Effects of adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine on rat islet function and metabolism

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Adenosine (1.0–100 μ M), *N*⁶-phenylisopropyladenosine (0.1–10 μ M) and 2-deoxyadenosine (10 mM) all produced a dose-dependent inhibition of glucose-stimulated insulin release. The inhibition of glucose-stimulated insulin release by adenosine and *N*⁶-phenylisopropyladenosine was abolished by 3-isobutyl-1-methylxanthine (0.1 mM), whereas 2-deoxyadenosine inhibited insulin release even in the presence of 3-isobutyl-1-methylxanthine. These adenosine nucleosides also inhibited the release of insulin induced by 4-methyl-2-oxopentanoate (20 mM), DL-glyceraldehyde (30 mM) and L-leucine (20 mM). Adenosine (10 μ M), *N*⁶-phenylisopropyladenosine (10 μ M) and 2-deoxyadenosine (10 mM) did not inhibit insulin biosynthesis or [U-¹⁴C]glucose oxidation at concentrations of the nucleosides that gave maximal inhibition of insulin release. However, adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine produced marked inhibition of the glucose-stimulated increases seen in islet cyclic AMP accumulation. Similar to its effects on insulin release, 3-isobutyl-1-methylxanthine (0.1 mM) antagonized the inhibitory effects of cyclic AMP accumulation produced by adenosine and *N*⁶-phenylisopropyladenosine, but had no effect on the inhibition of cyclic AMP accumulation seen with 2-deoxyadenosine. These results show that adenosine and its specifically modified analogues, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine, are strong inhibitors of insulin release from rat islets, a function that appears to be the consequence of their ability to inhibit the accumulation of cyclic AMP. It is proposed that the B cells, in common with many other tissues, may possess two different sites at which adenosine nucleosides interact to produce their biological effects; these are the so-called 'P' and 'R' sites first described by Londos & Wolff [(1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 5482–5486].

The adenosine nucleosides have diverse physiological effects on various tissues (see Arch & Newsholme, 1978, for a review). Thus they inhibit hormone-stimulated lipolysis by adipose tissue (Dole, 1961; Fain, 1973), they are potent vasodilators of coronary blood vessels (Berne, 1963), causing smooth-muscle relaxation (McKenzie *et al.*, 1977*a,b*), and they act as inhibitory neurotransmitters (Burnstock *et al.*, 1970, 1972).

The observation by Sattin & Rall (1970) that adenosine enhanced cyclic AMP accumulation in brain slices provided evidence for a unifying mechanism for the effects of this nucleoside. Indeed adenosine was subsequently shown to increase cyclic AMP in many other tissues, including platelets (Haslam & Lynham, 1972), neuroblastoma cells (Blume & Foster, 1975), adrenal cells and Leydig-tumour cells (Wolff & Cook, 1977). In contrast, adenosine inhibits cyclic AMP accumulation in

adipocytes treated with catecholamines (Fain *et al.*, 1972).

As in other tissues, adenosine has been shown to affect the physiology of the pancreatic islets, where micromolar concentrations of the nucleoside inhibit glucose-stimulated insulin release and adenylate cyclase activity (Ismail *et al.*, 1977). In contrast with that report, millimolar concentrations of adenosine have been shown to enhance glucose-stimulated insulin release (Feldman & Jackson, 1974; Taniguchi *et al.*, 1977; Andersson, 1978) and insulin biosynthesis in mouse islets (Andersson, 1978). However, Jain & Logothetopoulos (1977, 1978) found no effect of adenosine on glucose-stimulated insulin release or biosynthesis in rat islets.

We report here a detailed investigation into the effects of adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine on insulin secretion and biosynthesis. In addition, we have measured glucose

oxidation and islet cyclic AMP content in the presence of adenosine and its analogues.

