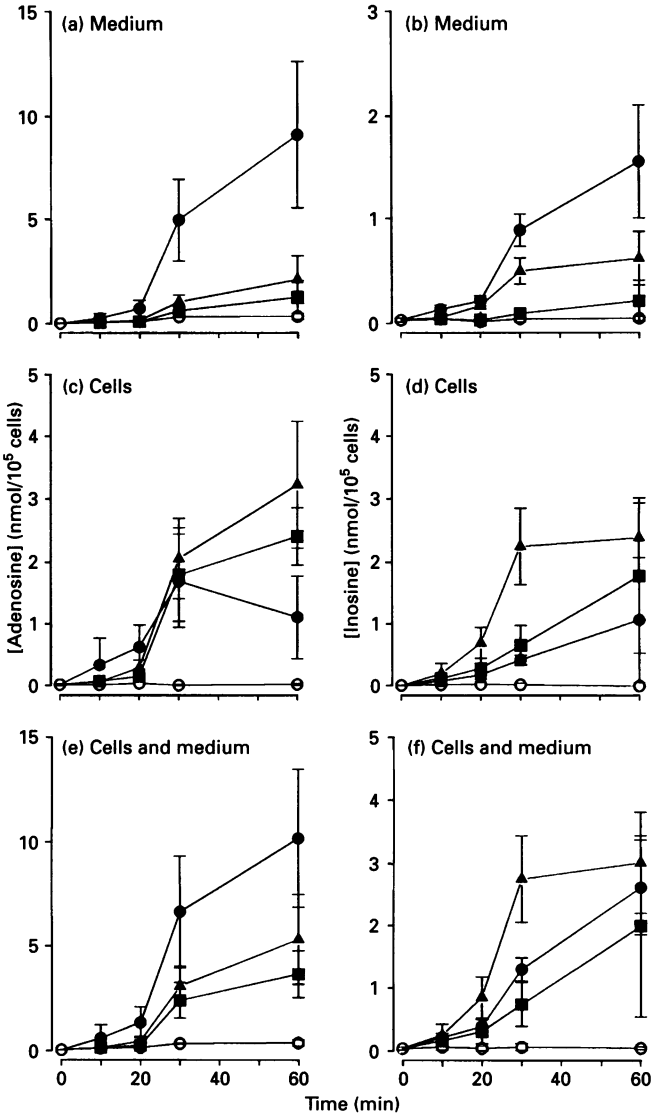

Figure 4 Nucleoside concentrations in 2-deoxyglucose- and oligomycin-treated adult rat ventricular myocytes

Myocytes [(7.57±1.18) × 10^4 cells/well] were incubated and metabolites measured as described in Figure 3.

compared with those of dipyridamole. With minor differences the effects of IBTA and dipyridamole were similar. As described previously (Meghji et al., 1985), dipyridamole almost completely prevented release of nucleosides to the medium. Dipyridamole also promoted a rise in intracellular adenosine concentration at 5 min, although not at 10 min. This effect of dipyridamole to decrease the total production of adenosine (Figure 2e) has been observed previously (Meghji et al., 1985) and can be explained by incomplete inhibition of adenosine deaminase or adenosine kinase.

Effects of IBTA and dipyridamole on adenosine formation by adult rat myocytes

Cell breakage did not generally exceed 5% under the experimental conditions used. However, when poisoned cells were incubated for 30–60 min it was sometimes greater than 5%, but never more than 10%. On average, cells remained 98.9±0.5% intact in the absence and 95.9±3.3% intact in the presence of 2-deoxyglucose and oligomycin at 60 min. In the additional presence of IBTA or dipyridamole, cells were 97.9±2.8% and 98.8±0.7% intact respectively ($n = 3$ in each case).

