Glucose Activates Both K_{ATP} Channel–Dependent and K_{ATP} Channel–Independent Signaling Pathways in Human Islets

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Insulin secretion by isolated islets of Langerhans from 19 human donors (9 women and 10 men) was studied in vitro to test the hypothesis that human islets contain both the K_{ATP} channel–dependent and the K_{ATP} channel–independent signaling pathways. The results demonstrated the presence of both of these major pathways of glucose signaling. Thus, insulin secretion was stimulated by high glucose concentrations, by the sulfonylurea tolbutamide, and by a depolarizing concentration of potassium chloride. Diazoxide, which activates the K_{ATP} channel, completely blocked the stimulation of release by glucose. Stimulation of insulin release by tolbutamide, which inhibits the K_{ATP} channel and depolarizes the β-cell, and inhibition of glucose-stimulated release by diazoxide, which activates the channel and repolarizes the β-cell, confirm the involvement of the K_{ATP} channel–dependent pathway in glucose signaling. The participation of the K_{ATP} channel–independent pathway in the stimulation of insulin release by glucose was demonstrated for the first time in human islets. This was done in two ways. The first method, in the presence of diazoxide, blocked the action of glucose on the K_{ATP} channel in combination with a depolarizing concentration of KCl to raise $[Ca^{2+}]_i$. Under these conditions, glucose stimulated insulin release. A second method to demonstrate the involvement of the K_{ATP} channel–independent pathway was to close the K_{ATP} channels with tolbutamide. Again, with no possibility of further action on the K_{ATP} channel, glucose stimulated insulin release. In a final series of experiments, glucose-stimulated insulin release was profoundly inhibited by somatostatin, clonidine, and prostaglandin E_2, but not by galanin. *Diabetes* 47:758–763, 1998

Studies of insulin secretion by human islets in vitro began in 1971, when islets were isolated from a pancreas surgically removed from a 4-year-old patient with idiopathic hypoglycemia (1). The islets responded to 16.7 mmol/l glucose with an increase in insulin release that was potentiated by caffeine. Despite the fact that these early findings were soon confirmed (2,3), knowledge of stimulus-secretion coupling in the human β-cell still lacks detail. This appears to be largely due to the limited availability of donor tissue and technical difficulties in the preparation of human islets for study. Thus, knowledge has been accumulated from in vivo studies, some in vitro studies, and extrapolations from what we know about (primarily) rodent islets in vitro. The presence and functionality of the K_{ATP} channel–dependent pathway of glucose signaling in humans has been demonstrated previously in vitro (4–14). In view of these and other findings, it has been assumed that stimulus-secretion coupling in the human β-cell would be similar to that of rodent and other animal β-cells that have been extensively studied. Although this may be largely true, it is clear from recent findings that there are major differences in human and animal β-cell function. One common assumption has been that glucose enters the β-cell via the glucose transporter GLUT2. This is true for rodent β-cells but may not be for human β-cells, where only very small amounts of GLUT2 can be found (15,16). As the human β-cell contains appreciable amounts of GLUT1 and GLUT3, which have different characteristics than GLUT2, the dynamics of glucose transport in these cells may be quite complex (17,18). There are also differences in ion channels between human and rodent tissues. Na^+ channels make a significant contribution to the action potential profile in human but not in rodent β-cells (13,19,20). Mouse β-cells possess only one type of voltage-activated Ca^{2+} channel (21) whereas both high- and low-voltage activated Ca^{2+} channels have been described in human isolated tissue (7,14). From these data, it can be seen that human and rodent islets are different. Consequently, it seems necessary to study all aspects of stimulus-secretion coupling in the human β-cell to develop a complete understanding of the control of insulin secretion in humans. This is especially important since defects in depolarization-response coupling have been described in isolated diabetic β-cells (12). In this study, we have addressed primarily the two major pathways by which glucose stimulates and augments insulin release. These are the K_{ATP} channel–dependent and the K_{ATP} channel–independent pathways of glucose signaling.

The concept of an action of glucose to stimulate insulin release by virtue of inhibition of the K_{ATP} channel began in 1984 with the discovery of an ATP-sensitive K^+ channel (22) and the demonstration that it was inhibited in the intact β-cell by exposure to a high glucose concentration (23). Subsequently, the K_{ATP} channel was cloned and found to be a complex of a K^+ channel (Kir 6.2) and an ATP-binding cassette protein (SUR1) that acts as a high-affinity receptor for sulfonylureas (24–26). It is now known that glucose closure of the channel,

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$[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; PGE_2, prostaglandin E_2.

