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Effect of type-selective inhibitors on cyclic nucleotide phosphodiesterase activity and insulin secretion in the clonal insulin secreting cell line BRIN-BD11

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1 The cyclic nucleotide phosphodiesterases (PDEs) present in an insulin secreting cell line, BRIN–BD11, were characterized using calcium/calmodulin, IGF-1, isoenzyme-selective PDE inhibitors and RT–PCR.

2 Calmodulin activated cyclic AMP or cyclic GMP PDE activity in pellet and was 3 fold ($P=0.002$) more potent in activating cyclic nucleotide hydrolysis in pellet compared with supernatant fractions.

3 The PDE1/PDE5 inhibitor zaprinast inhibited both cyclic AMP and cyclic GMP PDE activity in both pellet and supernatant fractions of cell homogenates by a maximum of around 25% (IC_{50} 1–5 μM), while rolipram (PDE4 selective) inhibited only cyclic AMP hydrolysis.

4 The PDE3-selective inhibitors Org 9935 (0.02–10 μM) and siguazodan (0.1–10 μM) inhibited cyclic AMP PDE activity in the pellet but not the supernatant fractions of cell homogenates, with a maximum inhibition of about 30%. IGF-1 (2–7.5 ng ml^{-1}) potently augmented this PDE activity.

5 RT–PCR using specific primers for PDE3B, but not for PDE3A, amplified, from BRIN–BD11 cell total RNA, a 351 base pair product that was >97% homologous with rat adipose tissue PDE3B.

6 IBMX, Org 9935, siguazodan and rolipram (1–50 μM), but not zaprinast, each augmented glucose-induced insulin secretion in the presence of 16.7 mM but not 1 mM glucose.

7 These findings, in a clonal insulin secreting cell line, are consistent with an important role for PDE3B in regulating the pool of cyclic AMP relevant to the modulation of glucose-induced insulin secretion.

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Abbreviations: ANOVA, analysis of variance; cyclic AMP, cyclic 3′5′-adenosine monophosphate, cyclic GMP; cyclic 3′5-guanosine monophosphate; EDTA, ethylenediaminetetraacetic acid; GLP-1, glucagon like peptide-1; IBMX, isobutylmethylxanthine; IGF-1, insulin like growth factor-1; PDE, phosphodiesterase; PCR, polymerase chain reaction; PMSF, phenylmethylsulphonyl fluoride; RT, reverse transcriptase

Introduction

Cyclic 3′,5′-adenosine monophosphate (cyclic AMP) is an important physiological amplifier of glucose-induced insulin secretion (Prentki & Matschinsky, 1987) and mediates the action of incretin factors such as glucagon like peptide-1 (GLP-1) on insulin release. The destruction of cyclic AMP is effected by hydrolysis catalysed by several different families of phosphodiesterases (PDEs), which differ in their kinetics, substrate specificity and susceptibility to inhibitors (Beavo, 1995).

As yet there is relatively little known about the PDEs responsible for cyclic AMP hydrolysis in the pancreatic islet β cell. However, we (Furman & Pyne, 1990; Shaffiee-Nick *et al*., 1995) suggested that PDE3 was the functionally important enzyme in relation to hydrolysis of the cyclic AMP pool relevant to insulin secretion in rat and human islets. PDE1, PDE4 and the cyclic 3′,5′ guanosine monophosphate (cyclic GMP)-specific PDE5 are all expressed in the pancreatic islet, as evidenced by the ability of type-selective enzyme inhibitors to reduce PDE activity (Shaffiee-Nick *et al*., 1995; El-Metwally *et al*., 1997). Moreover, others (Capito *et al*., 1986; Valverde & Malaisse, 1994) showed the presence of a calcium–calmodulin stimulated PDE activity, further indicating the presence of PDE1. However, glucose-induced insulin secretion in rat or human isolated islets was augmented only by selective inhibitors of PDE3 but not by inhibitors of PDE4 (rolipram, ICI63197) or PDE1/5 (zaprinast) (Furman & Pyne, 1990; Shaffiee-Nick *et al*., 1995). PDE3 is now known to exist as two isoforms (Manganiello *et al*., 1995), now referred to as PDE3A and PDE3B. It was shown recently that PDE3B was present in islet β-cells and that its activation mediates IGF-1 induced inhibition of insulin secretion (Zhao *et al*., 1997).