The changes in concentrations of nucleotides and nucleosides with time (0–60 min) and after the cells were poisoned with 2-deoxyglucose and oligomycin are shown in Figures 3 and 4. Addition of 2-deoxyglucose (30 mM) and oligomycin (2 μg/ml) produced a 70% fall in ATP concentration in cells in 30 min (Figure 3a). The initial fall in ATP concentration (1.4±0.3%/min) was very slow compared with that in neonatal myocytes and was unaltered in the presence of IBTA or dipyridamole. ADP concentrations were not markedly affected by 2-deoxyglucose and oligomycin treatment or by the additional presence of IBTA or dipyridamole (Figure 3b). The AMP concentrations initially decreased, followed by an increase (Figure 3c). IBTA, but not dipyridamole, tended to increase AMP concentrations after 60 min. IMP concentrations, in contrast, were increased continuously (Figure 3d), and both IBTA and dipyridamole tended to increase IMP concentrations after 30 min.

Adenosine and inosine production increased substantially in the presence of 2-deoxyglucose and oligomycin (Figure 4). Antiserum to rat liver ecto-5′-nucleotidase (1:100 dilution) did

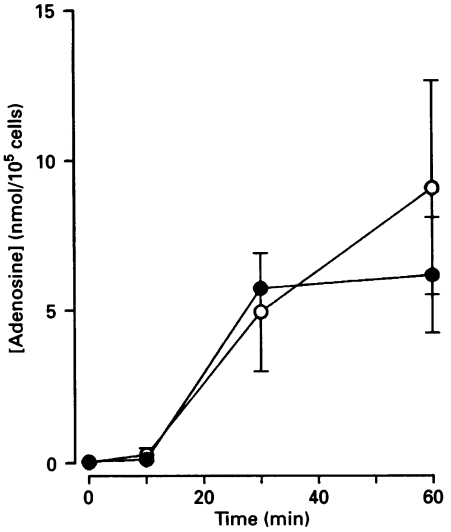

Figure 5 Effect of inhibition of ecto-5′-nucleotidase on adenosine concentrations in medium in 2-deoxyglucose- and oligomycin-treated adult rat ventricular myocytes

Myocytes [(6.41 ± 1.69) × 10^4 cells] were preincubated alone (○) or with anti-(rat liver ecto-5′-nucleotidase) serum (1:100 dilution in M-199 medium containing 4% fetal-calf serum) (●) for at least 30 min and then washed three times with Krebs Hepes solution before being incubated with 30 mM 2-deoxyglucose and 2 μg/ml oligomycin. In each experiment metabolites were measured in duplicate at zero time and at one of the indicated times because of the limited cell numbers available in each preparation. Experiments were repeated so that three measurements in duplicate were obtained for each time point; means ± S.D. are shown.

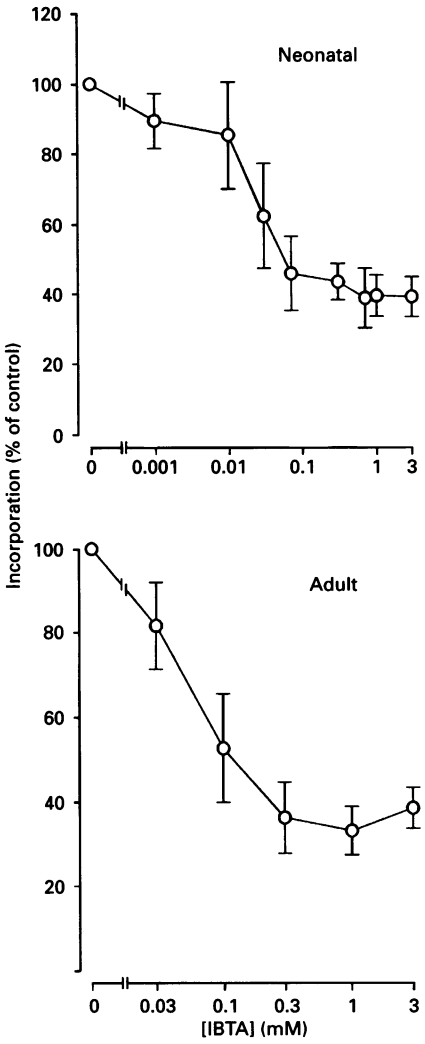

Figure 6 Effect of IBTA on adenosine incorporation into cellular nucleotides in neonatal and adult rat myocytes

