Glucose-induced Tyrosine Phosphorylation of p125 in Beta Cells and Pancreatic Islets

A NOVEL PROXIMAL SIGNAL IN INSULIN SECRETION*

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In this study, we demonstrate that stimulation of beta cells with carbachol and glucose causes increased tyrosine phosphorylation of a 125-kDa protein concurrently with increased insulin secretion. The effect was observed in two different insulin-secreting cell lines and in rat pancreatic islets. Tyrosine phosphorylation was largely calcium independent and occurred within 2 min after stimulation of beta cells with glucose and the muscarinic agonist carbachol. In islets, the effect of glucose was greatly diminished by the addition of mannheptulose, a seven-carbon sugar that inhibits glucokinase, suggesting that glucose metabolism is required for tyrosine phosphorylation of the protein to occur. Neither insulin nor insulin-like growth factor I significantly increased tyrosine phosphorylation of the 125-kDa protein, suggesting that it was not an autocrine effect. Depolarization of beta cells with glyburide or 50 mM potassium dramatically increased insulin secretion but had no significant effect on tyrosine phosphorylation. Addition of phorbol ester caused a less than 2-fold increase in tyrosine phosphorylation, whereas the calcium ionophore A23187 had no effect. Among the various fuel secretagogues tested, only d-glucose stimulated tyrosine phosphorylation, both alone and in combination with carbachol. Finally, the tyrosine kinase inhibitor AG879 inhibited both tyrosine phosphorylation and insulin secretion in a dose-dependent manner. Taken together, these data demonstrate the presence of a novel signaling pathway in glucose-induced insulin secretion: tyrosine phosphorylation of beta cell p125, which is a proximal step in insulin secretion. Our current working hypothesis is that glucose stimulation of beta cell p125 tyrosine phosphorylation is an essential step for insulin secretion.

Insulin secretion from beta cells can be stimulated by different types of secretagogues (1). d-Glucose, a fuel secretagogue, is the major physiological stimulus (2, 3). The mechanism of glucose-induced insulin release is incompletely understood, although glucose oxidation is essential (3–6). Glucokinase is thought to act as a glucose sensor, with phosphorylation of glucose to glucose 6-phosphate serving as the rate-limiting step in glucose oxidation (7). Although inhibition of glucose oxidation inhibits insulin release, the details of the mechanism coupling glucose oxidation to insulin secretion are less clear.

It is widely believed that oxidation of fuel secretagogues increases intracellular levels of ATP (8), although this view has been challenged by some groups (9). An increased ATP/ADP ratio is believed to close K+ATP channels at the plasma membrane, resulting in decreased K+ efflux and subsequent depolarization of the beta cell (10–12). Depolarization activates voltage-dependent Ca2+ channels, causing an influx of extra-cellular Ca2+ into the beta cell and increased intracellular Ca2+ (6, 13). In addition, it was recently shown that nutrient secretagogues increase beta cell malonyl-CoA levels, leading to increased cytosolic long chain acyl-CoA esters that positively modulate insulin secretion (14–16). Although increased intracellular Ca2+ activates protein kinases such as the Ca2+- and calmodulin-dependent protein kinase (17–21), it remains unclear how increases in intracellular Ca2+ lead to insulin release.

Because of these questions, the search for additional beta cell-specific pathways that result in insulin exocytosis has continued. It appears that other intracellular factors are also involved in regulating insulin release. These include arachidonate, guanine nucleotides, small monomeric GTP-binding proteins such as rab3A, and the heterotrimeric GTP-binding protein Goα (22–26).

In other secretory cell types, protein tyrosine phosphorylation has been shown to be important in modulating secretion (27–30). The role that tyrosine phosphorylation plays in insulin secretion is unknown. Tyrosine kinase activity has been shown to participate in interleukin 1β-induced nitric oxide synthase accumulation in beta cells (31). Recently, Rothenberg et al. (32) have demonstrated that in late passage β-TC3 cells glucose-induced insulin secretion activates the beta cell insulin receptor tyrosine kinase and its intracellular signal transduction pathway (32). In these experiments, exogenous insulin and insulin-like growth factor I (IGF-I) and agents that resulted in insulin release such as K+ caused the rapid tyrosine phosphorylation of the 97-kDa β subunit of the insulin receptor. Currently, the physiological significance of these findings with respect to glucose-induced insulin secretion is unknown.

