J. Membrane Biol. 152, 253–259 (1996)

The Journal of
Membrane
Biology

The ACh-evoked, Ca^{2+}-activated Whole-cell K^+ Current in Mouse Mandibular Secretory Cells. Whole-cell and Fluorescence Studies

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Received: 9 January 1996/Revised: 17 April 1996

Abstract. In our previous studies on sheep parotid secretory cells, we showed that the K^+ current evoked by acetylcholine (ACh) was not carried by the high-conductance voltage- and Ca^{2+}-activated K^+ (BK) channel which is so conspicuous in unstimulated cells, notwithstanding that the BK channel is activated by ACh. Since several studies from other laboratories had suggested that the BK channel did carry the ACh-evoked K^+ current in the secretory cells of the mouse mandibular gland, and that the current could be blocked with tetraethylammonium (TEA), a known blocker of BK channels, we decided to investigate the ACh-evoked K^+ current in mouse cells more closely. We studied whether the ACh-evoked K^+ current in the mouse is inhibited by TEA and quinine. Using the whole-cell patch-clamp technique and microspectrofluorimetric measurement of intracellular Ca^{2+}, we found that TEA and quinine do inhibit the ACh-evoked K^+ current but that the effect is due to inhibition of the increase in intracellular Ca^{2+} evoked by ACh, not to blockade of a K^+ conductance. Furthermore, we found that the K^+ conductance activated when ionomycin is used to increase intracellular free Ca^{2+} was inhibited only by quinine and not by TEA. We conclude that the ACh-evoked K^+ current in mouse mandibular cells does not have the blocker sensitivity pattern that would be expected if it were being carried by the high-conductance, voltage- and Ca^{2+}-activated K^+ (BK) channel. The properties of this current are, however, consistent with those of a 40 pS K^+ channel that we have reported to be activated by ACh in these cells [16].

Key words: K^+ currents — Acetylcholine — BK channels — Tetraethylammonium — Quinine — Ionomycin — Salivary glands

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Introduction

The occurrence of net K^+ efflux across the basolateral membranes of secretory cells during the early stages of evoked exocrine secretion has been extensively studied [1, 2, 9, 26, 28, 33, 39], and electrophysiological experiments have shown that acetylcholine (ACh) stimulation of salivary secretory endpiece cells increases the K^+ conductance of their basolateral membranes [29, 31] by increasing the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) [14, 29, 31]. The discovery that the basolateral membranes of salivary endpiece cells contain large numbers of the high-conductance, voltage- and Ca^{2+}-activated K^+ (BK) channel [23], and that channels of this type dominate the whole-cell current-voltage (*I–V*) relation of the unstimulated secretory cells of many salivary glands [3, 10, 17, 19, 24, 26] thus led to the hypothesis that BK channels carry the ACh-evoked K^+ efflux [30, 32]. This hypothesis was subsequently strengthened by the finding in cell-attached patch studies that BK channels are activated by the addition of ACh to the bath solution [10, 26, 36, 38] and the finding in mouse mandibular secretory cells that tetraethylammonium (TEA), a known blocker of BK channels, prevents the ACh activation of the whole-cell K^+ current [35].

The proposition that BK channels are the major carriers of the ACh-evoked K^+ current has recently been reassessed experimentally following the demonstration that the effects of TEA on salivary secretion can largely be attributed to its ability to inhibit the increase in cytosolic Ca^{2+} evoked by muscarinic stimulation [4, 34]. It is now clear that BK channels play little, if any role in the ACh-evoked K^+ current in sheep parotid [15], rat mandibular [18] and rat parotid secretory cells [22, 34]. This is despite the clear evidence that BK channels dominate the unstimulated whole-cell *I–V* relation in these cell types (*see above*), and that, in sheep [38] and rat [36] parotid cells, the addition of ACh to the bath solution activates the BK channels in cell-attached patches.

The finding that the ACh-evoked K^+ current is not carried by BK channels in the secretory cells of the sheep parotid [15], rat mandibular [18] or rat parotid glands [22, 34] conflicts with whole-cell [35] and cell-attached [10] patch-clamp studies in the secretory cells of the mouse mandibular gland, however, from which it had been concluded that BK channels are responsible for the ACh-evoked K^+ current.

