ATP-Sensitive K⁺ Channel–Dependent Regulation of Glucagon Release and Electrical Activity by Glucose in Wild-Type and SUR1⁻/⁻ Mouse α-Cells

Jesper Gromada,¹ Xiaosong Ma,² Marianne Høy,³ Krister Bokvist,¹ Albert Salehi,² Per-Olof Berggren,⁴ and Patrik Rorsman⁵

Patch-clamp recordings and glucagon release measurements were combined to determine the role of plasma membrane ATP-sensitive K⁺ channels (K\textsubscript{ATP} channels) in the control of glucagon secretion from mouse pancreatic α-cells. In wild-type mouse islets, glucose produced a concentration-dependent (half-maximal inhibitory concentration [IC\textsubscript{50}] = 2.5 mmol/l) reduction of glucagon release. Maximum inhibition (−50%) was attained at glucose concentrations >5 mmol/l. The sulfonylureas tolbutamide (100 μmol/l) and glibenclamide (100 nmol/l) inhibited glucagon secretion to the same extent as a maximally inhibitory concentration of glucose. In mice lacking functional K\textsubscript{ATP} channels (SUR1⁻/⁻), glucagon secretion in the absence of glucose was lower than that observed in wild-type islets and both glucose (0–20 mmol/l) and the sulfonylureas failed to inhibit glucagon secretion. Membrane potential recordings revealed that α-cells generate action potentials in the absence of glucose. Addition of glucose depolarized the α-cell by −7 mV and reduced spike height by 30% Application of tolbutamide likewise depolarized the α-cell (−17 mV) and reduced action potential amplitude (43%). Whereas insulin secretion increased monotonically with increasing external K⁺ concentrations (threshold 25 mmol/l), glucagon secretion was paradoxically suppressed at intermediate concentrations (5.6–15 mmol/l), and stimulation was first detectable at >25 mmol/l K⁺. In α-cells isolated from SUR1⁻/⁻ mice, both tolbutamide and glucose failed to produce membrane depolarization. These effects correlated with the presence of a small (0.13 nS) sulfonylurea-sensitive conductance in wild-type but not in SUR1⁻/⁻ α-cells. Recordings of the free cytoplasmic Ca²⁺ concentration ([Ca²⁺]) revealed that, whereas glucose lowered [Ca²⁺], to the same extent as application of tolbutamide, the Na⁺ channel blocker tetrodotoxin, or the Ca²⁺ channel blocker Co²⁺ in wild-type α-cells, the sugar was far less effective on [Ca²⁺], in SUR1⁻/⁻ α-cells. We conclude that the K\textsubscript{ATP} channel is involved in the control of glucagon secretion by regulating the membrane potential in the α-cell in a way reminiscent of that previously documented in insulin-releasing β-cells. However, because α-cells possess a different complement of voltage-gated ion channels involved in action potential generation than the β-cell, moderate membrane depolarization in α-cells is associated with reduced rather than increased electrical activity and secretion. Diabetes 53 (Suppl. 3):S181–S189, 2004

T he metabolic derangements associated with type 2 diabetes result from the combination of insulin deficiency and glucagon excess (1,2). Type 2 diabetes is associated with serious abnormalities of glucagon secretion. First, circulating levels of glucagon are elevated despite hyperglycemia, and a further rise in blood glucose fails to inhibit, or even, paradoxically, stimulate its release (3,4). Second, diabetic patients exhibit loss of adequate glucose counterregulation (i.e., they fail to respond with stimulation of glucagon release when blood glucose falls to low levels during insulin administration). Among the physiological glucose counterregulatory factors, an increment in circulating levels of glucagon (via increased hepatic glucose production) plays a primary role. Thus, it is essential to determine both how glucagon secretion is regulated in the hypoglycemic state and the mechanisms by which it is normally switched off when the blood glucose concentration increases.

The ability of high glucose concentrations to suppress glucagon release has been attributed to both direct actions on the α-cells (5–7) and paracrine effects exerted by compounds released by neighboring β- and δ-cells (5,8–12). Like β-cells, pancreatic α-cells are electrically excitable and generate Na⁺- and Ca²⁺-dependent action potentials in the absence of glucose (13–16). Glucagon release is a Ca²⁺-dependent process, and both capacitance and hormone release measurements have revealed a close relationship between N-type Ca²⁺ channels and secretion under basal (hypoglycemic) conditions (10,15). Surprisingly, pancreatic α-cells are equipped with ATP-sensitive K⁺ channels (K\textsubscript{ATP} channels) of the same type as those constituting the resting conductance in β-cells (14,17,18). Nevertheless, the role of these channels, if any, in gluca-

From the 1Lilly Research Laboratories, Hamburg, Germany; the 2Department of Physiological Sciences, Lund, Sweden; the 3Department of Medical Physiology, The Panum Institute, University of Copenhagen, Copenhagen, Denmark; the 4Department of Molecular Medicine, The Rolf Luft Center for Diabetes Research, Karolinska Institutet, Stockholm, Sweden; and the 5Diabetes Research Laboratories, Oxford Centre for Diabetes, Endocrinology and Metabolism, The Churchill Hospital, Oxford, England.

Address correspondence and reprint requests to Jesper Gromada, Lilly Research Laboratories, Essener Strasse 93, D-22419 Hamburg, Germany. E-mail: gromada@lilly.com.

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gon secretion remains controversial. Here we have applied patch-clamp recordings to isolated α-cells from wild-type and SUR1\(^{-/-}\) mice, which lack functional K\(_{ATP}\) channels (19), in parallel with free cytoplasmic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\)) imaging and glucagon secretion measurements to clarify the role of K\(_{ATP}\) channels in α-cells.

