6723 Journal of Physiology (1997), 504.2, pp.259–270

Inwardly rectifying, voltage-dependent and resting potassium currents in rat pancreatic acinar cells in primary culture

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1. In exocrine pancreatic acinar cells in primary culture an inwardly rectifying, a voltage-dependent and a permanent resting K^+ current were characterized.
2. Inwardly rectifying K^+ currents could be elicited by elevation of the extracellular K^+ concentration. The K^+ inward currents were almost completely blocked by 5 mM Ba^{2+}, whereas 10 mM TEA^+ had only a partial effect.
3. Depolarizing voltage steps from negative clamp potentials evoked transient activation of a voltage-dependent K^+ current. This voltage-dependent current could be blocked by 10 mM TEA^+ and 1 mM 4-aminopyridine, but not by 5 mM Ba^{2+}.
4. Neither the K^+ inward rectifier nor the voltage-dependent K^+ conductance produced a significant negative cell potential. Stable membrane potentials ($-38·7 \pm 2·3$ mV, $n = 38$) could only be recorded on cell clusters ($\geqslant 5$ cells).
5. Cell clusters, in contrast to single cells, had a permanent resting K^+ conductance in addition to the inward rectifier and the voltage-dependent current. This resting K^+ conductance was not blocked by TEA^+, Ba^{2+}, 4-aminopyridine or by the chromanol 293B.
6. Cytosolic alkalization by addition of NH_4Cl to the bath solution decreased the resting K^+ current. In parallel, electrical uncoupling of the cells and breakdown of the resting potential could be observed. The same effects could be produced when the cells were uncoupled by 0·2–1·0 mM n-octanol. It can be concluded that cell coupling is essential for maintenance of stable resting membrane potentials in pancreatic acinar cells.

Stimulation of pancreatic acinar cells with acetylcholine (ACh), cholecystokinin (CCK) or bombesin induces release of digestive enzymes into the acinar lumen and activates secretion of an isotonic NaCl-rich fluid. Current models for NaCl secretion suggest that Na^+ primarily flows paracellularly, whereas Cl^- is transported across the acinar cell by secondary active transport mechanisms in the basolateral plasma membrane (Zhao & Muallem, 1995) and a Ca^{2+}-dependent Cl^- conductance in the luminal membrane (Petersen, 1992). Since Cl^- passes the luminal membrane passively, Cl^- extrusion from the cytosol into the acinar lumen only occurs when the cell potential is more negative than the Cl^- equilibrium potential. Therefore, the standard model for hormone-evoked Cl^- secretion from exocrine acinar cells involves both, i.e. activation of a Ca^{2+}-dependent Cl^- conductance in the luminal membrane, which enables Cl^- efflux to the acinar lumen, and activation of a Ca^{2+}-dependent K^+ conductance in the basolateral membrane, which provides the necessary electrical driving force for Cl^- extrusion (Petersen, 1992). In accordance with this model, Ca^{2+}-dependent K^+ channels have been found in pancreatic acinar cells from various species, e.g. pig (Maruyama, Petersen, Flanagan & Pearson, 1983), guinea-pig (Suzuki & Petersen, 1988) and man (Petersen *et al.* 1985). However, there are also mammals in which NaCl secretion from the exocrine pancreas does not follow this model. Isolated pancreatic acinar cells from mice and rat respond to stimulation with ACh, CCK or bombesin with activation of a Ca^{2+}-dependent Cl^- conductance in the luminal and a non-selective cation conductance in the basolateral membrane. There is no evidence for the existence of either Ca^{2+}-dependent K^+ channels or any other hormone-activated K^+ conductances in this cell type (Randriamampita, Chanson & Trautman, 1988; Petersen, 1992; Schmid & Schulz, 1995). Nevertheless, an effective Cl^- extrusion could also be possible, even in the absence of a hormone-regulated K^+ conductance, as long as the cell potential is kept at values more negative than the Cl^- equilibrium potential. This could be accomplished by a permanently high resting K^+ conductance. However, previous whole-cell patch-clamp studies on freshly isolated acinar cells (Thorn & Petersen, 1994; Schmid & Schulz, 1995) have so far failed to demonstrate permanent K^+ currents that are large enough to maintain an outward driving force for Cl^- ions under hormonal stimulation.

A recent study on rat pancreatic acini reports resting potentials of about −49 mV in cell clusters (Slawik, Zdebik, Hug, Kerstan, Leipziger & Greger, 1996). This value is even more negative than the previously reported resting potentials of about −40 mV measured with microelectrodes

in mouse pancreatic tissue segments (Matthews & Petersen, 1973). The experiments on cell clusters and tissue segments indicate that cell-to-cell contacts might be important for development of stable resting membrane potentials. Enzymatic digestion and mechanical disruption of cell contacts during the cell isolation procedure could be the reason for an abnormal loss or downregulation of the resting potentials in single acinar cells (Schmid & Schulz, 1995). Therefore, to determine whether the isolated cells can recover normal resting K^+ conductances and cell potentials, we established primary cultures with freshly prepared acinar cells from newborn rats according to the method described by Anderson & McNiven (1995). In our electrophysiological experiments on the cultured pancreatic acinar cells we characterized three types of K^+ currents: a voltage-dependent and an inwardly rectifying K^+ current, as well as a permanent K^+ current. The experiments on cell clusters indicate that cell-to-cell coupling might play an important role for normal activity of the resting K^+ conductance and development of a stable cell potential in rat pancreatic acinar cells.

METHODS

Cell preparation and culturing of cells

Exocrine pancreatic acinar cells were prepared by a method similar to that described by Anderson & McNiven (1995). Briefly, the pancreata from 5- to 7-day-old rats killed by cervical dislocation, were taken and transferred to a calcium-free NaCl dissociation buffer (composition (mM): 130 NaCl, 5 KCl, 2 $MgCl_2$, 1·2 KH_2PO_4, 10 glucose, 20 Hepes; adjusted to pH 7·4 with NaOH; supplemented with 1% (v/v) basal amino acids (Gibco, 100× stock solution), 0·2% (w/v) bovine serum albumin, 0·1 mg ml^{-1} trypsin inhibitor, 100 units ml^{-1} penicillin and 0·1 mg ml^{-1} streptomycin). After mechanical removal of all adherent blood vessels and connective tissue the pancreata were subjected to enzymatic digestion at 37 °C for 50 min in the NaCl dissociation buffer supplemented with 100 units ml^{-1} collagenase type VII (Sigma). Enzymatic digestion was stopped by washing the tissue in Medium 199 (Gibco) containing 10% heat-inactivated horse serum (Gibco). The tissue was then pressed through a 100 μm mesh cell sieve. The resulting cell suspension was centrifuged for 5 min at 130 *g* and the pellet was resuspended in Medium 199 with 10% chicken embryo extract (Gibco) and 4% heat-inactivated horse serum. The cells were then seeded in the same medium onto glass coverslips in plastic dishes (~150000 cells (9·6 $cm^2)^{-1}$) and kept in cell primary culture for up to 21 days.

For patch-clamp experiments the glass coverslips were cut into small pieces (~4 mm × 6 mm) and transferred into a perfusion chamber mounted onto an inverted microscope.

