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ADENOSINE UPTAKE BY ISOLATED MOUSE PANCREATIC ISLETS

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Abstract—Adenosine uptake (consisting of both membrane transport and subsequent metabolism) was studied in mouse pancreatic islets in order to further evaluate the ability of adenosine to stimulate the function of B-cells. It was found that: (1) 0.05, 0.1 and 1 mM (U-^{14}C)-adenosine uptake was linear for approximately 20 min; (2) The curve describing the relationship between adenosine concentration and rate of uptake was hyperbolic; (3) (U-^{14}C)-adenosine uptake was higher than that of (8-^{14}C)-adenosine, possibly reflecting ribose-metabolism; (4) Dipyridamol strongly inhibited the uptake of 5 μM and 0.1 mM adenosine, but there was no effect on the uptake of 1 mM adenosine. This finding suggests the presence of both a low and a high K_m-transport system, and the occurrence of passive diffusion; (5) 1 mM inosine inhibited (8-^{14}C)-adenosine uptake; (6) (8-^{14}C)-adenosine uptake was energy-dependent, since both antimycin A and low temperature inhibited the uptake; (7) Glucose, glipizide, theophylline and starvation did not affect adenosine uptake, whereas 10 mM leucine stimulated (U-^{14}C)-adenosine uptake without affecting (8-^{14}C)-adenosine uptake; (8) Tissue culture of isolated islets in a medium containing high concentrations of adenosine decreased both adenosine uptake and adenosine oxidation, possibly reflecting saturation of the intracellular adenosine pools. On the other hand, culture at a high glucose concentration stimulated the uptake of adenosine; (9) Dipyridamol inhibited adenosine stimulated (pro)insulin biosynthesis without affecting that evoked by glucose. It is concluded that the characteristics of adenosine uptake in mouse pancreatic islets are similar to that of other tissues, mostly reflects nucleotide formation and is a prerequisite for adenosine stimulation of (pro)insulin biosynthesis.

INTRODUCTION

Adenosine has recently been shown to be a potent stimulator of insulin secretion and biosynthesis in both short- and long-term experiments [1]. These stimulatory effects of adenosine have been assumed to be due to its oxidative degradation [1–3]. On the other hand, recent studies have shown that fairly low concentrations of adenosine inhibit insulin secretion whilst decreasing the basal adenylate cyclase activity of the islets [4]. Against this background, it is quite evident that the intracellular concentration of adenosine in the B-cell may play a role in regulating insulin secretion but so far there is no information on mechanisms regulating the uptake of adenosine in pancreatic islets. In order to further elucidate this topic, we have studied the uptake of radioactively labelled adenosine in isolated pancreatic islets with special regard to the effects of certain known modulators of islet cell function.

MATERIALS AND METHODS

Chemicals. Collagenase (type CLS) was obtained from Worthington Biochemical Corp. (Freehold, NJ). Bovine albumin (fraction V), adenosine, adenine, inosine, L-leucine and antimycin A were supplied by Sigma Chemical Co. (St. Louis, MO). (U-^{14}C)-adenosine, (8-^{14}C)-adenosine and (6.6′(*n*)-^{3}H) sucrose and L-(4.5)^{3}H-leucine were purchased from The Radiochemical Centre (Amersham, U.K.). Hyamine hydroxide 10-X, Soluene and Instagel were from Packard Instruments (Downers Grove, IL). Econofluor was from New England Nuclear (Boston, MA), D-glucose was obtained from Mallinckrodt Chemical Works (St. Louis, MO). TCM 199, either glucose-free or containing 5.5 mM glucose, Hanks' solution and calf serum were obtained from Statens Bakteriologiska Laboratorium (Stockholm, Sweden). RPMI 1640 was supplied by Flow Laboratories Ltd (Irvine, U.K.). Penicillin and streptomycin were from Glaxo Laboratories Ltd. (Greenford, U.K.). Other chemicals used were of analytical grade from E. Merck (Darmstadt, West Germany). Glipizide was a gift from Carlo Erba S.p.A. (Milan, Italy). Dipyridamol was a gift from Boehringer (Ingelheim, West Germany).

Islet preparation. Pancreatic islets were isolated, by means of a collagenase digestion method [5], from male NMRI mice (Anticimex, Stockholm, Sweden), which had been starved overnight. In some experiments the starvation period was extended up to 65 hr with free access to drinking water.

