Implication of Glutamate in the Kinetics of Insulin Secretion in Rat and Mouse Perfused Pancreas

Pierre Maechler, Asllan Gjinovci, and Claes B. Wollheim

It is intriguing that the kinetics of glucose-stimulated insulin secretion from the in situ perfused pancreas differ between the rat and the mouse. Here we confirm that insulin release in the rat is clearly biphasic, whereas in the mouse glucose essentially elicits a transient monophasic insulin release. Glucose-derived glutamate has been suggested to participate in the full development of the secretory response. The present report shows that the expression of glutamate dehydrogenase is lower in mouse than in rat or human islets, paralleling the insulin secretion profile. Addition of glutamic acid dimethyl ester mainly enhances insulin release at an intermediate glucose concentration in the rat pancreas. In the mouse preparation, glutamic acid dimethyl ester induces a sustained secretory response, both at 7.0 and 16.7 mmol/l glucose. These results are compatible with a role for glucose-derived glutamate principally in the sustained phase of nutrient-stimulated insulin secretion. *Diabetes* 51 (Suppl. 1): S99–S102, 2002

The kinetics of insulin secretion from the perfused pancreas stimulated by glucose vary according to the animal model. This is particularly evident regarding the two most widely used species, i.e., the rat and the mouse (1,2). In rats, glucose stimulation results in biphasic insulin release, with a transient first phase followed, after 4–5 min, by a gradually increasing second phase (3). In contrast, the perfused mouse pancreas exhibits a weak second phase compared with the first phase in response to glucose stimulation (1,2). Only a few studies have addressed the mechanisms underlying these species differences. Among others, it has been proposed that the divergence may reside in the cellular levels of cyclic adenosine monophosphate (cAMP) (4) or inositol phosphate (5).

In the consensus model of glucose-stimulated insulin secretion, ATP is generated by mitochondrial metabolism, promoting an increase in cytosolic Ca^{2+} concentration, which constitutes the main trigger initiating insulin exocytosis (6–8). Glucose generates additional factors participating in the stimulation of insulin secretion (8–11). We have previously proposed that, during glucose stimulation, glutamate is generated by the mitochondria and plays a role in stimulus-secretion coupling (12,13). Under conditions of permissive, clamped cytosolic Ca^{2+} levels in permeabilized cells, glutamate directly stimulates insulin exocytosis independently of mitochondrial function, suggesting that glutamate acts as an intracellular factor coupling glucose metabolism to insulin secretion (12). Glutamate can be formed in the mitochondria from the tricarboxylic acid cycle intermediate α-ketoglutarate by glutamate dehydrogenase (GDH) (14). As a glucose-derived metabolic coupling factor, glutamate is postulated to participate mainly in the second phase of insulin secretion.

Glutamate does not penetrate efficiently into islet cells and is not insulinotropic (15), although induction of transient insulin release has been reported (16). Investigators have therefore used cell-permeant methyl ester derivatives. An insulinotropic action of glutamic acid dimethyl ester in the presence of intermediate glucose concentrations has been reported in the perfused rat pancreas (17). In the present study, we have examined the hypothesis that intracellular glutamate provision might be rate-limiting for full development of the second phase of insulin secretion. Deficient second phases are observed in the rat at intermediate glucose concentrations or in mice even at optimal glucose concentrations. Our results show that, in these incomplete secretory responses, glutamic acid dimethyl ester supplementation induces the development of a full, sustained phase of insulin secretion.

RESEARCH DESIGN AND METHODS

Insulin secretion. Male Wistar rats and BALB/c mice were anesthetized with sodium pentothal 100 mg/kg body wt i.p. and prepared for pancreas perfusion as previously described (18). The pancreas was perfused at 37°C with modified Krebs-Ringer HEPES buffer supplemented with the indicated concentrations of glucose. The perfusion was maintained at 5 ml/min for rats and 1.5 ml/min for mice. The pancreatic effluent of the first 30 min of perfusion with basal glucose (2.8 mmol/l) was not collected. After this equilibration period, the effluent was collected in 1-min fractions from a catheter placed in the portal vein. The insulin content of each fraction was determined by radioimmunoassay (12). Where mentioned, the area under the curve (AUC) was calculated after subtraction of basal release determined by the first 15 min of fraction collection. Differences in insulin secretion between groups were analyzed by Student's t test.

