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Interleukin-1β effects on cyclic GMP and cyclic AMP in cultured rat islets of Langerhans – arginine – dependence and relationship to insulin secretion

I. C. Green[1], C. A. Delaney[1], J. M. Cunningham[1], V. Karmiris[1] and C. Southern[2]

[1] Biochemistry Laboratory, School of Biological Sciences University of Sussex, Brighton, and
[2] Yamanouchi Research Institute, Littlemore Hospital, Oxford, UK

Summary. When islets were cultured with interleukin-1β (1 or 100 pmol/l) for 12 h in arginine-containing medium, cyclic GMP levels were increased 1.6- and 4.5-fold respectively. The arginine analogue, N-ω-nitro-L-arginine methyl ester, which blocks nitric oxide formation and partially reverses inhibition of insulin secretion by 100 pmol/l interleukin-1β, largely, but not completely, blocked generation of cyclic GMP. Treatment of islets with 100 pmol/l interleukin-1β for 12 h significantly decreased islet cyclic AMP generation in the absence of isobutylmethylxanthine (from 13.1 ± 0.7 to 9.3 ± 0.8 fmol/μg islet protein), this fall was arginine-dependent and may have resulted from an effect on a cyclic AMP phosphodiesterase, since it was masked if isobutylmethylxanthine was present. Isobutylmethylxanthine (0.4 mmol/l) reduced the inhibitory potency of interleukin-1β in 15 h slightly but significantly from 80.5 to 59.0%. The morpholinosydnonimine SIN-1, which is a nitric oxide donor, inhibited insulin secretion, raised islet cyclic GMP and lowered cyclic AMP; its effects were similar to those of interleukin-1β. However, 6-anilinoquinoline-5,8-quinone, [LY83583 (1–10 μmol/l)], inhibited insulin secretion, and significantly decreased cyclic GMP while 8-bromocyclic GMP stimulated insulin secretion. Both low- and high-dose interleukin-1β treatment give a large arginine-dependent and a small, yet significant, arginine-independent increase in cyclic GMP. The inhibitory effect of SIN-1 or interleukin-1β on insulin secretion seems to depend to a small extent on decreased islet cyclic AMP, though sustained increases in nitric oxide or depleted islet GTP may directly affect the secretory process.

Key words: Cyclic GMP, cyclic AMP, interleukin-1β, LY83583, SIN-1, 8-bromo cGMP.

Cytokines are known to modulate the function of the endocrine pancreas [1]. Direct inhibitory effects of interleukin-1 and -6 or combinations of interleukins with tumour necrosis factor-α (TNF) or interferon-γ have been observed in insulin-secreting cells [1], including human beta cells [2]. This accounts for the considerable interest in these pro-inflammatory agents in the development of insulin-dependent diabetes mellitus. The effects of cytokines on insulin secretion [3–7], DNA synthesis [8], glucose oxidation [9, 10] and cell viability are well documented [11, 12]. The mechanisms which have been investigated as triggering or mediating these events include increased prostaglandin production [11, 13], altered activities of inositol phosphate or protein kinase C [6, 14], inhibition of glucokinase [15] or aconitase [10], generation of free radicals [1, 16] or activation of proteases [7, 17–19]. These events may be accompanied by production of heat-shock proteins [20, 21], increased gene transcription [7, 22] and altered protein synthesis [23]. More recently, we have demonstrated that there is a role for L-arginine-derived nitric oxide in mediating part of the inhibitory effect of interleukin-1β (IL-1β) and in regulating the synergistic inhibition of beta cell function by combined interleukin-1β plus TNF treatment [24]. Our results demonstrating nitric oxide generation by IL-1β have been confirmed [10, 25]. An arginine analogue N-monomethyl-L-arginine (L-NMMA) completely reversed the inhibitory effects of IL-1β, though the structurally different analogue N-ω-nitro-L-arginine methyl ester (NAME), in our experiments, was only partially effective in attenuating inhibition [25], despite being used in arginine-free medium.

Islets may also be lysed following prolonged exposure to cytokines (2–6 days) and the stages preceding lysis may be mediated rather differently than those leading to inhibition of insulin secretion (6–48 h). Recently, nitric oxide derived from macrophages has been shown to promote islet cell lysis [26], an effect which can be blocked by arginine analogues. We are currently investigating islet localisation of nitric oxide synthase, but wherever nitric oxide originates – whether endocrine cells [10, 24, 25], non-endocrine islet cells [12, 26] or endothelial cells in perfused pancreas [27] – its mechanism of action merits examination.

Nitric oxide is a lipophilic, short-lived free radical which reacts with haem proteins and which elevates cyclic GMP through the activation of soluble guanylate cyclase [28, 29]. The effects of IL-1β were shown previously to be highly dose-dependent, low doses stimulated, while high doses inhibited insulin secretion [3]. In this study, we were interested in whether arginine-derived nitric oxide or nitric oxide-stimulated cyclic GMP mediates the dose-dependent effects of IL-1β on insulin secretion or whether there was a role for altered cyclic AMP levels [17, 30]. The aims of the study were therefore: a) to resolve the question of whether cyclic GMP is a necessary mediator of the stimulatory or inhibitory effects of IL-1β on insulin secretion; b) to see if cytokine effects could be reproduced by drug-induced elevation of nitric oxide or cyclic GMP; c) to determine whether IL-1β affects cyclic AMP as well as cyclic GMP production in adult rat islets in culture; and d) to conduct experiments which would show whether arginine-derived nitric oxide is necessary for altered cyclic nucleotide production and changes in insulin secretion rates.

Materials and methods

Female Sprague-Dawley rats weighing 150–220 g were used throughout the study; rats were fed ad libitum. Tissue culture RPMI-1640 complete medium, arginine-free RPMI or RPMI without glucose, arginine, inositol, glutamine, cysteine, methionine and leucine, but with supplements added as 100 × concentrates, were purchased from Gibco BRL (Paisley, UK). Cyclic nucleotide standards, cyclic nucleotide tyrosine methyl esters for iodination, 8-bromo cyclic GMP, collagenase type XI and N-ω-nitro-L-arginine methyl ester, (NAME) were from Sigma Chemicals Ltd. (Poole, Dorset, UK). IL-1β (code 86/552) was from the National Institute for Biological Standards and Control (Potters Bar, Herts., UK). 6-anilinoquinoline-5,8-quinone, LY83583, a guanylate cyclase inhibitor was from Novabiochem (Nottingham, Notts., UK). SIN-1, a nitric oxide donor, was a gift from Dr. R. Knowles (Wellcome Research Labs, Beckenham, Kent, UK).

