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Ion channels involved in insulin release are activated by osmotic swelling of pancreatic B-cells

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Abstract

Measurements of the membrane potential showed that osmotic swelling (−80 mosmol/l) of pancreatic B-cells led to a transient hyperpolarization followed by a more sustained depolarization of the cell membrane. Cell swelling triggers a transient activation of the K^+_{ATP} current and of an inward current, carried by Cl^-. This current was inhibited by DIDS, D600, and by omission of extracellular Ca^{2+}. The depolarization opens voltage dependent L-type Ca^{2+} channels, thereby increasing the intracellular Ca^{2+} activity ($[Ca^{2+}]_i$). This effect was blunted by D600 or abolished by omission of Ca^{2+}. Moreover, osmotic swelling transiently increased the amplitude of the Ca^{2+} currents. Replacement of NaCl by D-mannitol proved that the observed effects are due to an increase in cell volume and not to a reduction of extracellular Na^+ or $Cl-$. Our results suggest that regulatory volume decrease is achieved by activation of K^+ and Cl^- currents. The Cl^- current is responsible for the previously described depolarization and increase in insulin release induced by osmotic cell swelling.

Keywords: Pancreatic B-cell; Osmotic cell swelling; Anion channel; K_{ATP} channel

1. Introduction

Osmotic cell swelling is described to activate ion efflux mechanisms in B-cells responsible for regulatory cell volume decrease [1–4], to depolarize the cells [5], and to increase insulin release transiently [1,6,7]. It has been shown that osmotically swollen B-cells activate Rb^+ efflux [2,4] and decrease intracellular Cl^- content [2] to reduce their osmolarity, suggesting that K^+ and Cl^- currents are involved in cell volume regulation. Recently, it has been proved that osmotic swelling of B-cells indeed activates an inward current carried by Cl^- ($I_{Cl,\ islet}$) [8,9]. This current enables the cells to extrude anions during cell swelling and it readily explains the observed membrane depolarization and resulting insulin secretion under these conditions [1,5–7]. Furthermore, it has been shown that this current is involved in nutrient dependent activation of the B-cells [10]. The present study was performed to further elucidate ion currents evoked in pancreatic B-cells by osmotic cell swelling.

2. Material and methods

The experiments were performed on B-cells of islets of Langerhans from fed female NMRI mice

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(25–30 g), killed by cervical dislocation. Determination of the cell membrane potential with microelectrodes was performed with B-cells of intact islets as described by Meissner and Schmelz [11]. Osmotic swelling was induced by reduction of NaCl (or TEACl) by 40 mmol/l. For patch-clamp experiments, B-cells were isolated as described by Plant [12]. K^+_{ATP} currents were recorded using the following bath solution (in mmol/l): 140 NaCl, 5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 0.5 glucose, 10 Hepes, pH 7.4. Where indicated, NaCl was replaced isosmotically by TEACl. The pipette solution for conventional whole-cell measurements was composed of (in mmol/l): 120 KCl, 4 $MgCl_2$, 2 $CaCl_2$, 10 EGTA, 0.65 Na_2-ATP, 20 Hepes, pH 7.15 and for perforated patch measurements contained (in mmol/l): 10 KCl, 10 NaCl, 70 K_2SO_4, 4 $MgCl_2$, 2 $CaCl_2$, 10 EGTA, 20 Hepes, pH 7.15. Nystatin (150–250 μmol/l in pipette solution) was backfilled and pipette solution was sucked into the tip of the pipette. For determination of K^+_{ATP} currents, the cells were voltage-clamped at a holding potential of −70 mV. 300 ms pulses to −80 and −60 mV were applied at 15 s intervals. Currents measured under these conditions are almost entirely K^+_{ATP} currents, blockable by tolbutamide. K^+_{ATP} currents were quantified by the currents elicited by 10 mV depolarizing voltage steps. The swelling-induced inward current was measured as the holding current at −70 mV with the same bath and pipette solution as for the determination of K^+_{ATP} currents in the conventional whole-cell mode. At least a part of this inward current is $I_{Cl,islet}$ which was determined by I/V curves with voltage steps from −120 to +60 mV with a step width of 20 mV and the same solutions as used by Kinard and Satin [8]. L-type Ca^{2+} channel currents were determined in the perforated patch mode with Ba^{2+} as the charge carrier. Bath solution was composed of (in mmol/l): 115 NaCl, 20 TEACl, 10 $CaCl_2$ or $BaCl_2$, 1.2 $MgCl_2$, 0.1 tolbutamide, 15 glucose, 10 Hepes, pH 7.4, pipette solution contained (in mmol/l): 70 Cs_2SO_4, 10 NaCl, 10 KCl, 7 $MgCl_2$, and 10 Hepes, pH 7.4. The Ca^{2+} currents were

