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Insulin-stimulated Insulin Secretion in Single Pancreatic Beta Cells*

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Functional insulin receptors are known to occur in pancreatic beta cells; however, except for a positive feedback on insulin synthesis, their physiological effects are unknown. Amperometric measurements at single, primary pancreatic beta cells reveal that application of exogenous insulin in the presence or absence of nonstimulatory concentrations of glucose evokes exocytosis mediated by the beta cell insulin receptor. Insulin also elicits increases in intracellular Ca^{2+} concentration in beta cells but has minimal effects on membrane potential. Conditions where the insulin receptor is blocked or cell surface concentration of free insulin is reduced during exocytosis diminishes secretion induced by other secretagogues, providing evidence for direct autocrine action of insulin upon secretion from the same cell. These results indicate that the beta cell insulin receptor can mediate positive feedback for insulin secretion. The presence of a positive feedback mechanism for insulin secretion mediated by the insulin receptor provides a potential link between impaired insulin secretion and insulin resistance.

Glucose is the principal regulator of insulin secretion from pancreatic beta cells in islets of Langerhans (1, 2); however, intra-islet communication through paracrine interactions may also exert an important level of control over insulin secretion and ultimately glucose homeostasis. For example, glucagon secreted from islet alpha cells potentiates insulin secretion (3), whereas somatostatin secreted from delta cells is a potent inhibitor of glucose-stimulated insulin secretion (4). Although these paracrine interactions are well established, the potential autocrine action of insulin upon insulin secretion remains unclear.

Several lines of evidence support the possibility of an autocrine action of insulin on beta cells. Insulin binds to the surface of beta cells (5, 6), and functional insulin receptors and receptor substrates identical to those found in peripheral tissues have been identified in both clonal and primary beta cells (6–9). Glucose stimulation of beta cell lines activates the beta cell insulin receptor in the same way as application of exogenous insulin, suggesting that insulin secreted from beta cells binds to the insulin receptor eliciting a physiological response (9, 10). The complete physiological consequences of insulin receptor activation of the beta cell have yet to be completely elucidated, but at least one effect is initiation of protein synthesis at both transcriptional and translational levels (10–12).

Although functional insulin receptors have been identified on beta cells, the possible effects on insulin secretion mediated by beta cell insulin receptors have not been firmly established. Several reports have shown that glucose-stimulated insulin or C-peptide secretion from islets or perfused pancreas is suppressed in the presence of exogenous insulin, leading many to believe that insulin inhibits secretion in beta cells (13–20). Under similar conditions however, some reports have shown no effect of insulin on glucose-stimulated insulin secretion (21–28). Furthermore, these data are difficult to interpret as direct autocrine action of insulin because: 1) intact organs or islets possess neuronal and hormonal regulatory mechanisms that could interact with exogenous insulin, 2) maintenance of normoglycemia during the time course of the experiments is often difficult because of the addition of exogenous insulin, and 3) high glucose levels used to evoke stimulation likely leads to substantial activation of beta cell insulin receptors by secreted endogenous insulin, masking the effect of exogenous insulin.

Experiments with purified beta cells and beta cell lines have also generated conflicting evidence for insulin feedback. Glucose-stimulated insulin secretion from purified rat beta cells was inhibited by 20% at exogenous insulin concentrations above 1 μM (28). In contrast, measurements of the effect of insulin on C-peptide secretion in βTC3 cells failed to show direct evidence of secretory regulation by insulin (9). Furthermore, transfected βTC6-F7 cells in which the insulin receptor was overexpressed showed enhanced basal and glucose-stimulated insulin secretion, but fractional secretory levels (percentage of total releasable cell insulin secreted) remained unchanged at all glucose concentrations whereas cells expressing kinase negative (inactive) insulin receptors showed decreased glucose-stimulated insulin secretion (11). Recent studies have confirmed that manipulation of IRS-1 levels of beta cell lines affect levels of insulin synthesis and secretion (29, 30). These results suggest an autocrine pathway regulating one or more of the following: insulin secretion, insulin synthesis, and glucose sensing/utilization.