Neonatal rat myocytes [(4.2 ± 0.6) × 10^5 cells/well] or adult myocytes [(5.3 ± 0.5) × 10^4 cells/well] were washed three times with Krebs buffer and then preincubated alone or with different concentrations of IBTA for at least 30 min. Incorporation of [^{3}H]adenosine (10 μM) into cellular nucleotides was measured as described in the Materials and methods section. Each point is the mean ± S.D. from three different duplicate experiments. Values for the rate of incorporation of adenosine are expressed as a percentage of that obtained in the absence of inhibitor.

not alter the rate of appearance of adenosine in the medium (Figure 5), demonstrating that, as in the neonatal cells, adenosine was formed intracellularly under these conditions. The same dilution of antiserum inhibited the dephosphorylation of exogenous AMP (180 μM) by adult myocytes by 88 ± 7% (n = 3). As with the neonatal cells, IBTA effectively inhibited the release of adenosine and inosine from adult cardiomyocytes into the medium. IBTA also enhanced the cellular concentrations of both nucleosides. Dipyridamole behaved similarly to IBTA, though it was slightly more effective at enhancing the cellular concentrations but less effective at inhibiting release.

Adenosine incorporation into cellular nucleotides

Because the actions of IBTA in both neonatal and adult cells strongly suggested that it can act as a transport inhibitor, we investigated its effect on incorporation of adenosine into cellular nucleotides as an index of inward transport (Figure 6). In neonatal myocytes, adenosine was incorporated into cellular nucleotides at a rate of 510 ± 70 pmol/min per 10^7 cells. IBTA (3 mM) inhibited incorporation by 61 ± 6% (n = 3). Adult myocytes incorporated adenosine at a rate of 163 ± 51 pmol/min per 10^5 cells. This was inhibited by 61 ± 5% (n = 3) by IBTA (3 mM) and 69 ± 6% (n = 3) by dipyridamole (10 μM).

Cellular uptake of IBTA

The total water (^{3}H) space and extracellular ([^{14}C]mannitol) space were calculated for each cell pellet [(7.6 ± 0.3) × 10^5 cells/well] and the difference between these values was used as an estimate of intracellular water (n = 3). [^{14}C]Mannitol (0.7 ± 0.1%) and ^{3}H (1.2 ± 0.3%) were recovered in the cell pellet, giving 0.6 ± 0.3 μl/10^5 cells as an estimate of intracellular water. A much larger proportion of IBTA (0.1 mM) was recovered in the cell pellet (26.4 ± 5.1%), suggesting that this compound was also present in the lipid phase All the added IBTA was recovered unmetabolized, by h.p.l.c. Dipyridamole (30 μM) decreased the recovery of IBTA slightly but not significantly (22.7 ± 4.6%), suggesting that, although IBTA can inhibit nucleoside transport, it enters the cell principally by a pathway other than the nucleoside transporter.

DISCUSSION

Adenosine formation in neonatal rat heart cells in response to 2-deoxyglucose and oligomycin is not altered by blockade of ecto-5′-nucleotidase (Meghji et al., 1985). Furthermore, nucleoside-transport inhibitors (e.g. dipyridamole) decrease adenosine re-

lease from cells and increase the intracellular concentration of adenosine, demonstrating that adenosine is formed intracellularly in neonatal myocytes, as in numerous other cell types (see Meghji et al., 1989). In metabolically poisoned adult rat cardiomyocytes contradictory results have been reported. Adenosine 5′-[α,β-methylene]diphosphate, an inhibitor of ecto-5′-nucleotidase, inhibited adenosine release by 80% (Bukoski and Sparks, 1986). Nevertheless a nucleoside-transport inhibitor also decreased adenosine release by 60%. These results cannot be reconciled, since the ectoenzyme contains no membrane-spanning domain and is inaccessible to cytoplasmic nucleotides (Bailyes et al., 1990; Misumi et al., 1990) and cytosolic 5′-nucleotidases are not inhibited by adenosine 5′-[α,β-methylene]diphosphate (Newby et al., 1987).