Preparation and culture of isolated islets of Langerhans

Islets were isolated under aseptic conditions from collagenase digested pancreases of female Sprague-Dawley rats. Typically two-three rat pancreases (for freshly isolated) or three-four pancreases (for cultured islets) were used to make two isolates per day [24]. Islets were cultured in RPMI medium containing 5.5 or 11 mmol/l glucose, 5% fetal calf serum, penicillin (60 U/ml) and streptomycin (100 μg/ml), for a 48-h pre-culture period. The glucose content of RPMI medium has no effect on cytokine-induced cyclic nucleotide changes. The 2-day culture was followed by a 12–15 h treatment in RPMI with and without IL-1β. The treatment medium was either RPMI containing arginine (1 mmol/l) or arginine-free RPMI medium supplemented with NAME (1 mmol/l). In some specified experiments a low concentration of isobutylmethylxanthine (IBMX, 0.1–0.4 mmol/l) or forskolin (5 μmol/l) was added together with the cytokine to prevent degradation of cyclic nucleotides formed or to raise cyclic AMP levels respectively. Islets were also cultured with agents which raise or lower cyclic GMP levels i.e. 8-bromo cyclic GMP (0.1–10 μmol/l) or LY83583 (1–10 μmol/l). Islets were washed free of treatment medium and were used for insulin secretion and cyclic nucleotide measurements.

Islet incubation for insulin secretion

Cytokine – or other treated – islets were removed from culture medium and pre-incubated in Gey & Gey buffer [31] containing 2 mmol/l glucose and 1 mg/ml bovine serum albumin fraction V for 1 h. Insulin secretory responses were determined from groups of three or five islets challenged with buffer (1.0 ml) containing 2 or 20 mmol/l glucose with and without IBMX or test compounds e.g. LY83583 or SIN-1. At the end of a 30-min incubation, aliquots were removed for assay of insulin and, if required, acid ethanol (ethanol:concentrated HCl:H_2O; 23:0.45:7.0) was added in equal volume to the remaining islets and medium for extraction of insulin overnight. The extract was diluted (× 100), and stored at −20 °C prior to RIA. Insulin was assayed using rat insulin standard (Novo-Nordisk, Basingstoke, UK), guinea pig insulin antiserum and ^{125}I-bovine insulin, (both antibody and label were prepared in this laboratory [32]).

Cyclic GMP, cyclic AMP and protein estimation

Cytokine-treated islets were removed in groups of 15–40 to eppendorf tubes and the culture medium was removed. The islets were pre-incubated for 30 min, as for insulin secretion experiments, then incubated in buffer with and without IBMX (1.0 mmol/l) for 30 min. Freshly isolated islets were pre-incubated as above and incubated with test compounds for 30 min. In all cases the incubation medium was discarded, the islets were then boiled for 3 min in sodium acetate buffer (50 mmol/l) pH 6.2, centrifuged at 10000 × *g* for 3 min following which the supernatant was removed for RIA of cyclic nucleotides. The islet protein pellet was stored frozen, then dissolved in 0.2 ml of 0.1 mol/l NaOH, immediately prior to assaying protein in ELISA plates using the Bradford method [33].

Radioimmunoassay of cyclic GMP and cyclic AMP

Cyclic GMP was tested using an RIA kit (NEN-Dupont, Stevenage Herts., UK). The kit protocol was followed, except that 50 μl rather than 100 μl volumes of standard and unknown, as well as antibody and label were used. In other experiments cyclic GMP was tested by RIA using a protocol which was described originally [34] and employed in this lab previously for cyclic nucleotide measurement [35]. Identical results were obtained for cyclic GMP measurements using a purchased kit or our own assay. Antibody was prepared in this laboratory in rabbit against succinyl cyclic GMP conjugated to albumin (by Drs. Green and Ray; cross-reactivity of antibodies for other cyclic nucleotides was less than 0.1%). Cyclic GMP was used as standard and succinyl cyclic GMP tyrosyl methyl ester (TME) was iodinated in our laboratory. The labelled product was purified on paper chromatography, as previously described in detail [34]. Both kit and own assays used acetylated standards and unknowns, acetylation was performed with 2.5 μl of a mixture of triethylamine:acetic anhydride 2:1 in ethanol-washed soda glass tubes. An overnight incubation at 4 °C of 50 μl aliquots of sample, antibody and label was used. Bound and free label were separated using polyethylene glycol/gamma globulin mix, as for the insulin assay [32], values using this means of separation were similar to those obtained by the kit method, or by charcoal separation [35].

Cyclic AMP was assayed using the same method as described by Brooker [34]; antibody was a gift from Dr. M. Titheradge (Sussex University, UK), cyclic AMP standard was from Sigma Chemicals, succinyl cyclic AMP TME derivative was iodinated, as described for cyclic GMP. Fifty millilitres of standard or unknown was acetylated and 50 μl of both antibody and label were added to glass tubes and allowed to react overnight. Bound and free label were separated as for the cyclic GMP assay, and the cyclic nucleotide concentrations were determined using an RIA calc program on an LKB-Pharmacia multigamma counter.

