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ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 30 – 39
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Biphasic insulin secretion from freshly isolated or cultured, perifused rodent islets: comparative studies with rats and mice

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Received 16 April 2007; accepted 23 July 2007

Abstract

In the present report, we compared the insulin secretory responses of freshly isolated, perifused rat and mouse islets to glucose. Prestimulatory glucose levels were changed to assess their influence on the subsequent secretory responses. Additional studies included experiments with the incretin factor glucagon-like peptide-1 (GLP-1), the cholinergic agonist carbachol, and the α2 agonist epinephrine. Our findings demonstrate that under conditions where glucose (8.5-11.1 mmol/L) evokes a dramatic biphasic insulin secretory response from perifused rat islets, mouse islets exhibit little response. Increasing the prestimulatory glucose level to 8.5 mmol/L dramatically distorts subsequently measured glucose-induced insulin secretion from rat islets but allows the evocation of a modest but clear biphasic response from mouse islets in response to 30 mmol/L, but not 11.1 or 16.7 mmol/L, glucose. In the presence of a minimally effective glucose level (10 mmol/L), mouse islets remain exquisitely sensitive to the combined stimulatory effects of GLP-1 (2.5 nmol/L) plus carbachol (0.5 μmol/L) and to the inhibitory influence of epinephrine (10 nmol/L). Short-term culture of rat islets in CMRL 1066 containing 5.6 mmol/L glucose resulted in a significant decrease in the secretory response to 11.1 mmol/L glucose, whereas the same manipulation improved mouse islet responses. It is concluded that the process of collagenase isolating islets does not alter mouse islet sensitivity in any adverse way and that increasing the prestimulatory glucose level can indeed alter the pattern of insulin secretion in either a positive or negative manner depending upon the species being investigated. Prior short-term culture of rodent islets differentially affects secretion from these 2 species.

1. Introduction

Glucose-induced secretion from rat pancreatic β-cells, studied using a number of different preparations including the perfused pancreas [1-7] and perifused islets [8-10], is biphasic in nature. It is characterized by an initial burst of insulin, lasting several minutes, followed by a brief nadir and a slowly rising and sustained second-phase response. The magnitude of the peak second-phase response to sustained maximal hyperglycemic stimulation is on average about 20- to 40-fold greater than prestimulatory rates of secretion measured in the presence of substimulatory glucose concentrations. It is not yet clear which of the many metabolic, cationic, or second-messenger events underlie biphasicity, although a number of different studies [11-16] suggest that information flow in the phospholipase C (PLC)/protein kinase C (PKC) pathway may be particularly important.

The biphasic response of rat islets to hyperglycemic stimulation is well established, having been reported on and confirmed by numerous groups. However, the pattern of insulin release evoked from mouse β-cells during sustained stimulation with high glucose differs significantly from that usually noted from rat β-cells. It is characterized by a flat sustained second-phase response that is only modestly elevated above prestimulatory rates [5,6,12,13,17-20]. Recently, 2 separate studies have attempted to explain and/or reconcile the raison d'être for this species dichotomy in glucose-induced insulin release using a number of different approaches. The first report by Nunemaker and colleagues [21] suggested that part of this difference from rat islets may be a consequence of the isolation procedure. In the second report on this topic, Henquin and coworkers [22] suggested that the prestimulatory glucose level played a previously unrecognized but important role in determining the subsequent pattern of secretion in response to glucose. In these

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doi:10.1016/j.metabol.2007.07.020

reports, however, comparative studies using rat islets were not performed using identical protocols, thus excluding a direct comparison of rodent islet responses under similar experimental conditions from the same laboratory. In the present report, we compared glucose-induced insulin secretion from freshly isolated, noncultured, perifused rodent islets. Our findings emphasize the complexity of stimulus-response coupling patterns in rodent islets and the contribution of both the antecedent glucose concentration and culturing on their subsequent secretory responsiveness. Under identical experimental conditions, however, profound species differences to glucose stimulation were still evident.

2. Material and methods

The detailed methodologies used to assess insulin output from collagenase-isolated islets have been previously described [23,24]. Male Sprague-Dawley rats (weighing 325-425 g at the time of study) and male CD-1 mice (weighing 28-40 g at the time of study) were purchased from Charles River (Wilmington, MA) and used in all studies. All animals were treated in a manner that complied with the National Institutes of Health's *Guidelines for the Care and Use of Laboratory Animals*. The animals were fed ad lib. After anesthesia induced by Nembutal (pentobarbital sodium, 50 mg/kg; Abbott, North Chicago, IL), 20 to 30 mL of cold Hanks solution (without any added glucose) was used to distend the pancreas via the biliary system. Islets were isolated by collagenase digestion and handpicked, using a glass loop pipette, under a stereomicroscope into Krebs-Ringer bicarbonate supplemented with 3 mmol/L glucose. They were free of exocrine contamination.

2.1. Perifusion studies

Islets were loaded onto nylon filters (Sefar America, Kansas City, MO) immediately after the isolation, a process that took no longer than 90 minutes from the initiation of the pancreatectomy. The islets were perifused in a Krebs-Ringer bicarbonate buffer at a flow rate of 1 mL (±0.1 mL)/min for 30 minutes in the presence of various glucose concentrations to establish 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 figure legends and the Results section. In some experiments, GLP-1 and carbachol were included during the initial 30-minute period. The first-phase response was that observed during the initial 10 minutes of stimulation (not corrected for the dead space in the perifusion apparatus), and the second-phase response was that occurring during the final 30 minutes of stimulation. 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 [25]. At the termination of the experiment, total islet insulin content was measured; and in some figures, secretion is expressed as the fractional insulin secretion rate. This was calculated as the percentage of islet insulin content that was secreted per minute.