**RESEARCH DESIGN AND METHODS**

Islets were isolated from 8- to 10-week-old SUR1\(^{-/-}\) mice and either wild-type littermates or age-matched C57BL/6J mice as described elsewhere (19). The experimental procedures were approved by the local ethics committees in Copenhagen and Lund. For patch-clamp experiments, islets were dispersed into single cells by shaking in a Ca\(^{2+}\)-free medium and maintained in tissue culture for up to 3 days as described previously (10).

K\(_{ATP}\) currents and membrane potentials were recorded in the perforated patch whole-cell configuration using Axopatch 200B (Axon Instruments, Union City, CA) or EPC9 (Heka Elektronik, Lambrecht/Pfalz, Germany) patch-clamp amplifiers as reported previously (13,14). All electrophysiological experiments were performed at +33°C. The α-cells were functionally identified by cell capacitance (2.7 ± 0.1 pF, n = 98; range 2.2–3.4 pF) and the presence of tetrodotoxin (TTX)-sensitive Na\(^{+}\) current (13). Cells fulfilling these criteria were spontaneously active in the absence of glucose, which allows them to be distinguished from wild-type α- and β-cells (20).

Glucagon secretion was measured as previously described (7,21). The hormone release measurements were carried out at 37°C. All chemicals were purchased from Sigma (St Louis, MO), with the exception of TTX and thapsigargin, which were from Alomone Labs (Jerusalem, Israel).

Intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\)) in single α-cells was measured as outlined elsewhere (15).

Results are presented as means ± SE for the indicated number of experiments. Statistical significance was evaluated using Student’s t test or Dunnett’s test for multiple comparisons. For the analysis of the membrane potential changes, only α-cells exhibiting stable and regenerative electrical activity in the absence of glucose were included.

**RESULTS**

Glucose inhibits electrical activity in α-cells from SUR1\(^{-/-}\) mice. Figure 1A shows electrical activity recorded from a single wild-type α-cell using the perforated patch whole-cell configuration in the absence and presence of glucose. Regenerative electrical activity in the absence of glucose was observed in all tested cells (n = 3). Glucose stimulation (20 mmol/l) resulted in a slight depolarization of 7 ± 4 mV (n = 3) and reduced the amplitude of the action potentials. Figure 1B shows examples of action potentials in the absence of glucose (a) and after elevation of glucose (b). Under control conditions, the action potentials originated from a membrane potential of −67 ± 2 mV and peaked at 11 ± 3 mV. Following glucose stimulation, the action potentials originated from −55 ± 5 mV and peaked at −1 ± 5 mV (n = 3). Thus, the slight decrease in interspike voltage resulted in a 30% reduction of spike amplitude that we attribute to voltage-dependent inactivation of the voltage-gated membrane currents involved in action potential generation (7). The reduction in amplitude was paralleled by a 56% decrease in action potential frequency from 14 ± 2 to 6 ± 3 Hz in the absence and presence of glucose, respectively.

Regenerative electrical activity was likewise observed in SUR1\(^{-/-}\) α-cells (Fig. 1C). The most negative interspike membrane potential was −76 ± 21 mV, and the action potentials peaked at 21 ± 21 mV (n = 5). Thus, the amplitude of the α-cell action potentials was close to 100 mV. Increasing the extracellular glucose concentration to 20 mmol/l failed to depolarize SUR1\(^{-/-}\) α-cells (change −5 ± 6 mV; n = 5). Figure 1D shows examples of action potentials recorded from an α-cell obtained from SUR1\(^{-/-}\)-islets in the absence (c) and presence (d) of 20 mmol/l glucose. It is clear that although glucose stimulation was without effect on spike amplitude, frequency was reduced by 80%, from 9 ± 1 to 2 ± 1 Hz (P < 0.001; n = 5; Fig. 1D).

**SUR1\(^{-/-}\) α-cells contain no K\(_{ATP}\) currents.** We next measured K\(_{ATP}\) channel activity in α-cells using the perforated patch whole-cell configuration. In the absence of glucose, wild-type but not SUR1\(^{-/-}\) α-cells contained a small tolbutamide-sensitive current (Fig. 2A); the membrane conductance normalized to cell capacitance (= surface area) amounted to 0.18 nS/pF. Addition of 20 mmol/l glucose led to a marginal (15%, n = 5) reduction in whole-cell membrane conductance. The normalized conductance in 20 mmol/l glucose amounted to 0.15 nS/pF (not statistically different from that observed in the absence of glucose). Application of tolbutamide reduced the input conductance in wild-type α-cells by >65%, and the membrane conductance was reduced to 0.06 nS/pF (Fig. 2B, left). The effects of adding tolbutamide (0.1 mmol/l) on electrical activity in wild-type α- and β-cells are illustrated in Fig. 2C and D. It can be seen that whereas tolbutamide-induced depolarization triggers electrical activity in the β-cell, membrane depolarization in the α-cell leads to a reduction in both action potential amplitude and frequency.

The responses in α-cells from SUR1\(^{-/-}\)-islets were rather different (Fig. 2A, right). In these cells, the input conductance was low (0.06 nS/pF) already in the absence of glucose, and the addition of glucose had no additive effect. As expected and unlike the situation in wild-type

**FIG. 1.** Glucose reduces electrical activity in α-cells from wild-type and SUR1\(^{-/-}\) mice. A: Current-clamp recording of membrane potential (V) in a single α-cell from a wild-type (wt) mouse. Glucose (20 mmol/l) was applied during the period indicated by the horizontal line. B: Action potentials recorded at low (a) and high (b) glucose on an expanded time base. Records were taken from panel A as indicated by the letters. Data in A and B are representative of three experiments. C: As in A but using an α-cell from a SUR1\(^{-/-}\) mouse. Note that the inhibition of electrical activity is associated with a hyperpolarization of the plateau potential from which spiking occurs. D: As in B, but records were taken from C as indicated by letters c and d. The recording is representative of five separate experiments.
α-cells, tolbutamide (100 μmol/l) had no effect on membrane conductance in SUR1−/− α-cells (Fig. 2B, right).