In the present paper, we have used whole-cell techniques and microspectrofluorimetric $[Ca^{2+}]_i$ measurement to show that TEA and quinine block the ACh-evoked K^+ current in mouse mandibular secretory cells by preventing ACh from increasing $[Ca^{2+}]_i$, not by blocking K^+ channels directly. By using the Ca^{2+} ionophore, ionomycin, to increase $[Ca^{2+}]_i$, and thereby to bypass the muscarinic receptor, we have been able to show that the Ca^{2+}-activated K^+ current in these cells can be blocked directly by quinine but not by TEA.

Materials and Methods

Male Quackenbush strain mice were killed by cervical dislocation and the mandibular glands were removed and finely minced. To obtain single cells for patch-clamping, we incubated the minced mandibular glands for 30 min at 37°C in a shaking water bath in the Na^+-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 onto glass coverslips. For fluorimetry experiments, isolated endpieces were obtained by incubating the minced tissue in a 0.25 mg/ml collagenase solution (at 37°C for 30 min) following which the suspension was incubated with the acetoxymethyl ester of fura-2 (2 μmol/l) or fluo-3 (4.4 μmol/l) with 0.015% Pluronic for a further 30 min. The endpieces were then washed and stored on ice until ready for use.

Patch-clamp experiments were performed at room temperature in a 0.3 ml chamber that was perfused continuously. Secretory cells were distinguished from ductal cells on the basis of the marked differences in their appearance using phase contrast optics (duct cells are densely packed with exocytotic granules whereas secretory cells are not [6]) and the characteristics of their whole-cell current-voltage relations (duct cells have an inwardly rectifying current-voltage relation whereas secretory cells have an outwardly rectifying current-voltage relation [6]). Membrane currents of single secretory cells were measured in the standard whole-cell configuration using an Axopatch-1C amplifier (Axon Instruments, Foster City, CA). The membrane potential was held at −60 mV and a command pulse sequence repetitively applied at 380 msec intervals. The command pulse consisted of a hyperpolarization to −120 mV (40 msec duration), followed after a 120 msec interval by a second hyperpolarization to −80 mV (40 msec duration) and, after a further 120 msec interval, a depolarization to 0 mV (60 msec duration). In this protocol, 0 mV corresponds to the zero current potential for Cl^- and nonselective cation currents, and −80 mV is close to the zero current potential for K^+ currents, so the current at 0 mV is a relatively pure measure of the K^+ current and the current at −80 mV measures the Cl^- and nonselective cation currents with perhaps a small contribution from K^+ currents. In the mouse mandibular gland, the current at −120 mV measures only a mixture of the K^+ and Cl^- currents and so will not be presented in the results since it adds nothing to the study; we retained this clamp step in the command pulse sequence only so that the sequence would be identical to the one used previously in the sheep parotid where the clamp current at −120 mV corresponds to the current carried by an inwardly rectifying K^+ channel [15]. The resultant current was low-pass filtered at 500 Hz, sampled and digitized at 1 kHz using a MacLab8 data acquisition interface (AD Instruments, Sydney, Australia) attached to a Macintosh IIvx computer. 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.

For fluorimetric experiments, a small quantity of the suspension was placed on a coverslip coated with Cell-Tak (Collaborative Research, Bedford, MA) and left for approximately 10 min for the cells to adhere. The coverslip was then attached to the base of a superfusion chamber of approximately 300 μl volume. The chamber was then closed off with another coverslip, mounted on a microscope stage and perfused with the bath solution at a rate of 1.5 ml/min. All experiments were performed at 37°C using a Nikon Diaphot microscope equipped with a ×100 Fluor objective and a diaphragm to isolate single endpieces in the field of view.

In experiments in which fura-2 was used, the samples were irradiated using a Lambda-10 filter wheel (Sutter, Novato, CA) at 340 nm and 380 nm. The emitted light was passed through a 505 nm bandpass filter into a photomultiplier tube (Oriel, Stratford, CT) and the PMT output was recorded using a MacLab4 data-acquisition interface. The raw data were processed offline with in-house software. Fura-2 calibrations were performed as described previously [5]. In experiments in which fluo-3 was used, the same equipment was employed as for the fura-2 experiments but the samples were irradiated at 490 nm and emitted light measured at 505 nm.