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or closure by sulfonylureas, depolarizes the β-cell membrane and activates voltage-dependent Ca^{2+} channels. The result is an increase in the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, and the stimulation of insulin release. This pathway of glucose signaling is the K_{ATP} channel–dependent pathway of glucose stimulus-secretion coupling. In 1992, a second pathway of glucose stimulus-secretion coupling was demonstrated in studies on rat and mouse islets (27–29). It was found that when the K_{ATP} channel was clamped open with the K^+ channel activator diazoxide, glucose was still capable of stimulating insulin secretion. Thus, an additional glucose-signaling mechanism was present in addition to the K_{ATP} channel–dependent pathway. In the case of the diazoxide experiments, it was necessary to raise $[Ca^{2+}]_i$ by means of a depolarizing concentration of KCl to elicit the glucose response. This second pathway in glucose stimulus-secretion coupling is known as the K_{ATP} channel–independent pathway of glucose signaling (27–34). Physiologically, it depends on a rise in $[Ca^{2+}]_i$ and therefore operates in synergy with the K_{ATP} channel–dependent pathway. In the work presented here, we have looked at the activity of these two pathways, K_{ATP} channel–dependent and K_{ATP} channel–independent, in human β-cells in vitro. The presence of the K_{ATP} channel–independent pathway in the human β-cell is demonstrated here for the first time. Additionally, we have shown that insulin secretion from human islets is strongly inhibited by somatostatin, the α-adrenergic agonist clonidine, and prostaglandin E_2 (PGE_2), but not by galanin.

RESEARCH DESIGN AND METHODS

Isolation and treatment of human pancreatic islets. Over a 12-month period, we studied human islets obtained from 9 female and 10 male heart-beating cadaveric donors. The women ranged in age from 28 to 64, and the men from 28 to 60.

After surgery to remove the pancreases, the organs were initially perfused in situ with hyperosmolar citrate solutions at 4°C for transport. They were then processed by intraductal distension with a controlled semiautomated collagenase procedure, as fully described previously (35,36). Islets of Langerhans were subsequently separated using a Ficoll/Metrimizide-based continuous density gradient and placed in tissue culture (CMRL 1066). The islets were kept under tissue culture conditions in RPMI for 1 to 5 days before use.

Insulin release measurements. Insulin release was measured under static incubation conditions using batches of five islets. They were preincubated in Krebs-Ringer bicarbonate buffer containing 2 or 2.8 mmol/l glucose at pH 7.4 for 30 or 60 min, all at 37°C. Thereafter, they were washed once and then incubated for the indicated times in the presence and absence of test substances, as described in the text, for the measurement of insulin secretion. At the end of the incubations, aliquots of the Krebs-Ringer bicarbonate buffer were removed and kept at –20°C until radioimmunoassay was performed using a charcoal separation method (37). We used different glucose concentrations for basal (2.0 and 2.8 mmol/l) and stimulated conditions (16.7 and 20 mmol/l) in the course of this work because some of the islets were also used for electrophysiological experiments using these conditions, and it was useful for the interpretation of those experiments to have a record of the islet responsiveness.

Materials. Tolbutamide, diazoxide, somatostatin, clonidine, and PGE_2 were all obtained from Sigma (Poole, U.K.), whereas human galanin was from Peninsula Laboratories (St. Helens, U.K.). $[^{125}I]$Insulin was purchased from New England Nuclear (Boston, MA), and the human insulin standard with the reference number 66/304 was purchased from NIBSC, Hertfordshire, U.K.

Statistical analysis. Results are presented as means ± SE. Statistical analysis was by Student's *t* test for paired and unpaired data as appropriate.

RESULTS

K_{ATP} channel–dependent pathway

Glucose response. The data shown in Fig. 1 (right) are compiled from all the islets that were tested with 20 mmol/l glucose (n = 12). Insulin secretion under basal conditions was 2.3 ± 0.4 ng · 5 islets^{-1} · 30 min^{-1}. In the presence of 20 mmol/l glucose, the rate of insulin secretion was three times that of the basal rate, at 6.5 ± 1.6 ng · 5 islets^{-1} · 30 min^{-1} (P < 0.01, n = 12). Individual secretion rates ranged from 0.7 to 6.1 ng · 5 islets^{-1} · 30 min^{-1} under basal conditions and from 1.3 to 20.6 ng · 5 islets^{-1} · 30 min^{-1} with 20 mmol/l glucose. Insulin content was 44 ± 8 ng/islet (n = 12). These values for release and content compare favorably with those already in the literature for cultured human islets (3).

