Contrasting Effects of Nateglinide and Rosiglitazone on Insulin Secretion and Phospholipase C Activation

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When stimulated with 6 mmol/L glucose, a minimal, transient insulin secretory response was observed from perifused rat islets. The inclusion of 5 μmol/L nateglinide significantly amplified release. Elevating glucose to 8 or 10 mmol/L resulted in an increasing insulin secretory response that was again markedly potentiated by the further inclusion of nateglinide. The calcium channel antagonist, nitrendipine, abolished secretion to 8 mmol/L glucose plus nateglinide. Unlike nateglinide, rosiglitazone (5 μmol/L), troglitazone (1 to 10 μmol/L), or darglitazone (10 μmol/L), 3 peroxisome proliferator-activated receptor gamma (PPARγ) agonists, were without any acute stimulatory effect on insulin release in the simultaneous presence of 6 to 10 mmol/L glucose. Glucose (8 to 10 mmol/L) significantly increased inositol phosphate accumulation. Nateglinide amplified this response. Nitrendipine reduced inositol phosphate (IP) accumulation in response to the combination of 8 mmol/L glucose plus 5 μmol/L nateglinide. Rosiglitazone had no effect on IP accumulation. These results confirm the efficacy of nateglinide as a potent glucose-dependent insulin secretagogue that exerts its stimulatory effect, at least in part, through the activation of phospholipase C (PLC). No acute potentiating effect of rosiglitazone on either insulin secretion or IP accumulation could be detected in isolated rat islets.

IN AN ATTEMPT to augment the insufficient glucose-induced insulin secretory response of the pancreatic β cell that characterizes type 2 diabetes, a variety of pharmacologic tools have been utilized. Initially, sulfonylureas were used for this purpose.[1,2] A newer class of shorter-acting insulin secretagogues has been developed to provide an added impetus to insulin release from a decompensating β cell.[3-5] The glinide class of compounds, and, in particular, nateglinide, has achieved clinical significance in this regard. Most recently,[6] it has been reported that rosiglitazone, a member of the thiazolidinedione class of drugs that act as peripheral tissue insulin sensitizers,[7] also stimulates insulin release from the perfused rat pancreas. This previously unreported action of rosiglitazone has been suggested to play an important role in improving glucose tolerance. In most clinical studies, however, this action has not been routinely observed, and insulin levels usually decline during therapy with the glitazone class of compounds.[8-10]

Our interest in the factors that control the secretion of insulin from the β cell led us to perform comparative studies using nateglinide or rosiglitazone to augment glucose-induced release from isolated perifused rat islets. Because the activation of phospholipase C (PLC) and the attendant generation of a host of second messenger molecules appear to play an integral role in the regulation of glucose-induced secretion,[11-14] we explored the potential impact of these compounds on information flow in this signal transduction pathway. Our findings demonstrate that nateglinide evokes parallel increments in both glucose-induced PLC activation and insulin release from rat islets. Under conditions where marked acute insulin stimulatory effects of nateglinide were observed and in contrast to a recent study,[6] no effect on β-cell secretion was detected in comparable experiments with rosiglitazone.

MATERIALS AND METHODS

Islet Isolation

The detailed methodologies used to assess insulin output from collagenase-isolated islets have been previously described.[11] Male Sprague-Dawley rats (body weights at time of study 350 to 475 g) were purchased from Charles River (Wilmington, MA). All animals were treated in a manner that complied with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1985). The animals were fed ad libitum. After Nembutal (pentobarbital sodium; 50 mg/kg; Abbott, North Chicago, IL)-induced anesthesia islets were isolated by collagenase digestion and handpicked using a glass loop pipette under a stereo microscope. They were free of visible exocrine contamination.

Perifusion Studies

Groups of 14 to 18 isolated islets were loaded onto nylon filters (Sefar America, Briarcliff Manor, NY) and perifused in a Krebs-Ringer Bicarbonate (KRB) buffer at a flow rate of 1 mL/minute for 30 minutes in 3 mmol/L glucose to establish basal and stable insulin secretory rates. After this 30-minute stabilization period, they were then perifused with the appropriate compounds as indicated in the figure legends and Results section. Perifusate solutions were gassed with 95% O_2/5% CO_2 and maintained at 37°C. Insulin released into the medium was measured by radioimmunoassay (RIA).[15]