In this report, we show that stimulation of beta cells with glucose, carbachol, and various fuel secretagogues causes increased tyrosine phosphorylation of a 125-kDa protein. Neither insulin nor IGF-I significantly increased tyrosine phosphorylation of this protein, demonstrating that insulin secreted from

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1The abbreviations used are: IGF-I, insulin-like growth factor I; TPA, 12-O-tetradecanoylphorbol-13-acetate.
the beta cell is not responsible for the effect. Importantly, the tyrosine kinase inhibitor AG879 dose-dependently inhibited both insulin release and 125-kDa protein tyrosine phosphorylation, suggesting that tyrosine phosphorylation of the protein may be an essential step in insulin secretion.

**EXPERIMENTAL PROCEDURES**

**β-TC3 Cell Line Culture—**β-TC3 cells (passage 34) were obtained through the University of Pennsylvania Diabetes Center from Dr. D. Hanahan (University of California, San Francisco, CA). β-TC3 cells were cultured in 10-cm dishes in the presence of RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (75 μg/ml), streptomycin (50 μg/ml), and 2 mM L-glutamine as described (23, 33). Cells were passaged and subcloned weekly. Media were changed twice weekly and on the day prior to an experiment. Insulin secretory capacity of the β-TC3 cells in response to glucose and carbachol was regularly monitored. Cells were used exclusively between passages 40 and 55.

**Incubation of β-TC3 Cells for Insulin Secretion—**β-TC3 cells in 10-cm dishes were washed three times with 10 ml of Krebs’-HEPES buffer (25 mM HEPES, pH 7.40, 115 mM NaCl, 24 mM NaHCO3, 5 mM KCl, 2.5 mM CaCl2, 1 mM MgCl2, and 1% bovine serum albumin). Cells were preincubated in the same buffer for 30 min at 37 °C under an atmosphere of 95% air/5% CO2. The preincubation buffer was aspirated, and cells were incubated for 2–30 min at 37 °C under an atmosphere of 95% air/5% CO2 with 10 ml of fresh buffer supplemented with appropriate secretagogues. At the end of the incubation period, the supernatant was removed for insulin measurement by radioimmunoassay (26). Cells were processed for subsequent immunoprecipitation.

**Immunoprecipitation of Phosphotyrosyl Proteins—**All immunoprecipitation steps were performed at 4 °C. After removal of the supernatant, β-TC3 cells were washed twice with phosphate-buffered saline, pH 7.40. Following the second wash, phosphate-buffered saline was aspirated, and 1 ml of lysis buffer (50 mM HEPES, pH 7.40, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 5 mM EGTA, 20 mM NaF, 20 mM Na2P2O7, 1 mM NaVO4, 1 mg/ml bacitracin, 1 mM phenylmethysulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml aprotinin) was added to each dish. Cells were scraped, transferred into 1.5-ml screw-cap Eppendorf tubes, vortexed for 30 s to achieve complete lysis, and centrifuged at 10,000 × g for 10 s. The supernatant was transferred to a second tube, and phosphotyrosyl proteins were immunoprecipitated with 5 μg of mouse monoclonal antiphosphotyrosyl antibody (PY20; Transduction Laboratories, Lexington, KY) for 2 h on a rocker. After 2 h, 20 μl of protein A-Trisacryl beads (Pierce) preadsorbed with rabbit anti-mouse antibody (Sigma) were added to the tubes, and the incubation continued for an additional 1 h. At the end of the incubation, the beads were washed twice with Wash Buffer I (150 mM NaCl, 10 mM HEPES, pH 7.40, 1% Triton X-100, and 0.1% SDS) and once with Wash Buffer II (10 mM HEPES, pH 7.40, 1% Triton X-100, and 0.1% SDS). Twenty-five microliters of 2 × sample buffer (100 mM Tris, pH 6.80, 4% SDS, 20% glycerol, and 1 mg/ml bromphenol blue) was added to each tube. Samples were vortexed for 30 s, boiled for 3 min, and stored at −20 °C prior to analysis.

