Fast Reversibility of Glucose-Induced Desensitization in Rat Pancreatic Islets

Evidence for an Involvement of Ionic Fluxes

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The present study was done to achieve a better understanding of the role of ionic flux alterations in glucose-induced desensitization of pancreatic β-cells. Moreover, we investigated the reversibility of glucose-induced desensitization after different times of exposure to high glucose to ascertain the time necessary for desensitization reversal and to determine whether it depends on the length of high glucose exposure. Glucose desensitization was obtained by incubating rat pancreatic islets for 6 h in CMRL medium containing 16.7 mmol/l glucose. At the end of this period, insulin release, ^{86}Rb efflux, and ^{45}Ca uptake were measured in parallel experiments. In islets cultured at 16.7 mmol/l glucose, maximal glucose-induced insulin release was reduced (848 ± 97 pg · islet^{-1} · 30 min^{-1}) in comparison to islets incubated at 5.5 mmol/l glucose (1,436 ± 144, $n = 7$, $P < 0.01$). In contrast, insulin content was similar in the two groups, being 41.0 ± 2.7 and 47.8 ± 2.2 ng/islet in islets exposed to 16.7 or 5.5 mmol/l glucose, respectively ($P = 0.167$). The effect of glucose on both ^{86}Rb efflux and ^{45}Ca uptake was also significantly reduced in 16.7 mmol/l glucose–cultured islets. ^{86}Rb efflux was inhibited only 19 ± 4% in islets cultured at high glucose with respect to 56 ± 7% in control islets ($n = 5$, $P < 0.001$). ^{45}Ca uptake was 10.5 ± 1.7 pmol/islet (mean ± SE, $n = 9$) in islets cultured at high glucose with respect to 19.7 ± 2.4 pmol/islet in control islets ($P < 0.001$). In contrast, the effect of glyburide (10 μmol/l) on insulin release, ^{86}Rb efflux, and ^{45}Ca uptake was similar in islets exposed to 5.5 or 16.7 mmol/l glucose. All the abnormalities observed in islets cultured at 16.7 mmol/l glucose were promptly and simultaneously reversible after islets were transferred in culture medium at 5.5 mmol/l glucose; both insulin secretion and glucose effects on ^{86}Rb efflux and ^{45}Ca uptake returned to values similar to control islets within 5 min. Also, islets exposed to high glucose for a longer period (24 h) recovered from both secretory and ionic abnormalities after 5 min of incubation in CMRL medium at 5.5 mmol/l glucose. Reversal from glucose desensitization was slower (45–60 min) when islets were incubated at 5.5 mmol/l glucose in Krebs-Ringer HEPES buffer instead of CMRL medium. The present data suggest that ion flux and consequent membrane-potential changes play a key role in the mechanism leading to glucose-induced desensitization of pancreatic β-cells. Because a normal response to glyburide was observed in islets exposed to high glucose, a proximal signal defect for closure of K^+ channels rather than an intrinsic defect in the channel is likely. *Diabetes* 502–506, 1996

Prolonged exposure of pancreatic islets and isolated β-cells to elevated glucose concentrations induces a state of reduced responsiveness to glucose. This phenomenon has been called glucose desensitization and has been observed both in vivo and in vitro in rodent and human islets (1–8). The mechanisms underlying this defect are not completely understood (1–3,9,10), and this has contributed to some confusion in defining the phenomenon. The term desensitization should be used to indicate a temporary, readily induced, and reversible state of β-cell refractoriness, while the term glucotoxicity indicates an irreversible cellular damage that occurs after a more prolonged exposure to high glucose (11).

We have used an in vitro system to investigate the mechanisms responsible for the desensitization to glucose. The functional alterations caused by exposure to high glucose in vitro are likely to be distal to the first steps of glucose metabolism, since glucose transport and phosphorylation are not reduced in islets desensitized to glucose (12). Ionic fluxes are major candidates involved in the mechanism of islet desensitization; we previously observed that ^{86}Rb efflux (a marker of the ATP-dependent K^+ channels) is altered in rat islets exposed for 24 h to 16.7 mmol/l glucose (13). Moreover, recent studies have demonstrated that diazoxide, an agent that interacts with the ATP-dependent K^+ channels and blocks membrane depolarization, may prevent desensitization (14,15).

