J. Membrane Biol. 151, 19–27 (1996)

The Journal of
Membrane
Biology

The Ach-evoked Ca^{2+}-activated K^+ Current in Mouse Mandibular Secretory Cells. Single Channel Studies

T. Hayashi, J.A. Young, D.I. Cook
Department of Physiology, University of Sydney, NSW 2006 Australia

Received: 28 September 1995/Revised: 26 December 1995

Abstract. Although acetylcholine (ACh) is able to activate voltage- and Ca^{2+}-sensitive K^+ (BK) channels in mouse mandibular secretory cells, our recent whole cell studies have suggested that these channels, like those in sheep parotid secretory cells, do not contribute appreciably to the conductance that carries the ACh-evoked whole cell K^+ current. In the present study, we have used cell-attached patch clamp methods to identify and characterize the K^+ channel type responsible for carrying the bulk of this current. When the cells were bathed in a NaCl-rich solution the predominant channel type activated by ACh (1 μmol/l or 50 nmol/l) had a conductance only of 40 pS; it was not blocked by TEA but it was sensitive to quinine and it conducted Rb^+ to an appreciable extent. BK channels, which could be seen in some but not all patches from resting cells, also showed increased activity when ACh was added to the bath, but they were much less conspicuous during ACh stimulation than the 40-pS channels. When the cells were bathed in a KCl-rich rather than a NaCl-rich solution, a small-conductance K^+ channel, sensitive to quinine but not to TEA, was still the most conspicuous channel to be activated by ACh although its conductance was reduced to 25 pS. Our studies confirm that the ACh-evoked whole-cell K^+ current is not carried substantially by BK channels and show that it is carried by a small-conductance K^+ channel with quite different properties.

Key words: K^+ currents — Rb^+ — Acetylcholine — Tetraethylammonium — Quinine — BK channels

Introduction

Since it was recognized that the only K^+ channel commonly seen in the basolateral plasma membranes of unstimulated salivary endpiece cells was the large-conductance, voltage- and Ca^{2+}-activated K^+ (BK) channel [1, 7, 17, 21, 22, 24, 37], these channels have been widely held to be responsible for carrying the ACh-evoked K^+ current in salivary secretory cells [28, 29]. Two lines of evidence supported this belief. First, tetraethylammonium (TEA), a known blocker of BK channels, has been shown to inhibit the ACh-activated K^+ current in mouse mandibular secretory cells [33], as well as inhibiting ACh-evoked fluid secretion and ACh-evoked K^+ efflux in a variety of salivary gland types (*reviewed in* [2]). Second, cell-attached patch studies have shown that BK channel activity can be increased by the addition of ACh to the bath solution [7, 24, 34, 37].

Recent studies on secretory cells from sheep parotid [11], mouse mandibular [12], rat mandibular [15, 16] and rat parotid [18] glands, however, have suggested that BK channels do not carry the bulk of the ACh-activated K^+ current. These studies have shown that the TEA-sensitivity of the ACh-activated whole-cell K^+ current, as well as of ACh-evoked fluid secretion and ACh-evoked K^+ efflux, is due to TEA blocking the increase in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) evoked by muscarinic agonists as part of the normal signal-transduction mechanism [3, 12, 31], rather than to inhibition of K^+ channels in the plasma membranes of the secretory cells.

Although cell-attached patch clamp studies on salivary secretory cells have shown that the addition of ACh to the bath solution activates BK channels (*see above*), until now the channel type actually responsible for carrying the bulk of the ACh-activated K^+ current has not been identified. The available whole-cell patch clamp data suggest that this so far unidentified K^+ channel should be blocked by quinine (which also blocks BK channels [13]), but not by TEA, and that it should be activated by an increase in $[Ca^{2+}]_i$ and have a significant conductance for Rb^+ [11, 12, 15]. In the present paper, we report the results of experiments in which we used

Correspondence to: D.I. Cook

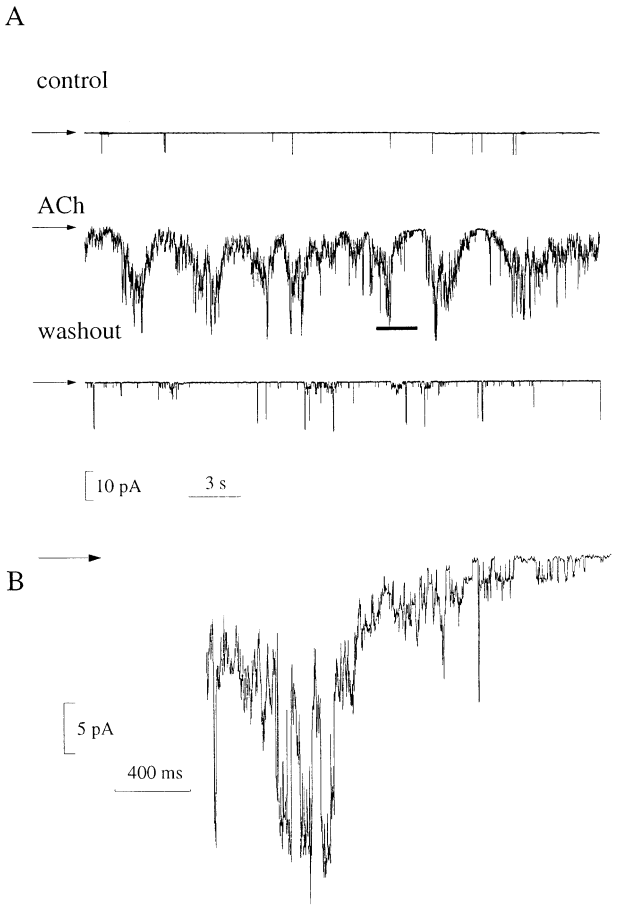

Fig. 1. (*A*) Cell-attached patch recording with a KCl-rich solution in the pipette and a NaCl-rich solution in the bath prior to addition of ACh (top tracing), during the addition of 1 μmol/l ACh (second tracing), and following washout of ACh (third tracing). (*B*) A recording on an expanded time scale of the period indicated by the dark horizontal bar on the second tracing in *A*. The larger of the single-channel transitions are due to a BK channel, the smaller to the 40-pS ACh-activated channel. The potential difference across the patch was held at the cell potential. The horizontal arrows indicate the zero current levels. Current flowing out of the cell is shown as an upward deflection.

cell-attached patch clamp methods to identify and characterize this channel.