Immunoblotting. Pancreatic islets were obtained from a human donor (12) or isolated from rats and mice by collagenase digestion as previously described (19). They were centrifuged, resuspended in lysis buffer, and sonicated before protein determination (Bradford's assay). Immunoblotting was performed after SDS-PAGE using 5 μg proteins of islet extract per lane or standard of GDH (Roche Diagnostics, Rotkreuz, Switzerland) on 11% gel. Proteins were transferred onto nitrocellulose membrane and incubated overnight at 4°C in the presence of rabbit anti-GDH polyclonal antibody (1:5,000) raised against bovine GDH (Rockland, Gilbertsville, PA). The membrane was then incubated for 1 h at room temperature with donkey anti–rabbit IgG

From the Division of Clinical Biochemistry, Department of Internal Medicine, University Medical Center, Geneva, Switzerland.

Address correspondence and reprint requests to pierre.maechler@medecine.unige.ch.

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AUC, area under the curve; dmGlut, L-glutamic acid dimethyl ester; GDH, glutamate dehydrogenase.

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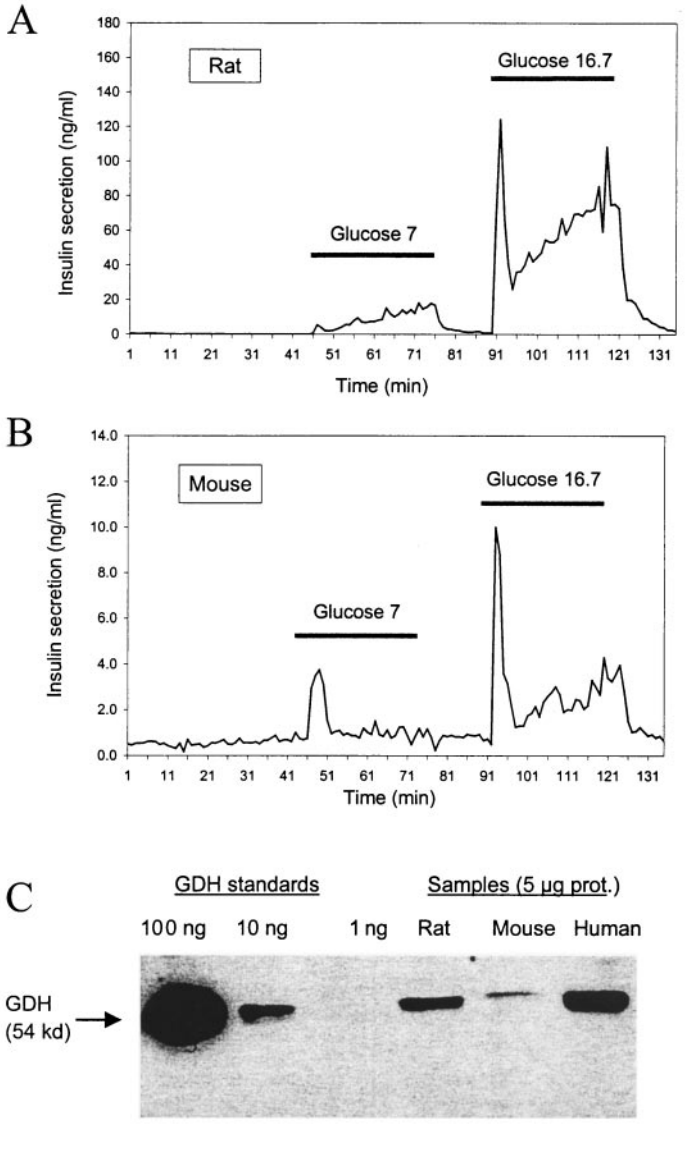

FIG. 1. *A* and *B*: Control in situ pancreatic perfusions. The pancreas was perfused at 5 ml/min for rat (*A*) and 1.5 ml/min for mouse (*B*). After a 30-min equilibration period at basal 2.8 mmol/l glucose, the effluent was collected in 1-min fractions from a catheter placed in the portal vein. The pancreas was perfused sequentially at different glucose concentrations, first at 2.8 mmol/l for 45 min, next at 7.0 mmol/l for 30 min, then again at 2.8 mmol/l for 15 min followed by a 30-min stimulation at 16.7 mmol/l, and finally at 2.8 mmol/l for 15 min. *C*: Immunoblotting for GDH. Islets from rats, mice, and a human donor were isolated and immunoblotting was performed after SDS-PAGE using 5 µg proteins of islet extract per lane or standard of GDH. The data presented in panels *A–C* are representative of three independent experiments each.

antibody (1:5,000) conjugated to horseradish peroxidase (ECL; Amersham, Zürich, Switzerland), and the GDH protein was revealed by chemiluminescence (Pierce, Rockford, IL).