Given the ambiguities of previous experiments, we have attempted to directly characterize the effect of exogenous insulin upon insulin secretion from single beta cells using amperometry (31, 32). In this technique, an amperometric electrode is positioned next to a single cell so that released secretory product can be detected with high sensitivity and temporal resolution. When secretion by vesicle fusion occurs, a current spike is recorded that corresponds to quantitative detection of packets of molecules released by exocytosis (31–33). Secreted insulin can be detected directly using a carbon fiber amperometric

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electrode that is modified with a mixed valent film of cyanoruthenate and ruthenium oxide (32). Alternatively, 5-hydroxytryptamine (5-HT)[1] secretion can be detected as a marker of exocytosis in beta cells (33–36). In this approach, 5-HT is allowed to accumulate into insulin containing secretory vesicles of beta cells, and 5-HT secretion is detected using an unmodified carbon fiber electrode. Unless stated otherwise, we used detection of 5-HT because it generated a higher signal to noise ratio and did not suffer from the possible interference of exogenous insulin on secretory measurements. This methodology allowed the measurement of exocytosis from single, isolated cells that were not affected by possible paracrine interactions of neighboring cells, thus allowing direct observation of the effect of insulin on regulation of beta cell exocytosis.

MATERIALS AND METHODS

Chemicals and Reagents—Bovine insulin, Type XI collagenase, HEPES, and tolbutamide were obtained from Sigma and used without further purification. Monoclonal anti-insulin, polyclonal anti-insulin receptor$_\alpha$ and IgG were obtained from BioDesign International (Kennebunk, ME) and were of rabbit origin. Unless otherwise stated, all chemicals for islet and cell culture were obtained from Life Technologies. All other chemicals were from Fisher unless noted and were of the highest purity available.

Isolation and in Vitro Culture of Mouse Islets and Beta Cells—Islets were isolated from 20–30 g CD-1 mice following ductal injection with collagenase and dispersed into single cells by shaking in dilute trypsin for 10 min at 37 °C (32, 37). Cells were cultured at 37 °C, 5% CO_2, pH 7.4, in RPMI 1640 containing: 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin and used on days 2 to 4 after isolation.

Isolation and in Vitro Culture of Canine, Porcine, and Human Islets and Beta Cells—Pancreatic islets were isolated from canine, porcine, or human pancreas using controlled collagenase (Boehringer Mannheim) perfusion via the duct, automated dissociation, and discontinuous Euro-Ficoll purification using the COBE 2991 blood cell processor as described previously (38, 39). Islets were dispersed into single cells the next day using a previously described procedure (32). Cells were cultured at 37 °C, 5% CO_2 in modified CMRL 1066 tissue culture media containing: 10% fetal bovine serum, 25 mM HEPES, 100 units/ml penicillin, and 100 μg/ml streptomycin, pH 7.4.

Data Collection and Analysis—Microelectrodes were constructed that consisted of carbon fiber disks sealed in glass micropipettes and were polished to 30–45° angle immediately prior to use (32, 33). Amperometry was performed using a battery to apply potential to a sodium saturated calomel electrode (SSCE) as described previously (33). For measurements of 5-HT secretion, dispersed beta cells were incubated in tissue culture media containing 0.5 mM 5-hydroxytryptamine and 1 mM 5-hydroxytryptophan for 16 h at 37 °C, 5% CO_2, pH 7.4 (33). Cells were used for secretion experiments immediately after loading. Measurements requiring direct detection of insulin were performed on beta cells that were not allowed to accumulate 5-HT prior to experimentation, and the microelectrode was chemically modified with a film of mixed valent cyanoruthenate and ruthenium oxide as described elsewhere (32). For detection of 5-HT the potential at the working electrode was 0.65 V, whereas for detection of insulin the potential was 0.85 V. Data were low pass filtered at 100 Hz and collected at 500 Hz using a personal computer (Gateway 2000 P5–166) via a data acquisition board (Axon Instruments, DigiData 1200B). For direct measurement of insulin using chemically modified microelectrodes, data were further high pass filtered following collection to remove the slow component of the background current associated with detection of the insulin stimulant, leaving the rapid current spikes unaffected.

Amperometric measurements were made by positioning microelectrodes ~1 μm from a cell and applying stimulant from a micropipette ~30 μm from the cell as described elsewhere (32, 33). All experiments were performed with cells incubated at 37 °C in pH 7.4 Kreb's Ringer buffer (KRB) containing (in mM): 118 NaCl, 5.4 KCl, 2.4 $CaCl_2$ (unless noted otherwise), 1.2 $MgSO_4$, 1.2 KH_2PO_4, 3 D-glucose, and 25 HEPES (24 $NaHCO_3$ for insulin measurements). Stimulant solutions (1 nM–1 μM insulin, 200 μM tolbutamide, 17 mM glucose) were prepared by dissolving the desired concentration of stimulant in KRB. 30 mM KCl stimulant was prepared as above but by removing an equal concentration of NaCl to maintain ionic strength. All means are reported ± 1 S.E. of the mean. Statistical differences between means were evaluated using a two-tailed Student's *t* test.

