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Extracellular Calcium and Glucose-Induced Insulin Release During Fasting in Isolated Perfused Rat Pancreas

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The effects of different concentrations of Ca^{2+} (2.5 or 7.5 mM) upon the kinetic and dynamic aspects of glucose-induced insulin release were investigated in the isolated perfused pancreas of fed and 4-day fasted rats. The curve relating the rate of insulin release to the glucose concentration (2.75-33.4 mM) showed a sigmoidal shape and was similar at both Ca^{2+} concentrations in the fed state. Pancreas of fasted animals showed a diminished insulin secretion and the kinetic pattern was linear throughout the whole range of glucose concentrations studied at 2.5 mM Ca^{2+}. Perfusion of pancreas from fasted rats with glucose at 7.5 mM Ca^{2+} resulted in an increase of insulin secretion but the kinetic behaviour continued to be linear. In contrast, pancreas of 4-day fasted plus 2-day refed rats showed a kinetic of glucose-induced insulin secretion with the cooperative mechanisms observed in fed control animals. It is concluded that changes in Ca^{2+} utilization by the pancreatic beta cell play little role in the decreased insulin release observed in the fasted state.

Stimulation of insulin secretion by glucose and by various other secretagogues depends on the presence of extracellular Ca^{2+} (7, 16, 18, 23). All insulinotropic agents so far investigated such as hexoses, glyceraldehyde, amino acids, cyclic AMP, theophylline, K^+ and sulphonylureas have been found to affect Ca^{2+} handling in the β-cell (4, 5, 14, 16) by causing an increased Ca^{2+} uptake and/or redistribution of intracellular Ca^{2+}. This cation is essential for the proper functioning of the secretory machinery of β-cell but the exact mode and site of its action within the process of stimulus-secretion coupling are not well known.

Ca^{2+} itself, in the absence of any other secretagogue, has been reported to elicit

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insulin release in the isolated perfused rat pancreas (8). However, an increased uptake of Ca^{2+} by pancreatic islets may not alone suffice to evoke insulin secretion. This suggestion arises from the observation that fructose, devoid of insulin-releasing activity, is as effective as glucose in stimulating Ca^{2+} uptake by islets of *ob/ob* mice (15). Furthermore, various α-keto-carboxylic acids stimulate $^{45}Ca^{2+}$ uptake by mouse pancreatic islets without stimulating insulin release (19). Therefore, other factors in addition to an increase of Ca^{2+} uptake may play an important role in insulin secretion. One such factor could be the intracellular distribution of Ca^{2+} after its initial entry in the β-cell.

Glucose-induced insulin release is decreased in the fasting state as shown by both *in vivo* and *in vitro* studies in man (6, 21) and animals (1, 17, 20, 22, 24, 30). In fasted rats, both phases of insulin secretion diminish and the glucose concentration-response curve shifts to the right with respect to fed controls (1, 31). This defect is abolished by refeeding (1, 10, 11, 30). However, the mechanism of the altered β-cell response to glucose in the fasted state is poorly understood. In this context, it has been reported that fasting causes a partial depletion of islet Ca^{2+} content (32). It is not known if there exists a relationship between islet Ca^{2+} handling and the depressing effect of fasting on β-cell response to glucose. As an approximation to this problem, the present work studies the effect of increasing extracellular Ca^{2+} concentrations on glucose-induced insulin release from isolated perfused pancreas of fed and fasted rats.

Materials and Methods

Male Wistar rats weighing between 280-300 g were divided into three groups. The first group (control) was fed a standard laboratory diet. The second group was starved for 4 days. The third group was starved for 4 days and then refed for 2 days. Water was supplied *ad libitum*. The pancreas was isolated as previously described (9) and placed in a perfusion chamber at 37° C. Perfusion medium consisted of Krebs-Ringer-Bicarbonate (KRB) buffer (pH 7.35) supplemented with 0.5% bovine serum albumin (BSA) (Behringwerke, Marburg-Lahn) and gassed with a mixture of O_2 and CO_2 (95:5). Perfusion flow rate was adjusted to 2.5 ml/min, which resulted in a perfusion pressure of 20-30 mm Hg. After a 10 min equilibration period, the effluent was collected at 2 min intervals and stored immediately at —20° C. The concentrations of glucose in the perfusion medium were 2.75, 5.5, 16.7 or 33.4 mM and those of Ca^{2+} were 2.5 mM (the normal level in KRB buffer) or 7.5 mM. The concentration of insulin (IRI) was measured as in (28) and expressed as mean μU IRI/min when studying dynamic aspects. Mean ± S.E.M. integrated insulin output per minute at each glucose concentration was represented for kinetic purposes. Student's *t* test was used to determine significance.