**Effects of glucose and KATP channel modulators on [Ca^{2+}]_{i} in wild-type α-cells.** The objective of electrical activity in the α-cell is to produce an elevation in [Ca^{2+}]_{i} that initiates glucagon secretion (15). Measurements of [Ca^{2+}]_{i} therefore provide a convenient and noninvasive readout of electrical activity in α-cells. Figure 3A shows a recording of [Ca^{2+}]_{i} from an individual α-cell initially exposed to glucose-free medium. The measured Ca^{2+} concentration in the absence of glucose averaged 191 ± 22 nmol/l (n = 36). Increasing the glucose concentration to 20 mmol/l resulted in a pronounced and reversible reduction of [Ca^{2+}]_{i} to 137 ± 23 nmol/l (n = 11; P < 0.05). The

![Graph](image-url)

FIG. 2. K<sub>ATP</sub> channels are active in pancreatic α-cells in the absence of glucose. A: Whole-cell current responses recorded from a wild-type (middle) or SUR1−/− (lower) α-cell under control conditions (left) and following addition of the K<sub>ATP</sub> channel blocker tolbutamide (0.1 mmol/l, right) in response to voltage steps going to −60 and −80 mV from a holding potential of −70 mV (top). B: Histogram summarizing measured conductance densities (membrane conductance normalized to cell capacitance; G/C) from five wild-type (●) and seven SUR1−/− (■) α-cells. Glucose (20 mmol/l) and tolbutamide were present or absent as indicated by the + and − signs below the histogram. *P < 0.05 and **P < 0.01. C: Tolbutamide inhibits electrical activity in the absence of glucose in wild-type mouse α-cells. The application of 100 μmol/l tolbutamide resulted in a (reversible) 17 mV depolarization followed by the cessation of electrical activity in wild-type mouse α-cells. The traces shown are representative for records from six different cells. D: Tolbutamide stimulates electrical activity in β-cells. Same conditions as in C. In C and D, the asterisks (*) indicate interruptions of the recording of ~1 min during the period of solution exchange. The segments selected for display were taken during steady state. Action potentials recorded from a wild-type α-cell in the absence (E) and presence (F) of tolbutamide on an expanded time base.

![Diagrams](image-url)

FIG. 3. Effects of glucose and channel modulators on [Ca^{2+}]_{i} in isolated wild-type and SUR1−/− α-cells. A and B: [Ca^{2+}]_{i} measured in a wild-type α-cell in the absence of glucose. Glucose (20 mmol/l), adrenaline (Adr.; 5 μmol/l), diazoxide (0.1 mmol/l), tolbutamide (0.1 mmol/l), and TTX (0.1 μg/ml) were added as indicated by the horizontal bars above the [Ca^{2+}]_{i} recording. C and D: As in A and B, but using an α-cell isolated from a SUR1−/− mouse and applying Co^{2+} (5 mmol/l). The traces are representative of 11–36 different cells.
ability of glucose to lower \([\text{Ca}^{2+}]_i\) confirms the identity of the \(\alpha\)-cell and is opposite to that observed in \(\beta\)-cells. This conclusion is reinforced by the transient elevation of \([\text{Ca}^{2+}]_i\) elicited by addition of 5 \(\mu\)mol/l adrenaline (22) (Fig. 3A).

Activation of KATP channels by diazoxide (0.1 \(\mu\)mol/l) promptly and reversibly reduced \([\text{Ca}^{2+}]_i\) in the \(\alpha\)-cell (145 ± 24 \(\mu\)mol/l; \(n = 6\)), an effect we attribute to membrane repolarization and suppression of regenerative electrical activity (14) (Fig. 3B). Paradoxically, tolbutamide (100 \(\mu\)mol/l) likewise produced a decrease in \([\text{Ca}^{2+}]_i\) (154 ± 21 \(\mu\)mol/l; \(n = 6\)). Electrical activity in mouse \(\alpha\)-cells depends on opening of TTX-sensitive voltage-gated Na\(^+\) channels (7). Indeed, application of TTX (0.1 \(\mu\)mol/ml) promptly reduced \([\text{Ca}^{2+}]_i\) (139 ± 13 \(\mu\)mol/l; \(n = 6\)) to the same extent as glucose, diazoxide, and tolbutamide (Fig. 3B).

