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Effects of type-selective phosphodiesterase inhibitors on glucose-induced insulin secretion and islet phosphodiesterase activity

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1 We examined various type-selective phosphodiesterase (PDE) inhibitors on glucose-induced insulin secretion from rat isolated islets, on islet PDE activity and on islet cyclic AMP accumulation in order to assess the relationship between type-selective PDE inhibition and modification of insulin release.

2 The non-selective PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX, $10^{-5}-10^{-3}$ M), as well as the type III selective PDE inhibitors SK&F 94836 ($10^{-5}-10^{-3}$ M), Org 9935 ($10^{-7}-10^{-4}$ M), SK&F 94120 ($10^{-5}-10^{-4}$ M) and ICI 118233 ($10^{-6}-10^{-4}$ M) each caused concentration-dependent augmentation (up to 40% increase) of insulin release in the presence of a stimulatory glucose concentration (10 mM), but not in the presence of 3 mM glucose.

3 Neither the type IV PDE inhibitor rolipram (10^{-4} M) nor the type I and type V PDE inhibitor, zaprinast ($10^{-4}-10^{-3}$ M) modified glucose-induced insulin release when incubated with islets, although a higher concentration of rolipram (10^{-3} M) inhibited secretion by 55%. However, when islets were *preincubated* with these drugs followed by incubation in their continued presence, zaprinast ($10^{-6}-10^{-4}$ M) produced a concentration-dependent inhibition (up to 45% at 10^{-4} M). Under these conditions, rolipram inhibited insulin secretion at a lower concentration (10^{-4} M) than when simply incubated with islets.

4 A combination of SK&F 94836 (10^{-5} M) and forskolin (5×10^{-8} M) significantly augmented glucose-induced insulin secretion (30% increase), although neither drug alone, in these concentrations, produced any significant effect.

5 Islet cyclic AMP levels, which were not modified by forskolin (10^{-6} M), SK&F 94836 (10^{-4} M) or Org 9935 (10^{-5} M) were significantly elevated (approximately 3.7 fold increase) by forskolin in combination with either SK&F 94836 or Org 9935.

6 Homogenates of rat islets showed a low K_m (1.7 μM) and high K_m (13 μM) cyclic AMP PDE in the supernatant fractions (from 48,000 *g* centrifugation), whereas the particulate fraction showed only a low K_m (1.4 μM) cyclic AMP PDE activity.

7 The PDE activity of both supernatant and pellet fractions were consistently inhibited by SK&F 94836 or Org 9935, the concentrations required to reduce particulate PDE activity by 50% being 5.5 and 0.05 μM respectively.

8 Rolipram ($10^{-5}-10^{-4}$ M) did not consistently inhibit PDE activity in homogenates of rat islets and zaprinast (10^{-4} M) consistently inhibited activity by 30% in the supernatant fraction, but not consistently in the pellet.

9 These data are consistent with the presence of a type III PDE in rat islets of Langerhans.

Keywords: Islets of Langerhans; insulin secretion; phosphodiesterase inhibitors; phosphodiesterase isoforms; forskolin; cyclic AMP

Introduction

Adenosine 3':5'-cyclic monophosphate (cyclic AMP) is generally accepted as an important amplifier of insulin release (Zawalich & Rasmussen, 1990; Holz & Habener, 1992). Glucose increases islet intracellular cyclic AMP levels, which may serve to amplify its own primary effect in stimulating insulin secretion. Agents that increase cyclic AMP levels by activating adenylyl cyclase (glucagon, forskolin), or by inhibiting cyclic AMP phosphodiesterase (isobutylmethylxanthine) markedly augment glucose-induced insulin release. Elevations in guanosine 3':5'-cyclic monophosphate (cyclic GMP) have been linked with decreases in insulin secretion (Vara & Tamarit-Rodriguez, 1991; Green *et al.*, 1993) but its role remains unclear (Verspohl & Ammon, 1989; Jones *et al.*, 1992). As in other tissues, phosphodiesterases in the islet β-cell hydrolyse cyclic AMP, which will thus be elevated only transiently in response to glucose and other stimuli. There are multiple isoforms of the PDE family with marked differences in their amino acid sequences and encoded by distinct genes (Beavo & Reifsnyder, 1990). The family comprises at least seven main types. Type I is a family of calcium/calmodulin-stimulated isoforms. These are inhibited by zaprinast (Shahid *et al.*, 1991) with relatively low potency (IC_{50} 10-50 μM). The type II isoform hydrolyses both cyclic AMP and cyclic GMP, cyclic AMP hydrolysis being activated by cyclic GMP. The type III and type IV isoforms are cyclic AMP-specific. The former is inhibited potently by cyclic GMP and by a number of selective drugs, including SK&F 94836 (Thompson, 1991) and Org 9935 (Shahid *et al.*, 1991), while the latter is selectively inhibited by Ro 20-1724 and rolipram (Beavo & Reifsnyder, 1990; Nicholson *et al.*, 1991; Thompson, 1991). There is also a cyclic GMP-specific enzyme (type V) inhibited potently by zaprinast ($IC_{50} < 1$ μM) (Nicholson *et al.*, 1991). Finally, there are type VI (retinal rod photoreceptor) and type VII PDEs (Beavo *et al.*, 1994).

