Insulin Secretion, Inositol Phosphate Levels, and Phospholipase C Isozymes in Rodent Pancreatic Islets

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During a dynamic perifusion, 20 mmol/L glucose, 20 mmol/L α-ketoisocaproate (KIC) or 20 mmol/L methyl pyruvate (MP) stimulate biphasic insulin secretory responses from collagenase-isolated rat islets. Peak first-phase insulin responses were comparable for all 3 nutrient agonists. The largest second-phase insulin secretory response was evoked by 20 mmol/L glucose (30-fold above basal release rates), and this response was more sustained than that observed with either 20 mmol/L KIC or 20 mmol/L MP. When mouse islets were perifused under similar conditions, KIC stimulated the largest first-phase insulin response, while comparable acute insulin secretion rates were obtained with glucose- or MP-stimulated islets. In contrast to rat islets, the sustained second phase of insulin secretion from mouse islets was minimal regardless of the nutrient secretagogue used. This anomalous response of mouse islets as compared with rat islets could not be ascribed to any obvious difference in the glucose usage rate or insulin content between these 2 species. Glucose, KIC, or MP stimulated significant increases in ^{3}H-inositol phosphates in rat islets. Significantly smaller increases were measured in mouse islets. Comparative Western blot analyses showed pronounced species differences in the expression of phospholipase Cβ1 (PLCβ1), PLCβ2, PLCβ3, and PLCδ1 but not PLCγ1 or protein kinase Cα (PKCα) between rat and mouse islets. PLCβ4 or PLCδ2 could not be identified in either species. These findings are consistent with the concept that the underexpression of the nutrient-activated PLC isozyme may account for the minimal inositol phosphate (IP) and second-phase insulin secretory response from mouse islets.

IN RESPONSE TO GLUCOSE stimulation, a variety of metabolic, ionic, and signal transduction events occur contemporaneously.[1-4] These events culminate in a rapid biphasic insulin secretory response from the perfused rat pancreas and from freshly isolated perifused rat pancreatic islets.[5-10] Human islets stimulated in vivo by a sustained increase in the plasma glucose level demonstrate a similar robust biphasic insulin secretory response,[11-13] suggesting that the signal transduction events which regulate insulin release are similar in both species.

Increasingly, transgenic mice are being generated to probe the contribution of the β cell in the pathogenesis of type 2 diabetes.[14] However, unlike the parallel insulin secretory characteristics documented to exist between rat and human islets, mouse islets deviate from these species in their response to glucose stimulation. For example, sustained stimulation of the perfused mouse pancreas preparation or freshly isolated perifused mouse islets with high glucose alone is not accompanied by a rising second-phase of insulin secretion.[15-21] Furthermore, and again in contrast to rat or human islets, prior short-term exposure to glucose does not potentiate subsequent insulin secretory responses.[17,20] The underlying explanation for this divergence in responsiveness to the hexose may reside, at least in part, in the inability of glucose to activate information flow in the phospholipase C/protein kinase C (PLC/PKC) signal transduction system to the same quantitative extent in mouse islets as it does in rat and, presumably, human islets as well.

Comparative studies with rat and mouse islets may provide us with some insight not only into how nutrients regulate insulin secretion but also into the potential contribution of the β cell to altered glucose homeostasis in transgenic models of diabetes.[14,22] To this end, we explored in more detail the capacity of several commonly used nutrient secretagogues to activate the β cell in terms of PLC-mediated phosphoinositide (PI) hydrolysis, monitored by the accumulation of inositol phosphates (IPs) and nutrient-induced insulin secretion monitored during a dynamic perifusion. In addition, glucose utilization rates of rat and mouse islets and their contents of insulin, PLC isozymes, and PKCα were assessed. A primary goal of these studies was to establish whether the anomalous behavior of mouse islets as compared with rat islets with regard to IP accumulation and insulin secretion is specific for glucose or is a trait shared with other nutrient secretagogues as well.

MATERIALS AND METHODS

Islet Isolation

The detailed methodologies used to assess insulin output from collagenase-isolated islets have been previously described.[23] Young adult male CD-1 mice (body weight at time of study, 25 to 37 g) or male Sprague-Dawley rats (body weight at time of study, 300 to 450 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, the islets were isolated by collagenase digestion and handpicked using a glass loop pipette under a stereomicroscope. They were free of exocrine contamination. After isolation, some groups of islets were directly analyzed for protein content using the Lowry method and bovine serum albumin as standard or insulin.

