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Sodium Channels Contribute to Action Potential Generation in Canine and Human Pancreatic Islet B Cells

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Summary. Pancreatic islet B cells depolarize and display trains of action potentials in response to stimulatory concentrations of glucose. Based on data from rodent islets these action potentials are considered to be predominantly Ca^{2+} dependent. Here we describe Na^+-dependent action potentials and Na^+ currents recorded from canine and human pancreatic islet B cells. Current-clamp recording using the nystatin "perforated-patch" technique demonstrates that B cells from both species display tetrodotoxin-sensitive Na^+ action potentials in response to modest glucose-induced depolarization. In companion "whole-cell" voltage-clamp experiments on canine B cells, the underlying Na^+ current displays steep voltage-dependent activation and inactivation over the range of −50 to −40 mV. The Na^+ current is sensitive to tetrodotoxin block with a K_I = 3.2 nM and has a reversal potential which changes with $[Na^+]_o$ as predicted by the Nernst equation. These results suggest that a voltage-dependent Na^+ current may contribute significantly to action potential generation in some species outside the rodent family.

Key Words Na^+ channels · action potentials · pancreatic islet B cell · islet cell electrophysiology

Introduction

When exposed to concentrations of glucose which provoke insulin secretion, pancreatic islet B cells depolarize and then often display periodic "bursts" of action potentials (for review *see* Petersen & Findlay, 1987). The action potentials have been studied almost exclusively in rodents where they are Ca^{2+} dependent; the voltage-dependent Ca^{2+} currents thought to underlie them have been described (Rorsman & Trube, 1986; Rorsman, Ashcroft & Trube, 1988; Satin & Cook, 1988). Voltage-dependent Na^+ currents have also been identified (e.g., Hiriart & Matteson, 1988; Plant, 1988) but their contribution to secretogogue-induced electrical activity has never been demonstrated.

By applying "perforated-patch" current-clamp recording (Horn & Marty, 1988; Falke et al., 1989) to canine and human B cells, we have recorded tetrodotoxin-sensitive, Na^+ action potentials during modest glucose-induced depolarization. In companion "whole-cell" voltage-clamp recording from B cells in canine islets we have identified the underlying voltage-dependent Na^+ currents. These observations suggest that in some species Na^+ currents could play a role in B cell stimulus-secretion coupling. They have recently been reported in abstract form (Pressel & Misler, 1990).

Materials and Methods

Purified human and canine islets were generous gifts of the Islet Transplantation Laboratory, Washington University (David Scharp, Director). Techniques for collagenase digestion of pancreases and Ficoll gradient separation of islets were previously described (Ricordi et al., 1988). Aliquots from most islet isolates used in these experiments were tested for insulin secretion by the Islet Transplantation Laboratory. They responded to a rise in perifusate glucose concentration from 3.3 to 16.6 mM (canine islets in the presence of 1 mM IBMX) with peak insulin secretion ≥3 times baseline. Islets were disrupted into small clumps of cells and occasional single cells by trituration and were then seeded on glass coverslips and cultured for 2–14 days as previously described (Misler et al., 1989). "Seeded" coverslips were transferred to a recording chamber containing an extracellular-like solution (ES) whose salt composition was (in mM): 144 NaCl, 5.5 KCl, 2.0 $CaCl_2$, 1 $MgCl_2$, and 20 HEPES titrated to pH 7.3 with NaOH. The composition of the ES was altered by isosmotically substituting a portion of the NaCl content with a test substance, or with an isosmotic NaCl solution containing the test substance.

Electrophysiology

Cells were recorded from using conventional whole-cell patch-clamp recording for voltage-clamp experiments (Hamill et al., 1981). Current-clamp recording was performed using the nystatin "perforated-patch" variant of whole-cell recording (Horn &

Marty, 1988; Falke et al., 1989). To perform conventional whole cell voltage clamp recording, micropipettes were filled with a "K^+-free" buffer solution containing (in mM): 111 CsCl, 20 NaCl, 5 EGTA, 3 $MgCl_2$, 3 MgATP, and 20 HEPES titrated to pH 7.3 with tetraethylamine hydroxide. Gigaohm resistance glass-to-membrane seals were made on isolated cells or cells within small clumps. Access resistances of 5–10 MΩ were routinely achieved when the cell-attached patch was disrupted by a pulse of suction. The voltage-clamp error during current flow of up to 1 nA was 5–10 mV. Series resistance compensation available via the patch-clamp amplifier (EPC-7, List Electronics, Darmstadt, FRG) was not used. The liquid junction potentials between the indifferent electrode and the bath were calculated to be less than 3.5 mV for all solutions; no corrections were made. Currents, filtered at 2500 Hz through an 8-pole Bessel filter, were digitized at 5000 samples per sec and recorded by a data acquisition and analysis system developed by L. Falke in our laboratory (*see* Misler et al., 1986). This system also generated and recorded the pulse sequences. Current/voltage (I/V) curves were plotted using this system as well. In these curves the clamping voltage, V_c, was corrected for the voltage drop across R_a.