2.2. Total islet insulin

After the perifusion, the islets still on filters were retrieved and placed in small glass vials. Hanks solution (240 μL) was gently added, and the samples were sonicated for 20 seconds. Aliquots were then frozen for the subsequent analysis of insulin content.

2.3. Cultured islet studies

In a number of experiments, isolated rodent islets were cultured for 3 hours in CMRL 1066 containing 5.6 mmol/L glucose. After this, they were perifused as described above; and their secretory responses to 11.1 mmol/L glucose were determined.

2.4. Reagents

Ice-cold Hanks solution without any added glucose 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 for the insulin assay was purchased from PerkinElmer Life Sciences (Boston, MA). Bovine serum albumin (radioimmunoassay grade), glucose, GLP-1, carbachol, epinephrine bitartrate salt, and the salts used to make the Hanks solution and perifusion medium were purchased from Sigma (St. Louis, MO). CMRL 1066 (Gibco no. 11530) was purchased from Invitrogen (Carlsbad, CA). Rat insulin standard (lot no. 615-ZS-157) was the generous gift of Dr Gerald Gold (Eli Lilly, Indianapolis, IN). Collagenase (type P) was obtained from Roche Applied Science (Indianapolis, IN).

2.5. Statistics

Statistical significance was determined using the Student *t* test for unpaired data or analysis of variance. A value of $P \leq .05$ was taken as significant. Values presented in the figures and the Results section represent means ± SEMs of at least 3 observations.

3. Results

3.1. Total insulin contents

The total insulin contents of the freshly isolated islets used in these studies, calculated as the amount contained in the islet at the end of the experiment and the amount secreted during the perifusion period, averaged 182 ± 8 (n = 44) and 190 ± 9 (n = 35) ng per islet for rat and mouse islets, respectively.

3.2. Stimulation of rodent islets with 8.5 mmol/L glucose

Most investigators, including ourselves, generally perifuse islets with substimulatory glucose concentrations for 20

to 30 minutes before stimulating them with higher glucose levels. This is done to establish basal, stable insulin secretory rates. The subsequent response to hyperglycemia can then be conveniently assessed and compared with prestimulatory rates of insulin secretion, and the magnitude of the response can be ascertained. Because the threshold for glucose-induced secretion is approximately 5.0 to 5.5 mmol/L [4,26,27], a level below this is most often used. In many previous studies with rodent islets, we have used 3 mmol/L (54 mg/dL) glucose. According to Henquin et al [22], the prestimulatory glucose level "can secondarily affect the magnitude and pattern of subsequent glucose-induced insulin secretion" [22]. As a first step to validate this concept with both rat and mouse islets, we initially established how islets from both species respond to 8.5-mmol/L (153 mg/dL) glucose stimulation when 3 mmol/L glucose was used during the 30-minute prestimulatory stabilization period. We chose 8.5 mmol/L glucose because Henquin and coworkers [22] cultured mouse islets with this glucose level for 18 hours before being studied. The results are given in Fig. 1 (top panel). The response of rat islets was most robust, and release rates increased about 20-fold from 20 ± 2 (n = 6) pg per islet per minute in the presence of 3 mmol/L glucose to 417 ± 33 pg per islet per minute after 40 minutes of stimulation with 8.5 mmol/L glucose (n = 6). A sharp dichotomy was noted when mouse islets, isolated and perifused using the exact same experimental protocols, were stimulated with 8.5 mmol/L glucose. Mouse islet responses were notable for their virtual lack of a response under this experimental condition. The most dramatic difference was the failure of mouse islets, in sharp contrast to rat islets, to mount a second-phase insulin secretory response. To allow a direct comparison between our data and that contained within the report of Henquin and coworkers [22], we also calculated the fractional insulin secretory rates of these islets; and these data are presented in Fig. 1 (bottom panel). Two points should be made. First, the secretory profile that emerges is identical to that where the actual secretion rates are presented (Fig. 1, top and bottom panels). Second, the amount of insulin secreted represents a very small fraction of the total insulin content. For example, even during the robust secretory response to 8.5 mmol/L glucose, less than 0.2%/min of the total insulin content is being mobilized.