Perifusion Studies

Groups of 14 to 18 freshly isolated rat or mouse islets were loaded onto nylon filters (Tetko, Briarcliff Manor, NY) and perifused in a Krebs-Ringer bicarbonate (KRB) buffer at a flow rate of 1 mL/min for 30 minutes in the absence of any added fuel, to establish basal and stable insulin secretory rates. After this 30-minute stabilization period, they were then perifused with the appropriate agonist or agonist combinations as indicated in the Figures and the Results. Perifusate solutions

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were gassed with 95% O_2/5% CO_2 and maintained at 37°C. Insulin release into the medium was measured by radioimmunoassay.[24]

Islet Labeling for IP Studies

After isolation, groups of 18 to 26 islets were loaded onto nylon filters, placed in a small glass vial, and incubated for 3 or 6 hours in a *myo*-[2-^{3}H]-inositol–containing KRB solution made up as follows: 10 μCi *myo*-[2-^{3}H]-inositol (specific activity, 16 to 23 Ci/mmol/L) was placed in a 10-mm × 75-mm culture tube. To this aliquot of tracer, 250 μL warmed (to 37°C) and oxygenated (KRB) medium supplemented with 5.0 mmol/L glucose was added. After mixing, 240 μL of this solution was gently added to the vial with islets. The vial was capped with a rubber stopper, gassed for 10 seconds with 95% O_2/5% CO_2, and incubated at 37°C. In islets labeled for 3 hours, the vials were again gently oxygenated after 90 minutes. In islets labeled for 6 hours, the vials were gently oxygenated every 90 minutes during labeling. After the labeling period, the islets still on nylon filters were washed with 5 mL fresh KRB.

IP Measurements

After washing, the islets on nylon filters were placed in small glass vials. KRB (400 μL) supplemented with 10 mmol/L LiCl, to prevent IP degradation, and the appropriate agonists as indicated were added gently to the vial. The vials were capped and gently gassed for 5 seconds with 95% O_2/5% CO_2. 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.[25,26]

Glucose Utilization Rates

In experiments using rat or mouse islets, glucose usage was measured by determining the rate of ^3H_2O formation from 5-^{3}H-glucose using methods previously described.[27] These islets were first perifused with the appropriate agonist and then incubated in 0.125 mL 3- or 20-mmol/L glucose supplemented with tracer 5-^{3}H-glucose. The ^3H_2O formed during the subsequent 1-hour incubation was separated from the unused ^{3}H-glucose as described previously.[27]

Studies With Carbachol-Stimulated Mouse Islets

Groups of mouse islets were incubated in a ^{3}H-inositol–containing medium for 3 hours as already described. After this, the islets were either batch-incubated for 30 minutes with 20 mmol/L glucose plus 100 μmol/L carbachol to measure IP accumulation or perifused to monitor the dynamics of insulin secretion in response to this stimulant combination.

Western Blot Analysis of Rodent Islets

Groups of islets were pelleted by centrifugation and then suspended in 25 to 50 μL homogenization buffer containing various protease inhibitors as described previously.[19,28] After sonication, triplicate aliquots were analyzed for protein content according to the Lowry method using bovine serum albumin as a standard. For the Western blots of PLCδ1 (20 to 23 μg), PLCδ2 (20 μg), PLCβ1 (20 μg), PLCβ2 (20 μg), PLCβ3 (20 μg), PLCβ4 (20 μg), PLCγ1 (15 μg), and PKCα (15 μg), protein sonicate from the islets was boiled for 90 seconds in 4× Laemmli sample buffer and separated by SDS-polyacrylamide gel electrophoresis using a 4% stacking gel and a 7% running gel at 12 and 16 mA, respectively. Gel-resolved proteins were electrotransferred onto an Immobilon PVDF membrane at 15 V for 20 hours. The Immobilon was stained with Ponceau S solution for protein, washed, and blocked for 2 hours in Tris-buffered saline supplemented with .05% Tween 20 and 5% milk powder. For PLCδ1 determinations, the membranes were incubated for 135 minutes with the primary anti-PLCδ1 antibody (1.0 μg/mL dilution), washed, incubated for 90 minutes with horseradish peroxidase (HRP)-conjugated IgG, and washed again. For PLCδ2 determinations, the membranes were incubated for 100 minutes with the primary anti-PLCδ2 antibody (0.5 μg/mL dilution), washed, incubated for 30 minutes with HRP-conjugated IgG, and washed again. For PLCβ1 determinations, the membranes were incubated for 60 minutes with the primary anti-PLCβ1 antibody (0.5 μg/mL dilution), washed, incubated for 45 minutes with HRP-conjugated IgG, and washed again. For PLCβ2 determinations, the membranes were incubated for 60 minutes with the primary anti-PLCβ2 antibody (0.5 μg/mL dilution), washed, incubated for 45 minutes with HRP-conjugated IgG, and washed again. For PLCβ3 determinations, the membranes were incubated for 60 minutes with the primary anti-PLCβ3 antibody (0.5 μg/mL dilution), washed, incubated for 45 minutes with HRP-conjugated IgG, and washed again. For PLCβ4 determinations, the membranes were incubated for 120 minutes with the primary anti-PLCβ4 antibody (0.5 μg/mL dilution), washed, incubated for 75 minutes with HRP-conjugated IgG, and washed again. For PLCγ1 determinations, the membranes were incubated for 135 minutes with the primary anti-PLCγ1 antibody (0.25 μg/mL dilution), washed, incubated for 90 minutes with HRP-conjugated IgG, and washed again. For PKCα determinations, the membranes were incubated for 45 minutes with the primary anti-PKCα antibody (0.5 μg/mL dilution), washed, incubated for 30 minutes with HRP-conjugated IgG, and washed again. The antigen-antibody complexes were visualized using the ECL system (Amersham, Arlington Heights, IL) and quantified densitometrically using the Visage 2000 (BioImage, Ann Arbor, MI). Samples to be compared were always analyzed in parallel. The optical density of rat islet samples was taken as 100%, and the content of the various enzymes measured in mouse islets is expressed as a percentage of rat islet content.