Whole-cell current recording

Current recordings were performed with an EPC-9 patch-clamp amplifier (Heka, Germany) in the tight seal whole-cell (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) and also in a few experiments in the perforated-patch configuration (Horn & Marty, 1988). In experiments on single cells, the cell capacitance (C_m) and series resistance were calculated with the software-supported internal routines of the EPC-9 and compensated at the beginning of each experiment. In current recordings on electrically coupled cell clusters, when the internal compensation routines of the EPC-9 failed to calculate reasonable values, the compensation for the slow capacitance component (C_{slow}) was disabled. The current–voltage relationship of the cells was investigated by applying voltage ramps and/or voltage pulses. When the cell capacitance could be compensated with the amplifier, voltage ramps lasting 200–400 ms from −100 to +40 mV were applied to the cell. Otherwise, the duration of the voltage ramp was prolonged to up to 2 s, to minimize artefacts of current flow due to the cell capacitance. During each voltage ramp 400–4000 data points were sampled and stored on hard disk. Voltage pulse protocols were used to investigate voltage-dependent K^+ currents and to trace changes in the electrical coupling within cell clusters. Analysis of voltage-dependent K^+ currents was only performed when the capacities in the measuring circuit could be compensated. For measuring the electrical coupling the C_{slow} compensation routine of the EPC-9 was disabled.

Solutions and materials

The patch pipette was filled either with a KCl (composition (mM): 145 KCl, 1 $MgCl_2$, 0·1 EGTA, 10 Hepes; pH 7·1 adjusted with KOH) or a potassium glutamate solution (composition (mM): 126 potassium glutamate, 15·5 NaCl, 0·1 EGTA, 10 Hepes; pH 7·1 adjusted with KOH). For experiments using the perforated-patch technique 200 μg ml^{-1} nystatin was added to the pipette solution. The standard bath solution contained (mM): 140 NaCl, 4·7 KCl, 1 $MgCl_2$, 1·3 $CaCl_2$, 10 glucose, 10 Hepes; pH 7·4 adjusted with NaOH. Ion substitution experiments were performed by replacing NaCl in the bath solution with equimolar concentrations of KCl, CsCl, RbCl, *N*-methyl-D-glucamine chloride (NMDG-Cl) or sodium methanesulphonate. When TEA-Cl, $BaCl_2$, NH_4Cl or sodium propionate was added to the NaCl bath solution osmolarity was balanced by omission of the respective amount of NaCl.

Glass pipettes were manufactured from borosilicate glass capillaries and fire polished before use. The input resistance of a pipette filled with KCl buffer was 1·5–4 MΩ and with potassium glutamate was 2·5–6 MΩ. All potentials refer to the cytosolic side of the membrane with the extracellular side connected to ground. Measurements were carried out at room temperature (21–26 °C). Mean values are given as means ± S.E.M. All chemical compounds were purchased from Sigma (Deisenhofen, Germany). Chromanol 293B was a kind gift from Hoechst AG (Frankfurt/Main, Germany).

RESULTS

Freshly prepared exocrine pancreatic acinar cells from 5- to 7-day-old rats possess the same morphological polarization as cells isolated from adult animals. The zymogen granules are tightly packed in the luminal part of the cytosol, whereas the nucleus is located in the basal cell pole, which is transparent under the light microscope. Within several hours of plating, the single cells or acini were loosely attached to the glass coverslips. First morphological changes could be observed about 10 h after plating when the cells flattened and became tightly attached to the coverslips. The zymogen granules, which initially were restricted to the luminal pole of the cell, became arranged near the nucleus, either surrounding it or in the form of parallel strings starting from one pole of the nucleus. During the next days flat cell clusters were formed by cell division. The pancreatic acinar cells could be held in primary culture for several weeks. However, 3 weeks after cell isolation the amount of

granules in the cells decreased and, furthermore, the incidence of polynuclear cells in the primary culture increased. Therefore, for the electrophysiological experiments the cells were used within 2 or maximally 3 weeks after plating.

The whole-cell current measurements with the patch-clamp technique revealed three types of potassium currents in the cultured pancreatic acinar cells: (1) an inwardly rectifying K^+ current, (2) a voltage-dependent K^+ current, and (3) a permanent resting K^+ conductance. The K^+ inwardly rectifying and the voltage-dependent K^+ current could be measured in single cells at the beginning of the cell culture as well as in cell clusters 3 weeks later. There was no indication that these currents change during cell culture. In contrast, a permanent resting K^+ conductance, which could produce cell potentials of about −40 mV, as reported from micropuncture studies on rat pancreatic tissue segments (Matthews & Petersen, 1973), could only be measured in electrically coupled cell clusters. In single cells as well as in freshly prepared cells the permanent K^+ conductance was negligible.

K^+ inward rectifier

Inwardly rectifying K^+ currents could be recorded when NaCl in the bath solution was replaced by KCl (Fig. 1*A*). However, the magnitude of the K^+ inward currents varied considerably from experiment to experiment. In twenty-two experiments on single cells inward currents ranging from −4 to −894 pA at a clamp potential of −54 mV were measured. The mean inward current of a single cell ($C_m = 28.6 \pm 3.7$ pF, $n = 22$) carried by K^+ influx was -142.0 ± 44.1 pA at −54 mV. This corresponds to a mean K^+ inward conductance of 2630 ± 817 pS per cell. Normalized to the cell capacitance a specific conductance of 97 ± 27 pS pF^{-1} can be calculated. In cell clusters, as shown in Fig. 1, for example, the K^+ inward current was increased due to electrical coupling of the cells.

The pharmacological profile of the K^+ inward rectifier in rat pancreatic acinar cells in primary culture was similar to that described for the inward rectifier in freshly prepared mouse pancreatic acinar cells (Schmid & Schulz, 1995). Ba^{2+} ions, added at a concentration of 5 mM to the extracellular KCl solution, almost completely suppressed the inward currents ($n = 4$), whereas 10 mM TEA^+ had only a partial effect (~10%, $n = 4$) (Fig. 1*A*). Neither 4-aminopyridine (4-AP, 1 mM, $n = 3$) nor the chromanol 293B (10 μM, $n = 3$) (Lohrmann *et al.* 1995) affected the K^+ inward rectifier. Furthermore, shifts in cytosolic pH in either direction, produced by acute addition of 10 mM NH_4Cl ($n = 3$) or 10 mM sodium propionate ($n = 3$), respectively, were without effect on the K^+ inward rectifier. A regulatory role of the cytosolic Ca^{2+} concentration on the K^+ inward conductance is unlikely, since addition of 1 mM EGTA to the pipette solution did not change the appearance of the inward rectifier (data not shown). Ion substitution experiments showed that Rb^+ ($n = 10$) and Cs^+ ions ($n = 10$) could also carry inward currents, although to a smaller extent than K^+ (Fig. 1*B*). The inward current in the presence of 140 mM Cs^+ or Rb^+ in the bath solution was about 20% of the K^+ inward current. In most experiments the current carried by Rb^+ ions was slightly larger than the Cs^+ inward current The permeability sequence for monovalent cations was $K^+ \gg Rb^+ > Cs^+ > Na^+$.