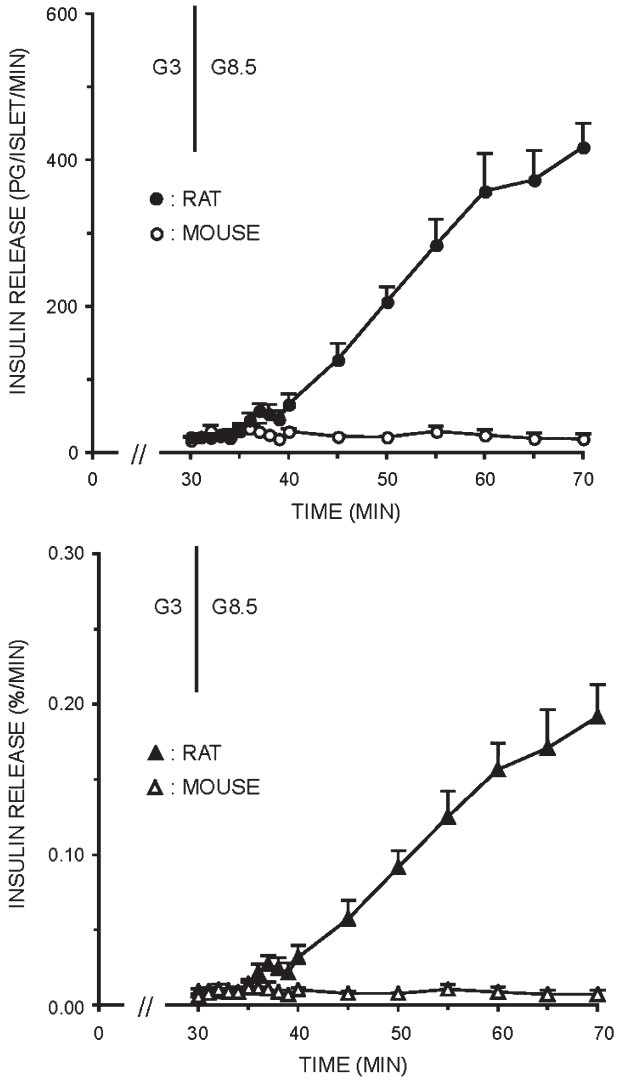

Fig. 1. Effects of prior 3.0 mmol/L glucose on insulin secretion from freshly isolated, perifused rodent islets stimulated with 8.5 mmol/L glucose. Groups of islets were isolated from rats or mice and perifused immediately after the isolation. For the initial 30 minutes of the perifusion, the glucose level was maintained at 3 mmol/L (G3). For the next 40 minutes (onset indicated by the vertical line), both groups of islets were stimulated with 8.5 mmol/L glucose (G8.5). The actual insulin secretion rates expressed as picograms per islet per minute (top panel) were measured, and the amount of insulin secreted was also calculated as the percentage of the total islet insulin content (bottom panel). The data point above the 30-minute mark on this and all subsequent graphs was collected from minutes 25 to 30 of the perifusion and reflects the secretory rate before switching to a higher glucose concentration. This and subsequent perifusion figures have not been corrected for the dead space, about 2.5 mL or 2.5 minutes with a flow rate of 1 mL/min. Mean values ± SEM of at least 3 experiments were performed under each condition.

3.3. Changing the stabilization and stimulatory glucose levels

In the comprehensive report by Henquin and coworkers [22] studying glucose-induced insulin secretion from cultured perifused mouse islets, the glucose level used during the prestimulatory stabilization period was varied from 3 to 8.5 mmol/L. In the next series of experiments, comparable studies were conducted with both rat and mouse islets. Freshly isolated islets were perifused with either 5 or 8.5 mmol/L glucose for 30 minutes before a 40-minute stimulatory period with 11.1, 16.7, or 30 mmol/L glucose, the exact same levels used in the previous study [22]. The results are given in Figs. 2-4.

When the glucose level was increased from 5 to 11.1 mmol/L, perifused rat islets responded with a dramatic biphasic insulin secretory response (Fig. 2, top left panel). Prestimulatory release rates of approximately 25 pg per islet

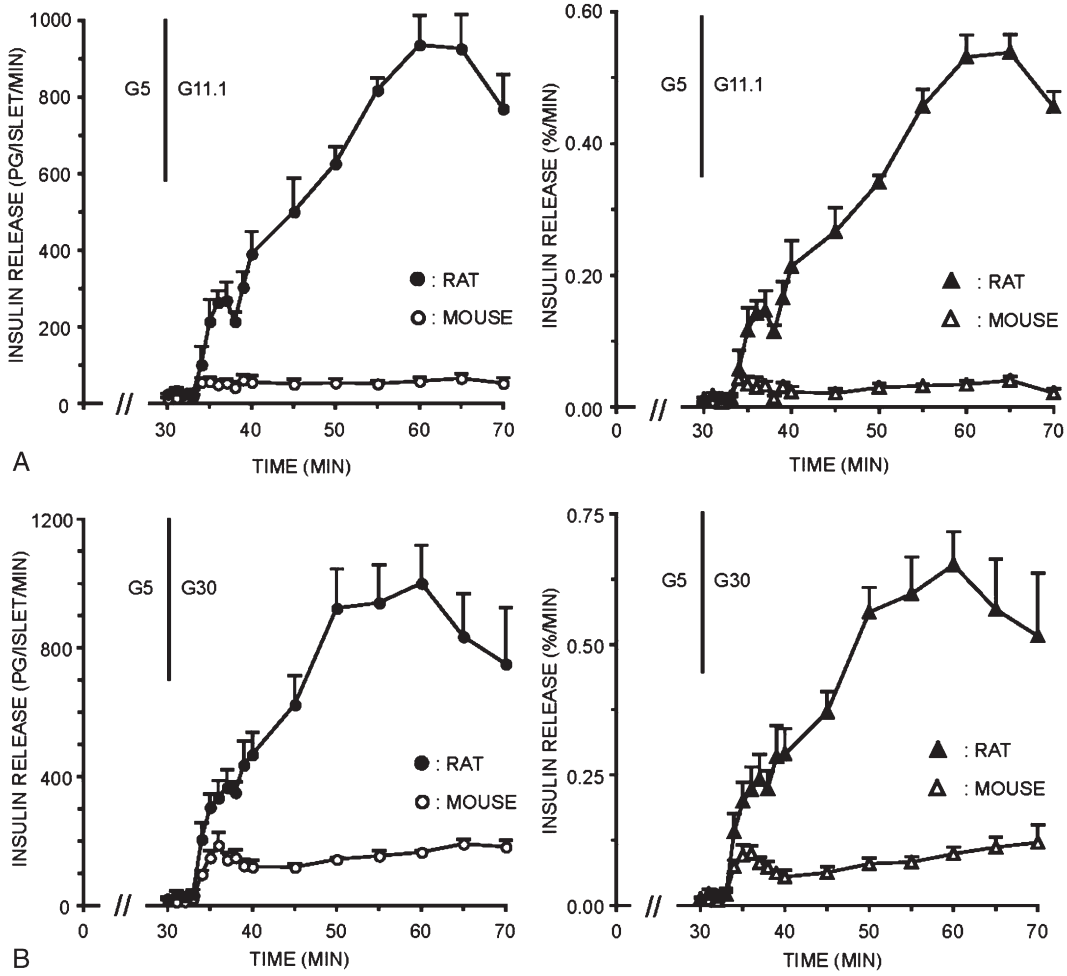

Fig. 2. Effects of prior 5.0 mmol/L glucose on insulin secretion from freshly isolated, perifused rodent islets stimulated with 11.1 and 30 mmol/L glucose. Groups of islets were isolated from rats or mice and perifused immediately after the isolation. For the initial 30 minutes of the perifusion, the glucose level was maintained at 5 mmol/L (G5). For the next 40 minutes (onset indicated by the vertical line), islets were stimulated with 11.1 or 30 mmol/L glucose (G11.1, G30). The actual insulin secretion rates expressed as picograms per islet per minute (left panels, top and bottom) were measured, and the amount of insulin secreted was also calculated as the percentage of the total islet insulin content (right panels, top and bottom).