Anti-insulin Receptor Antibody Experiments—Mouse beta cells were stimulated with 100 nM insulin, and exocytosis of 5-HT was detected by amperometry to establish viability. Following successful stimulation with 100 nM insulin, 10 nM anti-insulin receptor$_\alpha$ was added to the buffer and allowed to incubate for 5 min. The same cell was then stimulated again with 100 nM insulin in the presence of the antibody. Following insulin stimulations, the cell was stimulated with 30 mM K^+ in the presence of antibody to confirm cell viability.

Investigation of Autocrine Activation of Beta Cells—Direct autocrine activation of 5-HT loaded beta cells was investigated by first establishing cells to be responsive to 100 nM insulin stimulation by detecting exocytosis of 5-HT by amperometry. After establishment of viability, cells were stimulated with 30 mM K^+. Following K^+ stimulation, 25 nM anti-insulin receptor$_\alpha$ was added to the buffer and allowed to incubate for 5 min. Cells were again stimulated with 30 mM K^+, and the number of exocytosis events detected in the presence and absence of antibody was compared.

In a second series of experiments, 5-HT-loaded canine beta cells were bathed in KRB of varying H^+ and Zn^{2+} concentration. Buffer pH was adjusted by varying bicarbonate concentration to achieve the desired pH after bubbling with 5% CO_2. Extracellular Zn^{2+} concentration was adjusted by adding Zn^{2+} to the desired concentration. Ionic strength was held constant for all solutions. Cells were then stimulated with 200 μM tolbutamide dissolved in KRB of matching pH and Zn^{2+} concentration, and 5-HT secretion was detected by amperometry. The number of 5-HT spikes detected per stimulation was then compared at the various pH and Zn^{2+} concentrations.

Membrane Potential Measurements—Membrane potential measurements were made in the whole-cell perforated patch configuration at room temperature. Pipettes were pulled from borosilicate glass and had resistances between 4 and 6 megohm. Pipette solutions contained (in mM): 10 KCl, 76 K_2SO_4, 10 NaCl, 1 $MgCl_2$, 10 HEPES, and 200 μg/ml amphotericin B, pH 7.35. Data were low pass filtered at 100 Hz and collected at 500 Hz using Axopatch 200A patch-clamp amplifier and DigiData digitizer (Axon Instruments, Foster City, CA).

Extracellular Calcium Dependence of Insulin Stimulation—The extracellular calcium requirement for insulin stimulation was investigated using amperometry at 5-HT-loaded mouse beta cells. Cells were bathed in KRB containing 0 mM Ca^{2+} and stimulated with 100 nM insulin dissolved in KRB containing either 0 or 5 mM Ca^{2+}. All data come from paired experiments of cells stimulated with both 0 and 5 mM Ca^{2+}-containing stimulants.

Intracellular Calcium Measurements—Beta cells were incubated in 2 μM Fura-2/AM (Molecular Probes) dissolved in KRB at 37 °C, 5% CO_2 for 30 min. Dye solution was then replaced with KRB, and coverslips with adherent cells were placed into a coverslip dish for immediate use. The resulting fluorescence from individual cells was collected at 1 Hz through a Fluar × 40 oil immersion objective (Zeiss), band pass filter (510 ± 10 nm), and 20 μm pinhole aperture onto a photomultiplier tube using a SPEX CMX cation measurement system and DM3000M data acquisition software (Instruments SA).

RESULTS

Insulin-stimulated Insulin Secretion in Single Beta Cells—In view of the majority of prior results suggesting negative feedback of insulin on insulin secretion, it was surprising to find that application of bovine insulin to isolated mouse beta cells at nonstimulatory (3 mM) glucose concentrations stimulated exocytosis (Fig. 1*A*). This result was confined to beta cells as the cells that responded to insulin also exhibited exocytosis when stimulated with 17 mM glucose or 200 μM tolbutamide, stimulants known to act at beta cells (n = 8) (Fig. 1*B*). Furthermore, the effect did not result from a contaminant in the insulin-stimulatory solution as addition of anti-insulin to the stimulant solution abolished the secretory response elicited by insulin stimulation in all cases (n = 7) (Fig. 1*C*). Finally, this effect is not an artifact of measuring accumulated 5-HT instead of insulin, as it was possible to observe secretion by direct measurement of insulin at single beta cells that had not been allowed to accumulate 5-HT (n = 5) (Fig. 1*D*). The observation of insulin-stimulated insulin secretion is not unique to mouse

[1] The abbreviations used are: 5-HT, 5-hydroxytryptamine; KRB, Kreb's Ringer buffer.