**Western Blotting of Immunoprecipitates—**Samples were loaded onto 7.5% SDS-polyacrylamide gels. Colored rainbow molecular weight markers (Amersham Corp.) were run on each gel. Proteins were separated for 1 h at 175 V at room temperature using a Bio-Rad MiniPROTEAN II dual slab cell. Proteins were transferred to nitrocellulose membranes for 1 h at 175 V at room temperature using a Bio-Rad Mini Trans-Blot apparatus. Antibody markers (Amersham Corp.) were run on each gel. Proteins were separated and transferred to nitrocellulose membranes. Blots were probed with alkaline phosphatase-conjugated goat anti-mouse antibody for 1 h at room temperature, washed, and exposed to X-OMAT MR film (Kodak, Rochester, NY) according to the manufacturer’s instructions. Films were digitized using a Molecular Dynamics PhosphorImager and ImageQuant software. In each experiment, the amount of radioactivity in the control sample was set at 100%, and all other results were expressed as percentages of control.

**Incubation of Islets for Insulin Secretion and Protein Tyrosine Phosphorylation—**Islets were isolated from male Sprague-Dawley rats using collagenase digestion and Ficoll purification as described previously (26). Typically, this procedure provided 350–400 islets/rat. Islets were washed three times in Krebs’-HEPES buffer supplemented with 3 mM glucose. Islets (1000/condition) were incubated in 1 ml of the same buffer in silanized 13 × 100-mm round bottom tubes at 37 °C under an atmosphere of 95% O2/5% CO2. The preincubation buffer was aspirated, and islets were incubated for 5–30 min with Krebs’-HEPES buffer supplemented with the appropriate secretagogues at 37 °C under an atmosphere of 95% O2/5% CO2. At the end of the incubation period, the supernatant was removed for insulin measurement by radioimmunoassay (26). One ml of immunoprecipitation lysis buffer was added to each tube, and islets were homogenized on ice. Islet lysates were vortexed for 1 min and transferred to new 1.5-ml Eppendorf tubes and processed for subsequent immunoprecipitation as described above.

**Data Analysis—**Results are expressed as the mean ± S.E. Statistical analysis was performed using version 6.0 of SSPS for Windows. Data were analyzed by one-way analysis of variance, followed by multiple comparisons between means using the least significant difference test. A probability of p < 0.05 was considered statistically significant.

**RESULTS**

We first examined the effect of glucose and carbachol on protein tyrosine phosphorylation in β-TC3 cells. β-TC3 cells were incubated with either 0 mM glucose or 15 mM glucose and 0.5 mM carbachol. After incubation, cells were lysed and phosphotyrosyl proteins were immunoprecipitated and analyzed by Western blotting. Fig. 1 shows that stimulation of β-TC3 cells with 15 mM glucose and 0.5 mM carbachol markedly increased tyrosine phosphorylation of one protein of 125 kDa (p < 0.05 versus control). As early as 2 min after glucose and carbachol stimulation, tyrosine phosphorylation was increased by 290.2 ± 30.5%. Tyrosine phosphorylation continued to increase.

**FIG. 1. Glucose and carbachol increase tyrosine phosphorylation of a 125-kDa protein in β-TC3 cells.** β-TC3 cells were incubated for 2–30 min with either 0 mM glucose (G0) or 15 mM glucose and 0.5 mM carbachol (GIS/CCH). At the end of the incubation period, the supernatant was removed for insulin measurement. Cells were harvested, and phosphotyrosyl proteins were immunoprecipitated with antiphosphotyrosyl antibody. Proteins were separated and transferred to nitrocellulose, which was probed with antiphosphotyrosyl antibody. Bound antibody was detected with 125I-protein A. Blots were exposed on a PhosphorImager cassette, and radioactivity was quantitated using a Molecular Dynamics PhosphorImager and ImageQuant software. A, results from a representative experiment after stimulation of the cells with glucose and carbachol for 2–30 min. B, results from A shown as the mean ± S.E. (bars) from three independent experiments.