The standard Na^+-rich bath solution contained (in mmol/l): 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 K^+-rich pipette solution contained (in mmol/l): KCl (140), $MgCl_2$ (1), D-glucose (10), EGTA (0.5), and H-HEPES (10); and the pH was adjusted to 7.2 with KOH. The NaCl-rich bath solution used for fluorimetric experiments had the following composition (in mmol/l): NaCl (145), KCl (5), $CaCl_2$ (1), $MgCl_2$ (1), glucose (10) and H-HEPES (10); the pH was adjusted to 7.35 with NaOH. Fura-2-AM and fluo-3-AM were obtained from Molecular Probes (Eugene, OR). All chemicals were obtained from Sigma (St Louis, MO). Results are presented as means ± SEM with the number of individual observations (*n*) given in parentheses. Statistical significance was assessed using Student's unpaired *t*-test.

Results

The Effects of TEA on Whole-Cell Currents Evoked by ACh and Ionomycin

As noted above, a major component of the evidence suggesting a role for the BK channel in ACh-evoked secretion in mouse mandibular secretory cells was the observation that TEA inhibited ACh activation of the whole-cell K^+ current [35]. To study the whole-cell conductances activated by the addition of ACh to the bath, we used the whole-cell voltage-clamp protocol we had previously used to study the ACh-evoked currents in sheep parotid cells [15]. In unstimulated mouse mandibular cells voltage-clamped according to this protocol, the resting outward current at 0 mV was 482 ± 46 pA (n = 19), and the resting inward current at −80 mV was −170 ± 25 pA (n = 19). Exposure of the cells to ACh

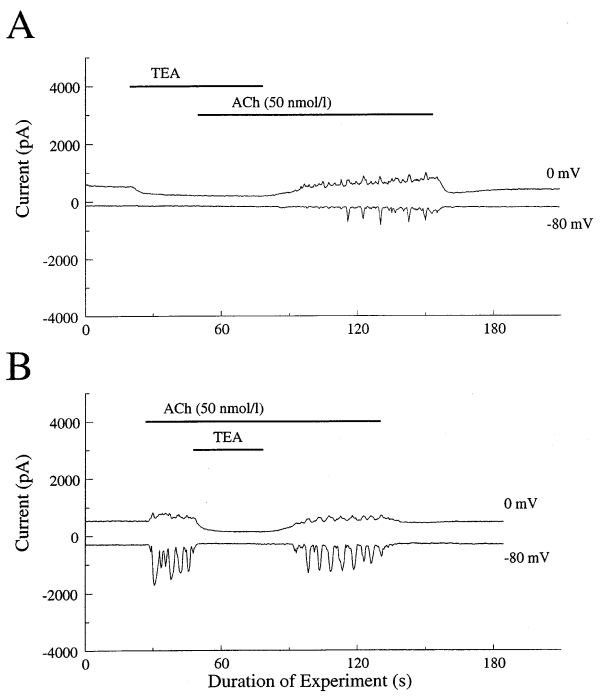

Fig. 1. Acetylcholine (50 nmol/l) evoked changes in membrane currents recorded from single mouse mandibular secretory cells under whole-cell clamp conditions. The voltage-clamp protocol used is described in Materials and Methods. The pipette contained the standard KCl solution and the bath contained the standard NaCl solution. (*A*) TEA (5 mmol/l) addition prior to ACh stimulation; (*B*) TEA addition during ACh stimulation.