There is little information concerning the identities of the phosphodiesterases present in the pancreatic islet β cell. Previous studies in the islets have shown the presence of a cytosolic calcium-calmodulin-activated PDE (Sugden & Ashcroft, 1981; Lipson & Oldham, 1983; Capito *et al.*, 1986) with high and low K_m components for both cyclic AMP and cyclic GMP,

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and a particulate form, which appeared insensitive to calcium-calmodulin. The enzymes do not appear to have been characterized further. The aim of the present work was to identify and characterize both pharmacological and biochemical properties of the PDE isoforms expressed in rat pancreatic islets. The effects of type specific PDE inhibitors on insulin secretion, islet PDE activity and cyclic AMP accumulation were examined, in order to assess the relationship between type-specific PDE inhibition and modification of glucose-induced insulin secretion. Some of this work has been published in abstract form (Shafiee-Nick *et al.*, 1993).

Methods

Preparation of islets

Male, Sprague-Dawley rats (250–300 g) were allowed free access to normal laboratory diet until the experiment. They were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and the pancreas was removed following distension with cold Krebs bicarbonate buffer (isolation Krebs) (containing (mM): $MgSO_4$ 0.9, KH_2PO_4 1.2, KCl 4.7, NaCl 94, $NaHCO_3$ 25, $CaCl_2$ 2.5, glucose 5.6) solution injected via the common bile duct. The tissue was chopped and digested at 37°C with collagenase (Lacy & Kostianovsky, 1967). Islets were hand-picked with a fine, siliconized Pasteur pipette and after two transfers in isolation Krebs, were transferred to Krebs bicarbonate buffer, containing glucose 3 mM, fumarate 5 mM, glutamate 5 mM, pyruvate 5 mM and bovine serum albumin 3 mg ml^{-1} (incubation Krebs).

Insulin secretion

Batches of 5 islets in 1.0 ml incubation Krebs were placed in vials and pre-incubated in a shaking incubator (60 oscillations min^{-1}) with continuous gassing (95% O_2/5% CO_2, pH 7.4, 37°C). The medium was removed and replaced by 1.0 ml fresh incubation Krebs solution containing drug or vehicle in the presence of 3 or 10 mM glucose. In some experiments (where indicated in the text) drugs (or vehicle) were additionally included in the preincubation medium. The islets were incubated for 60 min after which a sample of the medium was removed and frozen for subsequent determination of insulin. Immunoreactive insulin was determined with a commercial radioimmunoassay kit (ICN Biomedicals Inc., Thame, Oxfordshire) and either a human insulin standard or a pure crystalline rat insulin standard. Samples were diluted 1:5 with phosphate buffer (pH 7.4) before assay. Insulin secretion was expressed as μu islet h^{-1} (human insulin standard) or ng islet h^{-1} (rat insulin standard). Insulin secretion was also studied in a perifusion system, in which batches of 100 islets were placed in small chambers and perifused with Krebs bicarbonate buffer (95% O_2/5% CO_2, Po_2 about 600 mmHg, Pco_2 35–38 mmHg, pH 7.4, 37°C, 1.0 ml min^{-1}). Fractions were collected for 1 min at appropriate intervals for radioimmunoassay of insulin.

Islet phosphodiesterase activiy

Batches of 100 islets were incubated for 30 min in Krebs buffer at 37°C. Islets were pooled and washed twice at 4°C with Krebs and then with Tris buffer containing (mM concentration):- Tris 10, sucrose 250, EDTA 1.0, phenylmethylsulphonyl fluoride 0.01 and benzamidine 1.0. Islets were homogenized in the Tris buffer (1.0 μl per islet) and the homogenates were centrifuged (48,600 *g*, 20 min, 4°C). The pellet was resuspended in Tris buffer and both pellet and supernatant fractions were assayed for cyclic AMP PDE activity with 0.5 μM [^{3}H]-cyclic AMP used as substrate. Assays were performed by the two step radioactive assay (Thompson & Appleman, 1971) under linear rate formation of product and where less than 10% of substrate is utilised. Activity was expressed as pmol cyclic AMP min^{-1} ml^{-1} or as a percentage of control.

Kinetic analysis was carried out on the pellet and supernatant fractions with 11 substrate concentrations from 0.01–100 μM. Data were plotted as Eadie-Hoftsee plots (Cornish-Bowden, 1979) and K_m values determined according to Spears *et al.* (1971).

Determination of cyclic AMP

Batches of 100 islets were preincubated for 30 min in Krebs containing 3 mM glucose and then incubated for 15 min containing 3 or 10 mM glucose with/without drug. At the end of the incubation the medium was rapidly removed, replaced with 1 ml of cold absolute ethanol and the islets homogenized. The homogenate was centrifuged at 14,000 *g* for 10 min and the precipitate washed with 1.0 ml ethanol and recentrifuged. The supernates from both centrifugations were pooled and dried in a speed-vacuum overnight. Each sample was dissolved in 100 μl buffer (50 mM Tris/4 mM EDTA, pH 7.4) and assayed for cyclic AMP using [^{3}H]-cyclic AMP and bovine heart cyclic AMP-binding protein, which was purified according to Rubin *et al.* (1974).