**Effects of glucose and KATP channel modulators on \([\text{Ca}^{2+}]_i\) in \(\alpha\)-cells from SUR1\(^{-/-}\) mice.** The cytoplasmic \(\text{Ca}^{2+}\) concentration in \(\alpha\)-cells from SUR1\(^{-/-}\) mice also exhibited spontaneous oscillations in the absence of glucose (Fig. 3C). The average \([\text{Ca}^{2+}]_i\) measured in the absence of glucose was 261 ± 24 \(\mu\)mol/l (\(n = 47\)), significantly (\(P < 0.05\)) higher than in wild-type \(\alpha\)-cells (compare Fig. 3A and Fig. 3C). The \([\text{Ca}^{2+}]_i\) oscillations observed in the SUR1\(^{-/-}\) \(\alpha\)-cells were only partially suppressed following addition of glucose (Fig. 3C), and their amplitude fell from 442 ± 37 to 281 ± 27 \(\mu\)mol/l (\(P < 0.05\)). Application of adrenaline (5 \(\mu\)mol/l) elevated \([\text{Ca}^{2+}]_i\) transiently to a level comparable to that observed in wild-type \(\alpha\)-cells (Fig. 3C). As expected, diazoxide and tolbutamide (both 100 \(\mu\)mol/l) were ineffective in the SUR1-deficient \(\alpha\)-cells. The \([\text{Ca}^{2+}]_i\) oscillations involved \(\text{Ca}^{2+}\) influx through the plasma membrane, and addition of the broadband blocker of voltage-gated \(\text{Ca}^{2+}\) channels \(\text{Co}^{2+}\) (5 \(\mu\)mol/l) resulted in a prompt but reversible reduction in \([\text{Ca}^{2+}]_i\) (Fig. 3D). The \([\text{Ca}^{2+}]_i\) oscillations in SUR1\(^{-/-}\) \(\alpha\)-cells are likely to be secondary to bursts of \(\text{Na}^{+}\) channel–dependent action potentials, and the \(\text{Na}^{+}\) channel blocker TTX (0.1 \(\mu\)mol/ml) reduced \([\text{Ca}^{2+}]_i\) as strongly as \(\text{Co}^{2+}\) (Fig. 3D).

**Glucagon secretion.** We next correlated the electrophysiological and \([\text{Ca}^{2+}]_i\) measurements in islets lacking functional KATP channels with changes in glucagon secretion from intact pancreatic islets. Table I shows that increasing glucose from 0 mmol/l to 20 mmol/l reduced glucagon secretion by 50% in wild-type islets. Addition of the KATP channel inhibitors tolbutamide and glibenclamide as well as the activator diazoxide reduced glucagon secretion to the same extent as that obtained in response to elevation of glucose. The importance of voltage-gated \(\text{Na}^{+}\) channels for \(\alpha\)-cell electrical activity was underscored by the strong inhibitory action of the \(\text{Na}^{+}\) channel blocker TTX. The significance of \(\text{Ca}^{2+}\) influx through plasma membrane voltage-gated \(\text{Ca}^{2+}\) channels for glucagon secretion was illustrated by the 57 ± 9% inhibition obtained by 5 mmol/l \(\text{Co}^{2+}\). We also tested the effects of 4-AP, a blocker of a rapidly activating and inactivating TEA-resistant \(\text{K}^{+}\) current present in \(\alpha\)-cells (7). When applied at a concentration of 10 mmol/l, 4-AP reduced glucagon secretion at 1 mmol/l glucose from 39 ± 3 pg \(\cdot\) islet\(^{-1}\) \(\cdot\) h\(^{-1}\) to 22 ± 4 pg \(\cdot\) islet\(^{-1}\) \(\cdot\) h\(^{-1}\) (\(n = 10\), \(P < 0.001\); a 44 ± 9% reduction) and thus inhibited glucagon release to the same extent as increasing glucose to 20 mmol/l (22 ± 2 pg \(\cdot\) islet\(^{-1}\) \(\cdot\) h\(^{-1}\); \(n = 12\), \(P < 0.001\) vs. 1 mmol/l glucose). In KATP channel–deficient SUR1\(^{-/-}\) mice, the rate of glucagon secretion measured under basal conditions (0 mmol/l glucose alone) was reduced by ~30% relative to that observed in wild-type islets (Table I). Not unexpectedly, the KATP channel modulators tolbutamide, glibenclamide, and diazoxide were all ineffective in SUR1\(^{-/-}\) mice. However, both TTX and \(\text{Co}^{2+}\) remained potent inhibitors of glucagon secretion from knockout islets (35 ± 9% and 24 ± 6% inhibition, respectively; \(n = 5\), \(P < 0.01\)). Importantly, glucagon failed to inhibit glucagon secretion from islets lacking functional KATP channels. We also tested the effects of the incretin hormone glucagon-like peptide-1 (GLP-1) on glucagon secretion. Addition of 100 mmol/l GLP-1 reduced glucagon secretion in wild-type islets exposed to 1 mmol/l glucose by 44 ± 5% (\(P < 0.001\); \(n = 8\)) but was without effect in the SUR1\(^{-/-}\) islets (data not shown). Importantly, in SUR1\(^{-/-}\) islets, glucagon secretion in the presence of GLP-1 is 27 ± 9% higher than the corresponding value in wild-type islets. Thus, the failure of GLP-1 to suppress glucagon secretion in the SUR1\(^{-/-}\) islets is not simply because the release of the hormone is already maximally inhibited since glucagon secretion from SUR1\(^{-/-}\) islets can be further inhibited by \(\text{Co}^{2+}\) and TTX.

Figure 4 compares the effects of increasing glucose concentrations (0 to 20 mmol/l) on glucagon secretion measured from intact islets. Whereas glucose produced a concentration-dependent inhibition of glucagon secretion from wild-type islets with a half-maximal inhibitory concentration (IC\(_{50}\)) of ~2.5 mmol/l, no inhibition was detectable in islets lacking functional KATP channels, and the rate of secretion amounted to ~30 pg \(\cdot\) islet\(^{-1}\) \(\cdot\) h\(^{-1}\) at all glucose concentrations. It is pertinent to note that although glucagon secretion at low glucose concentrations is lower in SUR1\(^{-/-}\) islets than in wild-type islets, the opposite is true at concentrations ≥5 mmol/l.