Voltage-dependent K^+ current

Voltage ramps applied from a clamp potential of 0 mV normally yielded an almost linear current response of the cell. This changed when voltage ramps were applied from clamp potentials more negative than −40 mV. Then, in most experiments (> 90%, $n > 20$), an additional outward

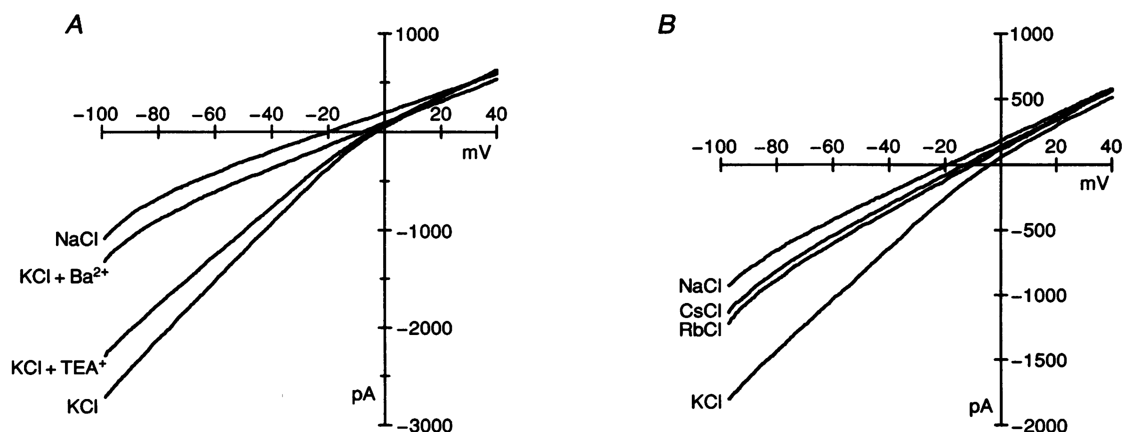

Figure 1. Inwardly rectifying K^+ currents in rat pancreatic acinar cells in primary culture

Whole-cell currents of a cell cluster consisting of 4 cells measured with KCl in the pipette. Voltage ramps from −100 to +40 mV were applied from 0 mV clamp potential. The membrane potential of the cell cluster was −16 mV. Replacement of NaCl in the bath solution with KCl caused depolarization of the cell cluster and increased inward currents at negative clamp potentials. The K^+ inward currents were largely suppressed by 5 mM Ba^{2+}. TEA^+ (10 mM) had a minor effect (*A*). Cs^+ and Rb^+ could partially substitute K^+ as charge carrier for inward currents at negative clamp potentials (*B*).

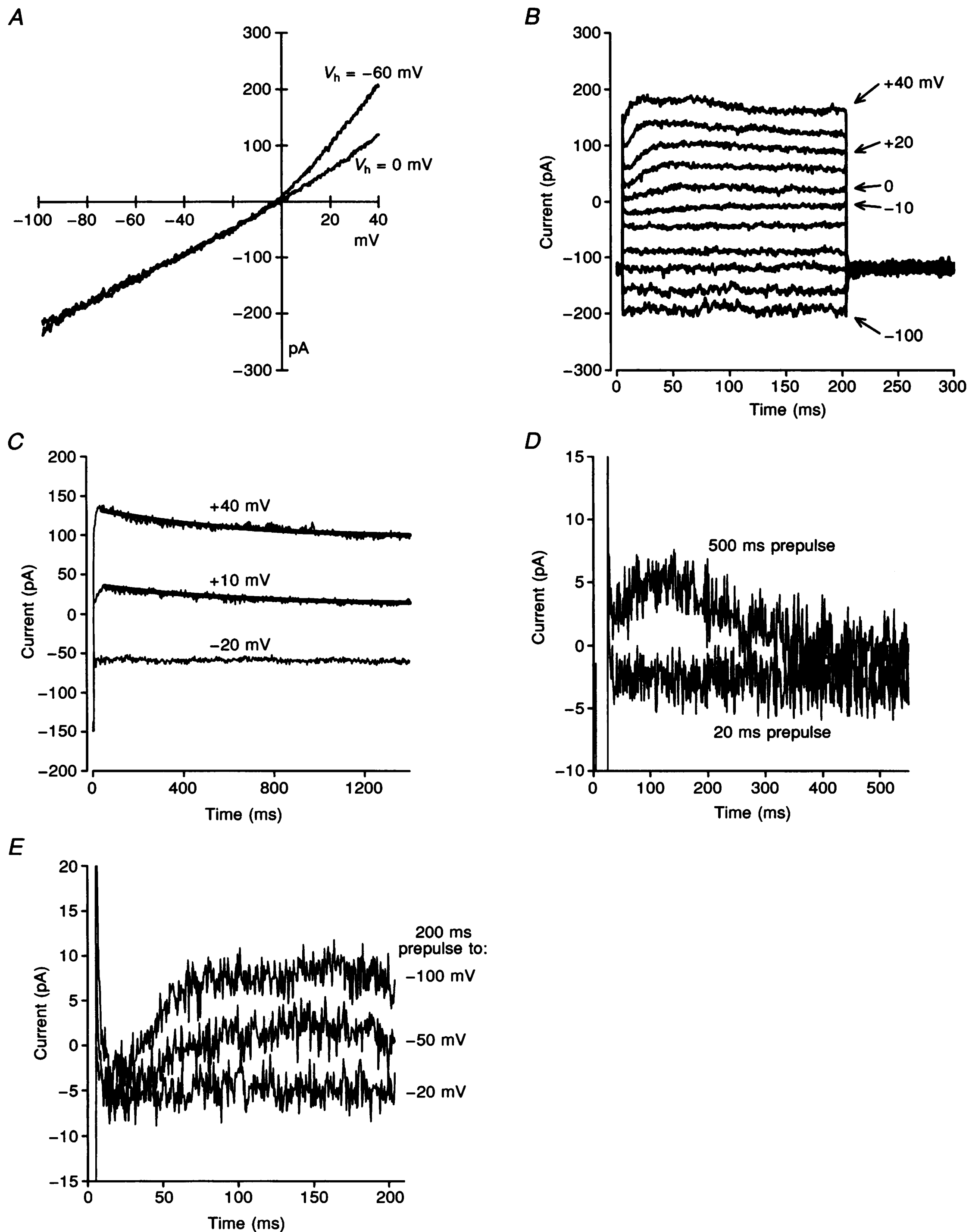

Figure 2. Voltage-dependent K^+ currents in rat pancreatic acinar cells in primary culture

A, current–voltage curves measured on a single cell with a KCl pipette solution. Voltage ramps from −100 to +40 mV lasting 200 ms were applied from a clamp potential (V_h) of either 0 or −60 mV. *B*, current response of the same cell to rectangular voltage pulses applied from a clamp potential of −60 mV. *C*, single exponential fits of the current decay after voltage jumps from −60 to +10 mV ($\tau = 594$ ms) and +40 mV ($\tau = 603$ ms). Depolarization-induced activation of voltage-dependent K^+ currents requires preceding membrane hyperpolarization. From 0 mV clamp potential voltage-dependent K^+ currents were preactivated by a 500 ms prepulse to −60 mV (*D*) or by 200 ms prepulses to −100 and −50 mV (*E*). When the clamp potential was set back to 0 mV positive outward currents indicate activation of K^+ currents. A 20 ms prepulse to −60 mV (*D*) or a 200 ms prepulse to −20 mV (*E*) was not sufficient to enable depolarization-dependent activation of the K^+ current.

current component could be observed at positive voltages (Fig. 2*A*). This current component is due to the transient activation of a voltage-dependent K^+ conductance. Figure 2*B* shows current recordings obtained from the same cell as shown in Fig. 2*A* in response to stepwise voltage changes from a clamp potential of −60 mV. Depolarization of the cell to +40 mV (uppermost trace) induced delayed activation of an outward current, which reached a maximum after about 20 ms (t_{max}) and then slowly declined again. The mean value of t_{max} after depolarization to +40 mV was 34·2 ± 8·0 ms ($n = 6$). t_{max} was increased when the magnitude of the depolarizing voltage step was reduced. When cells were depolarized to 0 mV, peak currents were reached after 72·7 ± 15·3 ms ($n = 6$). For activation of the delayed outward currents a depolarization of the cell membrane to values more positive than −20 mV was necessary. The decline of the outward current after the peak had been reached could be fitted with a single exponential decay (Fig. 2*C*). There was no significant difference in the time course of current decay after voltage jumps to +40 and 0 mV, respectively ($n = 6$). Hyperpolarization of the cell membrane was necessary to enable depolarization-dependent activation of the current. Both the duration and the magnitude of the hyperpolarization influenced preactivation of the voltage-dependent conductance. Neither very small hyperpolarizations (Fig. 2*E*) nor very short hyperpolarizing voltage pulses (Fig. 2*D*) were sufficient to promote depolarization-activated outward currents ($n = 6$).

