Molecular Identification and Physiological Roles of Parotid Acinar Cell Maxi-K Channels*

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The physiological success of fluid-secreting tissues relies on a regulated interplay between Ca2+-activated Cl− and K+ channels. Parotid acinar cells express two types of Ca2+-activated K+ channels: intermediate conductance IK1 channels and maxi-K channels. The IK1 channel is encoded by the Kcna3.1 gene, and the Kcna1.1 gene is a likely candidate for the maxi-K channel. To confirm the genetic identity of the maxi-K channel and to probe its specific roles, we studied parotid glands in mice with the Kcna1.1 gene ablated. Parotid acinar cells from these animals lacked maxi-K channels, confirming their genetic identity. The stimulated parotid gland fluid secretion rate was normal, but the sodium and potassium content of the secreted fluid was altered. In addition, we found that the regulatory volume decrease in acinar cells was substantially impaired in Kcna1.1-null animals. We examined fluid secretion from animals with both K+ channel genes deleted. The secretion rate was severely reduced, and the ion content of the secreted fluid was significantly changed. We measured the membrane potentials of acinar cells from wild-type mice and from animals with either or both K+ channel genes ablated. They revealed that the observed functional effects on fluid secretion reflected alterations in cell membrane voltage. Our findings show that the maxi-K channels are critical for the regulatory volume decrease in these cells and that they play an important role in the sodium uptake and potassium secretion process in the ducts of these fluid-secreting salivary glands.

In salivary glands, as in other secretory epithelia, muscarinic stimulation increases intracellular Ca2+, which activates the anion channels that drive fluid secretion. The activated anion channels allow an efflux of Cl− ions from the acinar cells into the lumen of the gland. This Cl− efflux generates a lumen negative voltage gradient sufficient to drive sodium ions into the lumen via a paracellular pathway. The net result is the secretion of sodium and chloride, with water following osmotically.

For the fluid secretion process to be sustained, an apical cell driving force for Cl− efflux must be maintained. This is accomplished by the hyperpolarizing influence of acinar cell K+ channels activated by intracellular Ca2+ (1). Two types of Ca2+-activated K+ channels are expressed in parotid acinar cells: a voltage-independent channel of “intermediate” single channel conductance (IK1) and a large conductance K+ channel (maxi-K) that is gated by voltage as well as by Ca2+ (2–6). In addition to their biophysical properties, these two types of channels can be distinguished by their pharmacological footprints. For example, IK1 channels are specifically inhibited by clotrimazole (7, 8) and maxi-K channels by paxilline (9).

A PCR screen of mouse parotid acinar cells revealed two candidate genes for encoding these two K+ channels: Kcna1.1 (also known as Kcma1.1) and Kcna3.1 (Kcna4) (10). Heterologous expression of these two genes produces channels with the biophysical and pharmacological profiles of the native maxi-K and IK1 channels (10). Although these results provide strong circumstantial evidence for the genetic identification of the parotid K+ channels, gene ablation studies would be definitive and help to uncover the physiological roles of these channels. Ablation of the Kcna3.1 gene eliminates expression of IK1 channels, confirming the molecular identity of the IK1 channel (11). However, parotid fluid secretion from Kcna3.1(−/−) mice is normal, perhaps because the remaining maxi-K channels are sufficient to maintain the necessary Cl− driving force and, hence, fluid secretion.

In addition to their ability to regulate fluid secretion, parotid acinar cells, like many other cells, also dynamically regulate their cell volume: a decrease in extracellular osmolality causes the cells to swell, as expected, but also activates K+ and Cl− channels. The resulting efflux of these ions reduces cell volume to near the initial resting value. The ion channels responsible for this regulatory volume decrease (RVD)3 generally include both K+ and Cl− channels, but the specific types vary among different cells (reviewed in Ref. 12). The molecular identity of the K+ channels involved in RVD is known for some cell types. For example, IK1 channels (encoded by the Kcma3.1 gene) are required for erythrocyte and T-lymphocyte RVD (11), and maxi-K channels participate in RVD in lacrimal cells (13), osteoblast-like cells (14), and a bronchial epithelial cell line (15). The channels relevant for RVD in parotid acinar cells are not known. Ablation of the Kcma3.1 gene has no effect on RVD in parotid acinar cells; however, inhibition of maxi-K channels by the specific antagonist paxilline substantially inhibits RVD.

3 The abbreviations used are: RVD, regulatory volume decrease; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; CCh, carbachol; pF, picofarads.
suggesting that this channel is required for RVD in these cells (11).