The present study was undertaken to further investigate ionic flux alterations and their role in β-cell glucose desensitization in vitro. Moreover, we investigated the time required for reversal of glucose-induced desensitization and whether it depends on the length of the exposure to high glucose. In parallel experiments, we measured insulin release, ^{86}Rb efflux, and ^{45}Ca uptake in islets exposed to high glucose for different periods of time before and after returning the glucose medium concentration to normal. A primary role of ion flux alterations in determining glucose-induced pancreatic islet desensitization in vitro and its reversibility emerges from these studies.

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RESEARCH DESIGN AND METHODS

Materials. Crude collagenase was obtained from Boehringer Mannheim (Mannheim, Germany). Culture medium CMRL-1066, heat-inactivated fetal calf serum, glutamine, and gentamycin were obtained from Gibco (Glasgow, U.K.). $^{45}CaCl_2$ (25 mCi/mg) and ^{86}Rb were obtained from Amersham (Amersham, Bucks, U.K.). Silicone oil (density 1.040) was obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

Islet preparation and culture conditions. Pancreatic islets were isolated by the collagenase method from 200- to 250-g fed male Wistar rats injected with 0.2 ml i.p. of a 0.2% pilocarpine solution 2 h before killing by decapitation (6). Purified islets were cultured overnight at 5.5 mmol/l glucose in CMRL-1066 medium containing 10% fetal calf serum, 2 mmol/l L-glutamine, and gentamycin at 37°C in a 95% air/5% CO_2 atmosphere and then at 5.5 or 16.7 mmol/l glucose for 6 or 24 h. In some experiments, diazoxide (100 μmol/l) was added together with 16.7 mmol/l glucose. After this incubation period, insulin secretion, rubidium efflux, and $^{45}Ca^{2+}$ uptake were studied in parallel experiments.

Insulin secretion in static experiments. Islets that had been cultured at 5.5 or 16.7 mmol/l glucose were washed twice in Krebs-Ringer HEPES buffer (115 mmol/l NaCl, 5.4 mmol/l KCl, 2.38 mmol/l $CaCl_2$, 0.8 mmol/l $MgSO_4$, 1 mmol/l Na_2HPO_4, 10 mmol/l HEPES, 0.5% BSA, pH 7.35) containing the same glucose concentration used during the culture period (5.5 or 16.7 mmol/l, respectively). Groups of five purified islets were then incubated with glucose (0–22 mmol/l) or glyburide (10 μmol/l) for 30 min at 37°C. Insulin in the medium and in the acid-alcohol extract of islets was then measured by radioimmunoassay (6). Results are expressed as insulin released in the medium (pg · islet^{-1} · 30 min^{-1}).

Insulin secretion and ^{86}Rb efflux in perifusion experiments. The $^{86}Rb^+$ efflux and the insulin release kinetic were studied in a perifusion system as previously described (13). Islets were perifused at a flow rate of 1 ml/min at 37°C in a Krebs-Ringer HEPES buffer containing 5.5 mmol/l glucose for 5 min. Then glucose concentration in the perifusion buffer was raised to 22.2 mmol/l or glyburide (10 μmol/l) was added. Effluent fractions were collected at 1-min intervals, and aliquots were analyzed for both $^{86}Rb^+$ radioactivity and insulin content. Insulin secretion was expressed as insulin released in the medium (picogram per islet per minute), and $^{86}Rb^+$ efflux was expressed as fractional efflux of $^{86}Rb^+$ ($^{86}Rb^+$ released per minute divided by $^{86}Rb^+$ retained in the islets at that time) (13).

