Rapid Publication

Chronic Exposure to High Glucose and Impairment of K^+-Channel Function in Perifused Rat Pancreatic Islets

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Prolonged exposure to high glucose levels impairs the ability of pancreatic islets to secrete insulin as a response to that stimulus. Because glucose, like other insulin secretagogues, elicits insulin secretion by inhibiting the ATP-sensitive K^+ channels, in this study, we investigated the effect of prolonged (24-h) exposure of rat pancreatic islets to high (16.7 mM) glucose concentration on ^{86}Rb efflux (used as a tracer for K^+). The data obtained indicate that islets exposed to high glucose concentration have impaired function of the glucose-sensitive K^+ channel, this phenomenon is temporarily related to a defective response of glucose-induced insulin release, and these alterations are reversible. *Diabetes* 39:397–99, 1990

Chronic exposure of pancreatic islets to high glucose concentrations leads to a desensitization of β-cells to further glucose stimulation. This desensitization has been shown in vivo in both laboratory animals and patients with non-insulin-dependent diabetes mellitus and in vitro with both a long-term perifusion system (1) and a static incubation model (2). However, the mechanism of impaired insulin secretion in desensitized islets is not known.

The inhibition of ATP-sensitive K^+ channels has been demonstrated to be an important step in the mechanisms leading to insulin secretion (3–6). The increase in ATP and/or other metabolites generated by glucose metabolism are believed to close the K^+ channels and, therefore, reduce β-cell permeability to K^+, causing cell depolarization and consequent insulin release. To clarify the possible role of impaired K^+ permeability in the mechanism of islet desensitization, we investigated the function of the ATP-sensitive K^+ channels in rat pancreatic islets cultured in vitro for 24 h with a high (16.7 mM) glucose concentration. In this model, insulin-secretion desensitization to glucose is associated with reduced efflux of the K^+ analogue ^{86}Rb.

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RESEARCH DESIGN AND METHODS

Male Wistar rats were purchased from Nossan (Milan, Italy), fed ad libitum, and killed by decapitation when they weighed 220–240 g. Islets were isolated by collagenase digestion (Boehringer Mannheim, Mannheim, FRG) and cultured in CMRL-1066 medium (Gibco, Grand Island, NY) containing either 5.5 (control islets) or 16.7 mM glucose for 24 h as previously described (2). The ^{86}Rb efflux (a marker of K^+ permeability) was measured according to the method described by Henquin (3). After the 24-h exposure to different glucose concentrations, separate groups of 100 islets were incubated for 2 h at 37°C in CMRL-1066 medium with 0.2 mM ^{86}Rb (sp act 1–8 mCi/mg, Amersham, Amersham, UK) then washed three times with fresh ^{86}Rb-free medium, placed in an Endotronics chamber in a BIOGEL-P2 matrix (Bio-Rad, Richmond, CA), and perifused at a flow rate of 1 ml/min. The perifusion medium (115 mM NaCl, 5.4 mM KCl, 2.38 $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM NaH_2PO_4, 10 mM HEPES, and 0.5 g/dl bovine serum albumin, pH 7.35, at 37°C) contained 1.4 mM glucose during the first 20 min to establish a stable basal secretory rate. Glucose content was then raised to 19.4 mM for a further 30 min to obtain maximal islet stimulation. Effluent fractions collected at 1-min intervals were analyzed for ^{86}Rb radioactivity and insulin content.

Results are expressed as fractional efflux of ^{86}Rb (^{86}Rb released per minute divided by ^{86}Rb remaining in the islets at measurement). Values are means ± SE. Statistical significance was determined by Student's *t* test for unpaired data.

RESULTS

In control islets, ^{86}Rb fractional efflux declined slowly during the initial period of perifusion with 1.4 mM glucose and then markedly decreased when the glucose concentration was

increased to 19.4 mM (Fig. 1*A*). The average fractional ^{86}Rb efflux declined from 0.012 ± 0.001% at the moment when glucose concentration was increased to 0.005 ± 0.0005% at the nadir value (n = 7; Δ of decrement −56.7 ± 3.1%). In the same perifusion fractions, insulin release was measured and found to be 8.5 ± 1.2 pg · min^{-1} · islet^{-1} at basal (unstimulated) conditions, 63.3 ± 5.7 pg · min^{-1} · islet^{-1} at the first phase of glucose-stimulated insulin release, and 75.0 ± 7.1 pg · min^{-1} · islet^{-1} at the second phase of insulin release (n = 7; Fig. 1*B*).

In pancreatic islets preexposed to 16.7 mM glucose for 24 h, ^{86}Rb efflux rate was significantly lower than in control islets when perifusion with glucose 1.4 mM was started (0.009 vs. 0.015%, $P < 0.01$). The efflux rate then gradually increased during the 20-min period of perifusion with 1.4 mM glucose, so that at the beginning of the stimulation with 19.4 mM glucose, the efflux rate was virtually identical compared with control islets (Fig. 2*A*). However, when glucose concentration was increased to 19.4 mM, only a small reduction of ^{86}Rb efflux was observed (from 0.011 ± 0.001 to 0.0070 ± 0.0007%, n = 7; Δ of decrement −36.8 ± 3.7%). Therefore, the decline of ^{86}Rb efflux was significantly less ($P < 0.05$) than in control islets (Fig. 2*A*).

