

Voltage-Gated Ion Channels in Human Pancreatic β -Cells: Electrophysiological Characterization and Role in Insulin Secretion

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OBJECTIVE—To characterize the voltage-gated ion channels in human β -cells from nondiabetic donors and their role in glucose-stimulated insulin release.

RESEARCH DESIGN AND METHODS—Insulin release was measured from intact islets. Whole-cell patch-clamp experiments and measurements of cell capacitance were performed on isolated β -cells. The ion channel complement was determined by quantitative PCR.

RESULTS—Human β -cells express two types of voltage-gated K^+ currents that flow through delayed rectifying ($K_{v2.1/2.2}$) and large-conductance Ca^{2+} -activated K^+ (BK) channels. Blockade of BK channels (using iberiotoxin) increased action potential amplitude and enhanced insulin secretion by 70%, whereas inhibition of $K_{v2.1/2.2}$ (with stromatocytin) was without stimulatory effect on electrical activity and secretion. Voltage-gated tetrodotoxin (TTX)-sensitive Na^+ currents ($Na_v1.6/1.7$) contribute to the upstroke of action potentials. Inhibition of Na^+ currents with TTX reduced glucose-stimulated (6–20 mmol/l) insulin secretion by 55–70%. Human β -cells are equipped with L- ($Ca_v1.3$), P/Q- ($Ca_v2.1$), and T- ($Ca_v3.2$), but not N- or R-type Ca^{2+} channels. Blockade of L-type channels abolished glucose-stimulated insulin release, while inhibition of T- and P/Q-type Ca^{2+} channels reduced glucose-induced (6 mmol/l) secretion by 60–70%. Membrane potential recordings suggest that L- and T-type Ca^{2+} channels participate in action potential generation. Blockade of P/Q-type Ca^{2+} channels suppressed exocytosis (measured as an increase in cell capacitance) by >80%, whereas inhibition of L-type Ca^{2+} channels only had a minor effect.

CONCLUSIONS—Voltage-gated T-type and L-type Ca^{2+} channels as well as Na^+ channels participate in glucose-stimulated electrical activity and insulin secretion. Ca^{2+} -activated BK channels are required for rapid membrane repolarization. Exocytosis of insulin-containing granules is principally triggered by Ca^{2+} influx through P/Q-type Ca^{2+} channels. *Diabetes* 57:1618–1628, 2008

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BK, Ca^{2+} -activated K^+ ; IbTX, iberiotoxin; I-V, current-voltage relationship; K_{ATP} channel, ATP-sensitive K^+ channel; TEA, tetraethylammonium; TTX, tetrodotoxin.

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Voltage-gated plasmalemmal ion channels play a fundamental role in stimulus secretion coupling in β -cells, and Ca^{2+} influx through voltage-gated Ca^{2+} channels triggers exocytosis of insulin-containing secretory granules (1). Voltage-gated Ca^{2+} channels are activated by coordinated fluctuations of the cell membrane potential (electrical activity), which are initiated by the glucose-induced closure of ATP-sensitive K^+ channels (K_{ATP} channels) (2,3) and dependent on voltage-gated Na^+ and K^+ channels (4). Due to the limited availability of human islets, few electrophysiological studies of voltage-gated ion channels in human β -cells have been published, and the identity of the β -cells was not unequivocally established (5–8), although 45% of normal human islets cells are non- β -cells (9). In some earlier studies (10–12), identification of β -cells was based on the presence of K_{ATP} currents. However, this is not unproblematic, as K_{ATP} channels are also found in non- β -cells (13,14).

In the present study, we have identified the voltage-gated ion channels expressed in human β -cells obtained from nondiabetic donors and characterized their role in glucose-induced insulin secretion. Our data illustrate that human β -cells differ from rodent cells in several important respects.

RESEARCH DESIGN AND METHODS

Islet isolation and cell culture. With appropriate ethical approval and clinical consent, pancreatic islets were isolated in the Diabetes Research and Wellness Foundation Human Islet Isolation Facility from human pancreata retrieved from nondiabetic, heart-beating donors. This study is based on 34 islet preparations. For patch-clamp experiments, islets were dispersed into single cells immediately after preparation by incubation in Ca^{2+} -free buffer followed by trituration. The cells were cultured in RPMI-1640 medium containing 10 mmol/l glucose and 2 mmol/l L-glutamine.

Materials. ω -Conotoxin GVIA, SNX482, ω -agatoxin IVA, and stromatocytin-1 were purchased from Alomone Labs (Jerusalem, Israel) or the Peptide Institute (Osaka, Japan). Iberiotoxin (IbTX) was from Bachem (St. Helens, U.K.). All other chemicals were purchased from Sigma-Aldrich.

Insulin secretion. Insulin secretion was measured as described elsewhere (15). Briefly, batches of 10–20 islets (in triplicates) were preincubated in 1 ml Krebs-Ringer buffer supplemented with 1 mmol/l glucose for 1 h followed by a 1-h incubation in 1 ml of test Krebs-Ringer buffer medium supplemented as indicated.

Electrophysiology. Patch-clamp experiments were performed using an EPC-9 amplifier and Pulse software (HEKA, Lamprecht, Germany). All electrophysiological experiments were performed at 32–33°C using the standard or perforated-patch whole-cell configurations.

Solutions. K^+ currents were recorded in an extracellular solution composed of (mmol/l) 138 NaCl, 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 5 HEPES, and 5 glucose (pH 7.4, adjusted with NaOH). For recording Na^+ and Ca^{2+} currents, 10 mmol/l tetraethylammonium (TEA) was added and NaCl correspondingly reduced. For Ca^{2+} current measurements, 0.1 μ g/ml tetrodotoxin was added