Experimental

Materials

Bovine serum albumin (fraction V), collagenase (type V), adenosine, 2-deoxyadenosine, 3-isobutyl-1-methylxanthine, DL-glyceraldehyde, 4-methyl-2-oxopentanoic acid, L-leucine and inosine were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. *N*⁶-Phenylisopropyladenosine was from Boehringer Corp. (London), Lewes, Sussex, U.K. CNBr-activated Sepharose 4B was from Pharmacia (G.B.), London W.5, U.K. Radiochemicals were supplied by Amersham International, Amersham, Bucks., U.K. Human insulin standard and purified human insulin and insulin-binding reagent were obtained from Wellcome Reagents, Beckenham, Kent, U.K. Cyclic AMP radioimmunoassay kits were purchased from New England Nuclear Corp., Southampton, U.K. Soluene 350 was obtained from Packard Instruments, Caversham, Berks., U.K. All other reagents and chemicals used in this study were of the purest grade commercially available.

Preparation of guinea-pig anti-insulin γ -globulin

Antiserum to human insulin was prepared in guinea pigs by the method of Robinson & Wright (1961). The crude γ -globulin fraction from this antiserum was then obtained by precipitation with $(\text{NH}_4)_2\text{SO}_4$ followed by dialysis against deionized distilled water and freeze-drying (Hales & Randle, 1963).

Isolation of islets

Fed male Sprague-Dawley rats (150–200 g) were killed by cervical dislocation, and the pancreas was removed. Islets were then isolated by the collagenase digestion procedure described in detail by Howell & Taylor (1968).

Insulin release

Groups of five islets were placed in tubes containing 0.4 ml of bicarbonate-buffered medium, pH 7.4 (Gey & Gey, 1936), previously gassed with O_2/CO_2 (19:1). The medium was supplemented with bovine serum albumin (1 mg/ml) and contained various additions as stated in the text or Tables and Figures. The tubes were then incubated at 37°C for 30 min with gentle shaking, after which time the medium was removed and stored at –20°C pending assay of insulin by radioimmunoassay, with human insulin as standard.

(Pro)insulin and total islet protein biosynthesis

Batches of 20 islets were incubated for 90 min at

37°C in 0.4 ml of the bicarbonate buffer containing bovine serum albumin (1 mg/ml), L-[4,5-³H]leucine (10 $\mu\text{Ci}/\text{ml}$; sp. radioactivity 130 Ci/mmol) and other additions as stated. After incubation the islets were washed twice in phosphate-buffered saline (50 mM- $\text{NaH}_2\text{PO}_4/0.9\%$ NaCl), pH 7.4, containing 1 mg of L-leucine/ml, and then disrupted in 150 μl of phosphate-buffered saline by ultrasonication (15 s at power output setting 3, with a Branson B-15 cell disruptor).

The incorporation of [³H]leucine into proinsulin and insulin [designated as (pro)insulin] was determined by the insulin-binding affinity method described by Berne (1975). By this method, 10 mg of freeze-dried crude guinea-pig anti-insulin γ -globulin was coupled to 1 g of CNBr-activated Sepharose 4B. The (anti-insulin γ -globulin)–Sepharose (AI–Sepharose) was suspended in the phosphate-buffered saline (10 mg/100 μl) and the binding capacity of 100 μl of this suspension was measured with iodinated human insulin.

Some 20 μl of the disrupted islet preparation was mixed with 1 ml of phosphate-buffered saline, containing 2 mg of bovine serum albumin/ml and 20 mg of AI–Sepharose. Controls of non-specific binding of labelled proteins were set up for each AI–Sepharose-treated sample as described above, except that 2 μg of human insulin was added. The immune-binding reaction was performed in 1.5 ml Beckman Microfuge tubes, which were rotated (20 rev./min) for 60 min at room temperature. The Sepharose was then washed three times with phosphate-buffered saline and solubilized overnight in 0.8 ml of Soluene 350.

For total islet protein synthesis, 20 μl of the sonicated islet preparation was added to 50 μl of phosphate-buffered saline containing 5 mg of bovine serum albumin/ml, and the preparation was mixed. The proteins were then precipitated by the addition of 0.5 ml of 10% (w/v) trichloroacetic acid, and the precipitate was collected by centrifugation (2000 g for 10 min). The precipitate was then washed twice with 5% trichloroacetic acid and then solubilized overnight in 0.3 ml of Soluene.

Finally, 20 μl of each sonicated preparation was removed for the determination of protein content (Lowry *et al.*, 1951), with crystalline bovine serum albumin as standard.