Reagents

Hanks 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. Other compounds were added where indicated, and the solution was gassed with a mixture of 95% O_2/5% CO_2. The ^{125}I-labeled insulin for the insulin assay, 5-^{3}H-glucose, and ^3H_2O were purchased from New England Nuclear (Boston, MA), and labeled *myo*-[2-^{3}H]-inositol was from Amersham. Bovine serum albumin (RIA grade), carbachol, glucose, α-ketoisocaproate (KIC), methyl pyruvate (MP), and the salts used to make Hanks solution and perifusion medium were purchased from Sigma (St Louis, MO). Rat insulin standard (lot #615-ZS-157) was a generous gift from Dr Gerald Gold of Eli Lilly (Indianapolis, IN). Collagenase (Type P) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Antibodies to PLCδ2, PLCβ1, 2, 3, and 4, and PKCα and the goat anti-mouse and goat anti-rabbit IgG-HRP antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to PLCγ1 and PLCδ1 were obtained from Upstate Biotechnology (Lake Placid, NY). Enhanced chemiluminescence (ECL) reagents and films were from Amersham.

Statistics

Statistical significance was determined using Student's *t* test for unpaired data or ANOVA in conjunction with the Newman-Keuls test for unpaired data. A *P* value of .05 or less was taken as significant. The results represent the mean ± SE of at least 3 observations.

RESULTS

Protein Content of Rodent Islets

The mean protein content of rat islets was 0.76 ± 0.03 μg/islet (n = 36). The mean protein content of mouse islets was 0.51 ± 0.036 μg/islet (n = 12). The insulin content of rat or

mouse islets was 0.163 ± 0.13 μg/islet (n = 6) or 0.135 ± 0.10 (n = 6), respectively. The values are within the range observed by other investigators.[18,29]

Nutrient-Induced Activation of Insulin Secretion From Rat or Mouse Islets

The insulin secretory responses of perifused rat and mouse islets to 20 mmol/L glucose, 20 mmol/L KIC, and 20 mmol/L MP are shown in Figs 1 to 3. With regard to the hexose, the response of rat islets was particularly robust and paralleled the finding made with the perfused rat pancreas.[5,7,18,30-32] For example, insulin release rates prior to stimulation were 47 ± 6 pg/islet/min (mean ± SE, n = 9), and in response to 20 mmol/L glucose, peak first-phase insulin secretory rates increased to 483 ± 120 pg/islet/min. Release rates measured 35 to 40 minutes after the onset of glucose stimulation increased approximately 30-fold to 1,352 ± 168 pg/islet/min. A similar peak first-phase response (565 ± 117 pg/islet/min, n = 5) was evoked from rat islets stimulated with 20 mmol/L KIC (Fig 2). Second-phase secretion rates increased for the next 20 minutes but declined as the perifusion progressed. Insulin release during the final 5 minutes of stimulation was 506 ± 42 pg/islet/min in response to sustained 20 mmol/L KIC stimulation. With regard to MP, peak first-phase release rates (572 ± 164 pg/islet/min, n = 8) from rat islets were comparable to those observed with either 20 mmol/L glucose or KIC (Fig 3). However, sustained secretory rates were considerably reduced and the amount of insulin released during the final 5 minutes of stimulation with MP was only 199 ± 53 pg/islet/min. This represents only 15% of the rate achieved with 20 mmol/L glucose and 40% of the rate achieved with KIC.