Voltage-dependent outward currents elicited by depolarization to 0 mV could be observed with potassium glutamate as well as with KCl in the pipette solution. When the extracellular Na^+ ions were replaced by K^+ the outward currents at 0 mV disappeared (data not shown). Therefore, it can be concluded that the voltage-dependent conductance in pancreatic acinar cells is selective for K^+ over Na^+. For further characterization of the voltage-dependent K^+ conductance, studies with typical K^+ channel blockers were carried out. TEA^+ ($n = 5$), at a concentration of 10 mM, and 1 mM 4-AP ($n = 5$) completely blocked the transient K^+ outward current. This could be seen in voltage pulse experiments as well as in voltage ramp experiments as shown in Fig. 3*A* and *B*. In contrast, 5 mM Ba^{2+} ($n = 6$), which effectively blocked the K^+ inward rectifier, had no obvious effect on the voltage-dependent K^+ current (data not shown).

Analogous to the K^+ inward rectifier, the voltage-dependent K^+ conductance could also be observed when the intracellular Ca^{2+} concentration was lowered by the addition of 1 mM EGTA ($n = 6$) to the pipette solution, and, furthermore, was not dependent on the extracelluar Ca^{2+} concentration (< 10^{-9} M to 1·3 mM). Shifts in the cytosolic pH induced by extracellular addition of either NH_4Cl or sodium propionate also did not influence the voltage-dependent K^+ currents. Furthermore, forskolin ($n = 4$, 5 μM), which increases the cytosolic cAMP level, also had no effect on this current.

Permanent resting K^+ conductance

Despite the presence of inwardly rectifying and voltage-dependent K^+ conductances single pancreatic acinar cells did not display significant resting potentials (Fig. 2), regardless of whether they were freshly prepared or kept in primary culture for 1 or 2 weeks. The membrane potentials measured in the whole-cell configuration with either KCl or

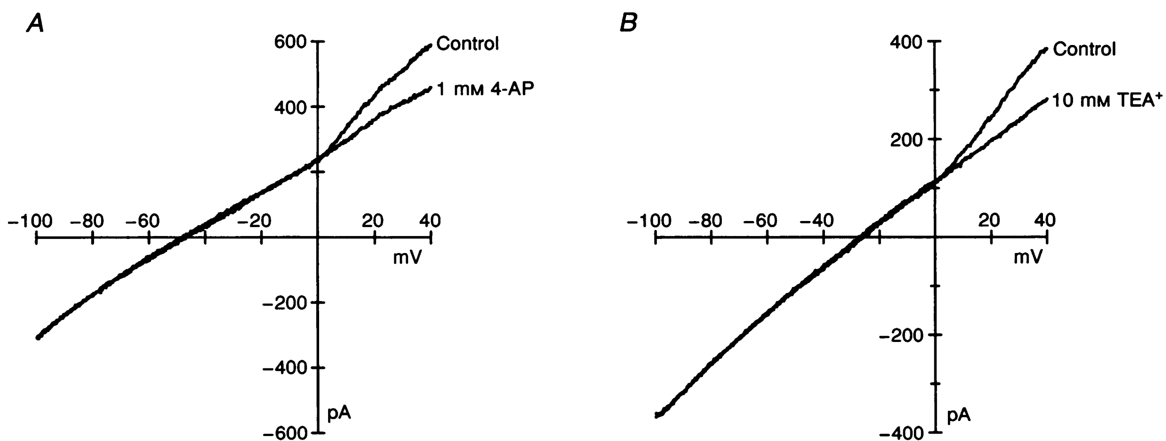

Figure 3. TEA^+ and 4-AP block the voltage-dependent but not the resting K^+ current in rat pancreatic acinar cells in primary culture

Current recordings on a cell cluster consisting of 5 cells. Voltage ramps (−100 to +40 mV), preceded by a 200 ms prepulse to −100 mV to allow activation of the voltage-dependent K^+ conductance, were applied from 0 mV clamp potential. The current recordings of the cell cluster comprise 2 components: a permanent resting K^+ conductance, which produced a negative membrane potential, and a voltage-dependent K^+ conductance, which led to increased outward currents at positive potentials. Both 10 mM TEA^+ and 1 mM 4-AP efficiently blocked the voltage-dependent K^+ current, whereas the resting K^+ conductance was unaffected.

potassium glutamate in the pipette solution were $-0\cdot5 \pm 0\cdot71$ mV ($n = 28$) and $-0\cdot1 \pm 0\cdot46$ mV ($n = 55$), respectively, indicating that in single cells under resting conditions the K^+ conductance of the plasma membrane is negligible. In contrast, as soon as cell clusters were formed, the relative fraction of the K^+ conductance in the plasma membrane of the cultured cells increased and negative membrane potentials could be measured (e.g. Figs 1 and 3). Cell clusters consisting of five or more cells displayed membrane potentials in the range of -15 to -75 mV, with a mean value of $-38\cdot7 \pm 2\cdot3$ mV ($n = 38$).

Ion substitution experiments proved that the negative membrane potentials in cell clusters mainly depended on K^+ conductance. Replacement of Na^+ with K^+ in the bath solution led to an almost complete depolarization of the cell cluster ($V_m = -3\cdot7 \pm 1\cdot2$ mV, $n = 33$; Fig. 4*A*). However, the difference between the theoretical K^+ equilibrium potential (approximately -85 mV) and the actual measured cell potentials with NaCl in the bath indicates that besides the K^+ conductance a further conductance exists, which carries inward currents at the cell resting potential and, thereby prevents development of a membrane potential on the level with the K^+ equilibrium potential. Superfusion of the cell clusters with NMDG-Cl instead of NaCl caused a hyperpolarization of the cells by $-20\cdot7 \pm 1\cdot9$ mV ($n = 6$) (Fig. 4*A*), whereas exchange of chloride with methane-sulphonate in the bath solution did not alter the membrane potential (not shown). In most experiments CsCl and RbCl (CsCl: 9 out of 14 experiments; RbCl: 5 out of 5 experiments) produced a small depolarization, probably due to inward currents through the K^+ inward rectifier (see also Fig. 1*A*). A membrane hyperpolarization by extracellular Cs^+ or Rb^+, which would argue for a highly Na^+-specific conductance, was never observed. Therefore, these data indicate that under resting conditions chloride conductances can only play a minor role, and that besides the K^+ conductance a non-selective cation conductance must be active. When the chloride permeability is neglected, from the mean membrane potential of $-38\cdot7$ mV a permeability ratio for K^+ over monovalent cations of $P_K/P_{cat} \approx 4\cdot1$ can be calculated. This value is probably underestimated due to non-specific leak currents flowing through the seal resistance.