Glucose oxidation

Groups of ten islets were incubated in 100 μl of the bicarbonate-buffered medium, containing bovine serum albumin (1 mg/ml), [U-¹⁴C]glucose (20 $\mu\text{Ci}/\text{ml}$; sp. radioactivity 260 Ci/mol) and other additions as stated. The concentration of non-radioactive glucose was 20 mM so that the specific radioactivity of the incubation medium became 1.0 Ci/mol.

The procedure for trapping and measuring ¹⁴CO₂

was based on the method of Sissons (1976), with minor modifications. The apparatus for $^{14}\text{CO}_2$ trapping consisted of a polypropylene T-connector (outside diameter 1.0 cm). The middle side-arm passed through a hole in a size 25 Suba-seal turnover seal to a scintillation vial in which was placed a 2.1 cm Whatman GF/C glass filter to which 0.1 ml of 1 M-KOH had been added. The top side-arm was sealed with another Suba-seal (size 29), in which the turnover had been removed. On the bottom side-arm was connected 2.0 cm of rubber tubing with an internal diameter of 8 mm; the incubation tube was connected to the apparatus via this rubber extension.

At the start of incubation, the tubes containing the islets were connected to the above apparatus and transferred to a metabolic shaker in a 37°C temperature-controlled room and incubated with gentle shaking for 60 min. At the end of this period, metabolism was arrested and the $^{14}\text{CO}_2$ liberated by injection of 100 μl of 1 M-HCl, through the top side-arm, with a micro-syringe. The efficiency of $^{14}\text{CO}_2$ trapping was determined by using $\text{NaH}^{14}\text{CO}_3$ (sp. radioactivity: 0.1 Ci/mol), and found to be greater than 97% after 4 h of gentle shaking at room temperature. At the completion of this further 4 h period the scintillation vials were removed for the counting of radioactivity. Control incubations without islets were performed as described, for each experimental condition investigated.

Determination of radioactivity

For measurement of (pro)insulin and total protein biosynthesis, 10 ml of toluene/2,5-diphenyloxazole scintillant (200:1, v/w) was added to each sample. For glucose oxidation experiments, 10 ml of 2,5-diphenyloxazole scintillant [5 g/litre of 40% (v/v) methoxyethanol in toluene] was added. The samples were then shaken and left for 30 min before radioactivity counting. In all cases radioactivity was determined by using an Intertechnique SL 3000 liquid-scintillation counter. Counting times were set to record at least 1000 counts.

Determination of islet cyclic AMP content

Groups of 50 islets were placed in 1.5 ml polypropylene tubes, and 0.4 ml of the bicarbonate buffer containing 1 mg of bovine serum albumin/ml, with other additions as stated, was added. These tubes were then incubated with gentle shaking at 37°C for 30 min, after which time the islets were inactivated by chilling at 0–4°C. The chilled islets were centrifuged at 2000 *g* for 1 min at 0–4°C and the incubation medium was removed and stored at –20°C pending assay of insulin.

To the islet pellet was immediately added 0.25 ml of ice-cold 20 mM-Tris/HCl buffer, pH 7.6, containing 1.0 mM-3-isobutyl-1-methylxanthine. The tubes

were then boiled for 5 min. The islets were disrupted by ultrasonication as previously described for (pro)insulin biosynthesis, and a sample of the sonicated preparation was removed and diluted in 50 mM-sodium acetate buffer, pH 6.2. The samples were assayed for cyclic AMP by radioimmunoassay (Steiner *et al.*, 1969) with commercially available kits.

Expression of results

Unless stated otherwise, all results are given as means $\pm$ S.E.M. for the numbers of observations in parentheses. The significance of differences between experimental groups was assessed by using Student's *t* test.