Materials and Methods

Male Quackenbush strain mice were killed by cervical dislocation and the mandibular glands were removed and finely chopped. To obtain single cells for patch clamping, we incubated the chopped tissue at 37°C for 30 min in a NaCl-rich bath solution (*see below*) to which 1 mg/ml collagenase (Worthington Type 4, Freehold, NJ) had been added. The cell suspension was then dispersed by trituration with a syringe, washed twice in fresh bath solution, filtered through nylon mesh (first with 200 μm mesh and then with 75 μm) and placed on glass coverslips.

Patch clamp experiments were performed at room temperature in a 0.3-ml chamber that could be perfused continuously. Single-channel currents, recorded with an Axopatch-1C amplifier (Axon Instruments, Foster City, CA), were filtered at 500 Hz and sampled at 1,000 Hz as previously described [6]. The reference electrode was a Ag/AgCl electrode placed directly into the bath. Potential differences are reported as the cell potential relative to the bath, and conventional current leaving the cell is defined as positive and shown as an upward deflection.

The standard NaCl-rich bath solution we used had the following composition (in mmol/1): NaCl (145), KCl (5), $CaCl_2$ (1), $MgCl_2$(1.2), NaH_2PO_4 (1.2), D-glucose (10), H-HEPES (7.5) and Na-HEPES (7.5); the pH was adjusted to 7.4 with NaOH. The standard KCl-rich pipette solution we used had the following composition (in mmol/l): KCl (140), $MgCl_2$ (1), D-glucose (10), EGTA (0.5) and H-HEPES (10); the pH was adjusted to 7.2 with KOH. In experiments in which the free Ca^{2+} concentration was to be buffered, we used a KCl-rich bath solution having the following composition (mmol/l): KCl (145), $MgCl_2$ (1.2), D-glucose (10), H-HEPES (10) (pH 7.2) and EGTA (5), with various concentrations of free Ca^{2+}. The free Ca^{2+} concentration was computed using a formula taking account of pH and the concentrations of EGTA, Ca^{2+} and Mg^{2+} [25]. The concentrations of Ca^{2+} used to produce the required free Ca^{2+} activities were as follows: 0.24 mmol/l (pCa = 8), 1.67 mmol/l (pCa = 7), 4.04 mmol/l (pCa = 6). All chemicals were obtained from Sigma (St Louis, MO) and were of the highest available grade; the purity of the EGTA (Sigma) was 96%.

Results are presented as means ± SEM., with the number of individual observations (*n*) given in parentheses. The differences of means were tested for statistical significance by calculating the probability levels (*P*) from Student's unpaired *t*-test.

Results

The Effects of ACh on Channel Activity in Cell-attached Patches

We studied the effects of ACh on channel activity in 60 cell-attached patches using a NaCl-rich bathing solution with the potential across the patch held at the cell potential (i.e., with the pipette potential held at 0 mV). Prior to exposure of the cells to ACh, BK channel activity was detected in 19 patches whereas 41 patches were silent even when the voltage was displaced by ±80 mV from the normal cell potential, except for the occasional opening in some patches of a small-conductance channel similar to that activated by ACh (*see below*). In 21 of the 60 cells studied, we first stimulated the cells with 50 nmol/l ACh and then with 1 μmol/l ACh; in the other 39 cells, we used only one concentration of ACh (1 μmol/l). Regardless of the ACh concentration used, in about half of those patches that responded to ACh, stimulation resulted in an oscillatory increase in channel activity (Fig. 1) which persisted for the duration of stimulation; in the other patches that responded to ACh, the increase in activity was steady throughout stimulation.

ACh stimulation caused BK channel activity to increase in some but not all of the patches showing resting BK channel activity, but it never evoked BK channel activity in patches that had been silent under resting conditions. Thus, of patches from 4 cells exposed to 50 nmol/l ACh which showed BK channel activity at rest,

none showed increased activity during stimulation, and of patches from 19 cells exposed to 1 μmol/l ACh which showed BK channel activity at rest, only 12 showed increased activity during stimulation.

The most striking effect of ACh, however, was not an increase in BK channel activity, but the activation of a smaller-conductance channel that was rarely seen in resting cells. Of 21 cells stimulated with 50 nmol/1 ACh, small-conductance channel activity was seen in 7 patches, and of 60 cells stimulated with 1 μmol/1 ACh, this kind of channel activity was seen in 44 patches.

In the 21 patches studied at two dose levels of ACh, we estimated the mean current carried by the channels in the patch over a 30-sec period. (This current is calculated as the difference between the mean current through the cell-attached patch over 30 sec and the baseline current recorded when channel activity is absent). In unstimulated cells with the potential across the patch held at the cell potential, the mean current carried by all channels present was 0.03 ± 0.01 pA ($n = 21$); in cells exposed to 50 nmol/l ACh it was 0.31 ± 0.16 pA ($n = 21$); and in cells exposed to 1 μmol/l ACh it was 1.78 ± 0.53 pA ($n = 21$).

Sensitivity of the ACh-activated Channels to Blockers

The inclusion of 10 mmol/l TEA in the KCl-rich pipette solution evidently inhibited all BK channel activity since channel openings attributable to BK channels were not observed prior to or during stimulation with 1 μmol/l ACh in any of 30 cell-attached patches studied. We did, however, observe apparently normal activation of the smaller-conductance channel by 1 μmol/l ACh (Fig. 2) in 21 of the 30 patches studied with 10 mmol/l TEA in the pipette solution. The single-channel current carried by the smaller-conductance channel at 0 mV with 10 mmol/l TEA in the pipette solution (1.93 ± 0.05 pA, $n = 21$) was significantly ($P = 0.027$) less than that observed when there was no TEA in the pipette solution (2.07 ± 0.04 pA, $n = 29$) although the reason for the small difference is unknown. The inclusion of quinine (1 mmol/l) in the pipette solution as well as 10 mmol/l TEA, markedly reduced the activity of the smaller-conductance channel (Fig. 3), although transitions due to it were still observed in 12 of the 25 cell-attached patches studied. Its single-channel current as 0 mV (1.82 ± 0.05 pA, $n = 12$) was not significantly different ($P = 0.144$) from that observed when the pipette solution contained only TEA.