The pharmacological profile of the permanent resting K^+ conductance in cell clusters differs from that of the K^+ inward rectifier and the voltage-dependent K^+ conductance. Ba^{2+} ($n = 19$; which, at a concentration of 5 mM, largely suppressed the inward rectifier) and TEA^+ (10 mM, $n = 19$: which inhibited the voltage-dependent K^+ current), had only little effect on the permanent K^+ conductance (Fig. 4*B*). 4-AP (1 mM, $n = 9$), the second effective inhibitor of the voltage-dependent K^+ current, and the chromanol 293B (10 μM, $n = 6$) (Lohrmann *et al.* 1995), which is known to inhibit slowly activating I_{sK} currents (Suessbrich *et al.* 1996), had no effect at all.

In a previous study on freshly prepared mouse pancreatic acinar cells it has been shown that cytosolic alkalization increased the activity of single K^+ channels measured in the whole-cell configuration (Schmid & Schulz, 1995). A more recent study on rat pancreatic acini reported K^+-dependent membrane hyperpolarization during cell acidification, which was interpreted as an increase in the fractional K^+ conductance (Slawik *et al.* 1996). We therefore tested whether changes in the cytosolic pH could influence the

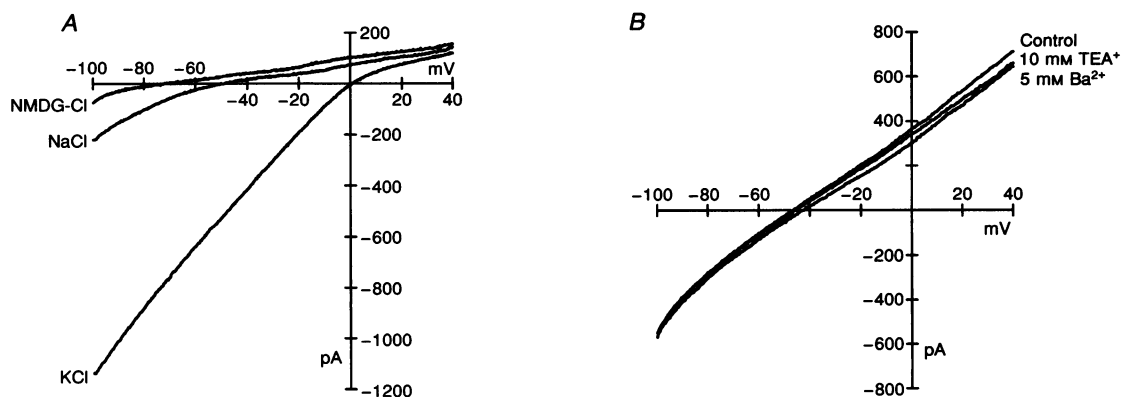

Figure 4. Ion selectivity and blocker sensitivity of the resting plasma membrane of rat pancreatic acinar cells in primary culture

A, voltage ramps from -100 to $+40$ mV were applied from 0 mV clamp potential. Replacement of extracellular Na^+ with K^+ caused depolarization of the cell cluster and increased inward currents due to the K^+ inward rectifier. The K^+-induced depolarization shows that the resting potential of the cell cluster was due to a K^+ conductance. In contrast, 140 mM extracellular $NMDG^+$ reduced inward currents and produced cell hyperpolarization, indicating that a non-selective cation conductance is also present. *B*, neither 5 mM Ba^{2+} nor 10 mM TEA^+ could effectively block the resting K^+ outward currents.

resting potential in cell clusters of rat pancreatic acinar cells in primary culture. Acute alkalization of the cytosol was accomplished by superfusion of the cell with NaCl buffer supplemented with NH_4Cl, whereas acute acidification was induced by addition of sodium propionate. Figure 5 shows that addition of 5 mM sodium propionate led to a slow increase in the outward current at a clamp potential of 0 mV ($n = 10$), whereas 5 mM NH_4Cl caused a decline of the whole-cell current ($n = 10$). Subsequent removal of the membrane-permeant base caused recovery of the outward current. When the extracellular fluid was replaced by KCl the outward current almost completely disappeared ($n = 28$). This demonstrates that the outward currents at a clamp potential of 0 mV were carried by K^+ efflux. Figure 5 also shows that 140 mM NMDG-Cl ($n = 7$), as with 5 mM sodium propionate, led to an increase in the net outward current. The time course of the current response produced by NMDG-Cl, however, was much faster than the current changes produced by addition of sodium propionate. With NMDG-Cl the rapid increase in the outward current finished within 10–15 s, which approximately corresponds to the time needed for the complete exchange of the bath solution, whereas with sodium propionate a steady state was reached after about 1–2 min.

Changes in the outward currents were accompanied by changes in the membrane reversal potential. This is demonstrated in Fig. 6*A* where single current–voltage ramps in control and in the presence of sodium propionate and NH_4Cl are presented. Cytosolic acidification with 10 mM sodium propionate led, in parallel to an increase in the total plasma membrane conductance, to a hyperpolarization of the cell cluster. The opposite effect could be observed when 10 mM NH_4Cl was added to the bath solution. Under these conditions the cells depolarized and the total membrane conductance decreased. The effect of sodium propionate and NH_4Cl were slowly reversible.

The increase and decrease in the whole-cell K^+ conductance could easily be interpreted as pH-dependent opening and closing of K^+ channels. However, a closer look at the current recordings revealed that, in parallel with changes in the conductance, changes in the electrical coupling of the cells also occurred (Fig. 6*B–D*). Cytosolic acidification ($n = 10$) improved electrical coupling of the cells within the cluster, which is indicated by slower current relaxation after a stepwise voltage change, whereas alkalization ($n = 10$) reduced electrical coupling of the cells, and caused a more rapid current relaxation. This means that the membrane area, which is accessible to electrical measurements, is increased when the cytosolic pH is shifted to more acidic values, whereas alkalization decreased the accessible membrane area. Changes in the electrical cell-to-cell coupling, therefore, directly alter the magnitude of the recorded currents, and a separate investigation of pH effects directly on the K^+ conductances in cell clusters is not possible in this way.

Another way to reduce electrical coupling between cells, without the use of membrane-permeant weak bases, is to treat the cells with the gap junction uncoupling agent octanol (0·2–1 mM). Figure 7 demonstrates that application

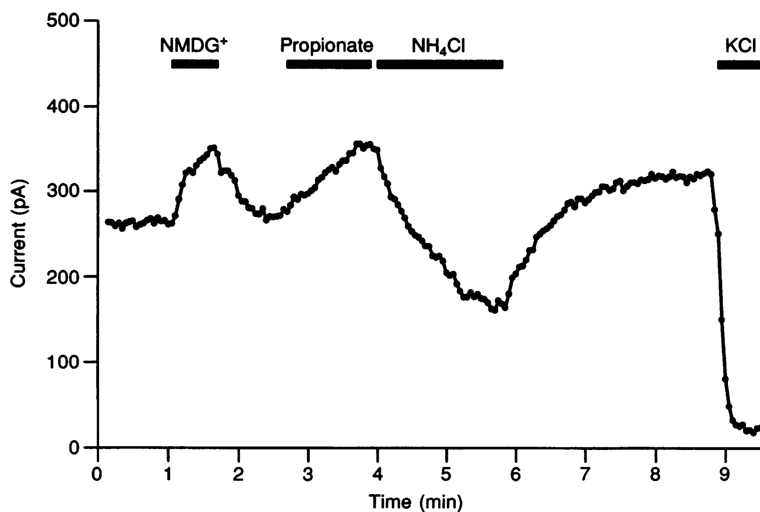

Figure 5. Effect of $NMDG^+$, NH_4Cl and propionate on K^+ outward currents in a cell cluster of rat pancreatic acinar cells in primary culture