Islet Labeling for Inositol Phosphate Studies

After isolation, groups of 18 to 26 islets were loaded onto nylon filters, placed in a small glass vial, and incubated for 3 hours in a myo-[2-H^3]-inositol-containing KRB solution made up as follows: 10 μCi myo-[2-H^3]-inositol (specific activity, 16 to 23 Ci/mmol/L) were placed in a 10 mm × 75 mm culture tube. To this aliquot of tracer 250 μL of warmed (to 37°C) and oxygenated (KRB) medium supplemented with 5.0 mmol/L glucose were added. After mixing, 240 μL of this solution was gently added to the vial with islets. The vials were capped with a rubber stopper. A 20-gauge needle was inserted through the stopper to act as an outlet port and another 20-gauge needle connected to a 95% O_2/5% CO_2 gas tank inserted through the stopper as well. The

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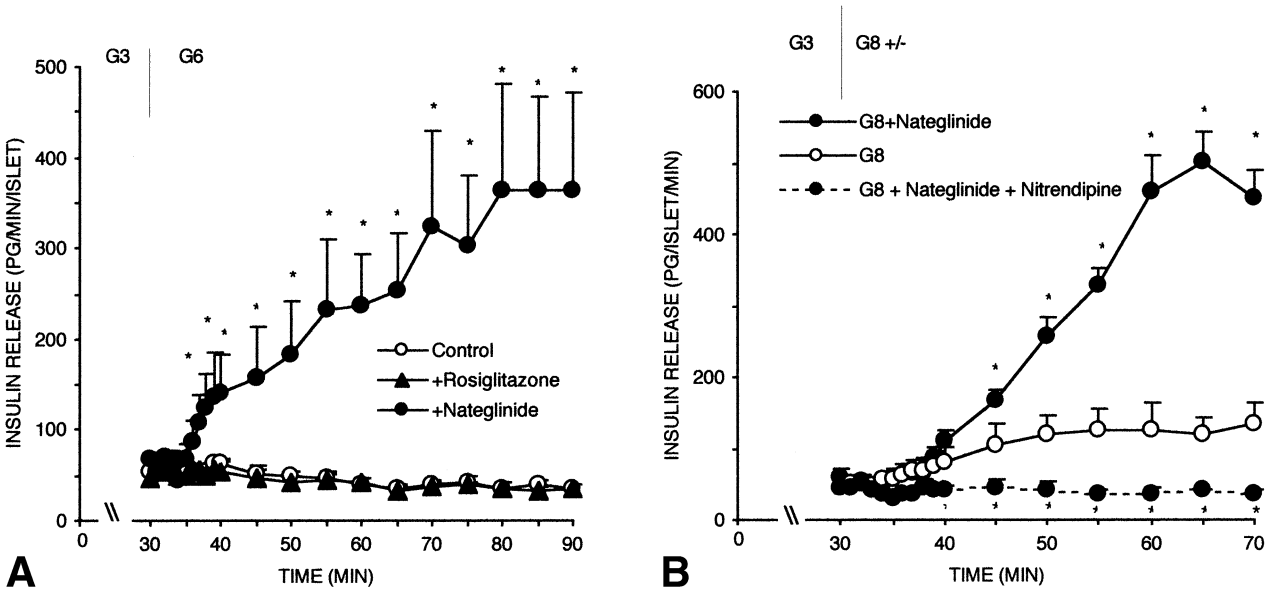

Fig 1. Insulin secretion from isolated perifused rat islets. Groups of 14 to 18 islets were isolated and perifused. For the initial 30 minutes, the islets were maintained with 3 mmol/L glucose (G3) to establish basal and stable insulin secretory rates. (A) They were then stimulated (indicated by the vertical line) for 60 minutes with 6 mmol/L glucose (G6) alone (○), 6 mmol/L glucose plus 5 μmol/L nateglinide (●, solid line), or 6 mmol/L glucose plus 5 μmol/L rosiglitazone (▲). (B) Islets were stimulated for 40 minutes with 8 mmol/L glucose (G8) alone (○), 8 mmol/L glucose plus 5 μmol/L nateglinide (●, solid line), or 8 mmol/L glucose plus 5 μmol/L nateglinide plus 1 μmol/L nitrendipine (●, dashed line). Mean values ± SE of at least 3 experiments are given. This and subsequent perifusion figures have not been corrected for the dead space in the perifusion apparatus, 2.5 mL or 2.5 minutes with a flow rate of 1 mL/min. Note change in insulin release scale for the different graphs. *Indicates a significant difference between the experimental group and control release in response to glucose alone.

atmosphere above the islets was gently aerated with this gas mixture for 5 seconds and incubated at 37°C. The vials were again gently oxygenated after 90 minutes. After the labeling period, the islets still on nylon filters were washed with 5 mL fresh KRB.