To determine more precisely the relative contributions of the BK channels and the smaller-conductance, TEA-insensitive channels to the ACh-evoked current response measured in cell-attached patches, we calculated the mean current flowing through channels in cell-attached patches with the potential across the patch held at the cell potential while the cells were exposed to ACh. As shown in Fig. 4, the inclusion of 5 or 10 mmol/l TEA in the pipette solution did not have a statistically significant inhibitory effect on the mean current when ACh was added to the bath. In the absence of TEA or quinine, the mean current for the 39 control patches was 2.32 ± 0.63 pA ($n = 39$), whereas in patches studied with 5 mmol/l TEA in the pipette solution it was 2.61 ± 1.03 pA ($n = 26$, $P = 0.803$), and in patches studied with 10 mmol/l TEA in the pipette it was 1.69 ± 0.52 ($n = 30$, $P = 0.461$). The addition of 1 mmol/l quinine as well as 10 mmol/l TEA to the pipette solution, however, significantly reduced the current (by about 85%) to 0.24 ± 0.07 pA ($n = 25$, $P = 0.014$ for comparison with the current recorded with 10 mmol/l TEA in the pipette solution).

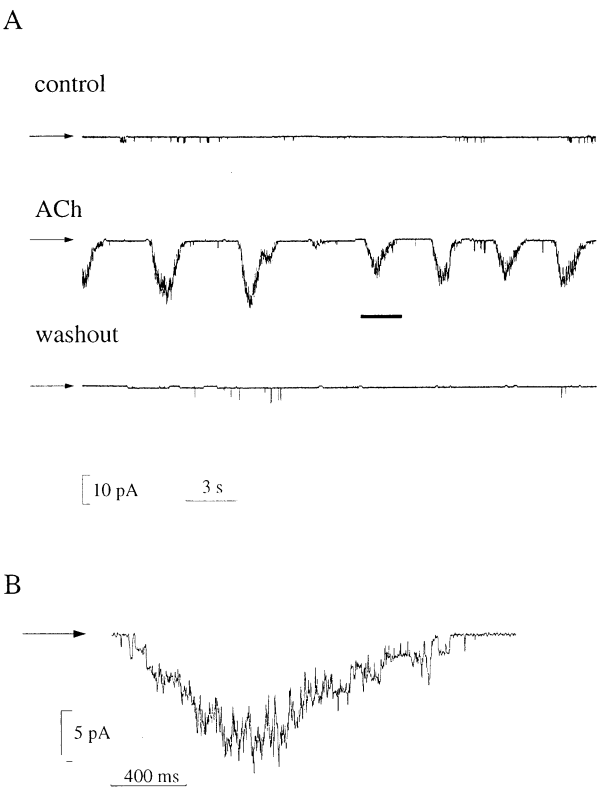

Fig. 2. (*A*) Cell-attached patch recording with a KCl-rich solution plus 10 mmol/l TEA in the pipette and a NaCl-rich solution in the bath prior to addition of ACh (top tracing), during the addition of 1 μmol/l ACh (second tracing), and following washout of ACh (third tracing). (*B*) A recording on an expanded time scale of the period indicated by the dark horizontal bar on the second tracing in *A*. The potential across the patch was held at the cell potential.

Characteristics of the Small-conductance ACh-activated Channel

We determined the current-voltage (*I–V*) relation of the ACh-activated channel in experiments in which we in-

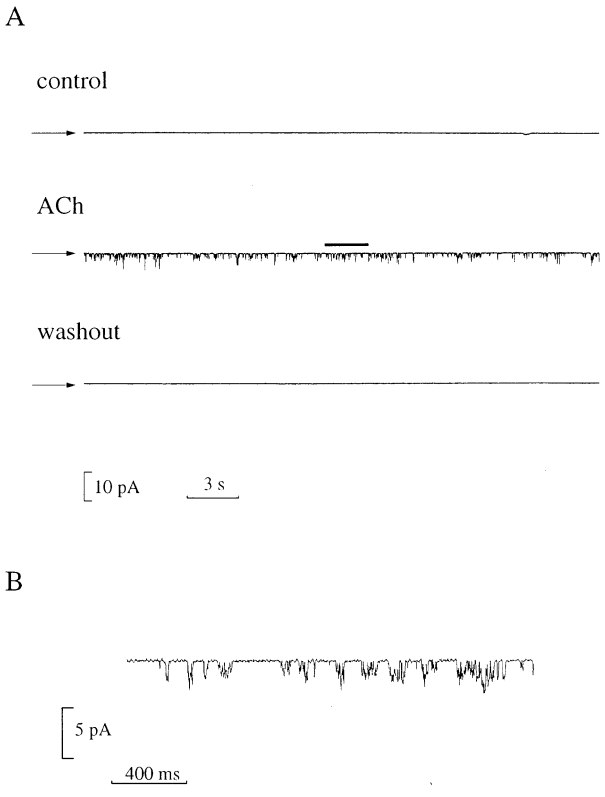

Fig. 3. (*A*) Cell-attached patch recording with a KCl-rich solution plus 10 mmol/l TEA and 1 mmol/1 quinine in the pipette, and a NaCl-rich solution in the bath prior to addition of ACh (top tracing), during the addition of 1 μmol/l ACh (second tracing), and following washout of ACh (third tracing). (*B*) A recording on an expanded time scale of the period indicated by the dark horizontal bar on the second tracing in *A*. The potential across the patch was held at the cell potential.

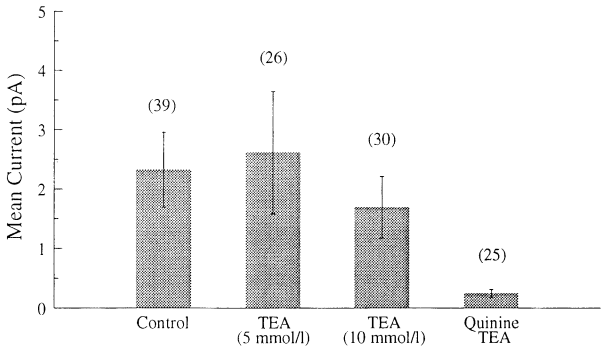

Fig. 4. Bar graph showing the mean current flow through cell-attached patches on cells stimulated with 1 μmol/l ACh. The bath contained a NaCl-rich solution and the pipette contained a KCl-rich solution plus TEA (5 or 10 mmol/l), alone or together with quinine (1 mmol/l) as marked. The potential across the patch was held at the cell potential. The baseline current (i.e., patch current when all channels were closed) was subtracted so that the mean current reported represents the mean current flow through open channels.

cluded 10 mmol/l TEA in the KCl-rich pipette solution to eliminate interfering transitions due to BK channels. The bath contained the NaCl-rich solution. We found that the *I–V* relation of the ACh-activated channel was linear (Fig. 5) with a slope conductance of 40.7 ± 1.6 pS ($n = 9$) and a zero current potential of +61.3 ± 3.5 mV ($n = 8$). Since the channel did not remain active in excised patches, it was necessary to determine its ion selectivity in cell-attached patches. We did this by determining the zero current potential of the ACh-activated channel when the pipette solution contained 72.5 mmol/l KCl and 72.5 mmol/l NaCl. As shown in Fig. 5, under these conditions the zero current potential was reduced to +47.6 ± 1.9 mV ($n = 9$), a shift (−13.7 ± 4.0 mV, 13 d.f.) not significantly different from that expected for a K^+-selective channel (−17.5 mV).