RESULTS

Control in situ pancreatic perfusions. Control experiments show the typical species-specific patterns of the dynamics of insulin secretion in the perfused pancreas stimulated with glucose (Fig. 1). In the rat, raising glucose from basal 2.8 to 16.7 mmol/l glucose induced a transient first phase followed by a sustained second phase with similar amplitudes (Fig. 1*A*). In the mouse, the same high glucose concentration (16.7 mmol/l) elicited a rapid first phase but only a very weak second phase (Fig. 1*B*). At 7.0 mmol/l glucose, the amplitudes of insulin release were smaller but the species difference was also observed, i.e., absence of the second phase in the mouse.

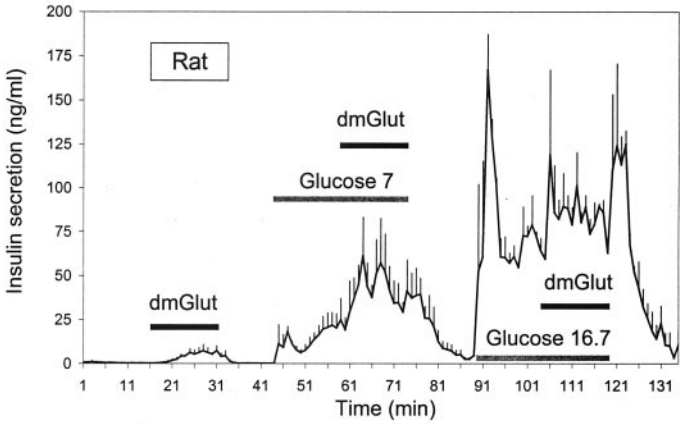

FIG. 2. Rat pancreatic perfusion. The pancreata from male Wistar rats were perfused at 5 ml/min, first for a 30-min equilibration period at basal 2.8 mmol/l glucose. The effluent was then collected in 1-min fractions from a catheter placed in the portal vein. The pancreas was perfused sequentially at different glucose concentrations without or with 5.0 mmol/l dmGlut. Each condition or combination was applied for 15 min. Values are means ± SE of three independent experiments.

GDH levels in pancreatic islets. Islets from rats, mice, and a human donor were isolated, and the equivalent of 5 µg protein was subjected to immunoblotting for quantification of GDH. Human and rat samples exhibited similar levels of GDH, whereas the signal from the mouse was noticeably weaker (Fig. 1*C*). Compared with the standards of GDH, the band from rat islet extracts was similar to the standard of 10 ng GDH. The intensity of the mouse band was situated between the standards 10 ng and 1 ng, the latter being undetectable. GDH concentration in rat islets could be estimated roughly at 0.2% of total proteins. This concentration is only indicative, since the reactivity of the antibody raised against bovine GDH may vary slightly between species, although this mitochondrial enzyme is extremely well conserved.

Effect of glutamic acid dimethyl ester on insulin secretion. Rat pancreatic perfusion was performed at the rate of 5 ml/min and resulted in a basal insulin release of 0.54 ± 0.34 ng/ml per minute at 2.8 mmol/l glucose. Addition of 5 mmol/l L-glutamic acid dimethyl ester (dmGlut) at low glucose (2.8 mmol/l) for 15 min induced only marginal, slow-onset insulin secretion over basal release (Fig. 2). Stimulation with 7.0 mmol/l glucose for 15 min resulted in biphasic insulin secretion with an AUC of 219.2 ± 76.6 ng/ml, and supplementation with 5 mmol/l dmGlut for the next 15 min induced a 3.6-fold increase in the AUC (786.2 ± 201.2 ng/ml, $P < 0.05$). After a 15-min period at low glucose (2.8 mmol/l), 16.7 mmol/l glucose stimulated insulin secretion, with a pronounced first and second phase (AUC = 1,194 ± 176 ng/ml for 15 min, 5.5-fold vs. 7.0 mmol/l glucose, $P < 0.05$). Application of 5 mmol/l dmGlut on top of high glucose for the subsequent 15 min resulted in a further modest 1.6-fold elevation of insulin release (AUC = 1,971 ± 218 ng/ml for 15 min, $P < 0.01$).