Currents were usually recorded immediately after rupture of the patch in order to minimize the contribution of a "leak conductance" which developed over minutes, especially in isolated cells. In lengthy experiments requiring several solution changes, peak currents recorded minutes into the experiment were "leak subtracted" using current values extrapolated to the given V_c. The extrapolated values were obtained by extending the nearly linear "leak" I/V curve obtained from a set of small depolarizing and hyperpolarizing voltage pulses from the holding potential.

Cells in the small aggregates or clumps were preferred to single cells for extended recording because of greater stability of electrical recording and more vigorous action potential activity. Cells of the intact rodent islet are reported to be electrically coupled (Eddleston et al., 1984). However, many of the "identified" B cells within cultured canine and human islet fragments exhibited values of cell capacitance (C_m = 4–9 pF), measured by capacity transient "nulling," within the range seen with isolated cells (3.5–7.5 pF). This and other criteria discussed below suggested that in our experiments, neighboring cells were often contributing little to the electrical behavior of the cell being monitored.

To perform current-clamp recording with the "perforated-patch" technique, 5–7 MΩ resistance micropipettes were filled in a two-stage process. The tip of each pipette was initially filled with a nystatin-free "high-K^+" solution containing (in mM): 67.5 KCl, 28.35 K_2SO_4, 11.8 NaCl, 1 $MgCl_2$, 0.5 EGTA, 47.2 sucrose, and 20.75 HEPES titrated to pH 7.3 with KOH. The remainder of the pipette was then backfilled with the high-K^+ solution also containing 150–300 μg/ml nystatin (Sigma, St. Louis, MO) dissolved in DMSO. The pipette was gently pressed against the surface of a cell, which was part of a small aggregate, and a gigaohm seal was formed by applying gentle suction to the interior of the pipette. Current-clamp recording was initiated when the access resistance (R_a) to the cell's interior had fallen to <100 MΩ. Membrane voltages were often directly recorded onto a chart recorder (Gould, Cleveland, OH). Voltage-clamp recording was sometimes attempted when R_a had fallen to <30 MΩ.

Cells were "physiologically" identified as B cells during the initial cell-attached patch recording. When the pipette was filled with the "high-K^+" solution, a cell was identified as a B cell if the cell-attached patch displayed characteristic unitary inward currents of 3.5–4.5 pA in amplitude at 0 mV clamping potential. Their frequency decreased, often preceding the onset of action current activity, on addition of glucose to the bath. Channel activity rapidly increased on addition of 3 mM sodium azide, a metabolic poison, to the bath (*see* Fig. 1*A* in Misler et al., 1989). These are convenient identifying features of the metabolically regulated, ATP_i-sensitive K^+ channel, which is well represented in B cells, but not in A cells, of the islet (Rorsman & Hellman, 1988). When the pipette was filled with the "K^+-free" solution containing a high concentration of Cs^+, openings of the K^+(ATP) channel could not be seen, as Cs^+ barely traverses this channel (Tabcharani, Falke & Misler, 1988). A cell was identified as a B cell if it displayed action current activity in the presence of 5 mM glucose or 20–40 μM tolbutamide, a depolarizing, hypoglycemic agent, and this electrical activity was reversibly blocked by addition of azide.

Results

Na^+-Dependent Action Potentials in Canine Islet B Cells

In our pilot experiments with cell-attached patches of canine islet cell membranes we noted that the biphasic action currents had consistently larger peak-to-peak amplitudes than those recorded from human or rat islet cell clumps of similar size (Pressel & Misler, 1989). This suggested that canine islets might be an excellent tissue for the investigation of the channel currents underlying the upstroke of the B cell action potential. Figure 1 demonstrates the activation of Na^+-dependent action potentials by bath-applied glucose, during "perforated-patch" recording from a "physiologically" identified B cell within a small clump of islet cells. A 20-gigaohm seal was formed between the nystatin-containing pipette and the cell surface. With the islet clump bathed in 5 mM glucose, openings of a 4-pA channel carrying inward current were seen at an imposed potential of 0 mV; these channel openings were interspersed with action currents (Fig. 1*a*). Immediately after addition to the bath of 3 mM sodium azide, as many as six simultaneous channel openings were seen (Fig. 1*b*). The channel had a slope conductance of 60–65 pS and a reversal potential of +65 mV (the pipette interior being taken as ground). These features are consistent with the channel being the metabolically regulated, ATP_i-sensitive K^+ channel previously described in rodent and human islet B cells.