Data are expressed as the mean ± S.E. Statistical analysis was performed using version 6.0 of SSPS for Windows. Data were analyzed by one-way analysis of variance, followed by multiple comparisons between means using the least significant difference test. A probability of p < 0.05 was considered statistically significant.
with time and was maximal (482.6 ± 122.3%) at 30 min (p < 0.05 versus control).

Addition of insulin or IGF-I had no significant effect on tyrosine phosphorylation in β-TC3 cells (Fig. 2), suggesting that secreted insulin was not responsible for the effect. IGF-I had no significant effect on insulin secretion from the β-TC3 cells. As a further control, when phosphotyrosine was added to the cell lysates, immunoprecipitation of the 125-kDa phosphotyrosyl protein was completely inhibited. Furthermore, treatment of the immunoprecipitates with λ-phosphatase decreased recognition of the 125-kDa phosphotyrosyl protein by the PY20 antibody. These observations strongly suggest that p125 is a phosphotyrosine-containing protein.

We next performed similar experiments in islets. Stimula-
tion of islets with 28 mM glucose and 0.5 mM carbachol resulted in increased tyrosine phosphorylation of the 125-kDa protein. As with β-TC3 cells, no other proteins underwent increased tyrosine phosphorylation. Tyrosine phosphorylation of the 125-kDa protein was increased by 192.5 ± 16.3% at 5 min (p < 0.05 versus control) and 184.1 ± 14.6% at 30 min (p < 0.05 versus control, n = 3). Insulin secretion was increased by 326.7 ± 160.5% at 5 min and 1247.5 ± 622.7% at 30 min (n = 3). As shown in Fig. 3, tyrosine phosphorylation of the protein was also stimulated with glucose alone in islets. At 5 min, 20 mM glucose increased tyrosine phosphorylation by 223.8 ± 31.0%.

The addition of mannoheptulose (an inhibitor of glucokinase
that inhibits islet beta cell glucose metabolism) completely inhibited glucose-induced increases in tyrosine phosphorylation and insulin secretion. These data suggest that glucose metabolism may be important for tyrosine phosphorylation of the 125-kDa protein. This concept was supported by the fact that the nonmetabolizable glucose analogs i-glucose and 2-deoxyglucose had no significant effect on protein tyrosine phosphorylation in β-TC3 cells (n = 4, data not shown).

As an additional control, the pattern of protein tyrosine phosphorylation was examined in another insulin secreting beta cell line, β-HC9 cells (Ref. 34 and Fig. 4). Stimulation of β-HC9 cells with 15 mM glucose and 0.5 mM carbachol for 30 min resulted in a 265.4 ± 33.2% increase in tyrosine phosphorylation of the 125-kDa protein and a concomitant increase (325.4 ± 47.5%) in insulin secretion.

The above experiments showed that tyrosine phosphorylation of a 125-kDa protein correlated with insulin secretion in islets as well as clonal beta cells. To determine Ca²⁺ dependence of tyrosine phosphorylation, β-TC3 cells were stimulated with glucose and carbachol in the presence and absence of extracellular Ca²⁺ (Fig. 5). In the presence of Ca²⁺, glucose and carbachol increased 125-kDa protein tyrosine phosphorylation by 349.7 ± 42.3% (p < 0.05 versus control). In the absence of Ca²⁺, glucose and carbachol were still able to increase tyrosine phosphorylation by 263.0 ± 28.1% (p < 0.05 versus control). At the same time, in the absence of Ca²⁺, stimulated insulin secretion was completely abolished, suggesting that tyrosine phosphorylation of the protein occurs independently of an increase in beta cell intracellular calcium.