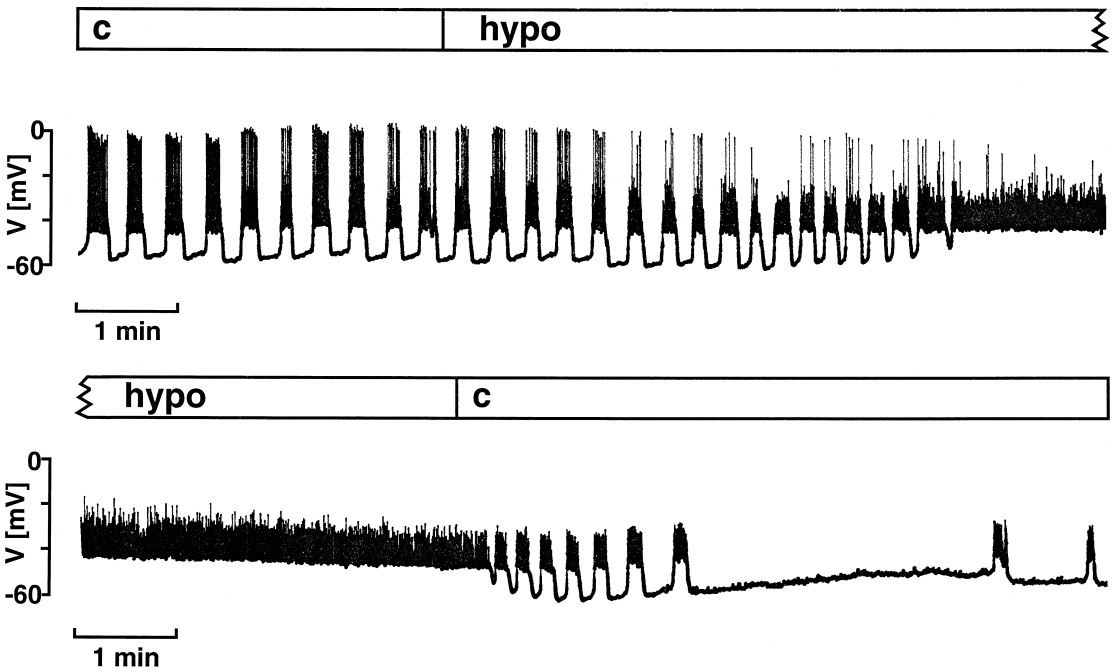

Fig. 1. Effect of osmotic cell swelling on the membrane potential recorded from pancreatic B-cells of intact islets. The experiments have been performed in the presence of 15 mmol/l glucose (c). Osmolarity was decreased by decreasing extracellular NaCl concentration by 40 mmol/l (hypo). Hypotonic solution was applied at the time indicated by the horizontal bar. The same applies to the other figures. Original recording representative of six experiments with similar results.

elicited by 50–300 ms voltage steps from −70 to 0 mV at 10–20 s intervals. For determination of intracellular free Ca^{2+} activity ($[Ca^{2+}]_i$) with fura-2 fluorescence, the same bath solutions as for the determination of K^+_{ATP} whole-cell currents were used. Prior to the measurements, single cells seeded on glass coverslips were incubated with the acetoxymethylester of fura-2 (fura-2-AM, 10 μmol/l, Molecular Probes, Eugene, Oregon, USA) for 30 min. Fluorescence measurements were performed at 340/380 nm excitation wavelength, as described by Grynkiewicz et al. [13]. Patch-clamp measurements were made at 25°C or 32°C, all other experiments at 37°C. DIDS (4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid) was from Sigma (Deisenhofen, FRG) and D600 was kindly provided by Knoll AG (Ludwigshafen, FRG). Data are presented as means ± SEM. Statistical analysis was carried out according to student's *t*-test or paired *t*-test and statistical significance was accepted at $p \leq 0.05$.

3. Results

3.1. Osmotic cell swelling leads to a biphasic alteration of the membrane potential

In isotonic extracellular fluid containing 15 mmol/l glucose the cell membrane potential recorded from B-cells of intact islets exhibited oscillations with a fraction of plateau phase (percentage of time with spiking activity) of 47 ± 6% ($n = 6$). A decrease in the bath osmolarity by −80 mosmol/l led to a transient hyperpolarization of the membrane by −8.2 ± 2.5 mV within the first 1–4 min and a significant decrease of the plateau phase to 32 ± 3% ($n = 6$),