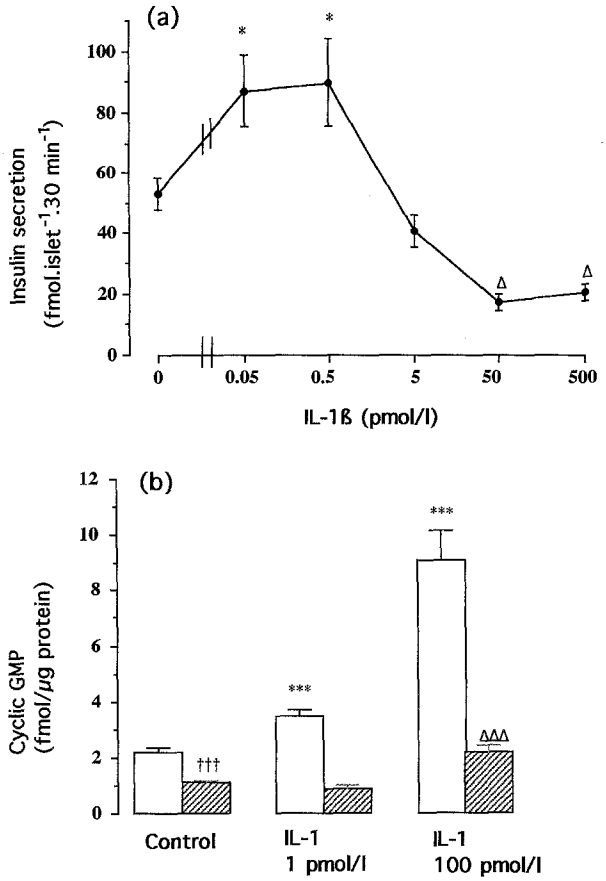

Fig. 1 a, b. Dose-dependent effect of interleukin-1β (IL-1β) on insulin secretion and cyclic GMP production. (**a**) Islets were cultured for 48 h in RPMI medium containing 11 mmol/l glucose followed by 12 h culture with and without IL-1β at the concentrations shown. 20 mmol/l glucose-induced insulin secretion from groups of three islets was determined as described in Materials and methods. Secretion in response to 2 mmol/l glucose was 9.7 ± 0.7 fmol · islet^{-1} · 30 min^{-1} (n = 16). Values are mean ± SEM of three islet isolates (n = 28): *$p < 0.01$ significantly greater than control; △$p < 0.001$ significantly less than control by Student's t-test. (**b**) Islets were cultured for 48 h in RPMI containing 11 mmol/l glucose and treated with two concentrations of IL-1β and a low concentration of IBMX (0.1 mmol/l) in medium containing 1 mmol/l arginine (□) or arginine-free medium containing 1 mmol/l N-ω-nitro-L-arginine methyl ester (NAME; ▨). Groups of 15 islets were removed and processed for cyclic GMP as described in the text. Values are mean ± SEM for four isolates (n = 11–14). *** $p < 0.001$ significantly greater than arginine control, ††† $p < 0.001$ significantly less than arginine control; △△△ $p < 0.001$ significantly greater than NAME control by Student's t-test

Statistical analysis

Data presented are usually means ± SEMs. The significance of differences between means was evaluated by Student's unpaired t-test, or by one way analysis of variance. In both instances the acceptance level of significance was $p < 0.05$ or better.

Results

When islets are treated for 12 h, in culture, with a range of concentrations of IL-1β and challenged afterwards for 30 min with medium containing 20 mmol/l glucose, the insulin secretory response ranged from stimulatory to inhibitory (Fig. 1 a). In islets treated with cytokine plus a low concentration of IBMX (0.1 mmol/l) in a 12-h culture period, 100 pmol/l IL-1β treatment resulted in a 4.3-fold rise in cyclic GMP; however, a low dose (1 pmol/l) also significantly raised cyclic GMP (1.6-fold) (Fig. 1 b). Cyclic GMP levels were reduced when islets were incubated in arginine-free medium containing NAME (1 mmol/l); cytokine-induced rises in cyclic GMP were significantly reduced, although a small and significant rise in cyclic GMP persisted (Fig. 1 b).

When islets were cultured in medium with 1 pmol/l or 100 pmol/l IL-1β for 15 h and then incubated for 30 min in medium containing IBMX (1 mmol/l) cyclic GMP levels were increased 3- and 8.5-fold, respectively, but there was no significant change in cyclic AMP production (Fig. 2 a). The glucose concentration in RPMI did not alter islet cyclic nucleotide generation. When arginine was substituted by the analogue NAME, the cytokine-induced rise in cyclic GMP for 1 pmol/l and 100 pmol/l was 40 % and 29 %,

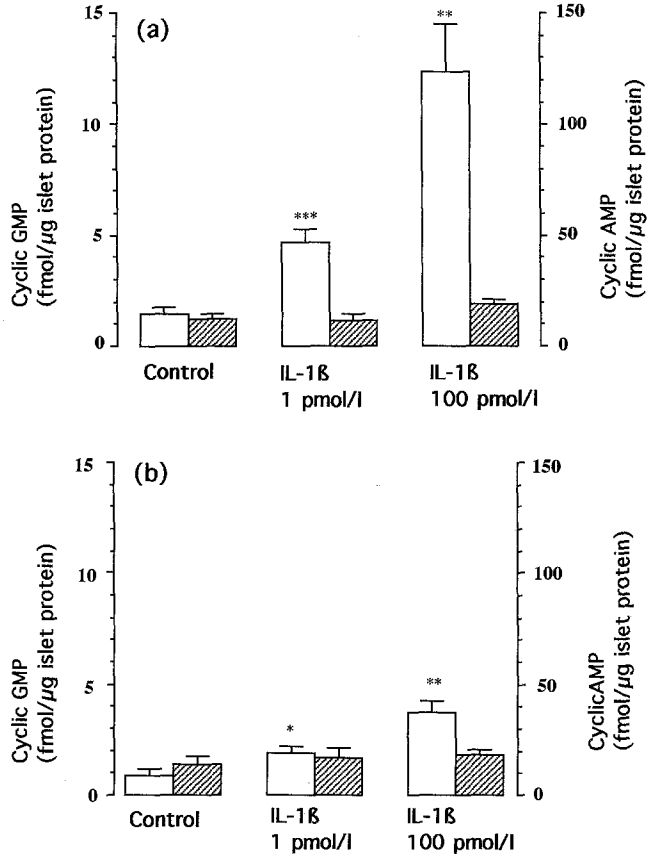

Fig. 2 a, b. Cyclic nucleotide production in response to interleukin-1β (IL-1β) in the presence and absence of arginine. Islets were cultured for 48 h in RPMI medium containing 5.5 mmol/l glucose and treated for 15 h with IL-1β in medium containing (**a**) arginine (1 mmol/l) or (**b**) arginine-free medium containing N-ω-nitro-L-arginine methyl ester (NAME; 1 mmol/l). Groups of 15 islets were then incubated for 30 min in buffer containing 20 mmol/l glucose + IBMX (1 mmol/l). Cyclic GMP (□) and cyclic AMP (▨) were extracted and assayed as described in Materials and methods. Values are mean ± SEM from four isolates (n = 10–12 for 1 pmol/l treatment; n = 6 for 100 pmol/l treatment): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$ significantly greater than control by Student's t-test

Table 1. Effect of culture with interleukin-1β (IL-1β) on cyclic AMP and cyclic GMP in islets. Islets were cultured for 48 h in RPMI medium containing 5.5 mmol/l glucose and were treated for 15 h with IL-1β in medium containing arginine (1 mmol/l) or arginine-free medium containing N-ω-nitro-L-arginine methyl ester (NAME; 1 mmol/l). After a pre-incubation period in buffer containing 2 mmol/l glucose, cyclic nucleotides were extracted from groups of 30–40 islets and assayed as described in Materials and methods