Stimulation of β-TC3 cells with the sulfonylurea glyburide or 50 mM extracellular potassium increased insulin secretion by 607.0 ± 80.0% (p < 0.05 versus control) and 1395.4 ± 197.7% (p < 0.05 versus control), respectively (Fig. 6). Increased insulin secretion occurs because these agents cause the beta cell to become depolarized, increasing intracellular Ca²⁺ levels. Tyrosine phosphorylation of the 125-kDa protein, however, was not significantly increased with either agent. This suggests that tyrosine phosphorylation of the 125-kDa protein is a proximal event in insulin exocytosis and not the result of depolarization or increased intracellular Ca²⁺.

Further support of this idea is provided by the data shown in Fig. 7. When β-TC3 cells were treated with A23187, a Ca²⁺ ionophore, there was a 1022.5 ± 107.9% increase in insulin secretion. No increased tyrosine phosphorylation was observed, suggesting that tyrosine phosphorylation of the 125-kDa protein is not an event mediated by an increase in intracellular Ca²⁺. When β-TC3 cells were stimulated with 12-O-tetradecanoylphorbol-13-acetate (TPA), which activates protein kinase C, insulin secretion was increased by 389.9 ± 48.3%. In contrast to A23187, TPA also increased tyrosine phosphorylation of the 125-kDa protein by 193.3 ± 39.4%, suggesting that its phosphorylation may occur partly through the action of protein kinase C. TPA, however, did not cause as great an increase in tyrosine phosphorylation of the 125-kDa protein as did glucose and carbachol (354.4 ± 15.9%, p < 0.05 versus control).

The effect of other secretagogues was next examined. As shown in Fig. 8, 15 mM glucose increased tyrosine phosphorylation 203.2 ± 18.5%, and 0.5 mM carbachol increased phosphorylation 270.1 ± 37.9%. A maximal increase in tyrosine phosphorylation was obtained when the β-TC3 cells were stimulated with both glucose and carbachol (467.5 ± 76.9%;
This also corresponded to the greatest increase in insulin secretion (605.0 ± 105.9%). Fructose, a metabolizable six-carbon sugar, did not stimulate tyrosine phosphorylation of the protein to as great an extent as did glucose (163.1 ± 1.4%). The combination of fructose and carbachol did not stimulate tyrosine phosphorylation of the protein to as great an extent as did glucose and carbachol (327.3 ± 51.4%). The effect of additional fuel secretagogues on tyrosine phosphorylation in β-TC3 cells was also examined. Leucine did not significantly increase tyrosine phosphorylation. Glyceroldehyde and α-ketoisocaproic acid increased phosphorylation to 150.4 ± 16.8 and 139.9 ± 11.6% of control, respectively. Addition of carbachol significantly increased tyrosine phosphorylation in the presence of α-ketoisocaproic acid but not glyceroldehyde or leucine. Of all agents tested, only glucose in combination with carbachol produced a significant increase in protein tyrosine phosphorylation and insulin secretion.

The above data suggested that tyrosine phosphorylation of the 125-kDa protein present in the beta cell may be an important event in insulin secretion. In the final series of experiments, the effect of a tyrosine kinase inhibitor, AG879, on insulin secretion and tyrosine phosphorylation was examined. AG879 had no effect on basal insulin secretion or protein tyrosine phosphorylation at concentrations as high as 50 μM (n =
Tyrosine phosphorylation of the 125-kDa protein in \( \beta \)-TC3 cells is maximal with the combination of glucose and carbachol compared with either secretagogue alone or the combination of fructose and carbachol. \( \beta \)-TC3 cells were stimulated for 15 min with 15 mM glucose (G15) or 15 mM fructose (FRUC) in the presence or absence of 0.5 mM carbachol (CCH). Insulin secretion and protein tyrosine phosphorylation were quantitated as in Fig. 1. G0, 0 mM glucose. A, results shown as the mean \( \pm \) S.E. (bars) from two to six observations per condition from three independent experiments. B, insulin secretion data that correspond to A. Maximally increased tyrosine phosphorylation of the 125-kDa protein is achieved by stimulation of the \( \beta \)-TC3 cells with the combination of glucose and carbachol.