**Effects of arginine and \(\text{K}^{+}\) on electrical activity and glucagon release.** If glucagon inhibits glucagon secretion by causing membrane depolarization and voltage-depen-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucagon release (pg (\cdot) islet(^{-1}) (\cdot) h(^{-1}))</th>
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<tbody>
<tr>
<td>Glucagon</td>
<td></td>
</tr>
<tr>
<td>0 mmol/l glucose</td>
<td>34 ± 3(^{\dagger})</td>
</tr>
<tr>
<td>0 mmol/l glucose + tolbutamide</td>
<td>33 ± 3(^{\circ})</td>
</tr>
<tr>
<td>0 mmol/l glucose + diazoxide</td>
<td>33 ± 3(^{\circ})</td>
</tr>
<tr>
<td>0 mmol/l glucose + glibenclamide</td>
<td>33 ± 3(^{\circ})</td>
</tr>
<tr>
<td>0 mmol/l glucose + TTX</td>
<td>22 ± 3(^{\circ})</td>
</tr>
<tr>
<td>0 mmol/l glucose + (\text{Co}^{2+})</td>
<td>26 ± 2(^{\circ})</td>
</tr>
<tr>
<td>20 mmol/l glucose</td>
<td>33 ± 4(^{\circ})</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE of six experiments. Glucagon release during 1 h was measured from batches of 10 size-matched freshly isolated islets at 0 or 20 mmol/l glucose, and effects of 100 mmol/l tolbutamide, 100 mmol/l diazoxide, 100 mmol/l glibenclamide, 5 mmol/l \(\text{Co}^{2+}\), or 0.1 \(\mu\)mol/ml TTX were investigated. *\(P < 0.01\) against control (0 mmol/l) in both wild-type or SUR1\(^{-/-}\) islets for comparisons within the same column. †\(P < 0.05\) vs. glucagon release measured in wild-type islet under the same experimental condition.
dent inactivation of the membrane currents involved in action potential firing, how can we explain the ability of arginine and elevation of extracellular K\(^+\) concentration from the basal 2.6 mmol/l to 15 mmol/l. In the presence of 15 mmol/l K\(^+\), the action potentials originated from a membrane potential of \(-31 \pm 2\) mV and peaked at \(-2 \pm 5\) mV. In addition, there was a 62% reduction in action potential frequency (from 3.7 \pm 1.1 to 1.4 \pm 0.1 Hz; \(P < 0.05; n = 3\)). These changes resemble those obtained in response to stimulation with glucose (Fig. 1A). In the presence of 70 mmol/l K\(^+\), the \(\alpha\)-cell was strongly depolarized, the membrane potential averaged \(-6 \pm 1\) mV, and the action potentials were small, if at all observed.

Addition of 10 mmol/l arginine produced a reversible increase in action potential frequency from \(4 \pm 1\) Hz under control conditions to \(7 \pm 1\) Hz in the presence of arginine (\(P < 0.05; n = 3\)). Figure 5B shows examples of action potentials in the absence of glucose (\(a\)) and after application of arginine (\(b\)). Under control conditions, the action potentials originated from a membrane potential of \(-55 \pm 9\) mV (most negative interspike voltage) and peaked at 16 \pm 8 mV. In the presence of arginine, the corresponding values were \(-37 \pm 3\) mV (most negative interspike voltage attained) and peaked at 6 \pm 7 mV. The observed 75% increase in action potential frequency correlated with a comparable stimulation of glucagon secretion; from \(39 \pm 3\) pg \(\cdot\) islet\(^{-1}\) \(\cdot\) h\(^{-1}\) under control conditions (0 mmol/l glucose) to 70 \pm 5 pg \(\cdot\) islet\(^{-1}\) \(\cdot\) h\(^{-1}\) in the presence of 10 mmol/l arginine (\(P < 0.001; n = 8\))—an enhancement of 80%.

Figure 5A also compares the effects of arginine on electrical activity with those obtained when increasing the extracellular K\(^+\) concentration from the basal 2.6 mmol/l to 15 mmol/l. In the presence of 15 mmol/l K\(^+\), the action potentials originated from a membrane potential of \(-31 \pm 2\) mV and peaked at \(-2 \pm 5\) mV. In addition, there was a 62% reduction in action potential frequency (from 3.7 \pm 1.1 to 1.4 \pm 0.1 Hz; \(P < 0.05; n = 3\)). These changes resemble those obtained in response to stimulation with glucose (Fig. 1A). In the presence of 70 mmol/l K\(^+\), the \(\alpha\)-cell was strongly depolarized, the membrane potential averaged \(-6 \pm 1\) mV, and the action potentials were small, if at all observed.

**FIG. 4.** Glucose-dependent inhibition of glucagon secretion in wild-type and SUR1\(^{-/-}\) mouse islets. Glucagon secretion was measured in wild-type (wt; ○) and SUR1\(^{-/-}\) islets (●) during 1-h incubations at 0, 2.5, 5, 10, and 20 mmol/l glucose. Data are means \pm SE of six experiments. In wild-type animals, glucagon secretion at all glucose concentrations \(\geq 2.5\) mmol/l is significantly (\(P < 0.01\)) inhibited with respect to release in the absence of glucose. Whereas glucagon secretion at 0 and 1 mmol/l is significantly (\(P < 0.05\)) reduced in SUR1\(^{-/-}\) islets relative to wild-type islets, glucagon secretion is enhanced (\(P < 0.05\)) at all glucose concentrations \(\geq 5\) mmol/l.