Inositol Phosphate Measurements

After washing, the islets on nylon filters were placed in small glass vials. Added gently to the vial was 400 μL KRB supplemented with 10 mmol/L LiCl to prevent inositol phosphate (IP) degradation and the appropriate compounds as indicated. The vials were capped and gently gassed for 5 seconds with 95% O_2/5% CO_2 and incubated at 37°C. After 30 minutes, the generation of IPs was stopped by adding 400 μL 20% perchloric acid. Total IPs formed were then measured using Dowex columns as described previously.[16,17]

Reagents

Hank's solution was used for the islet isolation. The perifusion medium consisted of 115 mmol/L NaCl, 5 mmol/L KCl, 2.2 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 24 mmol/L $NaHCO_3$, and 0.17 g/dL bovine serum albumin. The ^{125}I-labeled insulin used for the insulin assay was purchased from PerkinElmer Life Sciences (Boston, MA). The labeled myo-[2-H^3]-inositol was purchased from Amersham (Arlington Heights, IL). Bovine serum albumin (RIA grade), glucose, wortmannin, and the salts used to make the Hank's solution and perifusion medium were purchased from Sigma (St Louis, MO). Rat insulin standard (lot #615-ZS-157) was the generous gift of Dr Gerald Gold, Eli Lilly (Indianapolis, IN). Collagenase (Type P) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Nateglinide was the generous gift of Novartis Pharmaceuticals, Summit, NJ. Rosiglitazone was the generous gift of GlaxoSmithKline, Research Triangle Park, NC. Troglitazone was the generous gift of Sankyo, Tokyo, Japan. Darglitazone was generously supplied by Pfizer, Groton, CT. Stock solutions (10 mmol/L) of rosiglitazone, troglitazone, or darglitazone were made up in dimethyl sulfoxide (DMSO). Equivalent amounts of DMSO (0.1%) were added to control perifusion medium.

Statistics

Statistical significance was determined using the Student's *t* test for unpaired data or analysis of variance in conjunction with the Newman-Keuls test for unpaired data. A P value $\leq .05$ was taken as significant. Values presented in the figures and Results represent means ± SE of at least 3 observations.

RESULTS

Effects of Nateglinide on Glucose-Induced Insulin Secretion

In the initial series of studies, we confirmed the efficacy of nataglinide in amplifying glucose-induced secretion from collagenase isolated rat islets, islets that retain a level of glucose sensitivity comparable to the perfused pancreas preparation.[18,19] In Fig 1A, islets were stimulated with 6 mmol/L glucose alone or with the further addition of 5 μmol/L nateglinide. When compared with prestimulatory secretion rates in the presence of 3 mmol/L glucose, a small, transient and barely measurable increase in release was noted. For example, during the first 3 to 5 minutes after the onset of 6 mmol/L glucose stimulation, release rates increased from approximately 50 pg/islet/min to 63 pg/islet/min. As the perifusion progressed however, this small increment in release rapidly subsided. Release rates averaged only 35 ± 6 pg/islet/min (n = 4) during the final 5 minutes of the perifusion. The inclusion of 5 μmol/L nateg-

linide changed significantly the minimal secretory response to 6 mmol/L glucose alone. Both the kinetics and the amplitude of the response to 6 mmol/L glucose was altered. For example, during the final 5 minutes of stimulation with 6 mmol/L glucose alone, release rates averaged 35 ± 6 pg/islet/min. This increased to 365 ± 106 pg/islet/min (n = 4) with the addition of 5 μmol/L nateglinide.

Increasing the glucose level to 8 mmol/L resulted in a slowly increasing insulin secretory response from control islets (Fig 1B). During the final 5 minutes of a 40-minute stimulation period, a 3-fold to 4-fold increase over prestimulatory rates was observed. In the presence of 5 μmol/L nateglinide, a maximal 3-fold potentiating effect on 8 mmol/L glucose-induced release was noted (Fig 1B).

The impact of nateglinide on β-cell responses was dependent on both the influx of extracellular calcium and the glucose level. As shown in Fig 1B, the inclusion of 1 μmol/L of the calcium channel antagonist, nitrendipine, together with 8 mmol/L glucose attenuated the effect of nateglinide. In addition, nateglinide had no stimulatory effect on secretion if the glucose level was maintained at 3 mmol/L (results not shown).