Outward currents at 0 mV clamp potential measured on a cell cluster. Data points were taken from current–voltage ramps applied every 3 s. Replacement of NaCl with 140 mM NMDG-Cl caused a rapid increase in the outward currents. In contrast, the current increase produced by cytosolic acidification with 5 mM sodium propionate was much slower. Cytosolic alkalization by 5 mM NH_4Cl caused a decline of the whole-cell current. The outward currents at 0 mV clamp potential were mainly carried by K^+ ions since the currents almost completely disappeared when extracellular NaCl was replaced with KCl.

of octanol has a similar effect on the electrical properties of the cell cluster as NH_4Cl. Addition of octanol ($n = 6$) rapidly decreased outward currents at a clamp potential of 0 mV (Fig. 7*A*). After removal of the agent the outward current slowly recovered. The corresponding current–voltage curves (Fig. 7*B*) and the relaxation currents (Fig. 7*C* and *D*) show that the decrease in the outward current runs parallel with membrane depolarization and a complete uncoupling of the cells. A membrane depolarization by octanol could also be observed when the experiments were performed in the perforated-patch configuration ($n = 5$) to avoid dialysis of diffusible cytosolic factors. When the cytosolic pH of cells uncoupled with octanol was manipulated by additional application of either NH_4Cl or sodium propionate no significant changes in the cell conductance could be measured (data not shown). This is in accordance with whole-cell recordings on single cells (Schmid & Schulz, 1995), where manoeuvres that influenced the cytosolic pH, did not cause significant changes in the membrane potential.

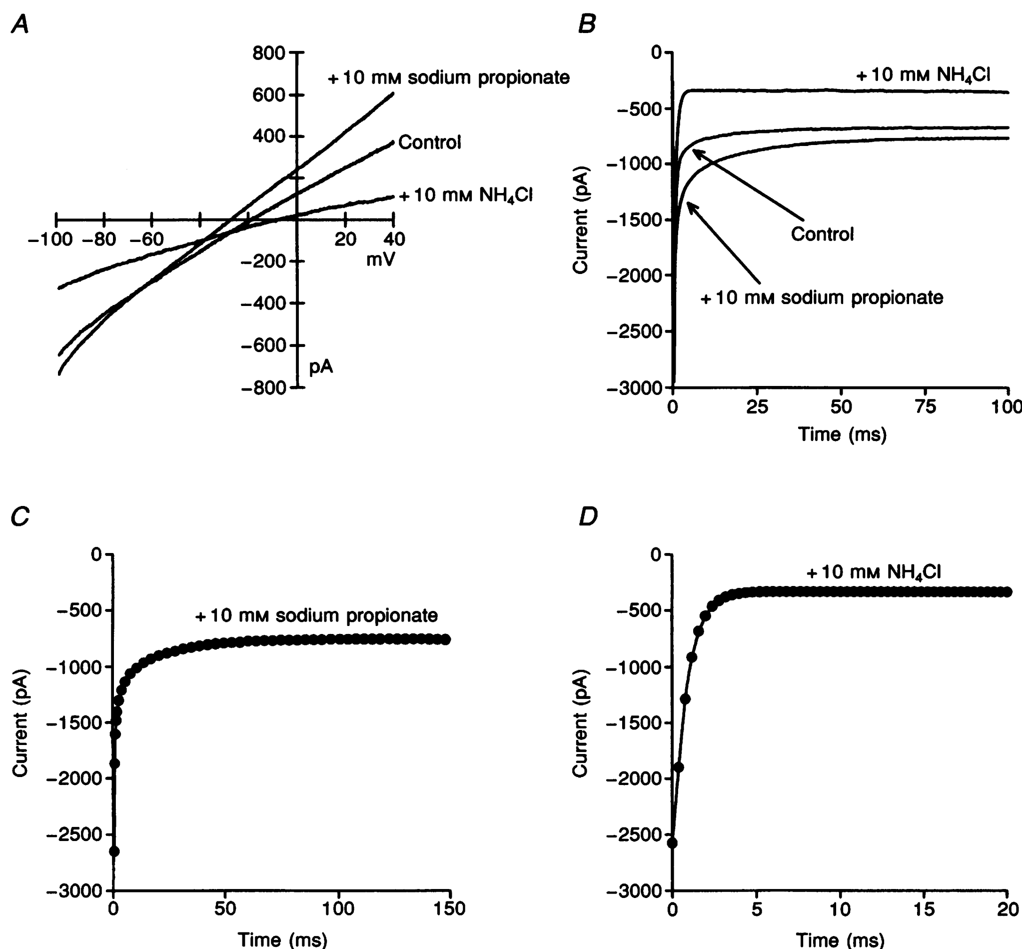

Figure 6. Effect of cytosolic acidification and alkalization on electrical cell-to-cell coupling and resting K^+ conductance in clusters of rat pancreatic acinar cells in primary culture

A, current–voltage curves of a cell cluster consisting of 5 cells under control conditions and in the presence of either 10 mM sodium propionate or 10 mM NH_4Cl. Acute addition of sodium propionate shifted the cell potential from −18·9 to −27·1 mV whereas NH_4Cl caused depolarization to −7·6 mV. *B*, whole-cell current responses to voltage jumps from −60 to −100 mV. NH_4Cl reduced the amplitude of the steady-state current at −100 mV and in addition accelerated current relaxation after voltage jumps. In contrast, sodium propionate prolonged the time constant for current relaxation and increased the steady-state current. *C*, the relaxation current in the presence of sodium propionate cannot be fitted with a single exponential function. At least 2 time constants must be employed to obtain a reasonable fit of the experimental data ($I_{01} = -2494$ pA, $\tau_1 = 0{\cdot}63$ ms, $I_{02} = -540$ pA, $\tau_2 = 16{\cdot}05$ ms, $I_{ss} = -761$ pA, where I_0 and I_{ss} are the peak and steady-state currents, respectively). The slow current relaxation indicates electrical coupling of many neighbouring cells through gap junctions. *D*, in the presence of 10 mM NH_4Cl the relaxation current could be fitted with a single time constant ($I_0 = -2578$ pA, $\tau_1 = 0{\cdot}81$ ms, $I_{ss} = -334$ pA). This means that cytosolic alkalization causes uncoupling of the pancreatic acinar cells.

DISCUSSION

In rat pancreatic acinar cells in primary culture we could characterize three distinct types of K^+ conductance: a Ba^{2+}-sensitive inwardly rectifying, a TEA^+ - and 4-AP-sensitive voltage-dependent and a Ba^{2+}-, TEA^+- and 4-AP-insensitive K^+ resting conductance.