Previous biochemical and pharmacological studies on PDEs in insulin secreting cells have used intact islets, which comprise a heterogeneous population of endocrine cells, only 60–70% of which are β-cells, together with vascular tissue and trapped blood cells. Thus, one hypothesis for the failure of certain type-selective PDE inhibitors to augment insulin release is that PDE1 and PDE4 are expressed in the non-β-cell population of the islets. Therefore the aim of the present study was to characterize the PDEs in a pure β cell population. In view of the difficulty in separating pure native β cells, the present study utilized a recently characterized, glucose-responsive clonal insulin-secreting cell line, BRIN–BD11 (McClenaghan *et al*., 1996; McClenaghan & Flatt, 1999) to determine for the first

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time the expression and properties of PDE isoforms in a β-cell line, as a model for native β-cells. The sensitivity of the PDEs to type-selective inhibitors was examined, as well as the ability of these drugs to modulate glucose-induced insulin secretion. Some of these data have been presented in abstract form (Ahmad *et al*., 1998; Flatt *et al*., 1998).

Methods

Cell culture

BRIN–BD11 cells were cultured in RPMI–1640 tissue culture medium containing 10% foetal calf serum, antibiotics (penicillin 5000 IU ml^{-1}; streptomycin 5 mg ml^{-1}) and 11.1 mmol l^{-1} glucose in vented tissue culture flasks at 37°C in an atmosphere of 5% CO_2 and 95% air using an LEEC incubator (Laboratory Technical Engineering, Nottingham, U.K.). The production and characterization of this cell line is described elsewhere (McClenaghan *et al*., 1996). Cells were used at passage 18-32.

PDE assay

Cells were scraped from the bottom of the flask, and suspended in culture medium and centrifuged ($600 \times g$) for 5 min. The supernatant fluid was removed and the pellet containing the cells was washed with 5 ml of isotonic buffer, pH 7.4, containing Tris-HCl 10 mM, sucrose 0.25 mM, phenylmethylsulphonyl fluoride (PMSF) 0.1 mM and benzamidine 2 mM. The suspension was centrifuged again and the cells homogenized by passing through an 0.24 mm gauge needle three times. After centrifugation at $36{,}400 \times g$ for 20 min at 4°C, the pellet and supernatant fractions were assayed for PDE using a two-step radiometric assay (Thomson & Appleman, 1971) and either ^{3}H-cyclic AMP or ^{3}H-cyclic GMP as substrates (0.5 μM).

Total RNA extraction

600 μl of Rneasy lysis buffer was added to cultured BRIN–BD11 cells and the lysate passed through a QIA shredder. Total RNA was extracted according to the Rneasy protocol. This includes incubating the extracted RNA with 4 units of DNAase at 37°C for 15 min to remove contaminating genomic DNA carried over during the RNA purification.

Reverse transcriptase (RT) reaction

First strand synthesis was carried out using 5 μg extracted total RNA and Superscript II reverse transcriptase. The reaction was primed using 500 ng of oligo (dT)18. This mixture was heated to 70°C for 10 min and quick-chilled on ice. The total assay volume of the reverse transcriptase reaction was 20 μl and included (final concentrations) (mM) Tris/HCl 50, pH 8.3, KCl 75, $MgCl_2$ 3, DTT 0.03 and dNTP mix 0.5 (0.5 mM dATP, dGTP, dCTP and dTTP) and 200 units of Superscript II reverse transcriptase. The mixture was incubated at 42°C for 90 min and terminated by heating at 7°C for 15 min.

PCR (polymerase chain reaction)

2 μl of BRIN–BD11 cDNA was used as a template for PCR. A sense primer (sequence GGAGATGAAGAAGCAACCCTGGGTC) was used in conjunction with an antisense primer (sequence TTCAGTGAGGTGGTGCATTAGTTGGC) to amplify the PDE3B. For PDE3A, the sequences of the sense and antisense primers were, respectively CTGGCCAACCTTCAGGAATC and CCCTCTTGGTTTCCCTTTCTC. A 100 μl PCR reaction mix contained (mM) Tris HCl 10, pH 8.8, $MgCl_2$ 1.5, KCL 50, 0.1% (v v^{-1}) Triton X-100, 0.2 mM dNTPs and 2.5 units of Dynazyme II DNA polymerase, 1 mM. The PCR reaction was carried out in a Perkin Elmer 480 Thermal Cycler using the following protocol: 95°C for 5 min and 35 cycles of 95°C for 1 min 30 sec, 55°C for 30 sec and 68°C for 1 min 40 sec. This was followed by a final extension of 5 min at 68°C. 15 μl of the product was electrophoresed on a 2% (w v^{-1}) agarose gel.