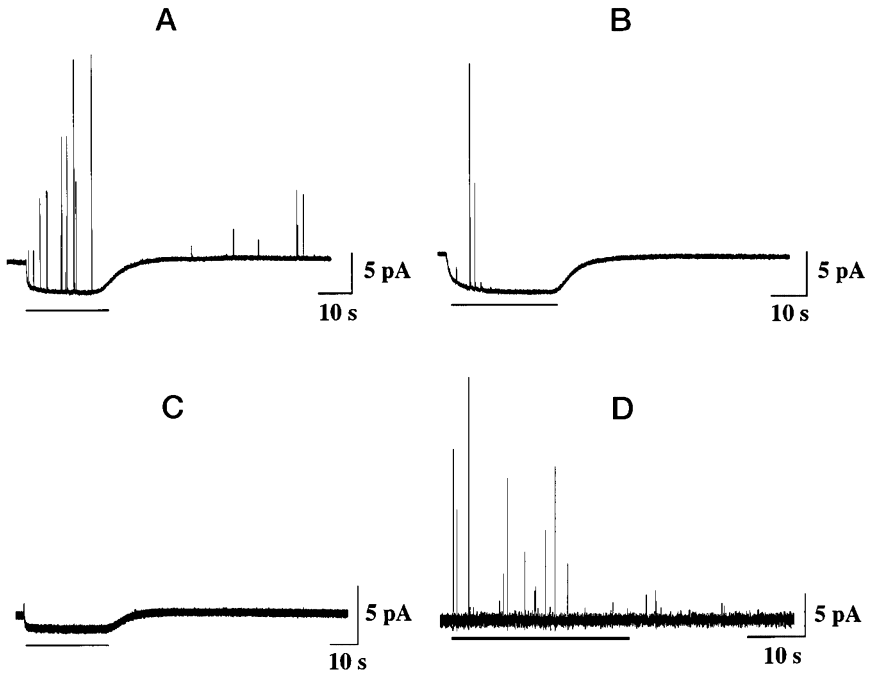

FIG. 1. **Detection of exocytosis at single mouse beta cells using carbon fiber microelectrode.** Detection of 5-HT upon application of 100 nM insulin (*A*) and 200 μM tolbutamide (*B*) (same cell as *panel A*). *C*, detection of 5-HT upon stimulation with 100 nM insulin with 100 nM anti-insulin. *D*, detection of insulin upon stimulation with 100 nM insulin with chemically modified microelectrode. In all cases, the *bar* under current traces represents application of stimulant.

cells as we observed similar stimulatory effects on insulin secretion following stimulation with exogenous insulin in human (n = 10), porcine (n = 7), and canine beta cells (n = 21) (Fig. 2).

Requirement of Beta Cell Insulin Receptor for Insulin-stimulated Exocytosis—To determine whether insulin-stimulated insulin secretion was mediated by the beta cell insulin receptor, we examined the antagonistic effect of anti-insulin receptor$_\alpha$ on insulin-stimulated insulin secretion (Fig. 3). For these experiments, cells were stimulated with 100 nM insulin to establish responsiveness, then incubated with 10 nM polyclonal anti-insulin receptor$_\alpha$ to block the insulin receptor, and stimulated again with 100 nM insulin. In no case was secretion detected following application of antibody (n = 4). Subsequent stimulation with 30 mM K^+ in the presence of anti-insulin receptor$_\alpha$ evoked secretion in all cases establishing cell viability following antibody treatment. In control experiments, addition of immunoglobulins had no detectable effect on insulin-stimulated exocytosis (data not shown). We also found that the insulin-stimulated insulin secretion was dependent upon the concentration of applied insulin in the range of 1–100 nM as illustrated in Fig. 3, *E* and *F*. It was found that higher concentrations of insulin (1 μM) did not induce a significantly different number of exocytosis events. (In evaluating the concentrations used to elicit secretion, it is important to realize that the concentrations reported are those present in the pipette. During stimulation, the solution is diluted by ill-defined amount as it is applied to the cell.)