As noted above, gene ablation studies confirmed that \( K_{Ca}3.1 \) encodes the IK1 channel and showed that \( K_{Ca}3.1 \) null mice have normal parotid gland fluid secretion and normal RVD in their parotid acinar cells. To confirm the molecular identity of the maxi-K channel and to probe its physiological roles, we studied mice in which the \( K_{Ca}1.1 \) gene was ablated (16). Parotid acinar cells from these animals lacked the time- and voltage-dependent, \( Ca^{2+} \)-activated maxi-K channel currents, confirming that these channels are indeed encoded by the \( K_{Ca}1.1 \) gene. Stimulated fluid secretion from the parotid glands of these animals was normal, but there were significant changes in the sodium and potassium content of the secreted fluid. RVD in \( K_{Ca}1.1 \)-null mice was substantially impaired. Fluid secretion from animals with both \( K^{+} \) channel genes deleted was severely reduced and accompanied by quite large alterations in the ion content of the secreted fluid. The functional effects on fluid secretion from the various gene-ablated animals correlated well with alterations in parotid acinar cell membrane potential levels. These results highlight the respective physiological roles of the \( Ca^{2+} \)-activated \( K^{+} \) channels in fluid-secreting salivary glands.

**EXPERIMENTAL PROCEDURES**

**Mouse Strains**—Several strains of mice were used, including three with one or more \( Ca^{2+} \)-activated \( K^{+} \) channel genes deleted. The \( K_{Ca}1.1 \)(+/−) mice (16) were a generous gift from R. W. Aldrich. Our generation of \( K_{Ca}3.1 \)(+/−) mice has been described previously (11) and has been maintained on a C57BL/6J background. Control animals used in experiments on \( K_{Ca}3.1 \)-null mice were wild-type littermates on this mixed background. Control animals used in experiments on \( K_{Ca}1.1 \)-null mice was normal, but there were significant changes in the sodium and potassium content of the secreted fluid. RVD in \( K_{Ca}1.1 \)-null mice was substantially impaired. Fluid secretion from animals with both \( K^{+} \) channel genes deleted was severely reduced and accompanied by quite large alterations in the ion content of the secreted fluid. The functional effects on fluid secretion from the various gene-ablated animals correlated well with alterations in parotid acinar cell membrane potential levels. These results highlight the respective physiological roles of the \( Ca^{2+} \)-activated \( K^{+} \) channels in fluid-secreting salivary glands.

**Acinar Cell Preparation**—Acinar cells were obtained by enzyme digestion as we described previously (10) with some small modifications. In brief, mice were anesthetized by exposure to \( CO_{2} \) gas and killed by exsanguination via cardiac puncture. The parotid glands were dispersed in digestion buffer (Eagle’s minimal essential medium (Biofluid Inc.), 0.17 mg/ml Liberase RI enzyme (Roche Applied Science), 1% bovine serum albumin) at 37 °C. The recovered mRNA (total RNA was extracted from 3-month-old wild-type, \( K_{Ca}1.1 \)-null, \( K_{Ca}3.1 \)-null, and double-null mice, and the tissue was immediately frozen in liquid \( N_{2} \). Total RNA was extracted from 3-bp 20 mg of tissue using the RNeasy kit (Qiagen Inc.) according to the manufacturer’s protocol. 100 \( \mu \)g of total RNA was further purified to mRNA by chromatography on oligo(dT) resin using an oligotex mRNA mini kit (Qiagen Inc.). The recovered mRNA (~1 \( \mu \)g) was fractionated by electrophoresis on 1% agarose gel containing 2 M formaldehyde and blotted on BrightStar-Plus positively charged nylon membrane (Ambion, Inc., Austin, TX) using 10X SSC buffer. Blots were UV-cross-linked, prehybridized with Church buffer for 60 min at 65 °C, and then hybridized overnight at 65 °C with the same buffer using gene-specific probes. Probes were amplified by PCR using gene-specific primers and \( K_{Ca}1.1 \) clones (GenBank accession number CB055960): 5′-ATGCAGTTTGATGACAGCATCG-3′ (forward) and 5′-CAGATCACCATAAACCACCA-3′ (reverse). The housekeeping ribosomal protein L32 (accession number NM_172086) was

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used to normalize gel loading. An L32 probe was generated by PCR using parotid cDNA and the following L32-specific primers: 5’-TTTCATGCGCAATGACGGC-3’ (forward) and 5’-ACACAAGCCATCTACTATCCGTTTG-3’ (reverse). Probes were labeled using the Random Primers DNA labeling system (Invitrogen) and purified with Centri-Sep columns (Princeton Separations, Inc., Adelphia, NJ). Marker sizes are based on 18 S and 28 S mRNA bands (1.9 and 4.7 kb, respectively). The $K_{\text{Ca}}$1.1 blot was exposed to Eastman Kodak imaging film for 3 days. The membrane was then stripped with boiling 0.5% SDS and reprobed with L32, and the 6-h exposure was overlaid with the $K_{\text{Ca}}$1.1 blot.

