Interleukin 1 Inhibits Insulin Secretion From Isolated Perifused Rat Islets

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SUMMARY

Preincubation of collagenase-isolated rat islets for 150 min with 100 U/ml purified human interleukin 1 (IL-1) altered their ability to secrete insulin. Whereas basal release rates with 4 mM glucose were comparable in control and IL-1–treated islets, both the first and second phases of release in response to 20 mM glucose were significantly reduced from IL-1–treated tissue. IL-1 pretreatment also impaired the secretory response to the combination of 100 nM cholecystokinin plus 7 mM glucose. However, the secretory response to 10 mM α-ketoisocaproate was comparable in control and IL-1–pretreated islets. Reducing the IL-1 exposure time to 60 min was accompanied by an augmented first phase of release to 20 mM glucose. Second phase secretion was diminished. The use of glucose measured after the perifusion was similar in control and IL-1–treated islets. Similar to other compounds that adversely impact on β-cell viability, the inhibitory effect of IL-1 on release may presage a cytotoxic action of monokine. DIABETES 1986; 35:1119–23.

The multiple metabolic effects of activated mononuclear phagocytes on host tissues appear to be mediated, at least in part, by the soluble protein messenger interleukin 1 (IL-1). The pleiotropic effects of this compound on various tissues have been summarized.[1] It was recently reported that pretreatment of cultured islets with IL-1 reduced insulin output in response to glucose stimulation.[2] Because an early event in the β-cytotoxic action of various compounds is an altered response to glucose stimulation,[3-5] the possibility that IL-1 may negatively impact on β-cell viability was suggested.[2] Our experiments were undertaken to examine in more detail the effects of acute IL-1 pretreatment on insulin release from perifused rat islets.

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The results demonstrate that IL-1 exposure reduces islet responsiveness to glucose and also to the combination of cholecystokinin plus 7 mM glucose. Secretion in response to 10 mM α-ketoisocaproate (KIC) was not altered by IL-1 pretreatment. The use of 20 mM glucose, assessed after the perifusion, was not adversely affected by IL-1 despite the reduction in insulin output. The results demonstrate that an early action of IL-1 on islet tissue is a selective impairment of glucose-stimulated and glucose-potentiated insulin output. Whether the sequence of events responsible for this inhibitory action on release presages a β-cytotoxic action of the monokine remains to be established.

MATERIALS AND METHODS

Male Sprague-Dawley rats purchased from Charles River (Wilmington, MA) were used in all studies. The animals were fed ad lib and weighed 300–400 g. After Nembutal-induced anesthesia (50 mg/kg), islets were isolated by collagenase digestion.[6] Usually, six groups of 12 islets were harvested from each rat, and three separate experiments with their appropriate controls were run in parallel. The islets were loaded onto nylon filters (Tetko, Elmsford, NY) and placed in small glass incubation vials. To the vials, 0.1 ml of a solution containing 115 mM NaCl, 10 mM KCl, 2 mM $MgCl_2$, 4.4 mM $CaCl_2$, 48 mM $NaHCO_3$, 16 mM glucose, and 0.34% bovine albumin was added. This solution was maintained at 37°C and gassed with 95% O_2/5% CO_2 for at least 10 min before use. In addition, 0.1 ml of purified human interleukin 1 (200 U/ml, lots 85CO1 and 85CO2), stabilized in 0.01% bovine albumin and 0.15 M NaCl, was added. (The acute nature of these studies prompted the use of much higher IL-1 levels than those used by Mandrup-Poulsen and co-workers.[2] However, their islets were exposed to IL-1 for days as opposed to hours under our experimental conditions. Obviously, detailed dose-response studies with IL-1 are required.) Control islets received 0.1 ml of 0.01% bovine albumin and 0.15 M NaCl. The glass vials were capped, gently gassed with 95% O_2/5% CO_2 for 30 s, and incubated for 60 or 150 min at 37°C in a Dubnoff shaker (60 strokes/min). Those islets incubated for 150 min