Within 30–35 min after seal formation the cell-attached membrane patch had permeabilized sufficiently that the access resistance (R_a) to the cell interior via the patch pipette had fallen to 100 MΩ, hence, allowing faithful recording of the cell's membrane potential. In 3 mM glucose ES, the cell's membrane potential, V_m, fluctuated but averaged −70 mV (Fig. 1*c*). Raising the glucose to the modest

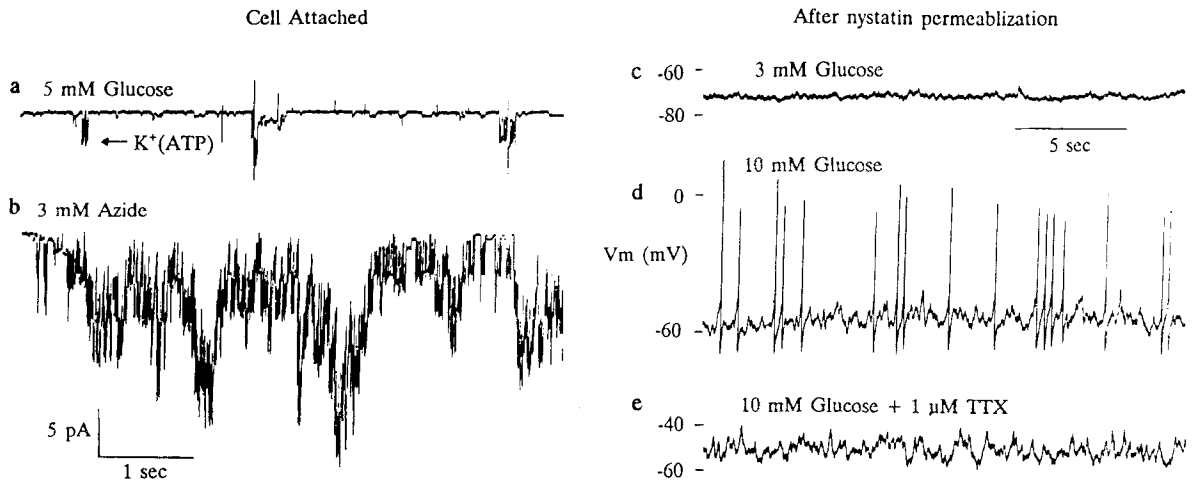

Fig. 1. Identification of B cells and glucose-induced electrical activity in a clump of cells from canine pancreatic islets. *Left column:* Cell-attached patch recording before patch perforation, "high-K^+" pipette. (*a*) K^+(ATP) channel currents and occasional action currents seen in the presence of 5 mM glucose. (*b*) Dramatically increased activity of K^+(ATP) channel and cessation of action currents seen after addition of 3 mM azide. *Right column:* Whole-cell current-clamp recording after patch perforation. R_a = 100 MΩ. (*c*) Resting membrane potential of −70 mV in 3 mM glucose. (*d*) Depolarization and onset of action potential activity after increasing bath glucose to 10 mM. (*e*) Block of action potentials following addition of 1 μM tetrodotoxin to the 10 mM glucose ES

stimulatory concentration of 10 mM depolarized the cell and initiated action potential activity (Fig. 1*d*). Action potentials, often overshooting 0 mV, began their trajectory at near −50 mV and occurred at a rate of 1–2 sec^{-1}. Action potentials often exhibited large afterhyperpolarizations. The action potentials were reversibly abolished, but average V_m was not affected, by the addition of 1.0 μM tetrodotoxin (TTX), a highly specific blocker of rapidly activating, voltage-dependent Na^+ currents (Fig. 1*e*). The action potentials were also abolished by isosmotically replacing the NaCl of ES with N-methylglucamine chloride, the salt of a bulky univalent cation which is impermeant to Na^+-selective channels (Hille, 1984). In some cells action potential frequency increased in a graded fashion with extracellular glucose concentration over the range of 3–10 mM.