Electrophysiology—Whole cell voltage clamp recordings were done at room temperature (20–22 °C) with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). Data acquisition was performed using a 12-bit analog/digital converter controlled by a personal computer. K⁺ channel currents were recorded with solutions in which almost all of the Cl⁻ was replaced with the rather impermeant glutamate ion. The external solution consisted of 135 mM sodium glutamate, 5 mM potassium glutamate, 2 mM CaCl₂, 2 mM MgCl₂, and 10 mM HEPES (pH 7.2). The internal solution contained 135 mM potassium glutamate, 10 mM HEPES (pH 7.2), 5 mM EGTA, and 3 mM CaCl₂, which established a free Ca²⁺ concentration of 250 nM (Ref. 19; see also www.stanford.edu/~cpton/maxx.html). The measured relevant junction potential in these recordings was <4 mV, sufficiently small that no correction to membrane voltage was made.

Parotid acinar cell membrane potentials were measured at room temperature (20–22 °C) with the current clamp mode of an Axopatch 200B amplifier using a Digidata 1320A digitizer running Clampex 9.2 (Axon Instruments); Clampfit 9.2 was used for analysis. These experiments utilized the perforated patch technique (20) with an external solution containing 150 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES (pH 7.2). The patch electrodes were front-filled with a solution consisting of 95 mM potassium methanesulfonate, 45 mM KCl, 15 mM NaCl, 1 mM MgCl₂, 5 mM BAPTA, and 10 mM HEPES (pH 7.2) and then back-filled with the same solution containing nystatin (250 µg/ml; Sigma) and the fluorescent dye Lucifer Yellow (2 mM; Sigma). Lucifer Yellow was used as an indicator of the integrity of the cell membrane under the patch. Cell fluorescence was monitored by epifluorescence microscopy with an enhanced green fluorescent protein filter cube. Only data from those cells in which the yellow dye was excluded from the cell interior were used. For these measurements, the bath was initially filled with the pipette solution, and with the pipette in this solution, the voltage was zeroed. After achieving a high resistance seal, the bath was perfused with the external solution. With this method, there will be an insignificant uncorrected junction potential.

Cell input resistance measurements were obtained in current clamp mode. We applied 500-ms long hyperpolarizing current steps of 10 pA, measured the resulting change in membrane voltage, and computed the membrane resistance via Ohm’s law. Several such measurements (usually five) were obtained over a 25-s period and averaged.

The membrane potential measurements were done with Corning 8161 glass electrodes; the voltage clamp recordings were made with quartz and Corning 8161 glass. The electrodes were not fire-polished and had resistance values between 2 and 6 megaohms when filled with the recording solutions. No leak corrections were made. Pooled current data were normalized to cell size determined as cell capacitance.

Most of the cells stimulated with carbachol (CCh) responded with oscillating membrane potentials (see below). Membrane potential data were recorded at 2.5 kHz, and the slowly oscillating signals (near 0.3 Hz) were resampled at 40-ms intervals. The sampled oscillating membrane voltage was averaged (omitting the initial “spike”; see below and Fig. 4) over the period of the CCh application (20–60 s). The membrane potentials of resting cells and the few that did not oscillate in CCh were averaged over a 20-s interval.

All data are presented as the mean ± S.E. Differences between means were determined by Student’s t test, with p < 0.05 considered significant. Along with reporting mean and S.E. values, we have included both the number of measurements and the number of mice. These are reported below or in the figure legends as appropriate.

RESULTS

$Ca^{2+}$-activated K⁺ Channels in Parotid Acinar Cells—As described in the Introduction, parotid acinar cells express two types of $Ca^{2+}$-activated K⁺ channels: the time- and voltage-independent IK1 channel and a maxi-K channel that is gated by voltage as well as by Ca²⁺. We have shown previously that heterologously expressed $K_{\text{Ca3.1}}$ channels have biophysical and pharmacological properties that are indistinguishable from those of native parotid IK1 channels and that ablation of the $K_{\text{Ca3.1}}$ gene eliminates the IK1 channel from the parotid cells, leaving only maxi-K channels (10, 11). These findings clearly identify the IK1 channel with the $K_{\text{Ca3.1}}$ gene.