Table 1 Effects of glucose and phosphodiesterase inhibitors on insulin secretion (ng10^6 $cells^{-1}$ 20 min^{-1}) from BRIN–BD11 cells

Treatment	*Concentration* μM	*Glucose* (mM)	*Insulin secretion*	P *value*[b]
Control	–	1.1	0.7±0.09	<0.001
Control	–	16.7	1.6±0.08	–
IBMX	1	16.7	1.9±0.91	NS
IBMX	10	16.7	2.2±0.2	<0.01
IBMX	50	16.7	3.2±0.4	<0.01
Org 9935	1	16.7	1.5±0.1	NS
Org 9935	10	16.7	2.7±0.7	NS
Org 9935	50	16.7	2.5±0.2	<0.01
Siguazodan	1	16.7	1.6±0.14	NS
Siguazodan	10	16.7	2.3±0.16	<0.01
Siguazodan	50	16.7	3.2±0.2	<0.001
Rolipram	1	16.7	1.6±0.06	NS
Rolipram	10	16.7	2.6±0.33	<0.05
Rolipram	50	16.7	2.5±0.24	<0.01
Zaprinast	1	16.7	1.9±0.18	NS
Zaprinast	10	16.7	1.8±0.27	NS
Zaprinast	50	16.7	1.9±0.4	NS

Each value is the mean±s.e.mean of eight observations. [b]vs control 16.7 mM glucose

Insulin secretion

Insulin release from clonal β-cells was determined using monolayers. The cells were harvested with the aid of trypsin/EDTA (Gibco Life Technologies Ltd, Paisley, Strathclyde, U.K.), seeded into 24-well plates (Nunc, Roskilde, Denmark) at a density of 2.5×10^5 cells per well and allowed to attach during overnight culture. Acute studies of insulin release were preceded by 40 min preincubation at 37°C in 1.0 ml Krebs bicarbonate buffer (mM) NaCl 115, KCl 4.7, $CaCl_2$ 1.28, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 10, 0.1% bovine serum albumin, pH 7.4) supplemented with 1.1 mM glucose. Test incubations were performed at 37°C using the same buffer supplemented with varying glucose concentrations and test agents as indicated in Table 1. After 20 min incubation, the buffer was removed from each well and aliquots were stored at −20°C for subsequent measurement of insulin by radioimmunoassay (Flatt & Bailey, 1981).

Drugs and chemicals

Org 9935 (Organon Laboratories, Newhouse, Lanarkshire, U.K.), siguazodan (SK&F 94836) (SmithKline Beecham, Harlow, Essex, U.K.), zaprinast (M&B 22948) (Rhone Poulenc Rorer, Dagenham, Essex, U.K.), rolipram (ZK 62711) (SmithKline Beecham King of Prussia, U.S.A.), and 3-isobutyl-1-methylxanthine, IBMX (Sigma Chemical Co., Poole, U.K.) were prepared as stock solutions in dimethylsulphoxide (DMSO, Sigma Chemical Co.) and diluted in buffer. The appropriate quantity of DMSO was used as control. RPMI 1640 tissue culture medium, foetal calf serum,

penicillin and streptomycin were purchased from Gibco Life Technologies Ltd. ^{125}I for iodination of insulin, ^{3}H-cyclic AMP and ^{3}H-cyclic GMP were obtained from Amersham International. Rat insulin was obtained from Novo Industria, Copenhagen, Denmark. Rneasy total RNA isolation kit, QIA shredder and Qiaquick spin DNA kits were purchased from Qiagen (Crawley, Sussex, U.K.). Superscript II reverse transcriptase and primers were purchased from Life Technologies. Dynazyme II DNA polymerase and DNAase, GFX PCR and gel purification kits, and dNTPs were purchased from Flowgen (Shenstone, U.K.), Promega (Southampton, U.K.), and Amersham Pharmacia Biotech (U.K.), respectively.

Statistical analysis

All data were expressed as mean ± s.e.mean. The quoted n values refer to the number of different cell homogenates used, with each homogenate being assayed in triplicate. Data were analysed using Student's *t*-test for unpaired observations or ANOVA followed by Dunnett's test where appropriate. Statistical significance was accepted where $P<0.05$.

Results

Effect of isobutylmethylxanthine (IBMX) on cyclic AMP hydrolysis

IBMX produced a concentration-dependent inhibition of cyclic AMP phosphodiesterase activity in both pellet and supernatant fractions of BRIN–BD11 cell homogenates with a maximum inhibition of around 90% and an IC_{50} of about 30 μM at 0.5 μM cyclic AMP (Figure 1).

Effect of calcium-calmodulin on PDE activity

In the presence of 100 μM added Ca^{2+}, calmodulin produced a concentration-dependent activation of cyclic AMP or cyclic GMP PDE in both pellet and supernatant fractions of BRIN–BD11 cell homogenates (Figure 2). Calmodulin was significantly ($P=0.002$ in each case) more potent in activating either cyclic AMP (Figure 2a) or cyclic GMP (Figure 2b) hydrolysis in the pellet (respective EC_{50} values ($n=5$) 1.8 ± 0.2 μM and 1.5 ± 0.4 μM) compared with the supernatant fractions (respective EC_{50} values ($n=5$) 4.8 ± 0.4 μM and 4.7 ± 0.6 μM).