per minute increased to 269 ± 47 (n = 4) pg per islet per minute during the first-phase response and increased to 700 to 900 pg per islet per minute during the final 20 minutes of stimulation. When compared with prestimulatory rates, a 25- to 30-fold response was evident. Mouse islet responses differed significantly from rat islet responses. A small first-phase response was noted, increasing from prestimulatory rates of approximately 25 to 69 ± 8 pg per islet per minute. In contrast to the large 25-fold increase in sustained second-phase release observed from rat islets, a small 3- to 4-fold response was noted from mouse islets stimulated with 11.1 mmol/L glucose (Fig. 2, top left panel). These second-phase responses were sustained but flat. Larger first-phase and second-phase responses were observed during stimulation of rat islets with 30 mmol/L glucose (Fig. 2, bottom left panel). At 30 mmol/L glucose, the emergence of a biphasic response from mouse islets was apparent, although the magnitude of the second-phase response was only about 20% of the rat islet response. When the amount of insulin released was expressed as percentage of total insulin content per minute, profiles similar to the actual secretion rates emerged (Fig. 2, bottom right panel).

If the glucose level used during the 30-minute stabilization period before stimulation with 11.1 or 30 mmol/L glucose was increased to 8.5 mmol/L glucose, an entirely different picture emerged when rat islets were examined (Figs. 3 and 4). The intensely stimulatory effect of 8.5 mmol/L glucose on rat islets during the initial 30-minute stabilization period was most obvious and distorted the subsequent secretory response to even higher glucose levels. For example, although stimulation with 11.1 mmol/L glucose after 5 mmol/L glucose resulted in an approximately 30-fold increment in sustained second-phase release rates, perifusion with 8.5 mmol/L glucose before 11.1 mmol/L glucose resulted in a maximal 2-fold response to 11.1 mmol/L glucose from 300 to 600 pg per islet per minute (Fig. 3, left

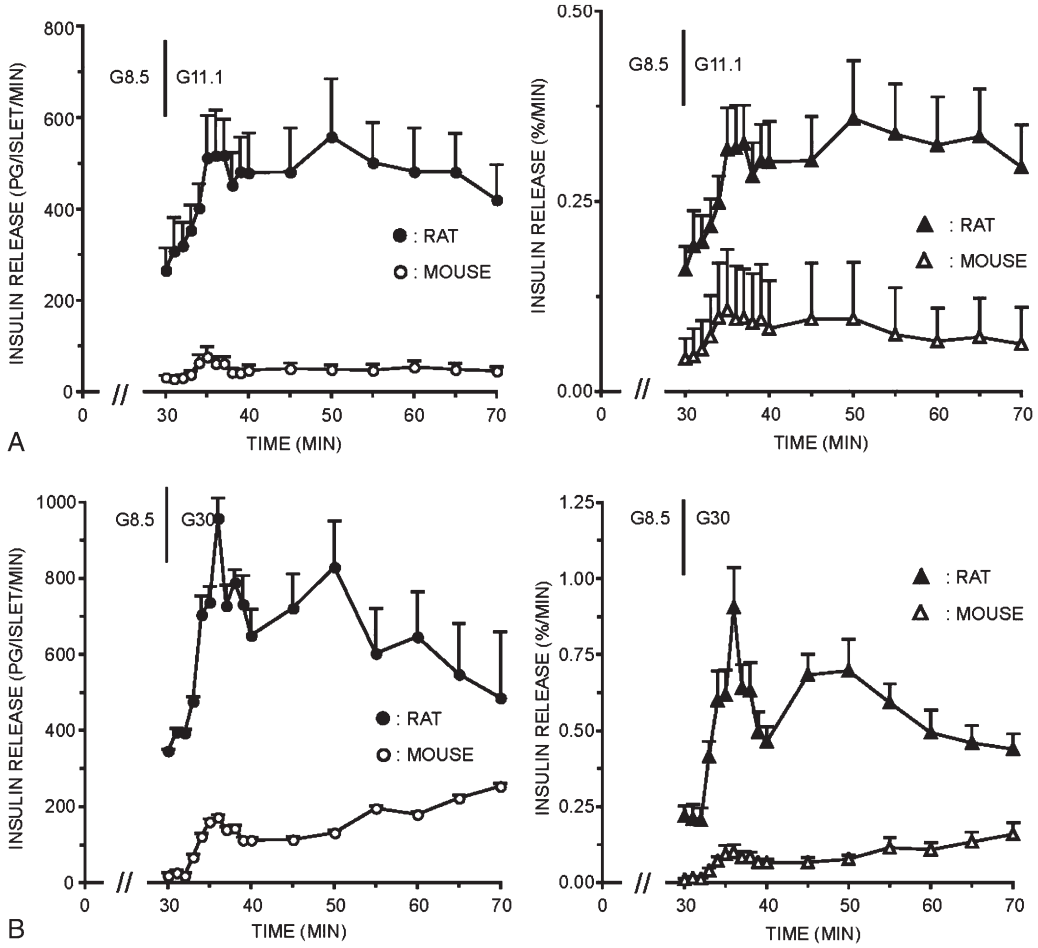

Fig. 3. Effects of prior 8.5 mmol/L glucose on insulin secretion from freshly isolated, perifused rodent islets stimulated with 11.1 and 30 mmol/L glucose. Groups of islets were isolated from rats or mice and perifused immediately after the isolation. For the initial 30 minutes of the perifusion, the glucose level was maintained at 8.5 mmol/L (G8.5). For the next 40 minutes (onset indicated by the vertical line), islets were stimulated with 11.1 or 30 mmol/L glucose (G11.1, G30). The actual insulin secretion rates expressed as picograms per islet per minute (left panels, top and bottom) were measured, and the amount of insulin secreted was also calculated as the percentage of the total islet insulin content (right panels, top and bottom).