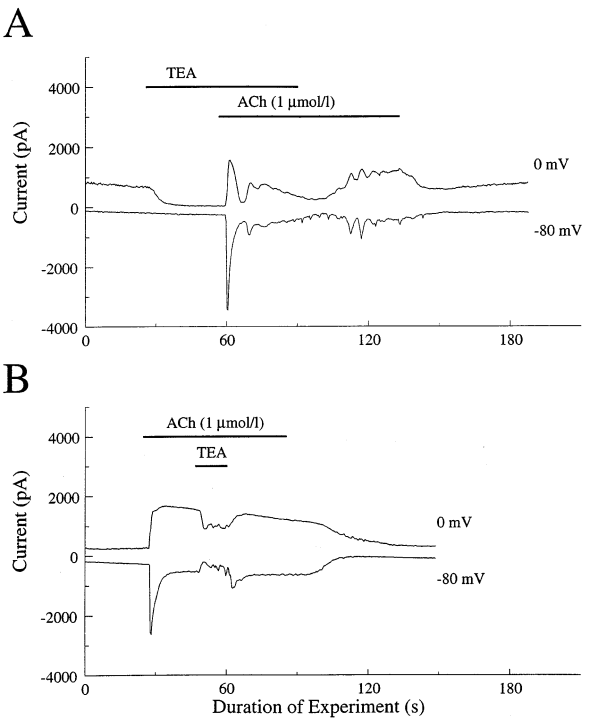

Fig. 2. Effects of TEA (5 mmol/l) on the membrane currents evoked by 1 μmol/l ACh on single mouse mandibular secretory cells. (*A*) TEA addition prior to ACh stimulation; (*B*) TEA addition during ACh stimulation. The experimental details are given in the legend to Fig. 1.

caused increases in both the outward and the inward currents (Fig. 1*B*). During exposure to 50 nmol/l ACh, the outward current at 0 mV increased by 547 ± 167 pA ($n = 4$) within 12.9 ± 3.3 sec ($n = 4$) of exposure to ACh, and the inward current at −80 mV increased by −1292 ± 504 pA ($n = 4$) within 24.5 ± 17.2 sec ($n = 4$). The currents fluctuated above the resting levels for as long as the exposure to ACh continued and returned to their resting levels following removal of ACh from the bath.

TEA has been reported to block both the resting and the ACh-evoked outward current in mouse mandibular cells [35]. We found that the addition of 5 mmol/l TEA to single unstimulated mouse mandibular secretory cells held in the whole-cell patch-clamp configuration reduced the outward current from 401 ± 50 pA ($n = 9$) to 75 ± 20 pA ($n = 9$), a reduction of 81%, but had no effect on the inward current at −80 mV (Fig. 1*A*, Fig. 2*A*). When 50 mmol/l ACh was added to the bath in the presence of 5 mmol/l TEA there was almost no change in either the outward or inward currents ($n = 4$) but upon removal of TEA, both currents were activated (Fig. 1*A*). TEA (5 mmol/l) also inhibited the currents at 0 mV and −80 mV when it was added to the bath during exposure of the cells to 50 nmol/l ACh (Fig. 1*B*).

This inhibition of the ACh-evoked currents by TEA could be overcome by increasing the concentration of ACh. Stimulation of the cells with 1 μmol/l ACh produced peak increases in the current at 0 mV of 2094 ± 396 pA ($n = 10$) and at −80 mV of −2535 ± 466 pA ($n = 10$) (Fig. 2*A*). Pretreatment with 5 mmol/l TEA had no significant effect on the peak increases in outward current (1461 ± 377 pA, $n = 5$, $P = 0.432$) or in inward current (−1898 ± 439 pA, $n = 5$, $P = 0.385$) evoked by 1 μmol/l ACh (Fig. 2*A*). When added after initiating stimulation with 1 μmol/l ACh, however, TEA did inhibit the currents at 0 mV and −80 mV by 75% ± 17 ($n = 3$) and 74% ± 8 ($n = 3$), respectively, indicating that even at 1 μmol/l ACh does not completely overcome the inhibitory effects of TEA (Fig. 2*B*).

To find out whether TEA inhibited the K^+ and Cl^- currents or interfered with the cholinergic signal transduction pathway, we used ionomycin to increase $[Ca^{2+}]_i$, thereby bypassing the muscarinic receptors. We found that, in the presence of ionomycin, TEA did not inhibit the activation of the currents at 0 mV or at −80 mV (Fig. 3). The addition of 5 mmol/l TEA to a cell already stimulated with 0.2 μmol/l ionomycin appeared to cause the current at 0 mV to decrease slightly by 11% ± 5 from 2870 ± 268 pA ($n = 5$) and the current at −80 mV to decrease by 9% ± 4 from −980 ± 298 pA ($n = 5$) but the decreases were not statistically significant.