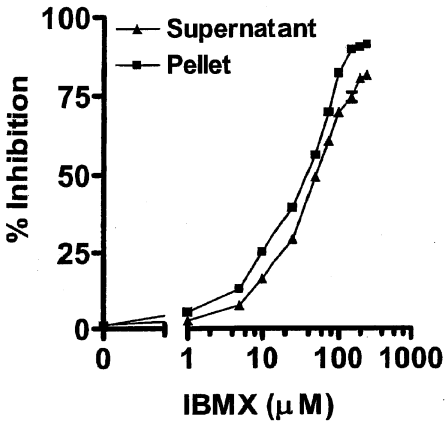


Figure 1 Effect of IBMX on cyclic AMP phosphodiesterase activity in pellet and supernatant fractions of BRIN–BD11 cell homogenates. Each value is the mean of seven observations. The s.e.mean values are smaller than the symbols and are not shown.

Effect of cyclic GMP on cyclic AMP PDE activity

Addition of cyclic GMP potently inhibited cyclic AMP PDE activity in both pellet and supernatant fractions with an approximate IC_{50} of 0.7 μM and a maximum inhibition of about 30–40% at around 2 μM (Figure 3). Concentrations of cyclic GMP above 10 μM produced a markedly increased inhibition which did not reach a maximum up to 100 μM, this concentration producing around 75% inhibition of cyclic AMP PDE activity (data not shown).

Effect of IGF-1 on PDE activity

Incubation of BRIN–BD11 cells for 10 min with IGF-1 produced a concentration-dependent activation of the enzyme in the cell homogenate (per cent increase over basal activity of 309 ± 8.2 pmol min^{-1} mg^{-1}; IGF-1 2.5 ng ml^{-1} 37.4 ± 3.3; 5 ng ml^{-1} 94.4 ± 6; 10 ng ml^{-1} 176 ± 6).

Effect of type-selective PDE inhibitors on PDE activity

The PDE1/PDE5 inhibitor zaprinast inhibited both cyclic AMP PDE (Figure 4a) and cyclic GMP PDE (Figure 4b) activity in pellet and supernatant fractions of cell homogenates by a maximum of around 25% with an IC_{50} in the range 1–5 μM.

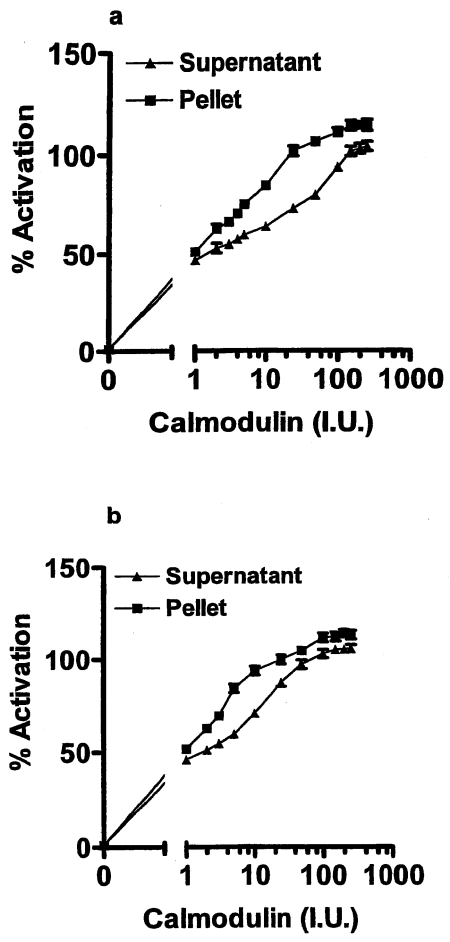


Figure 2 Effect of calmodulin on cyclic AMP phosphodiesterase activity (a) and cyclic GMP phosphodiesterase activity (b) in the presence of 100 μM added Ca^{2+} in pellet and supernatant fractions of BRIN–BD11 cell homogenates. Each value is the mean ± s.e.mean of five separate experiments.

The PDE3-selective inhibitors Org 9935 (0.02–10 μM, Figure 5a) and siguazodan (0.1–10 μM, Figure 5b) inhibited cyclic AMP PDE activity in the pellet but not the supernatant fractions of cell homogenates. The maximum inhibition produced by Org 9935 was about 35±2% and that produced by siguazodan was about 28±2% with respective IC_{50} values of 0.05 and 0.5 μM.