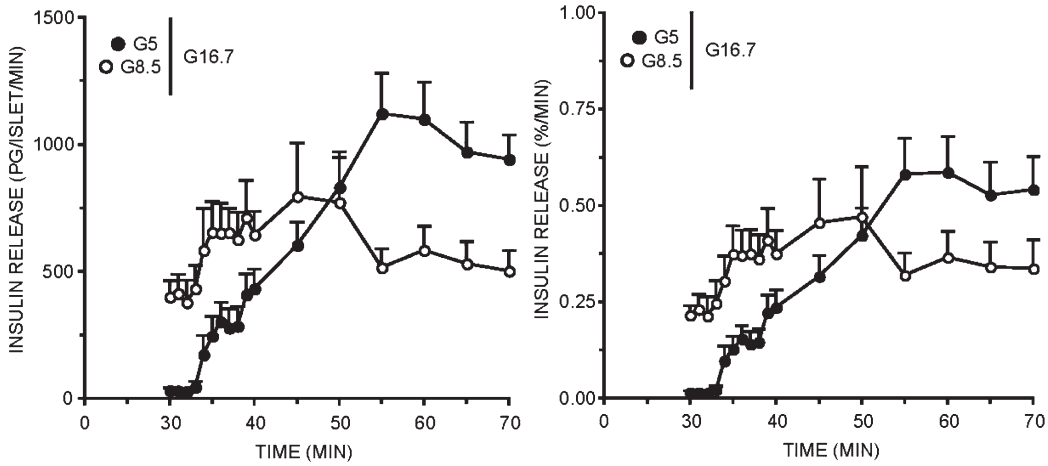

Fig. 4. Effects of prior 5.0 or 8.5 mmol/L glucose on insulin secretion from freshly isolated, perifused rat islets stimulated with 16.7 mmol/L glucose. Groups of rat islets were isolated and perifused immediately after the isolation. For the initial 30 minutes of the perifusion, the glucose level was maintained at either 5 mmol/L (G5) or 8.5 mmol/L (G8.5). For the next 40 minutes (onset indicated by the vertical line), islets were stimulated with 16.7 mmol/L glucose (G16.7). The actual insulin secretion rates expressed as picograms per islet per minute (left panel) were measured, and the amount of insulin secreted was also calculated as the percentage of the total islet insulin content (right panel).

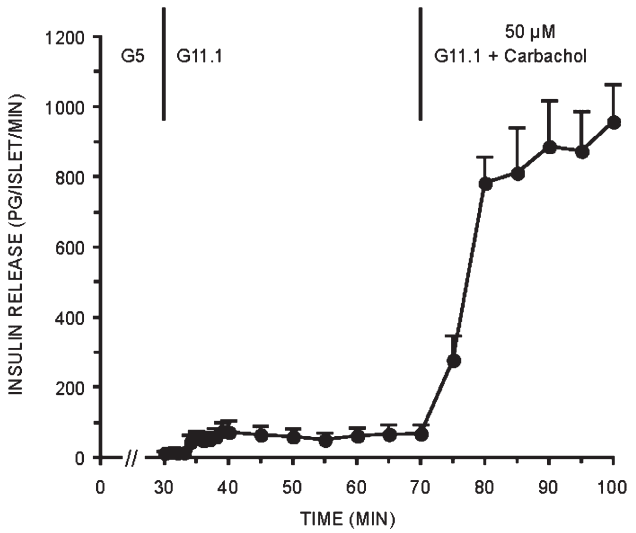

Fig. 5. Effects of carbachol on insulin secretion rates from mouse islets. Groups of mouse islets were perifused with 5 mmol/L glucose for 30 minutes and for an additional 70 minutes with 11.1 mmol/L glucose. After 40 minutes with 11.1 mmol/L glucose alone, the islets were subjected to an additional 30-minute stimulation period with 50 μmol/L carbachol.

panel). Unlike the response where the prestimulatory glucose level was 5 mmol/L, there was no rising second phase of insulin secretion; and the maximal secretory rates were reduced as well. This altered response pattern, a consequence of raising the prestimulatory glucose concentration to 8.5 mmol/L, was also noted if rat islets were subsequently stimulated with 30 (Fig. 3) or 16.7 mmol/L glucose (Fig. 4).

Mouse islet responses after an increase in glucose level during the stabilization period to 8.5 mmol/L are also given in Fig. 3. After 30 minutes in 8.5 mmol/L glucose, perifusion with 11.1 mmol/L glucose resulted in a small initial first-phase and a minimal sustained second-phase response (Fig. 3). A more vigorous response from mouse islets was observed during stimulation with 30 mmol/L glucose, a response that was somewhat biphasic with a slowly rising second-phase response.

After a 30-minute stabilization period with 5 mmol/L glucose, the secretory profile observed from rat islets in response to 16.7 mmol/L glucose was similar to that noted with 30-mmol/L glucose stimulation (Fig. 4). The sustained second-phase response averaged about 1000 pg per islet per minute (n = 5), a more than 30-fold increase from prestimulatory rates of insulin release. Again, the presence of 8.5 mmol/L glucose during the initial 30-minute stabilization period distorted the response of rat islets to 16.7-mmol/L glucose stimulation, as it did to the 11- or 30-mmol/L glucose responses.