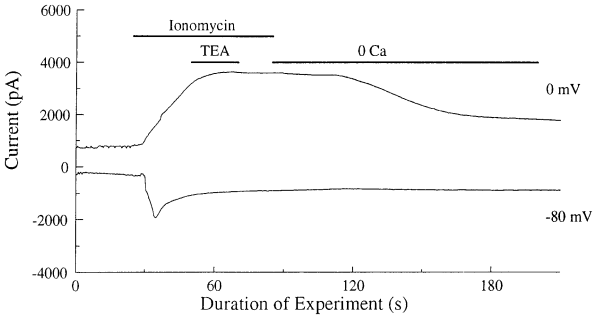

Fig. 3. Effect of TEA (5 mmol/l) on the increase in membrane currents evoked by 0.2 μmol/l ionomycin in single mouse secretory cells. The experimental details are given in the legend to Fig. 1.

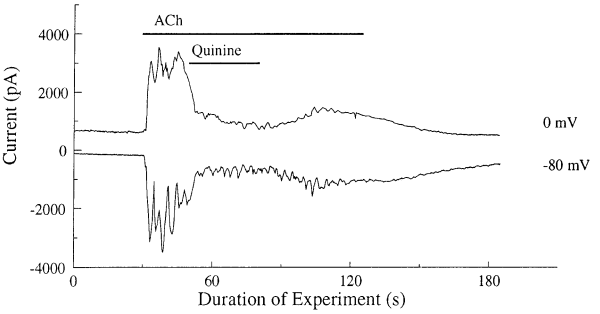

Fig. 4. Effect of quinine (1 mmol/l) on the acetylcholine (1 μmol/l) evoked changes in membrane currents recorded from a single mouse mandibular secretory cell under whole-cell clamp conditions. The experimental details are given in the legend to Fig. 1.

The Effects of Quinine on Whole-Cell Currents Evoked by ACh and Ionomycin

In our previous studies on the ACh-evoked current in sheep parotid cells, we had observed that quinine inhibited the ACh-evoked whole-cell K^+ current [15]. Consequently, we decided also to examine whether quinine blocked the ACh-evoked whole-cell K^+ current in mouse mandibular secretory cells. We found that 1 mmol/l quinine had no effect on the outward or inward currents in resting cells, but it reduced the current at 0 mV and −80 mV during ACh (1 μmol/l) by 82% ± 8 ($n = 4$) and 95% ± 2 ($n = 4$), respectively (Fig. 4).

Because we were concerned that quinine might have interfered with the intracellular Ca^{2+} signal evoked by ACh, we examined whether quinine blocked the whole-cell current response to ionomycin. When we used Cl^--rich solutions in both the bath and the pipette (Fig. 5*A*), we found that the current evoked by 0.2 μmol/l ionomycin at 0 mV was inhibited by 79% ± 5 ($n = 5$) by quinine, whereas the current evoked by ionomycin at −80 mV was increased by 78% ± 4 ($n = 5$). The effect of quinine on the ionomycin-evoked current at −80 mV was eliminated by replacement of all but 4 mmol/l of the Cl^- in the bath and pipette solutions by glutamate, a somewhat larger anion (Fig. 5*B*).

Ion Selectivity of the ACh-evoked K^+ Current

In five whole-cell experiments, we replaced the K^+ in the pipette solution by Rb^+. In these experiments, we found that the ACh-evoked current at 0 mV was significantly reduced (560 ± 165 pA, $n = 5$; $P = 0.001$) compared to that observed with the control KCl pipette solution (2015 ± 174 pA, $n = 4$), whereas the current at −80 mV was unaffected (−2356 ± 678 pA, $n = 5$ *vs.* −3361 ± 917 pA, $n = 4$; $P = 0.397$).

The Effects of TEA and Quinine on Changes in $[Ca^{2+}]_i$

The findings that TEA blocks both the ACh-evoked current at 0 mV and the ACh-evoked current at −80 mV, and