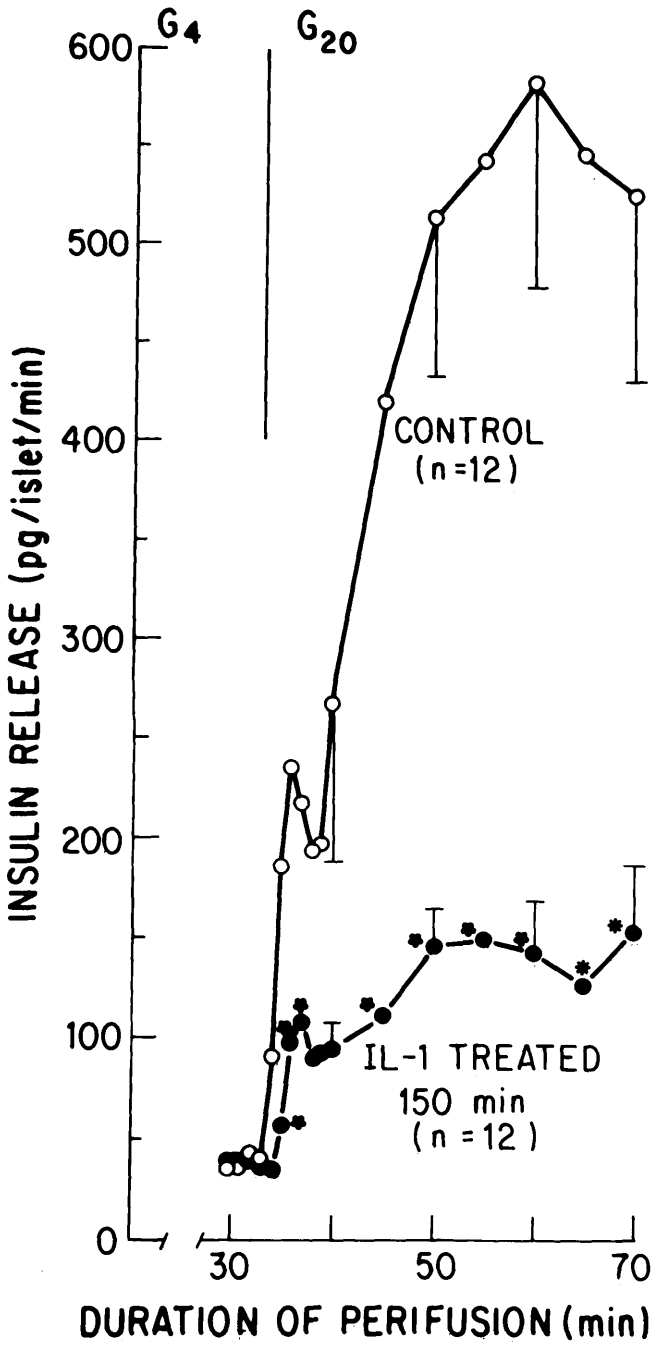

FIG. 1. Effects of IL-1 pretreatment (150 min) on glucuose-stimulated insulin secretion. Batches of islets were incubated for 150 min with IL-1 (100 μ/ml) and then perifused. After 30-min stabilization period with 4 mM glucose, islets were stimulated with 20 mM glucose. Mean values are given, and means ± SE of selected time points are presented for clarity. *Asterisk* indicates a significant ($P < .05$) group difference between release values at that time point.

were gently gassed again after the first 75 min. After the incubation, the islets, still attached to the nylon filter, were removed from the glass vial and perifused.[7] The basal perifusion medium consisted of 115 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2.2 mM $CaCl_2$, 24 mM $NaHCO_3$, and either 2.75 or 4.0 mM glucose.

After an initial 30-min perifusion period, in which stable release rates were achieved, islets were provoked with 20 mM glucose, 7 mM glucose plus 100 nM of the COOH-terminal 8-amino-acid cholecystokinin, fragment 26–33 amide, sulfated on the tyrosine residue (CCK8S), or 10 mM α-keto-isocaproate (sodium salt) plus 4 mM glucose. One-minute perifusate samples were collected for the first 10 min to monitor first-phase secretion, and 5-min samples were collected thereafter. Insulin content was measured by radioimmunoassay (RIA) with rat insulin (lot 615-D63-12-3, Lilly; Indianapolis, IN) as standard.[8]