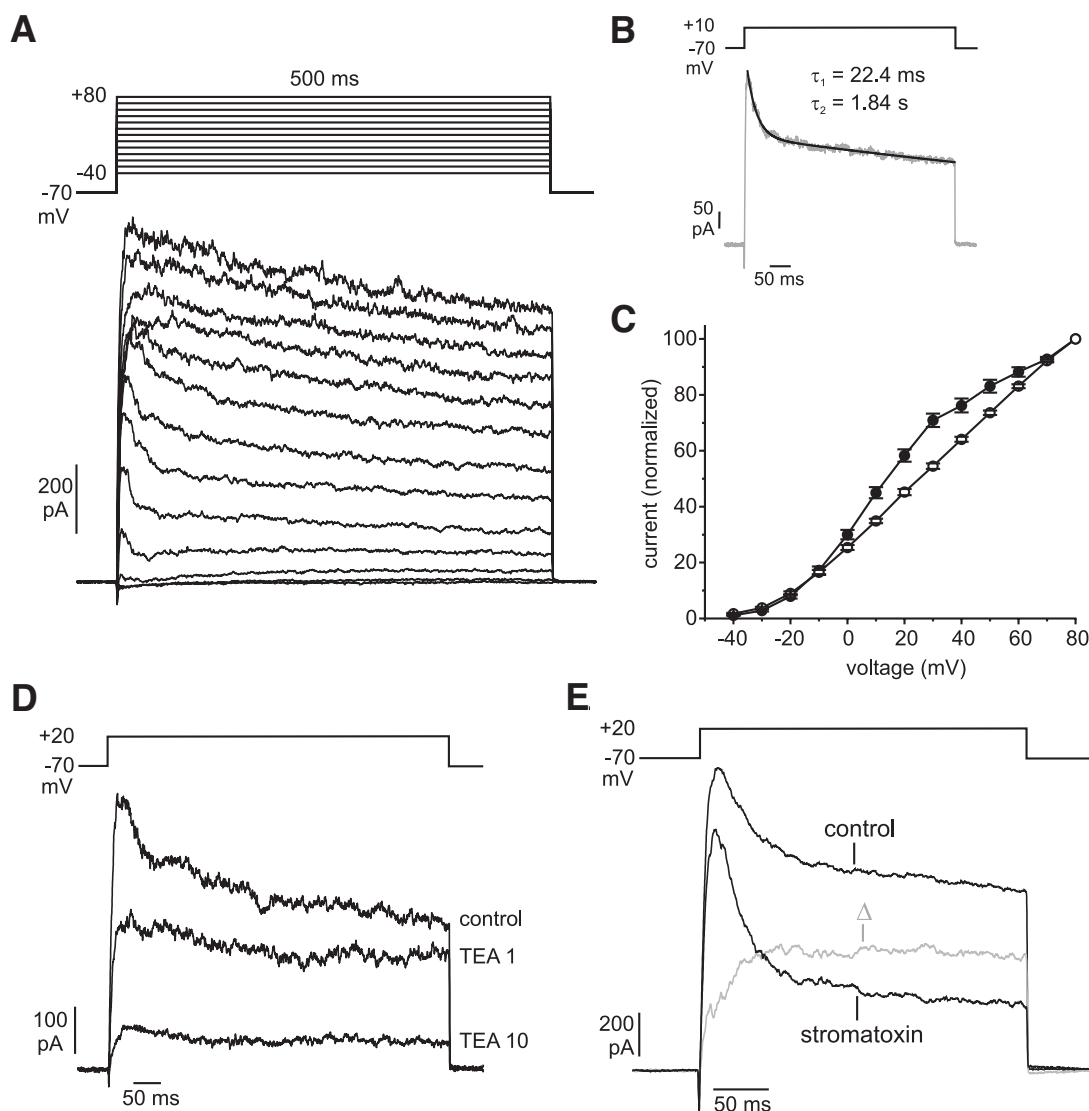


FIG. 1. Voltage-gated K^+ currents in β -cells. **A:** K^+ currents recorded during depolarizations to potentials between -40 and $+80$ mV (in 10 -mV steps). **B:** An exponential fit of the current decay using the time constants (τ) shown is superimposed on the current (same experiment as **A**). **C:** I-V relationship for peak (\bullet) and sustained (\circ) (measured at the end of 500 -ms depolarizations) current ($n = 23$). The currents are expressed as percent of the responses at $+80$ mV. **D:** Voltage-gated K^+ currents recorded under control conditions and in the presence of 1 or 10 mmol/l TEA. **E:** Voltage-gated K^+ currents recorded in the absence and presence of 100 nmol/l stromatoxin. The gray trace represents the difference current (Δ).

additionally. For Na^+ current recordings, extracellular $CaCl_2$ was replaced equimolarly with $MgCl_2$ and 1 mmol/l $CoCl_2$ included in the medium.

The intracellular solution for K^+ current measurements contained (mmol/l) 120 KCl, 1 $MgCl_2$, 10 EGTA, 1 $CaCl_2$, 10 HEPES, and 3 MgATP (pH 7.2 , KOH). Na^+ and Ca^{2+} current measurements were made after equimolar substitution of KCl by CsCl. For capacitance measurements, the pipette solution contained (mmol/l) 125 Cs-glutamate, 10 CsCl, 10 NaCl, 1 $MgCl_2$, 5 HEPES, 0.05 EGTA, 3 MgATP, and 0.1 cAMP (pH 7.2 , CsOH). Glucose- and tolbutamide-induced electrical activity was recorded in the perforated-patch whole-cell configuration as previously reported (16). Biotin (0.5 mg/ml; Invitrogen) was added to all pipette solutions to facilitate subsequent establishment of cell identity.

Immunocytochemistry. Immunolabeling with antibodies against insulin, glucagon, and somatostatin and detection of biocytin-loaded cells was performed essentially as described previously (17).

Quantitative RT-PCR. Gene expression profiling of ion-channel isoforms was performed by RT-qPCR on human islet total RNA (for details see the online appendix [available at <http://dx.doi.org/10.2337/db07-0991>]).

Data analysis. All data points are expressed as means \pm SE of indicated number of experiments. All experiments were performed using islets/cells from at least two different donors. Inhibitory effects of channel blockers on glucose-induced insulin secretion are given after subtraction of basal release. Statistical significance was evaluated using Student's t test. A more detailed description of the experimental procedures is provided in the online appendix.

RESULTS

Voltage-gated K^+ currents. The identity of all β -cells used for electrophysiological analysis in this study was confirmed by immunocytochemistry. Human β -cells thus identified had a membrane capacitance (C_{slow}) of 9.9 ± 0.3 pF ($n = 207$) (online appendix Fig. 1).

Voltage-gated K^+ currents were elicited by 500 ms voltage-clamp depolarizations from a holding potential of -70 mV. Outward K^+ currents became detectable during depolarizations to -30 mV and beyond (Fig. 1A). The activation kinetics of the K^+ current elicited by depolarization to $+10$ mV was estimated using a Hodgkin-Huxley m^4 model. The time constant (τ) of activation averaged 1.9 ± 0.1 ms ($n = 31$). In 26 of 31 cells, current inactivation was best described as the sum of two exponentials (Fig. 1B). The average time constants (τ) were 41 ± 7 ms for the fast (transient) component and 1.8 ± 0.1 s for the slowly inactivating (sustained) current. The transient component accounted for $28 \pm 2\%$ of the total current amplitude at

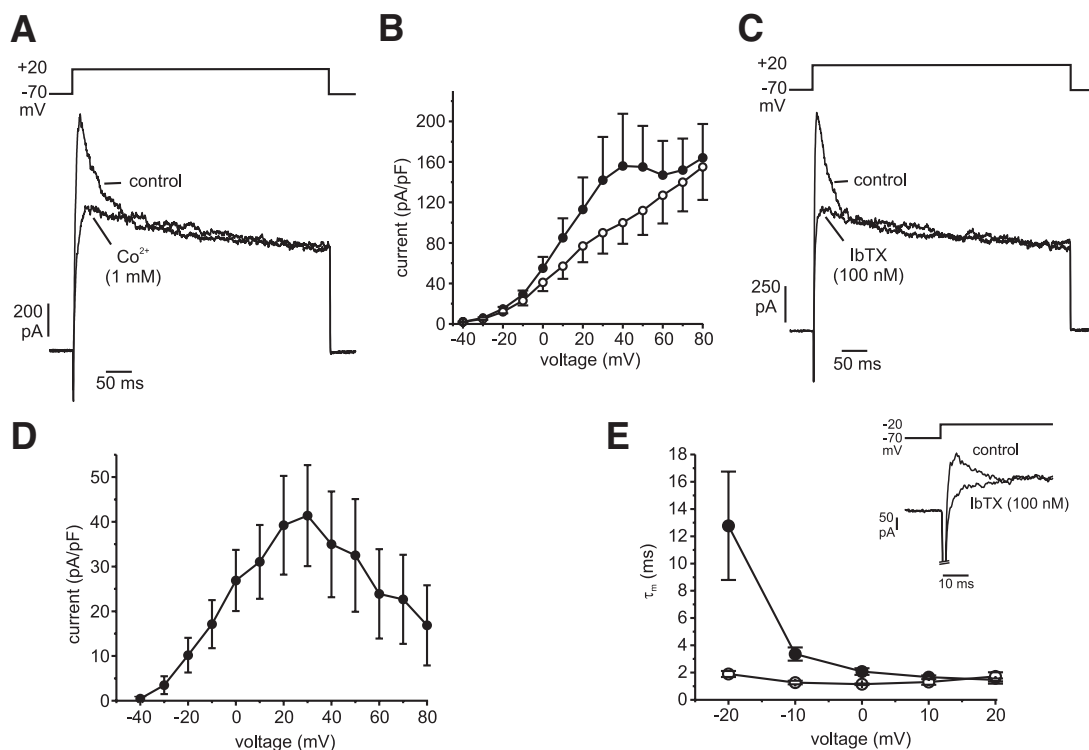


FIG. 2. Effects of Co^{2+} and IbTX on K^+ currents. **A:** K^+ currents recorded in the absence or presence of Co^{2+} . **B:** I-V relationship for peak outward currents (normalized to cell size) recorded in the absence (\bullet) and presence (\circ) of Co^{2+} ($n = 3$). **C:** K^+ currents recorded in the absence or presence of IbTX. **D:** I-V relationship of the IbTX-sensitive current ($n = 9$). **E:** Voltage dependence of the activation time constants τ_m of the IbTX-resistant (\bullet) and -sensitive (\circ) currents ($n = 7$). *Inset* shows currents evoked by depolarizations to -20 mV before and after application of IbTX on an expanded time base.