We investigated whether the 40-pS channel was conductive to Rb^+ by completely replacing the K^+ in the pipette solution with Rb^+. As shown in Fig. 6, the channel conducted Rb^+ at approximately 30% of the rate at which it conducted K^+ (14.6 ± 0.4 pS, $n = 4$, *vs.* 40.7 ± 1.6 pS, $n = 9$) and the zero current potential was shifted to +40.0 ± 0.8 mV ($n = 4$), suggesting that Rb^+ had a permeability relative to K^+ of 0.45.

We also examined whether the 40-pS K^+ channel could be activated by increasing cytosolic Ca^{2+}. As meantioned above, it was very rare to observe the ACh-activated channel once the patch had been excised. Of 24 inside-out excised patches in which we had previously observed channel activity while the patches were still attached to cells bathed in a KCl-rich solution, the channel was never observed when the cytosolic surface of the excised patch was bathed in a KCl-rich solution having a pCa of 8 or 7. When the Ca^{2+} concentration of the bath was raised to give a pCa of 6, however, the channel was reactivated in 3 of the 24 patches. Our failure to observe activation of the ACh-sensitive K^+ channel in most excised patches seems unlikely to have been due to the formation of patch vesicles, which is always a risk when one is studying excised patches, since we were able to observe Ca^{2+}-activation of BK channels in 4 of the patches even although there was no concomitant activation of the small-conductance channels.

Finally, we examined whether increasing intracellular Ca^{2+} with the Ca^{2+} ionophore, ionomycin, could activate the small-conductance channel in cell-attached patches on cells not exposed to ACh. The addition of 0.2 μmol/l ionomycin to the bath activated the small-conductance channel (Fig. 7) in 4 of 6 cell-attached patches studied with a NaCl-rich bath solution and a KCl-rich pipette solution not containing TEA.

The Influence of Bath Solution Composition on Channel Activity

A notable feature of the cell-attached patch data we have presented is that the BK channel appears to make a much

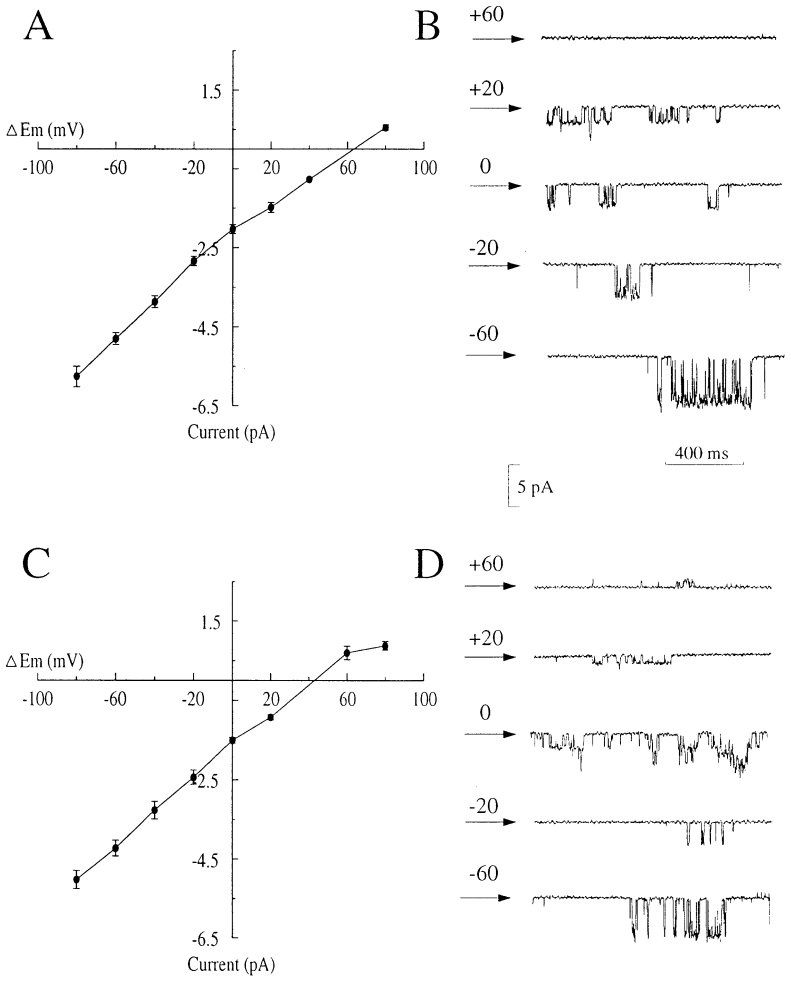

Fig. 5. Current-voltage relation and representative single-channel recordings in cell-attached patches of the small-conductance ACh-activated channel studied with a NaCl-rich bath solution. The pipette solution contained 10 mmol/l TEA with either 145 mmol/l KCl (panels *A* and *B*) or 72.5 mmol/l KCl plus 72.5 mmol/l NaCl (panels *C* and *D*). Potential differences are given as the bath potential relative to the pipette potential.

smaller contribution to the K^+ channel activity evoked by ACh than has been suggested by earlier reports [8,23]. It occurred to us that this might have been due, in at least some of the earlier studies, to the use of KCl-rich rather that NaCl-rich bath solutions. (It is also possible in these earlier studies that only patches exhibiting BK channel activity at rest were seleted for further study). We therefore repeated our studies using KCl-rich rather than NaCl-rich bath solutions. When we did this, we found, as previously reported [8, 23], that ACh only increased channel activity transiently in cell-attached patches. Nevertheless, the pattern of channel incidence we observed using the KCl-rich bath solution was similar to that observed with the NaCl-rich bath solution. Using the KCl-rich bath and holding the potential across the patch at −50 mV, we observed BK channels prior to ACh stimulation in only 6 of 22 cell-attached patches and the addition of ACh (1 μmol/l) to the bath increased the open probability of the BK channel in those patches but failed to induce BK channel activity in any of the other patches. In 19 of the 22 patches, however, 1 μmol/l ACh activated a smaller-conductance channel which had not been active prior to stimulation (Fig. 8).

