Glucose-stimulated Insulin Biosynthesis Depends on Insulin-stimulated Insulin Gene Transcription*

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Glucose stimulation of pancreatic β-cells leads to insulin secretion as well as up-regulation of insulin biosynthesis. The acute elevation in pro-insulin levels is thought to be exclusively because of the activation of translation of pre-existing prepro-insulin mRNA. Glucose-stimulated insulin gene transcription is believed to be a long term effect and should therefore not contribute to the acute elevation in pro-insulin levels. We have recently shown that glucose activates insulin gene transcription within minutes and that secreted insulin is one of the key factors triggering this process in an autocrine manner. We now provide evidence that 50% of the glucose-stimulated, acute pro-insulin biosynthesis within 30 min results from up-regulated insulin gene transcription. Our data led us to propose that glucose elevates pro-insulin levels by stimulating both transcriptional and post-transcriptional/post-translational events to an equal extent. Whereas the stimulatory effect on transcription is mediated by insulin secreted in response to glucose, glucose directly stimulates the post-transcriptional/post-translational processes.

The maintenance of glucose homeostasis in mammals is of vital importance. To keep blood glucose concentrations within narrow limits, strict regulation, and fast acting mechanisms that guarantee efficient insulin secretion and biosynthesis are necessary. In these processes, glucose itself has been shown to act as the major nutrient regulator by triggering a cascade referred to as the stimulus-response coupling. With regard to insulin biosynthesis, the commonly accepted view is that glucose exerts its immediate effect at the level of translation rather than at the level of insulin gene transcription. As a consequence of this view, glucose-stimulated insulin gene transcription should be uncoupled from glucose-stimulated insulin mRNA translation. Therefore, transcription is unlikely to have an impact on the immediately triggered insulin biosynthesis. The concept or dogma of a long term effect of glucose stimulation on insulin gene transcription is challenged by Efrat et al. (1) and by Leibiger et al. (2) through nuclear run-off experiments demonstrating that insulin gene transcription is up-regulated within minutes of glucose stimulation rather than hours. By studying the mechanisms that underlie the short term regulation of insulin gene transcription by glucose, we were able to show that insulin secreted in response to glucose stimulation is a key factor in glucose-stimulated insulin gene transcription (3).

Recent reports from several groups demonstrate that insulin indeed has a stimulatory role in pancreatic β-cell physiology (3–8). Xu et al. (4) reports a positive effect of insulin on β-cell protein biosynthesis and insulin-dependent activation of PHAS-1. The involvement of insulin in the regulation of basal pro-insulin and prepro-insulin mRNA levels is shown by Xu and Rothenberg (5). A similar observation is made in mice carrying a general knockout for insulin receptor substrate-2 (6). A β-cell-restricted knockout of insulin receptors resulted in an impaired first phase insulin secretion and a decrease in insulin content (7). Aspinwall et al. (8) demonstrates that insulin stimulates acute insulin secretion. Finally, Leibiger et al. (3) demonstrate the involvement of insulin signaling in the short term glucose-stimulated control of insulin gene transcription.

In this study, we showed that secreted insulin positively influences the acute pro-insulin biosynthesis by promoting transcription and translation. Our data provided evidence, for the first time, that short term regulated insulin gene transcription contributes to the immediate up-regulation of pro-insulin biosynthesis.

MATERIALS AND METHODS

Islet Isolation and Culture—Pancreatic islets were isolated from Wistar rats (200–300 g) by collagenase digestion (9). Islets were separated by a Ficoll gradient and then hand-picked. Islet cell suspensions were prepared and washed essentially as described in Ref. 10. Islets and cells of disaggregated islets were incubated overnight 95% air, 5% CO2 and 37 °C in RPMI 1640 medium supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and 10% heat-inactivated fetal calf serum. Islets and cell sets were pretreated in RPMI 1640 medium containing 3 mM glucose and they were supplemented as above for 90 min before the start of stimulation. Stimulation was for 15 min with either 16.7 mM glucose, 5 milliunits/ml insulin, 100 μM tolbutamide, 1 μM glibenclamide, or 50 mM KCl. Pharmacological inhibitors of voltage-gated L-type Ca2+-channels, such as 10 μM nifedipine (Calbiochem) or DNA-dependent RNA polymerase II, i.e. 5 μg/ml actinomycin D (Sigma), were added to the culture medium 30 min before stimulation and kept throughout stimulation. Anti-insulin antibodies (ICN) were added 30 min before stimulation to the culture medium (10 μg/ml) and kept throughout stimulation.

