Prostaglandin E\textsubscript{2} Mediates Inhibition of Insulin Secretion by Interleukin-1\textbeta*  

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Interleukin-1\textbeta (IL-1\textbeta) and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}), frequently co-participants in inflammatory states, are two well recognized inhibitors of glucose-induced insulin secretion. Previous reports have concluded that the inhibitory effects of these two autacoids on pancreatic \(\beta\) cell function are not related because indomethacin, a potent prostaglandin synthesis inhibitor, does not prevent IL-1\textbeta effects. However, indomethacin is not a specific cyclooxygenase inhibitor, and its other pharmacologic effects are likely to inhibit insulin secretion independently. Since we recently observed that IL-1\textbeta induces cyclooxygenase-2 (COX-2) gene expression and PGE\textsubscript{2} synthesis in islet \(\beta\) cells, we have reassessed the possibility that PGE\textsubscript{2} mediates IL-1\textbeta effects on \(\beta\) function. By using two cell lines (HIT-T15 and \(\beta\)HC13) as well as Wistar rat isolated pancreatic islets, we examined the ability of two COX-2-specific antagonists, NS-398 and SC-236, to prevent IL-1\textbeta inhibition of insulin secretion. Both drugs prevented IL-1\textbeta from inducing PGE\textsubscript{2} synthesis and inhibiting insulin secretion; adding back exogenous PGE\textsubscript{2} re-established inhibition of insulin secretion in the presence of IL-1\textbeta. We also found that EP3, the PGE\textsubscript{2} receptor subtype whose post-receptor effect is to decrease adenylyl cyclase activity and, thereby, insulin secretion, is the dominant mRNA subtype. We conclude that endogenous PGE\textsubscript{2} mediates the inhibitory effects of exogenous IL-1\textbeta on \(\beta\) cell function.  

Prostaglandin E\textsubscript{2} (PGE\textsubscript{2})\textsuperscript{1} is known to be an inhibitor of glucose-induced insulin secretion from studies in a \(\beta\) cell line (1, 2) and isolated and neonatal islets of Langerhans (3–6) as well as in vivo in both animal (7, 8) and human (9–11) studies. These findings have been reinforced by studies in which inhibitors of cyclooxygenase, hence PGE\textsubscript{2} synthesis, have augmented glucose-induced insulin secretion. The only discordant result in the latter category of studies has been observed when indomethacin was used as the cyclooxygenase inhibitor. This discrepant result can be attributed to other effects of indomethacin that would be expected to inhibit insulin secretion through adverse effects on exocytosis that are unrelated to its effects on prostaglandin synthesis (12).

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1 The abbreviations used are: PGE\textsubscript{2}, prostaglandin E\textsubscript{2}; HBSS, Hank’s balanced salt solution; PBS, fetal bovine serum; IL, interleukin; COX-2, cyclooxygenase-2; RT-PCR, reverse transcriptase-polymerase chain reaction; TAMRA, 6-carboxy-N,N,N’,N’-tetramethylrhodamine; FAM, 6-carboxyfluorescein.

Interleukin-1\textbeta has been reported to have major inhibitory effects on \(\beta\) cell function, especially under conditions of high glucose concentrations and prolonged exposure to this cytokine (13–16). This is an especially relevant observation because many reports suggest that IL-1\textbeta is an important force in the pathogenesis of diabetes mellitus (17, 18). Previously, studies have concluded that endogenous PGE\textsubscript{2} does not play a participatory role in the adverse effects of IL-1\textbeta on \(\beta\) cell function (5, 6). Ironically, however, the drug that was chosen to test this hypothesis and found not to reverse IL-1\textbeta inhibitory effects on insulin secretion was indomethacin, which itself has independent inhibitory actions on \(\beta\) cell exocytosis (12).