Treatment	Cyclic nucleotides (fmol/μg islet protein)			
	cyclic AMP		cyclic GMP	
	Arginine	NAME	Arginine	NAME
Control	13.1 ± 0.7 (5)	11.2 ± 3.0 (3)	2.7 ± 0.3 (5)	3.7 ± 0.7 (3)
IL-1β (1 pmol/l)	12.1 ± 2.9 (4)	11.1 ± 1.4 (5)	11.2 ± 2.3 (5)[b]	5.1 ± 0.8 (5)
IL-1β (100 pmol/l)	9.3 ± 0.8 (8)[a]	11.2 ± 1.8 (5)	14.9 ± 2.3 (8)[c]	4.9 ± 0.4 (6)

Values are mean ± SEM. Number of determinations from four islet isolates in parentheses.
[a] $p < 0.02$ significantly less than control; [b] $p < 0.01$, [c] $p < 0.001$ significantly greater than control by Student's *t*-test

Table 2. Effect of culture with and without interleukin-1β (IL-1β), IBMX or forskolin on insulin secretory response by islets. Islets were cultured for 48 h in RPMI medium containing 11 mmol/l glucose and were treated for 15 h with IBMX (0.4 mmol/l), IL-1β (100 pmol/l) and IBMX + IL-1β (experiment a) and forskolin (5 μmol/l), IL-1β (100 pmol/l) and forskolin + IL-1β (experiment b). Insulin secretion by groups of three islets incubated in buffer containing 2 mmol/l glucose and 20 mmol/l glucose with and without 1 mmol/l IBMX was determined as described in Materials and methods

	Insulin secretion (fmol · islet^{-1} · 30 min^{-1}) Incubation buffer		
	2 mmol/l glucose	20 mmol/l glucose	20 mmol/l glucose + IBMX
Culture treatment			
Experiment a			
None	46 ± 5	236 ± 24[a]	764 ± 150[a]
IBMX	45 ± 3	175 ± 17[a]	920 ± 146[a]
IL-1β	36 ± 3	46 ± 7	826 ± 174[a]
IL-1β + IBMX	40 ± 2	71 ± 5[abc]	748 ± 55[a]
Experiment b			
None	42 ± 5	159 ± 8[a]	874 ± 96[a]
forskolin	83 ± 10	110 ± 21	881 ± 107[a]
IL-1β	52 ± 33	86 ± 25	220 ± 38[a]
IL-1β + forskolin	72 ± 13	85 ± 16[d]	378 ± 69[a]

Values are mean ± SEM [$n = 6$–8 (experiment a) and 5–8 (experiment b)] each from two islet isolates.
[a] $p < 0.01$ significantly greater than 2 mmol/l glucose control; [b] $p < 0.05$ significantly greater than IL-1β-treated 20 mmol/l glucose value; [c] $p < 0.01$ significantly less than IBMX-treated 20 mmol/l glucose value; [d] $p > 0.05$ not significantly different from forskolin-treated 20 mmol/l glucose value by Student's *t*-test

respectively, of that in arginine-treated islets; cyclic AMP levels were unchanged (Fig. 2b). NAME (1 mmol/l) partially reversed IL-1β-inhibited insulin secretion [24], but had no effect on IL-1β-stimulated insulin secretion. The percent of stored insulin secreted in response to stimulation by 20 mmol/l glucose was increased in islets cultured with 1 pmol/l IL-1β in the presence of arginine to 2.9 from 2.1 %, $n = 18$, $p < 0.03$ and in the presence of NAME to 3.3 from 2.2 %, $n = 15$, $p < 0.03$ by analysis of variance, data from three experiments performed using islet isolates from rats of different size. Although NAME significantly reduced cyclic GMP production in untreated islets by comparison with levels in islets cultured in arginine-containing media, cyclic GMP was still increased in islets which were treated with cytokines in NAME-containing media (Fig. 2b).

In a further series of experiments measuring combined cyclic nucleotide production, no IBMX was used in the culture or post-culture incubations. In this instance (Table 1) 100 pmol/l IL-1β lowered cyclic AMP significantly, while raising cyclic GMP; 1 pmol/l IL-1β did not alter cyclic AMP. NAME blocked IL-1β-induced cyclic nucleotide changes.

In order to see if the inhibitory effect of IL-1β on glucose-induced insulin secretion could be reversed by maintaining islet cyclic AMP levels two treatments were used (Table 2). Islets, cultured for 15 h with IL-1β (0.1 nmol/l), were also treated with IBMX (0.4 mmol/l) or forskolin (5 μmol/l), subsequently, insulin secretory responses to glucose and IBMX (1.0 mmol/l) were measured. Long-term IBMX and forskolin treatment reduced the sensitivity of the islets to stimulation by glucose, thus altering the controls for the cytokine treatment and making interpretation less clear. The inhibitory effect of IL-1β after 15 h in the presence of IBMX expressed as a percent of the appropriate control was altered from 19.5 ± 2.9 % to 41 ± 2.8 %; the inhibitory effect of IL-1β in the presence of forskolin was also attenuated by 20 % when the response of 20 mmol/l glucose ± IBMX was considered.

Since cyclic GMP levels are altered to the greatest extent, predictably so as a result of nitric oxide stimulation of guanylate cyclase, the role of cyclic GMP in modifying insulin secretion was investigated. The treatment of islets with 8-bromo cyclic GMP for 6 h (Fig. 3a) or for 15 h (data not shown) resulted in stimulation of insulin secretion at a number of concentrations (0.1, 5 and 10 μmol/l). We also tested the effect of a guanylate cyclase inhibitor LY83583 and the nitric oxide donor SIN-1 on insulin secretion (Fig. 3b). Insulin secretion was inhibited both by LY83583 which lowered islet cyclic GMP (Table 3), and by SIN 1, which raised cyclic GMP (Table 3).