4). AG879, however, dose-dependently inhibited glucose- and carbachol-induced increases in insulin secretion and tyrosine phosphorylation, with almost complete inhibition at 10 \( \mu \)M (Fig. 9). In contrast, a structurally similar but inactive analogue, tyrphostin I, had no effect on either basal or stimulated insulin secretion or tyrosine phosphorylation at concentrations as high as 100 \( \mu \)M (n = 6). These data demonstrated that inhibition of 125-kDa protein tyrosine phosphorylation correlated with inhibition of insulin release, suggesting that tyrosine phosphorylation of the 125-kDa protein is an important event in this process.

**DISCUSSION**

We have demonstrated that stimulation of beta cells with glucose causes a 125-kDa protein present in beta cells to undergo markedly increased tyrosine phosphorylation. When phosphorylation was inhibited with the tyrosine kinase inhibitor AG879, insulin secretion was also inhibited, suggesting that this may be an important step in insulin release.

Tyrosine phosphorylation has been shown to be an important signal transduction mechanism in many different types of cells (27–30). For example, phospholipase \( \text{C}_\gamma \) becomes tyrosine phosphorylated following binding of various growth factors to different cell types (35). Phospholipase \( \text{C}_\gamma \) is activated by tyrosine phosphorylation and hydrolyzes phosphatidylinositol-4,5-bisphosphate to produce two important second messengers: diacylglycerol and myo-inositol-1,4,5-trisphosphate (35). Another well known example of tyrosine phosphorylation is that of the insulin receptor, which becomes tyrosine phosphorylated in cells following addition of insulin (36–39). The insulin receptor in turn phosphorylates insulin receptor substrate 1 on multiple tyrosine residues; tyrosine-phosphorylated insulin receptor substrate 1 then acts as a docking protein for numerous other proteins with SH2 domains that specifically bind phosphotyrosyl residues (37, 40, 41).

In a previous study, we demonstrated the presence of the insulin receptor 97-kDa \( \beta \) subunit in \( \beta \)-TC3 cells (32). Furthermore, exogenous insulin, as well as endogenous insulin released by glucose, and IGF-I caused tyrosine phosphorylation of the 97-kDa protein. Glucose-induced tyrosine phosphorylation of the insulin receptor was dependent on the presence of extracellular Ca\(^{2+}\). In the present study, neither insulin nor IGF-I increased tyrosine phosphorylation of the 125-kDa protein, indicating that insulin secretion from the beta cell is not responsible for this effect. Furthermore, glucose-induced tyrosine phosphorylation of the 125-kDa protein was calcium-independent. In these experiments, we could not document tyrosine phosphorylation of the insulin receptor in \( \beta \)-TC3 cells. The main difference between the two studies is the \( \beta \)-TC3 passage number: in the current study \( \beta \)-TC3 cells were used exclusively between passages 40 and 55, whereas in the former study \( \beta \)-TC3 cells were used at passages greater than 55. To test this possibility, we have reexamined the expression of the insulin receptor in beta \( \beta \)-TC3 cells as a function of passage number. Under our experimental conditions, we clearly detect glucose-induced tyrosine phosphorylation of the 97-kDa \( \beta \) subunit of the insulin receptor only in \( \beta \)-TC3 cells at a passage greater than 60 (data not shown).

Glucose-induced tyrosine phosphorylation of the 125-kDa protein was seen in at least two insulin-secreting transformed beta cell lines (\( \beta \)-TC3 and \( \beta \)-HC9) as well as in isolated pancreatic islets of Langerhans. The advantage of using beta cell lines is that they provide a pure population of beta cells without any other endocrine or passenger cells. A disadvantage is that many beta cell lines have glucose dose-response curves for insulin secretion that are shifted to the left compared with rat or mouse isolated islets. However, the \( \beta \)-HC9 line secretes insulin in response to glucose in a physiological fashion (34). The fact that glucose-induced tyrosine phosphorylation of the 125-kDa protein is observed in pure beta cell lines as well as in islets of Langerhans is strong evidence in favor of its localization to the beta cell.