We then examined the effects of inclusion of TEA (10 mmol/l) or quinine (1 mmol/l) plus TEA in the pipette solution on the effects of ACh on the mean current carried by channels in cell-attached patches from cells bathed in the KCl-rich solution which had been stimulated with 1 μmol/l ACh (Fig. 9). In the absence of TEA or quinine, the mean current for 22 patches was 3.25 ± 0.82 pA ($n = 22$) and for the 13 of these patches which did not contain BK channels but showed small-conductance channel activity, it was 2.05 ± 0.41 pA ($n = 13$). In 25 patches studied with TEA in the pipette solution, the mean current was 2.33 ± 0.55 pA ($n = 25$), a difference that was not statistically significant. In 19 patches studied with quinine (and TEA) in the pipette, however, there was a significant ($P = 0.002$, compared

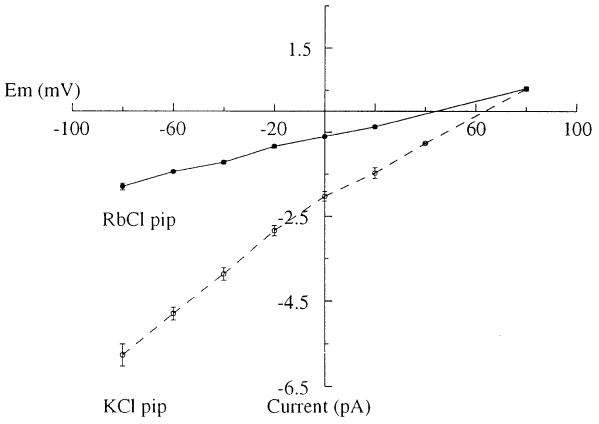

Fig. 6. Current-voltage relation in cell-attached patches of the ACh-activated channel with a NaCl-rich bath solution and a pipette solution containing 10 mmol/l TEA with either 145 mmol/l KCl or 145 mmol/l RbCl. Potential differences are given as the bath potential relative to the pipette potential.

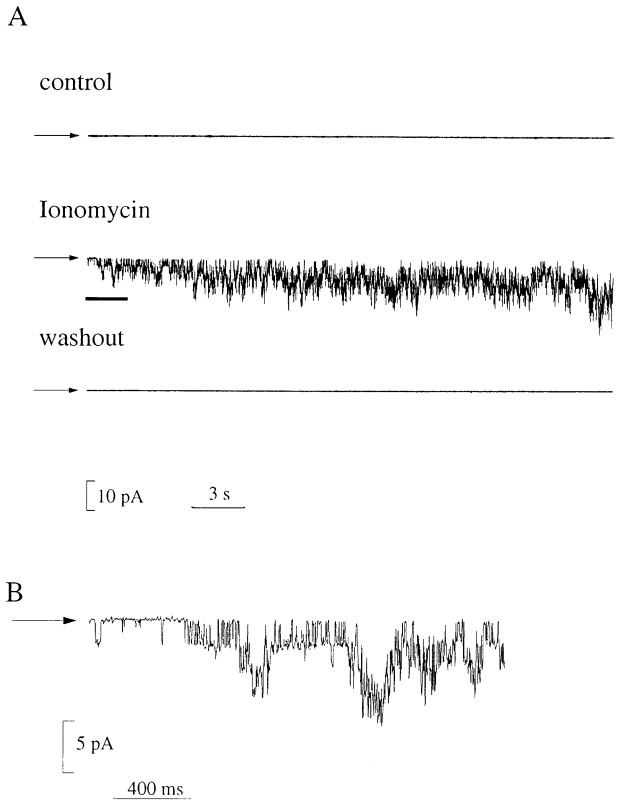

Fig. 7. (*A*) Cell-attached patch recording with a KCl-rich solution in the pipette and a NaCl-rich solution in the bath prior to addition of ionomycin (top tracing), during the addition of 0.2 μmol/l ionomycin (second tracing), and following washout of ionomycin (third tracing). (*B*) A recording on an expanded time scale of the period indicated by the dark horizontal bar in the second tracing in *A*. The potential across the patch was held at the cell potential.

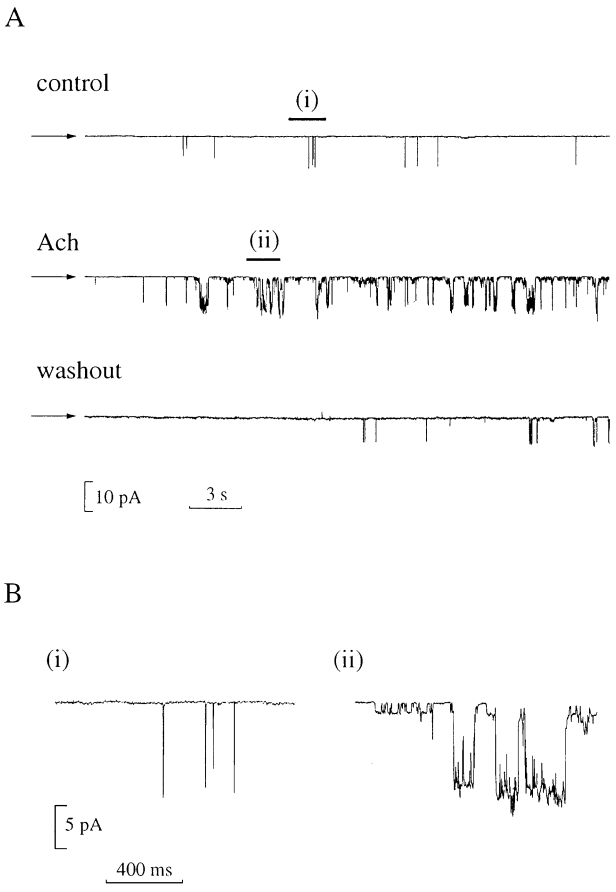

Fig. 8. (*A*) Cell-attached patch recording with a KCl-rich solution in the pipette and a KCl-rich solution in the bath prior to addition of ACh (top tracing), during the addition of 1 μmol/l ACh (second tracing), and following washout of ACh (third tracing). (*B*) Recordings on an expanded time scale of the periods indicated by the dark horizontal bars in the first and second tracings in *A*. The potential across the patch was held at −50 mV.

with the current recorded with 10 mmol/l TEA in the pipette solution) reduction of about 90% in the mean current to 0.17 ± 0.08 pA (n = 19) (Fig. 9).