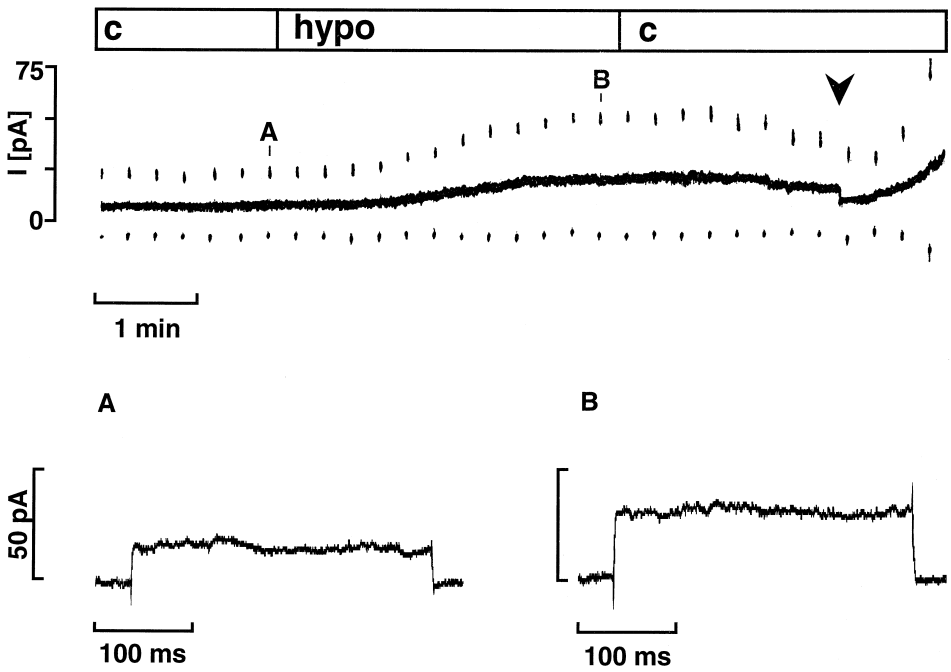

Fig. 2. Effect of osmotic cell swelling on K^+_{ATP} channel currents of pancreatic B-cells recorded in the perforated patch configuration. Upper panel: Currents shown at the holding potential of −70 mV (solid line) and during 300 ms steps to −80 and −60 mV (lower dashed trace and upper dashed trace, respectively). The arrow head marks the break of the patch into the conventional whole-cell configuration. Lower panel: Currents at A and B on an extended time scale. Original recording representative of six experiments with similar results.

which was followed by a depolarization to the plateau potential with continuous spike activity (Fig. 1). In all experiments the impalements were impaired (Fig. 1) or even lost after switching back to control solution presumably because of cell shrinkage and subsequent regulatory volume increase (RVI). In this context it is interesting to mention that a suppression of electrical activity was also observed after switching from control to hypertonic solution [5].

3.2. Swelling-induced increase of the K^+_{ATP} current

In the perforated patch configuration, a decrease in bath osmolarity led to a significant increase of the K^+_{ATP} current, evoked by a voltage step from -70 to -60 mV, from $+24 \pm 6$ pA under control conditions to $+66 \pm 17$ pA during perfusion with hypotonic bath solution ($n = 6$, Fig. 2). The arrow head indicates the break-through into the conventional whole-cell configuration which occurred in all experiments after switching back to control solution. In the standard whole-cell configuration a decrease in bath osmolarity also resulted in an increase in the K^+_{ATP} current, elicited by a 10 mV depolarizing voltage step from $+117 \pm 15$ pA under control conditions to $+147 \pm 30$ pA after switching to hypotonic solution ($n = 5$, Fig. 3(A)). Accordingly, the holding current at -70 mV tended to increase from $+84 \pm 13$ to $+96 \pm 20$ pA. However, in contrast to the perforated patch measurements, the increase in the outward current seemed to be transient (see below). One series of experiments was performed to determine the effect of isotonic NaCl reduction. The K^+_{ATP} current under control conditions was $+83 \pm 13$ and $+81 \pm 14$ pA