Recently, it has been appreciated that the pancreatic islet, and \(\beta\) cells in particular, constitutively and dominantly express cyclooxygenase-2 (COX-2) rather than COX-1, a situation just the opposite of most mammalian cells (19). Additionally, new drugs have become available that specifically inhibit COX-2 and consequently have replaced indomethacin as the model drug to examine the consequences of inhibition of endogenous prostaglandin synthesis (20, 21). These new developments prompted us to re-examine the possible interrelationships between PGE\textsubscript{2} and IL-1\textbeta as inhibitors of insulin secretion. Specifically, we asked the following: 1) whether IL-1\textbeta inhibits glucose-induced insulin secretion comparably in \(\beta\) cell lines and isolated islets; 2) whether IL-1\textbeta effects on insulin secretion can be prevented by pretreatment of cells and islets with specific inhibitors of COX-2 activity; and 3) whether the dominant PGE\textsubscript{2} receptor subtype in islets is one whose post-receptor action is likely to inhibit insulin secretion. We have found that the two structurally unrelated specific inhibitors of COX-2 activity and PGE\textsubscript{2} synthesis (NS-398 and SC-236) prevent IL-1\textbeta-induced inhibition of insulin secretion in \(\beta\) cell lines and isolated islets and that these preparations dominantly express the EP3 receptor subtype mRNA whose protein product would be predicted to decrease adenylyl cyclase activity and insulin secretion.

**Experimental Procedures**

**Cell Culture—HIT-T15 cells (passages 70–80) were grown in 5% CO\textsubscript{2}, 95% O\textsubscript{2} at 37 °C, maintained in RPMI 1640 culture medium supplemented with 10% fetal bovine serum (FBS), 11.1 mM glucose as described previously (22). βHC13 cells (passages 33–42) were maintained in Dulbecco’s modified Eagle’s culture medium containing 0.2% FBS, 0.2 mM glucose for 24 h.**

**Pancreatic Islet Isolation—Pancreata from male Wistar rats were infused with 10 ml of a 0.09% collagenase type V (Sigma), 1% FBS, and 2 units/ml RO1 DNase (Promega, Madison, WI) in Hank’s balanced salt solution (HBSS), pH 7.38. After surgical removal, the pancreas was incubated in the collagenase/HBSS solution for 20 min at 37 °C and then shaken for 15 min. Undigested tissue was washed twice with ice-cold HBSS followed by centrifugation at 250 × g for 4 min. The pellet was resuspended in 2 ml of 35% bovine serum albumin, and islets were separated using a dextran gradient.**

**Insulin Secretion Studies—Static insulin secretion in response to**
glucose was evaluated by plating cells (10⁶ cells per well) in a 12-well plate using medium containing 10% FBS (11.1 mM glucose for HIT cells and 22.2 mM glucose for βHC cells). The following day, cells were subcultured for 24 h in medium containing 0.2% FBS and IL-1β with or without drug (experimental medium). After the 24-h exposure to experimental medium, cells were incubated in Krebs-Ringer buffer (incubated in Krebs-Ringer buffer (KRB) (22)) containing IL-1β with or without drug for 2 h at 37 °C to measure insulin secretion. Islets were exposed to experimental medium for 24 h beginning the day after isolation, and the static incubation was performed the following day for a duration of 1 h. Insulin levels in the KRB buffer samples collected from the static incubations with cells and islets were measured by either radioimmunoassay as described previously (22) or by using a Sensitive Rat Insulin RIA kit (Linco Research Inc., St. Louis, MO).

Prostaglandin E₂ Levels—PGE₂ levels present in KRB buffer collected after the static incubations were measured using an enzyme immunoassay obtained from Amersham Pharmacia Biotech, according to the manufacturer’s protocol.