+10 mV. In the remaining five cells, the inactivation could be well described using a single time constant of 1.8 ± 0.3 s. In cells with a transient current component, a small “shoulder” in the current-voltage relationship (I-V) relationship was apparent at voltages between +40 and +60 mV (Fig. 1A and C).

The peak outward current was sensitive to TEA; 1 and 10 mmol/l of this broad-spectrum K^+ channel blocker inhibited $50 \pm 3\%$ ($n = 9$) and $83 \pm 3\%$ ($n = 14$), respectively (Fig. 1D). The $\text{Kv}2.1/2.2$ antagonist stromatocin-1 selectively inhibited the slowly inactivating outward current (Fig. 1E). In a series of eight experiments, the sustained current component (measured at the end of a 250-ms depolarization to +20 mV) was reduced by $45 \pm 6\%$ ($P < 0.001$), while the peak current decreased only by $15 \pm 4\%$ ($P < 0.01$).

The Ca^{2+} channel blocker Co^{2+} selectively abolished the transient current (Fig. 2A) and removed the shoulder in the I-V relationship (Fig. 2B). IbTX, a specific blocker of large-conductance Ca^{2+} -activated K^+ channels (BK channels), also selectively blocked the transient K^+ current (Fig. 2C), and its effect was indistinguishable from that of Co^{2+} (compare Fig. 2A and C). The IbTX-sensitive current was reconstructed by subtracting the current obtained in the presence of the blocker from that measured under control conditions. The isolated IbTX-sensitive current exhibited a bell-shaped voltage dependence with a peak of 41 ± 11 pA/pF ($n = 9$) at +30 mV (Fig. 2D), comparable with the 52 ± 23 pA/pF ($n = 3$) of the Co^{2+} -sensitive current (not shown).

Figure 2E (*inset*) shows the total and IbTX-resistant currents evoked by depolarization to -20 mV. Whereas the current before IbTX activated rapidly, the IbTX-insen-

sitive current developed more slowly. Figure 2E summarizes the relationship between voltage and time constants of activation (τ_m) of IbTX-sensitive and -resistant currents. The IbTX-sensitive and -insensitive currents elicited by depolarizations to +10 mV inactivated with time constants of 22 ± 6 ms ($n = 7$) and 1.6 ± 0.3 s ($n = 7$), respectively (not shown). These values are comparable with the time constants for the fast and slowly inactivating components under control conditions described above. The magnitude of the IbTX-sensitive component at +10 mV was $\sim 50\%$ of that of the IbTX-insensitive component (approximately one-third of the total), in good agreement with the fraction of the current showing rapid inactivation under control conditions (28%, see above).

The effects of the K^+ channel blockers on β -cell electrical activity are shown in Fig. 3. TEA (10–20 mmol/l) increased the peak voltage of the action potentials induced by 6 mmol/l glucose ($n = 3$) (Fig. 3A) or tolbutamide (100 $\mu\text{mol/l}$; $n = 4$) from -13 ± 5 mV to 4 ± 7 mV. The $\text{Kv}2.1/2.2$ blocker stromatocin had weak effects on electrical activity; a significant increase in spike height was observed in only one of seven cells (Fig. 3B) and the action potentials peaked at -21 ± 3 mV in both the absence and presence of the blocker ($n = 4$ for glucose, $n = 3$ for tolbutamide). Addition of IbTX significantly increased the amplitude of glucose-induced action potentials in two of five cells (Fig. 3C), whereas action potential firing was inhibited in the remaining three cells. The latter effect was accompanied by hyperpolarization and an increase in resting membrane conductance ($n = 2$) (not shown), presumably reflecting activation of K_{ATP} channels. When IbTX was tested in the presence of tolbutamide (100 $\mu\text{mol/l}$), it consistently increased the amplitude of action potentials ($n = 3$). Similar

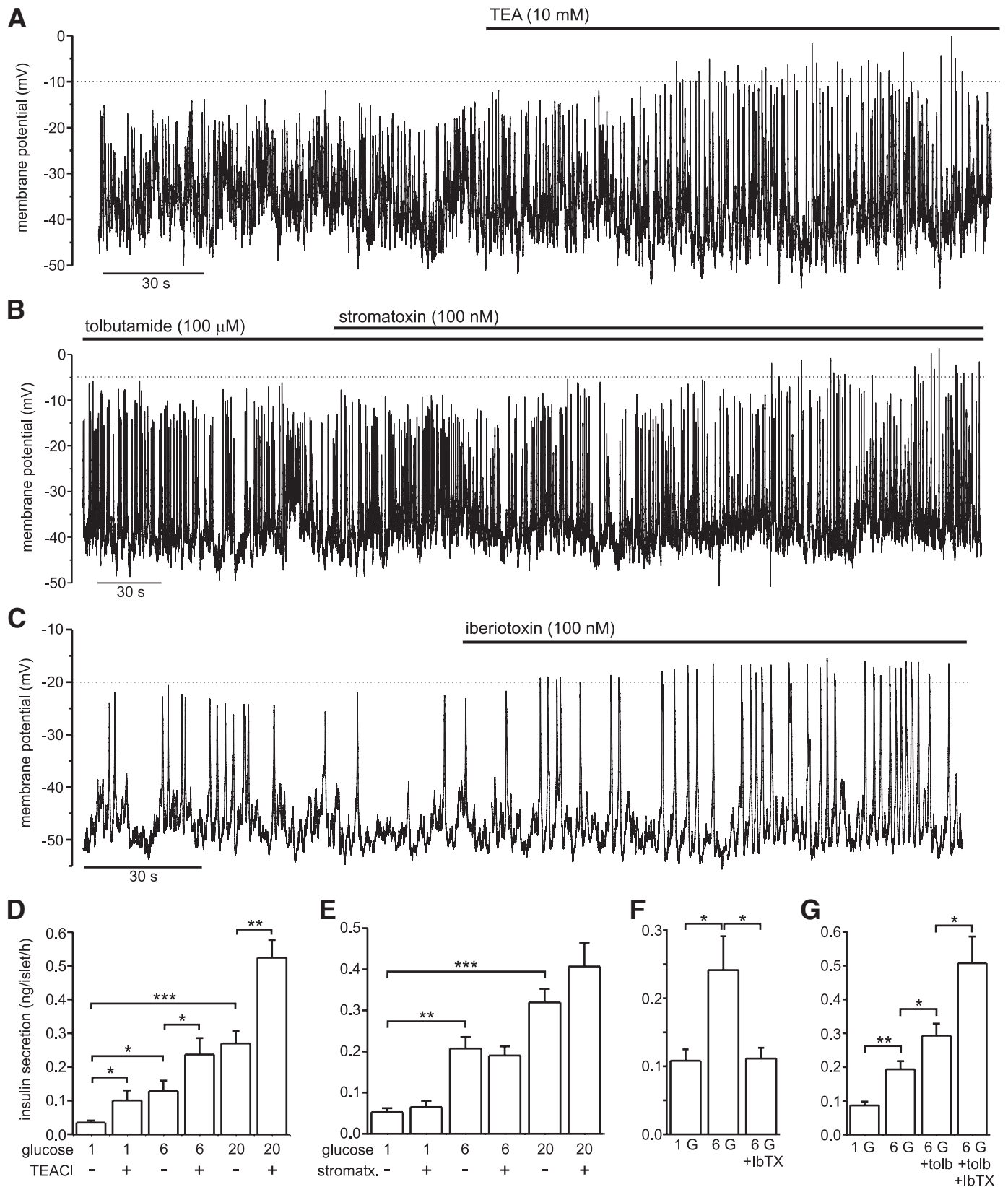


FIG. 3. Effects of K^+ channel blockers on β -cell electrical activity and insulin secretion. **A:** Effect of TEA on electrical activity evoked by 6 mmol/l glucose. In this experiment, the action potential peak voltage increased from -20 ± 1 to -13 ± 1 mV. **B:** Effect of stromatoxin on electrical activity triggered by 10 mmol/l glucose and 100 μ mol/l tolbutamide. Spike height in this experiment increased from -12 ± 1 to -8 ± 1 mV. **C:** Effect of IbTx on 6 mmol/l glucose-induced electrical activity. Action potentials peaked at -22 ± 1 and -14 ± 1 mV before and after addition of IbTx. Dashed horizontal lines have been inserted to facilitate detection of effects on action potential height. **D–G:** Insulin secretion measured at 1, 6, and 20 mmol/l glucose (as indicated) in the absence and presence of 10 mmol/l TEA (**D**), 100 nmol/l stromatoxin (**E**), 100 nmol/l IbTx (**F**), or 100 nmol/l IbTx and/or 0.1 mmol/l tolbutamide (**G**). $n = 8–9$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

effects of IbTX were observed in current-injection experiments (online appendix Fig. 2A).