**FIG. 5.** Effects of arginine and high K\(^+\) on electrical activity and glucagon release in \(\alpha\)-cells. A: Current-clamp recording of membrane potential (\(V\)) in a single mouse \(\alpha\)-cell. Arginine (10 mmol/l) and K\(^+\) (final concentrations 15 and 70 mmol/l) were applied during the periods indicated by the horizontal lines. The asterisks (*) indicate interruptions of the recording of \(-1\) min during the period of solution exchange. The segments selected for display were taken during steady state. B: Action potentials recorded at basal (\(a\)) and in the presence of arginine (\(b\)), 15 mmol/l (\(c\)), and 70 mmol/l K\(^+\) (\(d\)) on an expanded time base. Records were taken from \(A\) as indicated by the letters. The recording is representative of three separate experiments. C: Glucagon secretion was measured in mouse islets during 1-h incubations at increasing extracellular K\(^+\) concentrations (2.6–70 mmol/l) in the presence of 0 mmol/l glucose. The shaded bar shows glucagon release at 5.6 mmol/l K\(^+\) and 20 mmol/l glucose. D: As in \(C\), except that insulin secretion was measured. Data are means \pm SE of eight experiments. *\(P < 0.001\) vs. 2.6 mmol/l K\(^+\); †\(P < 0.01\) vs. 2.6 mmol/l K\(^+\) and 0 mmol/l glucose.
TABLE 2
Effects of thapsigargin on glucagon secretion in wild-type and K_{ATP} channel–deficient (SUR1^−/−) islets

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucagon release (pg • islet^−1 • h^−1)</th>
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<tbody>
<tr>
<td>1 mmol/l glucose</td>
<td>25 ± 2†</td>
</tr>
<tr>
<td>20 mmol/l glucose</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>1 μmol/l glucose + 10 μmol/l thapsigargin</td>
<td>26 ± 2†</td>
</tr>
<tr>
<td>20 μmol/l glucose + 10 μmol/l thapsigargin</td>
<td>26 ± 4</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SE of 8–12 experiments. Glucagon release during 1 h was measured from batches of 10 size-matched freshly isolated islets at 1 and 20 mmol/l glucose under control conditions and in the presence of 10 μmol/l thapsigargin (also present during the 30-min preincubation). *P < 0.01 against control (1 mmol/l) in both wild-type and SUR1^−/− islets. †P < 0.05 vs. wild-type islets under the same experimental condition. ‡P < 0.05 vs. wild-type islets exposed to 1 mmol/l glucose + thapsigargin.

We compared these effects on electrical activity with changes in glucagon secretion from intact wild-type mouse islets. Interestingly, increasing the external K^+ to concentrations up to 15 mmol/l resulted in a progressive inhibition of glucagon release (Fig. 5C). Again it is pertinent to note that the K^+–induced suppression of glucagon secretion was similar to that obtained in response to addition of 20 mmol/l glucose (shaded area in Fig. 5C). As K^+ was increased beyond 25 mmol/l, the inhibitory action was superseded by a progressive stimulation of glucagon secretion. This we attribute to the change in membrane potential being large enough to elicit openings of the high-threshold Ca^{2+} channels involved in glucagon secretion.

The effects of raising the external K^+ concentration on insulin secretion were rather different (Fig. 5D). Concentrations up to 15 mmol/l were without effect, whereas higher concentrations resulted in a monotonic stimulation of insulin secretion.

**Involvement of a Ca^{2+} store-operated membrane conductance.** Based on measurements of [Ca^{2+}], it has been proposed that a store-operated membrane conductance regulates α-cell electrical activity (23). However, the experiments were conducted in isolated α-cells, and glucagon secretion was not measured. Table 2 compares the ability of glucose to inhibit glucagon secretion in the absence and presence of thapsigargin, an inhibitor of SERCA (24), in wild-type and SUR1^−/− islets. It can be seen that increasing glucose from 1 to 20 mmol/l reduced glucagon secretion by 46% in wild-type islets and that this effect is partially (by one-third) counteracted by thapsigargin. In SUR1^−/− islets, neither glucose nor thapsigargin affected glucagon secretion.

**DISCUSSION**

Whereas the metabolic regulation of insulin secretion from the β-cell is fairly well understood (25), the control of glucagon secretion by glucose remains obscure. Studies on purified fractions of rat α-cells have revealed that they 1) are regulated by glucose metabolism (26); 2) express the glucose sensor enzyme glucokinase (26); 3) depend on the low-K_m glucose transporter protein Glut1 for transmembrane glucose transport, rather than Glut2, as in the β-cell (27); and 4) have a high ATP-to-ADP ratio already in the absence of glucose, and that it does not change upon glucose stimulation (28). It is also well established that the α-cells are electrically excitable and, unlike the β-cells, generate action potentials in the absence of glucose (15,16,20,29). The membrane conductances involved in action potential have been characterized in some detail for guinea pig (29,30), mouse (7), and rat (15) α-cells. These studies indicate that α-cells are equipped with voltage-dependent T-, N-, and L-type Ca^{2+} currents. They also contain a prominent voltage-gated and TTX-sensitive Na^+ current that is activated during the action potential and contributes to the fact that α-cells, in contrast to β-cells, produce overshooting action potentials (i.e., exceed 0 mV) (7,14,18). Curiously, the α-cells also contain K_{ATP} channels (14,18) of the same type as those that constitute the resting conductance of the β-cell.