Drugs and chemicals

ICI 118233 (6-[*p*-(3-methylureido)phenyl]-3[2H]-pyridazinone) (Imperial Chemical Industries, (now Zeneca) Macclesfield, Cheshire, England), SK&F 94836 (siguazodan) and SK&F 94120 (5-(4-acetamidophenyl)pyrazin-2-[1H]-one) (SmithKline Beecham, Harlow, Essex, England), Org 9935 (4,5 dihydro-6-(5,6-dimethoxy-benzo [β]thiophene-2-carboximidamide) HCl) (Organon Laboratories, Newhouse, Lanarkshire, Scotland), zaprinast (M&B 22948) (Rhone Poulenc Rorer, Dagenham, Essex), rolipram (ZK 62711) (SmithKline Beecham, King of Prussia, U.S.A.), forskolin, glucagon, and 3-isobutyl-1-methylxanthine, IBMX, (Sigma Chemical Co., Poole, Dorset) were prepared as stock solutions in dimethyl sulphoxide (DMSO, Sigma Chemical Co., Poole, Dorset) and diluted in Krebs buffer. The appropriate quantity of DMSO was used as control.

Table 1 Effects of SK&F 94120 (100 μM), SK&F 94836 (135 μM), IBMX (1000 μM), ICI 118233 (100 μM) and ORG 9935 (10 μM) on insulin release in the presence of 3 or 10 mM glucose

	Insulin secretion (μu/islet h^{-1})			
Treatment	*3 mM glucose*	n	*10 mM glucose*	n
Control	8.6±1.3	18	58.9±2.2	29
SK&F 94120	7.4±1.1	16	70.3±2.3	16
SK&F 94836	6.0±0.7	17	77.8±4.0	15
IBMX	10.2±1.9	7	78.7±3.9	11
ICI 118233	10.1±1.7	18	73.1±4.1	17
Org 9935	11.2±1.5	18	79.7±2.8	17

Batches of 5 islets were incubated with the drugs or vehicle (control) in either glucose concentration and insulin measured in the medium after 60 min. These are the concentrations producing maximum responses in 10 mM glucose. Values are mean±s.e.mean.

Statistical analysis

Data were expressed as mean ± s.e.mean. The quoted *n* values refer to 4–5 replicate experiments (batches of islets subjected to a treatment) from 3–5 different isolates, with two pancreata being used for each isolate. Statistical significance was determined by one way ANOVA followed by modified *t* test, (Bonferroni correction for multiple comparisons).

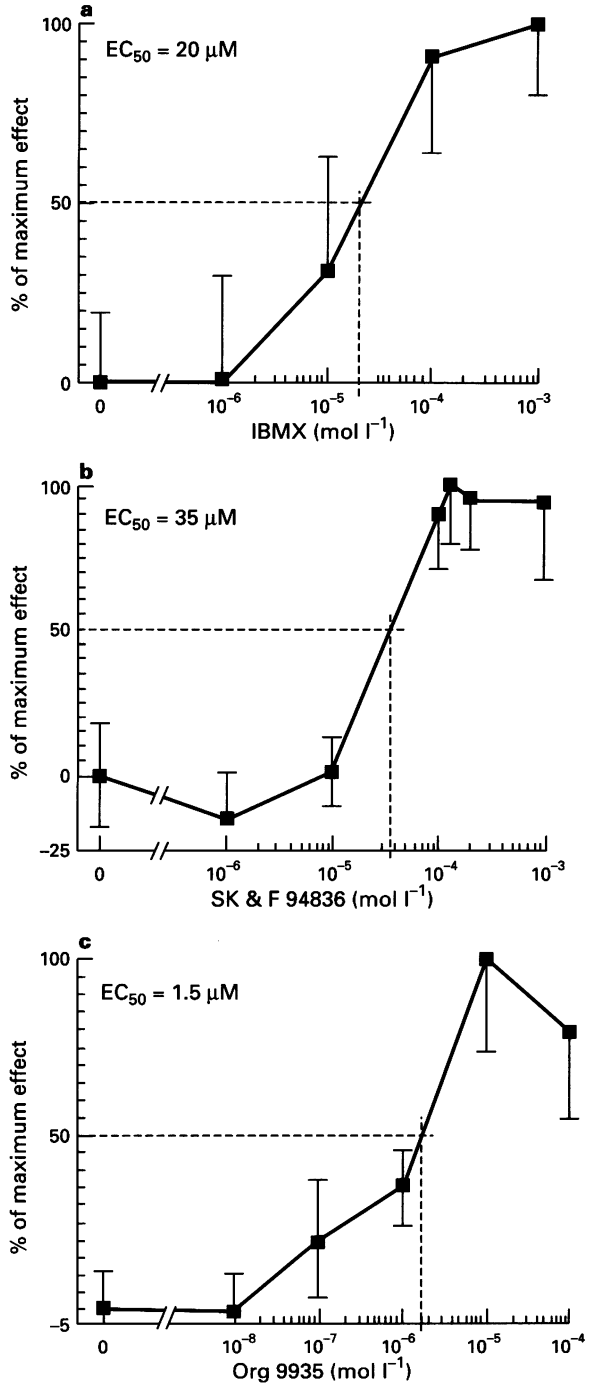

Figure 1 Effect of different concentrations of 3-isobutyl-1-methylxanthine (IBMX), SK&F 94836 (siguazadon) and Org 9935 on insulin secretion in response to 10 mM glucose from batches of 5 islets incubated for 60 min. Data are expressed as the % maximum insulin secretion values given in Table 1. Each value is the mean ± s.e.mean of 8–16 observations. The EC_{50} values, which were determined by eye, are derived from the mean dose-response curves and are the concentrations giving 50% maximum response.