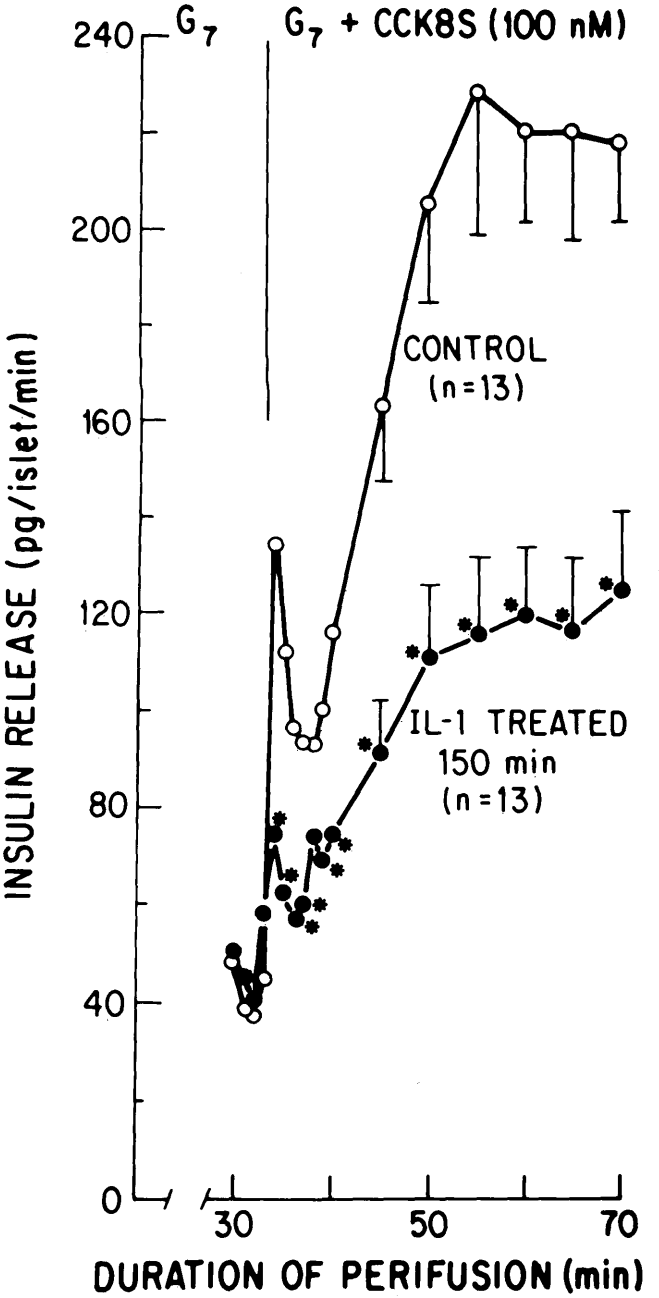

FIG. 2. Effects of IL-1 pretreatment (150 min) on CCK8S-stimulated insulin secretion. After IL-1 pretreatment, islets were perifused for 30 min in 7 mM glucose. After this, 100 nM cholecystokinin (CCK8S) was added to the medium and perifusion continued for additional 40 min. Mean values are given ± selected SE. *Asterisk* indicates a significant ($P < .05$) group difference between release values at that time point.