3.4. Effects of carbachol on 11.1 mmol/L glucose–induced secretion from mouse islets

It might be reasonably argued that mouse islets are indeed more vulnerable to the possible deleterious effects of the collagenase used to isolate them and that at least part of the disparity noted between these 2 species' response to glucose is a result of this. An additional experimental manipulation was designed to address this issue. Groups of mouse islets were stimulated with 50 μmol/L carbachol after first being stimulated with 11.1 mmol/L glucose. As shown in Fig. 5, although these islets were minimally responsive to glucose alone, a dramatic response to the cholinergic agonist in the presence of this weakly effective glucose concentration was seen. Thus, these islets clearly retain their sensitivity to stimulation; and peak response rates comparable with those evoked from rat islets stimulated by a maximally effective glucose stimulus were evoked (compare Figs. 2 and 5).

3.5. Rodent islet responses to GLP-1, carbachol, and epinephrine

In the final experiments, we explored the potential contribution of combined GLP-1 and cholinergic stimulation to evocation of biphasic secretion and the impact of the inhibitor epinephrine on these responses. In the report by Nunemaker et al [21], it proved possible to elicit biphasic secretion in vivo with hyperglycemic stimulation. However,

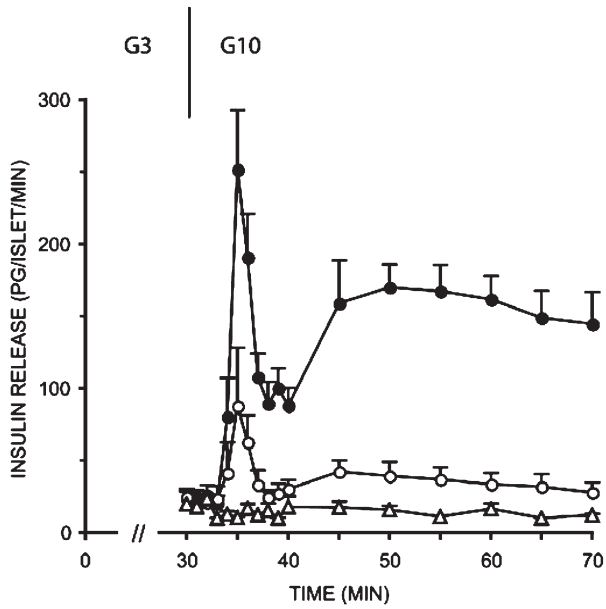

Fig. 6. Effects of GLP-1, carbachol, and epinephrine on 10 mmol/L glucose–induced insulin secretion from mouse islets. Three groups of islets were studied. The first group (n = 8, open circles) was perifused with 3 mmol/L glucose (G3) for 30 minutes before being stimulated for 40 minutes with 10 mmol/L glucose (G10, onset of stimulation indicated by the vertical line). The second group (closed circles, n = 9) was perifused for 30 minutes with 3 mmol/L glucose plus the combination of 2.5 nmol/L GLP-1 and 0.5 μmol/L carbachol. At this time and in the continued presence of GLP-1 and carbachol, the glucose level was increased to 10 mmol/L. The final group (open triangles, n = 3) was treated in a similar fashion to the second group except that epinephrine (10 nmol/L) was also included in the perifusate during the 40-minute stimulatory period with 10 mmol/L glucose, GLP-1, and carbachol.

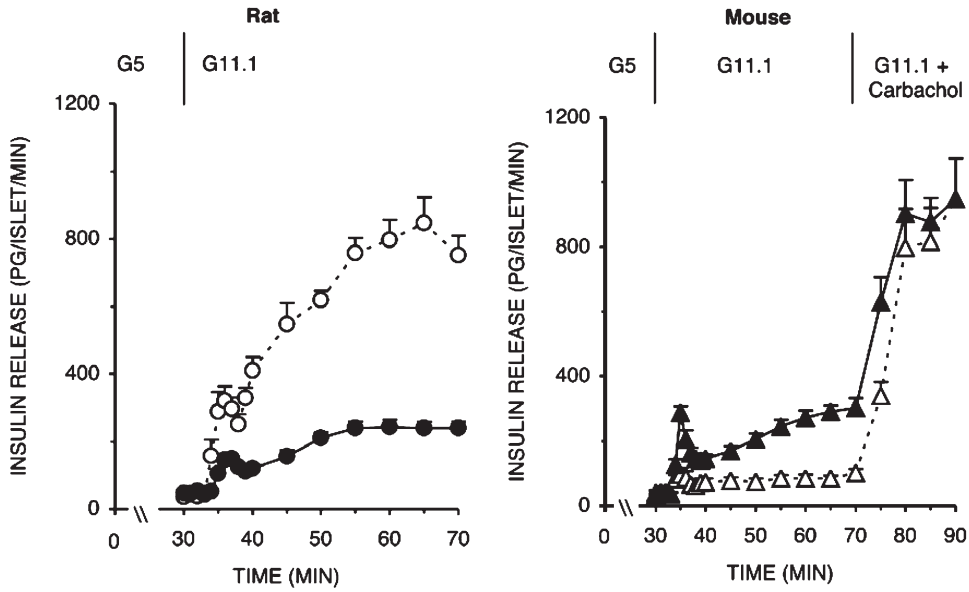

Fig. 7. Effects of short-term culture on glucose-induced insulin secretion from rodent islets. Groups of rat (left panel) or mouse (right panel) islets were isolated and perifused immediately (open circles and open triangles) or after a 3-hour incubation period in CMRL 1066 (closed circles, closed triangles). All islets were perifused for 30 minutes with 5 mmol/L glucose (G5) to establish stable insulin secretory rates and for an additional 40 minutes with 11.1 mmol/L glucose (G11.1). In mouse islets, an additional 20-minute stimulatory period was included where 50 μmol/L carbachol was included together with 11.1 mmol/L glucose.