The isolated islets were transferred to culture dishes containing culture media RPMI 1640 (11.1 mM glucose) or TCM 199 (3.3, 5.5 or 28 mM glucose) supplemented with 10 per cent (v/v) calf serum and antibiotics. The islets were cultured free-floating for 1–7 days [6] prior to the uptake studies.

Adenosine uptake. Islet uptake of adenosine was studied using a technique similar to that previously described by Hellman *et al.* [7]. Groups of 10 islets were incubated in 100 μl of a bicarbonate-buffered medium [8] containing 10 mM HEPES, (U-^{14}C) or (8-^{14}C)-adenosine (15–20 mCi/mmol) and test sub-

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stances as given below. The incubations were performed for 15 min at +37° in a 5 per cent CO_2-saturated atmosphere unless otherwise stated. After the incubation, the islets were washed 4 times at +4° in the above medium, except that labelled adenosine was omitted. After the final washing, the islets were transferred by means of a braking-pipette to a scintillation vial, for subsequent dissolution in 100 μl of Soluene. The radioactivity was estimated in a liquid scintillation counter after addition of 5 ml Econofluor. In 5 control experiments using ^{3}H-sucrose (10 μCi/ml) as an extracellular tracer, it was found that after the washing procedure only 5.2 ± 5.2 per cent of the radioactivity in the ^{14}C-channel was confined to the extracellular space, thus not allowing extracellular contamination to exceed 11 per cent. However, in order to monitor contamination from the washing buffer, drops of equal size from the final washing medium were treated in a similar way. Corrections were made in less than 10 per cent of the experiments, when these values exceeded 10 per cent of the actual uptake figures.

Oxidation experiments. These experiments were performed essentially as previously described [9]. Groups of 10 cultured islets (TCM 199; 3.3, 5.5 or 28 mM glucose) were incubated for 120 min in a bicarbonate buffered medium [10]. The radioactively labelled substrate used was (U-^{14}C)-adenosine (13.3 mCi/mmol). Unlabelled adenosine was added to give a final concentration of 0.1 mM.

(Pro)insulin biosynthesis. These experiments were performed essentially as previously described [1, 11]. Groups of 10 islets cultured for 2–5 days in RPMI 1640 were incubated for 120 min in a medium containing essential amino-acids, ^{3}H-leucine (100 μCi/ml) and supplements as given in Table 4.

RESULTS

The time-course of (U-^{14}C)-adenosine uptake is shown in Fig. 1. At all concentrations tested (1, 0.1, 0.05 mM), the uptake of adenosine was virtually

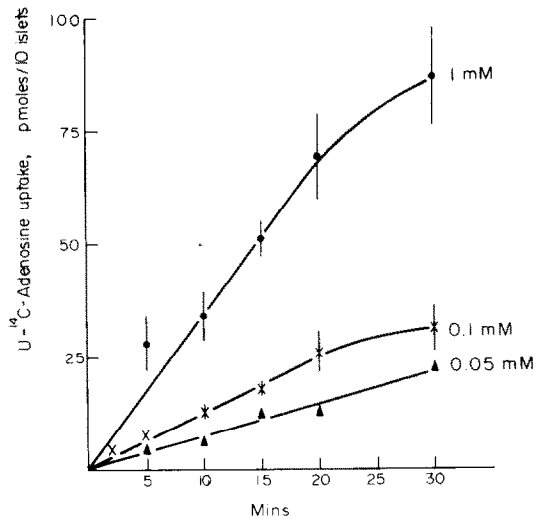

Fig. 1. Time-course of (U-^{14}C) labelled adenosine uptake at 0.05, 0.1 and 1 mM adenosine. The results are expressed as pmoles/10 islets and are the means ± S.E.M. of 3–9 observations.

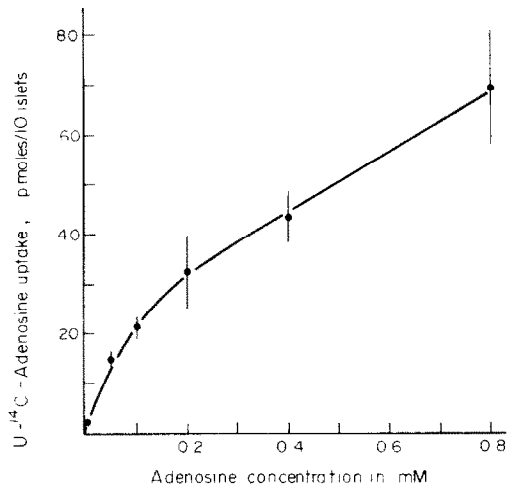

Fig. 2. Concentration dependence of (U-^{14}C)-adenosine uptake for 15 min. Each value is the mean ± S.E.M. of 5 observations and the results are expressed in pmoles/10 islets.