Results

Effects of adenosine, 2-deoxyadenosine and N^6 -phenylisopropyladenosine on insulin release

Over a wide range of concentrations, adenosine, 2-deoxyadenosine and N^6 -phenylisopropyladenosine did not alter the basal insulin release (caused by 2 mM-glucose) (see Fig. 1). In the presence of 20 mM-glucose, adenosine (Fig. 1a) caused a dose-dependent inhibition on potentiation of insulin release. Inhibition of insulin release was observed at the lower concentration of 1 μM -adenosine and was maximal at 100 μM -adenosine (47%, $P < 0.001$). In contrast, 10 mM-adenosine enhanced insulin release caused by 20 mM-glucose (28%, $P < 0.005$). At a concentration of 10 mM, 2-deoxyadenosine (Fig. 1b) significantly inhibited insulin release caused by 20 mM-glucose (53%, $P < 0.001$). In contrast, the adenosine analogue N^6 -phenylisopropyladenosine when tested for effects with 20 mM-glucose suppressed insulin release even at concentrations as low as 0.1 μM (Fig. 1c), with maximal inhibition occurring at 1 μM - N^6 -phenylisopropyladenosine. Finally, the purine ribonucleoside inosine (10 mM) significantly enhanced both basal and 20 mM-glucose-stimulated insulin release (Fig. 1d), although lower concentrations of the nucleoside were without effect.

The effects of adenosine, 2-deoxyadenosine and N^6 -phenylisopropyladenosine on the insulin release stimulated by 3-isobutyl-1-methylxanthine is shown in Table 1. In the presence of 3-isobutyl-1-methylxanthine, insulin release by 20 mM-glucose was almost doubled. Both adenosine and N^6 -phenylisopropyladenosine were without effect on insulin release by 20 mM-glucose in the presence of 3-isobutyl-1-methylxanthine, although both these compounds inhibited insulin release by 20 mM-glucose alone. In contrast, the inhibition of 20 mM-glucose-stimulated insulin release by 2-deoxyadenosine was not overcome by the addition of 3-isobutyl-1-methylxanthine.

Fig. 1. Effects of adenosine, 2-deoxyadenosine, *N*⁶-phenylisopropyladenosine and inosine on glucose-mediated insulin release from rat islets

Groups of five islets were incubated at 37°C for 30 min in a bicarbonate-buffered medium (see the Experimental section) containing 2 mM-glucose (●) or 20 mM-glucose (○) and with the indicated concentrations of adenosine (a), 2-deoxyadenosine (b), *N*⁶-phenylisopropyladenosine (c) or inosine (d). Insulin was assayed by radioimmunoassay. Values at each point represent means $\pm$ S.E.M. for at least six observations.

Table 1. Effects of adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine on insulin release stimulated by 3-isobutyl-1-methylxanthine (IBMX) from rat islets

Groups of five islets were incubated for 30 min at 37°C in a bicarbonate-buffered medium (see the Experimental section) and contained the additions stated. Insulin released into the medium was assayed by radioimmunoassay. Results are presented as means $\pm$ S.E.M. for the numbers of observations given in parentheses. *Values significantly greater than for 2 mM-glucose controls ($P \leq 0.05$); **values significantly greater than for samples without 3-isobutyl-1-methylxanthine ($P \leq 0.05$); ***values significantly lower than with 20 mM-glucose ($P \leq 0.05$).

Additions to medium	Insulin release (ng/30 min per five islets)	
	No IBMX	IBMX (0.1 mM)
2 mM-Glucose	0.70 $\pm$ 0.09 (5)	—
20 mM-Glucose	15.22 $\pm$ 1.10 (6)*	28.96 $\pm$ 2.40 (6)**
20 mM-Glucose and Adenosine (0.01 mM)	9.62 $\pm$ 1.41 (6)***	31.28 $\pm$ 1.06 (6)**
2-Deoxyadenosine (10 mM)	5.96 $\pm$ 0.56 (6)***	6.62 $\pm$ 1.10 (6)
<i>N</i> ⁶ -Phenylisopropyladenosine (0.01 mM)	6.85 $\pm$ 0.83 (5)***	27.71 $\pm$ 1.54 (5)**

The specificity for the inhibition of insulin release by adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine was tested in the presence of various secretagogues, the results of which are shown in Table 2. Adenosine (10 μ M) inhibited insulin release caused by 20 mM-4-methyl-2-oxopentanoic acid, 30 mM-DL-glyceraldehyde and 20 mM-L-leucine. Similarly, 2-deoxyadenosine

(10.0 mM) and *N*⁶-phenylisopropyladenosine (10 μ M) inhibited insulin release by these agents.