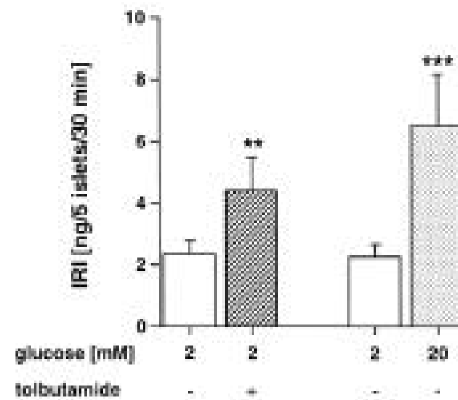

FIG. 1. The effects of 100 µmol/l tolbutamide and 20 mmol/l glucose on insulin secretion. Basal immunoreactive insulin secretion in the presence of 2 mmol/l glucose was 2.4 ± 0.5 ng · 5 islets^{-1} · 30 min^{-1} for the tolbutamide experiments (n = 5), and 2.3 ± 0.4 ng · 5 islets^{-1} · 30 min^{-1} for the 20 mmol/l glucose experiments (n = 12). **P < 0.02; ***P < 0.01.

Tolbutamide response. In Fig. 1 (left) are shown the effects of the sulfonylurea tolbutamide, which causes closure of the K_{ATP} channels, thus mimicking the action of glucose in this respect. Tolbutamide (100 µmol/l) almost doubled the rate of insulin release (P < 0.02, n = 12) in accord with its action to depolarize the β-cell and raise $[Ca^{2+}]_i$.

Diazoxide and KCl responses. In Fig. 2 (left), the effects of 16.7 mmol/l glucose to stimulate insulin release are shown together with the effect of 150 µmol/l diazoxide on glucose-stimulated release. Insulin secretion was raised by 16.7 mmol/l glucose from 3.0 ± 0.6 ng · 5 islets^{-1} · 30 min^{-1} under basal conditions to 7.9 ± 2.0 ng · 5 islets^{-1} · 30 min^{-1} (P < 0.01, n = 9). Diazoxide (100 µmol/l), the K^+ channel opener, completely eliminated the stimulation by glucose (P < 0.01, n = 9, compared with 16.7 mmol/l glucose alone). These results with glucose, tolbutamide, and diazoxide are in accord with the concept that the K_{ATP} channel plays a major role in the control of insulin release.

The response to a depolarizing concentration of KCl. As depolarization of the β-cell can be achieved by raising the extracellular K^+ concentration, we mimicked the depolarizing action of tolbutamide and glucose by exposing the islets to 30 mmol/l KCl. Under these conditions (Fig. 2, right), the rate of insulin secretion was more than doubled (basal secretion in 2 mmol/l glucose was 2.7 ± 0.3 ng · 5 islets^{-1} · 30 min^{-1} and in the presence of 30 mmol/l KCl was 6.3 ± 1.8 ng · 5 islets^{-1} · 30 min^{-1}; P < 0.05, n = 5). The secretion rate in the presence of 20 mmol/l glucose was 9.2 ± 1.2 ng · 5 islets^{-1} · 30 min^{-1} (P < 0.01, n = 5, compared with 2.0 mmol/l glucose alone).

K_{ATP} channel–independent pathway

Glucose response in the presence of diazoxide and KCl. Experiments were next carried out in the presence of 150 µmol/l diazoxide to completely activate the K_{ATP} channel. Figure 3 (left) shows the effects of 2.8 and 16.7 mmol/l glu-