Direct Autocrine Stimulation of Single Beta Cells—Next we investigated the possibility of direct autocrine action of insulin at single beta cells. Cells that had been established as insulin-responsive by detection of 5-HT secretion following insulin stimulation were stimulated with 30 mM K^+ in the presence and absence of 25 nM anti-insulin receptor$_\alpha$ to prevent autocrine activation of the beta cell insulin receptor. Stimulation resulted in 20.2 ± 4.5 spikes per stimulation preceding addition of antibody and was reduced to 9.6 ± 2.4 spikes per stimulation (n = 13) upon addition of antibody ($p < 0.05$), indicating that antibody could block released insulin from further enhancing release.

As further confirmation that insulin could contribute to direct positive feedback, we investigated the effects of conditions that reduce the free insulin concentration at the cell surface. We have previously shown that increasing H^+ and Zn^{2+} in the extracellular medium significantly decreases the extrusion rate of insulin from single secretory vesicles after vesicle fusion leading to a decrease in the concentration of free insulin at the cell surface in cells undergoing exocytosis (33, 34). Table I summarizes the amount of 5-HT secretion, measured as a number exocytosis events detected, evoked by 200 μM tolbutamide detected from cells incubated in control buffers and buffers containing varying H^+ and Zn^{2+} concentrations. The number of spikes detected by stimulation is significantly reduced at higher H^+ ($p < 0.01$) and Zn^{2+} ($p < 0.005$) concentrations. Thus, under conditions where the amount of free insulin is reduced at the cell surface after vesicle fusion, the number of exocytosis events is reduced. These results are consistent with the hypothesis that secreted free insulin activates further insulin secretion.

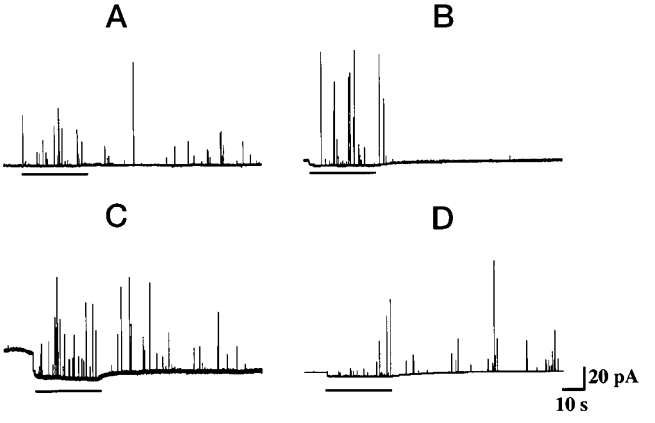

FIG. 2. **Insulin-stimulated exocytosis in beta cells from different species.** *Traces* represent detection of accumulated 5-HT with bare carbon fiber microelectrode from single, dispersed beta cells following application of 100 nM insulin dissolved in KRB as indicated by the *bars* underneath the trace. Detection is from canine (*A*), porcine (*B*), human (*C*), and mouse (*D*) beta cells.

Glucose Dependence of Insulin-stimulated Insulin Secretion—The primary physiological action of insulin is to stimu-