Following "perforation" of the patch to an R_a of <30 MΩ, it was often possible to switch from the current-clamp to the voltage-clamp mode. A rapidly activating, voltage-dependent inward current, often >1 nA in amplitude, was observed with a step depolarization positive to −40 mV. This current was abolished by nearly total substitution of Na^+ with N-methylglucamine or application of tetrodotoxin (1 μM) and was presumed to be a Na^+ current. The rapid inward "Na^+" current was usually followed by a more slowly developing outward current which became prominent at depolarizations positive to −10 mV. The latter is consistent with a delayed rectifier K^+ current. On replacement of the standard extracellular solution (*see* Materials and Methods) with one containing 15 mM $BaCl_2$, 20 mM TEA and 1 μM TTX, sustained inward currents were often seen. These currents were activated by steps of depolarization positive to −30 mV. They had peak amplitudes of 25–100 pA at 0 to +10 mV and were sometimes enhanced by the Ca^{+2} channel agonist BAY K 8644 (D.M. Pressel & S. Misler, *unpublished data*). The sustained inward current was presumed to traverse high threshold "L-type" Ca^{+2} channels previously seen in cell-attached patches (Pressel & Misler, 1989). Given the high access resistance and large magnitude of the fast inward current, a sizeable voltage drop occurred across the recording pipette. This precluded further quantitation of the presumed Na^+ current with "perforated-patch" recording.

Voltage-Activated Na^+ Currents in Canine Islet B Cells

Figure 2 presents more quantitative data for the fast inward Na^+ current obtained with conventional "whole-cell" recording from isolated B cells (upper panel) and those within a small islet clump (lower panel). In each case, pipette was filled with the "K^+-free" buffer solution. Typically action currents were noted immediately after gigaseal formation in the presence of a 5 mM glucose ES bath but ceased within seconds of addition of 3 mM sodium azide to the bath (*see* left column). Following rupture of the cell-attached patch to achieve "whole-

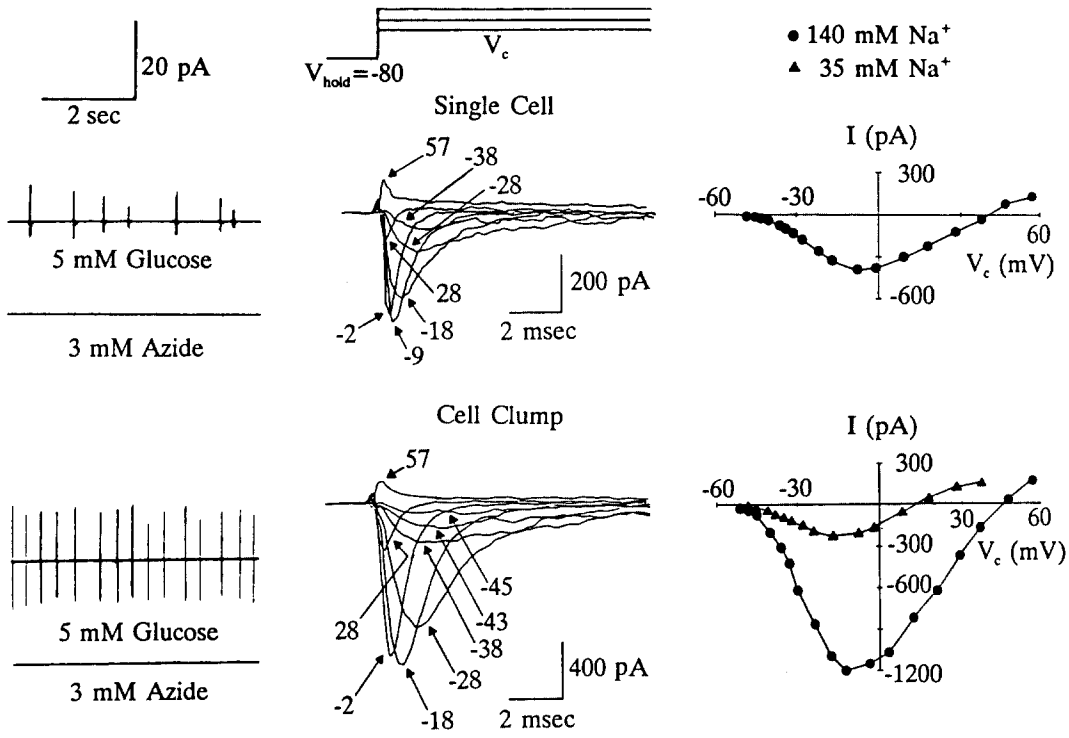

Fig. 2. Voltage-dependent Na^+ currents recorded from an isolated canine B cell (upper panel) and a cell within a canine islet clump (lower panel). "K^+-free" CsCl pipette was used. *Left column:* Cells were identified as B cells by their ability to generate action currents in the presence of glucose in the cell-attached patch configuration. These action currents were abolished by sodium azide. *Middle column:* Sample whole-cell currents recorded after a depolarizing step from −80 mV to the indicated clamping potential. *Right column:* Current/voltage relationship for the Na^+ currents in 140 mM Na^+ ES and 35 mM Na^+ ES accomplished by substitution of N-methyl-glucamine HCl for NaCl