The maxi-K channel is likely encoded by the $K_{\text{Ca1.1}}$ gene because RNA from this gene is expressed in parotid acinar cells, and heterologously expressed $K_{\text{Ca1.1}}$ channels have the same pharmacological and biophysical footprints as native maxi-K channels (10). To confirm the genetic identity of the maxi-K channel, we compared the parotid acinar cell K⁺ currents in wild-type mice and in mice in which the $K_{\text{Ca1.1}}$ gene was ablated. The Northern blot in Fig. 1A demonstrates the presence and absence of transcripts from the $K_{\text{Ca1.1}}$ gene in wild-type (WT) and $K_{\text{Ca1.1}}$-null animals, respectively.

If, as suspected, the time- and voltage-dependent maxi-K current is from the $K_{\text{Ca1.1}}$ gene, then parotid acinar cells from $K_{\text{Ca1.1}}$-null mice will have only the time- and voltage-independent IK1 channels. Recordings of K⁺ currents from a parotid acinar cell from a wild-type mouse are illustrated in Fig. 1B. As has been shown previously (11), immediately after achieving the whole cell configuration of the patch clamp, only time- and voltage-dependent maxi-K channels were observed. Within minutes (generally 2–5 min), the time-independent IK1 channels could be observed. Fig. 1B illustrates this behavior.

The insets in Fig. 1B show currents in response to a series of 40-ms voltage clamp steps recorded from a parotid acinar cell patched with a pipette solution containing Ca²⁺ buffered to a
value of 250 nM. Immediately after achieving the whole cell configuration (Early), only time- and voltage-dependent maxi-K channels were apparent. Shortly thereafter (Later), IK1 currents developed, contributing the time-independent component seen in the raw currents. The main part of Fig. 1B shows the currents measured at the end of the 40-ms test pulses. Immediately after patch breakthrough (○), the currents were entirely from maxi-K channels and exhibited the characteristic strong outward rectification. After stable development of IK1 channel currents (●), the current-voltage relation exhibited a relatively linear phase at negative potentials because of IK1 channels and a nonlinear outward-rectifying maxi-K contribution at more depolarized voltages.

There were no currents recorded from KCa1.1-null mice immediately after patch breakthrough and only time- and voltage-independent currents later (Fig. 1C, insets). At the end of the test pulses, there were very small currents recorded early on (○) and only a linear current-voltage relation later (●). Thus, as predicted, the voltage-gated time-dependent maxi-K currents of parotid acinar cells in wild-type mice are encoded by the KCa1.1 gene.

Fig. 1D illustrates the consequences of deleting both the KCa1.1 and KCa3.1 genes: almost the complete absence of currents. The second set of recordings (Later) was made after 12 min. In other experiments, no significant currents developed even as long as 23 min after achieving whole cell mode. As expected, KCa1.1 gene transcripts were absent in the double-null mice (Fig. 1A, (−/−)/(−/−)).

Maxi-K current density was quantitatively estimated as the time-dependent current over a 40-ms voltage pulse to +50 mV and was 135 ± 14 pA/picomfarads (pF) (14 cells from seven animals) in cells from wild-type mice. An identical analysis of KCa1.1-null and double-null mice revealed an extremely small residual current: 2.9 ± 0.6 pA/pF (11 cells from five mice) and 2.7 ± 0.25 pA/pF (nine cells from four mice), respectively. We also estimated the level of IK1 channel activity, in this case, as the amount of current at −110 mV, a voltage much too negative to allow maxi-K channel activation. Wild-type mice exhibited −48 ± 9.9 pA/pF (13 cells from six mice) of IK1 current at this potential, and the level in KCa1.1-null animals was not significantly different (p = 0.34) at −64 ± 14 pA/pF (eight cells from five mice). The current at −110 mV in the double-null mice was quite small at −1.0 ± 0.22 pA/pF (seven cells from four mice).

Parotid Fluid Secretion in KCa1.1-null Mice—As noted above, we have shown previously that deletion of IK1 channels has little or no effect on in vivo fluid secretion in parotid acinar cells (11). Thus, if IK1 channels are not important for fluid secretion, perhaps maxi-K channels are. However, the data in Fig. 2A show that fluid secretion elicited with the mixed agonist pilocarpine was not affected by ablation of the KCa1.1 gene.