Adult myocytes were less susceptible to metabolic poisoning than neonatal cells, equivalent fractional falls in ATP taking more than 10 times as long to achieve with the same treatment. More than half of the ATP catabolized in adult cells was converted into adenosine, and about a quarter yielded IMP and inosine, thus demonstrating less flux through the AMP deaminase pathway than in neonatal cells, where AMP and adenosine accounted for about one-third of the ATP degraded, but IMP and inosine accounted for almost 50%. Adenosine is also the predominant catabolite in 2-deoxyglucose- and oligomycin-poisoned human cardiomyocytes (Smolenski et al., 1992). In the present study, inhibition of ecto-5′-nucleotidase did not affect adenosine formation rates, suggesting an intracellular pathway as in the neonatal cells.

To characterize further the intracellular pathway of adenosine formation we used IBTA, as a selective inhibitor of the IMP-selective cytosolic 5′-nucleotidase. However, IBTA profoundly affected the distribution of adenosine between cells and medium, suggesting that it may also inhibit nucleoside transport (Pierré and Robert-Géro, 1979). We therefore tested IBTA as an inhibitor of adenosine incorporation and demonstrated concentration-dependent inhibition which was incomplete, reaching a maximum inhibition of approx. 60% at $\geqslant 0.5$ mM. Direct measurements of cellular uptake of IBTA suggest that it is distributed in the lipid phase within cells. The limited aqueous solubility may explain the apparent inability to inhibit adenosine incorporation completely. We then compared directly the effects of IBTA and dipyridamole at concentrations which inhibited incorporation of adenosine into myocyte adenine nucleotides to a similar degree. We can argue therefore that any additional effects of IBTA may be due to action on cytosolic 5′-nucleotidase.

IBTA, unlike dipyridamole, enhanced the rise in AMP concentration in either neonatal or adult myocytes. Both agents inhibited the release of adenosine into the medium surrounding either cell type. However, this was not exactly matched by an accumulation of adenosine in the cells, except in the case of IBTA action on the neonatal cells; and hence total adenosine concentration was decreased by both IBTA and dipyridamole. These reciprocal effects on AMP and adenosine concentration may be explained by increased rephosphorylation of adenosine due to the residual activity of adenosine kinase on the increased concentration of adenosine trapped inside cells. Except in adult myocytes at 60 min, there was no evidence for an additional inhibitory effect on adenosine formation produced by IBTA compared with dipyridamole that could be attributable to inhibition of cytosolic 5′-nucleotidase.

These data imply that the IMP-selective 5′-nucleotidase makes only a small contribution to adenosine production in adult myocytes or in neonatal myocytes. IBTA is only a weak inhibitor of the AMP-selective enzyme, which is known to be active in rat heart (Truong et al., 1988), and would have been expected to exert at most a 20% effect (Skladanowski et al., 1989). IBTA slowed the rate of inosine formation in both neonatal and adult cells as compared with dipyridamole. This suggests that the IMP-selective 5′-nucleotidase is responsible for inosine formation in both cell types. However, IBTA decreased the concentration of IMP in neonatal myocytes more than did dipyridamole, and the concentration of inosine also decreased, whereas in adult myocytes IBTA enhanced the rise in IMP concentration more than dipyridamole did. Total inosine production decreased in the presence of IBTA, but not of dipyridamole. The results in the neonatal heart cells, where the AMP deaminase pathway is more dominant, are consistent with an inhibition of AMP deaminase by IBTA, noted with the purified enzyme (Lawrence et al., 1980). However, adult rat heart AMP deaminase is not inhibited by IBTA (Skladanowski et al., 1989).

In conclusion, the site for adenosine formation in adult rat myocytes is predominantly intracellular, as in neonatal rat myocytes (Meghji et al., 1985) in response to metabolic poisoning. The release of nucleotides and extracellular production of adenosine noted in studies with perfused hearts may arise as a result of physical distortion not produced in experiments on cultured myocytes; alternatively, release may be from endothelial cells (Pearson and Gordon, 1979) or sympathetic neurones (Imai et al., 1989). The failure of IBTA to inhibit adenosine formation nevertheless implies a limited role for the IMP-selective cytosolic 5′-nucleotidase in these cells, in contrast with polymorphonuclear leucocytes. The effect of IBTA on the nucleoside transporter will limit its use to clarify the pathways of adenosine formation. Thus the need remains for more specific and potent compounds to study the intracellular catabolism of AMP and IMP.

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