Measurement of Pro-insulin Biosynthesis—Groups of 10 islets or 5 × 10^5 cells were used for each experiment. Stimulation for 15 min with either glucose, insulin, KCl, tolbutamide, or glibenclamide was performed in 2 ml of leucine-free RPMI 1640 medium containing 100 μCi of L-[4,5-3H]leucine (Amersham Pharmacia Biotech). After stimulation, the islets were washed with phosphate-buffered saline (136.9 mM NaCl, 1.5 mM KCl, 3.2 mM KH2PO4, 10.1 mM Na2HPO4, pH 7.4) and incubated for an additional 15 min in 2 ml of leucine-free RPMI 1640 medium containing 3 mM glucose and 100 μCi of L-[4,5-3H]leucine. After labeling, islets and cells were washed with ice-cold phosphate-buffered...
saline, harvested, resuspended in 100 µl of ice-cold immunoprecipitation buffer (25 mM Na2B4O7, pH 9.0, 3% (w/v) bovine serum albumin, 1% Tween 20, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM insulin was verified by discontinuous Tricine SDS-polyacrylamide gel electrophoresis (16.5% separating gel, 10% spacer gel, 4% stacking gel) for prepro-insulin mRNA translation is well accepted (reviewed in Ref. 13). Recently, we have shown that glucose stimulation for only 15 min resulted in a transient rise in insulin gene transcription and in a transient elevation of steady-state prepro-insulin mRNA levels (2). We were able to demonstrate that insulin, secreted in response to glucose stimulation, is a key factor involved in the up-regulation of insulin gene transcription (3). Because insulin has been demonstrated as a positive regulator of protein biosynthesis in several tissues (reviewed in Ref. 14) and in pancreatic β-cells (4, 5), we wanted to know whether secreted insulin also contributes to the stimulus-dependent elevation in pro-insulin biosynthesis.

To address the question of whether secreted insulin contributes to pro-insulin biosynthesis, we first compared the effects of short term glucose stimulation (15 min) with that of a short term insulin stimulation at substimulatory glucose concentrations (15 min) on acute overall protein biosynthesis and acute prepro-insulin biosynthesis in isolated pancreatic islets, i.e. within a 30-min period. Therefore, we performed protein labeling with [3H]leucine in response to the stimulation of pancreatic islets for 15 min with either 16.7 mM glucose or 5 milliunits of insulin/ml at 3 mM glucose. We then harvested the islets after culturing for another 15 min at 3 mM glucose (Fig. 1). Both the cultures and stimulations were performed in RPMI 1640 medium supplemented with 10% fetal calf serum. To show that the obtained effect on pro-insulin biosynthesis was a result of stimulation by either glucose or insulin rather than a result of recovery from a lack of glucose, growth factors, and amino acids, we deliberately used culture conditions where the medium was not depleted of serum, glucose, or insulin.

As shown in Fig. 1A, total protein biosynthesis was increased by the glucose stimulus up to 3-fold (average 2.08 ± 0.17, n = 11, p < 0.01) and by the insulin stimulus up to 2-fold (average 1.35 ± 0.13, n = 9, p < 0.05) within 30 min. Absolute prepro-insulin biosynthesis was elevated by glucose up to 6-fold (average 3.56 ± 0.48, n = 9, p < 0.01) and by insulin up to 3-fold (average 2.07 ± 0.34, n = 5, p < 0.05), respectively. The effect of glucose and insulin stimulation on specific prepro-insulin biosynthesis, i.e. after normalization to total protein biosynthesis, was up to 2-fold (average 1.6 ± 0.08, n = 10, p < 0.01) and up to 1.5-fold (average 1.32 ± 0.05, n = 9, p < 0.01), respectively (Figs. 1–3).
To evaluate whether the amount of insulin, which is necessary to stimulate pro-insulin biosynthesis, is within the physiological range, we generated a dose-response curve of exogenous insulin on pro-insulin biosynthesis. With regard to insulin gene transcription, we found a stimulatory effect when the endogenous insulin release was higher than 10 microunits/ml per 10 islets per 15 min. The range for endogenous release upon stimulation with 16.7 mM glucose was quite broad and could reach levels of up to 100 microunits/ml per 10 islets per 15 min. Therefore, we analyzed the necessary amounts of exogenous insulin to stimulate pro-insulin biosynthesis when cultivating islets in the medium without serum, i.e., medium supplemented with bovine serum albumin. As shown in Fig. 1B, the addition of 10 micromolars of insulin/ml of medium (i.e. 60 pmol) was necessary to significantly increase pro-insulin biosynthesis within a 30-min period. The addition of 30 micromolars/ml (i.e. 180 pmol) triggered the maximal response.