Discussion

The inhibitory effects of IL-1β on insulin secretion are accompanied by up to nine-fold increases in islet cyclic GMP. This elevation of cyclic GMP confirms that the observed increase in nitric oxide production in cultured islets leads to an anticipated stimulation of islet guanylate cyclase. The rise in cyclic GMP is greatly attenuated (to 30 % of control) if arginine is omitted from the culture medium and the arginine analogue, NAME, is substituted. This suggests that while cyclic GMP formation is largely arginine-dependent, there could be cyclic GMP generation which is arginine and nitric-oxide independent. The latter may arise from nucleotide modulation of cyclases [29]; in IL-1β-treated islets a possible decrease in ATP [30] may

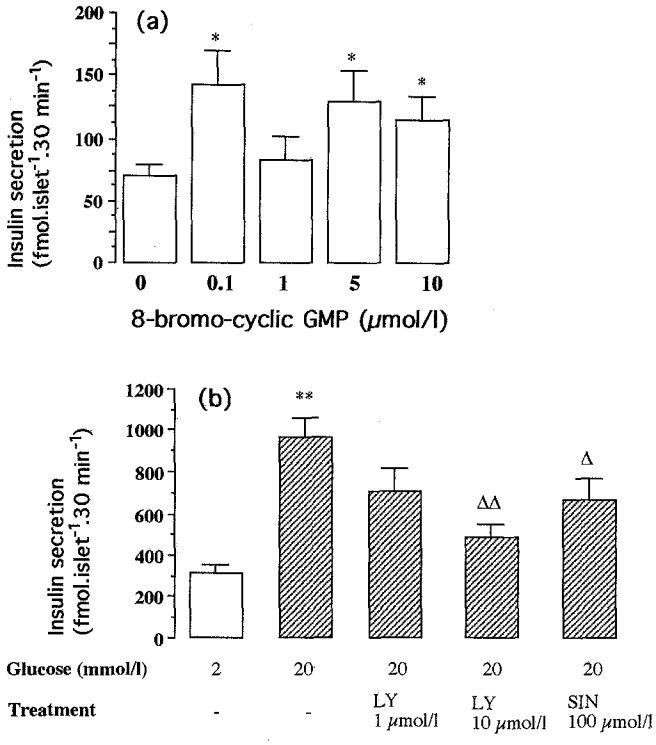

Fig. 3 a, b. Insulin secretion in response to agents which increase or decrease cyclic GMP. (**a**) Islets were cultured for 48 h in RPMI medium containing 11 mmol/l glucose followed by culture for 6 h with and without 8-bromo-cyclic GMP at the concentrations shown. Insulin secretion by groups of three islets was determined as described in Materials and methods. Values are mean ± SEM for islets from two isolates ($n = 11$–13): * $p < 0.05$ significantly different from control by Student's t-test. (**b**) Freshly isolated islets were pre-incubated in buffer containing 2 mmol/l glucose for 20 min and incubated in groups of three for 30 min in 0.6 ml buffer containing 2 mmol/l glucose (□) and 20 mmol/l glucose (▨) with the additions shown. Insulin secretion was determined as described in Materials and methods. Values are the means from a minimum of four islet isolates (n = a minimum of seven where additions were made): ** $p < 0.001$ significantly greater than 2 mmol/l glucose value; $\Delta p < 0.05$; $\Delta\Delta p < 0.001$ significantly less than 20 mmol/l glucose value by Student's t-test. In separate experiments values for insulin secreted (fmol · islet^{-1} · 30 min^{-1}) in the presence of 2 mmol/l glucose were 187 ± 27 (control), 155 ± 5 (+ 100 μmol/l SIN-1) and 123 ± 9* (+ 10 μmol/l LY) (mean values of 15–16 determinations ± SEM): * $p < 0.05$ significantly less than control value by Student's t-test

relieve ATP inhibition of guanylate cyclase [29]. However, there are several other unknown factors which might alter the interpretation of the results obtained with arginine analogues: the efficacy of arginine analogues in inhibiting islet nitric oxide synthase as opposed to macrophage, endothelial cell or brain nitric oxide synthase is not known. In addition, some analogues have little cellular infiltration, they may inhibit arginine *uptake* in whole cells, rather than inhibit the enzyme nitric oxide synthase [36]. The K_m of brain (constitutive) or macrophage (inducible) nitric oxide synthase for arginine is 5 and 2.8 μmol/l respectively [37, 38]; lowering arginine levels sufficiently to deny substrate to the enzyme, especially if islets are cultured in medium containing high levels of fetal calf serum, which contains arginine, may be difficult.

There is a poor correlation between islet cyclic GMP status and insulin secretory function [39–42]. In this study, low-dose IL-1β treatment raised cyclic GMP significantly, and under these circumstances insulin secretion was stimulated. Arginine-dependent stimulation of cyclic GMP formation, as well as of insulin secretion, has been demonstrated by others in short-term incubations in islets and RINm5F cells [40, 41] and in HIT-T15 cells [41]. In cultured islets in this study, arginine analogues have been found to lower islet cyclic GMP levels significantly; this led to decreased insulin secretion in short-term experiments with freshly isolated islets [40, 41] but decreased insulin secretion was not observed in other studies using freshly-isolated [42] or cultured islets [24]. It has also been demonstrated that when sodium nitroprusside or ascorbate are used to generate high levels of cyclic GMP that late – but not early – phase insulin secretion is inhibited in perifused islets in vitro [39]. However, when we attempted to raise islet cyclic GMP with 8-bromo cyclic GMP (0.1 to 10 μmol/l) this either stimulated or had no effect on insulin secretion; we failed to demonstrate any inhibitory effect. The nitric oxide donor SIN-1 was the most effective agent at mimicking the effects of IL-1β on insulin secretion and cyclic nucleotide generation. The time course for this effect was short (30 min) and we have found it difficult to induce long-term changes in cyclic GMP or insulin in 12-h culture periods with nitric oxide donors, perhaps due to the rapid effects or short half-life of the compounds we tested [43]. LY83583, a specific inhibitor of guanylate cyclase, reduced cyclic GMP levels by more than 30%, which confirms precisely the values obtained by Laychock and colleagues [40]. We also demonstrated that it had no effect on islet cyclic AMP production. LY83583 inhibited insulin secretion more potently than might be expected from the relatively modest reduction in cyclic GMP. Its inhibitory effect, even in the presence of 2 mmol/l glucose, suggests that it may have alternative effects on the secretory process. Thus, we have abandoned its use when designing experiments to evaluate the role of cyclic GMP in IL-1β modulation of insulin secretion.