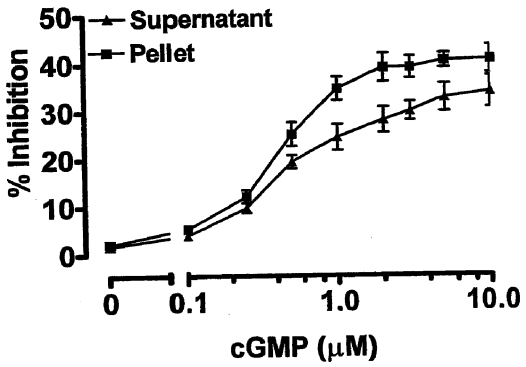
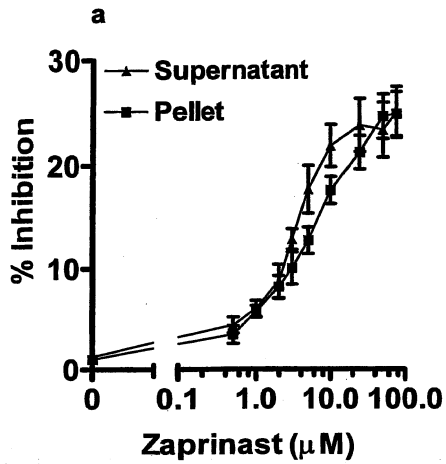


Figure 3 Effect of cyclic GMP on cyclic AMP phosphodiesterase activity in pellet and supernatant fractions of BRIN–BD11 cell homogenates. Each value is the mean±s.e.mean of five separate experiments.



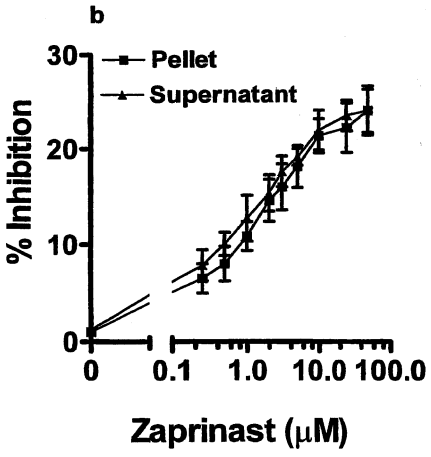
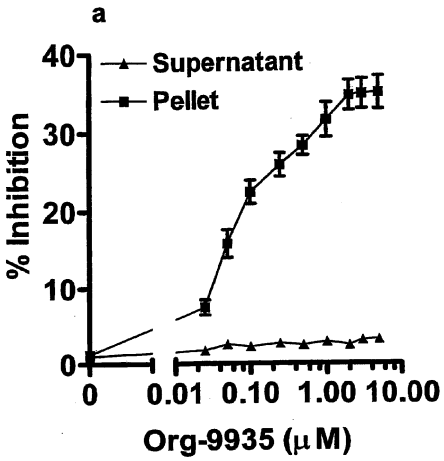


Figure 4 Effect of zaprinast on cyclic AMP phosphodiesterase activity (a) and cyclic GMP phosphodiesterase activity (b) in pellet and supernatant fractions of BRIN–BD11 cell homogenates. Each value is the mean±s.e.mean of five observations for cyclic AMP PDE activity and of eight observations for cyclic GMP PDE activity.

The PDE4 selective inhibitor rolipram potently inhibited cyclic AMP PDE activity in both pellet and supernatant fractions with a maximum inhibition around 25% and IC_{50} values of about 0.04 and 0.1 μM in the pellet and supernatant fractions, respectively (Figure 6). Rolipram



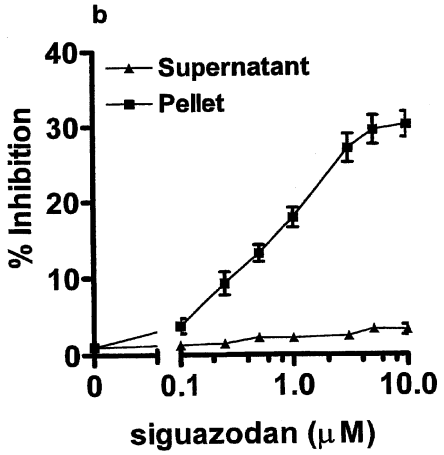


Figure 5 Effect of Org 9935 (a) and siguazodan (b) on cyclic AMP phosphodiesterase activity in pellet and supernatant fractions of BRIN–BD11 cell homogenates. Each value is the mean±s.e.mean of 15 separate experiments for Org 9935 and 10 for siguazodan.

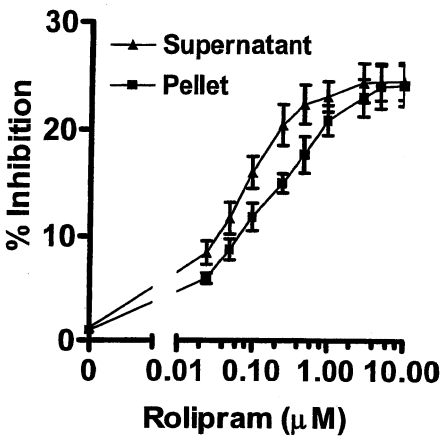


Figure 6 Effect of rolipram on cyclic AMP phosphodiesterase activity in pellet and supernatant fractions of BRIN–BD11 cell homogenates. Each value is the mean±s.e.mean of five separate experiments.