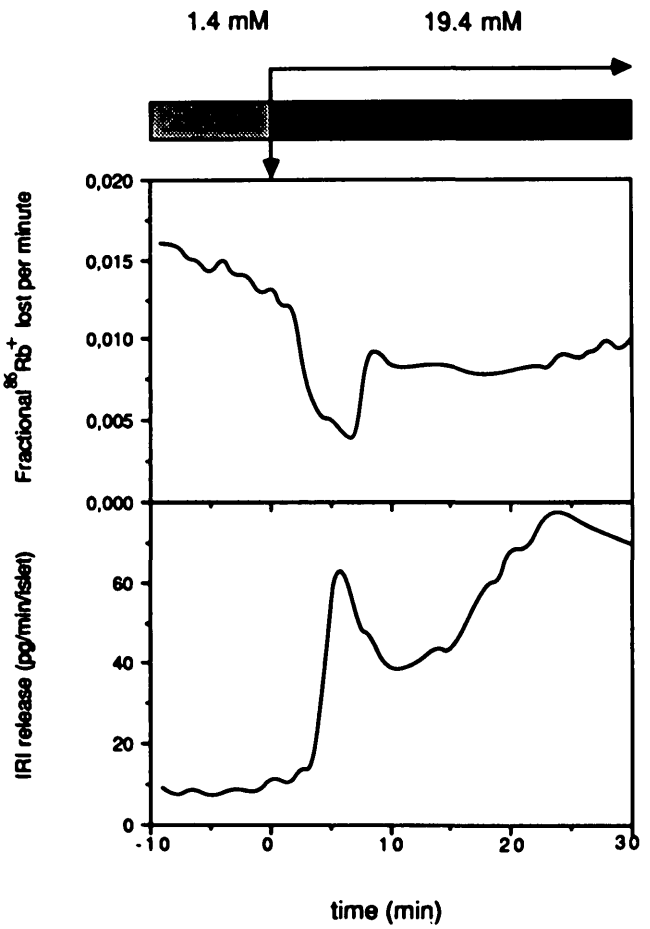

FIG. 1. Pancreatic islets were cultured for 24 h in CMRL-1066 medium containing 5.5 mM glucose. Islets were then incubated for 2 h with 0.2 mM ^{86}Rb, washed 3 times with ^{86}Rb-free medium, and perifused at flow rate of 1 ml/min with medium containing 1.4 mM glucose. At time 0, glucose concentration was raised to 19.4 mM. Fractional efflux of ^{86}Rb (*top*) was calculated by ratio of ^{86}Rb released per minute divided by ^{86}Rb remaining in islets at measurement. Insulin release (*bottom*) was measured by radioimmunoassay. Representative of 7 different experiments is shown.