Conflicting results for the role of cyclic GMP in insulin secretion may be resolved by considering the intracellular mechanism by which the nucleotide levels were modified. In this study, cytokine-induced increases in cyclic GMP occur via nitric-oxide-stimulated guanylate cyclase utilising endogenous islet GTP. Cytokine-induced nitric oxide

Table 3. Effect of LY83583 and SIN-1 on cyclic nucleotides in islets. Freshly isolated islets were pre-incubated in buffer containing 2 mmol/l glucose for 20 min and incubated for 30 min in groups of 30–40 in 1 ml buffer ± LY83583 (10 μmol/l) or SIN-1 (100 μmol/l). Islet cyclic nucleotides were extracted and assayed as described in Materials and methods

Treatment	Cyclic nucleotides (fmol/μg islet protein)	
	cyclic AMP	cyclic GMP
Control	18.3 ± 2 (5)	4.0 ± 0.3 (5)
LY83583	20.4 ± 3.3 (5)	2.7 ± 0.3 (5)[b]
Control	29.0 ± 4.3 (20)	1.6 ± 0.3 (11)
SIN-1	17.3 ± 3.3 (12)[a]	4.3 ± 0.8 (12)[c]

Values are from a minimum of four islet isolates. Number of determinations in parentheses.
[a] $p < 0.05$, [b] $p < 0.02$, [c] $p < 0.01$ vs control by Student's t-test

synthase also utilises GTP in the synthesis of tetrahydrobiopterin [44], a necessary co-factor for inducible nitric oxide synthase activity. Therefore, the net effect of cytokine induction of nitric oxide synthase may be to lower islet GTP, thus, cytokine-elevated cyclic GMP levels may indicate islet GTP levels which are rate-limiting for secretion. Depleted islet GTP has been shown to inhibit insulin secretion, possibly through impaired signal transduction [45], or by being present in amounts which are insufficient for necessary GTP-dependent functions e. g. microtubule assembly [46] or ATP synthesis and stimulation of adenylate cyclase [47], as shown in other cell types. GTP levels in islets have been shown to increase significantly in 30 min or less following a glucose challenge [48], and to increase by more than 100% in islets perifused for 150 min with medium containing 16.7 mmol/l glucose with GDP levels unchanged [49]. Conversely, GTP synthesis was inhibited by 81% using a synthesis inhibitor, mycophenolic acid [45], applied during an 18–20 h culture treatment. Work in our laboratory has shown that cyclic GMP formation in islets is rapid, peaking at 5–10 min, and by 30 min the levels have declined to near basal levels [35]. Since IL-1β treatment induces nitric oxide formation from arginine, guanylate cyclase may be subjected to continuous stimulation and therefore, it is theoretically feasible that GTP levels would fall significantly and perhaps in less time than we usually allow for cytokine treatment (12–15 h). Interpretation of GTP measurements is made difficult by problems of compartmentalisation within cells and by the knowledge that GTP can be free or protein-bound. There are no reports of the effects of IL-1β on islet GTP.

Treatment of islets with a low dose of IL-1β (1 pmol/l) results in a clear potentiation of insulin secretion which is accompanied by significant elevation of cyclic GMP and no change in cyclic AMP. Since we have shown that this stimulation of insulin release and cyclic GMP generation occurs whether or not arginine is present, it suggests that IL-1β is acting through a signalling pathway, not involving arginine-derived nitric oxide, when it exerts low-dose stimulatory effects on secretion. However, it should be noted that under some treatment regimens significant arginine-independent rises in cyclic GMP are caused by pmol/l amounts of IL-1β.

Modification of the prevailing cyclic AMP 'tone' is known to affect the degree of secretagogue-induced secretion [50]. In other cell types interleukin-1 is known to stimulate cyclic AMP production [51]. However, we have not been able to demonstrate under any conditions that cyclic AMP levels are increased in IL-1β-treated islets. We have demonstrated that cyclic AMP is not changed significantly by IL-1β when cyclic AMP and cyclic GMP phosphodiesterases are inhibited by IBMX. This further suggests that adenylate cyclase activity in islets is unchanged by IL-1β treatment. More importantly, our results support the hypothesis that increased cyclic GMP is accelerating breakdown of cyclic AMP in IL-1β-treated islets; a fall which does not occur when IBMX is used. Our results confirm observations of a decrease in cyclic AMP in IL-1β-treated adult rat islets [30] or fetal rat islets [17]. Since adenylate cyclase would appear to be unaffected by IL-1β, pertussis toxin alone may not reverse the effects of IL-1β as found in both fetal [17] and adult rat islets [23] especially if phosphodiesterase activity is increased [52]. This is supported by the finding that pertussis toxin combined with IL-1β treatment of fetal islets generated levels of cyclic AMP which were only equivalent to those in untreated islets, not pertussis toxin-treated control islets [17]. One could speculate that the phenomenon of lack of stimulation of insulin secretion by 1 μmol/l 8-bromo cyclic GMP may result if intra-islet concentrations of cyclic GMP reached a concentration range representing the K_d for the cyclic GMP-stimulated phosphodiesterase which accelerates cyclic AMP degradation, which in other cell types is 2 μmol/l [53]. However, cyclic AMP may make some contribution to the effects of IL-1β as stimulation of beta-cell secretory activity usually accelerates cytokine effects, but not when cyclic AMP is elevated [5]. Thus, culture of islets with IL-1β and IBMX or glucagon did not sensitise islets to the effects of IL-1β [5], while secretagogues such as tolbutamide, which have been reported to increase nitric oxide formation in islets, by constitutive nitric oxide synthase [41], accentuated cytokine inhibition of insulin secretion. If the HIT cell response is analagous to that of islets, it has been shown that a short-term (90-min) exposure to forskolin or glibenclamide is not sufficient to reverse inhibition by IL-1β [54]. Interpretation of our experiments using long-term treatment with IBMX for forskolin to attenuate the inhibitory effects of IL-1β are hampered by the changes these agents induce in the control islets; however, when cytokine inhibition is expressed as a percent of the appropriate control value, then raising cyclic AMP may have a small beneficial effect. The role of cyclic nucleotide phosphodiesterases in dictating a decrease in cyclic AMP when cyclic GMP levels are highly elevated has been shown in adrenal cells [52] and their possible role in cytokine inhibition of insulin secretion requires further analysis. To date, only a type 3, cyclic GMP-inhibitable phosphodiesterase and a less well-characterised type 4 phosphodiesterase have been shown to exist in rat islets [55]. The cyclic GMP-stimulated diesterase which could be involved in lowering islet cyclic AMP here is designated type 2 in other sytems [52, 56].

Low-dose IL-1β stimulation of insulin secretion is independent of arginine-related nitric oxide stimulation of guanylate cyclase. Stimulation by high-dose IL-1β of nitric oxide production in islets [24, 25] leads to an anticipated stimulation of guanylate cyclase, demonstrated in our preliminary report [57], and confirmed in RINm5F cells [58]. This phenomenon is reproduced here by a nitric oxide donor compound, SIN-1 and results in greatly increased cyclic GMP and lowered cyclic AMP. However, cyclic GMP-independent effects of IL-1β-induced nitric oxide may also be very important. Inhibition of insulin secretion by IL-1β has consistently been associated with decreased glucose oxidation, especially at the mitochondrial level [9, 10]. It is therefore relevant for inhibition of islet function and consistent with a role for nitric oxide in the effects of IL-1β that protein nitrosylation has been shown to occur in islets [25], and mitochondrial enzymes may be the targets [10]. Whether or not cytokines generate rate-limiting decreases in GTP levels in islets has not been investigated.