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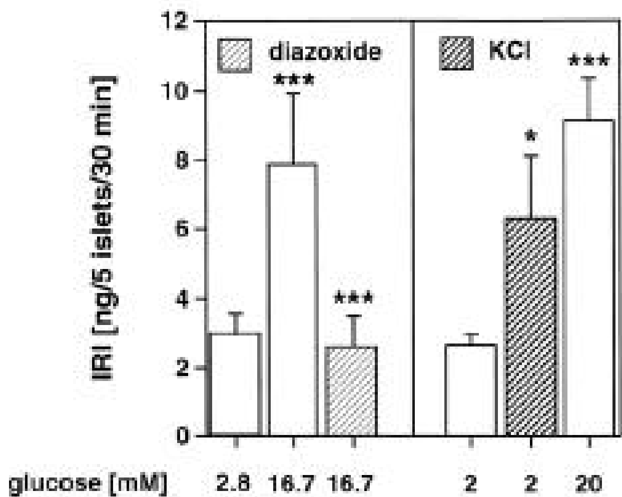

FIG. 2. The effect of 16.7 mmol/l glucose to stimulate insulin release and of 150 μmol/l diazoxide to block the stimulation of release (left); and the stimulatory effect of 30 mmol/l KCl in comparison with that of 20 mmol/l glucose (right). Basal insulin secretion in the presence of 2 mmol/l glucose was 3.0 ± 0.6 ng · 5 islets^{-1} · 30 min^{-1} for the diazoxide experiments (*n* = 9) and 2.7 ± 0.3 ng · 5 islets^{-1} · 30 min^{-1} for the KCl experiments (*n* = 5). **P* < 0.05; ****P* < 0.01.

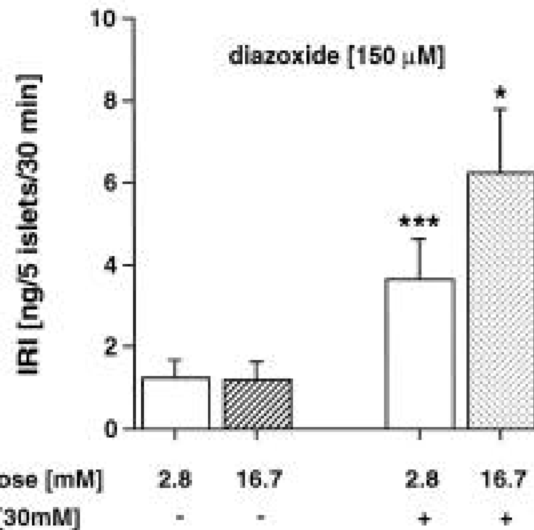

FIG. 3. The effect of 150 μmol/l diazoxide to block the effect of 16.7 mmol/l glucose; the effect of 30 mmol/l KCl to stimulate insulin release in the presence of diazoxide; and the effect of 16.7 mmol/l glucose to augment the KCl-stimulated release. All the experiments were performed in the presence of 150 μmol/l diazoxide throughout. Basal insulin secretion in the presence of 2.8 mmol/l glucose and 150 μmol/l diazoxide was 1.25 ± 0.45 ng · 5 islets^{-1} · 30 min^{-1} (*n* = 6). **P* < 0.02; ****P* < 0.01.

cose. As expected, 16.7 mmol/l glucose failed to stimulate insulin secretion under these conditions. On the right side of Fig. 3 is shown the effect of a depolarizing concentration of KCl (30 mmol/l). In the presence of 2.8 mmol/l glucose, KCl stimulated insulin secretion to a rate three times that of the basal rate with 2.8 mmol/l glucose alone (P < 0.01, n = 6). This KCl-stimulated insulin secretion was further increased by 16.7 mmol/l glucose (P < 0.02, n = 6). Thus, in the presence of diazoxide with no possible effect of glucose on the K_{ATP} channel, glucose is incapable of stimulating secretion. However, when $[Ca^{2+}]_i$ is raised by KCl and secretion is stimulated, glucose has an additional effect to augment the rate of insulin secretion. This additional effect is due to a K_{ATP} channel–independent pathway of glucose signaling.