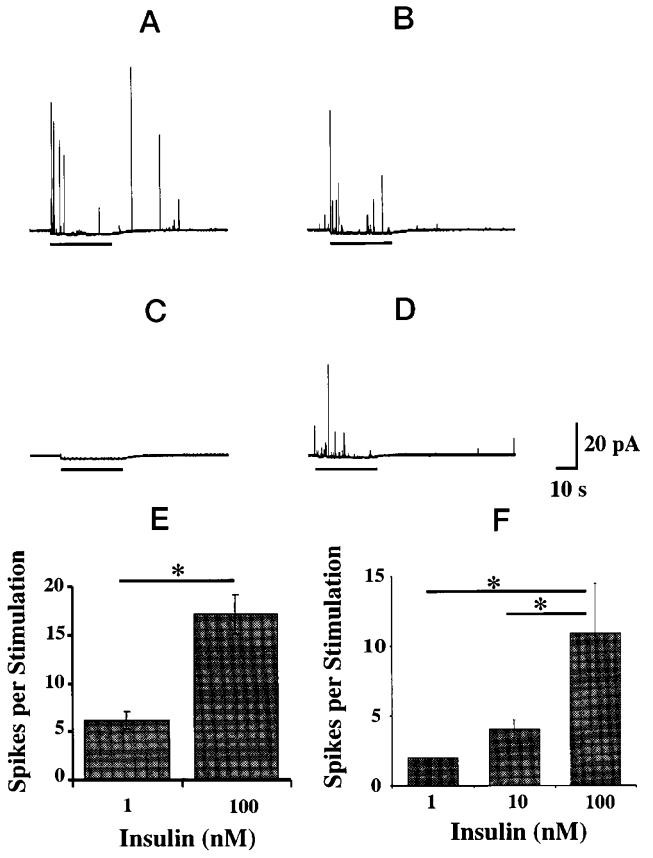

FIG. 3. **Receptor dependence of insulin-stimulated exocytosis in single mouse beta cells.** All data in *panels A–D* are detection of accumulated 5-HT from same cell. *A* and *B*, detection of exocytosis during stimulation with 100 nM insulin at 5-min time intervals. *C*, detection of exocytosis upon stimulation with 100 nM insulin following 5-min incubation in 10 nM polyclonal anti-insulin receptor$_\alpha$. *D*, detection of exocytosis upon stimulation with 30 mM K^+ in the presence of 10 nM polyclonal anti-insulin receptor$_\alpha$. *Bars* underneath traces represent application of stimulant. *E*, dependence of secretory activity, measured as number of exocytosis events detected, upon concentration of insulin in stimulant solution (n = 7 and 8 for 1 and 100 nM insulin, respectively) in mouse beta cells. Statistical significance for * is $p < 0.025$. *F*, dependence of secretory activity, measured as number of exocytosis events detected, upon concentration of insulin in stimulant solution (n = 4, 14, and 12 for 1, 10, and 100 nM insulin, respectively) in canine beta cells. Statistical significance for * is $p < 0.05$. Higher concentrations of insulin (1 μM) did not evoke a significantly different number of exocytosis events.

late glucose uptake and utilization, therefore we examined the interaction between exogenous insulin stimulation and extracellular glucose concentration by measuring the effect of insulin stimulation upon insulin secretion of beta cells bathed in 0, 3, and 20 mM glucose. At 0 and 3 mM glucose, application of 100 nM insulin evoked a similar number of exocytosis events per stimulation, 10.3 ± 2.2, n = 8, and 7.2 ± 1.4, n = 8, for 0 and 3 mM glucose, respectively (p = ns). In cells that were bathed in 20 mM glucose, application of 100 nM exogenous insulin further increased the frequency of detected exocytosis events from 0.168 ± 0.117 s^{-1} to 0.216 ± 0.084 s^{-1} (n = 5). This increase, however, was not statistically significant.

Effects of Insulin on Membrane Potential and Intracellular [Ca^{2+}]—The novelty of insulin as an insulin secretagogue prompted us to explore other effects of insulin on stimulus-secretion pathways. Many insulin secretagogues such as glucose, sulfonylureas, and K^+ depolarize the plasma membrane leading to opening of L-type voltage-gated Ca^{2+} channels and thereby allowing Ca^{2+} entry into the cell and initiation of exocytosis. Insulin however, did not depolarize the membrane

TABLE I

Effect of insulin complexation on autocrine feedback of beta cell insulin receptor

Cells were stimulated with 200 μM tolbutamide, and 5-HT secretion was detected by amperometry under control conditions and conditions where the amount of free insulin at the cell surface was reduced by slowing the dissociation and/or dissolution of the zinc:insulin complex. The number of stimulations is given as n.

pH	[Zn^{2+}]	Spikes per stimulation
	μM	
7.4	0	14.7 ± 2.64 n = 24
7.4[a]	15	5.45 ± 0.78 n = 40
6.4[b]	0	7.16 ± 1.21 n = 25

[a] Statistically different from control (pH = 7.4 and Zn^{2+} = 0 μM) with $p < 0.005$.
[b] Statistically different from control (pH = 7.4 and Zn^{2+} = 0 μM) with $p < 0.01$.

in beta cells that could be depolarized by 200 μM tolbutamide (Fig. 4*A*). The average change in membrane potential from base line to plateau (not including action potential) following tolbutamide stimulation was 32.5 ± 1.58 mV with the occurrence of action potentials although the average change following insulin stimulation was 1.47 ± 1.52 mV (n = 13). (Although some cells showed a small depolarization, such as that shown in Fig. 4*A*, others had no effect or slight hyperpolarization.) Insulin-stimulated secretion was not dependent upon extracellular Ca^{2+} (Fig. 4*B*) as the numbers of exocytosis events were not significantly different in cells bathed in Ca^{2+}-free KRB and stimulated with 100 nM insulin containing 5 mM or 0 mM Ca^{2+} (n = 10). This result is not an artifact of low levels of Ca^{2+} in the media as we have used similar protocols to demonstrate the Ca^{2+} dependence of glucose, tolbutamide, and K^+ stimulation (32). Although extracellular Ca^{2+} was not required to initiate secretion, stimulation with insulin did cause increases in intracellular calcium ($[Ca^{2+}]_i$) (n = 5) as seen in Fig. 4*C*. Also seen in Fig. 4*C*, the insulin-evoked $[Ca^{2+}]_i$ changes were smaller than those caused by K^+ but were of longer duration.