linear for the first 20 min, after which time no further increase of the uptake was recorded. Against this background, it was decided to adopt a 15 min incubation period as a standard protocol. Figure 2 shows the effects of the adenosine concentration on the (U-^{14}C)-adenosine uptake. Islets cultured for 1–4 days were used in most of the present studies. It was, however, evident that neither a prolonged culture period (7 days), nor a total lack of culture (freshly isolated islets) affected the uptake of U-^{14}C)-adenosine (results not shown). These studies also showed that the uptake of (U-^{14}C)-adenosine was greater than that of (8-^{14}C)-adenosine ($P < 0.05$).

The temperature dependence of (8-^{14}C)-adenosine uptake is shown in Fig. 3. The decrease in adenosine

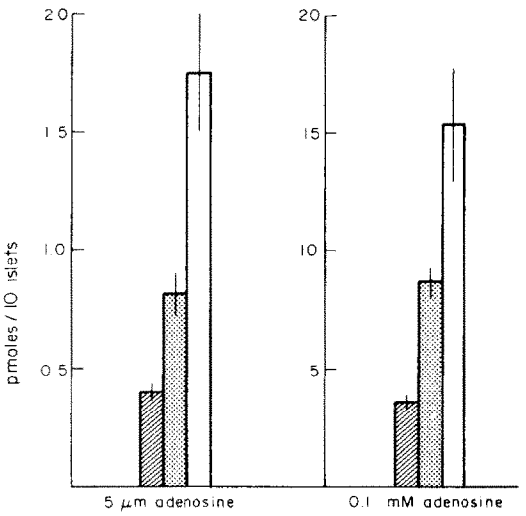

Fig. 3. Temperature dependence of (8-^{14}C)-adenosine uptake for 15 min at both 5 μM and 0.1 mM adenosine. The uptake at 4° is shown in the hatched column, the uptake at 24° is shown in the dotted column and the uptake at 37° is shown in the open column (means ± S.E.M. of 5 observations).

Table 1. (8-^{14}C)adenosine uptake in pmoles/10 islets/15 min

Addition to the medium	Adenosine concentration	
	5 μM	0.1 mM
None	1.76 ± 0.25	15.4 ± 2.43
10 μM antimycin A	0.65 ± 0.03*	8.34 ± 1.38‡
1 mM inosine	0.99 ± 0.17†	18.8 ± 1.99

Effects of 1 mM inosine and 10 μM antimycin A on (8-^{14}C)adenosine uptake. The incubations were carried out for 15 min and the results are expressed as pmol/10 islets. Each value is the mean ± S.E.M. of 4–6 observations.

* $P < 0.01$ for statistical significance against values obtained at 5 μM adenosine.

† $P < 0.05$.

‡ $P < 0.05$ when compared with 0.1 mM adenosine alone.

uptake with lowered temperature was almost identical at 5 μM and 0.1 mM adenosine. Evidence for an energy-dependence of the uptake is shown in Table 1. Thus, 10 μM antimycin A inhibited (8-^{14}C)-adenosine uptake at both adenosine concentrations 5 μM and 0.1 mM. Moreover, the uptake of adenosine was found to be influenced by the presence of other purine-nucleosides, since 1 mM inosine inhibited the (8-^{14}C)-adenosine uptake at 5 μM adenosine, whereas no effect was recorded at 0.1 mM adenosine (Table 1).

Table 2 shows the effects of dipyridamol, a known inhibitor of carrier-mediated adenosine transport, on (U-^{14}C)-adenosine uptake. The uptake of adenosine at both a low (5 μM) and a higher (0.1 mM) concentration was strongly inhibited by dipyridamol, in a concentration-dependent manner. At concentrations of 1 mM adenosine uptake was, however, not inhibited by the presence of 40 μM dipyridamol. On the other hand, the further addition of 1 mM adenine reduced adenosine uptake at 1 mM adenosine.

The effects of various known B-cell secretagogues on adenosine uptake were tested (detailed results are not shown). The uptake of (U-^{14}C)-adenosine was significantly increased by 10 mM 1-leucine ($P < 0.01$), whereas 16.7 mM glucose, 16.7 mM glucose + 5 mM theophylline and 16.7 mM glucose + 1 μg/ml glipizide had no effect. (8-^{14}C)-adenosine uptake was, however, not affected by the addition of 1-leucine. Starvation for 65 hr before islet isolation had no effect on either (U-^{14}C)-adenosine or (8-^{14}C)-adenosine uptake.