Results

At normal extracellular Ca^{2+} concentration (2.5 mM), the relationship between glucose concentration and the rate of insulin release was sigmoidal in pancreas of fed rats (fig. 1, top). Glucose concentrations below 5.5 mM did not practically influence the secretory rate. The largest increase in insulin secretion occurred between 5.5 and 16.7 mM glucose, indicating that β-cell is acutely sensitive to small changes in glucose concentration within the physiological range. The curve tended to reach a plateau between 16.7-33.4 mM glucose. Half-maximal rate of insulin secretion required about 15 mM glucose. Figure 2 shows the corresponding dynamics of glucose-induced insulin release.

In pancreas of fasted rats, the integrat-

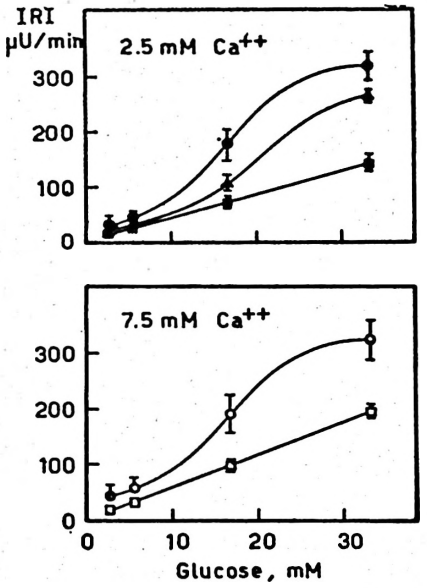

Fig. 1. *Effect of extracellular Ca^{2+} on the relationship between glucose concentration and insulin release.*
Isolated pancreas were perfused with glucose in KRB buffer containing 2.5 (upper pannel) or 7.5 (lower pannel) mM Ca^{2+}. Symbols correspond to fed (●, ○), 4-days fasted (■, □) and 4-days fasted plus 2-days refed (▲) rats. Each point is the mean ± S.E.M. integrated insulin output per minute at each glucose concentration and corresponds to 4-5 experiments.

ed insulin output diminished with respect to that of fed animals (fig. 1, top). More interesting, there was a linear relationship between the rate of insulin release and glucose concentration for the whole range tested. The corresponding dynamics of insulin release at 2.5 mM Ca^{2+} appear in figure 2. It is noticeable the characteristically modest and delayed response observed at 16.7 mM glucose.

In contrast to the fasting condition, pancreas of rats fasted for 4 and then refed for 2 days showed a pattern of insulin secretion that returned partially to control values (fig. 3). The shape of the curve representing the kinetic of insulin secretion was again sigmoidal (fig. 1, top).

When Ca^{2+} in the perfusion medium was increased from 2.5 to 7.5 mM, glucose-induced insulin release remained unchanged in pancreas from fed rats (fig. 4). There were no significant differences between the integrated insulin outputs at either 2.5 (fig. 1, top) or 7.5 (fig. 1, bottom) mM Ca^{2+}, as shown by the corresponding sigmoidal curves representing the kinetics of insulin release.

This was not the case in pancreas of 4 days fasted rats. In this condition, the insulin secretory response to glucose was higher when Ca^{2+} concentration in the perfusion medium increased from 2.5 to 7.5 mM. In fact, the integrated insulin outputs observed at 7.5 mM Ca^{2+} (fig. 1,

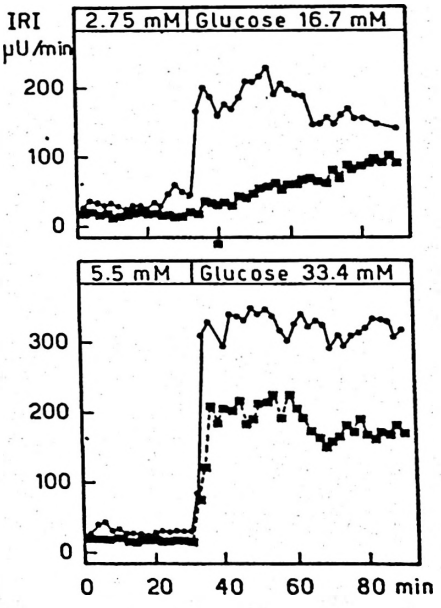

Fig. 2. *Dynamics of glucose-induced insulin release at 2.5 mM Ca^{2+} in the isolated perfused pancreas of fed and 4-days fasted rats.*
Upper panel: 2.75 and 16.7 mM glucose. Lower panel: 5.5 and 33.4 mM glucose. Symbols correspond to fed (●) and 4-days fasted (■) rats. Each point is the mean of 5 experiments.