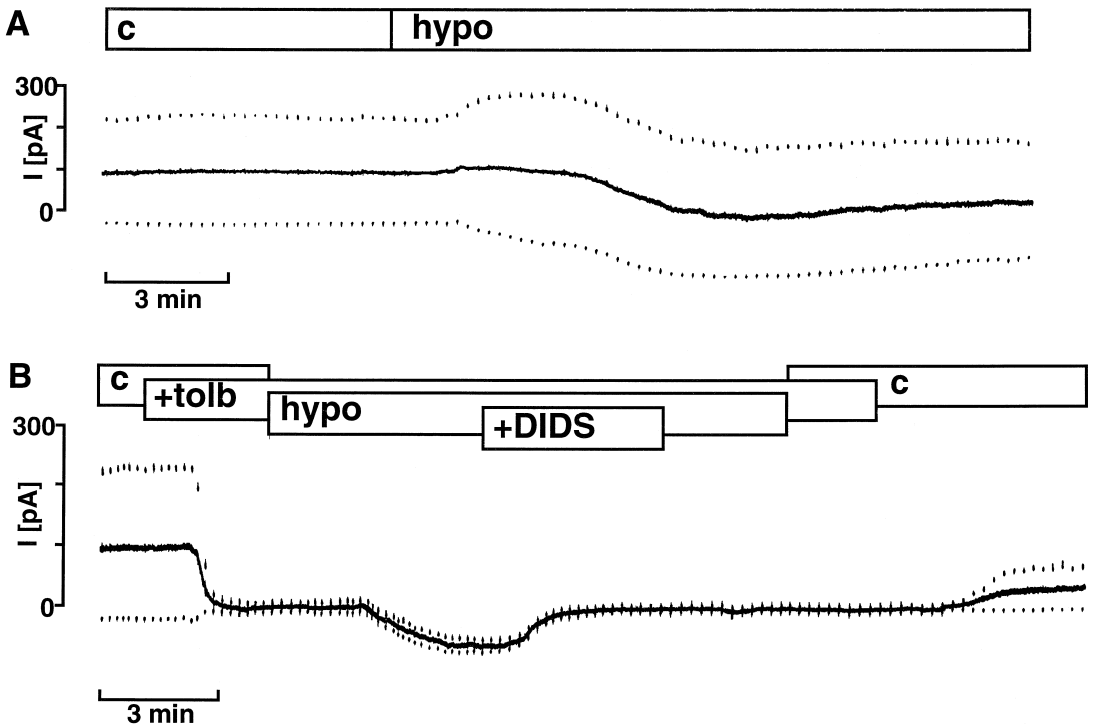

Fig. 3. Effect of osmotic cell swelling on conventional whole-cell currents in pancreatic B-cells. (A) Currents shown at the holding potential of -70 mV (solid line) and during 300 ms steps to -80 and -60 mV (lower dashed trace and upper dashed trace, respectively). Under control conditions the traces reflect K^+_{ATP} current. Hypotonic solution transiently increased K^+_{ATP} current and, as seen from the current trace recorded at -70 mV, induced a delayed inward current. Original record representative of five experiments with similar results. (B) Effects of 100 μmol/l DIDS (+DIDS) on the inward current induced by hypotonic solution (hypo). K^+_{ATP} current was inhibited by 100 μmol/l tolbutamide (+tolb). Trace representative of four experiments with similar results.

($n = 5$, not shown) after isosmotic replacement of 40 mmol/l NaCl by 80 mmol/l D-mannitol.

3.3. Osmotic cell swelling activates Ca^{2+}-dependent Cl^- currents

The swelling-induced increase in the outward current seemed to be transient because of the appearance of an inward current. The holding current at −70 mV changed from +84 ± 13 pA under control conditions transiently to +96 ± 20 pA and subsequently to −13 ± 20 pA ($n = 5$) during exposure to hypotonic solution (Fig. 3(A)). In another series of experiments inhibition of the K^+_{ATP} current by 100 μmol/l tolbutamide altered the holding current from +52 ± 14 to −15 ± 9 pA ($n = 4$, Fig. 3(B)). Subsequent reduction in bath osmolarity increased the inward holding current to −110 ± 21 pA ($n = 4$). Further addition of 100 μmol/l DIDS reduced the current to −18 ± 7 pA ($n = 4$, Fig. 3(B)). For further characterization of the inward current, the Ca^{2+} dependency was tested. To ensure that the current is not Na^+-dependent, Na^+ was replaced by TEA^+. The holding current at −70 mV changed from +93 ± 12 to −15 ± 4 pA ($n = 16$) after replacement of Na^+ by TEA^+ and inhibition of K^+_{ATP} channels by tolbutamide. Subsequent osmotic swelling increased the current to −119 ± 21 pA ($n = 9$, Fig. 4(A)). Omission of extracellular Ca^{2+} abolished this current (0 ± 1 pA, $n = 7$) (Fig. 4(B)). In four out of seven cells spontaneous transient inward currents as in (Fig. 4(B)) were observed after substituting TEA^+ for Na^+. They disappeared after removal of Ca^{2+} and may reflect electrical activity of depolarized and poorly clamped neighbours of the cell under investigation, when the patch pipette was occasionally sited on a small cluster instead of a single B-cell. The influence of D600 (10 μmol/l) on the inward current was also tested. Na^+ free solution containing tolbutamide and D600 decreased the holding current from +96 ± 17 to −11 ± 2 pA ($n = 12$). Reduction of the osmolarity in the continuous presence of D600 clearly inhibited the appearance of the swelling-induced inward current (−27 ± 6 pA, $n = 12$) (Fig. 4(C)). However, I/V curves and reversal potential measurements confirm the results of Kinard and Satin [8] and Best et al. [9] that the inward current induced by osmotic cell swelling is not carried by cations but by Cl^-. The reversal potential of the inward current was −33 ± 10 mV and shifted to −19 ± 11 mV when extracellular Cl^- was reduced from 110 to 18 mmol/l ($n = 3$, Fig. 5). These data are very similar to those obtained by Kinard and Satin [8]. According to Kinard and Satin [8] the deviation from the Cl^- equilibrium potential calculated according to the Nernst equation is due to aspartate in the pipette solution. They have calculated P_{asp}:P_{Cl} to be 0.17.