^{45}Ca uptake. $^{45}Ca^{2+}$ uptake was measured according to the method described by Henquin and Lambert (16). Islets were washed three times with buffer containing the same glucose concentration used during the culture period (5.5 or 16.7 mmol/l, respectively). Groups of 10 islets were transferred into a 50-μl Krebs-bicarbonate buffer modified (17) and layered on silicone oil. The uptake period was started by adding 50 μl of medium containing $^{45}Ca^{2+}$ (2.5 mmol/l), glucose (final concentration 5.5 or 22.2 mmol/l), or glyburide (10 μmol/l in the presence of 5.5 mmol/l glucose). The reaction was stopped by centrifuging the islets for 2 min in a microfuge (Beckman, Palo Alto, CA) through the layer of silicone oil. The bottoms of the 400-μl tubes (Beckman) were then cut and the radioactivity of the pellet counted by liquid scintillation. Tubes without islets were run as blanks. The uptake of [U-^{14}C]sucrose was measured to correct for label in the extracellular space.

Reversibility of the glucose-induced alterations. To study the reversibility of islet desensitization due to exposure to high glucose, at the end of the culture period, islets were washed twice and resuspended in CMRL at 5.5 mmol/l glucose. Islets were then incubated at 37°C in a 95% air/5% CO_2 atmosphere for various times, the shortest being 5 min. At the end of this period, insulin secretion, $^{86}Rb^+$ efflux, and $^{45}Ca^{2+}$ uptake were studied in parallel experiments.

Statistical analysis. Statistical significance was assessed by the Student's *t* test for unpaired comparison.

RESULTS

Insulin release. When pancreatic islets were exposed to 16.7 mmol/l glucose, a significant reduction of the maximally glucose-induced insulin release was observed after 6 h (Fig. 1). Insulin release stimulated by 22.2 mmol/l glucose was 1,436 ± 144 and 848 ± 97 pg · islet^{-1} · 30 min^{-1} (mean ± SE, n = 7) in islets exposed to 5.5 mmol/l glucose or 16.7 mmol/l glucose, respectively (P < 0.01). This secretory defect in islets exposed to high glucose was prevented by the contemporary presence of diazoxide (100 μmol/l) (Fig. 1). In contrast, insulin release in response to glyburide (in the presence of 5.5 mmol/l glucose) was similar in the two groups of islets (1,241 ± 78 and 1,233 ± 84 pg · islet^{-1} · 30 min^{-1}, n = 5). Insulin content was 41.0 ± 2.7 ng/islet (n = 19) in islets exposed to 16.7 mmol/l glucose and 47.8 ± 2.2 ng/islet in islets exposed to 5.5 mmol/l glucose (n = 16, P = 0.167 vs. islets cultured at 16.7 mmol/l).

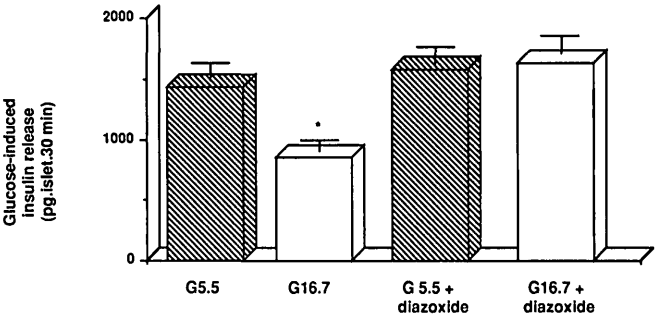

FIG. 1. Glucose-induced insulin release in pancreatic islets exposed for 6 h to 5.5 (▨) or 16.7 (□) mmol/l glucose in the presence or in the absence of diazoxide (100 μmol/l). Results represent the means ± SE of seven separate experiments.

^{86}Rb efflux. In control islets, the rate of $^{86}Rb^+$ efflux declined slowly during the initial period of perifusion with 5.5 mmol/l glucose. A sharp and marked decrease was observed when the glucose concentration was raised to 22.2 mmol/l (Fig. 2*A*). The average fractional $^{86}Rb^+$ efflux declined −56 ± 7% (n = 5). In the same perifusion fractions, insulin release increased from 12 ± 4 (basal condition) to 122 ± 9 pg · islet^{-1} · min^{-1} at the first phase peak and 120 ± 14 at the maximum level of second-phase insulin release (Fig. 2*B*). In contrast, in islets preexposed to 16.7 mmol/l glucose, the initial rate of $^{86}Rb^+$ efflux was significantly lower than in control islets (P < 0.05), even though $^{86}Rb^+$ loading was similar to control islets. Only a small and delayed decrease of $^{86}Rb^+$ efflux was observed when the glucose concentration was increased to 22.2 mmol/l (Fig. 2*B*). $^{86}Rb^+$ efflux decrement was −19 ± 4% (n = 5, P < 0.001 vs. control islets). The same perifusion fractions from islets cultured at high-glucose basal secretion were higher (P < 0.01), and both first- and second-phase glucose-stimulated insulin releases were markedly blunted (67 ± 4 and 62 ± 9 pg · islet^{-1} · min^{-1}, respectively; n = 5, P < 0.01 vs. control islets) (Fig. 2*B*).