Results

Insulin secretion

When incubated in 3 mM glucose, insulin release was not modified by ICI 118233, SK&F 94836, SK&F 94120, Org 9935 or 3-isobutyl-1-methylxanthine (IBMX) (Table 1). However, insulin release evoked by glucose (10 mM) was augmented in a concentration-dependent manner by ICI 118233, SK&F 94836, SK&F 94120, Org 9935 and IBMX (Table 1, Figure 1). Very high concentrations (above 10^{-4} M) of SK&F 94120 or ICI 118233 showed a trend to producing lower than maximum responses (data not shown). At concentrations up to 10^{-3} M zaprinast did not modify glucose-induced insulin secretion, whereas at 10^{-3} M but not at 10^{-4} M, rolipram was inhibitory (insulin secretion, μu/islet h^{-1}; control 58.4 ± 2.8 (n = 36), rolipram 10^{-3} M 26.3 ± 3 (n = 24); P < 0.01). Pre-incubation of islets (30 min) with zaprinast followed by incubation with the drug (60 min) resulted in a concentration-dependent inhibition of glucose-induced insulin release (insulin secretion, μu/islet h^{-1}; zaprinast 10^{-6} M 51.8 ± 2 (n = 10, NS); 10^{-5} M 41.6 ± 2.4 (n = 9, P < 0.05); 10^{-4} M 30.5 ± 5.3 (n = 8, P < 0.01)). Rolipram inhibited insulin secretion to 33 ± 1 μu/islet h^{-1} (P < 0.01) at 10^{-4} M (10 fold lower than previously) when preincubated (followed by incubation) with islets. This preincubation protocol did not modify the augmenting effect of SK&F 94836 (10^{-4} M) on insulin release.

In a perifusion system the islets showed a rapid secretory response to increasing the glucose concentration (from 3 to 10 mM) which rapidly declined on reverting to the low glucose concentration. SK&F 94836 augmented insulin release by perifused islets (Figure 2).

In view of the known adenosine blocking actions of IBMX, the effects of adenosine and adenosine deaminase were examined. Neither adenosine nor adenosine deaminase significantly modified insulin release when incubated with islets (for 60 min) in the presence of 10 mM glucose (insulin secretion ng/islet h^{-1}; control 3 mM glucose 1.3 ± 0.2 (n = 10); control 10 mM glucose 7.5 ± 0.4 (n = 18); adenosine (100 μM) 9.4 ± 0.7 (n = 14): adenosine deaminase (0.75 u ml^{-1}) 7.7 ± 0.6 (n = 13)).

Interactions of PDE inhibitors with forskolin

Forskolin (10^{-8}–10^{-5} M) produced a concentration-dependent augmentation of glucose-induced insulin secretion, with 10^{-6} M causing a maximum response (Figure 3). SK&F 94836 (10^{-4} M) and forskolin (10^{-6} M) each augmented glucose-induced insulin secretion but the combination of the two pro-

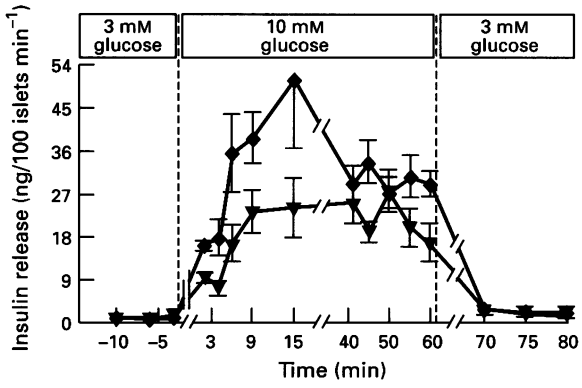

Figure 2 Effects of 10^{-4} M SK&F 94836 on insulin secretion in the presence of 3 mM and 10 mM glucose from batches of 100 islets perifused with Krebs solution (1 ml min^{-1}). Each value is the mean ± s.e.mean of 4 batches of islets from 4 different isolates: (◆) data from perifusion performed in the presence of SK&F 94836; (▼) control perifusions.

duced no greater effect than either alone. A combination of SK&F 94836 (10^{-4} M) and forskolin (10^{-7} M) also did not produce any greater stimulation than did each drug individually. The experiment was repeated with threshold concentrations of each drug. Neither SK&F 94836 (10^{-5} M) nor forskolin (5×10^{-8} M) significantly augmented glucose-induced insulin release. However, glucose-induced insulin secretion was significantly augmented by a combination of the two drugs.