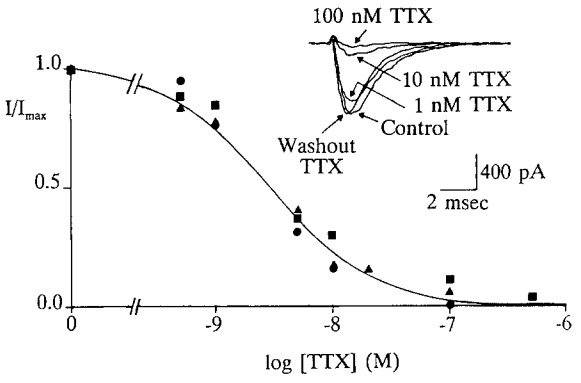

Fig. 3. Tetrodotoxin blockade of canine Na^+ currents. Normalized dose dependence of TTX block measured for three different cells at a clamping potential of −30 mV after stepping from a holding potential of −80 mV. The solid line was drawn from the best fit to Eq. (1) given in the text. K_I = 3.2 nM, n = 1.1. *Insert:* Sample whole-cell currents recorded in the presence of increasing concentrations of TTX

cell" recording, the membrane currents were recorded. The middle column depicts sample current traces recorded during the first 10 msec after depolarizing the cell from a sustained (1-sec) holding potential (V_{hold}) of −80 mV to a series of less negative clamping potentials (V_c).

The corresponding curves of the peak current as a function of V_c are presented in the right column. Detectable inward current is first noted at V_c = −50 mV. Peak inward current increases steeply with increasing depolarization over the range V_c = −40 to −20 mV and decreases with depolarizations to values positive to −10 mV. The zero current or "reversal potential" (E_{rev}) was determined in each case as +42 to +45 mV; this is not significantly different from the value of E_{rev} = +49 mV calculated for an exclusively Na^+-selective channel bounded by measured $[Na^+]_i$ and $[Na^+]_o$ of 20 and 140 mM, respectively. The lower right panel demonstrates a sample experiment where reducing $[Na^+]_o$ from 140 to 35 mM reduces the peak inward current and shifts E_{rev} negatively by 35 mV, as expected for a Na^+ current.

In both the isolated B cell and the B cell within a clump, the Na^+ conductance at a given clamping potential (G_{Na^+}), calculated as the chord conductance from the current/voltage curve, increases very steeply with V_c (nearly e-fold per 4-mV depolarization) over the range of −50 to −30 mV. Note

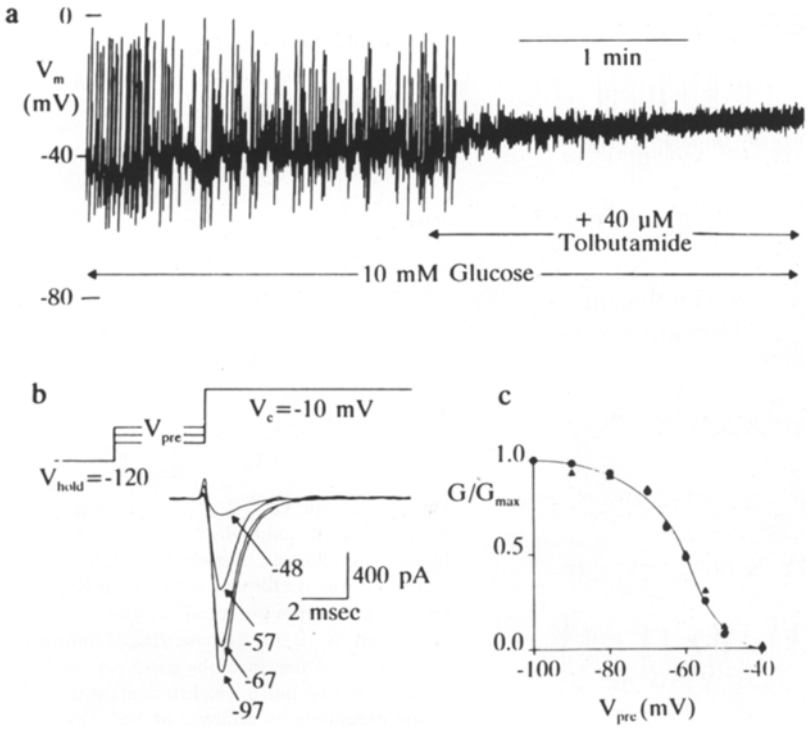

Fig. 4. Voltage-dependent inactivation of canine Na^+ action potentials and currents. (*a*) "Perforated-patch" recording, demonstrating the loss of glucose-induced spiking upon further depolarization of the B cell by addition of 40 μM tolbutamide to the bath. (*b*) Sample whole-cell currents recorded from within an islet clump showing inactivation following depolarizing prepulses to the indicated potential. (*c*) Normalized conductance as a function of prepulse for a single B cell (triangle) and for a B cell within an islet clump (circle). The solid line was fitted by eye