Effects of Rosiglitazone on Glucose-Induced Insulin Secretion

A most recent study using the perfused rat pancreas preparation reported that 0.045 to 4.5 μmol/L rosiglitazone amplified 6 mmol/L glucose-induced insulin secretion in a dose- and time-dependent fashion.[6] A maximum 3-fold increase was reported. As a prelude to determining the potential biochemical mechanisms involved in this reported stimulatory effect of rosiglitazone, we first attempted to repeat these observations by stimulating isolated islets with 6 mmol/L glucose plus 5 μmol/L rosiglitazone. Two different types of protocols were used. In the first type, islets were stimulated with 6 mmol/L glucose plus 5 μmol/L rosiglitazone for 60 minutes. These results are presented in Fig 1A. In contrast to the marked potentiating effect of nateglinide and most surprisingly considering a recent study,[6] we could not detect any positive impact of 5 μmol/L rosiglitazone on 6 mmol/L glucose-induced insulin secretion (Fig 1A). In the second protocol, islets were stimulated with 6 mmol/L glucose plus 5 μmol/L rosiglitazone for 40 minutes, after which time the medium was changed to one containing only 6 mmol/L glucose. Again, when compared with prestimulatory insulin secretory rates, no positive effect of rosiglitazone on secretion was noted (Fig 2).

Effects of Wortmannin on Glucose-Induced Insulin Secretion

In the report of Yang et al,[6] it was suggested that rosiglitazone amplified 10 mmol/L glucose-induced insulin secretion via a phosphatidylinositol 3-kinase (PI3-K)-dependent pathway. Inhibiting this pathway with the PI3K inhibitor, LY294002, abolished the potentiating effect of rosiglitazone. We previously reported,[20] in agreement with other studies,[21-23] that nanomolar levels of the PI3K inhibitor, wortmannin, amplify glucose-induced secretion, a response that could be duplicated by higher levels of LY294002. Additional studies were conducted using rat islets stimulated with 10 mmol/L glucose alone or 10 mmol/L glucose plus 5 μmol/L rosiglitazone. For comparative purposes, an additional group of islets was stimulated with 10 mmol/L glucose plus 5 μmol/L nateglinide. As shown in Fig 3, no stimulatory effect of rosiglitazone on 10 mmol/L glucose-induced secretion was noted. In contrast, a strong potentiating effect of nateglinide on 10 mmol/L glucose-induced release was observed (Fig 3). This effect, most dramatic during the initial 30 to 40 minutes of stimulation, waned as the perifusion progressed.

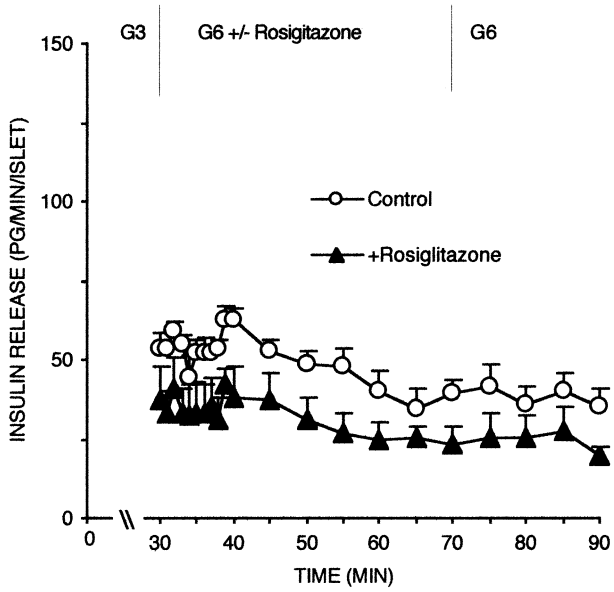

Fig 2. Effect of rosiglitazone on 6 mmol/L glucose-induced secretion. Two groups of islets were perifused for 30 minutes with 3 mmol/L glucose. For an additional 60 minutes 1 group (○) was stimulated with 6 mmol/L glucose alone (this is the same data as in Fig 1A). The second group (▲) was stimulated for 40 minutes with 6 mmol/L glucose plus 5 μmol/L rosiglitazone and then perifused for an additional 20 minutes with 6 mmol/L glucose alone.