Glucose at 6 mmol/l (a concentration attained postprandially in nondiabetic individuals [18]) and 20 mmol/l stimulated insulin secretion 3.7- and 7.7-fold over basal (1 mmol/l), respectively (Fig. 3D). TEA enhanced insulin secretion at 6 and 20 mmol/l glucose by 85 and 94%, respectively (Fig. 3D). By contrast, stromatocin had no significant effects on glucose-induced insulin secretion (Fig. 3E), even when tested at a concentration of 1 μ mol/l ($n = 3$ from one donor) (data not shown) (19). We ascertained that stromatocin remained a blocker of the delayed outward current under the experimental conditions used in the secretion assays (i.e., presence of BSA) (data not shown). Whereas IbTX was without effect at 20 mmol/l glucose (data not shown), it abolished the stimulatory effect of 6 mmol/l glucose (Fig. 3F). When tested in the simultaneous presence of 6 mmol/l glucose and 0.1 mmol/l tolbutamide, however, IbTX stimulated insulin secretion by 73% (Fig. 3G).

Voltage-gated Na^+ currents. When the pipettes were filled with a Cs^+ -containing medium, voltage-clamp depolarizations to 0 mV elicited inward currents consisting of a very transient (~ 2 ms) and a more slowly inactivating component (Fig. 4A). Whereas the sustained component was suppressed by removal of extracellular Ca^{2+} and addition of the broad-spectrum Ca^{2+} channel blocker Co^{2+} , the transient current was abolished by the selective Na^+ channel blocker tetrodotoxin (TTX). Voltage-gated Na^+ currents were activated by depolarizations to -30 mV and above and reached a maximal amplitude of 40 ± 7 pA/pF at 0 mV ($n = 8$) (Fig. 4B and C). The voltage dependence of inactivation was characterized using a two-pulse protocol (Fig. 4D). Inactivation was observed after prepulses positive to -80 mV. A Boltzmann fit yielded values for voltage of half-maximal inactivation (V_h) and slope factor (n_h) of -42 ± 2 mV and 6 ± 1 mV ($n = 9$), respectively (Fig. 4E).

We studied the effect of TTX on glucose-induced electrical activity (Fig. 4F). In the presence of 6 mmol/l extracellular glucose, the cell-generated action potentials originating from ~ -60 mV and reaching up to 0 mV. Addition of TTX reversibly reduced the peak voltage of the action potentials from -12 ± 2 mV to -24 ± 3 mV ($n = 2$). Similar effects were observed in current injection experiments ($n = 11$) (online appendix Fig. 2B). As shown in Fig. 4G, the TTX-sensitive Na^+ current is important for glucose-induced insulin secretion, and secretion elicited by 6 and 20 mmol/l glucose was reduced by 70 and 55%, respectively, in the presence of TTX.

Voltage-gated Ca^{2+} currents. Ca^{2+} currents became detectable during depolarizations to -50 mV and peaked at 0 mV (Fig. 5A), where the current density was 14 ± 1 pA/pF ($n = 41$). The activation kinetics of the Ca^{2+} current was described using a Hodgkin-Huxley m^2 model. At 0 mV, the current activated with a time constant of 0.41 ± 0.02 ms ($n = 28$). The inactivation of the current was biphasic in most cells. The time constants for the rapid (comprising $35 \pm 3\%$) and slow components ($43 \pm 3\%$) averaged 6.8 ± 0.4 ms ($n = 25$) and 65 ± 15 ms ($n = 28$), respectively.

The Ca^{2+} channel subtypes underlying the voltage-gated Ca^{2+} current were established using specific blockers. A representative recording is shown in Fig. 5B. In this experiment, the current was reduced by $>90\%$ by sequential application of first the L-type blocker isradipine alone, followed by a combination of isradipine and the P/Q-type

inhibitor ω -agatoxin IVA and finally isradipine together with the T-type antagonist NNC 55-0396 (see also online appendix Fig. 3). Table 1 summarizes the inhibitory effects of the Ca^{2+} channel blockers on the peak and integrated Ca^{2+} currents evoked by depolarizations from -70 to 0 mV. The cumulative inhibitory effects of isradipine, ω -agatoxin IVA, and NNC 55-0396 amounted to 91%. The N-type blocker ω -conotoxin GVIA and the R-type blocker SNX-482 exerted negligible effects.

The voltage-dependent inactivation of the T-type Ca^{2+} current was studied using a two-pulse protocol (Fig. 5C). The experiments were performed in the presence of isradipine and (in some cells) ω -agatoxin IVA to block L- and P/Q-type Ca^{2+} currents. A Boltzmann fit to the data points yielded values for V_h and n_h of -64 ± 2 mV and 8 ± 1 mV ($n = 13$), respectively.

The currents flowing through the different channel subtypes were isolated by subtracting the responses recorded after application of the respective blockers from those obtained before blocker application (Fig. 5D). I-V relationships for the total Ca^{2+} current and the individual components are shown in Fig. 5E. T-type currents activated at -50 mV and reached a peak between -40 and -30 mV. L-type currents were first seen during depolarizations to -40 mV and reached a maximum between -20 and -10 mV. P/Q-type currents became significant only at potentials beyond -20 mV and peaked at 0 mV. The effect of ω -agatoxin IVA showed a slow onset and was maximal only 3–4 min after addition (not shown) (20). Time-dependent rundown of T-type Ca^{2+} channels might therefore account for the apparent ω -agatoxin sensitivity of a current component at voltages more negative than -20 mV.

In insulin release experiments, isradipine inhibited glucose-stimulated (20 mmol/l) insulin release and reduced secretion below baseline levels (Fig. 6A). By contrast, both SNX-482 and ω -conotoxin GVIA were without inhibitory effect, whereas ω -agatoxin IVA exerted a moderate inhibitory effect (-31%) (Fig. 6B). The T-type channel blocker NNC 55-0396 was without effect on insulin secretion at 20 mmol/l glucose (not shown). When insulin secretion was evoked by 6 mmol/l glucose, addition of NNC 55-0396, ω -agatoxin IVA, and isradipine reduced glucose-stimulated release by 59, 71, and 100%, respectively (Fig. 6C and D), whereas ω -conotoxin remained ineffective (not shown).

Figure 6E shows a cell electrically active at 20 mmol/l glucose. In all cells tested, isradipine completely suppressed electrical activity induced by glucose (6 or 20 mmol/l) ($n = 3$) or tolbutamide ($n = 2$). The T-type antagonist NNC 55-0396 also reduced action potential amplitude and frequency (Fig. 6F). The peak voltage attained during the action potential was reduced from -28 ± 2 mV under control conditions to -33 ± 3 mV ($P < 0.05$) ($n = 3$) in the presence of NNC 55-0396. Data obtained in membrane potential recordings echo those in the current-injection experiments (online appendix Fig. 2C and D).