Peak first-phase insulin secretory responses from mouse islets to 20 mmol/L glucose (n = 9), 20 mmol/L KIC (n = 6), or 20 mmol/L MP (n = 4) were 220 ± 68, 613 ± 106, or 230 ± 52 pg/islet/min, respectively, with these agonists (Figs 1 to 3). A

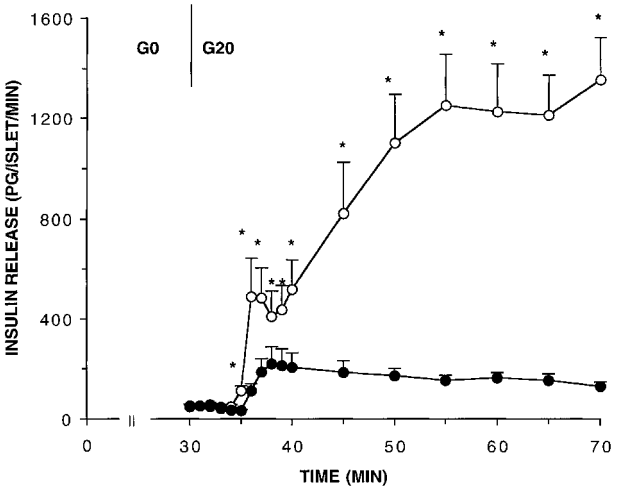

Fig 1. Secretory effects of 20 mmol/L glucose on β cells of rat or mouse islets. Groups of 15-18 islets were collagenase-isolated from Sprague-Dawley rats (○, n = 9) or CD-1 mice (●, n = 9). They were then perifused for 30 minutes without any added exogenous nutrients and for an additional 40 minutes with 20 mmol/L glucose (G20). *Significant (*P* < .05) differences between species. This and the 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.

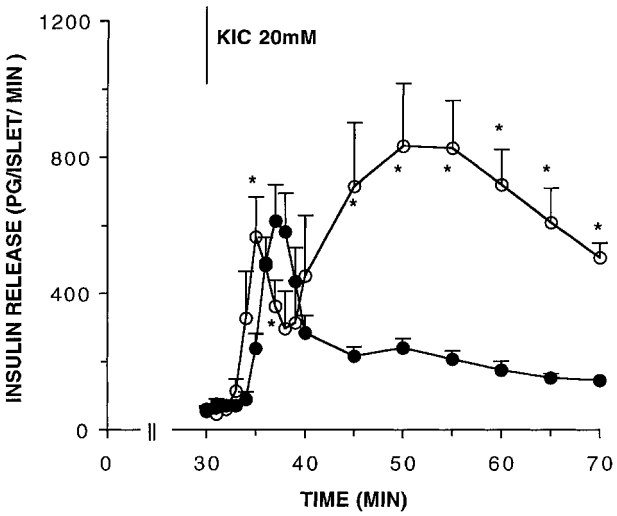

Fig 2. Secretory effects of 20 mmol/L KIC on β cells of rat or mouse islets. Groups of 15-18 islets were collagenase-isolated from Sprague-Dawley rats (○, n = 6) or CD-1 mice (●, n = 5). They were then perifused for 30 minutes without any added exogenous nutrients and for an additional 40 minutes with 20 mmol/L KIC. *Significant (*P* < .05) differences between species.

dichotomy was observed in the sustained secretory response between rat and mouse islets that was particularly evident when 20 mmol/L glucose was used to stimulate secretion. For example, while insulin release with 20 mmol/L glucose measured 35 to 40 minutes after the onset of stimulation was 1,352 ± 168 pg/islet/min from rat islets, the response from mouse islets at this time was only 129 ± 18 pg/islet/min. Less dramatic but still significant differences with KIC were also recorded. For example, 35 to 40 minutes after the onset of stimulation with 20 mmol/L KIC, release rates were 506 ± 42 pg/islet/min from rat islets but only 145 ± 12 pg/islet/min from mouse islets. With regard to MP, significant species differences were only evident at several time points during the perifusion (Fig 3). For example, 35 to 40 minutes after the onset of stimulation with 20 mmol/L MP, release rates were 199 ± 53 pg/islet/min from rat islets but only 71 ± 17 pg/islet/min from mouse islets.

Glucose Usage in Rat or Mouse Islets

There is little question that glucose metabolism regulates insulin secretion from rat islets.[27,33-36] We next determined if the reduction in glucose-induced insulin secretion from mouse islets as compared with rat islets is a consequence of reduced glucose usage rates. Significant increases in glucose usage rates in both rat and mouse islets resulted when the glucose concentration was increased from 3 mmol/L to 20 mmol/L. Compared with the rates observed at 3 mmol/L glucose, usage increased approximately 300% with 20 mmol/L glucose regardless of the species studied (Table 1).