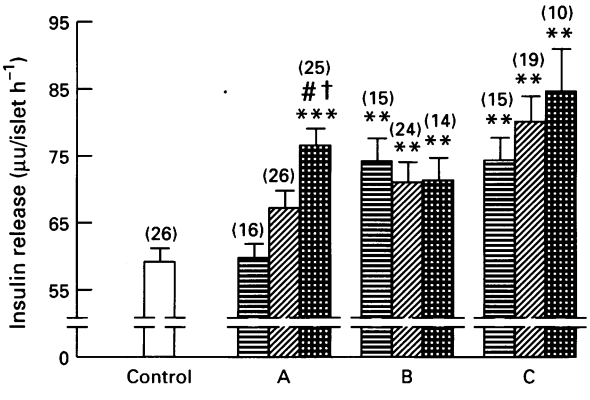

Figure 3 Effects of SK&F 94836 (lined column) and forskolin alone (hatched columns), or in combination (checkered columns), on insulin secretion in response to 10 mM glucose from batches of 5 islets incubated for 60 min. Open column, control insulin secretion in the presence of 10 mM glucose. The concentrations of SK&F 94836 and forskolin were, respectively, in experiment **A** 10^{-5} M and 5×10^{-8} M, experiment **B** 10^{-4} M and 10^{-7} M and in experiment **C** 10^{-4} M and 10^{-6} M. In a concentration of 10^{-5} M forskolin produced no greater insulin secretion than forskolin 10^{-6} M. Each value is mean ± s.e.mean of the numbers shown in parentheses. *Significant difference from control, $P<0.01$; **significant difference from control, $P<0.001$; #significant difference from forskolin, $P<0.05$; †significant difference from SK&F, $P<0.001$.

Islet phosphodiesterase activity

Cyclic AMP PDE activity in the homogenate and in supernatant and pellet fractions was assessed in the presence or absence of type-specific PDE inhibitors.

In the homogenate cyclic AMP-PDE activity was inhibited in a concentration-dependent manner by SK&F 94836, with a maximum % inhibition of 59.9 ± 1.9 at 10^{-4} M (Figure 4). In some experiments rolipram was without effect whereas in others cyclic AMP-PDE activity was inhibited by up to 20% (cyclic AMP-PDE activity, pmol min^{-1} mg^{-1}; control 44 ± 1.1; rolipram, 10^{-5} M 38.6 ± 0.6; rolipram 10^{-4} M 35.2 ± 0.7).

In the supernatant fraction, cyclic AMP PDE activity was inhibited concentration-dependently by SK&F 94836 and Org 9935 (Figure 4). The inhibition curve for Org 9935 appeared to be biphasic. The maximum percentage inhibitions at 10^{-4} M were 37.7 ± 2.9 and 54.4 ± 1.2, respectively. Zaprinast (10^{-4} M) produced a 30.7 ± 5.3% inhibition.

In the pellet fraction SK&F 94836 and Org 9935 each inhibited cyclic AMP-PDE activity by 74.2 ± 3.3% and 81.7 ± 4.4% respectively at 10^{-4} M (Figure 4). Zaprinast did not consistently inhibit cyclic AMP-PDE activity. However, in some experiments a 13.8 ± 1.6% inhibition was obtained with 10^{-5} M zaprinast ($n=4$) and 26 ± 2% inhibition with 10^{-4} M.

Kinetic analysis for cyclic AMP hydrolysis with Eadie-Hoftsee plots (Figure 5) appeared to show both low K_m and high K_m PDE activities in the supernatant fraction. More detailed analysis was carried out by the method of Spears *et al.* (1971). This showed a K_m of 2.02 (95% CL 1.54, 2.52) for the

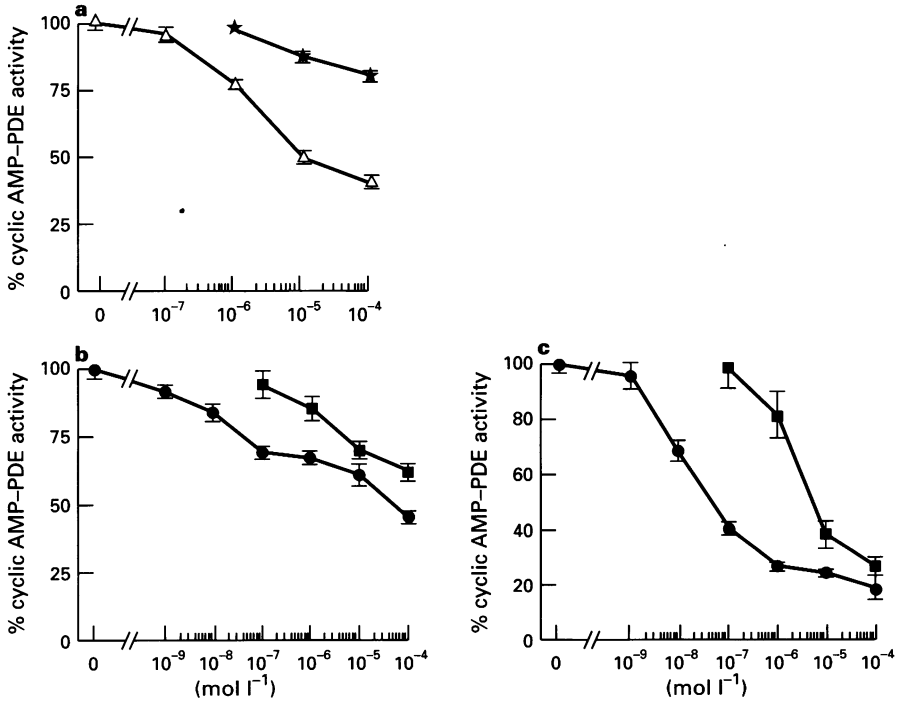

Figure 4 Effect of different concentrations of SK&F 94836 and Org 9935 on cyclic AMP-phosphodiesterase (PDE) activity in islet homogenates (a) and in supernatant (b) and pellet (c) fractions of homogenates of rat islets. The effect of rolipram (★, 10^{-6}–10^{-4} M) and SK&F 94836 (△) on cyclic AMP PDE activity in the homogenate is also shown in (a). In (b) and (c), effects of SK&F 94836 are shown by (■) and of Org 9935 by (●). The absolute values, in the absence of drug, were respectively for the pellet and supernatant fractions 31.5 ± 6.2 and 46.2 ± 10 pmol min^{-1} ml^{-1}. Each value is the mean ± s.e.mean of 8-10 observations for SK&F 94836 and 3 observations for Org 9935. The concentrations reducing the PDE activity in the pellet by 50% were for SK&F 94836 5.5 μM and for Org 9935 0.05 μM.