Real Time, Fluorescence-based RT-PCR of COX-2, COX-1, and the EP3 Receptor Subtype—Total RNA was extracted according to the method of Chomczynski and Sacchi (23). One-step RT-PCR was carried out using the Gold RT-PCR kit from Perkin-Elmer and an ABI Prism 770 sequence detector equipped with a thermocycler (Taqman® technology) and a cooled CCD camera to detect fluorescence emission over a range of wavelengths (500—650 nm). Briefly, reverse transcription was first performed using specific oligonucleotides and the MultiScribe™ reverse transcriptase at 48 °C for 30 min. Samples were then PCR-amplified using GoldTaq™ polymerase and oligonucleotide primers for COX-2, COX-1, EP3, and GAPDH (control for RNA quantity) described below for 40 cycles under the following conditions: denaturation at 95 °C for 15 s, annealing and extension at 60 °C for 1 min. The Taqman® technology used is based on the emission of a fluorescent signal from a reporter dye (6-carboxyfluorescein, FAM) linked to a quencher dye (6-carboxy-N,N,N,N-tetramethylrhodamine, TAMRA) and annealed to the template between both primers. Upon extension of the primers during PCR, the exonuclease activity of the polymerase releases the reporter dye from the quencher resulting in fluorescence emission. The emitted signal is detected over a range of wavelengths for each reaction at each cycle. This allows a complete curve of amplification over the course of 40 cycles to be established and the analysis to be carried out solely within the exponential range of amplification. Comparative analysis is then based upon the cycle number at which a significant increase in the amplification signal above base line is detected. Primer and probe sequences (5′-3′) are as follows: COX-1 forward primer, GCCGACAGACGACCTGCAAGCTTACGAGT-CTCG; COX-1 reverse primer, GTAGCCCGTGCGAGTACAATC; COX-2 probe, 6FAM-TCCATGGCCTGACCTCCCGGG-TAMRA; COX-2 forward primer, CAGTCCTCGGGTTCACTCTTACCTAC; COX-2 reverse primer, AAGCGCCAGCTTTATGCTGTGTT-CTGT; rat EP3 receptor probe, 6FAM-CCTAGGCGTCCTGAGGAGAGCTGACTAAGT-GGCTGGCTAGC; rat EP3 receptor forward primer, AAGCGAAAGGAGGTCACATTCC; rat EP3 receptor reverse primer, AGGAGATCATGACTTCGTT; rat EP3 receptor reverse primer, 6FAM-CAGGCGCATGTAGGTCGG-GCC-TAMRA; rat EP1 receptor probe, 6FAM-CAGGCGCATGTAGGTCGG-GCC-TAMRA; rat EP1 receptor forward primer, CCTGCTTGCCATCTG; rat EP1 receptor reverse primer, CAGTATACAGGGAGCACCA; rat EP2 receptor probe, 6FAM-CGCACGTAGGAGAGGACTGATGCTGTGCT-TAMRA; rat EP2 receptor forward primer, CGGACA-AGGTTGCAATGT; rat EP2 receptor reverse primer, CCTGCTGTTTTCGGTACCTA; rat EP4 receptor probe, 6FAM-TCTTGGCCTCC-GAGGCTGGTCCCTAC-TAMRA; rat EP4 receptor forward primer, CTCCTAATACGGCAGACCTA; rat EP4 receptor reverse primer, CATGCGGATCTGGGACAA.

Expression of Data and Statistics—Data are reported as mean ± S.E. when applicable. Statistical comparisons were performed using analysis of variance and the Bonferroni post hoc test with a p < 0.05 considered as significant.

Materials—The materials used were as follows: recombinant human IL-1β from R & D Systems, Minneapolis, MN; PGE₂, NS-398 from Biomol, Plymouth Meeting, PA; SC-236 from Monsanto Searle Co., Stokie, IL; and RT-PCR probes and primers from Perkin-Elmer.

RESULTS

Insulin Secretion in HIT-T15 and βHC13 Cells after Long Term Exposure to IL-1β—To determine the maximal effective concentration of IL-1β required to inhibit insulin secretion, HIT-T15 and βHC13 cells were exposed for 24 h to IL-1β at final concentrations ranging from 2.5 to 15 ng/ml. After the 24-h exposure, static incubations were done to measure glucose-stimulated insulin secretion. Pre-exposure of both HIT-T15 and βHC13 cells to IL-1β for 24 h significantly decreased subsequent insulin secretion in response to maximal glucose stimulation (11.1 or 22.2 mM glucose, respectively, for HIT-T15 and βHC cells) (Fig. 1). Maximal inhibition of insulin secretion required an IL-1β concentration of 5 ng/ml.