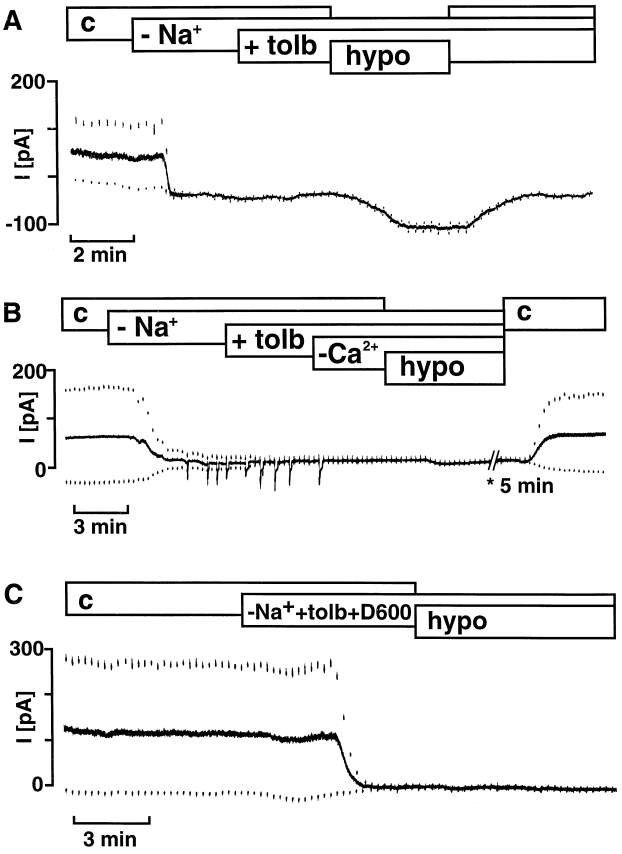

Fig. 4. Ca^{2+} dependency of the swelling-induced inward current. (A) Inward current induced by hypotonic solution (hypo) at −70 mV (solid line) after replacement of Na^+ by TEA^+ ($-Na^+$) and subsequent addition of 100 μmol/l tolbutamide (+tolb). The trace shown is representative of nine experiments. (B) Suppression of the inward current after additional removal of extracellular Ca^{2+} ($-Ca^{2+}$). The trace is representative of seven experiments. (C) Suppression of the inward current induced by hypotonic solution (hypo) in the presence of D600 (10 μmol/l). As in (A) and (B) Na^+ has been replaced by TEA^+ and tolbutamide was added ($-Na^+$ + tolb + D600). This record is representative of twelve experiments with similar results.