At odds with the blunted response to glucose, islets exposed to 16.7 mmol/l glucose exhibited, in response to glyburide, a pattern of $^{86}Rb^+$ efflux and insulin release that was similar to that in control islets exposed to 5.5 mmol/l glucose ($^{86}Rb^+$ efflux decrement of −47 ± 4% and −37 ± 2%, respectively; Fig. 3).

^{45}Ca uptake. In control islets, $^{45}Ca^{2+}$ uptake was 5.2 ± 0.6 pmol · islet^{-1} · 20 min^{-1} (mean ± SE, n = 9) under basal conditions (at 5.5 mmol/l glucose) and increased to 19.7 ± 2.4 at 22.2 mmol/l glucose and to 11.9 ± 0.9 in the presence of glyburide (10 μmol/l). In pancreatic islets preexposed to 16.7 mmol/l glucose, calcium uptake was slightly higher than in control islets under basal conditions (6.9 ± 2.4 pmol · islet^{-1} · 20 min^{-1}) but was significantly reduced at 22.2 mmol/l glucose (10.5 ± 1.7, P < 0.001 vs. control islets). In contrast, $^{45}Ca^{2+}$ uptake in response to glyburide was similar to control islets (12.6 ± 1.0 pmol · islet^{-1} · 20 min^{-1}).

Reversibility of the alterations caused by prolonged exposure to high glucose. After preincubation with either 5.5 or 16.7 mmol/l glucose for 6 h, islets were washed with