The normal fluid secretion observed in both KCa1.1-null and KCa3.1-null animals suggests that neither K+ channel is necessary for fluid secretion or that either one is sufficient to support normal secretion. To distinguish between these two possibilities, we crossed the KCa1.1-null and KCa3.1-null mice to ablate both K+ channel genes and tested for parotid gland fluid secretion in the double-null animals. First, however, it is important to note that this cross-breeding establishes a new wild-type strain and so may result in different fluid secretion rates. Apparently, this was the case, as this new wild-type strain exhibited a reduced fluid secretion rate (Fig. 2B, ○) compared with WT data).

It is not unusual for mice with different genetic backgrounds to exhibit differences in physiological function (e.g. Refs. 21–25), and the reduced secretion rate in these back-crossed animals serves to emphasize that these double-null animals must be compared with wild-type animals of the appropriate background. Shown in Fig. 2B (○) are the fluid secretion meas-
measurements from the double-null animals. It is clear from these results that fluid secretion is significantly reduced when both the KCa1.1 and KCa3.1 genes are ablated. Thus, some kind of K+ channel is critical for fluid secretion; one or the other K+ channel must be present to support robust fluid secretion.

We have shown previously that ablating the K+ channel KCa3.1 gene has a negligible effect on the ion content of the secreted fluid (11). In contrast, the data in Fig. 3 demonstrate significant changes in the ion content of saliva from the KCa1.1-null mice. Shown are the measured sodium, potassium, and chloride levels of the saliva and the associated osmolality values. These statistical box plots illustrate the mean values (■), the S.E. values (boxes), the medians (horizontal lines), and the S.D. values (error bars). Data from wild-type mice are represented by unfilled boxes, and the hatched boxes in Fig. 3A represent data from KCa1.1-null animals. The sodium and chloride content and osmolality of saliva were increased in the KCa1.1-null mice, and the potassium content was decreased. The differences were not dramatically large, but all were significant at the \( p = 0.05 \) level. As shown in Fig. 3B, the ion content of the saliva secreted from the wild-type strain appropriate for the double-null animals was different from that secreted from the KCa1.1-null wild-type strain (see "Discussion"). Ablating both K+ channel genes produced the same qualitative effects as knocking out only the KCa1.1 channel, but the magnitude of the changes was somewhat larger and, of course, was also statistically significant. The secreted values are listed in Table 1.

**Parotid Acinar Cell Membrane Potentials in Wild-type and K+ Channel-null Mice**—In the generally accepted fluid secretion model, the role of K+ channels in acinar cells is to provide an electrical driving force for Cl− efflux even in the face of an elevated Cl− conductance, which would tend to depolarize the membrane potential toward the Cl− Nernst potential \( E_{Cl} \). Thus, it is instructive to compare the parotid acinar cell membrane potentials in wild-type animals and those in which either or both K+ channel genes have been ablated. We measured cell membrane potentials with the noninvasive perforated patch technique (see “Experimental Procedures”) both in resting cells and in cells stimulated with low concentrations of the muscarinic receptor agonist CCh. Examples of such measurements are illustrated in Fig. 4.

Fig. 4A shows that the resting potential of parotid acinar cells from wild-type mice was near −60 mV in the absence of mus-
carinic stimulation with CCh. The mean value from the 45 cells examined was $-59 \pm 1.9 \text{ mV}$. Addition of 0.3 $\mu$M CCh induced a spike of depolarization to near $-25 \text{ mV}$, followed by rather regular oscillations between about $-70$ and $-50 \text{ mV}$. Muscarinic stimulation of salivary gland cells often induces oscillations of cytoplasmic Ca$^{2+}$ (26–29), resulting in the oscillatory activation of Ca$^{2+}$-activated Cl$^{-}$ and K$^{+}$ conductances (27, 29–31). The oscillations in membrane voltage observed in Fig. 4A were no doubt caused by the oscillations in the activities of these Cl$^{-}$ and K$^{+}$ channels. CCh-induced oscillations were observed in 74% of the cells from wild-type mice that were studied and in $>80\%$ of the cells from gene-ablated animals.

The oscillatory nature of the membrane potential during muscarinic stimulation complicates attempts at a quantitative analysis of possible differences among mice with K$^{+}$ channel genes ablated. Any one of several parameters could be measured, including the mean minimum potential during the oscillations or the mean maximum potential. Because we are attempting to understand the physiological implications of K$^{+}$ channel gene ablation, it is useful to choose a physiologically relevant membrane voltage parameter for analysis. The fluid secretion process involves a complex interplay between muscarinic receptor-activated intracellular Ca$^{2+}$ signals, Ca$^{2+}$-activated K$^{+}$ and Cl$^{-}$ channels, and ion fluxes driven by electrochemical gradients through these channels. Perhaps the simplest membrane potential parameter that has physiological implications is the time-averaged value.