Effects of adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine on (pro)insulin and protein biosynthesis (Table 3)

When the glucose concentration was increased from 2 to 20 mM, there was a stimulation of the

Table 2. *Effects of adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine on insulin release stimulated by various agents from rat islets*

Groups of five islets were incubated for 30 min at 37°C in a bicarbonate-buffered medium (see the Experimental section) with the additions stated. Insulin released into the medium was assayed by radioimmunoassay. Results are presented as means $\pm$ S.E.M. for six observations. *Values significantly greater than the 2 mM-glucose control ($P \leq 0.05$); **values significantly less than with the corresponding compound alone ($P \leq 0.05$).

Additions to medium	Insulin release (ng/30 min per five islets)
2 mM-Glucose	0.65 ± 0.09
20 mM-4-Methyl-2-oxopentanoate	$8.62 \pm 0.77^*$
20 mM-4-Methyl-2-oxopentanoate and Adenosine (0.01 mM)	$4.76 \pm 0.62^{**}$
2-Deoxyadenosine (10 mM)	$1.48 \pm 0.20^{**}$
N ⁶ -Phenylisopropyladenosine (0.01 mM)	$2.86 \pm 0.34^{**}$
30 mM-DL-Glyceraldehyde	$2.72 \pm 0.15^*$
30 mM-DL-Glyceraldehyde and Adenosine (0.01 mM)	$2.00 \pm 0.09^{**}$
2-Deoxyadenosine (10 mM)	$1.42 \pm 0.13^{**}$
N ⁶ -Phenylisopropyladenosine (0.01 mM)	$1.64 \pm 0.17^{**}$
20 mM-L-Leucine	$5.30 \pm 0.39^*$
20 mM-L-Leucine and Adenosine (0.01 mM)	3.19 ± 0.15
2-Deoxyadenosine (10 mM)	$2.11 \pm 0.29^{**}$
N ⁶ -Phenylisopropyladenosine (0.01 mM)	$2.33 \pm 0.29^{**}$

Table 3. *Effects of adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine on (pro)insulin and protein biosynthesis stimulated by glucose in rat islets*

Groups of 25 islets were incubated for 90 min at 37°C in a bicarbonate-buffered medium containing albumin (2 mg/ml), [4,5-³H]leucine (10 μ Ci/ml) and the additions stated. (Pro)insulin and protein biosynthesis was determined as described in the Experimental section. The results are expressed as a percentage of the rates found with 20 mM-glucose in the same experiment, and are given as means $\pm$ S.E.M. for five observations. All values are significantly greater than the 2 mM-glucose control ($P < 0.001$).

Additions to medium	(Pro)insulin synthesis	Protein synthesis	(Pro)insulin protein
2 mM-Glucose	10.3 ± 1.3	48.6 ± 3.6	0.08 ± 0.006
20 mM-Glucose	100.0	100.0	0.37 ± 0.01
20 mM-Glucose and Adenosine (0.01 mM)	105.0 ± 3.4	102.8 ± 9.1	0.36 ± 0.03
2-Deoxyadenosine (10 mM)	97.5 ± 2.7	93.0 ± 2.9	0.38 ± 0.01
N ⁶ -Phenylisopropyladenosine (0.01 mM)	93.0 ± 5.2	99.8 ± 2.5	0.34 ± 0.01

incorporation of [4,5-³H]leucine into both (pro)insulin (10-fold) and trichloroacetic acid-precipitable proteins (2-fold), and the ratio of (pro)insulin to protein synthesis increased from 0.08 to 0.37. The addition of adenosine, 2-deoxyadenosine or N⁶-phenylisopropyladenosine (at a concentration known to inhibit glucose-stimulated insulin release) did not alter the biosynthetic response of the islets caused by 20 mM-glucose.

Effects of adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine on glucose oxidation

To rule out the possibility that the actions of adenosine, 2-deoxyadenosine or N⁶-phenylisopropyladenosine are mediated via inhibition of glucose metabolism, the effects of the nucleosides on

glucose oxidation were examined (see Table 4). Incubation of the islets with 20 mM-glucose caused a 9-fold increase in the rate of glucose oxidation, compared with 2 mM-glucose. This increase in glucose oxidation was not appreciably altered in the presence of adenosine, 2-deoxyadenosine or N⁶-phenylisopropyladenosine.