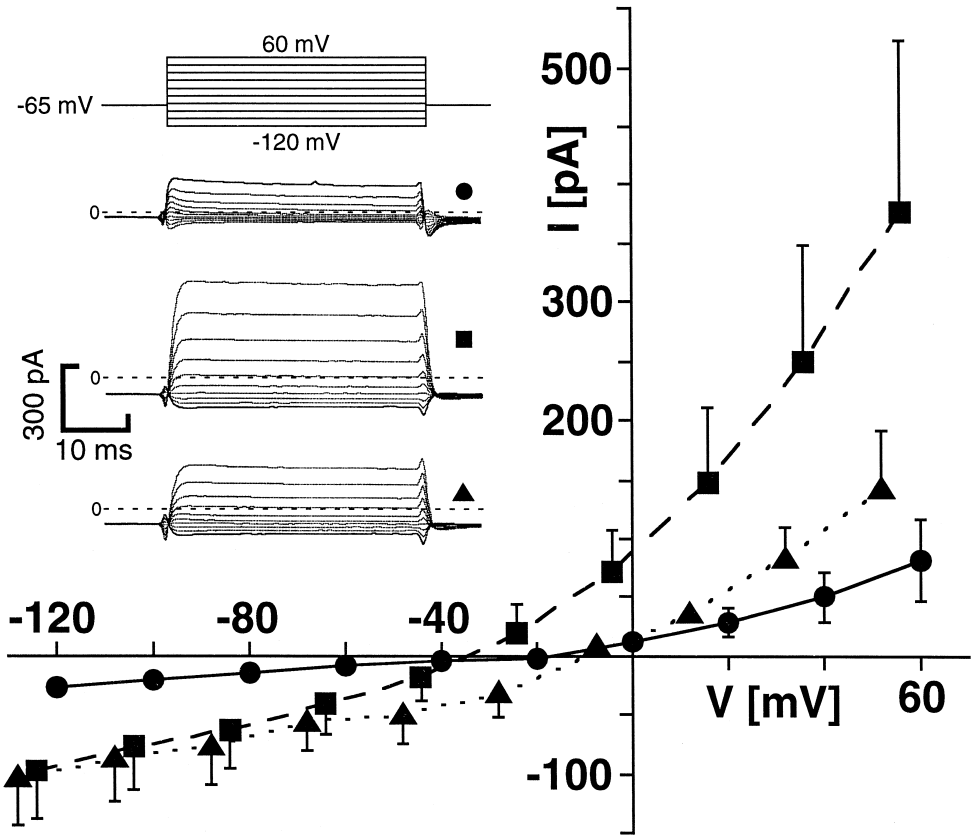

Fig. 5. Effect of cell swelling on current-voltage relationships. I/V curves were recorded during 50 ms pulses at +20 mV increments from −120 to +60 mV in isotonic control solution (●), hypotonic solution (■) and in hypotonic solution with reduced extracellular Cl^- concentration (from 110 to 18 mmol/l) (▲). In this series of experiments the same solutions have been used as described by Kinard and Satin [8]. The I/V curves are corrected for liquid junction potentials. The data are mean values of three experiments. The inset shows the pulse protocol and the corresponding currents under the different experimental conditions for one experiment.

3.4. Influence of osmotic cell swelling on L-type Ca^{2+} currents and intracellular Ca^{2+} activity

In the perforated patch configuration a small but statistically significant transient increase in the Ba^{2+} current through voltage-dependent Ca^{2+} channels was observed. Under control conditions the current elicited by a voltage step from −70 to 0 mV was -117 ± 19 pA and transiently increased to -133 ± 20 pA ($n = 5$) during the exposure to hypotonic solution. Subsequently, the current amplitude decreased within 5 min to -107 ± 24 pA ($n = 5$). After switching back to control solution the Ba^{2+} current was -113 ± 16 pA ($n = 5$, Fig. 6).

Intracellular free Ca^{2+} activity showed a reversible increase from 66 ± 4 nmol/l under control conditions to 349 ± 66 nmol/l during exposure to hypotonic bath solution ($n = 4$, Fig. 7(A)). To investigate the influence of the Na^+:Ca^{2+} exchanger on the swelling induced increase of $[Ca^{2+}]_i$, 40 mmol/l NaCl was isosmotically replaced by 80 mmol/l D-mannitol. This set of experiments showed that reduction of Na^+ did not alter $[Ca^{2+}]_i$ (80 ± 6 nmol/l under control conditions, 83 ± 7 nmol/l in the D-mannitol containing solution ($n = 4$)). Subsequent omission of D-mannitol increased $[Ca^{2+}]_i$ to 169 ± 8 nmol/l ($n = 4$). In the presence of the Ca^{2+} channel blocker D600 (10 μmol/l), reduction of the bath osmolarity increased $[Ca^{2+}]_i$ only weakly from 57 ± 5 to 83 ± 6 nmol/l ($n = 5$, Fig. 7(B)). To verify whether cell swelling provokes Ca^{2+}-influx across the plasma membrane hypotonic solution was tested in the absence of external Ca^{2+}. In this series of experiments first K^+ was transiently increased from 5 to