Intermediates between the extreme levels associated with oscillations. Thus, the amount of K$^{+}$ and Cl$^{-}$ conductances activated by these intermediary Ca$^{2+}$ levels might be expected to be generally intermediate between the extreme levels associated with oscillations. Thus, the amount of K$^{+}$ and Cl$^{-}$ conductances activated by these intermediary Ca$^{2+}$ levels might be expected to be similar to the value averaged over time during oscillations. In any case, as noted above, there was no statistical difference between oscillating and non-oscillating cells, and because the latter constitutes a minor component, we included all cells in our analyses. Thus, the mean membrane potential during CCh stimulation observed in all wild-type mice was $-65 \pm 1.2 \text{ mV}$ ($n = 39$).

The resting membrane potential of parotid acinar cells from $K_{Ca1.1}$-null mice was slightly (but not statistically significantly) depolarized compared with data from wild-type animals: $-53 \pm 2.5 \text{ mV}$ ($n = 28$). As illustrated in Fig. 4B, CCh also induced oscillations in the membrane potential of cells from these mice. The mean $V_m$ value under these conditions was $-64 \pm 1.9 \text{ mV}$ ($n = 12$), not significantly different from wild-type values.

We also examined the resting and stimulated membrane potentials in $K_{Ca3.1}$-null mice, an example of which is illustrated in Fig. 4C. The resting potential of cells from these animals was not statistically significantly different from the value in cells from wild-type mice: $-63 \pm 2.9 \text{ mV}$ ($n = 18$). CCh also induced $V_m$ oscillations in cells from these animals (Fig. 4C); the mean value during CCh treatment ($-55 \pm 2.4 \text{ mV}$, $n = 17$) was slightly but significantly more depolarized than in wild-type cells. As noted earlier, parotid fluid secretion from these
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$K_{Ca}3.1$-null mice is normal (11), so it would appear that this small depolarization has little or no effect on fluid secretion.

We also recorded the membrane potentials in mice with both $K^+$ channel genes ablated, an example of which is illustrated in Fig. 4D. The resting membrane potential of cells from double-null mice was not significantly different from that of cells from wild-type mice ($−56 \pm 1.9$ mV, $n = 18$), suggesting that neither of these $K^+$ channels is responsible for setting the cell resting potential. This is not surprising because neither channel is likely to be significantly activated at these negative voltages at the low Ca$^{2+}$ levels of non-stimulated cells. We have made no further investigation of the channels that establish the cell resting potential. The total number (and conductance) of such channels must be quite low because the cell conductance, in the absence of the $K_{Ca}1.1$ and $K_{Ca}3.1$ channels, is extremely low (see Fig. 1 and associated discussion).

Muscarnic stimulation of parotid cells from double-null mice, as from all of the other strains, induced robust membrane potential oscillations as illustrated in Fig. 4D. The mean value in CCh was $−41 \pm 2.3$ mV ($n = 10$), significantly more depolarized than the value in CCh for all of the other animals. Thus, it appears that either of the $K^+$ channels is sufficient to maintain a relative negative membrane potential during CCh stimulation but that ablating both $K^+$ channels allows the activation of Cl$^-$ channels to produce a significant depolarization. These more depolarized $V_m$ levels in the acinar cells from the double-null animals will reduce the driving force for Cl$^-$ efflux. With a reduced efflux of Cl$^-$ into the ducts, there will be a reduced transepithelial potential and so less Na$^+$ entering the ducts. The reduced NaCl content of the acinar secretion means that less water is needed for osmotic balance. The net result is a plasma-like primary saliva flowing at a reduced rate. The duct cells will operate on this fluid to alter the final sodium, potassium, and chloride levels (see “Discussion”) but at a reduced saliva flow rate (Figs. 2B and 3).

Fig. 5 summarizes the mean membrane potentials in the absence (Fig. 5A) and presence (Fig. 5B) of muscarinic stimulation with CCh. These statistical box plots paint a picture of a resting membrane potential quite insensitive to the presence or absence of either or both the Ca$^{2+}$-activated $K^+$ channels. Ablating either $K^+$ channel gene had, at most, a small effect on the membrane potential during muscarinic stimulation. Only with both $K^+$ channels removed was the stimulated membrane potential greatly different from the membrane potentials of wild-type and single-null mice.