Capacitance measurements. Capacitance measurements were used to investigate depolarization-evoked exocytosis in human β -cells. While 20-ms depolarizations usually failed to evoke a clear (>10 fF) response, longer depolarization pulses triggered progressively larger capacitance increases (Fig. 7A). In many β -cells, exocytosis continued beyond the depolarization. Figure 7B plots the increase evoked by the depolarization against pulse dura-

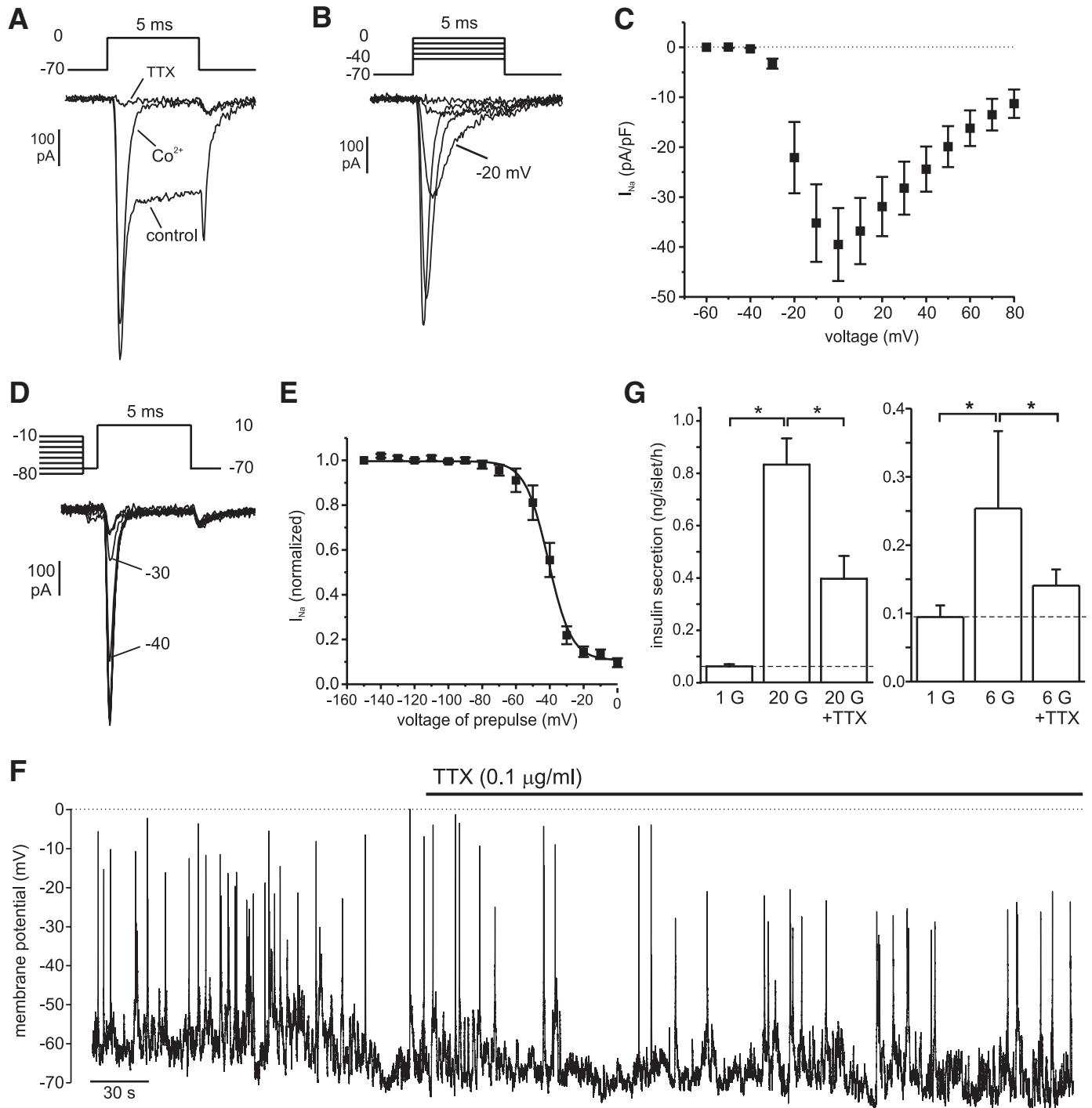


FIG. 4. Voltage-gated Na^+ -currents. **A:** Inward currents recorded in the presence of TEA (control), after addition of Co^{2+} (1 mmol/l) and after additional application of TTX (0.1 $\mu\text{g/ml}$). **B:** Na^+ currents elicited by depolarizations to voltages between -40 and 0 mV. **C:** I-V relationship for peak Na^+ currents (I_{Na} ; $n = 8$). **D:** Na^+ current inactivation. The peak current was measured during a test pulse to $+10$ mV preceded by a 50-ms conditioning prepulse to different voltages (see schematic above current traces). The responses following prepulses to -40 and -30 mV are indicated by lines. **E:** Steady-state Na^+ current (I_{Na}) inactivation measured as in **D** ($n = 9$). A Boltzmann fit to the data is superimposed. **F:** Effects of TTX (0.1 $\mu\text{g/ml}$) on electrical activity evoked by 6 mmol/l glucose. Action potentials peaked at -14 ± 2 mV before and -27 ± 1 mV after TTX. Dashed horizontal line has been inserted to facilitate detection of effect on action potential height. **G:** Insulin secretion measured at 1, 6, and 20 mmol/l glucose (1G/6G/20G) with or without TTX (0.1 $\mu\text{g/ml}$), as indicated ($n = 6-8$). * $P < 0.05$.

tion. The average response during 500-ms depolarizations was 41 ± 5 fF/pF.

Figure 7C shows the relationship between the integrated Ca^{2+} current and exocytosis. Exocytosis was small for integrated Ca^{2+} currents with a charge of <0.4 pC/pF but then increased supralinearly with charge entry. The relationship was well described by a fourth-order polynomial

function, compatible with cooperative binding of Ca^{2+} to the Ca^{2+} sensor of exocytosis (21).

We studied the voltage dependence of exocytosis by applying depolarizing pulses from -70 mV to voltages between -40 and $+40$ mV (Fig. 7D). Depolarizations to -20 mV or below were largely ineffective, but the responses then increased steeply, with a maximum being