The *I–V* relation of the small-conductance channel activated by ACh, with 10 mmol/1 TEA in the pipette solution and with a KCl-rich bath solution, was slightly rectifying (Fig. 10) with a slope conductance of 25.4 ± 0.4 pS (n = 8) at negative membrane potentials and of 16.5 ± 1.8 pS (n = 8) at positive membrane potentials. It had a zero current potential of −3.3 ± 1.0 mV (n = 8). When the pipette solution contained 72.5 mmol/l KCl and 72.5 mmol/l NaCl, the zero current potential was displaced to −19.9 ± 1.0 mV (n = 7), a shift not significantly different from that expected for a K^+-selective channel (Fig. 10).

Discussion

In this paper, we demonstrate for mouse mandibular secretory cells that the addition of ACh to the bath acti-

vates not only the BK channel, but also a smaller-conductance K^+ channel (Figs. 1 and 8). When the cells are bathed in a NaCl-rich solution, this channel has a conductance of 40 pS and conducts Rb^+ (Fig. 6), is insensitive to TEA (Figs. 2 and 4), is blocked by quinine (Figs. 3 and 4) and is activated by increased $[Ca^{2+}]_i$ (Fig. 7). When the cells are bathed in a KCl-rich solution, the channel has a conductance of only 25 pA but its properties otherwise seem to be unaltered. Our inability to reactivate the small-conductance channel in most inside-out patches exised into a KCl-rich solution, even when the free Ca^{2+} bathing the cytosolic surface of the patch is as high as 1 μmol/l (a value some 4 times the maximum $[Ca^{2+}]_i$ measured in these cells during ACh stimulation [5, 12]), suggests that Ca^{2+} may regulate these channels by acting in synergy with some other cytosolic constituent. An alternative explanation might be that the free Ca^{2+} in the region of the membrane where the channels are localized is far higher than is indicated by fura-2 methods, although this seems unlikely, given the relatively modest increases in BK channel activity observed in the present studies during ACh stimulation. Finally, we find that when the cells are bathed in a NaCl-rich solution, the small-conductance, TEA-insensitive channel carries the bulk (at least 70%) of the ACh-evoked current through most cell-attached patches (Fig. 4).

We consider it unlikely that the 40-pS K^+ channel is a subconductance state of the BK channel because the BK channel, unlike the 40-pS K^+ channel, is sensitive to TEA [13, 19]. That the 40-pS K^+ channel conducts Rb^+ also distinguishes it from BK channels which, in the mouse mandibular gland (unlike the BK channels in the sheep parotid [37]), do not conduct Rb^+ [9].

The cell-attached patch studies carried out on cells bathed in a KCl-rich solution showed that the ACh-activated small-conductance K^+ channel had a lower conductance than when the bath was NaCl-rich. The difference in conductance of the ACh-activated K^+ channel observed with these two bath solutions cannot be attributed to differences in the transmembrane potential in the two experimental protocols since the pipette potentials were chosen to maintain similar transmembrane potentials. Since the pattern of blocker sensitivities we have observed for the ACh-activated K^+ channels, *viz.*, sensitive to quinine but insensitive to TEA, was the same with the two bath solutions, we believe that the different single-channel conductances we measured cannot be attributed to activation of different channel types under the two conditions. Why the channel conductance should be lower when the KCl-rich bath is used is unclear, although a change in channel properties might have resulted from a change in intracellular pH secondary to inactivation of Na^+-H^+ exchange when the KCl-rich bath solution was used (*see* for example [27]).

The present studies show that the 40-pS K^+ channel we have identified has properties similar to the ACh-activated K^+ conductance we demonstrated previously in whole-cell studies on the same cell type [12], i.e., it is conductive to Rb^+, insensitive to TEA, blocked by quinine and activated by increased $[Ca^{2+}]_i$. It thus seems likely that the bulk of the ACh-activated K^+ current in mouse mandibular secretory cells is attributable to activation of the 40-pS K^+ channels. Furthermore, given that the ACh-activated K^+ conductances in sheep parotid and rat mandibular glands have also been reported to be insensitive to TEA but blocked by quinine [11, 15], it seems quite possible that the 40-pS K^+ channel may underlie the ACh-activated K^+ current in these tissues also.

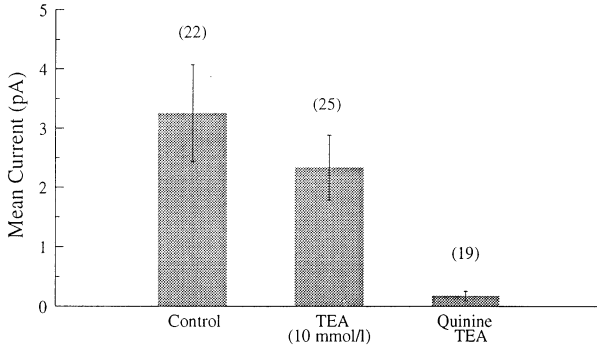

Fig. 9. Bar graph showing the mean current flow through cell-attached patches on cells stimulated with 1 μmol/l ACh. The bath contained a KCl-rich solution and the pipette contained a KCl-rich solution plus TEA (10 mmol/l) or TEA (10 mmol/l) and quinine (1 mmol/l), as marked. The potential across the patch was held at −50 mV. The baseline current (i.e., patch current when all channels were closed) was subtracted so that the mean current reported represents the mean current flow through open channels.

The ACh-activated K^+ channel we have observed in mouse mandibular secretory cells has very similar properties to a K^+ channel seen in HSG cells, a cell line derived from human submandibular glands [14, 20, 32], which has been said originate from the intercalated ducts [20, 30]. The channel in HSG cells is muscarinically activated and is Ca^{2+} sensitive, with a conductance of about 23 pS in symmetrical K^+ solutions. It conducts Rb^+ at 30% of the rate at which it conducts K^+, is blocked by quinine and, in whole-cell studies, is insensitive to TEA [14, 20]. The implication for intercalated duct function of the presence of similar Ca^{2+}-activated K^+ channels in the HSG cell line and mouse mandibular secretory cells is unclear although it is worth pointing out that both cell types are thought to develop from cells comprising the originally nonsecretory terminal tubules [38].