the inclusion of additional agonists for secretion present in vivo but not in vitro may have contributed to this response. In an attempt to address this issue, we perifused mouse islets with 3 mmol/L glucose alone or with 3 mmol/L glucose plus the combination of 0.5 μmol/L carbachol and 2.5 nmol/L GLP-1. The presence of these 2 compounds had no impact on basal release rates at this glucose level (Fig. 6). However, upon increasing the glucose level to 10 mmol/L, their presence profoundly altered the subsequent secretory response to 10 mmol/L glucose. For example, mouse islets exposed to 10 mmol/L glucose alone exhibited an immediate first-phase and a small, flat second-phase secretory response (Fig. 6). The presence of GLP-1 and carbachol markedly amplified the first-phase response to 10 mmol/L glucose and also resulted in a rising second-phase response. Further attesting to the retention of the physiologic integrity of the collagenase-isolated islet preparation, the addition of epinephrine (10 nmol/L) totally abolished the amplified secretory response to this combination of agonists.

Additional studies using rat islets were also performed. After 30 minutes with 3 mmol/L glucose alone or with 3 mmol/L glucose plus the combination of 0.5 μmol/L carbachol and 2.5 nmol/L GLP-1, these islets were then stimulated for 40 minutes. In this case, however, islets were stimulated with 6.5 mmol/L glucose alone or in the continued presence of 0.5 μmol/L carbachol and 2.5 nmol/L GLP-1. A biphasic insulin secretory response occurred in response to this combination of agonists but not to 6.5 mmol/L glucose alone. Similar to mouse islets, these responses were abolished by 10 nmol/L epinephrine (results not shown).

3.6. Impact of short-term culture on rodent islet responses to 11.1 mmol/L glucose

We considered the possibility that prior culture of islets may be a contributory factor to the disparate secretory response patterns observed from these 2 species. To address this issue, islets were cultured in CMRL 1066 for 3 hours after their isolation. They were then perifused for 30 minutes with 5 mmol/L glucose before stimulation with 11.1 mmol/L glucose. No significant impact on prestimulatory secretory rates in the presence of 5 mmol/L glucose was observed in either species. However, as shown in Fig. 7, a differential effect of culturing was noted when islets were stimulated with 11.1 mmol/L glucose. For example, when compared with the responses of freshly studied islets, a significant decrease was observed from rat islets to 11.1-mmol/L glucose stimulation. In contrast, an improvement in mouse islets to this glucose level was observed after the same experimental manipulation. Closer scrutiny of the data in Fig. 7 also shows a convergence of the glucose's stimulatory effect in these 2 species under this condition; the secretory profiles from cultured rodent islets, unlike the clear and significant deviation noted from freshly studied islets, are almost superimposable.

4. Discussion

A remarkable disparity exists in the glucose responsiveness of the many preparations used to study insulin secretion. At the 2 extremes are tumoral cell lines, many of which are minimally responsive to glucose, and the perfused rat

pancreas preparation, a model that displays a robust biphasic insulin secretory response. Profound species differences also exist between the 2 species most commonly used to experimentally dissect out the factors that control insulin exocytosis. This topic has been the subject of 2 recent studies that have attempted to provide the possible reasons for this species dichotomy. Nunemaker et al [21] suggested 2 possibilities for these species differences. First, the collagenase isolation procedure somehow altered vulnerable response elements in mouse islets. The potential nature of the lesion was not addressed, nor was any reason provided as to why this occurs uniquely with mouse islets but not with rat islets. Second, based on their studies with the intact animal, they suggested the possibility that the absence of in vivo input to the isolated islet preparation may account for the lack of biphasicity. These inputs might include acetylcholine released from the vagus nerve or circulating incretin factors such as GLP-1. Experimental attempts to duplicate the in vivo observation of biphasicity with in vitro perifusion of mouse islets were not performed in this report, so the potential nature of the added stimulants remains to be clarified.

On the other hand, Henquin and coworkers [22] argued that the prestimulatory glucose level may be a key component responsible for the anomalous behavior of mouse islet, when compared with rat islet, responses to glucose. Unfortunately, both studies used only mouse islets to address this issue of species disparity; and comparable studies with rat islets, pretreated and studied under the identical conditions, were not provided. In the present study, we have attempted to fill this void. Although our studies demonstrate, in agreement with many previous reports [5,6,10,12,17,20,28], that profound species difference does exist, they also demonstrate that it is indeed possible to evoke a biphasic pattern of secretion from mouse islets under selected conditions as proposed by Henquin et al [22]. Moreover, our results also suggest that culturing may also significantly influence the secretory responses observed from rodent islets.

When stimulated with 8.5 mmol/L glucose, after a stabilization period of 30 minutes with 3 mmol/L glucose, freshly isolated rat islets exhibit a brisk biphasic secretory response. When compared with prestimulatory secretion rates in the presence of 3 mmol/L glucose, about a 20-fold increase in secretion is evoked by 8.5 mmol/L glucose. Even greater secretory responses were recorded with higher glucose levels (11.1-30 mmol/L) that are more pharmacologic than physiologic. However, these findings with collagenase-isolated, perifused rat islets agree well with comparable studies using the perfused rat pancreas and attest to the retention of the glucose sensitivity of these islets after the isolation procedure.

Very different responses were observed from mouse islets when studied under conditions that culminated in robust secretory responses from rat islets. No rising second phase of secretion using stimulatory glucose levels of 8.5 to 16.7 mmol/L, levels that evoke 20- to 30-fold increases in insulin release from rat islets, could be elicited from mouse islets.