Effects of adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine on the islet cyclic AMP content (Table 5)

The physiological effects of adenosine on a number of tissues appears to be the result of alterations in the intracellular content of cyclic AMP. We have examined whether there is a link between the inhibition of insulin release and the islet

Table 4. *Effects of adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine on glucose oxidation by rat islets*
Groups of ten islets were incubated for 60 min at 37°C in a bicarbonate-buffered medium containing albumin (2 mg/ml), [U-¹⁴C]glucose (sp. radioactivity 1.10 Ci/mol) and the additions stated. Glucose oxidation was determined as described in the Experimental section. Results are given as means $\pm$ s.e.m. for the numbers of observations in parentheses. All values are significantly greater than the 2 mM-glucose controls ($P < 0.001$).

Additions to medium	Glucose oxidized (pmol/60 min per ten islets)
2 mM-Glucose	65.5 $\pm$ 5.9 (6)
20 mM-Glucose	580.6 $\pm$ 22.3 (8)
20 mM-Glucose and Adenosine (0.01 mM)	588.9 $\pm$ 47.4 (6)
2-Deoxyadenosine (10 mM)	569.4 $\pm$ 48.9 (5)
N ⁶ -Phenylisopropyladenosine (0.01 mM)	579.8 $\pm$ 31.8 (5)

Table 5. *Effects of adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine on cyclic AMP content in rat islets*
Groups of 50 islets were incubated for 30 min at 37°C in a bicarbonate-buffered medium containing albumin (2 mg/ml) and the additions stated. Islet cyclic AMP was determined as described in the Experimental section. Both insulin and cyclic AMP were assayed by radioimmunoassay. Results are presented as means $\pm$ s.e.m. for the numbers of observations in parentheses. *Values significantly greater than for 2 mM-glucose control ($P \leq 0.05$); **values significantly less than with 20 mM-glucose ($P \leq 0.05$); ***values significantly greater than with 20 mM-glucose ($P \leq 0.05$).

Additions to medium	Insulin release (ng/30 min per 50 islets)	Cyclic AMP content (pmol/30 min per 50 islets)
2 mM-Glucose	3.3 $\pm$ 0.17 (5)	1.1 $\pm$ 0.0 (4)
20 mM-Glucose	42.3 $\pm$ 1.5 (5)*	2.0 $\pm$ 0.1 (5)*
20 mM-Glucose and Adenosine (0.01 mM)	32.4 $\pm$ 1.3 (8)**	1.2 $\pm$ 0.0 (8)**
2-Deoxyadenosine (10 mM)	24.6 $\pm$ 1.4 (7)**	1.0 $\pm$ 0.0 (7)**
N ⁶ -Phenylisopropyladenosine (0.01 mM)	32.5 $\pm$ 1.2 (7)**	1.1 $\pm$ 0.0 (7)**
20 mM-Glucose + 3-isobutyl-1-methylxanthine (0.1 mM)	75.8 $\pm$ 3.5 (5)***	2.7 $\pm$ 0.1 (5)***
20 mM-Glucose + 3-isobutyl-1-methylxanthine (0.1 mM) and Adenosine (0.01 mM)	76.7 $\pm$ 5.3 (6)***	2.7 $\pm$ 0.0 (6)***
2-Deoxyadenosine (10 mM)	32.1 $\pm$ 3.9 (6)	1.2 $\pm$ 0.0 (6)**
N ⁶ -Phenylisopropyladenosine (0.01 mM)	74.9 $\pm$ 1.9 (4)***	2.7 $\pm$ 0.1 (5)***

cyclic AMP content caused by adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine. Islets incubated in the presence of 20 mM-glucose showed an almost 2-fold increase in cyclic AMP content compared with those incubated with 2 mM-glucose. However, adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine all blocked this increase in cyclic AMP caused by 20 mM-glucose. 3-Isobutyl-1-methylxanthine, in addition to enhancing insulin release by glucose, also augmented intracellular cyclic AMP. Neither adenosine nor N⁶-phenylisopropyladenosine altered the cyclic AMP accumulation caused by glucose plus 3-isobutyl-1-methylxanthine. In marked contrast, in the presence of 2-deoxyadenosine, cyclic AMP remained at basal concentrations when islets were incubated with 20 mM-glucose and 3-isobutyl-1-methylxanthine.