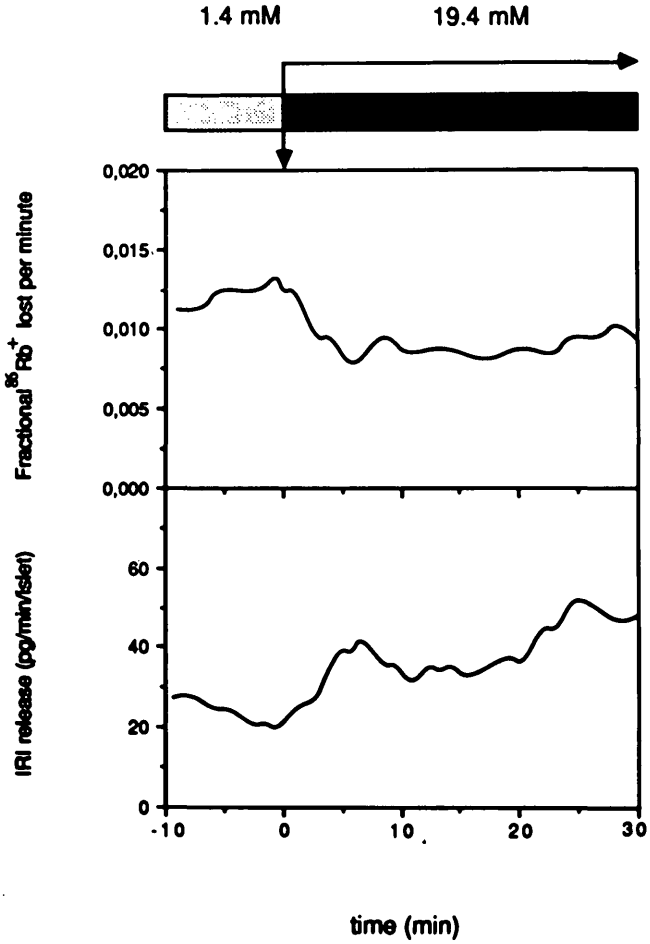

FIG. 2. Pancreatic islets were cultured for 24 h in CMRL-1066 medium containing 16.7 mM glucose. Islets were then incubated for 2 h with 0.2 mM ^{86}Rb, washed 3 times with ^{86}Rb-free medium, and perifused at flow rate of 1 ml/min with medium containing 1.4 mM glucose. At time 0, glucose concentration was raised to 19.4 mM. Fractional efflux of ^{86}Rb (*top*) was calculated by ratio of ^{86}Rb released per minute divided by ^{86}Rb remaining in islets at measurement. Insulin release (*bottom*) was measured by radioimmunoassay. Representative of 7 different experiments is shown.

In islets preexposed to high glucose, basal insulin release was significantly higher than in control islets (22.0 ± 2.6 pg · min^{-1} · islet^{-1}, $P < 0.05$). In addition, both first- and second-phase glucose-stimulated insulin secretion were diminished (42.3 ± 3.1 and 49.8 ± 4.1 pg · min^{-1} · islet^{-1}, respectively; $P < 0.05$ vs. control islets).

The decreased inhibition of ^{86}Rb efflux and the reduced insulin release observed in islets preexposed to 16.4 mM glucose was not a consequence of islet damage or depletion of intracellular insulin. In fact, when islets preexposed to high (16.7 mM) glucose were subsequently cultured for an additional 24 h with 5.5 mM glucose, both ^{86}Rb efflux rate and insulin release returned to values similar to those observed in control islets preexposed to 5.5 mM glucose for 24 or 48 h (Table 1).

DISCUSSION

Our findings indicate that both ^{86}Rb permeability and insulin release are altered in rat pancreatic islets "desensitized" by

TABLE 1
Effect of culture conditions on islet ^{86}Rb efflux and insulin release

Glucose	^{86}Rb efflux (%)	Glucose-stimulated insulin release (pg · min^{-1} · islet^{-1})
5.5 mM for 24 h	−56.7 ± 3.1	63.3 ± 5.7
16.7 mM for 24 h	−36.8 ± 3.7*	42.3 ± 3.1*
5.5 mM for 48 h	−58.3 ± 3.4	66.5 ± 3.5
16.7 mM for 24 h + 5.5 mM for 24 h	−60.0 ± 4.0	69.7 ± 2.6

Pancreatic islets were first cultured for 24 h in CMRL-1066 medium containing 5.5 or 16.7 mM glucose. Culture medium was then replaced with fresh medium containing 5.5 mM glucose, and islets were cultured for an additional 24 h. ^{86}Rb efflux and insulin release were measured at 24 and 48 h in perifused islets, and values are given as the Δ of decrement.
*$P < 0.05$ vs. islets cultured in 5.5 mM glucose.

24 h of exposure to a high (16.7 mM) glucose concentration. Our data do not demonstrate a cause-effect relationship between the two phenomena; however, this is a possibility, because it is known that the closure of ATP-sensitive K^+ channels and the subsequent membrane depolarization is one of the first events leading to insulin release. In addition, in our experiments, the alteration of K^+ permeability and insulin release appears to be both temporally and quantitatively related. Finally, when islets preexposed to high glucose concentrations were returned to control conditions (5.5 mM glucose), both abnormalities were reversed, thus indicating that no irreversible damage had occurred and also further supporting the correlation between the two phenomena. Therefore, these data suggest that the altered function of the K^+ channels may be at least in part responsible for the glucose-induced desensitization. In addition, because various insulin secretagogues induce the closure of K^+ channels (7,8) and also induce desensitization in vitro (9), these data suggest that alteration in ATP-sensitive K^+ channels may be a general mechanism leading to the desensitization of pancreatic β-cells. As for control islets, we have observed two different abnormalities of ^{86}Rb efflux and insulin release in the pancreatic islets chronically exposed to high glucose concentrations. One abnormality is the reduced ^{86}Rb efflux and the increased insulin secretion under basal (unstimulated) conditions. A second abnormality is the reduced decline of ^{86}Rb efflux and the impaired insulin release observed after a maximal glucose stimulation. Both abnormalities may reflect an abnormal intracellular availability of ATP or other glucose-metabolism derivatives responsible for a change in K^+-channel function and/or a chronically reduced islet cell permeability to K^+ with consequent membrane depolarization. Therefore, further stimulation of these islets by a maximal glucose concentration will find them in a refractory state, according to a model that is widely accepted in the physiological behavior of other systems, which has also been recently proposed for insulin secretion (third or depressed phase of insulin secretion; 9).

Because similar abnormalities of insulin release are present in non-insulin-dependent diabetic patients, an in vivo model of chronic pancreatic exposure to high glucose, a better understanding of these mechanisms may prove useful in searching for the treatment for impaired insulin secretion in these patients.

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