that the Na^+ currents recorded from the isolated B cell (C_m = 5 pF) and the B cell within the clump (C_m = 8 pF) show very similar kinetics of activation and inactivation. Neither set of current traces displays delayed activation of current at clamping voltages near threshold, or complex "ringing" of the current trace; either feature would be suggestive of poor space clamping. The currents from the cell within the clump, however, were threefold larger, which is consistent with the larger action currents recorded from the cell-attached patch. They also persisted at steady-state levels for many minutes after the Na^+ currents from the single cell became severely contaminated with "leak currents." Recording from cells within clumps proved to be more stable than recording from a single cell in that many more solution changes could be performed for each experiment.

Taking advantage of the recording stability afforded by "whole-cell" voltage clamp from apparently well space-clamped B cells with islet cell clumps, we examined the effect of tetrodotoxin on Na^+ currents. Figure 3 demonstrates that as little as 10 nM TTX reduces peak inward current by more than half. The time course and voltage dependence of the remaining current is not noticeably altered. We assumed that TTX interacts with a Na^+ channel in the following manner:

$$\text{channel (open)} + n \cdot \text{TTX} \rightleftarrows \text{channel (blocked)} \cdot \text{TTX}_n. \quad (1)$$

Based on this scheme, we pooled data from three experiments, in which I^+_{Na} evoked at a given V_c was measured in the presence of increasing concentrations of TTX. The solid line in Fig. 3 was drawn from a theoretical curve

$$(I_{TTX}/I_{control}) = 1/(1 + \{[TTX]/K_d\}^n). \quad (2)$$

From the fit we obtained a binding constant (K_d) of 3.2 nM and a Hill coefficient (n) of 1.1, which suggests that a single toxin molecule binds to the Na^+ channel.

An often encountered feature of the electrogenesis of the canine B cell is its ability to repetitively fire action potentials from V_m's of −50 to −45 mV but not from V_m's of −40 to −35 mV. In some cells this is seen during exposure of large concentrations to tolbutamide (Fig. 4*a*) or the passage of depolarizing current. In principle this behavior might arise from the inactivation of the Na^+ current of these cells. Figure 4*b* demonstrates that Na^+ current inactivation is indeed very steep over this voltage range. Na^+ channel inactivation was measured by a three-pulse protocol. First a sustained (1-sec) step to a hyperpolarized holding potential of −120 mV was imposed to remove all channel inactivation. This was followed by a prepulse step of 1 sec to a less negative potential (V_{pre}). Current was measured during a test pulse to −10 mV, a potential at which Na^+ channel activation is maximal. Figure 4*b* shows sample current traces of Na^+ current inacti-