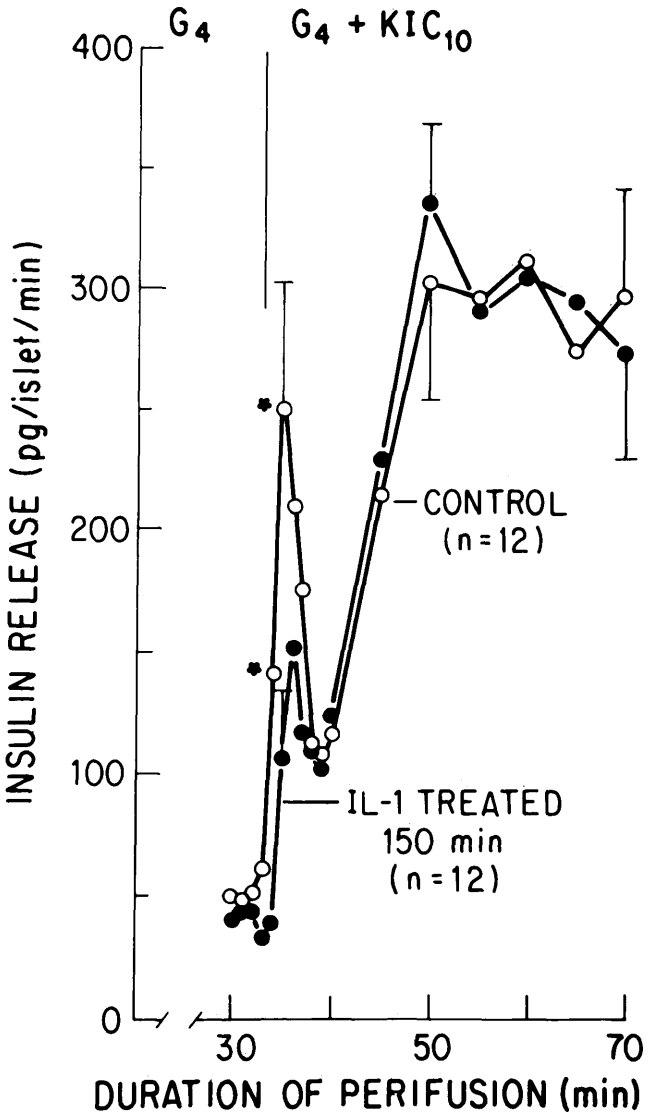

FIG. 3. KIC-stimulated insulin output after IL-1 pretreatment (150 min). Islets were incubated in IL-1 and then perifused with 4 mM glucose for 30 min. They were then stimulated with 10 mM α-ketoisocaproate (sodium salt; KIC) for 40 min and release monitored. Similar secretory profiles were noted during perifusion.

After the perifusion, the islets were removed from the chamber and glucose use assessed by the formation of ^3H_2O from [5-^{3}H]glucose.[9,10] The medium for the use studies was identical to that used during the stimulatory phase of the perifusion, except for the presence of tracer [5-^{3}H]glucose.

Reagents. The salts, albumin (RIA grade), cholecystokinin (C-9271), and α-ketoisocaproate were purchased from Sigma (St. Louis, MO). The isotopes used to measure glucose usage (^3H_2O and [5-^{3}H]glucose) and the ^{125}I-labeled insulin were purchased from New England Nuclear (Boston, MA). Interleukin 1 (purified human) was purchased from Cistron Technology (Pine Brook, NJ).

Statistics. Statistical significance was determined with the Student's *t* test for unpaired data, and *P* values <.05 were considered significant. Values in figures represent means ± SE of the specified number of observations.

RESULTS

After a 150-min incubation, both control and IL-1–treated islets released comparable amounts of insulin during the 30-min stabilization period of the perifusion (Fig. 1). With the addition of 20 mM glucose to the medium, control islets responded with a brisk biphasic output of insulin. Peak release during the final 20 min of stimulation averaged well over 500 pg · islet^{-1} · min^{-1}. Although a similar biphasic pattern of insulin output was observed in the IL-1–pretreated islets, the response was delayed somewhat and dramatically reduced. Release rates were significantly lower than control rates during most of the perifusion and achieved an average peak value of ~150 pg · islet^{-1} · min^{-1} (Fig. 1). A similar reduction in insulin output in response to the glucose-dependent secretagogue CCK8S (in the presence of 7 mM glucose) was