Studies were also conducted with rat islets stimulated with 10 mmol/L glucose plus 50 nmolL wortmannin or the combination of wortmannin and rosiglitazone. At a level of 50 nmol/L, wortmannin markedly potentiated 10 mmol/L glucose-induced insulin secretion (Fig 4), confirming previous studies.[20-24] The further addition of 5 μmol/L rosiglitazone was without any impact on the potentiated response to 10 mmol/L glucose plus wortmannin (Fig 4).

Effects of Troglitazone or Darglitazone on Glucose-Induced Insulin Secretion

Both positive[25,26] and negative[27,28] effects of troglitazone have been reported in a variety of preparations, including tumoral cell lines and islets. In light of our negative findings with rosiglitazone, we decided to re-examine troglitazone's effects on insulin secretion in the presence of 6 mmol/L glucose. Concentrations of 1 μmol/L (data not shown) or 10 μmol/L troglitazone had no stimulatory effect on insulin secretion from perifused rat islets in the presence of 6 mmol/L glucose (Fig 5). Confirmation of their sensitivity was established by a subsequent perifusion of these islets with 20 mmol/L glucose. Islets that were unresponsive to troglitazone responded with a marked increase in insulin release when

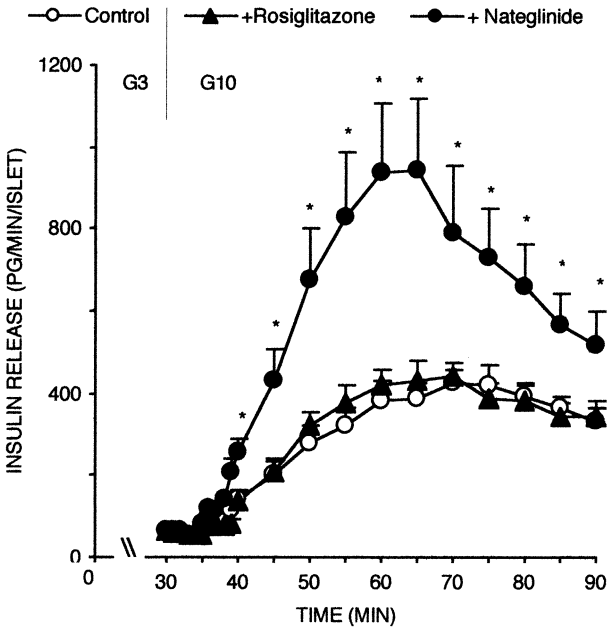

Fig 3. Impact of nateglinide and rosiglitazone on 10 mmol/L glucose-induced insulin release. Groups of 14 to 18 islets were isolated and perifused. For the initial 30 minutes, the islets were maintained with 3 mmol/L glucose (G3) to establish basal and stable insulin secretory rates. For the next 60 minutes (onset indicated by the vertical line), they were stimulated with 10 mmol/L glucose (G10) alone (○), 10 mmol/L glucose plus 5 μmol/L nateglinide (●), or 10 mmol/L glucose plus 5 μmol/L rosiglitazone (▲). *Indicates a significant difference between the experimental group and control release in response to 10 mmol/L glucose alone.

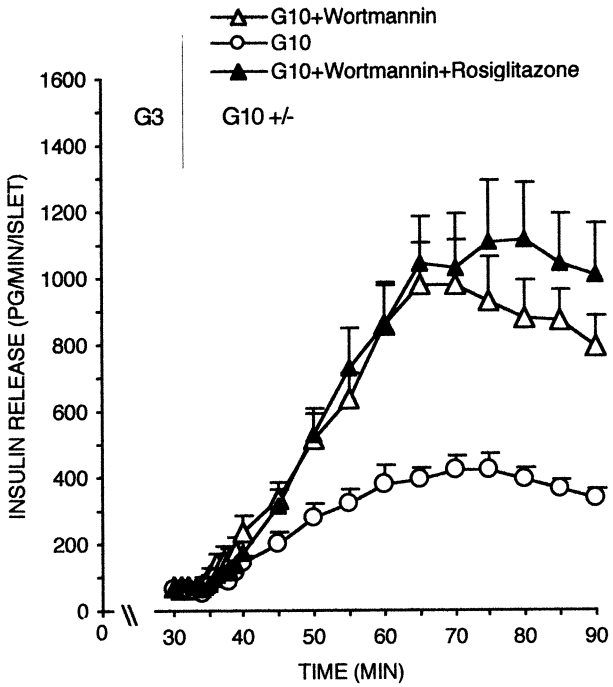

Fig 4. Effects of wortmannin and rosiglitazone on 10 mmol/L glucose-induced insulin secretion. Groups of 14 to 18 islets were isolated and perifused. For the initial 30 minutes, the islets were maintained with 3 mmol/L glucose (G3) to establish basal and stable insulin secretory rates. For the next 60 minutes, they were stimulated with 10 mmol/L glucose (G10) alone (○, same data as given in Fig 3), 10 mmol/L glucose plus 50 nmol/L wortmannin (△), or 10 mmol/L glucose plus 50 nmol/L wortmannin plus 5 μmol/L rosiglitazone (▲).