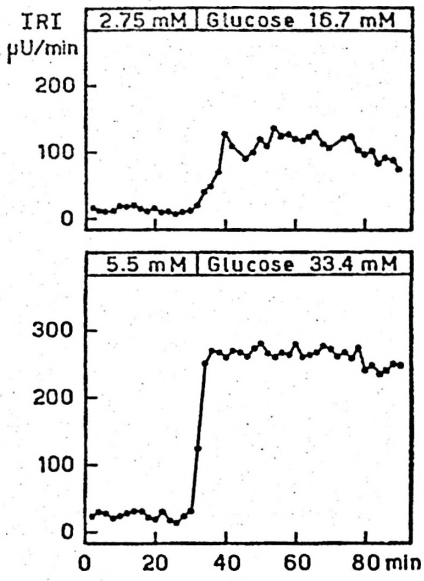

Fig. 3. *Dynamics of glucose-induced insulin release at 2.5 mM Ca^{2+} in the isolated perfused pancreas of 4-days fasted plus 2-days refed rats.*
Upper panel: 2.75 and 16.7 mM glucose. Lower panel: 5.5 and 33.4 mM glucose. Each point is the mean of 4 experiments.

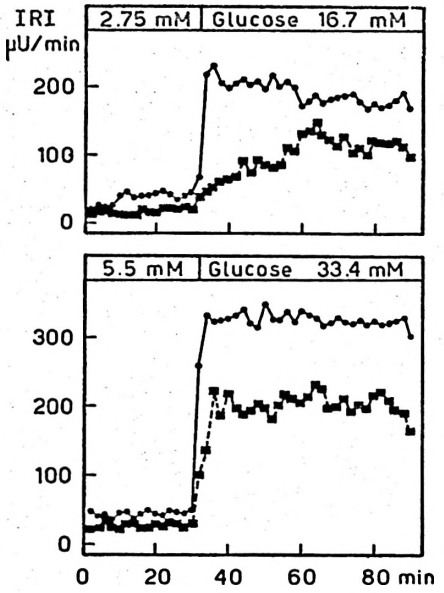

Fig. 4. *Dynamics of glucose-induced insulin release at 7.5 mM Ca^{2+} in the isolated perfused pancreas of fed and 4-days fasted rats.*
Upper panel: 2.75 and 16.7 mM glucose. Lower panel: 5.5 and 33.4 mM glucose. Symbols correspond to fed (●) and 4 days fasted (■) rats. Each point is the mean of 4 experiments.

bottom) were significantly higher than those at 2.5 mM Ca^{2+} (fig. 1, top) at 16.7 ($p < 0.05$) and 33.4 ($p < 0.025$) mM glucose. In spite of this increase, glucose-induced insulin secretion at 7.5 mM Ca^{2+} showed a linear kinetic as it did at 2.5 mM Ca^{2+}. Figure 4 represents the dynamics of insulin release in that condition.

Discussion

Current concepts concerning the mechanism by which glucose initiates insulin release propose that: *a)* glucose is metabolized with the formation of specific metabolites or cofactors which initiate insulin secretion, or *b)* specific receptors for glucose exist on the pancreatic β-cell membrane (see 12 for a recent review). Though no definitive conclusion can be given and both possibilities are not mutually exclusive, a considerable body of evidence indicates that the glucose molecule has to be metabolized in the β-cell to initiate insulin release. In fact, the curves relating, the rate of insulin release and glucose utilization by the β-cell to the extracellular concentration of glucose possess the same sigmoidal relationship (3) as it do other metabolic parameters in the islets such as glucose oxidation (2), glucose-6-phosphate content (2), lactate formation (29) and oxygen consumption (13) and other glucose-dependent islet functions including membrane electrical activity (27) and uptake of $^{45}Ca^{2+}$ (26).