Effect on PGE₂ Production after Exposure to IL-1β With or Without NS-398 or SC-236—To determine if the decrease in insulin secretion occurred concomitantly with an increase in PGE₂ production following exposure to IL-1β, KRB buffer samples collected during the static incubations were also evaluated for PGE₂ levels. Exposure to 5 ng/ml IL-1β for 24 h increased PGE₂ production significantly over control levels (Fig. 2). Concurrent treatment with either 0.01 mM NS-398 or 25 ng/ml SC-236 decreased PGE₂ to levels not significantly different from control (Fig. 2). Similar to observations in experiments using the HIT and βHC cells, PGE₂ production by islets increased significantly from control levels following exposure to IL-1β. Concurrent treatment with IL-1β and 0.01 mM NS-398 decreased levels (Fig. 2 legend).

Effect of NS-398 and SC-236 on IL-1β-dependent Decreases in Insulin Secretion—To determine if the IL-1β-dependent decrease in insulin secretion involved the activation of COX-2, cells were exposed to IL-1β and either 0.01 mM NS-398 or 25 ng/ml SC-236, both of which inhibit COX-2 specifically when these concentrations are used (20). Both NS-398 and SC-236 prevented inhibition of insulin secretion by IL-1β (Fig. 3). Add-back experiments with exogenous PGE₂ (10⁻⁴ M) to the cells treated with IL-1β and either NS-398 or SC-236 reproduced the inhibition of insulin secretion observed in cells treated with IL-1β alone (Fig. 3). There were no nonspecific drug or vehicle effects at the concentrations used since treatment with either drug or vehicle (without IL-1β) did not inhibit insulin secretion. Indomethacin, the nonspecific COX inhibitor known to have more effect on COX-1 than COX-2, did not prevent inhibition of insulin secretion by IL-1β, as previously reported (6, 24) (Fig. 3).

Effect of IL-1β On Insulin Secretion in Wistar Rat Islets—To support the physiological importance of the results observed in the cell lines, the IL-1β effect and role of COX-2 on insulin secretion were evaluated with Wistar rat islets. IL-1β at a final concentration of 5 ng/ml was used because it consistently decreased insulin secretion in both the HIT and βHC cells with-

![Fig. 1. The inhibitory effect of IL-1β on glucose-induced insulin secretion from the pancreatic islet β cell lines, HIT-T15 and βHC13. Cells were preincubated in RPMI 1640 containing 10% FBS and no or increasing concentrations of IL-1β for 24 h and then incubated in KRB buffer for 2 h at 37 °C in the presence of the same IL-1β concentration and a maximally stimulating concentration of glucose (11.1 mM for HIT-T15 and 22.2 mM for βHC13). The maximal inhibitory effect of IL-1β was found with a final concentration of 5 ng/ml. Data are mean ± S.E. from three separate experiments, each performed in duplicate; *p < 0.05 comparing 0 versus 2.5 and 5 ng/ml IL-1β.](image-url)
out toxic or nonspecific effects. After 24 h of pre-exposure to IL-1β, insulin secretion in response to 22.2 mM glucose was decreased significantly. Control islets secreted insulin at a rate of 1027 ± 178 microunits/ml, whereas insulin secretion from IL-1β-treated cells was significantly decreased to 184 ± 5 microunits/ml (p < 0.05 compared with control). Concurrent treatment with either 0.01 mM NS-398 or 25 ng/ml SC-236 blocked the IL-1β effect and restored insulin secretion to levels not significantly different from control. Pretreatment with indomethacin had no preventive effect on the IL-1β-dependent decrease in insulin secretion. Addition of exogenous PGE₂ with IL-1β and either NS-398 or SC-236 caused decreases in insulin secretion (Fig. 4). Insulin secretion was expressed as fold response instead of microunits/ml to account for differences in islet mass on the different experiment days.