The inwardly rectifying K^+ current in cultured pancreatic acinar cells displayed, with respect to the ion selectivity and blocker sensitivity, the same characteristics as the K^+ inward rectifier recently described on freshly prepared pancreatic acinar cells (Schmid & Schulz, 1995). In both preparations the K^+ inward conductance varied considerably from cell to cell. In cultured cells the mean K^+ inward conductance was 2630 pS, which is about four times higher than the K^+ inward conductance found in freshly prepared cells (620 pS per cell). However, when the conductance was normalized to the cell capacitance, there was only a slight difference between the cells kept in primary culture (97 ± 27 pS pF^{-1}) and the freshly prepared acinar cells (58 ± 10 pS pF^{-1}). Further similarities in the

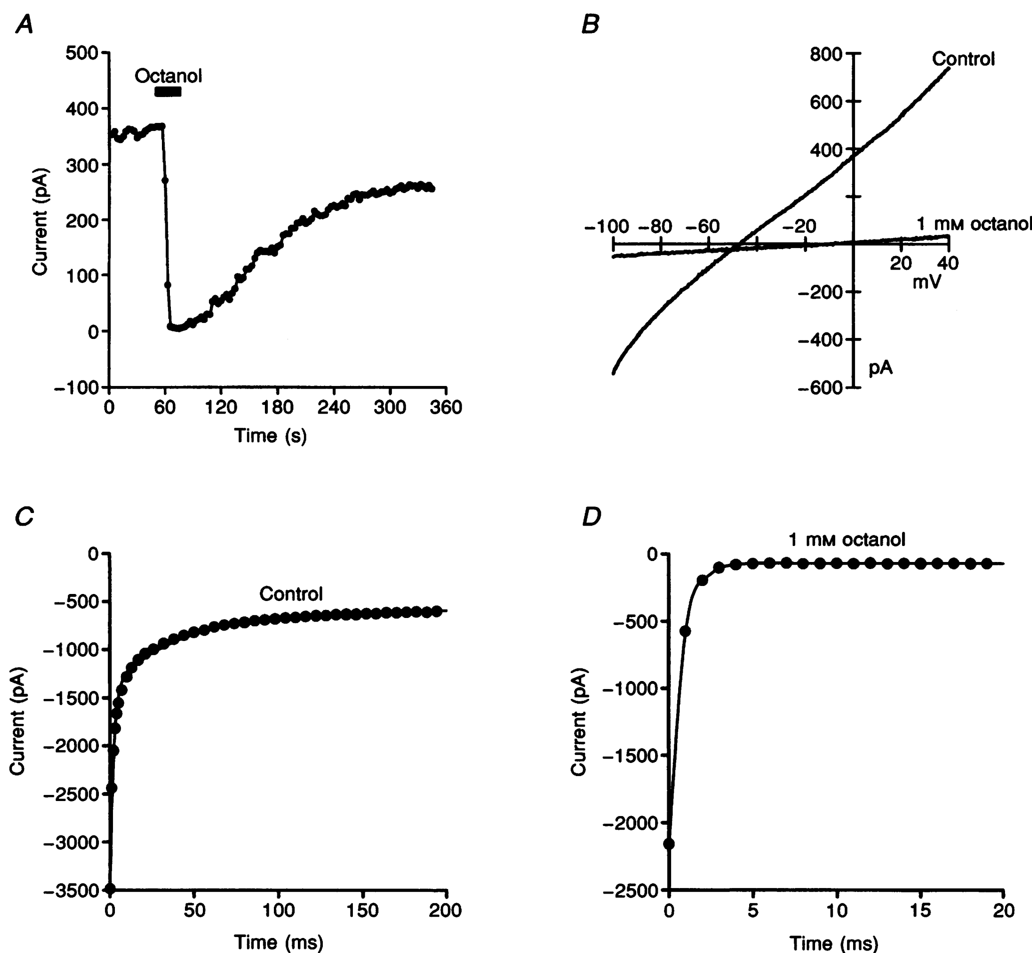

Figure 7. Effect of octanol on electrical cell-to-cell coupling and resting K^+ conductance in clusters of rat pancreatic acinar cells in primary culture

A, outward currents measured from a cell cluster with a KCl pipette solution at 0 mV clamp potential. Data points were taken from current–voltage ramps applied every 3 s. Application of 1 mM octanol (bar) reversibly decreased the K^+ outward current. *B*, current–voltage curves in absence (Control) and presence of 1 mM octanol. With octanol the membrane reversal potential is shifted from −47 to −7 mV. *C* and *D*, uncoupling of the cell cluster by octanol can be demonstrated by voltage jumps from 0 to −100 mV. The current response under control conditions can only be fitted assuming at least a double exponential decay with two time constants ($I_{01} = -2002$ pA, $\tau_1 = 2{\cdot}09$ ms, $I_{02} = -800$ pA, $\tau_2 = 43{\cdot}7$ ms, $I_{ss} = -584$ pA). The longer time constant indicates that under control conditions in the measuring circuit the membrane capacitance of neighbouring cells is connected in parallel to the directly accessed cell capacitance by gap junctional series resistances. In the presence of 1 mM octanol current relaxation follows a single exponential decay ($I_{01} = -2089$ pA, $\tau_1 = 0{\cdot}71$ ms, $I_{ss} = -69{\cdot}5$ pA). From the time constant (τ_1), the peak current ($I_0 = I_{01} + I_{ss}$) and the steady-state current (I_{ss}) a cell capacitance of about 15·8 pF can be calculated. This is approximately the membrane capacitance of a single cell.

K^+ inward conductance of the two cell preparations were found when possible physiological modulators of K^+ currents were investigated. In both preparations the inward conductance was not changed when the intracellular pH was shifted by extracellular application of NH_4Cl or sodium propionate, and also there was no difference when the intracellular solution was buffered by the addition of the Ca^{2+} chelator EGTA to the pipette solution. The strong inwardly rectifying 17 pS K^+ channel, which was characterized in cell-attached experiments on freshly prepared pancreatic acinar cells (Schmid & Schulz, 1995), has not yet been seen in cultured cells. Further experiments will be necessary to verify whether a 17 pS K^+ channel in cultured cells is responsible for the K^+ inward currents.

Voltage-sensitive transient K^+ currents have been found previously in freshly prepared pancreatic acinar cells (Thorn & Petersen, 1994) and this study demonstrates similar currents in cultured cells. In both preparations, the transient outward currents could be elicited when the cells were depolarized from negative clamp potentials. However, there are clear differences in the time course of activation and inactivation of the voltage-sensitive currents in the different cell preparations. In freshly prepared mouse pancreatic acinar cells peak currents were reached in less than 20 ms after depolarization to positive clamp potentials (Figs 1, 3 and 4 in Thorn & Petersen, 1994). In contrast, t_{max} in cultured rat acinar cells was 72·7 ms when the cell membrane was depolarized to 0 mV and 34·2 ms when voltage jumps to +40 mV were applied. Also the spontaneous current inactivation was much slower in cultured cells as compared with the freshly prepared cells from mouse. The time constant (τ_{inact}) for current relaxation in cultured cells was about 600 ms, whereas τ_{inact} in mouse pancreatic acinar cells was in the range of 29 to 37 ms. Besides the different gating behaviour there were also differences in the blocker sensitivity. The current in cultured cells from the rat was inhibited by both 4-AP and TEA^+, whereas the current in mouse acinar cells was only sensitive to 4-AP. Similarities in the voltage-sensitive currents were found when possible physiological modulators were investigated. In both preparations, the voltage-sensitive K^+ current was neither affected by cAMP nor by reduction of the intracellular Ca^{2+} concentration by buffering with EGTA or BAPTA. Since we could find voltage-sensitive K^+ currents with the same gating properties not only in cultured cells but also in the freshly prepared cells before seeding them for primary culture (data not shown), it is most likely that the differences in gating behaviour, described by Thorn & Petersen (1994) and found in our experiments, are not due to the culturing of the pancreatic acinar cells, but reflect either species-dependent differences between mouse and rat or age-dependent differences between newborn and adult animals.