Another question arising from this study concerns the role of BK channels in the normal function of salivary secretory cells. As mentioned previously, BK channels dominate the resting whole-cell *I–V* relation of

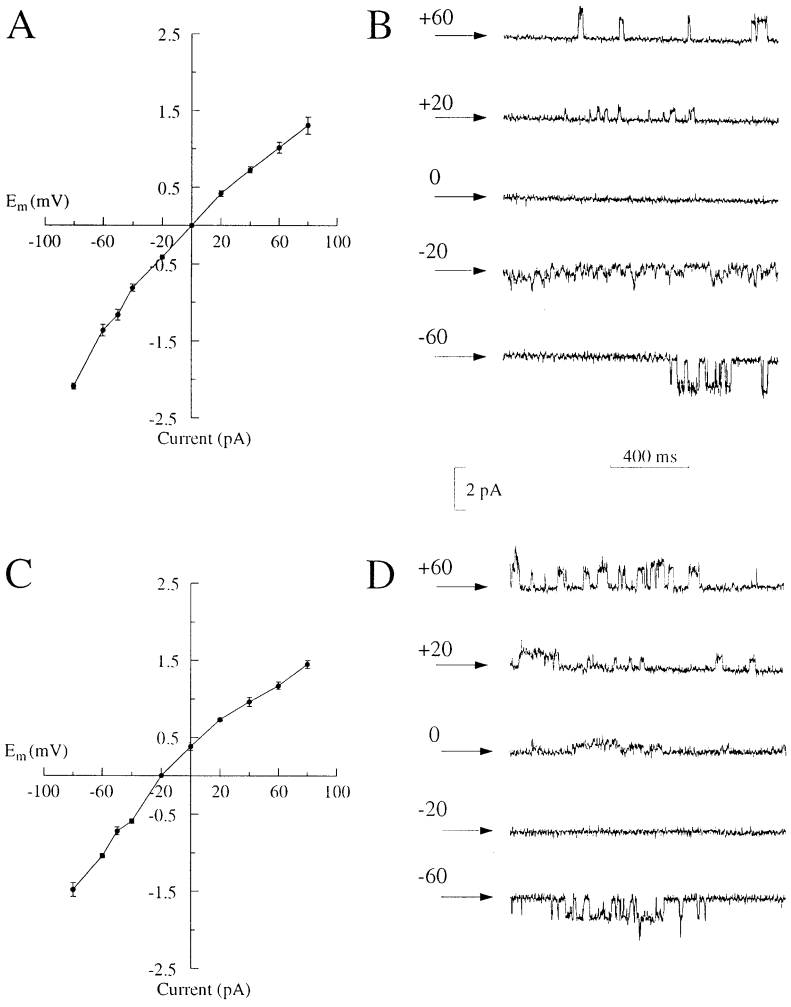

Fig. 10. Current-voltage relation and representative single-channel recordings in cell-attached patches of the small-conductance ACh-activated channel studied with a KCl-rich bath solution. The pipette solution contained 10 mmol/l TEA with either 145 mmol/l KCl (panels *A, B*) or 72.5 mmol/l KCl plus 72.5 mmol/l NaCl (panels *C, D*). Potential differences are given as the bath potential relative to the pipette potential.

mouse mandibular secretory cells [7], but, although their activity does increase during ACh stimulation [7, 8] (*present study*), they are not a major component of the ACh-activated K^+ conductance [12]. It may be that their function is to support the initial phase of secretion, when the increased $[Ca^{2+}]_i$, which is confined to the apical pole of the secretory cells in rat lacrimal cells [35, 36] (and possibly also in parotid cells [10]), would lead to activation of the apical anion channels without concomitant activation of the basolateral K^+ conductance. This role of BK channels would however be diminished to the extent that there is a Ca^{2+}-activated K^+ conductance in the apical membrane of the secretory cells [1, 35]. Their function is thus unclear although recent studies in rat lacrimal cells have suggested that they may have a role in regulatory volume decrease [26].

This project was supported by the Australian Research Council.

References

1. Cook, D.I., Gard, G.B., Champion, M., Young, J.A. 1988. Patch-clamp studies of the electrolyte secretory mechanism of rat mandibular gland cells stimulated with acetylcholine or isoproterenol. *In:* Molecular Mechanisms in Secretion (25th Alfred Benzon Symposium 1987). N.A. Thorn, M. Treiman, O.H. Petersen and J.H. Thaysen, editors. pp. 131–151. Munksgaard, Copenhagen
2. Cook, D.I., Roberts, M.L., Van Lennep, E.W., Young, J.A. 1994. Secretion by the major salivary glands. *In:* Physiology of the Gastrointestinal Tract, Volume 2, 3rd Edition. L. Johnson, J. Christensen, M. Jackson, E. Jacobson and J. Walsh, editors. pp. 1065–1107. Raven Press, New York
3. Cook, D.I., Wegman, E.A., Ishikawa, T., Poronnik, P., Allen, D.G., Young, J.A. 1992. Tetraethylammonium blocks muscarinically evoked secretion in the sheep parotid gland by a mechanism additional to its blockade of BK channels. *Pfluegers Arch.* **420:**167–171
4. Cook, D.I., Young, J.A. 1989. Effect of K^+ channels in the apical