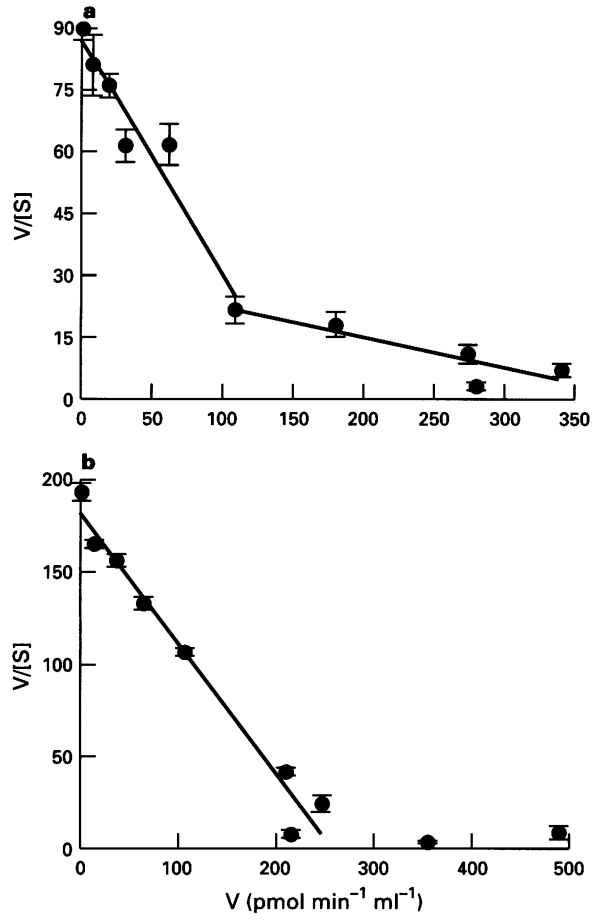

Figure 5 Typical Eadie-Hoftsee plots for cyclic AMP phosphodiesterase (PDE) activity in supernatant (a) and pellet (b) fractions of rat islet homogenates obtained at different substrate concentrations. The lines were fitted by computer using linear regression. Each point is the mean ± s.e.mean, of 3-6 observations.

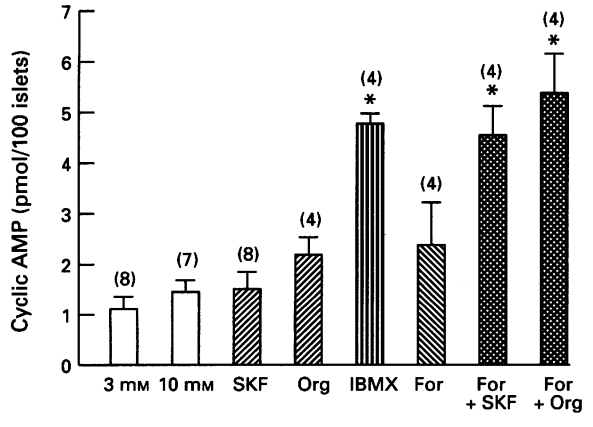

Figure 6 Islet cyclic AMP content in batches of 100 islets incubated for 15 min in the presence of 3 mM glucose, 10 mM glucose alone or 10 mM glucose together with SK&F 94836 (SKF, 10^{-4} M), Org 9935 (Org, 10^{-5} M), 3-isobutyl-1-methylxanthine (IBMX 10^{-4} M), forskolin (For, 10^{-6} M) or combinations of forskolin (10^{-6} M) plus SK&F 94836 (10^{-4} M) or forskolin (10^{-6} M) plus Org 9935 (10^{-5} M). Each column is the mean ± s.e.mean of the number of observations shown in parentheses. *Indicates significantly different from control, $P<0.001$.

low K_m component and 23.1 (95% CL 18.6, 27,6) for the high K_m component. The pellet clearly showed a low K_m PDE (1.4 μM) with no evidence of a high K_m isoform.

Incubation of intact islets with insulin (0.32; 3.2, 32 nM) for 15 min prior to homogenization did not modify cyclic AMP PDE activity in the islet homogenates (activity, pmol min^{-1} mg^{-1}; control 44.3 ± 2.2 ($n=12$), insulin 0.32 nM, 45.4 ± 2.7 ($n=9$); insulin 3.2 nM, 42.0 ± 1.2 ($n=6$); insulin 32 nM 46.8 ± 1.4 ($n=9$)). Moreover, incubation with insulin as above did not modify the inhibitory effect of SK&F 94836 (10^{-5} M) added to the homogenate (PDE activity pmol min^{-1} mg^{-1}; control 44.3 ± 2.2 ($n=12$) SK&F 94836 23.8 ± 1 ($n=12$ $P<0.01$ vs control) insulin (32 nM) + SK&F 94836 25.2 ± 2.7 ($n=9$)).