stimulated with this hexose level. In additional studies using troglitazone, the glucose level was increased to 10 mmol/L. The inclusion of 10 μmol/L troglitazone together with 10 mmol/L glucose had no effect above that observed with 10 mmol/L glucose alone (Fig 6).

A final series of perifusion studies was conducted with the newer, more potent thiazolidinedione, darglitazone.[29,30] Similar to the findings made with rosiglitazone and troglitazone, it also failed to stimulate secretion in the presence of 6 mmol/L glucose (Fig 5) or 10 mmol/L glucose (results not shown).

IP Responses to Nateglinide and Rosiglitazone

The calcium-dependent activation of islet PLC, monitored in most studies by the accumulation of Ips,[13,14,16,31-33] parallels the insulin secretory response to glucose.[11,12,34] We next assessed the impact of nateglinide and rosiglitazone on IP accumulation in islets whose phosphoinositide pools were labeled with ^{3}H-inositol. Stimulation with 8 mmol/L glucose increased significantly IP accumulation above levels observed in the presence of 3 mmol/L glucose (Table 1). The addition of 5 μmol/L nateglinide potentiated this response to 8 mmol/L glucose. Blocking calcium influx with the calcium channel antagonist, nitrendipine (1 μmol/L) reduced IP accumulation to the combination of 8 mmol/L glucose plus 5 μmol/L nateglinide. Increasing the glucose level to 10 mmol/L was accompanied by a further increase in IP accumulation, and the addition

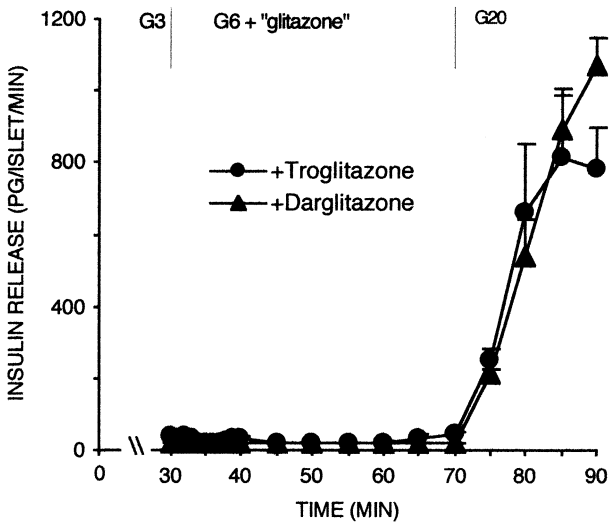

Fig 5. Effect of troglitazone or darglitazone on 6 mmol/L glucose-induced secretion. Two groups of islets were perifused for 30 minutes with 3 mmol/L glucose. For an additional 40 minutes, 1 group was stimulated with 6 mmol/L glucose plus 10 μmol/L troglitazone (●). The second group was stimulated for 40 minutes with 6 mmol/L glucose plus 10 μmol/L darglitazone (▲). Both groups were then perifused for an additional 20 minutes with 20 mmol/L glucose alone. Three experiments were conducted under each condition.

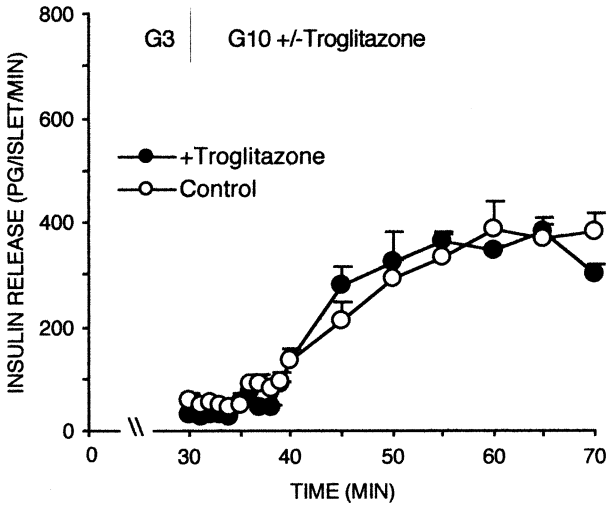

Fig 6. Effect of troglitazone on 10 mmol/L glucose-induced insulin secretion. Two groups of islets were perifused for 30 minutes with 3 mmol/L glucose. For an additional 40 minutes, onset indicated by the vertical line, they were stimulated with 10 mmol/L glucose alone (○) or 10 mmol/L glucose plus 10 μmol/L troglitazone (●).