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References

1. Mandrup-Poulsen T, Helqvist S, Wogensen LD et al. (1990) Cytokines and free radicals as effector molecules in the destruction of pancreatic β-cells. In: Baekkeshov S, Hansen B (eds) Human diabetes – genetic, environmental and autoimmune etiology. Springer, Berlin Heidelberg New York, pp 169–193
2. Rabinovitch A, Sumoski W, Rajotte RV, Warnock G (1990) Cytotoxic effects of cytokines on human pancreatic islet cells in monolayer culture. J Clin Endocrinol Metab 71: 152–156
3. Spinas GA, Mandrup-Poulsen T, Molvig J et al. (1986) Low concentrations of interleukin-1 stimulate and high concentrations inhibit insulin release from isolated rat islets of Langerhans. Acta Endocrinol (Copenh) 113: 551–558
4. Comens P, Wolf BA, Unanue ER, Lacy PE, McDaniel ML (1987) Interleukin 1 is a potent modulator of insulin secretion from isolated rat islets of Langerhans. Diabetes 36: 963–970
5. Johannesen J, Helqvist S, Nerup J (1990) Not all insulin secretagogues sensitize pancreatic islets to recombinant interleukin 1β. Acta Endocrinol (Copenh) 123: 445–452
6. Zawalich WS, Zawalich KC, Rasmussen H (1989) Interleukin 1a exerts glucose-dependent stimulatory and inhibitory effects on islet cell phosphoinositide hydrolysis and insulin secretion. Endocrinology 124: 2350–2357
7. Eizirik DL, Bendtzen K, Sandler S (1991) Short exposure of rat pancreatic islets to IL-1β induces a sustained but reversible impairment in β cell function: influence of protease activation, gene transcription and protein synthesis. Endocrinology 128: 1611–1616
8. Sandler S, Bendtzen K, Eizirik DL, Sjoholm Å, Welsh N (1989) Decreased cell replication and polyamine content in insulin-producing cells after exposure to human interleukin-1. Immunol Lett 22: 267–272
9. Eizirik DL, Sandler S, Hallberg A, Bendtzen K, Sener A, Malaisse WJ (1989) Differential sensitivity to β-cell secretagogues in cultured rat pancreatic islets exposed to human interleukin-1β. Endocrinology 125: 752–759
10. Welsh N, Sandler S (1992) Interleukin-1β induces nitric oxide production and inhibits the activity of aconitase without decreasing glucose oxidation rates in isolated mouse pancreatic islets. Biochim Biophys Res Commun 182: 333–340
11. Rabinovitch A, Baquerizo H, Sumoski W (1990) Cytotoxic effects of cytokines on islet β-cells: evidence for involvement of eicosanoids. Endocrinology 126: 67–71
12. Bergmann L, Kroncke K-D, Suschek C, Kolb H, Kolb-Bachofen V (1992) Cytotoxic action of IL-1β against pancreatic islets is mediated via nitric oxide formation and is inhibited by N-G-monomethyl-L-arginine. FEBS Lett 299: 103–106
13. Hughes JH, Easom RA, Wolf BA, Turk J, McDaniel M (1989) Interleukin 1-induced prostaglandin E2 accumulation by isolated pancreatic islets. Diabetes 38: 1251–1257
14. Welsh N, Nilsson T, Hallberg A, Arkhammar P, Berggren P-O, Sandler S (1989) Human interleukin-1β stimulates islet insulin release by a mechanism not dependent on changes in phospholipase C, protein kinase C activities or Ca^{2+} handling. Acta Endocrinol (Copenh) 121: 698–704
15. Beggs M, Beresford G, Clarke J, Metz R, Espinal J, Hammonds P (1990) Interleukin-1β inhibits glucokinase activity in clonal HIT-T15 β-cells. FEBS Lett 267: 217–220
16. Sumoski W, Baquerizo H, Rabinovitch A (1989) Oxygen free radical scavengers protect rat islet cells from damage by cytokines. Diabetologia 32: 792–796
17. Sjöholm Å (1991) Inhibition of fetal rat pancreatic β-cell replication by interleukin-1β in vitro is not mediated through pertussis toxin-sensitive G-proteins, a decrease in cyclic AMP or protease activation. FEBS Lett 289: 249–252
18. Borg LAH, Sandler S, Eizirik DL (1991) Interleukin-1β increases the activity of superoxide dismutase in rat pancreatic islets. Diabetologia 34 [Suppl 2]: A8 (Abstract)
19. Welsh N, Bendtzen K, Sandler S (1991) Influence of protease on inhibitory and stimulatory effects of interleukin 1β on β-cell function. Diabetes 40: 290–294
20. Helqvist S, Hansen BS, Johannesen J, Andersen HU, Nielsen JH, Nerup J (1989) Interleukin 1 induces new protein formation in isolated rat islets of Langerhans. Acta Endocrinol (Copenh) 121: 136–140
21. Helqvist S, Polla BS, Johannesen J, Nerup J (1991) Heat shock protein induction in rat pancreatic islets by recombinant human interleukin 1β. Diabetologia 34: 150–156
22. Hughes JH, Colca J, Easom RA, Turk J, McDaniel ML (1990) Interleukin 1 inhibits insulin secretion from isolated rat pancreatic islets by a process that requires gene transcription and mRNA translation. J Clin Invest 86: 856–863
23. Eizirik DL, Hallgren IB, Forsbeck E, Sandler S (1991) Interleukin-1β actions on β-cells are mediated by type 1 IL-1 receptors and gene transcription. Diabetologia 34 [Suppl 2]: A9 (Abstract)
24. Southern C, Schulster D, Green IC (1990) Inhibition of insulin secretion by interleukin-1β and tumour necrosis factor-α via an L-arginine-dependent nitric oxide generating mechanism. FEBS Lett 276: 42–44
25. Corbett JA, Lancaster JR Jr, Sweetland MA, McDaniel ML (1991) Interleukin-1β-induced formation of EPR-detectable iron-nitrosyl complexes in islets of Langerhans. J Biol Chem 266: 21351–21354
26. Kroncke KD, Kolb-Bachofen V, Berschick B, Burkart V, Kolb H (1991) Activated macrophages kill pancreatic syngeneic islet cells via arginine-dependent nitric oxide generation. Biochim Biophys Res Commun 175: 752–758
27. Jansson L, Sandler S (1991) The nitric oxide synthase II inhibitor N-nitro-L-arginine stimulates pancreatic islet insulin release in vitro, but not in the perfused pancreas. Endocrinology 128: 3081–3085
28. Moncada S, Palmer RMJ, Higgs EA (1991) Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev 43: 109–142
29. Waldman SA, Murad F (1987) Cyclic GMP synthesis and function. Pharmacol Rev 39: 163–196
30. Sandler S, Bendtzen K, Eizirik DL, Strandell E, Welsh M, Welsh N (1990) Metabolism and B-cell function of rat pancreatic islets exposed to human interleukin-1β in the presence of a high glucose concentration. Immunol Lett 26: 245–252
31. Gey GO, Gey MH (1936) Maintenance of human normal cells and tumor cells in continuous culture. Am J Cancer 27: 45–76
32. Green IC, Perrin D, Penman E, Ray K, Howell SL (1983) Effect of dynorphin on insulin and somatostatin secretion, calcium uptake and c-AMP levels in isolated rat islets of Langerhans. Diabetes 32: 685–690
33. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254
34. Brooker G, Harper JF, Terasaki WL, Moylan RD (1979) Radioimmunoassay of cyclic AMP and cyclic GMP. Adv Cyclic Nucleotide Res 10: 1–33
35. Green IC, Ray K, Perrin D (1983) Opioid peptide effects on insulin release and c-AMP in islets of Langerhans. Horm Metab Res 15: 124–128
36. Bogle RG, Moncada S, Pearson JD, Mann GE (1992) Identification of inhibitors of nitric oxide synthase that do not interact with the endothelial cell L-arginine transporter. Br J Pharmacol 105: 768–770