Nutrient-Induced IP Accumulation in Rat or Mouse Islets

After labeling PI pools for 3 hours, islet responses to these nutrient activators of the β cell were determined. In agreement with previous reports,[37,38] the addition of 20 mmol/L glucose or KIC significantly increased IP accumulation in rat islets (Table 2). A smaller response was observed with 20 mmol/L MP. From

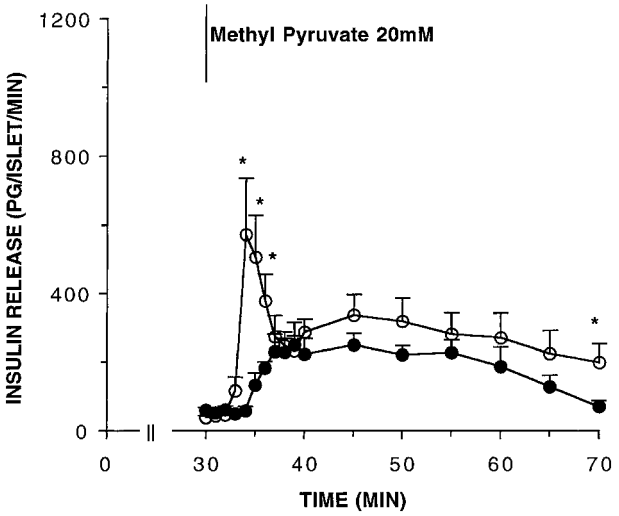

Fig 3. Secretory effects of 20 mmol/L MP on β cells of rat or mouse islets. Groups of 15-18 islets were collagenase-isolated from Sprague-Dawley rats (○, n = 8) or CD-1 mice (●, n = 4). They were then perifused for 30 minutes without any added exogenous nutrients and for an additional 40 minutes with 20 mmol/L MP. *Significant ($P < .05$) differences between species.

a quantitative perspective, the largest effects were observed with KIC (517% increase above basal control IP levels) and glucose (443% increase), with MP (199% increase) being least effective in this regard.

A dichotomy in the IP response between mouse and rat islets was observed when mouse islets were stimulated under identical conditions (Table 2). IP responses in this case were significantly smaller regardless of the nutrient stimulant used. Compared with basal control levels (803 ± 105 cpm/30 min/40 islets), the increases with 20 mmol/L glucose were 68%, with 20 mmol/L KIC 106%, and with 20 mmol/L MP 22%.

Additional studies were conducted with mouse islets labeled with ^{3}H-inositol for 6 hours. IP accumulation in the absence of any added substrate was 1,670 ± 125 cpm/30 min/40 islets (n = 3). In response to 20 mmol/L glucose stimulation, IP accumulation was 2,698 ± 402 cpm/30 min/40 islets, a 62% increase (n = 3).

At least 2 signals, one metabolic in nature and the other calcium, are required for glucose-induced activation of PLC in rat islets.[3,39] Since glucose metabolism was comparable in islets isolated from both species, we considered the possibility that reduced calcium availability resulted in impaired PLC activation. To address this issue, ^{3}H-inositol–labeled mouse islets were stimulated with the combination of 20 mmol/L glucose plus depolarizing levels (30 mmol/L) of potassium chloride. By maintaining the patency of voltage-regulated calcium channels, the monovalent cation stimulates calcium influx. Its addition together with 20 mmol/L glucose slightly but insignificantly increased IP above the levels observed with 20 mmol/L glucose alone in mouse islets (Table 2).

Table 1. Glucose Usage Rates of Rat and Mouse Islets (pmol/islet/h)

Islets	3 mmol/L Glucose	20 mmol/L Glucose
Rat	35.9 ± 2.5 (n = 8)	147.6 ± 9.8 (n = 6)
Mouse	28.7 ± 2.4 (n = 3)	111.9 ± 14.5 (n = 7)

NOTE. Groups of rat or mouse islets were isolated and perifused for 70 minutes. One group was maintained for the duration of the perifusion at 3 mmol/L glucose. The second group was first perifused for 30 minutes in the absence of glucose and for an additional 40 minutes with 20 mmol/L glucose. Usage rates (mean ± SE) were measured using the amount of $^{3}H_2O$ formed from 5-^{3}H-glucose.