In cultured pancreatic acinar cells neither the K^+ inward rectifier, nor the voltage-dependent K^+ conductance carried significant outward currents under resting condition. It is therefore unlikely that these two K^+ conductances can produce stable negative cell membrane potentials. Our experiments on single cells in primary culture indeed showed that, despite the presence of both inwardly rectifying and voltage-sensitive K^+ conductances, single cells commonly do not display significant resting potentials. This is consistent with previous observations on freshly prepared single pancreatic acinar cells from rat and mouse that also demonstrated membrane potentials near 0 mV (Randriamampita *et al.* 1988; Schmid & Schulz, 1995).

In further experiments we have shown that cultured pancreatic acinar cells can produce stable negative resting potentials as soon as electrically coupled cell clusters were formed. The membrane potential of electrically coupled cell clusters ($V_m = -39$ mV) was similar to the membrane potential previously described for pancreatic tissue segments ($V_m = -37$ mV; Matthews & Petersen, 1973; Nishiyama & Petersen, 1974) and isolated pancreatic acini ($V_m = -49$ mV; Slawik *et al.* 1996). The resting potential in cultured cell clusters was due to activity of a Ba^{2+}-insensitive permanent K^+ conductance. This is consistent with observations of Slawik *et al.* (1996), who noted that 5 mM Ba^{2+} was without effect on the resting K^+ conductance in rat pancreatic acini.

The membrane potentials of cell clusters in primary culture varied considerably from experiment to experiment. However, in all recordings the membrane potential was below the theoretical equilibrium potential for K^+. This indicates that in the resting cell in addition to the K^+ conductance there is either a chloride or a non-selective cation conductance active, which forces the membrane potential to less negative values. Since anion replacement did not influence the membrane potential of the cell clusters, a significant role of a chloride conductance on the resting potential can be excluded. On the other hand, replacement of extracellular Na^+ with $NMDG^+$ caused rapid hyperpolarization of the cell, which was accompanied by a reduction in the total membrane conductance. The $NMDG^+$-induced cell hyperpolarization was reversible and, in contrast to the effects of sodium propionate, had a much faster time course. Furthermore, intracellular acidification with sodium propionate led to an increase in the total conductance, whereas $NMDG^+$ caused a decrease in the membrane conductance. It is therefore unlikely that the effect of $NMDG^+$ on cultured acinar cells is based on a reduction of a pH-dependent Cl^- conductance by intracellular acidification, as suggested by experiments on freshly prepared pancreatic acini (Slawik *et al.* 1996). From our data we have to conclude that, in resting pancreatic acinar cells kept in cell culture, a non-selective cation conductance is active. In cell-excised experiments we were able to characterize a 27 pS channel (data not shown), which shared all fundamental properties with the non-selective cation channel seen in freshly prepared pancreatic acinar cells (Maruyama & Petersen, 1982). The channel did not discriminate between K^+ and Na^+, but was impermeable to

$NMDG^+$ and anions. The channel activity could be suppressed when the cytosolic surface of the membrane was exposed to a Ca^{2+}-free bath solution. It is most likely that this channel, which in freshly prepared pancreatic acinar cells produces hormone-induced cell depolarization (for review see Petersen, 1992), is also responsible for the relative low membrane potentials that we have found in the cultured acinar cells.

In a previous study we have shown that cytosolic alkalization by NH_4Cl increased the activity of a Ba^{2+}- and TEA^+-insensitive K^+ channel in the plasma membrane of freshly prepared mouse pancreatic acinar cells (Schmid & Schulz, 1995). Therefore, it was a surprise to see that in cell clusters cytosolic alkalization produced cell depolarization and a decrease in the membrane conductance. However, further experiments then clearly proved that the pH-dependent changes in the total conductance of cell clusters were due to changes in the electrical coupling between the cells. Cytosolic alkalization caused electrical uncoupling, whereas acidification led to an improved cell-to-cell coupling. This is in contrast to observations on other cell types, where gap junctional coupling was reduced by intracellular acidification (Spray, White, de-Carvalho, Harris & Bennett, 1984; Rorig, Klausa & Sutor, 1996). However, similar results were obtained on isolated rat pancreatic acini, which displayed cytosolic acidification and, in parallel, cell uncoupling after application of 15 mM NO_2^- (Loessberg-Stauffer, Zhao, Luby-Phelps, Moss, Star & Muallem, 1993; Zhao, Xu, Ujiie, Star & Muallem, 1994).

Cytosolic alkalization not only reduced the measured total membrane conductance but also caused depolarization of the cells. The decrease in the membrane conductance at least partially reflects the electrical uncoupling of the cells. Furthermore, the membrane depolarization indicates that, in the uncoupled cell, which is directly accessed by the patch pipette, the fraction of the K^+ conductance on the total cell conductance must be reduced compared with the relative fraction of the K^+ conductance in electrically coupled cell clusters. This could be due to a pH-dependent reduction in the K^+ conductance. However, the same depolarizing effect could be observed when the cell clusters were uncoupled with octanol, which does not produce cell alkalization (Meda, Bruzzone, Knodel & Orci, 1986; Pappas, Rioult & Ransom, 1996). Furthermore, manoeuvres that shifted cytosolic pH had no effect on the membrane potential in the presence of n-octanol. This shows that membrane depolarization is caused by cell uncoupling itself and not by a direct effect of the cytosolic pH on the K^+ conductance.

There are several ways to explain membrane potential breakdown during cell uncoupling. Gap junctional uncoupling not only reduces the electrically accessible surface area to the membrane area of a single cell, but also increases the relative influence of non-specific currents, which are flowing through the seal resistance. It is evident that these leak currents could cause a partial membrane depolarization when the neighbouring cells are electrically disconnected from the cell that is directly accessed by the patch pipette. However, a complete breakdown of the membrane potential, as observed in the experiments with octanol, suggests that at least the directly accessed cell has lost not only the gap junctional conductance but also any significant resting K^+ conductance. One explanation for this phenomenon could be that a diffusible factor, which is necessary for maintenance of a normal K^+ conductance activity, is washed out of the cytosol from the cell under investigation by dialysis with pipette solution after the fast whole-cell configuration has been established. However, membrane depolarization after cell uncoupling with octanol could also be observed in experiments using the perforated-patch configuration, which avoids the diffusion of cytosolic factors out of the cell. Furthermore, in fast whole-cell experiments on single cells significant cell potentials could not be measured, even at the moment when the direct access to the cytosol was established. Together, these observations argue against the hypothesis that washing out of a putative diffusible cytosolic factor alone can explain breakdown of the recorded membrane potential during cell uncoupling. Our experimental data suggest rather that there is a direct regulatory cross talk between gap junctional and resting K^+ conductance. There is so far no information on whether such a cross talk involves regulatory proteins shared by both conductances or a direct interaction between gap junctions and K^+ conductance. Immunohistochemical studies investigating co-localization of gap junctions and K^+ channels are also lacking.

From our data we conclude that electrical coupling of pancreatic acinar cells in cell clusters is essential for maintenance of a stable negative resting potential in the individual cell. Furthermore, during hormonal stimulation of the pancreatic acinus, current flow through gap junctional channels between cells can stabilize the outward driving force for oscillatory Cl^- extrusion (Kasai & Augustine, 1990; Kasai, Li & Miyashita, 1993; Thorn, Alison, Smith, Gallacher & Petersen, 1993) from an individual cell within the cell cluster.

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Acknowledgements

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB-246/A9). We thank Bärbel Kohler for excellent technical assistance and Dr Fatima Pfeiffer and Dr Elmar Krause for helpful suggestions and critical discussions.

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Received 14 March 1997; accepted 27 June 1997.