Expression of COX-2, COX-1, and the EP3 Prostaglandin Receptor Subtype in Wistar Rat Islets—As described previously with Syrian hamster islets, we found that the major isoform of COX expressed in Wistar rat islets is COX-2 with substantially less expression of COX-1 (Fig. 5A). The major PGE₂ receptor subtype expressed was EP3, with lesser amounts of EP1, EP2, and EP4 also being detected (Fig. 5B). RT-PCR analysis of epididymal fat isolated from the same animals also showed that EP3 was the most abundant receptor type expressed (data

FIG. 2. PGE₂ levels in the buffer samples obtained after the static incubations with HIT-T15 or βHC13 cells in the presence of IL-1β (5 ng/ml) as described in the legend for Fig. 1. The addition in the buffer of either NS-398 (0.01 mM) or SC-236 (25 ng/ml), two specific inhibitors of COX-2 activity, prevented the increase in PGE₂ synthesis induced by IL-1β. *p > 0.01 comparing IL-1β versus control, NS-398, or SC-236. The effect of IL-1β on synthesis of PGE₂ by isolated Wistar rat pancreatic islets was also evaluated. Islets were preincubated in RPMI media containing IL-1β (5 ng/ml) for 24 h followed by 2 h of static incubations in KRB buffer containing IL-1β and a maximal stimulatory concentration of glucose (17.6 mM). IL-1β increased the synthesis of PGE₂ in the absence (13.3 ± 3.7 pg/ml versus 5.6 ± 2.1 pg/ml control, *p < 0.05, n = 3 experiments) but not in the presence of the two specific COX-2 inhibitors, NS-398 (0.01 mM) or SC-236 (25 ng/ml), 9.6 ± 3.8 and 7.7 ± 3.8 pg/ml, respectively.

FIG. 3. The preventive effect of NS-398 or SC-236 on the IL-1β (5 ng/ml)-induced decrease in glucose-stimulated insulin secretion from HIT or βHC cells. Pretreatment with either NS-398 (0.01 mM) or SC-236 (25 ng/ml) prevented IL-1β from inhibiting insulin secretion, whereas indomethacin (0.8 mM) did not. Adding back exogenous PGE₂ (10⁻⁶ mM) decreased insulin secretion to levels observed with cells treated with IL-1β alone. Mean data were from four separate experiments for HIT-T15 cells and three for βHC13 cells. *, p < 0.05; **, p < 0.01 versus control.

FIG. 4. The preventive effect of NS-398 or SC-236 on IL-1β-induced inhibition of glucose-induced insulin secretion from isolated Wistar rat pancreatic islets. IL-1β (5 ng/ml) significantly inhibited insulin secretion (*, p < 0.05 versus control), an effect that was prevented when islets were preincubated in media for 24 h with either NS-398 (0.01 mM) or SC-236 (25 ng/ml). Indomethacin (0.8 mM) pretreatment did not have this preventive effect. When exogenous PGE₂ (10⁻⁶ mM) was added back to the incubates of cells treated with both IL-1β and the specific COX-2 inhibitors, decreases in insulin secretion to the level seen in cells treated with IL-1β alone were observed. *, p < 0.05 versus control.

FIG. 5. Detection by RT-PCR using Taqman™ technology of COX isoform, as well as EP receptor subtype, gene expression in isolated Wistar rat pancreatic islets. ∆Rn designates change in expression compared to control. A, COX-2 is expressed more highly than COX-1 with C values of 26 and 30, respectively. B, the EP3 receptor subtype was the most highly expressed followed by EP2, EP1, and EP4 (C, values of 25, 28, 30, and 34, respectively). Representative amplification plots were from experiments performed in triplicate on two separate occasions.
not shown). These observations correlated with those from rat studies performed by Boie et al. (25) using Northern analysis.

**DISCUSSION**

These studies were designed to assess the hypothesis that endogenous PGE$_2$ mediates the inhibitory effect of IL-1$\beta$ on glucose-induced insulin secretion. We observed the following:

- exogenous IL-1$\beta$ stimulated synthesis of endogenous PGE$_2$ and inhibited glucose-induced insulin secretion from HIT-T15 and $\beta$HC13 cells as well as from Wistar rat isolated islets; two structurally unrelated inhibitors of COX-2 activity, NS-398 and SC-236, significantly decreased PGE$_2$ production by the cell lines and islets; this blockade of PGE$_2$ production prevented the inhibitory effect of exogenous IL-1$\beta$ on insulin secretion and was re-established when exogenous PGE$_2$ was provided; and EPO gene receptor expression is significantly greater than the other three receptor subtypes in islets. These observations support the hypothesis that endogenous PGE$_2$ mediated the inhibitory effect of IL-1$\beta$ on $\beta$ cell function.