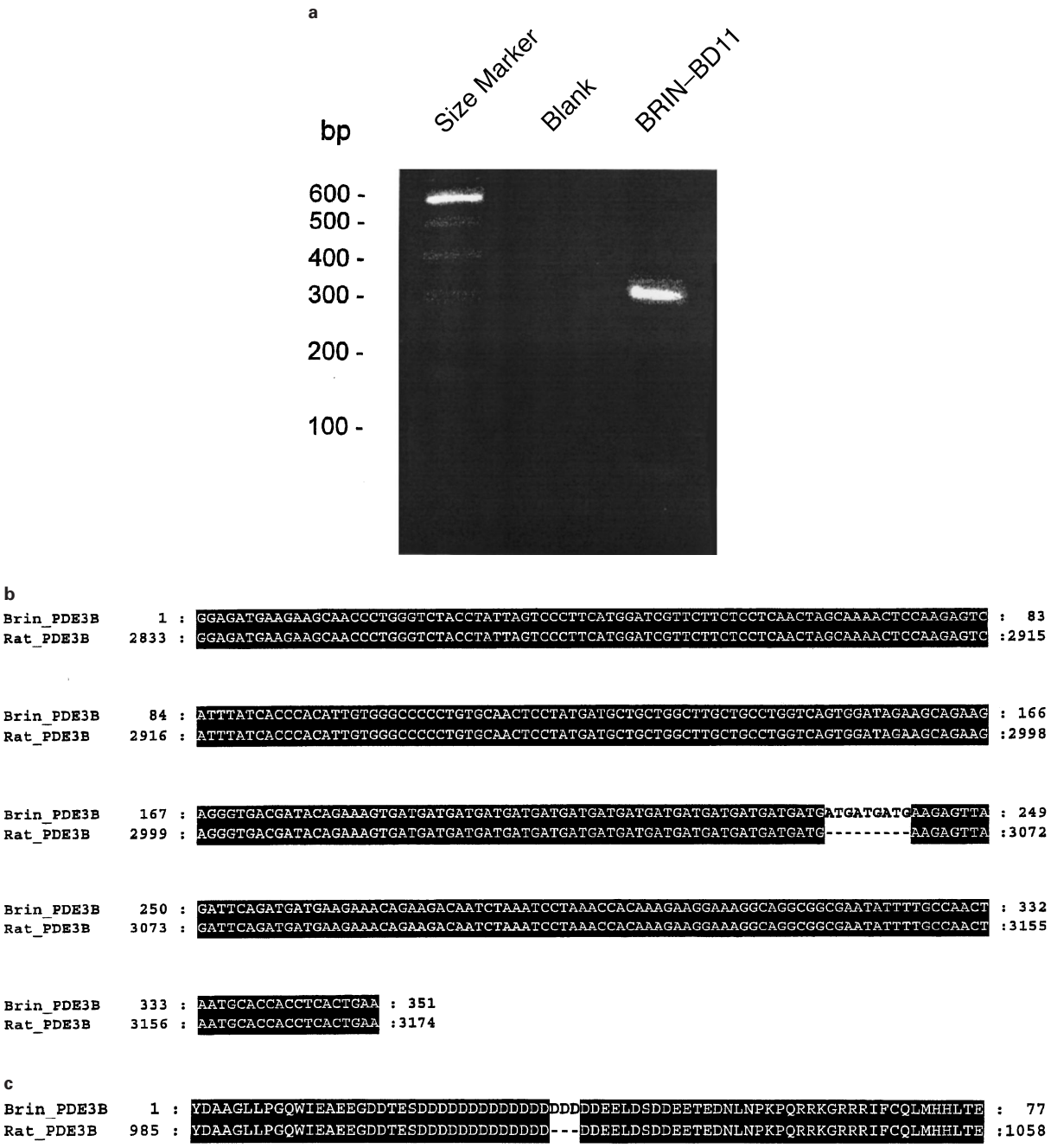


Figure 7 Reverse transcriptase polymerase chain reaction analysis showing (a) amplification of a 351 bp product when primers for PDE3B were used (b) the sequence of the product and (c) the corresponding amino acid sequence.

failed to modify cyclic GMP hydrolysis in concentrations as high as 100 μM.

PCR

RT–PCR of BRIN–BD11 cells with specific primers for PDE3B amplified a 351 base pair product from BRIN–BD11 total RNA which was homologous with the corresponding region in rat and human PDE3B (Figure 7). Comparison of the cDNA and predicted amino-acid sequence of this product with the corresponding region in rat adipocyte PDE-3B showed >97% similarity. In contrast, no product was obtained when specific primers for PDE3A were used or, when reverse transcriptase was omitted from the reaction. The primers for PDE3A amplified a PDE3A transcript from rat pulmonary artery (data not shown).