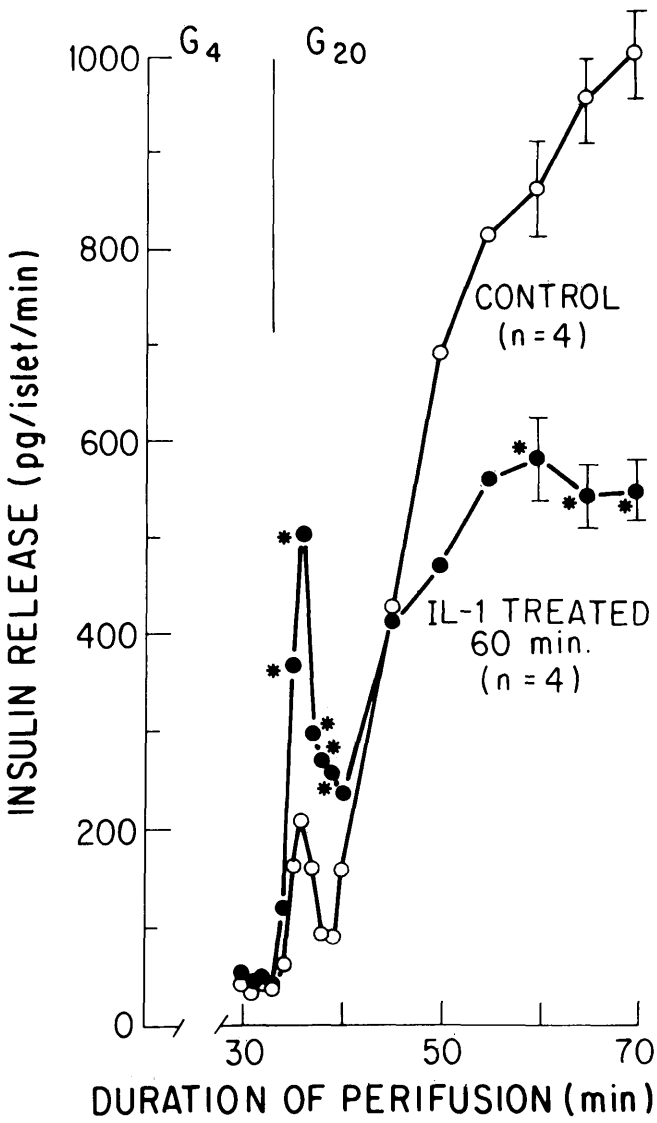

FIG. 4. Effects of IL-1 pretreatment (60 min) on glucose-stimulated insulin secretion. Islets were preincubated for 60 min in IL-1 and then perifused with 4 mM glucose for 30 min, followed by 40-min provocation with 20 mM glucose. IL-1–treated islets released significantly ($P < .05$, indicated by *asterisk*) more insulin during the first phase of release and significantly less during the final 15 min of stimulation.

TABLE 1
Glucose usage rates from control and IL-1–treated islets

Perifusion protocol	N	Duration of IL-1 treatment (min)	Glucose usage* (pmol · islet^{-1} · h^{-1})
$G_4 \longrightarrow G_{20}$	4		125 ± 19
$G_4 \longrightarrow G_{20}$	4	60	124 ± 10.0
$G_4 \longrightarrow G_{20}$	12		127 ± 10.3
$G_4 \longrightarrow G_{20}$	12	150	137 ± 8.5
$G_7 \longrightarrow G_7$ + CCK8S(100 nM)	13		66 ± 4.3
$G_7 \longrightarrow G_7$ + CCK8S(100 nM)	13	150	73 ± 3.8
$G_4 \longrightarrow G_4 + KIC_{10}$	12		40.2 ± 3.1
$G_4 \longrightarrow G_4 + KIC_{10}$	12	150	45.1 ± 3.9

After the perifusion, islets were incubated for another 60 min in a medium identical to that used during the final phase of the perifusion, except for the presence of tracer (1–2 μCi) [5-^{3}H]glucose. The amount of ^3H_2O formed served as the index of hexose usage. The glucose (G) and α-ketoisocaproate (KIC) concentrations given in subscript are millimolar.
*Means ± SE.

also noted (Fig. 2). In sharp contrast to the reduced response noted with 20 mM glucose or 100 nM CCK8S plus 7 mM glucose after IL-1, the ability of 10 mM KIC to augment secretion was comparable in both control and IL-1–treated islets (Fig. 3). Only during the first few minutes of stimulation was insulin output greater from control tissue. Second-phase responses were virtually identical.

In a limited series of experiments, the effect of a 60-min exposure to IL-1 on the response to 20 mM glucose was examined. The results are shown in Fig. 4. Reducing the exposure time to IL-1 was accompanied by a reduced second-phase response to glucose stimulation, a result similar to that noted in the longer incubation (150-min) experiments. However, the first-phase secretory response from IL-1–treated islets was significantly greater than that noted from control islets. Peak first-phase secretion averaged slightly >500 pg · islet^{-1} · min^{-1} from IL-1–treated islets as opposed to a value of ~200 pg · islet^{-1} · min^{-1} from control islets.

After the perifusion, the capacity of the islets to use glucose was measured (Table 1). Compared to control glucose usage rates, IL-1 pretreatment had no adverse impact on glucose metabolism measured over a 60-min incubation period.

DISCUSSION

Multiple metabolic actions have been ascribed to the monokine IL-1.[1] Among other effects, this compound appears to mediate fever produced by exogenous pyrogens,[11] to induce skeletal muscle proteolysis,[12] and to promote IL-2 receptor production from T-lymphocytes.[13] Our study implicates the β-cell of the islet of Langerhans as another target system for the polypeptide. Although it is currently difficult to assess the physiologic or pathophysiologic implications of these observations, primarily because in vivo levels of the compound are difficult to determine, several comments seem warranted.