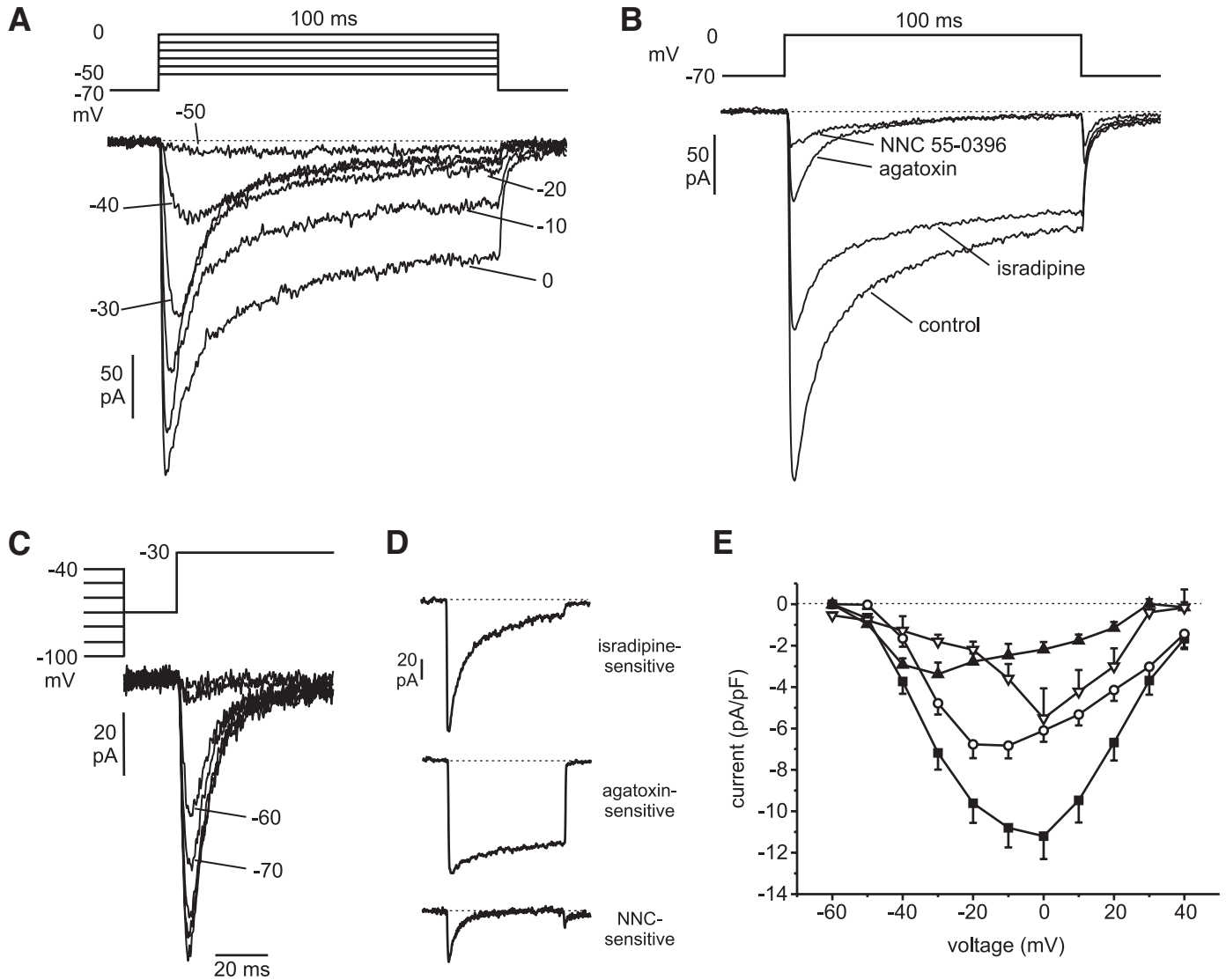


FIG. 5. Voltage-gated Ca^{2+} currents. *A*: Ca^{2+} currents elicited by depolarizations to voltages between -50 and 0 mV. *B*: Effect of the sequential application of isradipine ($10 \mu\text{mol/l}$), ω -agatoxin IVA (200 nmol/l), and NNC 55-0396 ($1 \mu\text{mol/l}$). Isradipine was present in all blocker-containing solutions, while ω -agatoxin was applied only temporarily as its effects showed no reversibility upon washout. *C*: Steady-state inactivation of T-type Ca^{2+} currents measured with a two-pulse protocol, consisting of a test pulse to -30 mV preceded by a 500 -ms conditioning pulse to voltages between -100 and -40 mV. The T-type Ca^{2+} current was isolated by blocking L- and P/Q-type Ca^{2+} currents. The responses obtained after conditioning pulses to -70 and -60 mV are indicated by lines. *D*: L-, P/Q-, and T-type Ca^{2+} currents obtained by subtracting currents recorded in the presence of isradipine, ω -agatoxin IVA, and NNC 55-0396 from currents observed in the absence of the respective antagonist. *E*: Current-voltage relationship for total Ca^{2+} current (\blacksquare), L-type (\circ) (isradipine sensitive), P/Q-type (∇) (ω -agatoxin sensitive), and T-type (\blacktriangle) (NNC 55-0396 sensitive) Ca^{2+} currents ($n = 5-13$).

TABLE 1
Effects of Ca^{2+} channel blockers on Ca^{2+} current charge and peak amplitude

Antagonist	Concentration ($\mu\text{mol/l}$)	Inhibition (%)		<i>n</i>
		Charge	Peak current	
Isradipine	10	$38 \pm 3^*$	$49 \pm 3^*$	30
ω -Agatoxin IVA	0.2	$46 \pm 5^\dagger$	$24 \pm 3^\dagger$	10
NNC 55-0396	1	$6 \pm 1^*$	$18 \pm 2^*$	14
ω -Conotoxin GVIA	0.1	2 ± 3	5 ± 3	7
SNX-482	0.1	0 ± 1	$4 \pm 1^\dagger$	6

Data are means \pm SE, unless otherwise indicated. Ca^{2+} currents were measured during 100 -ms depolarizations from -70 to 0 mV. The data are expressed as percent inhibition of control responses ($*P < 0.001$; $^\dagger P < 0.01$).

attained at 0 mV and a secondary reduction at more positive voltages. The observed voltage dependence of exocytosis most closely resembles that of the P/Q-type Ca^{2+} channels (cf. Figure 5E).

We studied the effects of Ca^{2+} channel blockers on depolarization-evoked exocytosis. Whereas ω -agatoxin exerted a strong inhibitory action on exocytosis evoked by a 500 -ms depolarization to 0 mV, the effect of isradipine was weak (Fig. 7E). On average, isradipine and ω -agatoxin reduced exocytosis by $31 \pm 15\%$ (NS) ($n = 6$) and $80 \pm 3\%$ ($P < 0.001$) ($n = 5$), respectively.

Expression profiling of human islets. We quantified mRNA expression of voltage-gated ion channels in nine human islet preparations (online appendix Table 1). All known isoforms of Na^+ channels were screened. Of the α -subunits, $\text{Na}_v1.7$ and $\text{Na}_v1.6$ dominated, while type 1β constitutes 80% of the total β -subunit expression. Of the