We have reported previously that treatment of pancreatic $\beta$ cells with exogenous PGE$_2$ causes a decrease in insulin secretion in vitro (1–6) and in vivo (7–11) and that inhibitors of endogenous prostaglandin synthesis augment glucose-induced insulin secretion in vitro (3, 26) and in vivo (9–11). The single exception to the latter generalization has been indomethacin. The discordance of the effects of indomethacin with other cyclooxygenase inhibitors has been attributed to non-cyclooxygenase-related drug effects of indomethacin that themselves would be predicted to decrease insulin secretion (12). More recently, by using Northern analysis and RT-PCR, we have reported that, unlike other cells and organelles, pancreatic $\beta$ cells dominantly express COX-2 rather than COX-1 (19) and that exogenous IL-1$\beta$ stimulates islet COX-2 gene expression and PGE$_2$ production (19). The PGE$_2$ levels reported in the current study are lower than those we reported earlier (19) due to differences in experimental conditions (incubation in KRB buffer without FBS in the current study versus incubation in cytokine-containing culture media and fetal bovine serum). This ability of IL-1$\beta$ to inhibit insulin secretion and to stimulate synthesis of PGE$_2$ sets up the prediction that the inhibitory effect of exogenous IL-1$\beta$ on insulin secretion might be mediated by endogenous PGE$_2$. However, two groups of investigators have previously assessed this hypothesis by examining whether indomethacin would prevent IL-1$\beta$ inhibitory effects on the $\beta$ cell. Since indomethacin failed to reverse the effects of IL-1$\beta$, they concluded that endogenous PGE$_2$ does not mediate the inhibitory effect of IL-1$\beta$ on insulin secretion (5, 6). We confirmed this negative finding with indomethacin in our current studies. However, with the recent availability of specific inhibitors of COX-2 activity, it is now apparent that the previously published indomethacin experiments led to an erroneous conclusion, likely because of the independent effects of this drug to inhibit insulin secretion. Our add-back experiments with exogenous PGE$_2$, which re-established the inhibitory action of IL-1$\beta$ on insulin secretion, reinforce the hypothesis that endogenous PGE$_2$ mediates the negative effects of IL-1$\beta$ on $\beta$ cell function.

The current study also establishes that the dominant PGE$_2$ receptor subtype in the islet is EP3 whose post-receptor action is to decrease adenyl cyclase activity. This novel observation is consistent with the concentration-dependent relationship between PGE$_2$ concentrations and diminished cAMP accumulation in HIT cells we earlier reported (1, 2). In the current study, we used RT-PCR with probes and primers specific for the rat prostaglandin receptor subtypes to evaluate the expression levels of the four major subtypes in Wistar rat islets. We limited this part of our study to Wistar islets because neither the complete genomic sequence nor cDNA sequence for hamster receptor subtypes (HIT-T15 cells were derived from Syrian hamster) was available for design of the Taqman$^\text{TM}$ probes and primers. All the other EP receptor subtype genes (EP$_2$, EP$_3$, and EP$_4$) were also expressed but to a lesser degree. As controls and as validation of the Taqman$^\text{TM}$ technology for the characterization of these receptor subtypes, the expression of each receptor subtype was also evaluated in non-islet tissues (data not shown) and found to be consistent with published reports. Although the expression of the EP receptor subtypes cannot be quantitatively compared due to the possible differences in the efficiency of RT-PCR using different probe and primer sets, the differences in the $C_t$ value between each subtype mRNA signifies 2$^n$-fold difference in expression level with $n$ being the difference between the $C_t$ values. A difference in $C_t$ of 3 would, therefore, designate an 8-fold difference in mRNA expression levels.