Glucose response in the presence of tolbutamide. A second way to demonstrate K_{ATP} channel–independent effects of glucose is to pharmacologically block the K_{ATP} channel so that glucose can no longer affect it and then seek for additional effects of glucose on secretion. The results of such experiments are shown in Fig. 4 (right). A supermaximal concentration of tolbutamide (500 μmol/l), which was used to completely close the K_{ATP} channels, doubled the rate of insulin secretion in the presence of 2.8 mmol/l glucose (P < 0.01, n = 6). The basal secretion rate was also more than doubled by 16.7 mmol/l glucose (P < 0.01, n = 6). However, in the presence of tolbutamide, the rate of secretion increased again to twice, and more than twice, the rates with 16.7 mmol/l glucose and 500 μmol/l tolbutamide alone, respectively (both P < 0.02, n = 6). As tolbutamide had closed the K_{ATP} channels, this latter effect of glucose could not be exerted by any action on K_{ATP} channels and is, by definition, K_{ATP} channel–independent. In addition to these studies with tolbutamide, paired experiments were performed using diazoxide and KCl (Fig. 4, left). As anticipated, 16.7 mmol/l glucose had no effect in the presence of diazoxide. However, as can be seen from the results with 30 mmol/l KCl and basal glucose and with KCl plus 16.7 mmol/l glucose, the K_{ATP} channel–independent augmentation of insulin secretion by glucose (P < 0.01, n = 6) was comparable with that of 16.7 mmol/l glucose with tolbutamide. Thus, the two methods of demonstrating the K_{ATP} channel–independent effect of glucose gave similar data.

Physiological inhibition of secretion. During the course of this work, three inhibitors of insulin secretion, somatostatin, the α_2-adrenergic agonist clonidine, and PGE_2, were tested to see if they inhibited glucose-stimulated insulin secretion in human islets. As can be seen from the results in Fig. 5, 20 mmol/l glucose stimulated insulin release (P < 0.01), and somatostatin, clonidine, and PGE_2 completely inhibited the stimulated release (all P < 0.01, n = 4–7).

In the final series of experiments, the effect of galanin was examined. No inhibitory effect of galanin was detected. Basal insulin secretion at 2 mmol/l glucose was 0.38 ± 0.1 ng · 5 islets^{-1} · 30 min^{-1}, and this was stimulated more than 2.5-fold by 20 mmol/l glucose to 0.96 ± 0.3 ng · 5 islets^{-1} · 30 min^{-1} (P < 0.05, n = 5). This stimulated release rate was unaltered by 100 nmol/l galanin at 0.90 ± 0.2 ng · 5 islets^{-1} · 30 min^{-1}. The basal and stimulated secretion rates by these batches of islets were extremely low when compared with the other experiments reported in this article. We have no explanation for this other than that variability is seen in different batches of human islets after transport and incubation under tissue culture conditions.

DISCUSSION

The results of this work demonstrate the functional activity of two pathways of glucose signaling in human β-cell stimulus-secretion coupling. One is the well-known K_{ATP} channel–dependent pathway by which glucose depolarizes the β-cell, activates voltage-dependent Ca^{2+} channels, and raises $[Ca^{2+}]_i$ (6,7,22–25). The other is the less well-known K_{ATP} channel–independent pathway (27–29). These two pathways work in synergy, one stimulating insulin secretion via an

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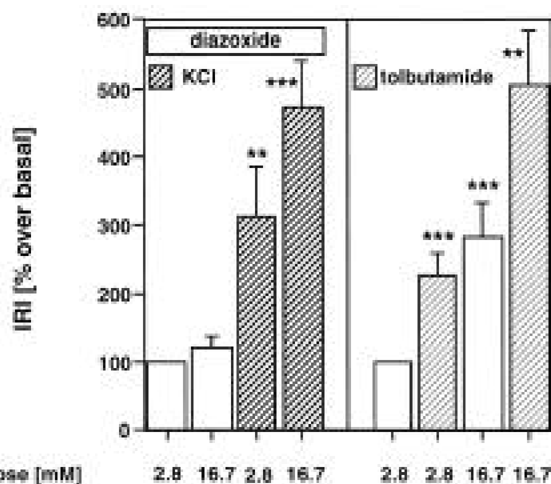

FIG. 4. Augmentation effects of 16.7 mmol/l glucose in the presence of either 150 μmol/l diazoxide and 30 mmol/l KCl (left) or 500 μmol/l tolbutamide (right). The basal rate of insulin secretion was 1.1 ± 0.5 ng · 5 islets^{-1} · 30 min^{-1} (n = 6). ***P* < 0.02; ****P* < 0.01.