DISCUSSION

We have demonstrated for the first time that insulin can stimulate insulin secretion in pancreatic beta cells (Fig. 1*D*). This effect is mediated by the beta cell insulin receptor as evidenced by the antagonistic effect of anti-insulin receptor (Fig. 3). Furthermore, the insulin concentrations necessary for this effect are in the nanomolar range, which is reasonable because the EC_{50} of the beta cell insulin receptor is ~4 nM (9). Insulin released from a single cell is present at a sufficient level to activate the receptor and enhance secretion as demonstrated by the following results: 1) decreased secretion stimulated by K^+ when the insulin receptor is blocked by anti-insulin; 2) decreased tolbutamide-evoked secretion when the cell surface concentration of free insulin is reduced by increases in H^+ and Zn^{2+} extracellular concentration (Table I); and 3) the relatively minor enhancement of secretion by exogenous insulin during stimulation with 20 mM glucose, a condition in which released endogenous insulin may be expected to activate receptors to near maximal levels. Our observation that released insulin can activate insulin autoreceptors is in agreement with previous results which demonstrated that beta cell insulin receptors are activated by glucose (9). Taken together, these results suggest that a portion of secretion that is normally observed from single beta cells is because of positive autocrine feedback upon beta cell exocytosis acting through the beta cell insulin receptor.

Although insulin evokes secretion, it does not evoke membrane depolarization and subsequent Ca^{2+} entry to cause se-