In agreement with the report of Henquin and coworkers [22], it was possible, by manipulating the antecedent glucose concentration, to improve mouse islet responses. In their report, increasing the glucose level before stimulation to 8.5 mmol/L resulted in a biphasic response to 30 mmol/L glucose. We confirmed this effect here and also showed that a less salient biphasic response could also be evoked by 30 mmol/L glucose after a prestimulatory period with 5 mmol/L glucose. In the previous report by Henquin et al [22], they used 8.5 mmol/L glucose during the stabilization period because the in vivo glucose level at the start of their hyperglycemic clamp studies was 8.5 mmol/L. In addition, their islets were also cultured for 18 hours with 8.5 mmol/L glucose as well. It was not determined in these studies how culturing influenced the subsequently measured secretory responses; but as discussed below, this has to be taken into consideration. It is also unlikely that mouse islets in vivo in their natural state would be subjected to such a sustained hyperglycemic stimulus. Notwithstanding these caveats, it was indeed possible to evoke a clear biphasic insulin secretory response from freshly isolated mouse islets.

Although the in vivo and in vitro studies of Henquin and colleagues [22] established the significance of antecedent hyperglycemia on the elicitation of biphasic secretion, they did not address its potential effect on rat islet responses. However, this type of manipulation when performed on rat islets totally distorted their response to glucose, again leading to a pronounced species dichotomy. The intensely stimulatory effect of 8.5 mmol/L glucose on rat β-cells, shown in Figs. 1-4, resulted in secretory responses to 11.1, 16.7, or 30 mmol/L glucose that were altered in a major way. In particular, the robustness, as well as the kinetics, of the responses to 11, 16.7, or 30 mmol/L glucose was profoundly reduced by prior exposure to 8.5 mmol/L glucose. The adverse impact of prior hyperglycemia on rat islet responses has been observed previously, and potential mechanisms have been discussed in other reports [29-34].

Despite the recognition that the patterns of glucose-induced insulin secretion from rats and mice deviate in such a major way, there is as yet no general agreement as to the underlying cause for this difference. Previous studies have demonstrated that glucose utilization rates of rat and mouse islets are similar [13,35], thus excluding any gross derangement in metabolism as the underlying cause. Moreover, this dichotomy cannot be explained by differences in insulin content but most likely reflects differences in signal transduction events. Based on a large number of observations using a variety of agonists, we have suggested that differences in information flow in the PLC/PKC may in part explain these species differences. For example, glucose alone induces a 400% to 500% increase in ^{3}H-inositol phosphate accumulation in rat islets, a barometer of PLC activation [10,13,36]. On the other hand, mouse islet responses to the same manipulation are minimal, about a

25% to 50% increase in ^{3}H-inositol phosphate levels to glucose stimulation [13,37]. The underexpression of a nutrient-activated PLC isozyme may be responsible for this lack of responsiveness to glucose [12,13].

If the difference in these species' responses to glucose is indeed the result of this biochemical anomaly, how does stimulation with 30 mmol/L glucose result in the evocation of biphasic secretion, especially after prior prolonged exposure to 8.5 mmol/L glucose? There are at least several possibilities. First, it should be recalled that mouse islet PLC is activated, albeit significantly less than in rat islets, by glucose. It may well be that the intensely stimulatory effects of 30 mmol/L glucose allow for the accumulation of sufficient PLC-derived second-messenger molecules to support biphasic secretion. Alternatively, this maneuver may culminate in an increase in intracellular calcium that facilitates increased activation of PLC itself or downstream signaling proteins such as calcium-sensitive PKC isozymes. In this context, it should be noted that the PKC activator phorbol 12-myristate 13-acetate is a most potent inducer of second-phase secretion in both rat and mouse islets [38,39]. The translocation of this calcium-sensitive enzyme, motivated by a sustained small increase in PLC activation by glucose in mouse islets, may provide an added impetus for biphasic secretion in response to sustained stimulation with 30 mmol/L glucose.

Because many investigators culture islets before assessing secretory integrity, we also explored whether this manipulation influences rodent islet sensitivity to glucose stimulation. We did not attempt to optimize culture conditions, by using various media or altering the glucose level, to improve the maintenance of secretory responsiveness. Disparate species responses to short-term culture in CMRL 1066 media supplemented with 5.6 mmol/L glucose were observed. Mouse islet responses to glucose increased, but rat islet responses were reduced. Enzyme induction, their altered activation, or the more complete elimination of any prior in vivo augmenting or inhibitory factors should be considered as possible candidates to explain these findings. For example, it is known that within the same time frame used in these culture studies, alterations in either protein synthesis by cycloheximide or enzyme induction by glucocorticoids can exert profound effects on islet responses [40-43]. Other biochemical or cationic alterations should be considered as well. In support of these findings with normal mouse islets, it has been reported [44] most recently that culturing dramatically enhances glucose-induced secretion from Sur1KO islets as well.

In conclusion, several salient points emerge from these studies. First, rat and mouse islets contain similar amounts of insulin. Second, the amount of insulin secreted during intense stimulation with glucose in either species is at most only a small fraction of the total insulin content. Third, irrelevant of the glucose levels used during the initial stabilization phase of the perifusion, clear species differences are still evident between freshly studied rat and mouse islet responses. Fourth, it is possible to convert the flat second-phase secretory response of mouse islets to glucose to a rising one. Fifth, freshly studied rat and mouse islets retain their exquisite sensitivity to membrane effective agonists including GLP-1, carbachol, and epinephrine. Sixth, via mechanisms that remain to be explored in much greater detail, short-term culture influences subsequently measured responses to glucose stimulation in both species. Finally, as suggested by our group in previous reports [10,24,45], comparative studies using these species may help us identify those pathways that are quantitatively most important in the regulation of glucose-induced secretion.

Acknowledgment

These studies were supported by National Institutes of Health grant 41230.

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