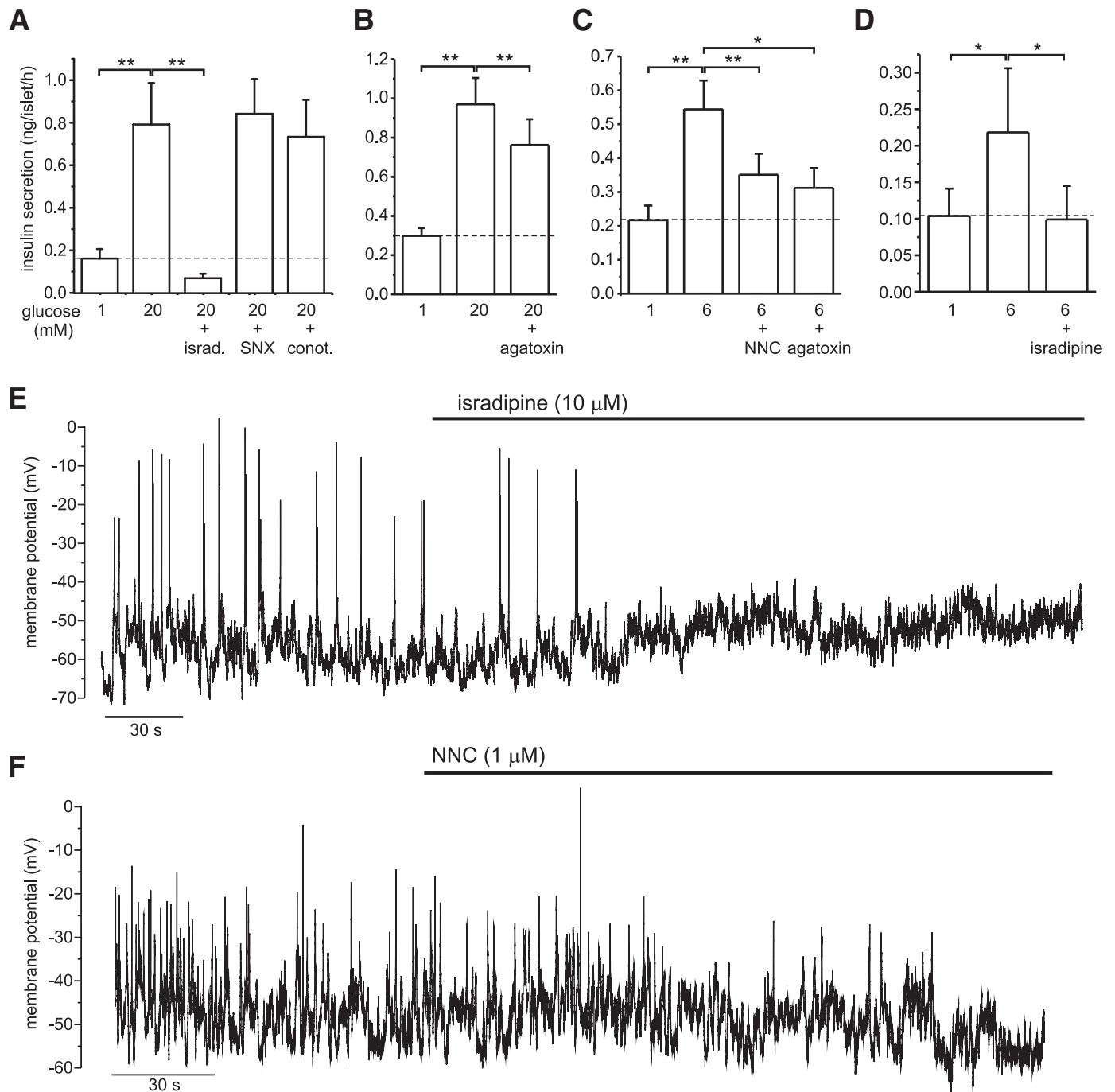


FIG. 6. Effects of Ca^{2+} channel blockers on insulin secretion and electrical activity. *A–D*: Effects of isradipine (10 $\mu\text{mol/l}$), SNX482 (0.1 $\mu\text{mol/l}$), ω -conotoxin GVIA (0.1 $\mu\text{mol/l}$), ω -agatoxin IVA (0.2 $\mu\text{mol/l}$), and NNC55-0396 (1 $\mu\text{mol/l}$) on insulin secretion at the indicated glucose concentrations. *A*: $n = 9$; *B*: $n = 13$; *C*: $n = 7–9$; *D*: $n = 9$. $*P < 0.05$; $**P < 0.01$. *E* and *F*: Effects of isradipine (*E*) and NNC 55-0396 (*F*) on electrical activity elicited by 20 mmol/l glucose (*E*) or 10 mmol/l glucose supplemented with 0.1 mmol/l tolbutamide (*F*).

Ca^{2+} channels, $\text{Ca}_v3.2$ (α_{1G} ; T type), $\text{Ca}_v1.3$ (α_{1D} ; L type), and $\text{Ca}_v2.1$ (α_{1A} ; P/Q type) isoforms were most abundant and account for 48, 28, and 12% of the transcript numbers, respectively. A selection of voltage-gated K^+ channels based on Yan et al. (22) was screened. The expression of K_v channels was dominated by $\text{K}_v2.1$ and $\text{K}_v2.2$ (24 and 73%, respectively). The α -subunit of the large conductance Ca^{2+} -activated K^+ channels (BK channels) was highly expressed, and of the β -subunits β_2 predominated (80%).

DISCUSSION

We have characterized the voltage-gated membrane currents in identified human β -cells, their molecular composition, and their involvement in exocytosis, electrical activity, and glucose-induced insulin secretion. This was facilitated by the access to novel and selective blockers. Based on these data, we provide a model that outlines the respective roles of the different voltage-gated ion channels in stimulus secretion coupling in human β -cells.

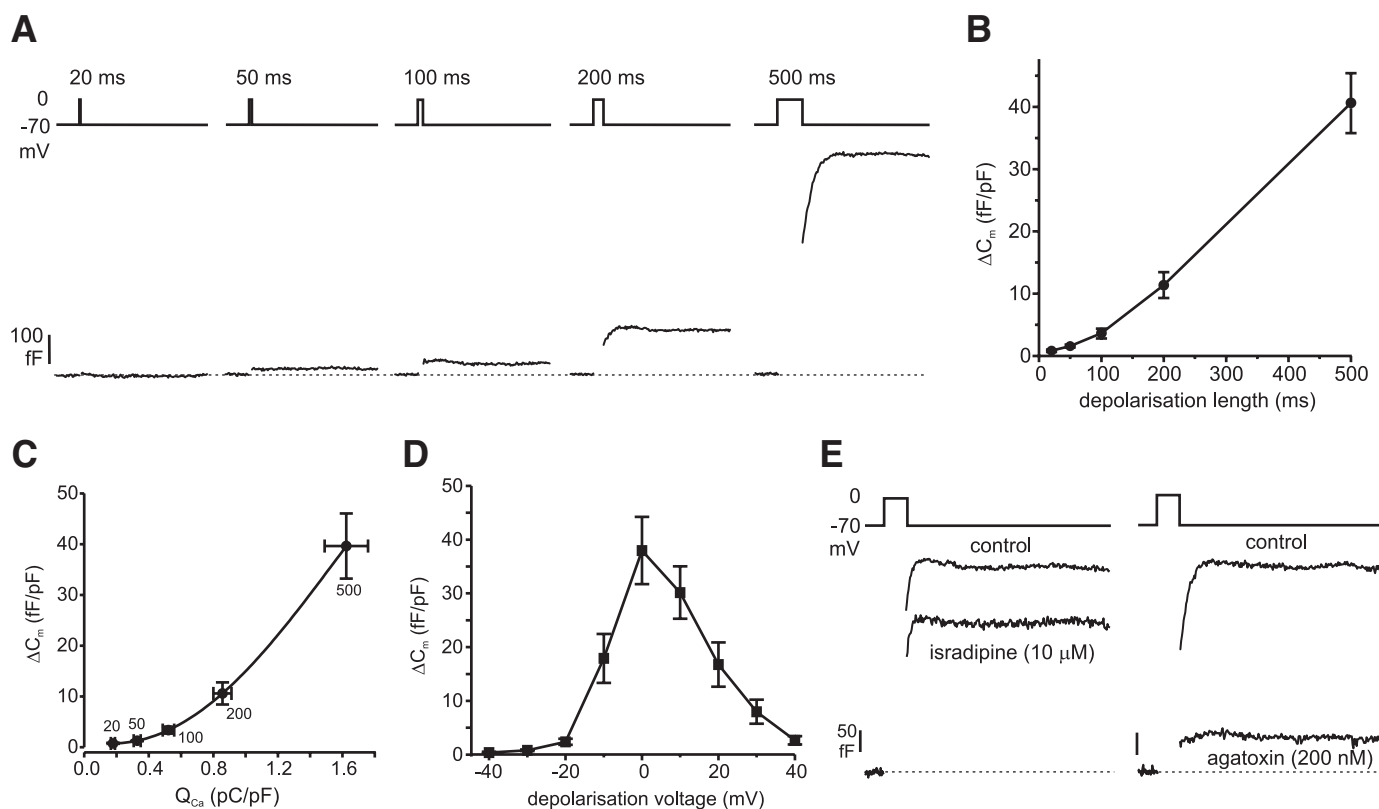


FIG. 7. Depolarization-evoked exocytosis. **A:** Capacitance increase evoked by progressively longer depolarizations to 0 mV (applied at 15-s intervals). **B:** Relationship between pulse length and the total (including postpulse response) capacitance increase (ΔC_m). Exocytotic responses have been normalized to cell capacitance ($n = 16$). **C:** Relationship between Ca^{2+} -current charge (Q_{Ca}) and ΔC_m , both normalized to cell capacitance ($n = 12$). The numbers next to the symbols indicate the length of the depolarization pulse (ms). The curve was obtained by fitting the data with a fourth-order polynomial function ($R^2 = 1$). **D:** ΔC_m (normalized to cell capacitance) in response to 500-ms depolarizations plotted against the voltage during the pulse ($n = 13$). **E:** Effects of isradipine (10 μ mol/l) and ω -agatoxin IVA (200 nmol/l) on depolarization-evoked (500 ms to 0 mV) capacitance increase.