Studies With Carbachol-Stimulated Mouse Islets

Our working hypothesis is that the robust activation of PLC, monitored by IP accumulation, and the increasing second-phase insulin secretory response of rat islets to 20 mmol/L glucose stimulation are related events, and the failure of mouse islets to develop a second-phase insulin response of similar magnitude is the result, at least in part, of the minimal activation of PLC by 20 mmol/L glucose. However, it might be argued that these 2 events in these 2 species are not related. In an attempt to address this issue, we provided increased amounts of PLC-generated signals by stimulating mouse islets with the cholinergic agonist carbachol. This compound activates an isozyme of PLC distinct from the nutrient isozyme activated by glucose.[28] The addition of 100 μmol/L carbachol together with 20 mmol/L glucose resulted in a dramatic increase in IP accumulation in mouse islets (Table 2). In the simultaneous presence of 20 mmol/L glucose, carbachol elicited a large and sustained insulin secretory response as well (Fig 4). This observation agrees with our previous studies[19] and other investigations using cholinergic agonists.[40,41]

Western Blot Studies

In an attempt to further characterize the PLC isozyme profile in rat and mouse islets, islet sonicates from each species were probed with antibodies directed against several previously uncharacterized isozymes. We also compared the PKCα content and repeated some of our previously published studies probing the species expression of PLC isozymes.[19] We could not detect PLCδ2 or PLCβ4 in rat or mouse islets (data not shown). However, PLCβ2 and PLCβ3 were present in both species (Fig 5). Despite the analysis of comparable amounts of islet protein (20 μg), the bands found in mouse islets were lighter than the corresponding rat islet bands. In 2 separate experiments, the optical density of PLCβ2 and PLCβ3 bands was only 22% or 31%, respectively, of the corresponding rat islet bands. Islet expression of PKCα was comparable between the 2 species. In 3 separate experiments, mouse islet PKCα content was 107% ± 22% of that found in rat islets. In addition to reduced expression of PLCβ2 and PLCβ3 and in agreement with our prior report, mouse islets also underexpress PLCβ1 (35% ± 13% of rat) and PLCδ1 (1.5% ± 0.5% of rat). PLCγ1 expression was comparable between the species.

DISCUSSION

When stimulated with 20 mmol/L glucose, our perifused rat islet preparation responded with a brisk biphasic insulin secretory response comparable in magnitude to that observed with the perfused rat pancreas preparation (Fig 1). The peak first-phase insulin secretory responses of rat islets to KIC or MP were similar in magnitude to those observed with 20 mmol/L glucose stimulation. Sustained second-phase responses measured 35 to 40 minutes after the onset of stimulation with these stimulants were less than those observed with 20 mmol/L

Table 2. Effects of 20 mmol/L Glucose, 20 mmol/L KIC, or 20 mmol/L MP on IP Accumulation in Rat or Mouse Islets of Langerhans

Stimulatory Condition	IP Accumulation (cpm/40 islets/30 min)	
	Rat Islets	Mouse Islets
1. No added stimulant	2,883 ± 271	803 ± 105
2. 20 mmol/L glucose	15,664 ± 1,713 (+443%)	1,352 ± 250 (+68%)
3. 20 mmol/L KIC	16,628 ± 682 (+517%)	1,654 ± 172 (+106%)
4. 20 mmol/L MP	8,627 ± 689 (+199%)	981 ± 130 (+22%)
5. 20 mmol/L glucose plus 30 mmol/L KCl	ND	1,682 ± 191 (+109%)
6. 20 mmol/L glucose plus 100 μmol/L carbachol	ND	9,589 ± 740 (+1,094%)

NOTE. Groups of rat or mouse 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 the additions as indicated. Also included during the 30-minute stimulatory period was 10 mmol/L LiCl to prevent the dephosphorylation of IPs. At least 4 experiments were conducted under each condition and the mean ± SE are presented. Statistical analysis: rat islets, results for conditions 2 to 4 are all statistically different *v* condition 1; mouse islets, results for conditions 2, 3, 5, and 6 are different *v* condition 1. Values in parentheses are the percentage increase above IP accumulation measured in the absence of added stimulant (condition 1).

glucose. In the case of MP, it was only about 15% to 20% of the response found with 20 mmol/L glucose. In the case of KIC, a significant decline in release was noted after about 20 minutes of stimulation. The precise reason for this reduction in secretion is not clear.

The findings obtained with mouse islets stimulated with glucose, KIC, and MP are also shown in Figs 1 to 3. An immediate first-phase response to each of these nutrient secretagogues was observed. However, the sustained insulin secretory response to glucose was seriously compromised in mouse islets when compared with the corresponding rat islet responses. The sustained secretory response to KIC was also reduced compared with the corresponding rat islet responses. With regard to MP, the differences in release rates were less dramatic but still significantly different at several time points.