Islet cyclic AMP content

Neither SK&F 94836 (10^{-4} M) nor Org 9935 produced any significant elevation in islet cyclic AMP (in the presence of 10 mM glucose), whereas IBMX (10^{-4} M) produced a 3 fold increase (Figure 6). Forskolin 10^{-6} M produced no significant increase in islet cyclic AMP. When combined with either SK&F 94836 (10^{-4} M) or Org 9935 (10^{-5} M), forskolin (10^{-6} M) produced a marked and significant increase in cyclic AMP.

Discussion

The role of cyclic AMP in regulating insulin secretion is quite well-established. Glucose-stimulated insulin secretion is not primarily mediated by cyclic AMP and does not involve protein kinase A (Henquin & Meissner, 1984a; Persaud *et al.*, 1990). However, islet cyclic AMP is increased in response to glucose and agents that elevate islet cyclic AMP augment glucose-induced insulin secretion. The role of phosphodiesterases in terminating cyclic AMP activity in the islets has been largely adduced from the ability of IBMX to promote islet cyclic AMP accumulation and to augment insulin release (Prentki & Matschinsky 1987). However, in addition to its non-selective PDE inhibitory properties, IBMX blocks adenosine receptors (Fredholm, 1980), which may complicate interpretation since adenosine may inhibit insulin release (Bertrand *et al.*, 1989). It has also been shown to depolarize the islet β cell membrane, an effect that cannot be attributed to cyclic AMP, since it was demonstrated in the presence of low glucose concentrations (3 mM) (Henquin & Meissner, 1984b).

The nature of the phosphodiesterases present in the insulin-secreting cells is unclear but the present data suggest the functional importance of the type III isoenzymes. Among a broad range of structurally dissimilar phosphodiesterase inhibitors, only the non-selective inhibitor IBMX and the selective type III inhibitors, ICI 118233 (Pyne *et al.*, 1987; Nicholson *et al.*, 1991), SK&F 94836 (Thompson, 1991), SK&F 94120 (Reeves *et al.*, 1987; Nicholson *et al.*, 1991), and Org 9935 (Shahid *et al.*, 1991) augmented glucose-induced insulin secretion, without modifying basal release. This effect was clearly not an artefact of static incubation, as it was seen in a dynamic perifusion system, in which glucose-induced insulin release was augmented by SK&F 94836. At high concentrations, these drugs may inhibit other PDE isoforms. For example, Org 9935 at concentrations above 1 μM can exert effects on type IV and type V isoforms (Shahid *et al.*, 1991). However, the type IV inhibitor rolipram (Beavo & Reifsnyder, 1990) did not modify insulin secretion, except at the highest concentration, which was inhibitory. A ten fold lower concentration of rolipram was inhibitory when islets were *pre*incubated with rolipram. We have also shown previously that glucose-induced insulin secretion was not modified by another type IV inhibitor, ICI 63197 (Furman & Pyne, 1990). The significance of the effect of the very high concentration of rolipram is unclear. Zaprinast, an inhibitor of both type I and type V PDEs, failed to enhance insulin release when incubated with the islets and actually *inhibited* secretion following *pre*incubation. The different effect of preincubation with zaprinast may indicate re-

lative slow penetration of the intact islet. Zaprinast, in addition to inhibiting cyclic AMP hydrolysis is a potent inhibitor of the cyclic GMP specific type V PDE isoform (Beavo & Reifsnyder, 1990). We have found a low K_m (< 1 μM) enzyme component for cyclic GMP hydrolysis in the supernatant fraction, which probably equates with the expression of this particular PDE (unpublished observations). Accumulation of cyclic GMP following zaprinast treatment may explain the inhibitory effect on insulin secretion as cyclic GMP may be associated with inhibition of insulin release (Green *et al.*, 1993). However, this requires further exploration.

The pharmacological data on insulin secretion are in broad agreement with data obtained on enzyme inhibition. Thus SK&F 94836 and Org 9935 each inhibited cyclic AMP PDE activity in both pellet and supernatant fractions of islet homogenates. It is noteworthy that neither drug produced complete inhibition of islet PDE activity, even at the maximum concentrations used in the insulin secretion studies. Each drug produced a larger % inhibition (up to 85%) and was more potent in the pellet. This was particularly noticeable with Org 9935. These data are consistent with earlier observations showing a Ca^{2+}-calmodulin insensitive phosphodiesterase in the particulate fraction of homogenates of mouse islets (Capito *et al.*, 1986). Moreover, the low K_m for cyclic AMP in the pellet and inhibition of islet PDE activity by cyclic GMP (Furman & Pyne, 1990) is compatible with the presence of the type III isoform (Nicholson *et al.*, 1991). Rolipram did not produce any consistent inhibition, suggesting the relative unimportance of the type IV isoenzyme. The 30% inhibition produced by zaprinast in the supernatant fraction, but not consistently in the pellet, also supported the previous demonstration of an islet cytosolic Ca^{2+}-calmodulin sensitive (type I) PDE (Capito *et al.*, 1986). Indeed, a high K_m cyclic GMP degrading enzyme was also found in the supernatant fraction (Shafiee-Nick *et al.*, unpublished observations). The importance of this enzyme in regulating islet cyclic AMP levels remains to be determined. When interpreting the function of PDE isoforms in relation to insulin secretion, it must be remembered that islets comprise at least four cell types, with the insulin secreting β cells making up about 60–70%. Thus, the presence of an isoform in islet homogenates does not necessarily indicate that it is present or functional in the β cells. The relationship between enzyme inhibition and augmentation of insulin secretion is supported since Org 9935 was much more potent as a PDE inhibitor than SK&F 94836 and was also more potent in promoting insulin secretion. Both drugs were more potent as PDE inhibitors (IC_{50} values 50 nM and 5.5 μM, respectively) compared with their effects on insulin release (EC_{50} values 1.5 μM and 35 μM respectively), the potency ratio being about 30 for Org 9935 and 6 for SK&F 94836. The difference in relative potencies between the two drugs may reflect differences in their ability to penetrate the intact islet, although *preincubation* with SK&F 94836 did not appear to increase its potency.