K⁺ currents. The voltage-gated K⁺ current in human β -cells consists of at least two different components. A transient component activates rapidly upon membrane depolarization, is dependent on Ca²⁺ influx, and is blocked by IbTX. These properties suggest that it is carried by large-conductance Ca²⁺-activated K⁺ channels (BK channels). BK currents exhibited a typical bell-shaped voltage dependence, with decreasing amplitude at potentials >30 mV due to reduced Ca²⁺ entry. It can be noted that the peak BK current is observed at a potential ~30 mV more positive than the peak Ca²⁺ current (compare Figs. 2D and 5E). This rightward shift reflects both the intrinsic voltage dependence of the channels, with (at fixed Ca²⁺ levels) increased open probability at depolarized potentials (23), and the increasing driving force for K⁺ entry. Similar to studies in chromaffin cells (24) and mouse β -cells (25), BK currents were recorded despite the presence of a high concentration of EGTA in the intracellular solution. This suggests that BK channels and voltage-gated Ca²⁺ channels are closely colocalized (26). In mouse β -cells, BK channels do not play a major role in glucose-induced electrical activity or insulin secretion (27). By contrast, in human β -cells blockade of BK channels increases spike amplitude. In some cells, addition of IbTX suppressed action potential firing. This effect appeared to result from an unexpected ability of IbTX to activate K_{ATP} channels, which also accounts for the inhibition of glucose-induced insulin secretion. When insulin secretion was instead measured in the presence of tolbutamide, IbTX, as ex-

pected from the electrophysiological data, stimulated insulin secretion.

The second component of the K⁺ current developed with a delayed time course. This component was unaffected by Co²⁺ and IbTX but inhibited by stromatocin and TEA; inhibition by the latter compound was half maximal at ~1 mmol/l. The current is likely to be due to delayed rectifying K⁺ channels. K_V2.1 channels, which are dominant in rodent cells (28), are also expressed in human β -cells (22). However, at the mRNA level K_V2.2 channels are more abundant in human islets. In rodent cells, blockade of K_V2.1/2.2 channels stimulates insulin secretion (19,28). By contrast, in human islets the K_V2.1/2.2 blocker stromatocin had no major effect on insulin release and electrical activity. It has been suggested that human β -cells also express A-type K⁺ currents (29), but we have so far detected such currents only in non- β -cells in human islets (not shown). Collectively, these data suggest that BK channels are particularly important for spike repolarization. Their activation kinetics and voltage dependence make them ideally suited for this task.

Na⁺ currents. In agreement with previous studies in human β -cells (10,12), but in marked contrast to mouse β -cells (30–32), the voltage-gated Na⁺ current could be activated from physiologically relevant membrane potentials (–70 mV and more positive). Using TTX, we could demonstrate the significance of these channels for action potential generation and insulin secretion. At the mRNA level, human islets express approximately equal amounts

of $\text{Na}_v1.6$ and $\text{Na}_v1.7$. The latter channels are involved in nociception (33), and $\text{Na}_v1.7$ -specific antagonists are considered as analgesics. The possibility that such channels are involved in insulin secretion suggests that the use of such drugs may cause impaired insulin secretion as a side effect.

Ca^{2+} currents and exocytosis. In agreement with previous studies (5,11), we show that L-type Ca^{2+} channels are expressed in human β -cells. Blockade of L-type Ca^{2+} channels using the selective antagonist isradipine leads to complete inhibition of glucose-induced insulin secretion. Our data suggest that the importance of L-type channels predominantly results from their essential role in the generation of electrical activity, whereas their role in exocytosis is modest ($\sim 30\%$). The significance of L-type channels for the generation of electrical activity may reflect their voltage dependence: activation commences at voltages as negative as -40 mV (Fig. 5E). The PCR data suggest that the L-type Ca^{2+} channel is of the $\text{Ca}_v1.3$ ($\alpha 1D$) subtype. The fact that the isradipine-sensitive current activates at more negative voltages in human than in mouse β -cells is consistent with this observation (34).

Although expression of P/Q-type Ca^{2+} channels in human β -cells has been reported earlier (8), their relative contribution to the total Ca^{2+} current and their function in stimulus secretion coupling have been unclear. We now show that P/Q-type Ca^{2+} channels account for $\sim 45\%$ of the integrated whole-cell Ca^{2+} current and that they play a critical role in depolarization-evoked exocytosis and glucose-induced insulin secretion, especially at low glucose concentrations (6 mmol/l). At variance with mouse β -cells (35), we found no electrophysiological evidence for the presence of R-type Ca^{2+} channels. In fact, they are not at all expressed in human islets (online appendix Table 1).

We further confirm that T-type Ca^{2+} channels are also expressed (5) and contribute to electrical activity (10) in human β -cells. Using a recently developed, more selective antagonist (NNC55-9036) (see online appendix Fig. 3), we obtained evidence that T-type channels are involved in insulin release evoked by 6 mmol/l glucose but not by 20 mmol/l glucose. We acknowledge that NNC55-9036 is not ideal in inhibiting non-T-type Ca^{2+} currents also. However, the nonspecific effects (unlike those on the T type) were reversible. For secretion studies, islets were therefore preincubated for 15 min with the blocker, followed by a 10-min washout phase. In patch-clamp experiments using the same protocol, only the peak (T-type) Ca^{2+} current was significantly reduced after pretreatment with NNC55-9036, whereas the sustained current (non-T type) was unaffected (see legend to online appendix Fig. 3). PCR analysis suggests that the T-type Ca^{2+} current in human β -cells is of the $\text{Ca}_v3.2$ subtype.

A model for electrical activity in human β -cells. Based on the findings in the present and previous studies, we propose that at physiological glucose concentrations (~ 6 mmol/l) the closure of K_{ATP} channel depolarizes the β -cell membrane to potentials above -55 mV. The activation of T-type (at voltages above -60 mV) and L-type Ca^{2+} channels (above -50 mV) (Fig. 5E) initiates the action potential. During the upstroke of the action potential, voltage-gated Na^+ channels also open (above -40 mV) (Fig. 4B and C), leading to a further acceleration of the upstroke and sufficient depolarization to activate P/Q-type Ca^{2+} channels (above -20 mV). Ca^{2+} influx via P/Q-type (and to a lesser extent L-type) Ca^{2+} channels directly triggers exocytosis of insulin granules. The β -cell is repo-

larized by the activation of Ca^{2+} -activated BK channels, with $\text{K}_v2.1/2.2$ channels playing only a minor role. The scenario outlined above is consistent with the observation that the inhibitory actions of TTX (see also 10), NNC55-0936, and ω -agatoxin are weaker at 20 than at 6 mmol/l glucose. As shown in Figs. 4F and 6F, inhibition of T-type Ca^{2+} channels and Na^+ channels reduces the action potential amplitude. A reduction of spike height by 15–20 mV (from the normal peak voltage of -10 to 0 mV) will result in $>50\%$ reduction of both the P/Q-type Ca^{2+} current (Fig. 5E) and exocytosis (Fig. 7D). Our data suggest that P/Q-type Ca^{2+} channels are more tightly coupled to exocytosis than L-type Ca^{2+} channels. Indeed, the exocytotic responses were small during depolarizations to -20 mV, a voltage associated with the maximum activation of the L-type Ca^{2+} channels (Fig. 5E).

Concluding remarks. It is evident that human and mouse β -cells differ in many respects. Thus, some channels which are not considered functionally important in mouse β -cells (like the BK channels, T- and P/Q-type Ca^{2+} channels, and voltage-gated Na^+ channels) play critical roles in human β -cells. Conversely, R-type Ca^{2+} channels and $\text{K}_v2.1$ channels appear less important in human cells than suggested by previous work in mice. As discussed above, this may have an impact on drug development. It seems unlikely that the differences between human and rodent β -cells are confined to ion channels. Indeed, there are examples of such differences in the literature. Whereas mouse β -cells depend on Glut2 for transmembrane glucose transport, human β -cells instead depend on Glut1 (36). These discrepancies might also be relevant to the understanding of the genetics of type 2 diabetes. Clearly, a gene polymorphism associated with increased risk of type 2 diabetes is more likely to affect insulin secretion if the gene is expressed in human β -cells and vice versa.

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