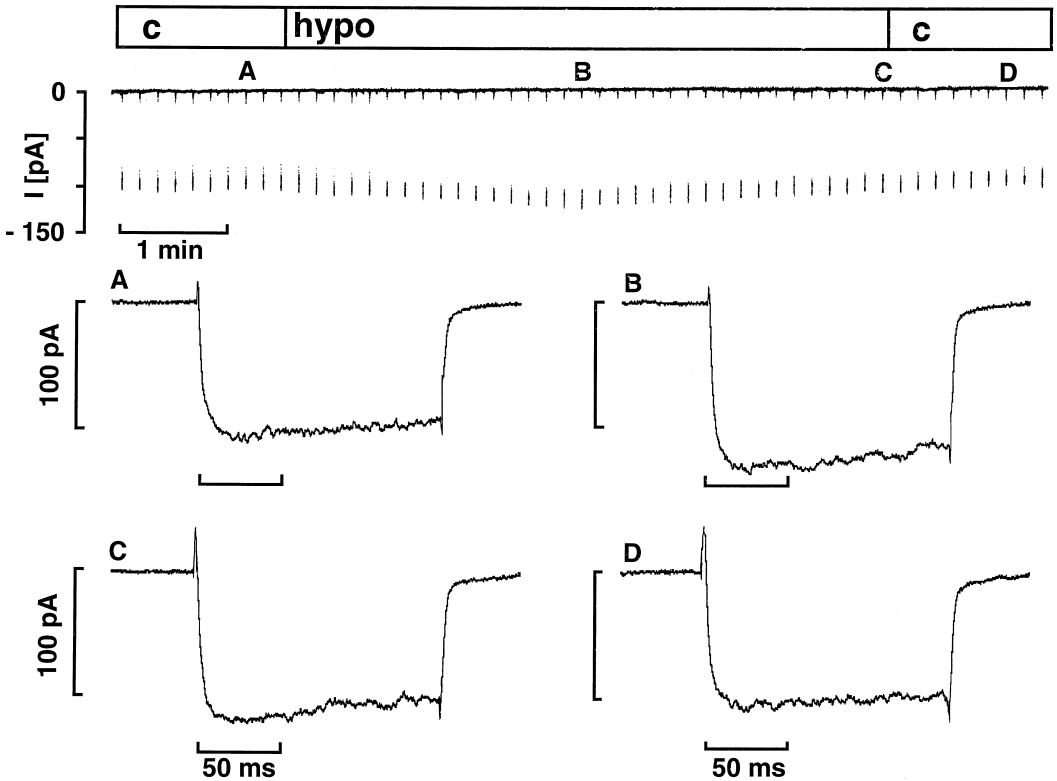

Fig. 6. Effect of osmotic cell swelling on voltage-dependent Ca^{2+} currents. Currents were recorded in the perforated patch configuration with Ba^{2+} as charge carrier. The upper panel shows a continuous recording. Currents were elicited every 10 s by 150 ms voltage steps from the holding potential of −70 to 0 mV. The lower panels show currents at A, B, C and D on an extended time scale. The records are representative of five experiments with similar results.

25 mmol/l to test the responsiveness of the cells. Under these conditions $[Ca^{2+}]_i$ was raised from 64 ± 17 to 373 ± 36 nmol/l ($n = 5$, Fig. 7(C)). After removal of external Ca^{2+} from the control solution $[Ca^{2+}]_i$ amounted to 75 ± 24 nmol/l. After addition of hypotonic solution $[Ca^{2+}]_i$ was 82 ± 26 nmol/l and after switching back to control solution 109 ± 23 nmol/l ($n = 5$, Fig. 7(C)).

4. Discussion

Osmotic swelling of pancreatic B-cells has long been shown to lead to a transient increase in insulin release [1,6,7]. Our data strongly suggest that this increase in insulin release is owing to the increase in $[Ca^{2+}]_i$ (Fig. 7(A)) which in turn is the result of a swelling-induced depolarization and increase in electrical activity (Fig. 1 and [5]). However, Blackard et al. [6] have found an enhancement of insulin secretion by hypoosmolarity in nominally Ca^{2+}-free solution. Osmotic swelling of B-cells is counteracted by regulatory volume decrease (RVD) [1–4], i.e. the extrusion of anions, cations and water. In the present paper ion currents are described which underlie these changes in ion content and which directly influence the membrane potential and electrical activity [5].

The hyperpolarization in the first phase of osmotic cell swelling (Fig. 1 and [5]) can be explained by an increase in K^+_{ATP} current (Figs. 2 and 3(A)). In the perforated patch mode this increase is moderate and reversible (Fig. 2). However, in the standard whole-cell clamp the rise in outward current is counteracted by the appearance of a large inward current (Fig. 3(A)). In the perforated patch mode the cells are likely still able to support RVD and thus the current changes are small. To the contrary, in the conventional whole-cell mode the cells are unable to regu-