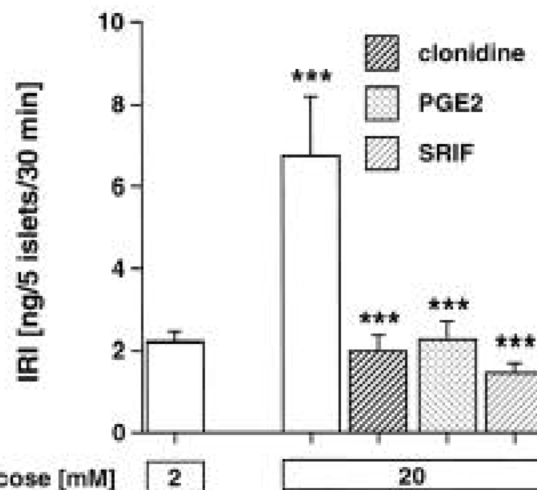

FIG. 5. The effects of 100 nmol/l somatostatin, 10 μmol/l clonidine, and 100 nmol/l PGE_2 to inhibit insulin release stimulated by 20 mmol/l glucose. Basal insulin secretion in the presence of 2 mmol/l glucose was 2.2 ± 0.3 ng · 5 islets^{-1} · 30 min^{-1} (n = 4–7). ****P* < 0.01.

increase in $[Ca^{2+}]_i$, the other augmenting the secretory response to the increased $[Ca^{2+}]_i$. Both pathways are essential to the development of the full biphasic secretory response to glucose (34). This is obvious from the fact that sulfonylureas, which inhibit K_{ATP} channel activity like glucose, fail to give the same magnitude of response as glucose (38). Also, the sulfonylureas fail to produce the characteristic biphasic response of glucose-stimulated insulin release, i.e., a rapid release of insulin to a peak rate followed by a nadir, which completes the first phase of release, and a subsequent prolonged rising second phase of release (38). The rapid rise in $[Ca^{2+}]_i$ in the K_{ATP} channel–dependent pathway appears to be responsible for the first phase of insulin secretion. Elevated $[Ca^{2+}]_i$ due to the K_{ATP} channel–dependent pathway and augmentation by the K_{ATP} channel–independent pathway appear to be responsible for the second phase of insulin release (31,34). Although it is possible to ascribe the greater part of the insulin release to prolonged glucose stimulation of the K_{ATP} channel–independent pathway, it must be borne in mind that this pathway is critically dependent on the elevation of $[Ca^{2+}]_i$ and therefore requires that the K_{ATP} channel–dependent pathway is active. It is also likely that the K_{ATP} channel–independent pathway is the underlying mechanism whereby glucose induces time-dependent potentiation or priming of insulin release (34). Although the biochemical mechanisms underlying the K_{ATP} channel–independent pathway are not known, we favor the idea that the anaplerotic–malonyl CoA pathway as proposed by the work of Corkey, Prentki, and co-workers (39–41) is involved. They provide strong evidence that a pathway originating in the mitochondria with the production of citrate leads to increased levels of malonyl CoA. The rise in malonyl CoA inhibits carnitine palmitoyl transferase I, reduces the entry of fatty acids into the mitochondria—thus decreasing their oxidation—and leads to an increase in cytosolic long-chain acyl CoA esters. These esters are presumed to be the source of the signaling molecules, for example diacylglycerol, that increase the rate of insulin release. Other possible mechanisms include a putative mitochondrial signal distinct from increased citrate (42) and cytosolic NADPH produced by the mitochondrial pyruvate malate shuttle as suggested by the work of MacDonald (43). The two signaling pathways of glucose and the potential underlying mechanisms for the K_{ATP} channel–independent pathway are presented in Fig. 6.

The additional studies performed here on the inhibition of insulin release confirm that somatostatin, the α-adrenergic agonist clonidine, and PGE_2 are all powerful inhibitors of insulin release. Galanin, which was reported to inhibit insulin release in vitro (44) but not in vivo (45–49), failed to inhibit insulin secretion in our studies. We think that the weight of evidence now is against the idea that galanin has any direct effect to inhibit insulin release in the human islet, despite its well-characterized and powerful effect on rodent islets and cell lines derived from rodents (50). The low rate of insulin release seen in these experiments with galanin, relative to the rates seen in most of the experiments reported here, is a good example of the variability seen with human islets in vitro. We have no explanation for the low values seen in the five batches of islets used for these experiments. In general, the reasons for the variability lie partly in the difference between individual batches of human islets, but mostly, we believe, in the isolation conditions, treatment, and duration of tissue culture before experimentation. The decreased rates of stimulated secretion that occur during tissue culture have been well documented for rat islets (51).