It might be argued that the deficient mouse islet insulin secretory responses to glucose stimulation reflect differences in insulin content between the species. Previous studies, confirmed herein, have shown that mouse islet insulin contents are similar to rat islets.[18,42,43] Moreover, considering the fact that the cumulative amount of insulin secreted from mouse islets in response to glucose alone is only several nanograms per islet, insulin stores would not appear to be rate-limiting for glucose-induced secretion. Finally, the fact that the addition of carbachol (Fig 4) or TPA together with high glucose results in a large insulin secretory response argues against the concept that insulin content limits the secretory response of mouse islets.[19]

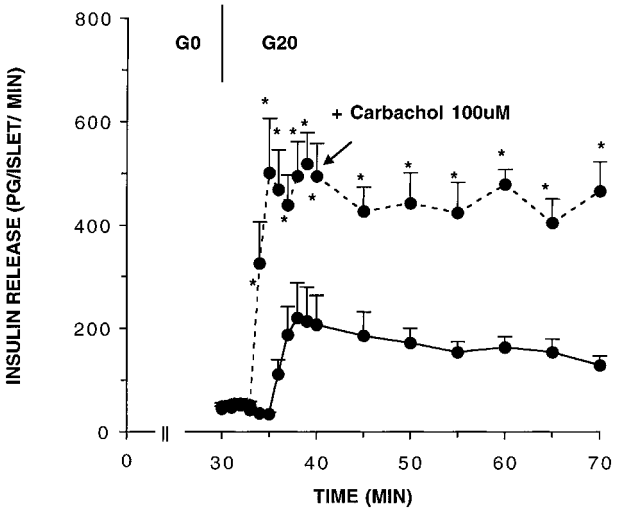

Fig 4. Insulin secretory responses from mouse islets stimulated with the combination of carbachol and glucose. Two groups of mouse islets were perifused for 30 minutes in the absence of any glucose. One group (n = 9) was then stimulated with 20 mmol/L glucose alone (——, the same mouse data as in Fig 1) while the second group (n = 6) was stimulated with 20 mmol/L glucose plus 100 μmol/L carbachol (– – – –). *Significant ($P < .05$) differences between the responses.

It might be suggested that the inability of mouse islets to respond to glucose stimulation with a large second-phase insulin secretory response is a consequence of aberrant glucose usage by this species. This was not the case, and in agreement with previous rat[27] and mouse[44] studies, the glucose usage rate increased significantly as the hexose level was elevated. With both species, the increase in glucose usage was approximately 300% when the hexose level was increased from 3 mmol/L to 20 mmol/L.

Our results support the concept that PLC activation may play a role in the species-dependent insulin secretory responses observed. For example, significant 200% to 500% nutrient-induced increases in IP accumulation were measured in rat islets, a finding that supports the activation of PLC by these agonists. Compared with the responses of rat islets, minimal IP responses from mouse islets were recorded. This biochemical deviation is also paralleled by a much smaller sustained insulin secretory response from mouse islets. We could not significantly increase IP accumulation in mouse islets above that observed with 20 mmol/L glucose alone by costimulation with 20 mmol/L glucose plus 30 mmol/L potassium, a manipulation that depolarizes the β cell, opens voltage-regulated calcium channels, and increases intracellular calcium. This finding would appear to eliminate altered calcium availability as the underlying biochemical anomaly.

We considered the possibility that poor incorporation of label into PI pools may have contributed to the minimal IP responses in mouse islets stimulated with various fuels. To circumvent this potential shortcoming in mouse islets, the labeling period was extended to 6 hours in some experiments. While basal IP levels approximately doubled under this condition, a modest IP response (62% increase), paralleling the findings with 3-hour labeled mouse islets, was observed in response to 20 mmol/L glucose stimulation. Most importantly, however, and attesting to the fact that PI pools were labeled during the 3-hour