Insulin secretion

Insulin release from BRIN–BD11 cells was augmented by 16.7 mM glucose. IBMX, Org 9935, siguazodan and rolipram (1–50 μM), but not zaprinast, each augmented glucose-induced insulin secretion (Table 1) in the presence of 16.7 mM glucose. None of the drugs affected insulin release at 1.1 mM glucose (data not shown).

Discussion

These data show that a glucose-responsive, insulin-secreting cell line expresses a number of cyclic nucleotide phosphodiesterase activities. The potent activation of both cyclic AMP and cyclic GMP PDE by calmodulin suggest the presence of PDE1

(Beavo, 1995). This is further supported by the effect of zaprinast, which produced a 25% maximum inhibition of cyclic AMP PDE. The activation by calmodulin and the inhibition by zaprinast was seen in both pellet and supernatant fractions, although calmodulin appeared to be significantly more potent in activating enzyme activity in the pellet. We previously found zaprinast to inhibit PDE activity in both pellet and supernatant fractions of rat islet homogenates, although inhibition was inconsistent in the pellet (Shafiee-Nick *et al*., 1995). Zaprinast is more potent in inhibiting PDE5 compared with PDE1 but PDE5 does not hydrolyse cyclic AMP, further supporting the involvement of PDE1. PDE1 has generally been reported in other tissues as a soluble enzyme activity, although there are some reports of a particulate activity (Wang *et al*., 1990). Several earlier studies in rat and mouse islets (Sugden & Ashcroft, 1981; Lipsom & Oldham, 1983; Capito *et al*., 1986) have shown the presence of a calcium–calmodulin stimulated PDE activity but this was found only in the soluble fraction (Capito *et al*., 1986). There is little evidence for the functional significance of this enzyme in regulating islet β-cell function. However, the reduction in forskolin-stimulated cyclic AMP content by Ca^{2+} in RINm5F cells (Ullrich & Wolheim, 1988) and its enhancement by verapamil (Rajan *et al*., 1989) are compatible with a role for Ca^{2+} in the regulation of β-cell cyclic AMP levels *via* activation of PDE1. On the other hand, the failure of zaprinast to augment insulin secretion in the present study or in earlier work in islets (Shafiee-Nick *et al*., 1995) argues against a functional role for PDE1 that is relevant to insulin release. Interestingly, a very recent paper published just at the time of submission of the present manuscript, suggested that PDE1C might be important in modulating insulin secretion in a different cell line (βTC3 insulinoma; Han *et al*., 1999). PDE1C was clearly expressed as a soluble enzyme and insulin secretion was augmented by 8-methoxy-isobutylmethylxanthine (8MM-IBMX), a relatively selective inhibitor of PDE1C, as well as by zaprinast. 8MM-IBMX, albeit at high concentrations (500 μM) also augmented glucose-induced insulin secretion in islets, although zaprinast was not studied.

The potent inhibitory effect of cyclic GMP on BRIN–BD11 cell PDE activity, along with the potent inhibitory effects of the selective PDE3 inhibitors Org 9935 and siguazodan, suggest that PDE3 is expressed in BRIN–BD11 cells. Moreover, the RT–PCR studies suggested that this is PDE3B, as found by Zhao *et al*. (1997) in native islet β-cells. We were able to confirm using RT–PCR, that transcripts for PDE-3B are expressed in BRIN–BD11 cells. The primers used were designed to amplify a region of the C-terminal portion of the protein, which contains the catalytic site. The product, like that corresponding to the rat adipocyte form, contained a repeating codon sequence, GAT, corresponding to aspartate. In the rat adipocyte form there are 15 repeating aspartates, while the BRIN–BD11 form has 18. Interestingly, PDE-3B from BRIN–BD11 cells has a nine base pair insertion, which encodes the additional three aspartates. The functional role of this stretch of aspartates is unknown. However, this could confer a polyanionic region which might prevent insertion of the cytoplasmic C-terminal tail of the protein into membranes. The presence of three additional asparates in the BRIN–BD11 form would confer a stronger acidic nature to this region that may be important in determining a slightly different membrane topography of the enzyme. The selective PDE3 inhibitors inhibited PDE activity maximally by around 30–40% in the pellet fraction suggesting that about 30–40% of BRIN–BD11 cell particulate PDE activity is PDE3. This broadly supports our previous study in islets (Shafiee-Nick *et al*., 1995), the drugs showing similar potencies in inhibiting PDE. However, there are two differences from islets. First, around 70% of islet PDE activity is inhibited by Org 9935 or siguazodan. Second, although there is clearly PDE3 activity in both pellet and supernatant fractions of islet homogenates (Shafiee-Nick *et al*., 1995), no inhibition of supernatant-fraction PDE was obtained with either of the selective PDE3 inhibitors up to 100 μM, suggesting that there was no soluble PDE3 present. This was also found in the recent paper by Han *et al* in βTC3 insulinoma cells (1999). Interestingly, previous work in other tissues shows PDE3B expression to be predominantly particulate, whereas PDE3A expression seems to be cytosolic (Manganiello *et al*., 1995; Liu & Maurice, 1998). Thus, the soluble PDE3 that we demonstrated previously in islets may have been PDE3A located in blood vessels or other cell types present in islets. It is possible that the soluble PDE3 present in islets represented particulate enzyme displaced from the membranes. The inhibition by cyclic GMP of both supernatant and pellet PDE activities by similar amounts and with broadly similar potencies, might be partly explained by competitive inhibition of PDE1.