**Different mechanisms control glucagon secretion in different species.** With so much information available, one would expect the metabolic regulation of glucagon secretion to be already elucidated. Unfortunately, we are still far from achieving this goal, but several hypotheses have been postulated over the years. These include that Zn^{2+} cosecreted with insulin exerts a paracrine inhibitory action on mouse pancreatic α-cells (9). Based on measurements of [Ca^{2+}], in isolated mouse α-cells, it has recently been suggested that a store-operated membrane conductance plays a pivotal role in its regulation (23). At low glucose, intracellular Ca^{2+} stores are empty, leading to activation of the depolarizing conductance with resultant initiation of α-cell electrical activity and stimulation of glucagon secretion. Following an increase in glucose concentration, metabolism is accelerated and the intracellular Ca^{2+} stores are filled, leading to reduction of conductance, membrane repolarization, and suppression of glucagon secretion. In wild-type islets, application of 10 μmol/l thapsigargin partially (<30%) antagonized the glucose inhibitory action, whereas this Ca^{2+} ATPase inhibitor had no effect in SUR1^−/− islets. Nevertheless, the presence of a small store-operated conductance regulated by glucose may explain the reduction in action potential frequency (Fig. 1) and decreased amplitude of the [Ca^{2+}] oscillations in SUR1^−/− α-cells (Fig. 3C). Finally, paracrine inhibition of glucagon secretion by somatostatin released by neighboring δ-cells (11) or GABA secreted by β-cells (8,12,31) has been proposed to contribute to the glucose inhibitory action. The latter mechanism appears quantitatively important in rat islets, but it is probably less significant in mouse islets, which contain much lower levels of GABA than rat and human islets (32). Overall, it seems that different mechanisms may control glucagon secretion in different species, and some of the existing controversies may simply reflect interspecies variation.

**A low activity of K_{ATP} channels is required for glucagon secretion.** Studies on isolated inside-out patches have revealed that the pancreatic α-cells paradoxically contain a high density of K_{ATP} channels (14). In the pancreatic β-cells, glucose inhibits the K_{ATP} channels and thus leads to stimulation of electrical activity and insulin secretion (33,34). Yet, if closure of K_{ATP} channels in β-cells results in stimulation of insulin secretion, how can closure...
of the same channels in α-cells inhibit glucagon secretion? We have proposed that the answer to this conundrum lies in the distinct electrophysiological properties of the α- and β-cells (Fig. 6; see also Fig. 2). Thus, whereas β-cell electrical activity and secretion are principally dependent on L-type Ca\(^{2+}\) channels, α-cell action potential firing involves voltage-gated Na\(^{+}\) channels. Unlike the L-type Ca\(^{2+}\) channels, the Na\(^{+}\) channels undergo voltage-dependent inactivation (i.e., they enter a nonconducting state when the voltage becomes too positive) (7). Closure of the K\(_{ATP}\) channels with resultant membrane depolarization will, therefore, contrary to what is observed in β-cells, result in reduction of action potential firing. A low K\(_{ATP}\) channel density combined with a relatively high ATP-to-ADP ratio inside the α-cell already at low glucose concentrations (28) is indeed a requirement to keep the membrane potential sufficiently depolarized to allow regenerative electrical activity and yet prevent voltage-dependent inactivation of the ionic conductances involved in action potential firing. The concept that membrane depolarization due to closure of K\(_{ATP}\) channels is involved in glucose-induced inhibition of glucagon secretion is supported by the findings that addition of a low concentration of K\(^{+}\) leads to a moderate membrane depolarization but a marked reduction in spike amplitude and frequency and decreases glucagon secretion to the same extent as a maximally inhibitory concentration of glucose.

We demonstrate here that metabolic, hormonal, and pharmacological regulation of glucagon secretion is severely compromised in SUR1\(^{-/-}\) islets. At least five important conclusions can be drawn from the data: 1) some K\(_{ATP}\) channels are active in wild-type α-cells in the absence of glucose (Fig. 2), 2) closure of the K\(_{ATP}\) channels leads to inhibition of glucagon secretion (Table 1), 3) the effects of closing K\(_{ATP}\) channels resemble those obtained by blocking voltage-gated Na\(^{+}\) and Ca\(^{2+}\) channels (Fig. 3 and Table 1), 4) overactivation of K\(_{ATP}\) channels using diazoxide inhibits glucagon secretion, indicating that glucagon secretion only occurs within a certain window of K\(_{ATP}\) channel activity (Fig. 3 and Table 1), and 5) functional K\(_{ATP}\) channels are required for glucose and GLP-1 to inhibit glucagon secretion.

We also observed that 4-AP, an inhibitor of voltage-activated A-type K\(^{+}\) channels, inhibited glucagon secretion almost as effectively as glucose or TTX. This finding raises the interesting possibility that A-type K\(^{+}\) channels are required to restore the negative membrane potential after each action potential. This in turn is necessary for channels inactivated during the depolarization to be reactivated. In this context, it is of interest that A-type K\(^{+}\) channels undergo voltage-dependent inactivation at more negative voltages than the Na\(^{+}\) channels (half-maximal inactivation occurring at −68 mV and −47 mV for the A-type K\(^{+}\) current and Na\(^{+}\) current, respectively) (7). Pharmacological A-type K\(^{+}\) current inactivation will therefore have the same effect as inhibition of the Na\(^{+}\) channels on glucagon secretion and vice versa.