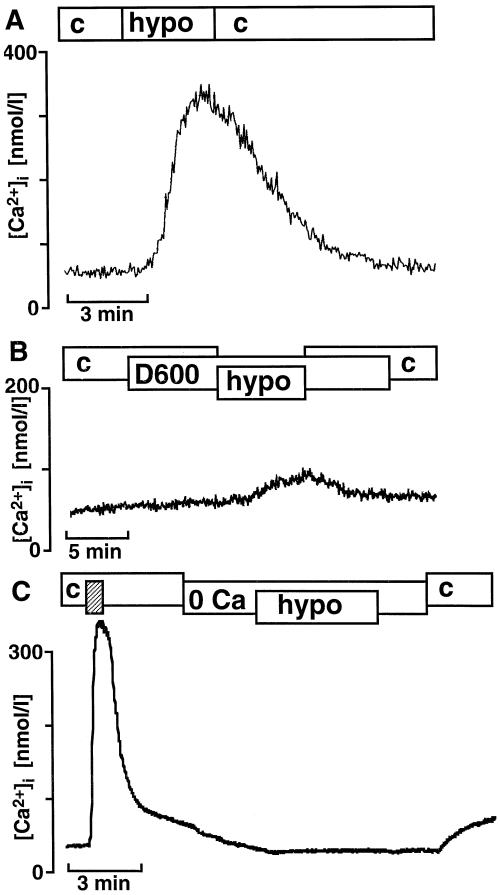

Fig. 7. Effect of osmotic cell swelling on $[Ca^{2+}]_i$. (A) Effect of hypotonic solution (hypo) on $[Ca^{2+}]_i$. The plot shown is representative of four experiments. (B) Effect of hypotonic solution (hypo) on $[Ca^{2+}]_i$ in the presence of 10 μmol/l D600 (D600). The trace is representative of five experiments. (C) Effect of hypotonic solution (hypo) on $[Ca^{2+}]_i$ in Ca^{2+}-free extracellular solution. 1 mmol/l EGTA has been added to the Ca^{2+}-free bath solution. At the time indicated by the hatched bar the K^+ concentration of the bath solution has been raised from 5 to 25 mmol/l. The trace is representative of five experiments with similar results.

late their volume and therefore the maximal possible currents develop. Isotonic reduction of 100 mmol/l NaCl leads to a similar transient hyperpolarization [14] as the reduction of osmolarity by omitting 20 mmol/l or 40 mmol/l NaCl ([5] and this paper), although the changes in the pattern of electrical activity induced by the two manoeuvres in the steady state are different. Nevertheless, one might argue that not cell swelling but the reduction of the NaCl concentration interferes with K^+_{ATP} current presumably via changes in pH_i by affecting the Na^+/H^+ exchanger which is present in B-cells [15]. However, the mechanisms by which isotonic and hypotonic NaCl reduction hyperpolarize B-cells transiently seems to be different since isotonic NaCl replacement did not affect the K^+_{ATP} current.

Fig. 3(B) shows that during blockade of the K^+_{ATP} current osmotic cell swelling induces an inward current which is most likely responsible for the observed depolarization and increase in electrical activity (Fig. 1 and [5]). Since this current is almost entirely inhibited by DIDS (Fig. 3(B)) and has the characteristics and reversal potential of the earlier described $I_{Cl,islet}$ [8] we conclude that it is a Cl^- current. However, in contrast to the findings of Best et al. [9] that this current is independent from Ca^{2+} we observed that it is dependent on Ca^{2+} influx. Ca^{2+} dependence of cell volume regulation has been described for a variety of cell types [16]. Fig. 4 shows that the current still exists in the absence of extracellular Na^+ (Fig. 4(A)) but that omission of extracellular Ca^{2+} abolishes it (Fig. 4(B)). Additionally, the swelling-induced Cl^- current as well as the increase in $[Ca^{2+}]_i$ is markedly inhibited by D600 (Fig. 4(C)) and Fig. 7(B)). Moreover, the rise in $[Ca^{2+}]_i$ induced by hypotonic solution is suppressed in Ca^{2+}-free external medium (Fig. 7(C)). These data suggest that Ca^{2+} influx is a prerequisite for Cl^- channel activation which presumably occurs through L-type Ca^{2+} channels. Although this seems unlikely at a membrane potential as negative as −70 mV, it has been shown previously that Ca^{2+} entry through voltage-dependent L-type Ca^{2+} channels exists at resting membrane potential [17]. Alternatively, we cannot rule out that D600 directly blocks the Cl^- current or another Ca^{2+} current. That Ca^{2+} influx activates a Cl^- current involved in RVD which depolarizes the membrane has been shown e.g. for mesangial cells [18]. However, in these cells Ca^{2+} influx does not occur via L-type Ca^{2+} channels.

At present it is difficult to reconcile the different observations regarding the Ca^{2+} dependence of the Cl^- current in B-cells. Inhibition of Ca^{2+} entry through L-type Ca^{2+} channels by Cd^{2+} [8] or Co^{2+} [9] may be incomplete and chelation of intracellular Ca^{2+} by BAPTA [9] may not prevent a local Ca^{2+} increase directly beneath the membrane which might be sufficient to activate the current.

In conclusion, the data presented here indicate that osmotic swelling activates K^+_{ATP} channels, L-type Ca^{2+} channels, and Ca^{2+}-dependent Cl^- channels. This leads to the release of cellular KCl and thus in turn to a decrease in intracellular osmolarity and RVD. The accompanying depolarization of the membrane increases electrical activity and $[Ca^{2+}]_i$ which finally leads to insulin secretion.

Acknowledgements

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