Although the stimulated membrane potential of cells from double-null mice ($−41$ mV) was significantly more depolarized than the comparable values of cells from wild-type and single-null cells, it was still significantly more negative than the Cl$^-$ ion equilibrium potential ($−24$ mV). Normally, it is the activity of $K^+$ channels that prevents the activation of Cl$^-$ channels to dominate the $V_m$ values. But the double-null animals lack these $K^+$ channels, and so what prevents Cl$^-$ channels from dominating the $V_m$ values? One possibility is the electrogenic Na$^+/K^+$ pump. The absence of the $K^+$ channels might be expected to result in a higher membrane resistance and so allow the electrogenic Na$^+/K^+$ pump current to generate a significant hyperpolarization. These ideas may be tested with the Na$^+$ pump-specific inhibitor ouabain.

An example of the actions of ouabain on the membrane potential of cells from double-null animals is shown in Fig. 4D. In the presence of ouabain, CCh caused the $V_m$ to closely approach the Cl$^-$ equilibrium potential. The mean value in five cells (three mice) was $−26 \pm 1$ mV, only 2 mV more hyperpolarized than the $−24$ mV Cl$^-$ equilibrium potential and 15 mV more depolarized than the $−41$ mV level in the absence of ouabain. The average membrane voltage during CCh stimulation of cells from wild-type animals was $−57 \pm 2$ mV (seven cells from four animals) in the presence of ouabain, 8 mV more depolarized than the $−65$ mV level in the absence of ouabain.

The membrane resistance of double-null cells in CCh was $0.67 \pm 0.067$ gigaohms (three cells from two mice), about twice that of wild-type cells ($0.31 \pm 0.04$ gigaohms; eight cells from three animals). Thus, the absence of the Ca$^{2+}$-activated $K^+$ channels increased membrane resistance during CCh stimulation by about a factor of 2, and so the contribution of the Na$^+$ pump to the membrane potential would be expected to be about twice as large in the double-null animals. Indeed, inhibition of the Na$^+$ pump by ouabain produced twice the membrane depolarization in the double-null animals compared with the wild-type mice (15 mV compared with 8 mV).

$RVD$ in Parotid Acinar Cells from $K_{Ca}1.1$-null Mice—Upon exposure to a hypotonic bathing medium, many cells first swell
and then shrink because of the process known as RVD. As noted in the Introduction, many cells use K⁺ and Cl⁻ channels in this process (reviewed in Ref. 12), including salivary gland acinar cells (32). We have shown previously that ablating the \( \text{KCa3.1} \) gene inhibits RVD in red blood cells but not in parotid acinar cells; however, the maxi-K channel-specific antagonist paxilline inhibits RVD in parotid acinar cells (11), suggesting that this Ca\(^{2+} \)-activated K⁺ channel may play an important role in this process. Thus, we measured RVD in parotid acinar cells from \( \text{KCa}1.1 \)-null mice and from the appropriate wild-type strain.

Shown in Fig. 6 are the pooled results of the relative cell volume of acinar cells from wild-type (■) and \( \text{KCa}1.1 \)-null (○) mice in response to a 30% reduction in solution osmolality. After the expected increase in volume, there was a relative rapid, spontaneous decrease in the volume of the cells from wild-type animals, the RVD response. The cells from \( \text{KCa}1.1 \)-null mice, like those from wild-type animals, increased in volume in response to the reduction in solution osmolality but, unlike wild-type cells, had a rather poor RVD response. The RVD rate was quantitatively estimated from the slope of these data during the early part of the volume recovery (lines). This analysis revealed RVD rates of 0.015 min\(^{-1} \) for cells from wild-type animals and 0.0058 min\(^{-1} \) for cells from \( \text{KCa}1.1 \)-null mice, a 2.6-fold reduction.

**DISCUSSION**

We found that mice lacking the \( \text{KCa}1.1 \) gene also lacked the time- and voltage-dependent \( \text{Ca}^{2+} \)-activated maxi-K channel normally expressed in parotid gland acinar cells (Fig. 1C). RVD of the parotid acinar cells was substantially inhibited in the \( \text{KCa}1.1 \)-null animals (Fig. 6). Pilocarpine-stimulated parotid gland fluid secretion was not significantly altered in these mice (Fig. 2A), but there were significant changes in the ion content of the secreted fluid (Fig. 3A). The secreted sodium level increased by \( \sim \)37% in the \( \text{KCa}1.1 \)-null mice, and the secreted potassium level was reduced to less than half of that in wild-type animals. Mice with both the \( \text{KCa}1.1 \) and \( \text{KCa3.1} \) genes ablated had essentially no \( \text{Ca}^{2+} \)-activated K⁺ channel current (Fig. 1D) and a severely impaired fluid secretion rate (Fig. 2B). These animals showed the same alterations in the ion content and osmolality of the secreted fluid as did the \( \text{KCa}1.1 \)-null mice, but the changes were more severe. The membrane potentials of the acinar cells elicited by muscarinic stimulation paralleled the basic findings with fluid secretion (Figs. 4 and 5), i.e. there was very little change in time-averaged stimulated membrane voltage in wild-type, \( \text{KCa}1.1 \)-null, and \( \text{KCa3.1} \)-null mice. The biggest difference was 10 mV between the wild-type and \( \text{KCa3.1} \)-null animals. However, the double-null mice had a stimulated membrane voltage almost 25 mV more depolarized compared with the wild-type animals.