As in islets, either of the PDE3 inhibitors augmented insulin secretion in the presence of stimulatory (16.7 mM) glucose concentrations, but not in the presence of low glucose concentrations. This is compatible with our hypothesis (Shafiee-Nick *et al*., 1995) that PDE3 regulates the cyclic AMP pool responsible for modulating insulin release. This has been supported by others using selective PDE3 inhibitors (Parker *et al*., 1995; Han *et al*., 1999). Inhibition of insulin release by IGF-1 was attributed to its activation of PDE3B in the β-cell (Zhao *et al*., 1997). The present study confirms that the BRIN–BD11 cell PDE activity is markedly enhanced by IGF-1, showing that the enzyme activity in the cell line is regulated similarly to that found in islets.

The potent inhibitory effect of rolipram on the hydrolysis of cyclic AMP, but not cyclic GMP, suggests the presence of a PDE4 in the BRIN–BD11 cells and the maximum per cent inhibition suggests that this accounts for about 25% of the total PDE activity in supernatant and pellet fractions. This corresponds to the previous findings in islets using rolipram (Shaffiee-Nick *et al*., 1995) or ICI16397 (Furman & Pyne, 1990). However, in contrast to findings in islets (Shafiee-Nick *et al*., 1995; Parker *et al*., 1995) rolipram augmented glucose-induced insulin secretion. Perhaps PDE4 is compartmentalized or the expression level is decreased in native β-cells compared with clonal β-cells, although we have no evidence for these possibilities. It is noteworthy that we reported that rolipram-inhibition of cyclic AMP PDE activity in islets was not consistent among different experiments (Shafiee-Nick *et al*., 1995). The data of Han *et al*. (1999) are consistent with our present findings in BRIN–D11 cells and our previous findings in islets as they also showed rolipram to augment insulin secretion only in their cell line.

Overall, the present study suggests that cyclic AMP PDE activity in BRIN–BD11 cells can be largely accounted for by PDE1, PDE3 and PDE4, with the PDE3 and PDE4 regulating the cyclic AMP levels relevant to insulin secretion. About 10% of total cyclic AMP PDE activity was not inhibited by maximal concentrations of IBMX or by the concurrent application of Org 9935, rolipram and zaprinast in maximal inhibitory concentrations (data not shown). Thus these cells may also express the high affinity, IBMX insensitive PDE7 and PDE8 enzymes, although there are currently no selective inhibitors for these. There are clearly some differences from islets in the proportion of the different PDEs expressed and in their functional roles. Explanations

for these differences remain speculative but may relate to the transformed nature of the BRIN–BD11 cell. Additionally, an islet is a complex structure, comprising at least four types of endocrine cells, along with vascular smooth muscle and endothelial cells. The biochemical analysis of enzymes in an islet cannot therefore reflect the situation present in a β-cell. In this sense, the BRIN–BD11 cell is clearly advantageous, as it is provides a homogeneous cell population. However, a monolayer of BRIN cells must lack the interactions among cells that probably occurs in an islet. Although such interactions are poorly understood, their importance is exemplified by the observation that isolated β-cells show impaired secretory function relative to intact islets (Pipeleers *et al*., 1994). Moreover, recent work has shown that when another insulin secreting cell line (Min6) is grown on gelatin the cells form smooth, rounded 'pseudoislets' which show markedly superior insulin secretion compared with the same cells grown as monolayers (Haughe-Evans *et al*., 1999). The absence of an islet structure in monolayer cultures could influence the expression and/or activity of different PDE isoenzymes and this hypothesis is currently under investigation.

In conclusion, the present findings obtained in a clonal insulin secreting cell line support the hypothesis for an important role for PDE3 in regulating the pool of cyclic AMP relevant to the modulation of glucose induced insulin secretion, although PDE4 may also contribute.

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