of nateglinide potentiated this response as well. On the other hand and consistent with its lack of effect on 10 mmol/L glucose-induced insulin release, 5 μmol/L rosiglitazone had no additional effect on labeled IP accumulation in isolated islets above that noted with 10 mmol/L glucose alone.

DISCUSSION

Glucose-induced insulin secretion is regulated by a complex interaction between glucose metabolism, a host of second messenger signaling molecules, and by a number of ion channels as well.[35-37] Failure of these cells to compensate for insulin resistance by augmenting insulin secretion plays a major role in the pathogenesis of type 2 diabetes. Hence, unraveling how these signaling circuits are wired and their interconnections assumes more than passing scientific interest; it is an area replete with important clinical overtones. In the present studies, we focused our attention on 2 widely used clinical compounds utilized to improve glucose homeostasis in glucose intolerant patients. One, nateglinide, positively impacts secretion by affecting the activity of the adenosine triphosphate (ATP)-sensitive potassium channel.[3] The other, rosiglitazone, is a PPARγ agonist[7] that enhances insulin sensitivity.[10] However, recently it has been reported that it stimulates insulin secretion as well, an action mediated via PI3K activity.[6] Our goal was to define in more detail the nature of the signaling events that potentially contribute to their insulinotropic effects.

Initially, our attention was directed to nateglinide, one of a class of newly designed, shorter-acting insulin secretagogues.[3-5] That it is a potent glucose-dependent agonist for secretion was readily confirmed. Nateglinide markedly amplified insulin secretion in the presence of 6 mmol/L glucose or higher levels of the hexose. Lowering the glucose level to 3 mmol/L abolished its effect on release. Moreover, blocking calcium influx into the β cell also abrogated its positive insulin secretory action.

While there is little doubt that the glinide class of compounds modulates the ATP-sensitive K channel,[3] additional actions of the nateglinide have to be considered in its insulin stimulatory effect. In the present experiments, nateglinide amplified the activation of islet PLC that normally accompanies glucose-stimulated insulin secretion. This activation process paralleled the potentiated insulin secretory response and, like insulin secretion, was itself reduced by the further addition of nitrendipine. These observations suggests that nateglinide, like tolbutamide and glibenclimide,[38-40] exerts at least part of its insulin stimulatory effect on release through the increased generation of phosphoinositide-derived second messengers. These include both IPs and the protein kinase C activator, diacylglycerol.[41,42]

We failed to confirm that rosiglitazone potentiates glucose-induced secretion. In the presence of either 6 mmol/L or 10 mmol/L glucose, the same concentrations of hexose used by Yang et al[6] in studies using the perfused pancreas preparation, the addition of 5 μmol/L rosiglitazone was without any acute additional impact on the secretory process above that noted to glucose alone. In addition, we could not document any stimulatory effect on glucose-induced secretion from islets acutely stimulated with troglitazone or darglitazone. Previous studies with troglitazone have led to conflicting reports in the literature. For example, Masuda et al[25] reported that 1 to 10 μmol/L troglitazone potentiated 11.1 mmol/L glucose-induced secretion from both statically-incubated rat islets and HIT cells, while 100 μmol/L inhibited secretion. On the other hand, in the presence of 7 mmol/L glucose, Ohtani et al,[26] also using HIT cells, reported no stimulatory effect of 1 μmol/L troglitazone and a potentiating effect at 100 μmol/L. In studies with rat islets, Shimabukuro et al[27] found no effect of a 48-hour culture

Table 1. IP Responses of Rat Islets to Nateglinide and Rosiglitazone

	IP Accumulation (cpm/40 islets)
1. G3	3,179 ± 544 (n=4)
2. G8	6,700 ± 732 (n=6)
3. G8 plus nateglinide	11,785 ± 1,081 (n=7)
4. G8 plus nateglinide plus nitrendipine	5,036 ± 552 (n=7)
5. G10	10,032 ± 1,143 (n=6)
6. G10 plus nateglinide	16,821 ± 1,529 (n=6)
7. G10 plus rosiglitazone	10,418 ± 668 (n=4)