As it was expected, the curve relating the kinetic of insulin release to the glucose concentrations used in pancreas of fed rats in the present work was a sig-

moid. This result agrees with the substrate-site model of insulin release but it does not exclude the receptor or regulator-side model (12), as discussed above. The reduction of insulin release and the shift to the right in the glucose concentration-response curve observed in pancreas of 4 days fasted animals can consequently be explained by a decreased activity of enzymes of islet glycolytic pathway (25) or by a decreased affinity of the hypothetical glucoreceptor molecule on the surface of the β-cell membrane. However, modifications of other factors such as adenylate cyclase-cyclic AMP system and related protein phosphorylation-dephosphorylation (11, 31) or handling of Ca^{2+} by the islets (14, 18) cannot be excluded of having a role in the decreased insulin release observed in the fasted state.

It is interesting to note the linearity of the kinetic of insulin release observed in pancreas of fasted rats throughout the whole range of glucose concentrations studied (2.75-33.4 mM). It means a lack of cooperative phenomena in contrast to results obtained in fed rats. Refeeding of fasted animals resulted in the recuperation of insulin secretory activity as well as of sigmoidal kinetic. These findings suggest that fasting dramatically affects the affinity characteristics of the system that initiates insulin release.

The precise function of Ca^{2+} in the insulin-discharge mechanism remains to be elucidated. Current concepts suggest that a rise in intracellular Ca^{2+} concentration is a major controlling influence in stimulus-secretion coupling in β-cell (18, 23). A sufficient supply of extracellular Ca^{2+} is thus required for maintenance of the Ca^{2+} cellular pool mediating insulin release. This dependence indicates a permissive role for extracellular Ca^{2+}, further supported by present results on that glucose-induced insulin secretion in pancreas of fed rats was similar at 2.5 and 7.5 mM Ca^{2+} in the perfusion medium. Pancreas of fasted rats, in contrast to those of fed animals, did secrete more insulin at high Ca^{2+} concentration.

These results indicate that fasting increases the threshold concentration of Ca^{2+} at which glucose elicits insulin secretion. Then, high extracellular Ca^{2+} concentrations may sufficiently increase the amount of Ca^{2+} accumulated in the β-cell to eventually trigger insulin release, as previously suggested by others (18, 23). It is also interesting to note that islet Ca^{2+} appears to be diminished in the fasted state (32).

The kinetic of glucose-induced insulin release in pancreas of fasted rats did not show cooperative effects even at a level of Ca^{2+} in the perfusion medium as high as 7.5 mM. This suggests that the diminished secretory response of β-cell to glucose in the fasted state is primarily due to a defect in recognition of glucose and/or in the normal glucose-dependent signal responsible for the stimulation of insulin release. Then, changes in Ca^{2+} handling by the β-cell appear to play little role in the pattern of glucose-induced insulin secretion observed in pancreas of fasted rats. This conclusion is similar to that reported recently (22) for changes in islet adenylate cyclase-cyclic AMP system and its related protein phosphorylation system observed in the same fasting condition.

Resumen

Se han estudiado los aspectos cinéticos y dinámicos de la secreción de insulina inducida por glucosa a diferentes concentraciones de Ca^{2+} (2,5 ó 7,5 mM) en páncreas aislado y perfundido de ratas controles alimentadas o sometidas a 4 días de ayuno. La curva que relaciona la secreción de insulina con las diferentes concentraciones de glucosa utilizadas (2,75-33,4 mM) es de tipo sigmoidal en la situación de alimentación, no existiendo diferencias entre las dos concentraciones de Ca^{2+}. Los páncreas de animales sometidos a ayuno muestran una secreción de insulina disminuida y el modelo cinético es lineal a lo largo de todo el rango de concentraciones de glucosa

utilizadas en presencia de Ca^{2+} 2,5 mM. La perfusión de páncreas de animales sometidos a ayuno con glucosa en presencia de Ca^{2+} 7,5 mM da lugar a un incremento de la secreción de insulina, aunque la cinética continúa siendo lineal. En contraste, los páncreas de ratas sometidas a 4 días de ayuno y realimentadas a continuación durante 2 días dan lugar a una cinética de secreción de insulina con los mecanismos cooperativos típicos de los animales controles alimentados. No parece que los cambios en la utilización del Ca^{2+} por la célula beta pancreática jueguen un papel importante en la secreción disminuida de insulina que se observa en condiciones de ayuno.

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