plasma membrane on epithelial secretion based on secondary active Cl^- transport. *J. Membrane Biol.* **110:**139–146
5. Dinudom, A., Poronnik, P., Allen, D.G., Young, J.A., Cook, D.I. 1993. Control of intracellular Ca^{2+} by adrenergic and muscarinic agonists in mouse mandibular ducts and endpieces. *Cell Calcium* **14:**631–638
6. Dinudom, A., Young, J.A., Cook, D.I. 1994. Ion channels in the basolateral membrane of intralobular duct cells of mouse mandibular glands. *Pfluegers Arch.* **428:**202–208
7. Gallacher, D.V., Morris, A.P. 1986. A patch-clamp study of potassium currents in resting and acetylcholine-stimulated mouse submandibular acinar cells. *J. Physiol.* **373:**379–395
8. Gallacher, D.V., Morris, A.P. 1987. The receptor-regulated calcium influx in mouse submandibular acinar cells is sodium dependent: a patch-clamp study. *J. Physiol.* **384:**119–130
9. Gallacher, D.V., Maruyama, Y., Petersen, O.H. 1984. Patch-clamp study of rubidium and potassium conductances in single cation channels from mammalian exocrine acini. *Pfluegers Arch.* **401:**361–367
10. Gromada, J., Jørgensen, T.D., Tritsaris, K., Nauntofte, B., Dissing, S. 1993. Ca^{2+} signalling in exocine acinar cells: the diffusional properties of cellular inositol 1,4,5-trisphosphate and its role in the release of Ca^{2+}. *Cell Calcium* **14:**711–723
11. Hayashi, T., Hirono, C., Young, J.A., Cook, D.I. 1995. The ACh-induced whole-cell currents in sheep parotid secretory cells. Do BK channels really carry the ACh-evoked whole-cell K^+ current? *J. Membrane Biol.* **144:**157–166
12. Hayashi, T., Poronnik, P., Young, J.A., Cook, D.I. 1995. The ACh-evoked, Ca^{2+}-activated whole-cell K^+ current in mouse mandibular secretory cells. Whole-cell and fluorescence studies. *J. Membrane Biol.* (*Submitted*)
13. Ishikawa, T., Cook, D.I. 1993. Effects of K^+ blockers on inwardly and outwardly rectifying whole-cell K^+ currents in sheep parotid secretory cells. *J. Membrane Biol.* **133:**29–41
14. Ishikawa, T., Cook, D.I. 1995. A Ca^{2+}-activated 23-pS K^+ channel in a human submandibular duct cell line (HSG). *Cell. Physiol. Biochem.* **5:**232–242
15. Ishikawa, T., Murakami, M. 1995. Tetraethylammonium-insensitive, Ca^{2+}-activated whole-cell K^+ currents in rat submandibular acinar cells. *Pfluegers Arch.* **429:**748–750
16. Ishikawa, T., Murakami, M., Seo, Y. 1994. Basolateral K^+ efflux is largely independent of maxi-K^+ channels in rat submandibular glands during secretion. *Pfluegers Arch.* **428:**516–525
17. Ishikawa, T., Wegman, E.A., Cook, D.I. 1993. An inwardly rectifying potassium channel in the basolateral membrane of sheep parotid secretory cells. *J. Membrane Biol.* **131:**193–202
18. Iwatsuki, N., Maruyama, Y., Matsumoto, O., Nishiyama, A. 1985. Activation of Ca^{2+}-dependent Cl^- and K^+ conductances in rat and mouse parotid acinar cells. *Jpn. J. Physiol.* **35:**933–944
19. Iwatsuki, N., Petersen, O.H. 1985. Action of tetraethylammonium on calcium-activated potassium channels in pig pancreatic acinar cells studied by patch-clamp single-channel and whole-cell current recording. *J. Membrane Biol.* **86:**139–144
20. Izutsu, K.T., Fatherazi, S., Wellner, R.B., Herrington, J., Belton, C.M., Oda, D. 1994. Characteristics and regulation of a muscarinically activated K current in HSG-PA cells. *Am. J. Physiol.* **266:**C58–C66
21. Maruyama, Y., Gallacher, D.V., Petersen, O.H. 1983. Voltage and Ca^{2+}-activated K^+ channel in basolateral acinar cell membranes in mammalian salivary glands. *Nature* **302:**827–829
22. Maruyama, Y., Nishiyama, A., Izumi, T., Hoshimiya, N., Petersen, O.H. 1986. Ensemble noise and current relaxation analysis of K^+ current in single isolated salivary acinar cells from rat. *Pfluegers Arch.* **406:**69–72
23. Morris, A.P., Fuller, C.M., Gallacher, D.V. 1987. Cholingergic receptors regulate a voltage-insensitive but Na^+-dependent calcium influx pathway in salivary acinar cells. *FEBS Lett.* **211:**195–199
24. Morris, A.P., Gallacher, D.V., Fuller, C.M., Scott, J. 1987. Cholingeric receptor-regulation of potassium channels and potassium transport in human submandibular acinar cells. *J. Dent. Res.* **66:**541–546
25. Oiki, S., Okada, Y. 1987. Ca-EGTA buffer in physiological solutions. *Seitanokagaku* **38:**79–83
26. Park, K.P., Beck, J.S., Douglas, I.J., Brown, P.D. 1994. Ca^{2+}-activated channels are involved in regulatory decrease in acinar cells isolated from the rat lacrimal gland. *J. Membrane Biol.* **141:**193–201
27. Park, K., Brown, P.D. 1994. Intracellular pH modulates ion channel activity in lacrimal gland acinar cells. *Jpn. J. Physiol.* **44:**S255–S259
28. Petersen, O.H. 1992. Stimulus-secretion coupling: cytoplasmic calcium signals and the control of ion channels in exocrine acinar cells. *J. Physiol.* **448:**1–51
29. Petersen, O.H., Maruyama, Y. 1984. Calcium-activated potassium channels and their role in secretion. *Nature* **307:**693–696
30. Sato, M., Hayashi, Y., Yoshida, H., Yanagawa, T., Yura, Y., Nitta, T. 1984. Search for specific markers of neoplastic epithelial duct and myoepithelial cell lines from human salivary glands and characterization of their growth in vitro. *Cancer Philadelphia* **54:**2959–2967
31. Shigetomi, T., Hayashi, T., Ueda, M., Kaneda, T., Tokuno, H., Takai, A., Tomita, T. 1991. Effects of Ca^{2+} removal and of tetraethylammonium on membrane currents induced by carbachol in isolated cells from the rat parotid gland. *Pfluegers Arch.* **419:**332–337
32. Ship, J.A., Patton, L.L., Wellner, R.B. 1990. Muscarinic regulation of potassium transport in a human submandibular epithelial cell line. *Am. J. Physiol.* **259:**C340–C348
33. Smith, P.M., Gallacher, D.V. 1992. Acetylcholine and caffeine evoked repetitive transient Ca^{2+} activated K^+ and Cl^- currents in mouse submandibular cells. *J. Physiol.* **449:**109–120
34. Soltoff, S.P., McMillian, M.K., Cantley, L.C., Cragoe, E.J., Talamo, B.R. 1989. Effects of muscarinic, alpha-adrenergic, and substance-P agonists and ionomycin on ion transport mechanisms in the rat parotid acinar cell. The dependence of ion transport on intracellular calcium. *J. Gen. Physiol.* **93:**285–319
35. Tan, Y.P., Marty, A., Trautman, A. 1992. High density of Ca^{2+}-dependent K^+ and Cl^- channels in the luminal membrane of lacrimal acinar cells. *Proc. Natl. Acad. Sci. USA* **89:**11229–11233
36. Toescu, E.C., Lawrie, A.M., Petersen, O.H., Gallacher, D.V. 1992. Spatial and temporal distribution of agonist-evoked cytoplasmic Ca^{2+} signals in exocrine acinar cells analysed by digital image microscopy. *EMBO J.* **11:**1623–1629
37. Wegman, E.A., Ishikawa, T., Young, J.A., Cook, D.I. 1992. Cation channels in the basolateral membrane of sheep parotid secretory cells. *Am. J. Physiol.* **263:**G786–G794
38. Young, J.A., Van Lennep, E.W. 1978. The Morphology of Salivary Glands. Academic Press, London