In the mouse, pancreatic perfusion of 5 mmol/l dmGlut at 2.8 mmol/l glucose did not modify basal insulin release

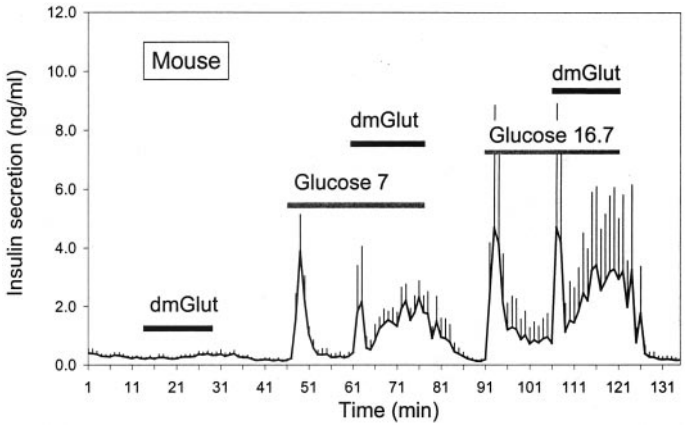

FIG. 3. Mouse pancreatic perfusion. The pancreata from male BALB/c mice were perfused at 1.5 ml/min, first for a 30-min equilibration period at basal 2.8mmol/l glucose. The effluent was then collected in 1-min fractions from a catheter placed in the portal vein. The pancreas was perfused sequentially at different glucose concentrations without or with 5.0 mmol/l dmGlut. Each condition or combination was applied for 15 min. Values are means ± SE of three independent experiments.

(Fig. 3). In the presence of 7.0 mmol/l glucose, there was a transient, first phase–like stimulation of insulin secretion (AUC = 8.4 ± 4.0 ng/ml for 15 min), without establishment of a second phase. Addition of dmGlut (5 mmol/l) in the continuous presence of 7.0 mmol/l glucose induced biphasic, sustained insulin release (AUC = 2.5-fold compared with 7.0 mmol/l glucose alone, $P < 0.05$). Stimulation with 16.7 mmol/l glucose resulted in a similar pattern of insulin secretion both in the absence and the presence of dmGlut.

DISCUSSION

The kinetics of insulin secretion from the in situ perfused pancreas were studied in rats and mice. Our control experiments confirmed the species difference in insulin release, with typical transient first and sustained second phases in rats compared with a transient first phase followed by only a very weak second phase in mice (1,2). Biphasic insulin secretion in response to stimulatory glucose is also observed in humans (20), rendering the mouse questionable as a relevant model. However, the lack of a sustained second phase in mice provides a useful tool for the dissection of mechanisms involved in the establishment of prolonged and robust insulin release. This might also be useful in the search for new treatments of impaired insulin secretion.

Studying the species difference, it has been proposed that a larger production of cAMP in rat β-cells may account for the pronounced second phase of insulin secretion compared with mice (4). Zawalich et al. (5) have shown that the increase in inositol phosphates upon glucose stimulation was much more marked in rat compared with mouse islets, correlating with phospholipase C expression. Differences between rat and mouse pancreatic islets were also reported regarding membrane potential oscillations and changes in cytosolic Ca^{2+} concentration in response to glucose (21).

In the present report, we have shown that the expression of GDH is lower in islets isolated from mice compared with those of rats or humans. This mitochondrial enzyme can generate glutamate from the tricarboxylic acid cycle intermediate α-ketoglutarate (14). In conditions of permissive cytosolic Ca^{2+} concentrations, glutamate stimulated insulin exocytosis in permeabilized insulinoma cells (12). This led to the proposal that glutamate plays a role as an intracellular factor in the β-cell (22). Glucose-derived glutamate might participate in the potentiation of insulin secretion rather than its initiation caused by a rise in cytosolic Ca^{2+}. In the perfused rat pancreas, in the absence of glucose, non-nutrient stimulation of insulin secretion by a sulfonylurea was defective, but it was restored by the addition of dmGlut (17). It was further proposed that dmGlut might bypass glucose metabolism to enhance the insulinotropic action of sulfonylureas (23).

We have shown here that the incomplete second phase of insulin secretion observed in the perfused rat pancreas at intermediate glucose concentration was overcome by dmGlut supplementation. In mice, insulin secretion upon glucose stimulation was essentially transient, with a very weak second phase even at optimal glucose concentrations. Addition of dmGlut caused a biphasic release pattern at permissive glucose concentrations but had no effect at basal glucose. The reason for the biphasic response induced by dmGlut in the mouse, contrasting with the monophasic profile in the rat, is unclear. It could be speculated that the readily releasable pool of insulin granules accumulates and is larger in the mouse than in the rat because of the marked difference in the glucose-induced insulin secretion rate.

The present results are in accordance with a role for glutamate as a metabolic coupling factor potentiating rather than initiating insulin secretion. Conflicting data about the glutamate levels in insulin-secreting cells stimulated by glucose exist in the literature (22). Therefore, future work should attempt to clarify the role of intracellular glutamate in the rat and mouse β-cell.

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