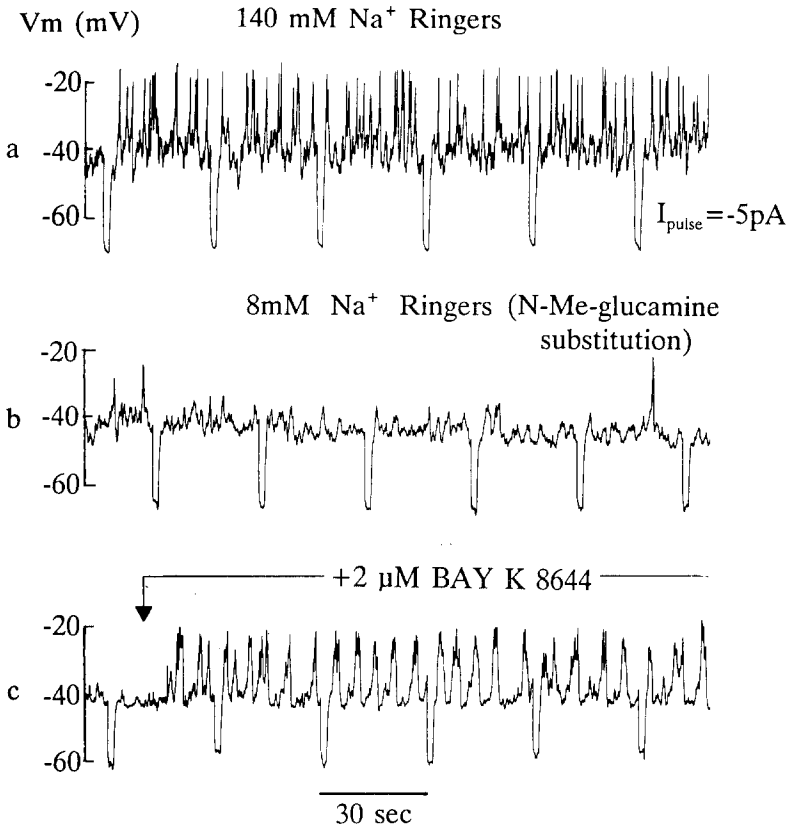

Fig. 5. Na_o^+ and Ca_o^{2+}-dependent action potentials recorded from a cell within a human islet clump. "Perforated-patch" recording was performed with a high-K^+ pipette. (*a*) Action potential activity stimulated by 10 mM glucose ES. Membrane resistance was measured by a −5 pA hyperpolarizing pulse. (*b*) Elimination of action potentials by removal of Na_o^+. (*c*) Emergence of Ca_o^{2+}-dependent electrical activity after addition of 2 μM BAY K 8644

vation for a cell within an islet clump. Figure 4*c* illustrates that the normalized Na^+ conductance (G/G_{max}) displays a sigmoidal decrease with increasingly depolarized V_{pre}. This "inactivation" of the conductance mechanism is complete at a V_{pre} of −40 mV. Note that the characteristic voltage dependence of inactivation is identical for both the cell within an islet clump and the single isolated cell.

Evidence for Na^+-Dependent Component of the Action Potential in Human Islet B Cells

Human islet cells often display trains of rapid upstroke, large amplitude action potentials early in their course of depolarization by glucose (<10 mM), or tolbutamide (10–20 μM); they arise from "rumbling" membrane potentials of −55 to −45 mV and peak at near −10 to 0 mV. These action potentials usually disappear when the baseline membrane potential approaches −35 to −30 mV. They can again be evoked by "anodal break" (i.e., the termination of current pulses which hyperpolarize the membrane to values negative to −60 mV).

By analogy with canine islet action potentials, we tested whether these action potentials might be Na_o^+ dependent. Figure 5 presents typical membrane voltage traces obtained in one of four cells which displayed very graded depolarization and rapid upstroke action potentials in response to small-to-moderate concentrations of glucose (3–10 mM) (*see* Fig. 5*a*). These action potentials persisted at low frequencies for many minutes hence permitting examination of their ionic basis. Note, in Fig. 5*b*, that the action potential activity was almost completely abolished by replacement of the NaCl content of ES with N-methylglucamine HCl, although the underlying V_m and membrane resistance (R_m) were unaffected. Broader and somewhat lower amplitude action potentials appeared with addition of BAYK 8644 (*see* Fig. 5*c*) which shifts the activation characteristic of high threshold Ca^{2+} channels negatively along the voltage axis (McCleskey et al., 1986). The effect of BAY K 8644 in permitting the expression of these coarser action potentials was abolished by reducing the $CaCl_2$ concentration of ES to 0.1 mM by isosmotic replacement with $MgCl_2$.

Discussion

To date, the ionic dependence of electrogenesis and insulin secretion in pancreatic islet B cell has been studied almost exclusively in rodents. The contribu-

tion of voltage-dependent Na^+ currents to action potential generation has not been reported in rodents, while the role of Na^+ channels in insulin secretion has remained uncertain. On the one hand, glucose-induced insulin release has been reported to be increased by Na^+ channel enhancer drugs (e.g., veratridine) and decreased by Na^+ channel blocker drugs (e.g., TTX) (Pace, 1979; Hiriart & Matteson, 1988). Na^+ channel currents have been recorded from both normal B cells (Hiriart & Matteson, 1988; Plant, 1988; Satin & Cook, 1988) and clonal insulinoma cells (Rorsman, Arkhammer & Berggren, 1986; Satin & Cook, 1988). On the other hand, in the best-studied species, the mouse, intracellular recording has not revealed a Na^+-dependent component to the B cell action potential (*see* Ribalet & Beigelman, 1981). In fact, Na^+ removal appears to increase the frequency and amplitude of action potentials (Meissner & Preissler, 1980). Also, tetrodotoxin depolarizes rodent B cells while action potentials persist, while veratridine, a Na^+ channel agonist, hyperpolarizes these cells (Tarvin & Pace, 1981). In addition, there is disagreement as to whether Na^+ currents recorded in the conventional "whole-cell" voltage-clamp configuration are totally inactivated in the physiological range of membrane potentials; results of experiments on mouse suggest that they are (Plant, 1988) while data obtained from rats suggest that they are not (Hiriart & Matteson, 1988).