Next we wanted to know the necessary amount of exogenous insulin to stimulate pro-insulin biosynthesis when cultivating islets in fully supplemented medium. As illustrated in Fig. 1C, here also the addition of 10 micromolars of insulin/ml of medium was necessary to significantly increase pro-insulin biosynthesis within a 30-min period. Addition of exogenous insulin above the physiological range did not lead to a further increase in pro-insulin biosynthesis.

To test whether endogenous insulin that secreted upon stimulation had a similar effect as exogenous insulin, we next stimulated the isolated pancreatic islets for 15 min with 50 mM KCl at 3 mM glucose. This allowed insulin secretion without the additional effects of glucose metabolism. Indeed, KCl-stimulated insulin secretion resulted in a similar 55% elevation of specific pro-insulin biosynthesis within 30 min (Fig. 2A). To
exclude an unspecific effect of KCl and to further demonstrate that insulin secreted by the β-cell promotes pro-insulin biosynthesis, we tested the effects of classical insulin secretagogues, i.e. the sulfonylurea compounds, tolbutamide and glibenclamide, on acute pro-insulin biosynthesis. Indeed, stimulation with either 100 μM tolbutamide or 1 μM glibenclamide at 3 mM glucose led to a similar amount of increase in pro-insulin biosynthesis as obtained by the addition of exogenous insulin (Fig. 2B) or stimulation with 50 mM KCl (Fig. 2A).

To test the effects of glucose metabolism without the additional effects of secreted insulin, we stimulated islet cells for 15 min with 16.7 mM glucose but blocked the depolarization-mediated influx of Ca2+ via voltage-gated L-type Ca2+ channels and the subsequent insulin secretion by treatment with 10 μM nifedipine. This protocol has been shown to abolish the Ca2+ influx via L-type Ca2+ channels and to completely block insulin secretion (15). As shown in Fig. 2C, nifedipine treatment decreased glucose-stimulated, pro-insulin biosynthesis by roughly 50%.

So far our data suggest that neither secreted insulin nor glucose metabolism per se can account for the total effect on acute glucose-stimulated, pro-insulin biosynthesis, but each of the stimuli had an impact of approximately 50%. If both effects were independent, they should be additive. To test this hypothesis, we stimulated pancreatic islets with 16.7 mM glucose and blocked secretion of endogenous insulin with 10 μM nifedipine but substituted the insulin effect by administering 5 milli-units/ml exogenous insulin together with the sugar. As shown in Fig. 2C (16.7 glc + nif + insulin), exogenous insulin could restore glucose-induced, pro-insulin biosynthesis in the presence of nifedipine, indeed demonstrating that the effects of glucose metabolism and insulin feedback were additive.

To finally prove that insulin secreted by the pancreatic β-cell is promoting pro-insulin biosynthesis, we stimulated insulin secretion by 16.7 mM glucose but absorbed the secreted insulin with anti-insulin antibodies. The antibodies were added 30 min before stimulation and were kept throughout stimulation in the culture medium (10 μg/ml). This approach resulted in approximately a 50% reduction in pro-insulin biosynthesis (Fig. 2D).

Next we wanted to test the impact of the short term glucose/insulin-stimulated insulin gene transcription on the immediate glucose/insulin-stimulated pro-insulin biosynthesis. As shown in Fig. 3A, already 15 min after glucose stimulation, prepro-insulin mRNA steady state levels were elevated by 40%, increasing further with time. By performing a nuclear run-off assay on islets, we found a 3.5-fold elevation in insulin gene transcription initiation 45 min after the start of stimulation (data not shown). To evaluate the importance of insulin gene transcription, we combined glucose/insulin stimulation with actinomycin D treatment. This approach allowed stimulus-dependent stimulation of the endoplasmic reticulum, the initiation, and the elongation in prepro-insulin mRNA translation, is in agreement with earlier reports (reviewed in Ref. 13). Although we cannot totally exclude a direct effect of glucose/glucose metabolism on insulin gene transcription, the activation of glucose metabolism is mediated via glucose-stimulated exocytosis of insulin and the positive paracrine feedback of insulin as described previously (3).

Our data demonstrate for the first time that glucose-stimulated insulin gene transcription directly contributes to the immediately triggered pro-insulin biosynthesis. The involvement of insulin signaling in stimulation-dependent pro-insulin biosynthesis supports the new evolving concept that the pancreatic β-cell itself is a target for insulin and that the positive autocrine feedback loop contributes to β-cell physiology.

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