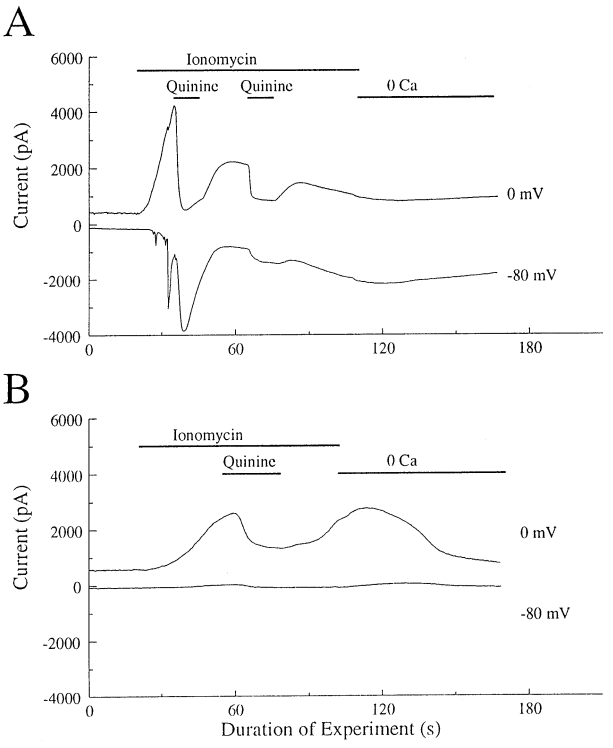

Fig. 5. Effect of quinine (1 mmol/l) on the increase in membrane currents evoked by 0.2 μmol/l ionomycin in single mouse secretory cells. The voltage-clamp protocol is described in Materials and Methods. (*A*) The pipette contained the standard KCl solution and the bath contained the standard NaCl solution; (*B*) The pipette and bath contained solutions in which all but 4 mmol/l Cl^- had been replaced with glutamate.

that it fails to block the currents evoked by ionomycin, are consistent with the possibility that TEA blocked the ACh-evoked increase in $[Ca^{2+}]_i$, rather than by acting as a channel blocker. To check whether this was the case, we determined whether 5 mmol/l TEA inhibited the $[Ca^{2+}]_i$ response to ACh. The mouse mandibular end-

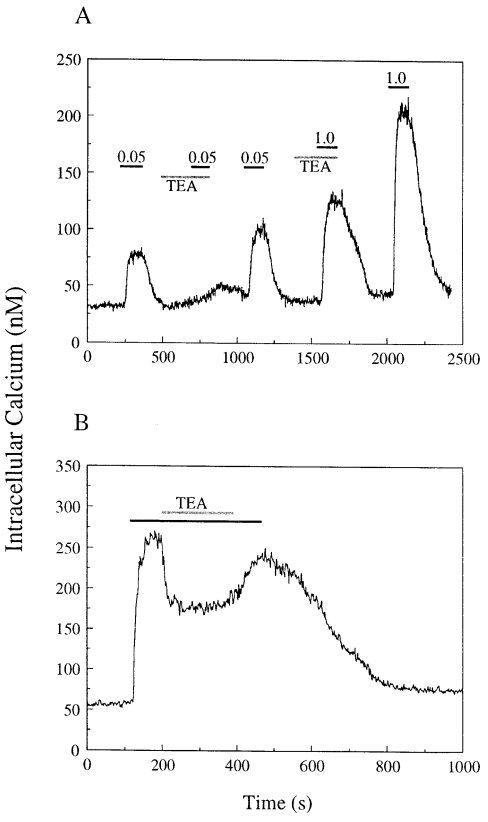

Fig. 6. (*A*) Effects of ACh (50 nmol/l and 1 μmol/l) on cytosolic free Ca^{2+} in mouse mandibular secretory endpieces in the presence and absence of 5 mmol/l TEA. In control experiments, studied with the same protocol of agonist washout and reapplication, the endpieces gave reproducible increases in $[Ca^{2+}]_i$ in response to repeated stimulation with ACh. (*B*) The effect of TEA (5 mmol/l) on the sustained increase in $[Ca^{2+}]_i$ induced by 1 μmol/l ACh. In each panel the duration of the exposure to ACh is shown by the black bars (with the concentration of acetylcholine used in μmol/l), and the exposure to TEA by the grey bars.