We have examined the contribution of tetrodotoxin-sensitive Na^+ currents to B cell electrogenesis in two non-rodent species, dog and man. Our data from "perforated-patch" current-clamp recording from canine islet cells indicate that Na^+ currents contribute substantially to action potential generation during the initial phases of secretogogue-induced depolarization. Na^+-dependent action potentials also occur during sustained depolarizations where the underlying cell membrane potential remains negative to −45 mV. In companion "whole-cell" voltage-clamp recording, the Na^+ currents show steep voltage-dependent activation beginning at ~−50 mV; they also show steep voltage and time-dependent inactivation at V_m's positive to −60 mV. These Na^+ currents are totally abolished by maintenance, for 1 sec, of a V_m equal or positive to −40 mV. Their voltage dependence, kinetics and TTX sensitivity resemble those of Na^+ currents found in a variety of neurons and neuroendocrine cells. In one series of experiments, 15 of 16 cells, which were identified as B cells during cell-attached patch recording by their display of action currents in response to glucose or tolbutamide, were found to have Na^+ currents during subsequent "whole-cell" voltage-clamp recording. Our less extensive data for human islet B cells suggest that in one-third to one-half of these cells, TTX-inhibitable voltage-dependent Na^+ currents also contribute to action potential generation. Together these results suggest that, in contrast to the case of several rodent species, in dog and man Na^+ currents play a significant role in action potential generation by the B cell.

In our experiments on islet cells in clumps we selected cells that appeared to be well space clamped. Our data indicate that these cells have the same range of membrane capacitance as isolated cells. In addition, we have demonstrated that voltage-dependent Na^+ currents recorded from both isolated B cells and B cells within islet clumps display similar activation and inactivation characteristics. These three features suggest that the Na^+ currents we analyzed were generated by single cells which were not discernibly electrically coupled with other cells in the clump. In another group of recordings made from "physiologically" identified B cells in islet clumps, Na^+ currents were clearly seen but there was evidence of poor space clamping. This latter observation suggests that at least a subset of cells within islet clumps were electrically coupled. The effect of maintenance of cells in tissue culture on the degree of electrical coupling among islet cells is unknown. Interestingly, we consistently have observed that extracellular action currents and whole-cell Na^+ currents recorded from "uncoupled" cells in islet clumps are 2–3 times larger than corresponding currents from single cells. This suggests that paracrine interactions within an islet may be important as a modulator of B cell electrogenesis.

Our results, which suggest a significant role for Na^+ currents in canine and human B cell excitability, reopen the question of the role of Na^+ currents in insulin secretion in these cells. This issue shall only be settled through correlative studies on the effects of extracellular Na^+ and Ca^{2+}, as well as pharmacological modifiers of their entry into cells, on insulin secretion in these species. These studies are in progress. To date, however, we have observed that the majority of canine and human islet cells which display either Na^+-dependent action potentials and/or sizeable voltage-dependent Na^+ currents, also display sustained, voltage-dependent inward currents in the presence of Ca^{2+} and Ba^{2+} (D.M. Pressel, L. Falke, K. Gillis & S. Misler, *unpublished data*). These high threshold-type "Ca^{2+}" currents are evoked by depolarization positive to −40 mV, and peak at ~0 mV. Ca^{2+} currents from human and dog B cells are of roughly similar amplitudes as those recorded under similar conditions in mouse and rat B cells (e.g., Rorsman et al., 1988; Falke et al., 1989). In rodent B cells these Ca^{2+} currents are presumed to result in the enhanced cytosolic Ca^{2+} associated with triggered insulin secre-

tion (Petersen & Findlay, 1987; Findlay et al., 1989).

Based on our observations, we suggest the following hypothesis for the interaction of Na^+ currents and high threshold Ca^{2+} currents in secretagogue-induced insulin secretion. Na^+ currents, activatable by small depolarizations from rest, allow B cells to initiate Na^+-dependent action potentials at membrane potentials (~ -50 mV) insufficient to open high threshold, voltage-dependent Ca^{2+} channels. The large excursion Na^+ action potentials, with peaks ranging from -20 to $+10$ mV, produce brief pulses of Ca^{2+} entry and insulin secretion. Graded increases in the frequency of Na^+-dependent action potentials at more depolarized V_m's (up to -40 mV) should therefore increase Ca^{2+} entry. Similar interactions of Na^+ and Ca^+ currents promote depolarization-secretion coupling seen at nerve terminals of high safety synapses such as the stellate ganglion of the squid (Llinas, Steinberg & Walton, 1981). Na^+ currents would cease to contribute to canine B cell excitability when the cell is depolarized to membrane potentials positive to -40 mV where Na^+ channels are almost totally inactivated.

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We dedicate this work to the memory of our friend Alex Mauro—inspired teacher of biophysics, champion of humanity, and ideal for a life in science.

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