These findings, coupled with our previous study showing that heterologously expressed \( \text{KCa}1.1 \) channels exhibit the same biophysical and pharmacological properties as the native maxi-K channels (10), confirm that these channels in parotid acinar cells are encoded by the \( \text{KCa}1.1 \) gene. This study has also shown that the \( \text{KCa}1.1 \) channel plays an important role in RVD of these cells.

It is tempting to conclude that the maxi-K channel plays no role in fluid secretion because there was no alteration in the fluid secretion rate in the \( \text{KCa}1.1 \)-null mice. The fact that fluid secretion was also normal in \( \text{KCa3.1} \)-null mice but substantially reduced in double-null animals argues for a redundant role for these two channels. However, the situation may be more complex because we have shown previously that activation of the IK1 channels (\( \text{KCa3.1} \) gene) inhibits maxi-K channels (31). In addition, although the use of pilocarpine to stimulate in vivo fluid secretion from salivary glands has been quite common for many years (33, 34) and successfully used in gene ablation studies (35), a continuous systemic application of this agent may not accurately simulate all of the subtleties in the physiologically relevant, nerve-evoked, fluid secretion process.

The salivary gland primary acinar secretion is a plasma-like fluid very high in sodium and very low in potassium, but the final secreted saliva has much reduced sodium and increased potassium levels (33, 34). Thus, the ducts in these glands contain sodium uptake and potassium secretion mechanisms. As a consequence, the secreted sodium and potassium (and chloride) levels depend upon flow rate: the lower the flow rate, the more time is available for the sodium uptake/potassium secre-
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The sodium, potassium, and chloride content of saliva from $K_{Ca}1.1$-null (and double-null) mice was significantly altered, but the pilocarpine-stimulated fluid secretion rate was no different from that in matched wild-type animals. Thus, the differences in ion content cannot be explained by a flow rate change and so must represent changes in ion uptake/secretion in the ducts. There is little information on K+ channels in salivary gland ducts; but IK1 channels are not expressed there (10), so maxi-K channels are likely alone in supporting the K+ secretion process in these cells. The presence of maxi-K channels in the duct cells would be expected to support a negative membrane potential and so provide a driving force for Na+ uptake (likely through epithelial Na+ channels). Ablation of maxi-K channels would be expected to cause cell depolarization and a reduced driving force for Na+ uptake and result in a higher level of secreted sodium, exactly as seen with the $K_{Ca}1.1$-null (and double-null) mice (Fig. 3).

Perhaps our most surprising result was the relatively small (25 mV depolarization) effect on the muscarinic receptor-stimulated membrane potential in the double-null mice. As shown in Fig. 1D, these animals had essentially no K+ channel current and so, even with moderate activation of Cl− channels, no apparent means to maintain a membrane voltage more negative than the Cl− equilibrium potential. However, in the presence of the Na+/K+ pump inhibitor ouabain, the membrane potential did move to the Cl− equilibrium potential (Fig. 4). Thus, this electrogenic pump, operating through an increased membrane resistance (due to the absence of the K+ channels) can apparently supply enough of a hyperpolarization to support at least minimum fluid secretion (Fig. 2B).

In summary, we have shown that the $K_{Ca}1.1$ gene encodes the Ca2+-activated maxi-K channel in the parotid gland. This channel is necessary for the RVD response of these cells, and it plays an important role in sodium reabsorption/potassium secretion in the ducts. Although fluid secretion is unaffected by ablation of either the $K_{Ca}1.1$ or $K_{Ca}3.1$ gene, removing both genes severely impairs fluid secretion. Although the straightforward interpretation of this result is that these two K+ channels have redundant roles in fluid secretion, their apparent interaction and distinct properties suggest more subtle issues in fluid secretion than we are able to discern with the in vivo pilocarpine assay. Understanding these more subtle features of fluid secretion and the precise interplay between the two K+ channels will be the subjects of future studies.

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