pieces had a resting $[Ca^{2+}]_i$ of 59 ± 9 nmol/l ($n = 13$) and, following exposure to 50 nmol/l ACh, $[Ca^{2+}]_i$ rose rapidly by 63 ± 5 nmol/l ($n = 9$) (Fig. 6*A*). In response to 1 μmol/l ACh, $[Ca^{2+}]_i$ rose by 262 ± 43 nmol/l ($n = 11$) (Fig. 6*A*). Pre-exposure of the endpieces to 5 mmol/l TEA reduced the rise in $[Ca^{2+}]_i$ following exposure to 50 nmol/l ACh by 92% to 8 ± 4 nmol/l ($n = 6$) (Fig. 6*A*). Following exposure to 1 μmol/l ACh in the presence of 5 mmol/l TEA, the increase in $[Ca^{2+}]_i$ was reduced by 42% to 178 ± 30 nmol/l ($n = 5$, $P = 0.236$), compared to the 1 μmol/l ACh control (Fig. 6*A*). When TEA (5 mmol/l) was added to the endpieces during sustained exposure to 1 μmol/l ACh, $[Ca^{2+}]_i$ fell by 46% ± 4 ($n = 4$) (Fig. 6*B*) and it returned to its previous stimulated level following the withdrawal of TEA (Fig. 6*B*).

It was not possible to use fura-2 to investigate the effects of quinine on the Ca^{2+} signal evoked by ACh because quinine interferes with the fluorescence signal.

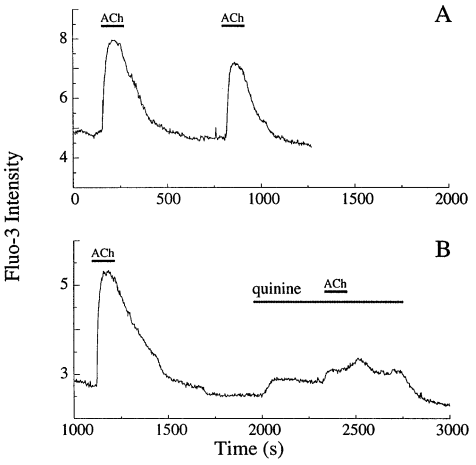

Fig. 7. Effects of ACh (1 μmol/l) and quinine (1 mmol/l) on the cytosolic-free Ca^{2+} as measured by fluo-3 fluorescence. The experimental details are given in the legend to Fig. 6.

Instead, we used the non-ratiometric Ca^{2+}-sensitive dye, fluo-3. In paired experiments, we found that 1 mmol/l quinine reduced the increase in the fluo-3 signal evoked by 1 μmol/l ACh from 2.84 ± 0.2 ($n = 3$) to 0.4 ± 0.1 ($n = 3$; Fig. 7*B*). An increase in the fluo-3 signal during the addition of quinine to the bathing solution, which is evident in Fig. 7*B*, is due to quinine itself increasing intracellular free Ca^{2+}, and could be prevented by the inclusion of EGTA in the bath solution.

Discussion

In this paper we demonstrate that the ACh-evoked whole-cell K^+ current in mouse mandibular cells is not directly blocked by TEA. Previous reports by Smith and Gallacher [35] that this current could be blocked by TEA can best be explained in terms of the ability of TEA to act as a muscarinic blocker [4, 34] antagonizing the action of ACh, which was used in a relatively low concentration (50 nmol/l) in Smith and Gallacher's experiments [35]. This possibility was probably not considered by Smith and Gallacher [35] because their experiments were performed under Cl^--free conditions which would have prevented their observing the telltale inhibition of the ACh-evoked whole-cell Cl^- current by TEA. Our conclusion is supported by the finding that TEA blocks the increase in cytosolic-free Ca^{2+} evoked by 50 nmol/l ACh completely (Fig. 6*A*), but has a smaller effect on that evoked by 1 μmol/l ACh (Fig. 6*A*). Furthermore, when we used ionomycin to activate the Ca^{2+}-sensitive whole-cell K^+ currents, we found that TEA had no effect (Fig. 3), indicating that BK channels in these cells do not make a significant contribution to the Ca^{2+}-activated K^+ conductance. It would thus appear that the mouse mandibular

gland conforms to the pattern found in the sheep parotid gland [15] in which BK channels play little or no role in the ACh-evoked K^+ current. A similar pattern seems also to be found in the rat mandibular [18] and the rat parotid [22, 34] glands.