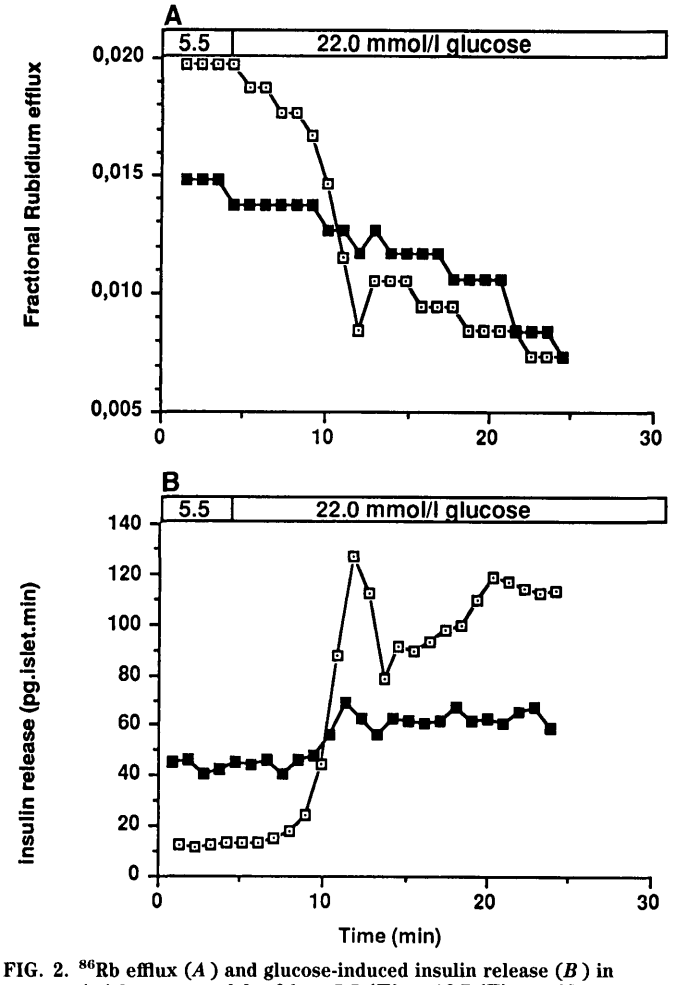

FIG. 2. ^{86}Rb efflux (*A*) and glucose-induced insulin release (*B*) in pancreatic islets exposed for 6 h to 5.5 (□) or 16.7 (■) mmol/l glucose. Groups of 150 islets were incubated for 2 h in CMRL-1066 medium with 0.2 mmol/l $^{86}Rb^+$ at 37°C. After extensive washing, islets were perifused at a flow rate of 1 ml/min at 37°C in $^{86}Rb^+$-free medium. After a 5-min perifusion with buffer containing 5.5 mmol/l glucose to equilibrate the system, the islets were stimulated with 22.2 mmol/l glucose. Perifusate samples were collected at 1-min intervals, and 400-μl aliquots were analyzed for $^{86}Rb^+$ radioactivity (*A*) and insulin release (*B*). A representative of five separate experiments is shown.

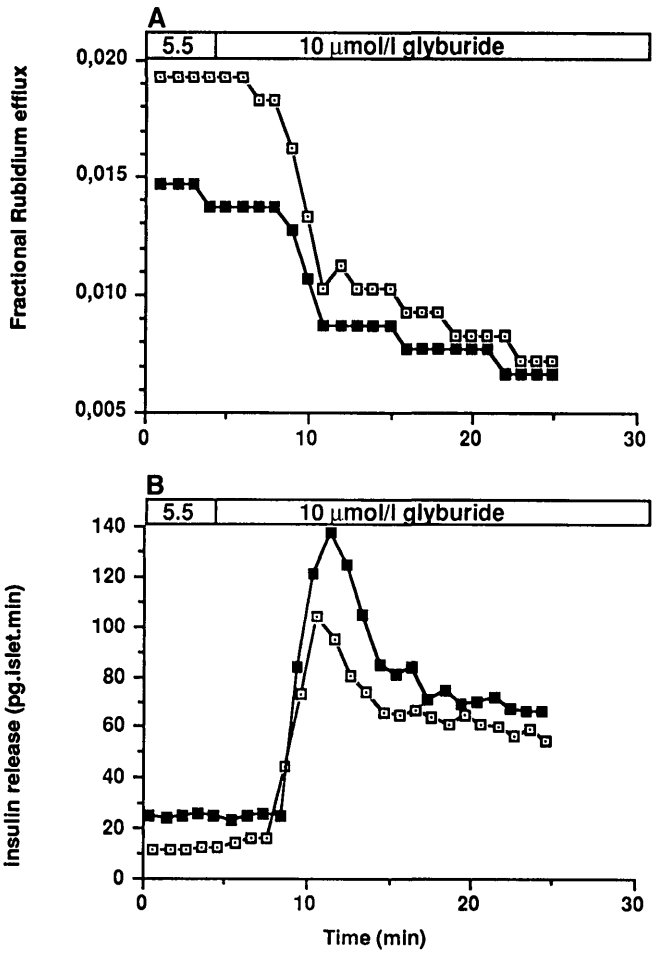

FIG. 3. ^{86}Rb efflux (*A*) and glyburide-induced insulin release (*B*) in pancreatic islets exposed for 6 h to 5.5 (□) or 16.7 (■) mmol/l glucose. Groups of 150 islets were incubated for 2 h in CMRL-1066 medium with 0.2 mmol/l $^{86}Rb^+$ at 37°C. After extensive washing, islets were perifused at a flow rate of 1 ml/min at 37°C in $^{86}Rb^+$-free medium. After a 5-min perifusion with buffer containing 5.5 mmol/l glucose to equilibrate the system, the islets were stimulated with 10 μmol/l glyburide. Perifusate samples were collected at 1-min intervals, and 400-μl aliquots were analyzed for $^{86}Rb^+$ radioactivity (*A*) and insulin release (*B*). A representative of four separate experiments is shown.