The importance of the K_{ATP} channel–independent pathway of glucose signaling lies in its potential involvement in *1*) the development of the second phase and full response to glucose; *2*) the induction of time-dependent potentiation; *3*) some forms of NIDDM due to defects in this pathway; *4*) β-cell hypersecretory states such as persistent hyperinsulinemic hypoglycemia of infancy, e.g., in those cases where the defect may not to be in either SUR1 or Kir 6.2 (36); and *5*) as a target for novel drug therapy because each molecular interaction in the signaling pathway is a potential site for drug action. Sulfonylureas and Ca^{2+}-channel blockers are common examples of drugs that act on the K_{ATP} channel–dependent pathway. No therapeutic drugs are yet known to definitively act on the K_{ATP} channel–independent pathway, although there are several candidates. Among these would be any compounds that

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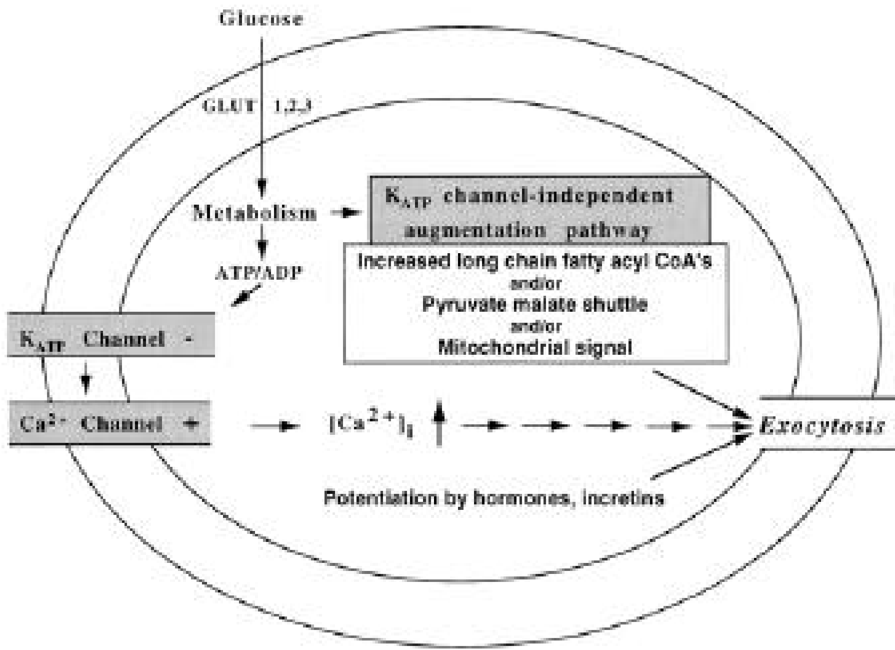

FIG. 6. Scheme of the K_{ATP} channel–dependent and the K_{ATP} channel–independent pathways of glucose signaling. Shown is glucose entry into the β-cell via the glucose transporters GLUT1, GLUT2, and GLUT3, as well as metabolism to give an increased ATP:ADP ratio and closure of the K_{ATP} channel. K_{ATP} channel closure leads to depolarization, activation of voltage-dependent Ca^{2+} channels, increased Ca^{2+} entry, and increased $[Ca^{2+}]_i$. The latter triggers exocytosis and stimulates insulin release (this is the K_{ATP} channel–dependent pathway). Augmentation of the stimulated release is by the K_{ATP} channel–independent pathway. This is illustrated in this scheme as the result of an increase in malonyl CoA, inhibition of CPT1, increased cytosolic long-chain fatty acyl CoA esters (38–40), the pyruvate malate shuttle (43), and/or an as-yet-unknown mitochondrial factor (42), and subsequent messenger moieties. The two pathways of glucose signaling work in a coordinated synergy. Included in the figure is an indication that hormones and incretins also potentiate secretion by a "distal" action.

block carnitine palmitoyl transferase I and inhibit fatty acid oxidation. These, like glucose, would increase the concentration of cytosolic long-chain acyl CoA esters and second messengers that are thought to augment the rate of exocytosis. Other possibilities include the imidazoline derivatives that like some sulfonylureas, both block K_{ATP} channels and act on distal signaling events to stimulate Ca^{2+}-dependent exocytosis in β-cells (41–44). Understanding the mechanisms responsible for the K_{ATP} channel–independent pathway in human β-cells should provide rich rewards both in our understanding of disorders of insulin release and in our ability to treat them.

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