The finding that TEA inhibits almost completely the resting K^+ conductance (Figs. 1 and 2) is consistent with the conclusion of earlier whole-cell studies which have suggested that BK channels dominate the resting K^+ conductance of mouse mandibular secretory cells [6, 10] and the finding that BK channels are the predominant ion channel observed in cell-attached patch studies on unstimulated mouse mandibular secretory cells [10, 11, 16]. The failure of quinine, which has also been reported to inhibit BK channels [17, 21], to block the resting K^+ conductance may be attributable to the quinine block of BK channels being voltage-dependent, as has been reported in type II pneumocytes [27]. It may also be attributable to the K^+ current at 0 mV in unstimulated mouse mandibular cells being carried by channels other than BK channels. The failure of single-channel studies to reveal this hypothetical TEA-sensitive, quinine-insensitive K^+ channel [11, 16] would require that the channel have a single-channel conductance below that resolvable by single channel methods (*ca.* 4 pS) or that it be localized in the apical membranes of the secretory cells.

We also show that the use of quinine to investigate the role of K^+ channels in the ACh-evoked K^+ current is complicated by its action as a muscarinic antagonist [13, 25], as is evident from its inhibition of both the K^+ current and the anion current activated by ACh (Fig. 4), and its inhibition of the $[Ca^{2+}]_i$ response evoked by ACh (Fig. 7*B*). We believe, however, that quinine also blocks the ACh-evoked K^+ current in mouse mandibular secretory cells directly, because when we used ionomycin to activate this current directly by increasing intracellular free Ca^{2+}, the K^+ conductance was blocked by quinine (Fig. 5). The recent report that the ACh-evoked K^+ efflux from rat mandibular glands is sensitive to quinine [20] may have been confounded by the muscarinic actions of quinine, although, in the rat mandibular gland, as in the mouse mandibular gland, whole-cell patch-clamp studies have shown that increasing the $[Ca^{2+}]_i$ activates a quinine-sensitive K^+ current [18]. In sheep parotid cells, however, there is direct evidence that the ACh-evoked K^+ current is blocked by quinine [15] since the failure of quinine to block the ACh-evoked anion current [15] indicates that its action as a muscarinic antagonist is not important at least in this tissue. Thus, in mouse mandibular, rat mandibular and sheep parotid secretory cells, the ACh-activated K^+ channel appears to be blocked by quinine.

In addition to its actions as a muscarinic antagonist (*see above*), quinine increases $[Ca^{2+}]_i$ (Fig. 7) and potentiates the activation of the inward current by ionomycin (Fig. 5*A*). The mechanism of this potentiation is unknown. In sheep parotid cells, quinine may also activate the ACh-evoked current at −80 mV [15] although the apparent increase we reported in that study failed to reach statistical significance. Thus the phenomenon may not be unique to the mouse mandibular gland. The anion replacement data (Fig. 5*B*) suggest that quinine may be activating an anion current, although recent reports that intracellular Cl^- controls the activity of a variety of membrane transport systems [7, 8] including Ca^{2+}-activated nonselective cation channels [37] indicates that this conclusion should be regarded as tentative.

The finding that Rb^+ has a significant conductance through the ACh-activated K^+ conductance further distinguishes it from the BK channel seen in mouse mandibular secretory cells, which, unlike that in sheep parotid secretory cells [38], does not have a significant conductance for Rb^+ (T. Hayashi and D.I. Cook, *unpublished data; see also* ref. [12]). The ACh-evoked K^+ current in mouse mandibular cells thus resembles the Ca^{2+}-activated TEA-insensitive K^+ current in rat mandibular cells, which also has a significant conductance for Rb^+ [18].

The ACh-activated K^+ conductance in mouse mandibular secretory cells is thus: (i) not directly blocked by TEA; (ii) blocked by quinine; (iii) conductive to Rb^+. These three properties match those of the 40 pS K^+ channel that is activated by ACh in mouse mandibular secretory cells [16]. This 40 pS K^+ channel is not active in cell-attached patches on unstimulated mouse mandibular secretory cells, but during stimulation with ACh it accounts for the bulk (80% or more) of the K^+ current through cell attached patches [16]. The results of these single-channel studies [16], taken together with whole-cell studies in the present paper thus indicate that this 40 pS K^+ channel underlies the ACh-activated K^+ conductance in mouse mandibular cells.

This project was supported by the Australian Research Council.

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