The long-term effects of different glucose and adenosine concentrations on the islet uptake of adenosine are shown in Table 3. The uptake of (U-^{14}C)-adenosine was generally higher than that of (8-^{14}C)-adenosine ($P < 0.05$) in all groups of islets. The addition of 10 mM adenosine to the low glucose

Table 2. (U-^{14}C)adenosine uptake in pmoles/10 islets/15 min

Addition to the medium	Adenosine concentration		
	5 μM	0.1 mM	1 mM
None	2.73 ± 0.41	20.8 ± 1.80	53.1 ± 4.68
4 μM dipyridamol	0.69 ± 0.07*	12.3 ± 1.60†	–
40 μM dipyridamol	0.33 ± 0.05*	5.53 ± 0.85‡	41.6 ± 6.83
40 μM dipyridamol + 1 mM adenosine	–	3.89 ± 0.30‡	29.6 ± 6.03§

Effects of two different concentrations of dipyridamol and 1 mM adenine on (U-^{14}C)adenosine uptake by isolated pancreatic islets. The studies were performed at three different adenosine concentrations (5 μM, 0.1 mM and 1 mM adenosine) and the incubations were carried out for 15 min. The results are expressed as pmoles/10 islets. Each value is the mean ± S.E.M. of 4–7 observations.

* $P < 0.01$ when compared with values for 5 μM adenosine alone.

† $P < 0.05$.

‡ $P < 0.001$ when compared with values for 0.1 mM adenosine alone.

§ $P < 0.05$ when compared with values for 1 mM adenosine alone.

Table 3. ^{14}C-labelled 0.1 mM adenosine uptake and oxidation.

Culture conditions	0.1 mM (8-^{14}C) adenosine uptake	0.1 mM (U-^{14}C) adenosine uptake	0.1 mM (U-^{14}C) adenosine oxidation
3.3 mM glucose (control)	10.0 ± 1.0	14.6 ± 0.8	32.9 ± 3.8
3.3 mM glucose + 10 mM adenosine	6.82 ± 0.72*	9.96 ± 0.66†	16.1 ± 2.5§
5.5 mM glucose	10.1 ± 1.3	15.1 ± 1.5	27.1 ± 3.5
28 mM glucose	14.0 ± 1.2*	19.1 ± 1.7‡	32.1 ± 4.8

The effects of long-term culture (7 days), in medium TCM 199 at different glucose and adenosine concentrations, on U- or (8-^{14}C)adenosine uptake and (U-^{14}C)adenosine oxidation. The uptake (15 min) and oxidation (120 min) studies were performed at 0.1 mM adenosine. Values are expressed in pmoles/10 islets (means ± S.E.M. of 5–7 observations).

* $P < 0.05$ when compared with control (8-^{14}C) adenosine uptake.

† $P < 0.01$ and ‡ $P < 0.05$ respectively, when compared with U-^{14}C adenosine uptake of control culture (3.3 mM glucose).

§ $P < 0.01$ when compared with control (U-^{14}C) adenosine oxidation.

Table 4. Effects of adenosine and dipyridamol on (pro)insulin biosynthesis

Additions to the medium	(a) 0	(b) 16.7 mM glucose	(c) 16.7 mM glucose + 40 μM dipyridamol	(d) 0.1 mM adenosine	(e) 0.1 mM adenosine + 40 μM dipyridamol	(f) 1 mM adenosine	(g) 1 mM adenosine + 40 μM dipyridamol	(h) 1 mM adenosine + 40 μM dipyridamol + 1 mM adenine
PI-I	0.9 ± 0.2	8.9 ± 1.0***	6.6 ± 0.8	2.3 ± 0.5*	0.5 ± 0.2*	10.7 ± 2.0***	1.7 ± 0.6***	1.4 ± 0.4
TCA	26.0 ± 3.3	72.8 ± 8.1***	54.3 ± 4.2	28.7 ± 3.9	15.1 ± 2.7	68.9 ± 5.6***	23.8 ± 3.9***	21.3 ± 3.7
Per cent	3.6 ± 0.7	12.5 ± 1.3***	12.5 ± 1.4	7.6 ± 0.8**	3.6 ± 1.1*	15.1 ± 1.9***	6.5 ± 1.4**	5.9 ± 1.1