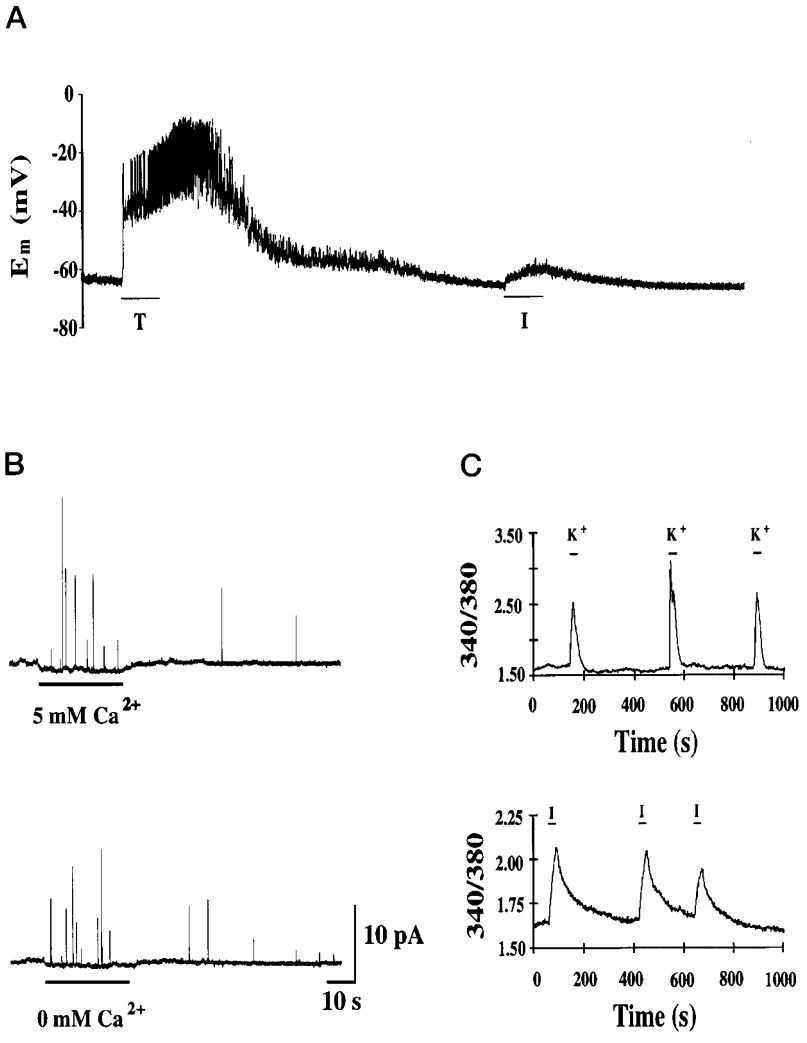

FIG. 4. **Effect of insulin on membrane potential and $[Ca^{2+}]_i$ in single mouse beta cells.** *A*, membrane potential measurements following stimulation with 200 μM tolbutamide (*T*) and 100 nM insulin (*I*) at a single beta cell. *B*, detection of exocytosis during application of 100 nM insulin to cells incubated in Ca^{2+}-free KRB. Stimulant contained 5 mM $CaCl_2$ or 0 mM $CaCl_2$ as indicated. Data are from the same cell. *C*, Fura-2 microfluorimetry measurements of $[Ca^{2+}]_i$ changes following application of 30 mM K^+ or 200 nM insulin (*I*) dissolved in KRB containing 2.4 mM Ca^{2+}. For all traces, *bar* represents application of stimulant for 30 s.

cretion as evidenced by the minimal effect on membrane potential (Fig. 4*A*) and independence of the insulin-stimulatory effect on extracellular Ca^{2+} (Fig. 4*B*). Insulin-stimulated insulin secretion is not mediated by glucose or increases in glucose utlization as the effect occurs even at 0 mM glucose. Insulin does, however, evoke a rise in $[Ca^{2+}]_i$. These results are consistent with a mechanism of stimulation in which insulin evokes release of intracellular calcium stores to initiate exocytosis reminiscent of ATP binding to P_{2y} receptors in beta cells (40, 41).

Autoreceptor effects on hormone or neurotransmitter secretion are well known; however, most autoreceptors mediate negative feedback on secretion. The beta cell-insulin system appears to be a rare example of positive feedback on secretion. The interplay of this positive feedback effect with other regulatory mechanisms to control insulin secretion and glucose homeostasis *in vivo* is likely complex. It is reasonable to speculate that positive feedback would cause augmented secretion during the initial stages of elevated glucose levels giving rise to a greater bolus of insulin release; however, other mechanisms must eventually take over to suppress release. Such a sequence could contribute to the rapid increase observed in first phase insulin secretion and the sustained, lower secretion during second phase. The possibility that insulin has a local effect on secretion also raises the possibility of novel regulatory mechanisms that might occur within islets. For example, because Zn^{2+} and H^+ can control the cell-surface concentration of insulin after vesicular fusion (33), these ions could play a role in regulating insulin secretion by affecting positive feedback. Serum concentrations of Zn^{2+} are in the range of 15–25 μM (42), which would be sufficient to have a large effect on free insulin level around the beta cell.

The existence of positive feedback may allow explanation of several phenomena in beta cells. For example, cultured beta cells in contact with other beta cells have been shown to secrete more insulin than isolated cells (43, 44). This result has not been explained but could be mediated by insulin from one cell stimulating further release in neighboring cells. In addition, insulin secretion from islets has been demonstrated to be oscillatory in nature and many models for oscillation have assumed some form of positive feedback by a diffusible factor released from beta cells (45, 46). No compound has been satisfactorily identified that could serve this role; however, these results indicate insulin as a possible candidate. Oscillations in insulin release are of significant interest because loss of oscillatory release is an early symptom of type-II diabetes (47). Finally, it has been demonstrated that many type II diabetics have a marked reduction in first phase insulin secretion (48), which to date has remained unexplained but could be envisioned as involving lack of positive feedback from the beta cell insulin receptor. Supporting the possibility that autocrine activation of the beta cell insulin receptor is involved in early secretory responses is recent work in which mice with knockouts of the beta cell insulin receptor have impaired early insulin secretion and concomitant glucose intolerance (49).

Perhaps most importantly, these results suggest a possible

link between impaired insulin secretion and insulin resistance, both of which can lead to hyperglycemia and are considered hallmarks of type-II diabetes (50). Considerable controversy surrounds the issue of which of these deficiencies is the primary cause of diabetes. In some studies, the earliest observed defect is dysfunctional secretion (45, 47) and in others insulin resistance appears to be the first detectable problem (51). The observation that insulin receptors on beta cells mediate insulin secretion and synthesis (10, 11, 12), in addition to the well known role in activating glucose utilization, leads to the possibility of a direct link between dysfunctional insulin secretion and insulin resistance. Such a link is supported by evidence that disruption of the beta cell insulin receptor (49) or beta cell receptor substrates (11, 29, 30) induces defects in secretion whereas disruption in insulin receptor substrates induces insulin resistance (52, 53).

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