Mouse pancreatic α-cells are equipped with T-, L-, and N-type Ca\(^{2+}\) channels (7,13). Glucagon secretion triggered by hypoglycemia alone depends principally on Ca\(^{2+}\) influx through N-type Ca\(^{2+}\) channels (21). The high-threshold Ca\(^{2+}\) channels require strong depolarization to activate, and capacitance measurements of exocytosis in α-cells within intact pancreatic islets have revealed that exocytosis at −1 mV (the peak of the action potential in the presence of 20 mmol/l glucose or 15 mmol/l K\(^{+}\)) is only 77 and 50% of that observed at 6 and 16 mV (the peak of the action potentials observed in the presence of arginine and in the absence of glucose), respectively (21). We believe that these features are central to the understanding of how glucose regulates glucagon secretion. We postulate that the N-type Ca\(^{2+}\) channels and stimulation of exocytosis is therefore only obtained for a brief period around the peak of the action potential when the voltage exceeds 0 mV. Any membrane depolarization will, via inactivation of the Na\(^{+}\) channels, cause a reduction of the spike amplitude and a corresponding decrease in the time during which voltage exceeds that required to open the Ca\(^{2+}\) channels and trigger exocytosis. For stronger depolarizations, inactivation of the Na\(^{+}\) channels eventually becomes so pro-

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**FIG. 6.** Glucose regulation of the mouse pancreatic α-cell. A: At low glucose, K\(_{ATP}\) channel activity is low (but greater than zero) so that membrane potential is at the threshold for action potential firing. Opening of voltage-gated T- and N-type Ca\(^{2+}\) channels and TTX-sensitive Na\(^{+}\) channels triggers electrical activity, Ca\(^{2+}\) influx, and glucagon release. Opening of A-type K\(^{+}\) channels restores the negative membrane potential after the action potential, thus facilitating reactivation of channels inactivated during depolarization. B: At high glucose, K\(_{ATP}\) channels close completely, leading to greater membrane depolarization. The voltage-gated Ca\(^{2+}\) and Na\(^{+}\) channels therefore become inactivated and enter a nonconductive state, and electrical activity ceases. In turn, Ca\(^{2+}\) influx is reduced and glucagon release is suppressed.
nounced that the action potential does not extend into the range of voltages associated with Ca2+ channel opening or fail completely with resultant inhibition of glucagon secretion. This scenario is fully consistent with the observations that the Na+ channel antagonist TTX and the KATP channel blocker tolbutamide are stronger inhibitors of glucagon secretion than glucose. This is because they lead to complete inhibition of the Na+ channels although the mechanisms of action are slightly different (channel block vs. inactivation). Collectively, the effects of glucose on spike frequency (−44%) and height (−30%), combined with the voltage dependence of exocytosis (exocytosis at −1 mV 50% of that at 16 mV), predict that 20 mmol/l glucose should inhibit exocytosis by ∼50%, close to that observed experimentally (Table 1).

It may appear that this scenario fails to account for the ability of arginine to stimulate glucagon secretion (+75%). Application of arginine did in fact increase action potential frequency by 80%, but this effect associated with a reduction in spike height (peak potential 6 mV instead of 16 mV). Given that exocytosis at the more negative voltage is only 77% of that seen at 16 mV, we would expect the arginine-induced stimulation to be limited to 25%, only one-third of that actually observed. However, it may be that the reduction in spike height is overestimated in the electrophysiological measurements, as any damage of the cell, which actually observed. However, it may be that the reduction in spike height is overestimated in the electrophysiological measurements, as any damage of the cell, which certainly not only occurs during seal establishment, will tend to depolarize the α-cell. Alternatively, arginine may (e.g., via generation of nitric oxide [35]) modulate glucagon secretion by a direct effect on the secretory machinery.

Controversies. We acknowledge that the theory that closure of KATP channels underlies glucose-dependent inhibition of glucagon secretion is not uncontested. It has been reported that only a small fraction of isolated mouse α-cells respond to tolbutamide with elevation of [Ca2+]i (23). This suggests that although the α-cell contains KATP channels, these are not active. It has also been reported that tolbutamide lacks effects on [Ca2+]i in intact mouse islets exposed to 3 mmol/l glucose (36). It should be noted, however, that our measurements of glucagon release indicate that α-cell secretion is fully inhibited already at 5 mmol/l glucose (Fig. 4). If KATP channels are involved in the regulation of glucagon secretion by glucose, then the channels are likely to be almost completely blocked in the presence of 3 mmol/l of the sugar, and it is therefore not unexpected that the response to addition of tolbutamide is limited.

Our data are also in disagreement with those of Miki et al., who reported that glucose retains an inhibitory action on glucagon secretion in Kir6.2-deficient mice (37), which like SUR1−/− mice lack functional KATP channels (38). Moreover, in contrast to our findings in SUR1−/− islets, glucagon secretion from Kir6.2−/− islets at 1.7 mmol/l glucose was enhanced compared with that from wild-type mouse islets. Our finding that glucose remained capable of suppressing [Ca2+]i and reducing spike frequency in isolated α-cells from SUR1−/− mouse (Fig. 3C and D) is in fact consistent with the data obtained in Kir6.2−/− islets. These differences between the two KATP channel−deficient mice are puzzling, but it is important to emphasize that at least in the case of SUR1, the biological effects of the protein do not seem to be confined to being a subunit of the KATP channel (39). It may also seem surprising that the α-cells from the SUR1-deficient mice remain capable of generating action potentials and that the membrane potential remained so negative despite the low K+ conductance. This suggests the presence of compensatory mechanisms and that the SUR1−/− α-cells possesses a conductance compensating for the lack of KATP channels, the identity of which remains to be elucidated.

Concluding remarks. Glucagon release from the pancreatic α-cells contributes to postprandial hyperglycemia in both type 1 and type 2 diabetes (40,41), and substantial improvement of glycemia is obtained if endogenous glucagon production is suppressed. The sulfonylurea compound tolbutamide suppresses glucagon secretion in normal and type 2 diabetic subjects (42). Furthermore, glibenclamide inhibits glucagon response in diabetic patients under hypoglycemic conditions (43). Thus, the same metabolic defects that lead to a reduced insulin secretion from the β-cells (34) might be associated with failure of glucose to inhibit glucagon secretion from the α-cells. Greater insight into the physiological and pharmacological mechanisms controlling glucagon secretion is not without implications for diabetes therapy.

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