37. Knowles RG, Palacios M, Palmer RMJ, Moncada S (1990) Nitric oxide synthase in the brain. In: Moncada S, Higgs EA (eds) Nitric oxide from L-arginine. A bioregulatory system. Excerpta Medica International, Amsterdam, pp 139–147
38. Stuehr DJ, Cho HJ, Kwon NS, Nathan CF (1992) The cytokine-induced macrophage nitric oxide synthase is an FAD- and FMN-containing flavoprotein. 2nd International Meeting "The Biology of nitric oxide" Part II Enzymology Biochemistry and Immunology, September 1991. Portland Press, London
39. Laychock SG (1981) Evidence for guanosine 3′5′-monophosphate as a putative mediator of insulin secretion from isolated rat islets. Endocrinology 108: 1197–1205
40. Laychock SG, Modica ME, Cavanaugh CT (1991) L-arginine stimulates cyclic guanosine 3′,5′-monophosphate formation in rat islets of Langerhans and RINm5F insulinoma cells: evidence for L-arginine: nitric oxide synthase. Endocrinology 129: 3043–3052
41. Schmidt HHW, Warner TD, Ishi K, Sheng H, Murad F (1992) Insulin secretion from pancreatic B cells caused by L-arginine derived nitrogen oxides. Science 255: 721–723
42. Jones PM, Persaud SJ, Howell SL (1992) Is cyclic GMP involved in insulin secretion from rat islets? Diabetic Med 9 [Suppl 1]: 12 A (Abstract)
43. Southam E, Garthwaite J (1991) Comparative effects of some nitric oxide donors on cyclic GMP levels in rat cerebellar slices. Neurosci Lett 130: 107–111
44. Gross SS, Levi R (1992) Synthesis of tetrahydrobiopterin is a requirement for induction of nitric oxide synthesis by LPS/interferon in vascular smooth muscle. In: 2nd International Meeting "Biology of nitric oxide" September 1991. Portland Press, London
45. Metz SA, Rabaglia M, Pintar TJ (1992) Selective inhibitors of GTP synthesis impede exocytotic insulin release from intact rat islets. J Biol Chem 267: 12517–12527
46. Terry BJ, Purich DK (1980) Assembly and disassembly properties of microtubules formed in the presence of GTP, 5′ guanylyl imidodiphosphate, and 5′-guanylyl methylenediphosphate. J Biol Chem 255: 10532–10536
47. Iyengar R, Abramowitz J, Bordelon-Riser M et al. (1980) Hormone receptor-mediated stimulation of adenylyl cyclase systems. Nucleotide effects and analysis in terms of a simple two state-model for the basic receptor-affected enzyme. J Biol Chem 255: 3558–3564
48. Meglasson MD, Nelson J, Nelson D, Erecinska M (1989) Bioenergetic response of pancreatic islets to stimulation by fuel molecules. Metabolism 38: 1188–1195
49. Hoenig M, Matschinsky FM (1987) HPLC analysis of nucleotide profiles in glucose-stimulated perifused rat islets. Metabolism 36: 295–301
50. Howell SL, Green IC, Montague W (1973) A possible role of adenylate cyclase in the long term dietary regulation of insulin secretion from rat islets of Langerhans. Biochem J 136: 343–349
51. Mizel SB (1990) Cyclic AMP and interleukin 1 signal transduction. Immunl Today 11: 390–394
52. MacFarland RT, Zelus BD, Beavo J (1991) High concentrations of a cyclic GMP-stimulated phosphodiesterase mediate ANP-induced decreases in cAMP and steroidogenesis in adrenal glomerulosa cells. J Biol Chem 266: 136–142
53. Stroop SD, Beavo JA (1991) Structure and function studies of the cGMP-stimulated phosphodiesterase. J Biol Chem 266: 23802–23809
54. Hammonds P, Beggs M, Beresford G, Espinal J, Clarke J, Metz RJ (1990) Insulin-secretory β-cells possess specific receptors for interleukin-1β. FEBS Lett 261: 97–100
55. Furman BL, Pyne NJ (1990) Islet phosphodiesterase isoenzymes and insulin secretion. Diabetic Med 7 [Suppl 1]: 19 A (Abstract)
56. Beavo JA, Reifsnyder DH (1990) Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. TiPS 11: 150–155
57. Southern C, Schulster D, Green IC (1991) Nitric-oxide stimulated cGMP, relationship to cytokine effects on insulin secretion. Diabetic Med 8 [Suppl 1]: 1 A (Abstract)
58. Corbett JA, Tilton RA, Chang K et al. (1992) Aminoguanidine, a novel inhibitor of nitric oxide formation, prevents diabetic vascular dysfunction. Diabetes 41: 552–556

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Dr. I. C. Green
Biochemistry Department
School of Biological Sciences
University of Sussex
Brighton BN1 9QG
Sussex
UK