Groups of 10 cultured islets were incubated for 120 min in a balanced salt solution supplemented with essential amino acids and ^{3}H-leucine [1]. Further additions to the media are given in the table. The values for (pro)insulin biosynthesis (PI-I) and total protein biosynthesis (TCA) are expressed in cpm $\times 10^{-3}$/10 islets and 120 min. The percentage figures (per cent) denote the values for the ratio between the radioactivity in (pro)insulin and in TCA-precipitable proteins for each individual incubation. The values are means ± S.E.M. for 8 experiments. The statistical significances of differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) refer to the following comparisons: (b) vs (a), (d) vs (a), (f) vs (a), (e) vs (d) and (g) vs (f).

culture medium gave a substantial reduction of both the (U-^{14}C)- and the (8-^{14}C)-adenosine uptake. On the other hand, culture at a high glucose concentration induced an increase of the uptake of both labelled compounds. The formation of $^{14}CO_2$ from 0.1 mM (U-^{14}C)-adenosine was examined in the different groups of cultured islets. The adenosine exposed islets produced less $^{14}CO_2$ than the three other groups of islets (Table 3). The difference disappeared however, following a 2 hr preincubation in the absence of adenosine (results not shown).

Table 4 shows the effects of dipyridamol and adenine on adenosine-stimulated (pro)insulin and total protein biosynthesis. The stimulatory effect of 1 mM adenosine was similar to that of 16.7 mM glucose, whereas that of 0.1 mM adenosine was much smaller ($P < 0.001$) and confined to that of (pro)insulin biosynthesis. This in turn meant that the percentage figure, expressing (pro)insulin biosynthesis as a percentage of total protein biosynthesis in each individual experiment, was significantly increased already with the lower adenosine concentration. 40 μM dipyridamol inhibited both (pro)insulin and total protein biosynthesis when added to 0.1 or 1.0 mM adenosine but glucose-stimulated biosynthesis was unaffected. The further addition of 1 mM adenine to 40 μM dipyridamol and 1.0 mM adenosine was without inhibitory effect.

DISCUSSION

The cellular uptake of adenosine has been studied in several cell-systems, including erythrocytes [12], endothelial cells [13], myocardium [14], leukemia cells [15], blood platelets [16] and HeLa cells [17]. Thus a saturable, specific uptake mechanism has been demonstrated. Since the total uptake process involves both membrane transport and metabolism within the cell, adenosine uptake might be influenced by either or both of these processes. Membrane transport has been assumed to be rate-limiting, since adenosine never accumulates within the cells. This idea has been confirmed in a recent paper by Lum *et al.* [15], who measured transport for very short time periods in leukemia cells with inhibited metabolism. They found, however, that conventional uptake experiments performed at concentrations of approximately 0.1 mM adenosine do not represent transport alone, since there was a most significant release of labelled metabolic products of adenosine into the surrounding medium. Only by conversion to nucleotides will the labelled adenosine molecule be retained within the cell, whereas inosine, hypoxanthine and adenine can easily be released from the cells. Therefore, only uptake experiments performed at very low adenosine concentrations will mainly represent transport, since adenosine phosphorylation is very active at these concentrations. The rapid deamination of adenosine operating at higher adenosine concentrations gives uptake values which represent transport only to a very low extent. The aim of this study was primarily to relate total adenosine uptake to our previous investigation of both islet adenosine metabolism and its effects on the concomitant specific functions of the B-cells [1]. In this respect, the total uptake studies would be of

much greater interest than those of pure transport alone.

Two different systems for specific adenosine transport have been suggested, one with a low K_m-value and one with a high K_m-value [13, 16]. Furthermore, passive diffusion has been suggested to be important at high adenosine concentrations [12, 13], although others have questioned its existence [15]. However, our present finding of a hyperbolic dose-response curve (Fig. 2), which confirms earlier data [12, 13, 16], is in good agreement with the hypothesis of passive diffusion. Thus the rapid increase in adenosine uptake at low concentrations may be due to the low K_m-transport system, and the almost linear second phase mediated via both passive diffusion and the high K_m-transport system. Dipyridamol (40 μM) has been reported to almost completely block the low K_m-transport system [15]. In our study, 40 μM dipyridamol strongly inhibited the uptake of 5 μM adenosine, whereas the uptake at 0.1 mM adenosine was inhibited by 70 per cent and the uptake at 1 mM adenosine was not affected. Further addition of 1 mM adenine, known to inhibit the high K_m-transport system [16], did significantly inhibit 1 mM adenosine uptake, thus producing an effect of dipyridamol at this adenosine concentration. This finding suggests the presence of a high K_m-transport system in pancreatic islets. The non-dipyridamol and non-adenine inhibited component could be passive diffusion, which would be expected to be linear and non-energy dependent.