After a 150-min incubation period, IL-1, at least at the concentration used in our study, reduces the first and second phases of glucose and glucose-dependent insulin output. A shorter (60-min) exposure to the monokine is likewise accompanied by a reduction in the second phase of secretion. However, the initial phase of secretion is more than doubled after IL-1 treatment. Our study thus confirms and extends the original observations of Mandrup-Poulsen and co-workers.[2] The inhibitory effect on insulin output is not paralleled by any perceptible derangement in overall glucose metabolism by the tissue, an event thought by most investigators[14–16] to be a key determinant in the insulin secretory response. This finding implies perhaps that the coupling between glucose metabolism and secretion has been altered. The locus of the impairment has not been pinpointed by the present analysis, but it might be speculated that a second messenger system, possibly calcium, cAMP, or a product of phosphatidylinositol (PI) metabolism, may be involved. The finding that the stimulatory effect of CCK8S (in the presence of 7 mM glucose) is also reduced after IL-1 pretreatment appears to support the contention that PI metabolism may be involved. It is known, for example, that glucose induces PI turnover in perifused islets,[17] and similar effects have been described for CCK8S.[18] Specific binding sites for CCK8S have been shown to exist on the islet, and their occupancy appears related to insulin secretion.[19,20] Because both CCK8S and IL-1 most likely interact with the β-cell membrane (if not exclusively, then at the least initially), it is interesting to speculate that IL-1 may interfere somehow with CCK8S or glucose-mediated membrane PI metabolism. As a possible first step along those lines, the direct effects of IL-1 on insulin secretion and PI metabolism should be assessed.

The finding that the second phase of secretion in response to KIC remains intact after IL-1 treatment emphasizes several points. First, the acute effects of IL-1 on the islet appear, at least initially, to be specific in nature and not the result of a generalized destructive process. This idea is further supported by the finding of basically normal glucose usage rates in all groups of islets after IL-1 treatment despite markedly different secretory responses. The possibility that a more generalized defect occurs with prolonged IL-1 cannot be excluded, but for now at least, glucose and glucose-dependent CCK8S secretion appear most vulnerable. Second, if the inhibitory effects of IL-1 are mediated via impaired PI turnover, KIC-stimulated insulin output, at least to a large extent, is not mediated by PI turnover. It may also be proposed that KIC-generated second messengers are able to break through the inhibitory effects of IL-1 on PI metabolism. The observation that the first phase of release to KIC was reduced by IL-1 would seem to indicate that release to this secretagogue is not totally immune from the inhibitory effects of monokine. Future studies should be directed at both the time-course and dose-response effects of IL-1 on KIC-induced insulin output.

The results of our study may assume particular clinical significance if it can be demonstrated that IL-1 participates in some manner in the immunologic destruction of the β-cell, a situation thought to characterize type I insulin-dependent diabetes. It is known, for example, that mononuclear infiltration occurs during inflammation of the islet (insulitis) in both humans[21] and the BB/W rat.[22] Consequently, the acute inhibitory effects of the compound on stimulated secretion may presage a β-cytotoxic action of the compound. In this regard, IL-1 would appear to be similar to a variety of established β-cell toxins, including streptozocin,[3] alloxan,[4] the rodenticide Vacor,[23] and islet cell antibodies,[24] compounds whose earliest

effect appears to be on the sensitive insulin secretory apparatus of the islet.

Our results provide little insight into the mechanism of IL-1 action on the islet. In neutrophils, IL-1 is thought to activate phospholipase A_2 (PLA_2), which in turn generates arachidonic acid from membrane phospholipids.[25] The further metabolism of the arachidonic acid to various prostaglandins, prostacyclins, or leukotrienes is thought to mediate the ensuing metabolic responses. Because PLA_2 is calcium activated[26] and because the calcium ionophore A23187 mimics many of the actions of IL-1,[27] an ionophoretic mechanism for the monokine has been proposed.[28] However, similar to other membrane-active compounds (e.g., hormones and neurotransmitters), the elevation in intracellular calcium may be an indirect effect of IL-1 mediated by second messengers generated via phospholipid metabolism, e.g., inositol phosphates generated during PI turnover.[29–31] How the products of arachidonic acid metabolism, if indeed these are the mediators of IL-1 action, might impair stimulated release is not known. It has been demonstrated that arachidonic acid and some of its metabolites actually increase insulin output from islet tissue.[32,33] However, the effects are extremely small despite the high levels used, and most important, the reversible nature of arachidonic acid–stimulated release has not been determined. Although the basis for the inhibitory effects of IL-1 on insulin secretion remains a fertile area for speculation, the isolated-islet preparation would appear to be a suitable target system to further analyze the metabolic impact of IL-1 on β-cell responsiveness.

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