NOTE. Groups of rat islets were collagenase isolated. They were then incubated for 3 hours in KRB solution supplemented with 5 mmol/L glucose plus tracer ^{3}H-inositol (10 μCi). After washing with fresh KRB to remove unincorporated label, they were then incubated for 30 minutes with 3 mmol/L (G3), 8 mmol/L (G8), or 10 mmol/L glucose (G10) plus or minus 5 μmol/L nateglinide, 5 μmol/L rosiglitazone or 1 μmol/L nitrendipine as indicated. Also included during the 30-minute stimulatory period was 10 mmol/L LiCl to prevent the dephosphorylation of the IPs. Statistical analyses: line 1 *v* lines 2 and 5, line 2 *v* line 3, line 3 *v* line 4, line 5 *v* line 6, significant at $P < .05$; line 5 *v* line 7, not significant.

Abbreviations: IP, inositol phosphate; KRB, Krebs-Ringer bicarbonate.

period with 10 μmol/L troglitazone on 23 mmol/L glucose-induced release from lean +/+ Zucker diabetic fatty (ZDF) islets. An inhibitory effect of high levels of this compound on 11 mmol/L glucose-induced secretion was reported by Conget and Malaisse.[28] We have no adequate explanation for these discrepancies except to note that different preparations, different concentrations, and different methodologies may be contributory factors. Our failure to observe any stimulatory effect of troglitazone, darglitazone, or rosiglitazone on insulin secretion is in accord with the established clinical observations made with this class of compounds. During therapy with them, insulin levels generally decrease.[8-10,43] Finally, it has most recently been reported that a 24-hour exposure of MIN-6 and INS-1 cells to troglitazone increased glucokinase gene expression.[44] The functional significance of this observation remains unclear, because insulin secretion from these cells was not determined. In in vivo studies, however, 24 hours of treatment of obese rats with rosiglitazone significantly reduces circulating insulin levels,[45] a finding in accord with many clinical studies.

In the study by Yang et al,[6] it was reported that the PI3K inhibitor, LY294002, abolished the stimulatory effect of rosiglitazone on 10 mmol/L glucose-induced insulin release. They concluded that PI3K activition mediated the potentiating effect of rosiglitazone on glucose-induced secretion. Previous studies using rat islets, mouse islets, or MIN-6 cells have demonstrated that the PI3K inhibitors, wortmannin or LY294002, potentiate glucose-induced secretion.[20-23,46] We conducted additional experiments using the PI3K inhibitor, wortmannin, alone or together with rosiglitazone on islets stimulated with 10 mmol/L glucose. We confirmed what we[20] and other groups[21-23] have previously reported using wortmannin: it is a most effective potentiator of glucose-induced insulin release. This and other observations[21,47] have been interpreted to support the concept that insulin signaling in β cells functions normally as a negative feedback inhibitor of the β-cell secretory response, and that PI3K mediates this effect. While it may be argued that wortmannin's action may be mediated by biochemical mechanisms other than PI3K inhibition, one thing remains clear, its impact on release is substantial. From a quantitative perspective, its stimulatory effect is comparable to that observed using traditional potentiators of secretion, such as forskolin, theophylline, or carbachol.[37,48,49] As shown here as well with 10 mmol/L glucose, the efficacy of 50 nmol/L wortmannin on the insulin secretory process is comparable to 5 μmol/L nateglinide. The additional inclusion of rosiglitazone to wortmannin-plus glucose-stimulated islets had no effect.

In conclusion, we have confirmed that nateglinide is a potent glucose-dependent insulin secretagogue and documented that under conditions where it amplifies secretion, it also potentiates PLC activation. Both processes are inhibited by blocking calcium influx into the β cell with nitrendipine. We also confirmed that the PI3K inhibitor, wortmannin, like nateglinide, significantly potentiates glucose-induced secretion and that from a quantitative perspective, its stimulatory effect rivals that of other classically used potentiators of secretion. Finally, we failed to confirm the observation that rosiglitazone has any acute positive impact on glucose-induced secretion. While we have no adequate explanation for this failure, we are left to conclude, in agreement with prior clinical studies, that rosiglitazone exerts its actions on peripheral tissues and unlike the sulfonylureas or glinides, it has no acute impact on the insulin secretory process.

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