the appropriate glucose media and then incubated in CMRL medium at 5.5 mmol/l glucose. All the abnormalities induced by exposure to high glucose were reversible within 5 min of islet exposure to 5.5 mmol/l glucose (Table 1). Insulin secretion was similar to that of control islets. The initial rate of $^{86}Rb^+$ efflux (previously significantly lower in desensitized islets) was similar in the two groups of islets after incubation for 5 min at 5.5 mmol/l glucose, irrespective of the previous exposure to either 5.5 or 16.7 mmol/l glucose (0.020 ± 0.003 and 0.019 ± 0.003, n = 5). In addition, in both groups of islets, the pattern of $^{86}Rb^+$ efflux and the fractional efflux decrement in response to 22.2 mmol/l glucose was similar (−59 ± 3 and −58 ± 6%, respectively, n = 5; Fig. 4). Under the same experimental conditions, glucose-stimulated $^{45}Ca^{2+}$ uptake was also restored (Table 1).

We next investigated whether the time of recovery from desensitization was dependent on the time of exposure to high glucose. We compared the recovery time in islets preexposed to 16.7 mmol/l glucose for 6 h or 24 h. Similar abnormalities of glucose-induced insulin release, $^{86}Rb^+$ efflux, and $^{45}Ca^{2+}$ uptake were observed in islets exposed to 16.7 mmol/l glucose for either 6 or 24 h (Table 2). After 5 min of incubation in CMRL medium at 5.5 mmol/l glucose, the recovery of secretory and ionic-flux behavior in islets exposed to high glucose for 24 h was similar to that of islets exposed to high glucose for 6 h (Table 2), indicating that within the times studied, the length of the period at high glucose did not affect the recovery time.

Finally, when islets previously exposed to 16.7 mmol/l glucose were incubated at 5.5 mmol/l glucose in Krebs-Ringer HEPES buffer instead of CMRL medium, the recovery from desensitization was slower. Complete reversibility was observed only after 45–60 min (data not shown).

DISCUSSION

The present data confirm that islet desensitization due to exposure to high glucose is associated with alterations in the pattern of K^+ and Ca^{2+} flux through the plasma membrane. In addition, they demonstrate that ion flux variations as well as the insulin release impairment are promptly and simultaneously reversible. They suggest, therefore, that changes induced in cultured islets by chronic exposure to high glucose are functional and that because of the concomitant and simultaneous pattern of ionic flux changes and secretion impairment, a cause-effect relationship exists between the

TABLE 1
Effects of glucose concentrations in the culture medium on insulin release, $^{86}Rb^+$ efflux, and $^{45}Ca^{2+}$ uptake in rat pancreatic islets

Culture conditions	Insulin release at 22.0 mmol/l glucose (pg · islet^{-1} · 30 min^{-1})	$^{86}Rb^+$ efflux at 22.0 mmol/l glucose (% of decrease)	$^{45}Ca^{2+}$ uptake at 22.0 mmol/l glucose (pmol · islet^{-1} · 20 min^{-1})
5.5 mmol/l glucose for 6 h	1,436 ± 144	−56 ± 7	19.7 ± 2.4
16.7 mmol/l glucose for 6 h	848 ± 97*	−19 ± 4†	10.5 ± 1.7†
5.5 mmol/l glucose for 6 h + 5.5 mmol/l for 5 min	1,605 ± 108	−59 ± 3	20.1 ± 1.5
16.7 mmol/l glucose for 6 h + 5.5 mmol/l for 5 min	1,465 ± 146	−58 ± 6	23.6 ± 2.5

Data are means ± SE. *$P < 0.01$; †$P < 0.001$.

two phenomena. Finally, our studies indicate that the time required for the recovery from this functional change is not dependent on the length of exposure to high glucose (at least within the limit of 24 h). These results suggest, therefore, that ion-flux (and the consequent membrane-potential) changes play a key role in the mechanism leading to glucose-induced desensitization of pancreatic islets. Because a normal response to glyburide was observed in islets exposed to high glucose, a proximal signal defect for closure of K^+ channels rather than an intrinsic defect in the channel is likely. In contrast, islet insulin content is essentially unmodified by the 6 h exposure to high glucose; an exhaustion of insulin storage, therefore, is not a likely explanation of the abnormal secretory pattern of islets exposed to high glucose.