Discussion

As in many other tissues, adenosine, 2-deoxyadenosine and N⁶-phenylisopropyladenosine have

been shown here to exhibit marked effects on islet-cell function as judged by insulin release. This appears to be a consequence of such nucleosides altering the islet content of cyclic AMP.

In agreement with the observations of others, micromolar concentrations of adenosine were found to inhibit glucose-stimulated insulin release (Ismail *et al.*, 1977), whereas 10 mM-adenosine slightly enhanced insulin release caused by high glucose concentrations (Taniguchi *et al.*, 1977). In contrast with these results (but in agreement with Jain & Logothetopoulos, 1978), in our experiments 10 mM-adenosine failed to affect basal insulin release, which is at variance with the observations noted by some other authors (Feldman & Jackson, 1974; Andersson, 1978). It is known that marked species differences exist in the response of the islets to the purine nucleoside inosine (Campbell & Taylor, 1977). Such differences, as well as differences in experimental techniques, could account for the results noted here.

The purine-modified analogue of adenosine, N⁶-phenylisopropyladenosine, was similar in its dose-

effectiveness to adenosine. Thus it inhibited insulin release in the micromolar range, although the minimum concentration at which inhibition of glucose-stimulated insulin release was mediated by this analogue is an order of magnitude less than that for adenosine (Fig. 1). The ribose-modified adenosine analogue, 2-deoxyadenosine, was quite dissimilar to adenosine or *N*⁶-phenylisopropyladenosine, only inhibiting insulin release at a concentration of 10 mM, with micromolar concentrations not altering the response. A further striking difference between the physiological effects of 2-deoxyadenosine and adenosine or *N*⁶-phenylisopropyladenosine is seen in the presence of 3-isobutyl-1-methylxanthine. The inhibitory effects of adenosine or *N*⁶-phenylisopropyladenosine on insulin release were completely abolished by the methylxanthine, whereas the inhibition mediated by 2-deoxyadenosine was not altered (Table 1). Inosine (which is formed from the deamination of adenosine by adenosine deaminase) failed to mimic the effects of micromolar concentrations of adenosine on glucose-stimulated insulin release, and indeed 10 mM-inosine greatly enhanced insulin release by both low and high glucose concentrations, which is in accordance with previous observations (Campbell & Taylor, 1977). This result, together with the fact that *N*⁶-phenylisopropyladenosine (which is similar in its effects on insulin release to adenosine) may not be metabolized (Fain & Malbon, 1979), supports the conclusion that the action of adenosine is specific for the nucleoside and not the result of a metabolic by-product.

In addition to glucose-stimulated insulin release, adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine also inhibited insulin release caused by 4-methyl-2-oxopentanoate, L-leucine and DL-glyceraldehyde. The broad nature of this inhibition by adenosine and its analogues suggests that the basis for their actions is at a critical site in the stimulus coupling mechanism for insulin release, which is common to a wide variety of secretagogues. More general and non-specific actions of adenosine, *N*⁶-phenylisopropyladenosine and 2-deoxyadenosine are not apparent, as glucose-induced insulin biosynthesis and glucose oxidation were unaffected by these nucleosides, at concentrations that inhibit insulin release.