Tyrosine phosphorylation of p125 in beta cells was stimulated by glucose. This effect was specific for d-glucose and was dependent on glucose metabolism, since mannheptulose blocked glucose-induced tyrosine phosphorylation of p125. Our data suggest that glucose-induced tyrosine phosphorylation of p125 is an early step in the signal transduction pathway of glucose-induced insulin secretion for the following reasons: 1) most of the fuel secretagogues tested had a modest or no effect on p125 tyrosine phosphorylation compared with that obtained with glucose alone; 2) glucose-induced p125 tyrosine phosphorylation did not require extracellular calcium; 3) agonists that stimulate an increase in intracellular calcium, such as calcium

![Fig. 8. Tyrosine phosphorylation of the 125-kDa protein in \( \beta \)-TC3 cells is maximal with the combination of glucose and carbachol compared with either secretagogue alone or the combination of fructose and carbachol. \( \beta \)-TC3 cells were stimulated for 15 min with 15 mM glucose (G15) or 15 mM fructose (FRUC) in the presence or absence of 0.5 mM carbachol (CCH). Insulin secretion and protein tyrosine phosphorylation were quantitated as in Fig. 1. G0, 0 mM glucose. A, results shown as the mean \( \pm \) S.E. (bars) from two to six observations per condition from three independent experiments. B, insulin secretion data that correspond to A. Maximally increased tyrosine phosphorylation of the 125-kDa protein is achieved by stimulation of the \( \beta \)-TC3 cells with the combination of glucose and carbachol.](http://www.jbc.org/)

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ionophores, depolarizing agents, and sulfonylureas, did not affect p125 tyrosine phosphorylation, even though they caused robust insulin secretion; and 4) the muscarinic agonist carbachol, which stimulates phospholipase C, also stimulated p125 tyrosine phosphorylation. Although these findings are consistent with a proximal localization of p125 tyrosine phosphorylation in the cascade of events resulting in insulin secretion, the identity of the coupling signal(s) between beta cell glucose recognition and p125 tyrosine phosphorylation is unknown.

The identity of beta cell p125 is unknown. Two-dimensional gel electrophoresis followed by immunoblotting with antiphosphotyrosine antibody demonstrates one single band. Preliminary experiments using commercially available antibodies to known phosphotyrosine proteins with a similar molecular weight show that beta cell p125 is not Fak, Jak1, Jak2, or Cbl (42–44). Studies are ongoing to purify beta cell p125.

The role of glucose-induced p125 tyrosine phosphorylation in insulin secretion remains to be fully determined. The experiments with the tyrosine kinase inhibitor AG879 suggest that p125 tyrosine phosphorylation may be required for insulin secretion, since inhibition of p125 tyrosine phosphorylation correlated with inhibition of insulin secretion. Furthermore, tyrphostin 1, an inactive analogue, had no effect on insulin secretion and tyrosine phosphorylation. However, since it is

![Fig. 9. The tyrosine kinase inhibitor AG879 inhibits insulin secretion and tyrosine phosphorylation of the 125-kDa beta cell protein in a dose-dependent manner. β-TC3 cells were stimulated for 15 min with either 0 mM glucose (G0) or 15 mM glucose and 0.5 mM carbachol (G15/CCH) in the presence of increasing concentrations of AG879. In these experiments, all incubation solutions contained 1% Me2SO. Insulin secretion and protein tyrosine (TYR-1) phosphorylation were quantitated as in Fig. 1. A, results from a representative experiment. B, results shown as the mean ± S.E. (bars) from two independent experiments. C, insulin secretion data that correspond to B.](http://www.jbc.org/)

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likely that AG879 inhibits all beta cell tyrosine kinase activities, these findings should be interpreted with caution in the absence of a selective inhibition of beta cell p125 function.

In summary, we have shown that glucose stimulation of insulin-secreting beta cells and isolated pancreatic islets results in the rapid tyrosine phosphorylation of a beta cell protein, p125, which is proximal in the signaling pathway resulting in insulin secretion. Our current working hypothesis is that glucose stimulation of beta cell p125 tyrosine phosphorylation is a novel and essential step for insulin secretion.

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