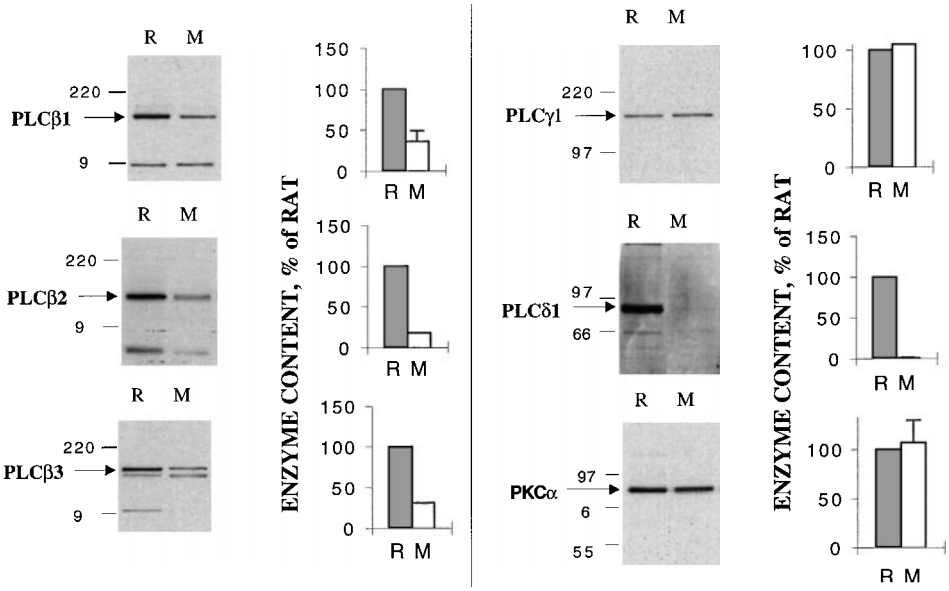

Fig 5. Comparison of PLC isozyme and PKCα expression in rat (R) and mouse (M) islets. Groups of rat or mouse islets were isolated and sonicated, and equivalent amounts of islet protein analyzed for the presence of PLCβ1 (n = 3), PLCβ2 (n = 2), PLCβ3 (n = 2), PLCγ1 (n = 1), PLCδ1 (n = 3), and PKCα (n = 3). Arrow indicates position of appropriate isozyme band. Left, actual blots; right, enzyme content of mouse islets, expressed as a percentage of rat islet content.

incubation period is the observation that significant increases in IP accumulation could be stimulated in mouse islets if, in addition to 20 mmol/L glucose, the cholinergic agonist carbachol was included in the medium. Furthermore, this IP response was paralleled by a dramatic enhancement of second-phase insulin secretion from these islets. These studies demonstrate 3 important points: first, mouse islets remain viable and PLC-responsive PI pools are labeled during the 3-hour incubation period with ^{3}H-inositol; second, mouse islets are capable of large IP responses if they are stimulated with an agonist whose mechanism of action bypasses the nutrient-activated PLC isozyme; and third, mouse islets are capable of responding with a large sustained second-phase response under the appropriate conditions. Studies such as these also reinforce the concept that in mouse islets (compared with rat islets) the reduced capacity of glucose to stimulate IP accumulation and second-phase insulin secretion may be linked phenomena.

Similar to the situation with PKC, the inherent complexity of PLC activation is only now being fully appreciated. This is related, in part, to the increasing number of PLC isozymes identified and their unique activation characteristics.[45-47] We initially reported and confirm herein that rat and mouse islets contain the 3 major PLC isozymes: β1, δ1, and γ1.[28] In the present report, we have identified the presence of β2 and β3 PLC isozymes, but not β4 or δ2 isozymes. Despite the analysis of similar amounts of islet protein, the intensity of Western blot bands for all β isozymes was lower in mouse islet sonicates compared with rat islets. However, the most significant species difference exists in the expression of PLCδ1. In the present Western blot studies, this deviation in isozyme content was even more severe than we previously reported.[19] Since this enzyme is exquisitely sensitive to calcium,[47,48] and since nutrient-induced PLC activation in islets is regulated by the cation as well,[38,49] PLCδ1 may be the relevant isozyme activated during the metabolic transformation of fuel secretagogues. Future studies should address the possibility of overexpressing PLC isozymes in mouse islets using approaches previously described for glucokinase.[50] It should also be noted that the underexpression of PLC isozymes in mouse islets compared with rat islets was not universal. The contents of PLCγ1 were comparable between the 2 species, indicating the selective nature of the species differences observed.

To summarize, rat and mouse islets contain comparable amounts of insulin and use comparable amounts of glucose when stimulated with 20 mmol/L of the hexose. However, when compared with rat islets studied under similar conditions, both the PLC-mediated hydrolysis of islet PI pools and the generation of an increasing and sustained second-phase insulin secretory response, particularly to glucose and KIC, were significantly less in mouse islets. When stimulated with the combination of glucose and carbachol, a muscarinic agonist that activates an isozyme of PLC different from the one activated by glucose, mouse islets responded with marked increases in IP accumulation and second-phase secretion. Since the major biochemical effect of carbachol is the receptor-mediated stimulation of PLC in islets,[40,51] these findings suggest that in the presence of 20 mmol/L glucose, the provision of PI-derived signals by carbachol is responsible for amplified second-phase insulin secretion. Moreover, this finding supports the concept that the failure of 20 mmol/L glucose alone to stimulate a large second-phase response in perifused mouse islets may be a result, at least in part, of a reduction in PI-derived signals. While it remains to be established as to how the various PLC isozymes are activated, comparative studies with mouse and rat islets may facilitate this process. Furthermore, and as previously posited,[18] these results also suggest caution in the extrapolation of the experimental findings made with mouse islets to rat or human islets, particularly when fuel molecules are used as stimulants. Finally, the possible contribution of species differences in β-cell glucose sensitivity to the phenotypic expression of impaired glucose tolerance in transgenic animals should also be considered.[14,22]

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