In the present paper, evidence is also provided which suggests that adenosine and its derivatives alter the cyclic AMP content of the islets. Fluctuations in the intra-islet cyclic AMP may be important in mediating the effects of a number of agents on insulin release (Howell & Montague, 1975; Sharp, 1979); for example glucose, inosine, 4-methyl-2-oxopentanoate and *N*-acetylglucosamine, which are initiators of insulin release, have all been shown to elevate the islet content of cyclic AMP

(Campbell & Taylor, 1981). In the present work, adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine all blocked the increase in islet cyclic AMP caused by 20 mM-glucose. Furthermore, the changes in islet cyclic AMP caused by these compounds, and in the presence of 3-isobutyl-1-methylxanthine, also correlated with insulin release from the islets. When 3-isobutyl-1-methylxanthine blocked the inhibitory effects of adenosine and *N*⁶-phenylisopropyladenosine on glucose-stimulated insulin release, the islet cyclic AMP content was increased. On the other hand, the inability of the methylxanthine to alter 2-deoxyadenosine-inhibited insulin release was also reflected in decreased cyclic AMP within the islet. These results imply that the inhibition of insulin release by adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine results primarily from the ability of these compounds to inhibit the accumulation of cyclic AMP in the islets.

The role of cyclic AMP in the short-term regulation of insulin biosynthesis is not clear. However, cyclic AMP may be more significant in the long-term regulation of insulin synthesis (Campbell *et al.*, 1982). The lack of effect of adenosine and adenosine analogues on glucose-induced insulin biosynthesis could be seen as an argument against cyclic AMP being a major regulatory factor in the short term for this process. Nevertheless, minute-to-minute variations in the rate of insulin biosynthesis have not been specifically studied in these experiments, particularly in relation to cyclic AMP content.

At this point, it is relevant to discuss the molecular basis for the actions of adenosine nucleosides. It is clear from our experiments with the islets of Langerhans that adenosine, 2-deoxyadenosine and *N*⁶-phenylisopropyladenosine by inhibiting insulin release and intracellular cyclic AMP accumulation closely resemble their effects on adipocytes, where hormone-stimulated lipolysis is inhibited in concert with cyclic AMP concentrations (Fain *et al.*, 1972; Schwabe *et al.*, 1973). Further correlations can be drawn between the effects of adenosine and its analogues on islets and many other tissues (see Fain & Malbon, 1979). There exists a marked difference between the effects of *N*⁶-phenylisopropyladenosine and 2-deoxyadenosine, in terms of both dose dependency and effects of methylxanthines. This implies that the mechanism of action of these analogues is also different. It is therefore suggested, as has been proposed for other tissues (Londos & Wolff, 1977; Fain & Malbon, 1979), that the islets of Langerhans possess an adenylate cyclase-linked 'P'- and 'R'-site system. In this system the 'P' site has structural specificity for the intact purine ring of adenosine, and analogues modified in the ribose moiety serve as agonists, e.g. 2-deoxyadenosine. The 'R' site has structural specificity for

the native ribose moiety, and agonists are adenosine- or purine-modified analogues such as *N*⁶-phenylisopropyladenosine. Thus in islets, the binding of either analogue to its receptor would be expected to lead to inhibition of adenylate cyclase, and therefore insulin release, in the presence of an initiator such as glucose. Support for this concept comes from the work of Ismail *et al.* (1977), who showed that micromolar concentrations of adenosine inhibit adenylate cyclase activity in rat islet homogenates.

The two-site model may also best explain the methylxanthine sensitivity for the effects of adenosine nucleosides on the islets. It has been observed that methylxanthines antagonize the inhibitory action of purine-modified ('R' site) analogues on adenylate cyclase activity in rat adipocyte membranes (Londos *et al.*, 1978). This effect of methylxanthines was not observed when adenylate cyclase activity was inhibited with ribose-modified ('P' site) adenosine compounds. Thus in the islets the methylxanthine antagonism of the inhibition of cyclic AMP by *N*⁶-phenylisopropyladenosine and adenosine may result from the interaction of the methylxanthine with the 'R' site, blocking the binding of the nucleoside. In contrast, the methylxanthine does not interact with the 'P' site and fails to affect the inhibitory actions of 2-deoxyadenosine.

The current study adds support to the suggestion of Ismail *et al.* (1977) that adenosine may be important as a physiological regulator of insulin release via its effects on islet adenylate cyclase. The effective concentrations of adenosine are low in those experiments and in the present work. Nevertheless, little is known of the intracellular concentration of this nucleoside in islets, nor whether it is released into the extracellular milieu, or finally whether the compartmentation of adenosine can be modified by the substances which normally regulate insulin release. It may be of some importance to determine adenosine concentrations within islets in future experiments.

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