These studies uniquely establish the important roles that COX-2 and endogenous PGE$_2$ play in the mechanism of action leading to $\beta$ cell dysfunction induced by IL-1$\beta$. This interrelationship raises the possibility of using COX-2-specific inhibitors for the prevention of $\beta$ cell dysfunction under inflammatory conditions. In this regard, consideration of COX-2-based therapy for the disease of type 1 diabetes mellitus is especially intriguing since endogenous IL-1$\beta$, as well as other cytokines, have been reported to be major contributors to the $\beta$ cell dysfunction and destruction that is associated with this form of diabetes (17, 18).

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**REFERENCES**

1. Robertson, R. P., Tsai, P., Little, S. A., Zhang, H. J., and Walseth, T. F. (1987) Diabetes 36, 1047–1053.
2. Seaquist, E. R., Walseth, T. F., Nelson, D. M., and Robertson, R. P. (1989) Diabetes 38, 1439–1445.
3. Metz, S. A., Robertson, R. P., and Fujimoto, W. Y. (1981) Diabetes 30, 551–557.
4. Burri, I. M., and Sharp, R. (1974) Endocrinology 94, 835–839.
5. Hughes, J. H., Rasom, R. A., Wolf, B. A., Turk, J., and McDaniel, M. L. (1989) Diabetes 38, 1251–1257.
6. Sjoholm, A. (1996) Biochim. Biophys. Acta 1313, 106–110.
7. Robertson, R. P., Cavarecki, D. J., Porte, D., Jr., and Bierman, E. L. (1974) J. Clin. Invest. 54, 310–315.
8. Sasa, L., Perez, G. B., Peng, Y., Pascucci, I., and Cordubrelli, M. (1975) Acta Endocrinol. 79, 266–274.
9. Chen, M., and Robertson, R. P. (1979) Prostaglandins 18, 557–567.
10. Robertson, R. P., and Chen, M. (1977) Trans. Assoc. Am. Physicians 90, 353–365.
11. Giugliano, D., Di Pinto, P., Torrella, R., Frascolla, N., Saccomanno, F., Passeriello, N., and D’Onofrio, F. (1983) Am. J. Physiol. 245, E591–E597.
12. Robertson, R. P. (1983) Diabetes 32, 231–234.
13. Engler, D. L., Bendtzen, K., and Sandler, S. (1991) Endocrinology 128, 1611–1616.
14. Palmer, J. P., Helquist, S., Spinas, G. A., Molvig, J., Mandrup-Poulsen, T., Andersen, H. U., and Nerup, J. (1989) Diabetes 38, 1211–1216.
15. Zawalich, W. S., Zawalich, K. C., and Rasmussen, H. (1989) Endocrinology 124, 2350–2357.
16. Comens, P. G., Wolf, B. A., Unanue, E. R., Lacy, P. E., and McDaniel, M. L. (1987) Diabetes 36, 963–970.
17. Helquist, S. (1994) Dan. Med. Bull. 41, 151–166.
18. Mandrup-Poulsen, T. (1996) Diabetesology 3, 1005–1029.
19. Sorli, C. H., Zhang, H. J., Armstrong, M. B., Rajotte, R. R., Maclouf, J., and Robertson, R. P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1788–1793.
20. Gierse, J. K., McDonald, J. J., Hausser, S. D., Rangwala, S. H., Koholdt, C. M., and Seibert, K. (1996) J. Biol. Chem. 271, 15810–15814.
21. Kawai, S., Nishida, S., Kato, M., Furumaya, Y., Okamoto, R., Koshino, T., and Mizushima, Y. (1989) Eur. J. Pharmacol. 162, 87–94.
22. Zhang, H. J., Walseth, T. F., and Robertson, R. P. (1989) Diabetes 38, 44–48.
23. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
24. McDaniel, M. L., Kwon, G., Hill, J. R., Marshall, C. A., and Corbett, J. A. (1996) Proc. Soc. Exp. Biol. Med. 211, 24–32.
25. Boie, Y., Stocco, R., Sawyer, N., Slepitz, D. M., Ungrin, M. D., Neuschafer-Rube, F., Paschel, G. P., Metters, K. M., and Abramovitz, M. (1997) Eur. J. Pharmacol. 340, 227–241.
26. Fujimoto, W. Y., and Metz, S. A. (1984) Diabetes 33, 872–878.