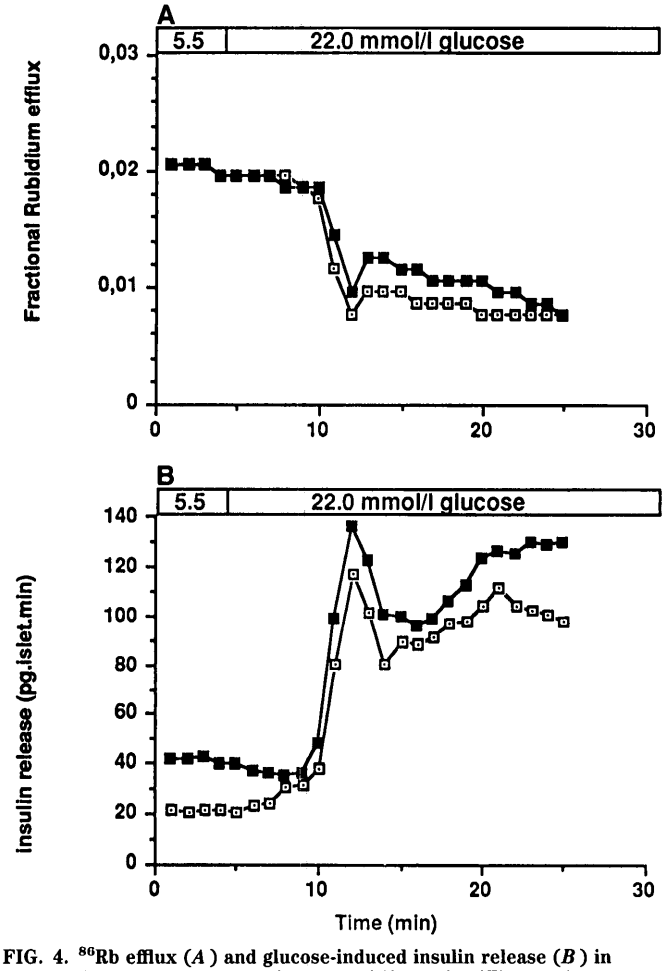

FIG. 4. ^{86}Rb efflux (*A*) and glucose-induced insulin release (*B*) in pancreatic islets exposed for 6 h to 5.5 (□) or 16.7 (■) mmol/l glucose and then washed twice and resuspended in CMRL at 5.5 mmol/l glucose for 5 min. Groups of 150 islets were incubated for 2 h in CMRL-1066 medium with 0.2 mmol/l $^{86}Rb^+$ at 37°C. After extensive washing, islets were perifused at a flow rate of 1 ml/min at 37°C in $^{86}Rb^+$-free medium. After a 5-min perifusion with a buffer containing 5.5 mmol/l glucose to equilibrate the system, the islets were stimulated with 22.2 mmol/l glucose. Perifusate samples were collected at 1-min intervals, and 400-µl aliquots were analyzed for $^{86}Rb^+$ radioactivity (*A*) and insulin release (*B*). A representative of five separate experiments is shown.

After exposure to high glucose, islet K^+ efflux is decreased, indicating that as a consequence of the constant glucose stimulation, ATP-dependent K^+ channels are, at least in part, inhibited. Moreover, in these islets, the inhibition of K^+ efflux in response to an acute glucose stimulation is reduced, while a normal responsiveness to the sulfonylurea glyburide is maintained, indicating that the channels are functionally intact but selectively unresponsive to glucose. This might be due to changes in glucose metabolism affecting ATP production or activation of the ATP-dependent K^+ channels. In animal models of NIDDM, this defect has been found and attributed to an intracellular defect of glucose metabolism distal to glucokinase (18). Alternatively, the channels may be less sensitive to ATP because of a change in the ATP-to-ADP ratio. The prolonged exposure to high glucose, in fact, on one hand increases ATP production but on the other hand will decrease it (and enhance ADP) because of the energy expenditure necessary for granule exocytosis. Because a decreased ATP-to-ADP ratio has been shown to reduce the K^+ channel sensitivity to ATP (19,20), their selective reduced responsiveness to glucose under these conditions might be explained. Finally, a reduced sensitivity to ATP has also been reported after exposure to high concentrations of the nucleotide (21).