The finding of a substantially decreased adenosine uptake in islets cultured in the presence of adenosine might well be due to a decreased diffusion gradient, which could in turn depend on increased intra-cellular pools of adenosine and its metabolites. In support of this hypothesis, we found that the (U-^{14}C)-adenosine oxidation of the islets cultured at a high adenosine concentration was significantly lower than that of islets cultured at different glucose concentrations. The decreased oxidation observed may therefore reflect saturated intracellular pools of adenosine, which caused a dilution of isotopic label in islets cultured in adenosine.

The temperature dependence of both adenosine transport and uptake is well documented [15, 18]. In our system, we observed a strong decrease in the uptake of both 5 μM and 0.1 mM (8-^{14}C)-adenosine when the temperature was lowered. At 4° both enzymatic reactions and transport carriers are considerably slowed, and the uptake values therefore approached those observed after inhibition with 40 μM dipyridamol and 1 mM adenine (Table 2).

Antimycin A was found to inhibit (8-^{14}C)-adenosine uptake at adenosine concentrations of both 5 μM and 0.1 mM. This finding suggests that adenosine uptake is ATP-dependent, which would be expected if uptake is taken to represent nucleotide formation. Indeed, t.l.c. of TCA-extracted (2-^{3}H)-adenosine labelled islets showed that about 90 per cent of the radioactivity was confined to the nucleotide pools (M. Welsh, unpublished work). There is also evidence for a low purine-specificity in the transport system, since 1 mM inosine slightly inhibited 5 μM adenosine uptake. This inhibition could however, possibly reflect an excessive IMP formation from inosine, which would in turn dilute the radioactive nucleotide pool and be recorded as a decreased uptake. It is worthy of note, in this context, that the fraction of nucleotides formed from inosine is low [15].

The recent demonstrations of the presence in pancreatic islets of nucleoside phosphorylase (EC 2.4.2.1) [19, 20] may explain our present finding of a higher uptake of (U-^{14}C)-adenosine than of (8-^{14}C)-adenosine. Nucleoside phosphorylase splits inosine to ribose-1-phosphate and hypoxanthine. The former will enter carbohydrate metabolism and stay inside the cell, whereas the latter will either be released to the medium [15] or metabolized into IMP by hypoxanthine phosphoribosyl transferase (EC 2.4.2.8). The release of hypoxanthime could therefore cause a difference in the estimated adenosine uptake when (8-^{14}C)-adenosine is used as a marker instead of the uniformly labelled adenosine.

Our studies on the effects of different B-cell secretagogues on adenosine uptake, showed that there was no effect of either 16.7 mM glucose, theophylline of glipizide. However, 10 mM l-leucine caused a significant increase in (U-^{14}C)-adenosine uptake, an effect which was not seen when (8-^{14}C)-adenosine uptake was studied. This finding suggests that leucine stimulates adenosine uptake by affecting ribose-1-P metabolism. On the other hand, we have previously reported that starvation increased (U-^{14}C)-adenosine oxidation in isolated islets [2]. In the present study however, we were unable to demonstrate an effect of starvation on the uptake of either (U-^{14}C) or (8-^{14}C)-adenosine. Our present finding that high-glucose cultured islets displayed an enhanced rate of adenosine uptake, nevertheless indicates that the functional state of the islets may influence the uptake of nucleosides. This increased uptake was almost entirely confined to the uptake of adenosine labelled in the 8-position, suggesting that this particular group of islets have larger nucleotide and nucleoside pools. Indeed, we have previously demonstrated increased ATP concentrations in high glucose cultured islets [21].

In a further attempt to demonstrate a correlation between rates of adenosine uptake and islet B-cell function, we investigated the influence of dipyridamol on adenosine-stimulated (pro)insulin biosynthesis. Indeed, dipyridamol was found to inhibit markedly this process leaving that of glucose-stimulated biosynthesis unaffected. Obviously, at 1.0 mM adenosine the rate of biosynthesis was more decreased than that of adenosine uptake, which, however, may be due to the difference in incubation time. The finding that the further addition of adenine did not potentiate the dipyridamol induced inhibition can at least partly be explained by our recent demonstration that adenine itself has a weak stimulatory effect on (pro)insulin biosynthesis [1].

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