Insulin activates type III PDE in adipocytes (Rahn *et al.*, 1994). As the islets are exposed to a high concentration of insulin in incubation experiments it was considered that this may modulate the measured enzyme activity. However, incubation of islets with insulin, even in high concentrations, did not modify PDE activity in the subsequently produced homogenate and did not modify the inhibitory effect of submaximum concentration (10 μM) of SK&F 94836 added to the homogenate.

The similarity of the effects of IBMX and four different type III PDE inhibitors on insulin release, the lack of augmentation of insulin secretion by zaprinast and rolipram and the relative potencies of Org 9935 and SK&F 94836 on insulin secretion and PDE inhibition all suggest that inhibition of type III PDE is the common action that explains their effects on insulin secretion. However, while IBMX markedly elevated islet cyclic AMP, we were unable to demonstrate unequivocally any elevation with either Org 9935 or SK&F 94836 in the presence of 10 mM glucose. Similar discrepancies between functional effects of selective PDE inhibitors and their effects on cyclic AMP accumulation have been reported in other systems. For example, in ventricular cardiomyocytes, compounds with PDE inhibitory activity increased cyclic AMP accumulation only at concentrations that produced maximal response on contractile function and enoxamine, the selective PDE III inhibitor, failed to produce any increase in cyclic AMP (Kelso *et al.*, 1993). In contrast, the type IV selective PDE inhibitor Ro 20-1724 failed to increase contractile activity of cardiomyocytes, despite producing marked increase in cyclic AMP (Kelso *et al.*, 1993). The failure of type III inhibitors alone to increase islet cyclic AMP levels could be due to cyclic AMP hydrolysis by other, non-inhibited PDE isoenzymes. Thus, the rate of cyclic AMP degradation may remain sufficiently fast to prevent a net increase in measurable cyclic AMP. On the other hand, the augmentation of islet cyclic AMP levels by a combination of forskolin with either SK&F 94836 or Org 9935 indicates that these drugs do, indeed, inhibit cyclic AMP hydrolysis in the intact islet sufficiently to allow its accumulation when turnover is accelerated by forskolin. The observation that a combination of threshold concentrations of SK&F 94836 and forskolin produced an augmentation of glucose-induced insulin release significantly greater than that of either drug alone provides further support for the functional importance of the type III PDE. The inability of the combinations of higher concentrations to show synergism in augmenting insulin secretion, may indicate that only a relatively small accumulation of cyclic AMP, or increase in its turnover, is necessary to produce a maximal cyclic AMP effect on insulin release. There is a relatively narrow window between glucose-induced insulin secretion in the absence of drug and the presence of maximally effective concentrations of either forskolin or the PDE inhibitors. In this context, although 10^{-6} M forskolin produced a maximum effect on glucose-induced insulin secretion, maximum adenylyl cyclase activation in islets or purified β cells requires about 50–100 μM forskolin (Henquin & Meissner, 1984a; Schuit & Pipeleers, 1985).

It remains possible that other cyclic AMP-independent actions may be involved, as suggested for the effect of PDE inhibitors on ventricular cardiomyocytes (Kelso *et al.*, 1993). Blockade of adenosine receptors, referred to above, is unlikely to be implicated in the actions of IBMX or of the other PDE inhibitors since, in the present work, adenosine either did not significantly modify insulin secretion or tended to increase it. Moreover, the lack of effect of a high concentration of adenosine deaminase suggested that endogenous adenosine does not modulate insulin secretion.

In conclusion, selective inhibitors of type III phosphodiesterase augment glucose-induced insulin secretion whereas rolipram, a selective inhibitor of the type IV PDE and zaprinast a type I/V PDE inhibitor, do not. This is consistent with our preliminary results showing type III PDE activity in homogenates of rat islets (Furman & Pyne, 1990). We have recently shown that SK&F 94836 stimulates insulin release and potently inhibits phosphodiesterase in human islets, suggesting that human islets also possess a functionally important type III PDE isoform (Shafiee-Nick *et al.*, 1994). Selective inhibition of the relevant islet β cell PDE may lead to novel agents for the treatment of type II diabetes mellitus. In support of this idea, a novel aryl piperazine was found to be hypoglycaemic (Johnston *et al.*, 1991), to stimulate insulin secretion and to be a phosphodiesterase inhibitor (Leibowitz *et al.*, 1995).

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