After exposure to high glucose, islet Ca^{2+} uptake is also decreased, paralleling the secretory defect. Because Ca^{2+} uptake and the subsequent increase of the intracellular calcium concentrations are key steps in the mechanism leading to insulin secretion, it is conceivable that the reduced uptake might be responsible for the reduced secretory response to glucose. We have studied Ca^{2+} uptake and not cytosolic $[Ca^{2+}]_i$ concentrations because the latter studies are better performed in purified β-cells and we were interested in the function of intact islets. On the basis of the present data, we believe that a likely explanation of the results is that the persistent glucose stimulation induces a partial closure and decreased sensitivity to glucose and/or ATP of the ATP-dependent K^+ channels. This will, in turn, decrease calcium influx through the voltage-dependent Ca^{2+} channels, and this mechanism is directly responsible for the β-cell unresponsiveness to glucose. K^+ channels (and consequently Ca^{2+} uptake) in response to glyburide are normal in parallel with the unaltered secretory response. Being a functional abnormality, a normal channel competence is quickly restored as soon as the excessive glucose stimulation ends.

Previous data have been published to support a role of K^+

TABLE 2
Effects of glucose concentrations in the culture medium on insulin release, $^{86}Rb^+$ efflux, and $^{45}Ca^{2+}$ uptake in rat pancreatic islets

Culture conditions	Insulin release at 22.0 mmol/l glucose (pg · islet^{-1} · 30 min^{-1})	$^{86}Rb^+$ efflux at 22.0 mmol/l glucose (% of decrease)	$^{45}Ca^{2+}$ uptake at 22.0 mmol/l glucose (pmol · islet^{-1} · 20 min^{-1})
5.5 mmol/l glucose for 24 h	1,187 ± 134	−50.5 ± 2.9	22.6 ± 1.9
16.7 mmol/l glucose for 24 h	712 ± 98*	−19.5 ± 1.4†	13.5 ± 2.6†
5.5 mmol/l glucose for 24 h + 5.5 mmol/l for 5 min	1,229 ± 141	−53.7 ± 2.2	23.3 ± 1.9
16.7 mmol/l glucose for 24 h + 5.5 mmol/l for 5 min	1,312 ± 186	−55.0 ± 2.1	24.6 ± 1.9

Data are means ± SE. *$P < 0.01$; †$P < 0.001$.

and Ca^{2+} flux alterations in glucose desensitization in various experimental models in vivo and in vitro. In the Goto-Kakizaki rat, a genetic model of NIDDM, the impaired insulin response to glucose has been associated with a reduced sensitivity to glucose of the ATP-dependent K^+ channels (18). Moreover, transgenic mice overexpressing K^+ channels showed impaired glucose-induced Ca^{2+} oscillations and insulin release (22). Iwashima et al. (23) found a significant reduction in the mRNA levels of Ca^{2+} channels in islets from 48-h glucose-infused rats. These results, although obtained in in vivo experimental models largely different from each other and from our own, demonstrate that altered K^+ or Ca^{2+} flux may cause an abnormal secretory pattern. In an in vitro model similar to that in this study, Okamoto et al. (24) showed that in islets cultured at high glucose for 24 h, the ability of glucose to raise the intracellular calcium levels was significantly reduced. In contrast, a previous paper by Bolaffi et al. (25) found no difference in Ca^{2+} uptake in islets incubated at 11.0 mmol/l glucose. Finally, in different experimental models, diazoxide, an agent that inhibits insulin secretion by blocking the ATP-dependent K^+ channels, is able to prevent islet desensitization to glucose (14,15, this study). Reversibility of desensitization has been previously reported (26). However, we observed that the reversal was slower if Krebs-Ringer HEPES buffer was used for the islet recovery step instead of CMRL culture medium, indicating the requirement for a (yet unidentified) medium constituent(s). The comprehension of the biochemical mechanisms leading to the abnormalities observed in glucose desensitized pancreatic islets may be of crucial importance to provide a better understanding of the mechanisms regulating the metabolic control in NIDDM patients and a potential way to better treat them.

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