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The ACh-induced Whole-Cell Currents in Sheep Parotid Secretory Cells. Do BK Channels Really Carry the ACh-evoked Whole-Cell K^+ Current?

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Abstract. As in other salivary glands, the secretory cells of the sheep parotid have a resting K^+ conductance that is dominated by BK channels, which are activated by acetylcholine (ACh) and are blocked by tetraethylammonium (TEA). Nevertheless, perfusion studies indicate that TEA does not inhibit ACh-evoked fluid secretion or K^+ efflux from intact sheep parotid glands. In the present study, we have used whole-cell patch clamp techniques to show that ACh activates K^+ and Cl^- conductances in sheep parotid secretory cells by increasing intracellular free Ca^{2+}, and we have compared the blocker sensitivity of the ACh-evoked whole-cell K^+ current to the previously reported blocker sensitivity of the BK channels seen in these cells.

The ACh-induced whole-cell K^+ current was not blocked by TEA (10 mmol/l) or verapamil (100 μmol/l), both of which block the resting K^+ conductance and inhibit BK channels in these cells. Quinine (1 mmol/l) and quinidine (1 mmol/l), although only weak blockers of the resting K^+ conductance, inhibited the ACh-evoked current at 0 mV (K^+ current), by 68% and 78%, respectively. 4-Aminopyridine (10 mmol/l) partially inhibited the ACh-induced K^+ current and caused it to fluctuate. It also caused the resting membrane currents to fluctuate, possibly by altering cytosolic free Ca^{2+}. Ba^{2+} (100 μmol/l), a blocker of the inwardly rectifying K^+ conductance in sheep parotid cells, had no effect on the ACh-induced K^+ current.

We conclude that the ACh-induced K^+ conductance in sheep parotid cells is pharmacologically distinct from both the outwardly rectifying (BK) K^+ conductance and the inwardly rectifying K^+ conductance seen in unstimulated cells. Given that in vitro perfusion and K^+ efflux studies on other salivary glands in which BK channels dominate the resting conductance (e.g., the rat mandibular, rat parotid and mouse mandibular glands) have revealed an insensitivity to TEA, suggesting that BK channels do not carry the ACh-evoked K^+ current, we propose that BK channels do not contribute substantially to the K^+ current evoked by ACh in the secretory cells of most salivary glands.

Key words: K^+ and Cl^- currents — Tetraethylammonium — Verapamil — Quinine — 4-Aminopyridine — BK channels

Introduction

Beginning with the earliest patch clamp studies [21, 27], large-conductance, voltage- and Ca^{2+}-activated K^+ channels (BK channels) have frequently been reported in resting salivary secretory cells [4]. Thus, in whole-cell studies on unstimulated salivary secretory cells, BK channels have been shown to be the predominant outward conductance [3, 12, 22, 31] and in cell-attached patch studies they have been shown to be activated by acetylcholine (ACh) [9, 24, 25]. They have thus widely been assumed to underlie the ACh-activated K^+ conductance seen in salivary secretory cells [27]. Nevertheless, there are studies in which the ACh-activated K^+ conductance has been investigated in salivary secretory cells by whole-cell patch clamp techniques [17] or, indirectly, by observation of the effect of K^+ channel blockers on the rate of salivary secretion [7, 37] or K^+ efflux [6, 18, 32, 37], which have given results that suggest that BK channels do not carry the outward K^+ current evoked by ACh.

The secretory cells of the sheep parotid exhibit an outwardly rectifying K^+ current [16], an inwardly rectifying K^+ current [16] and a Ca^{2+}-evoked Cl^- current [14]. The inwardly rectifying K^+ current has been shown to be carried by the classical "inward rectifier" [16] and

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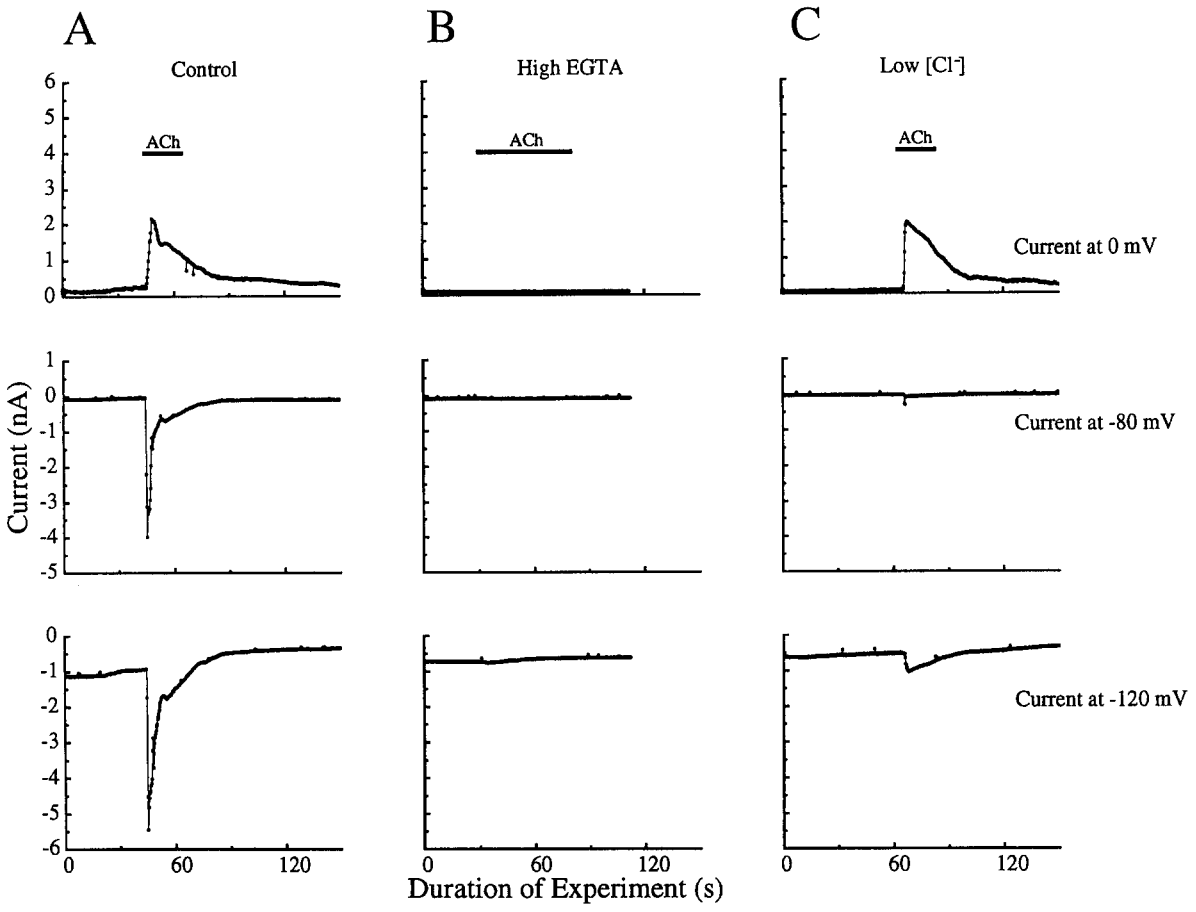

Fig. 1. (*A*) Acetylcholine (1 μmol/l) induced changes in membrane currents recorded from a single sheep secretory cell under whole-cell clamp conditions. The holding potential was −60 mV and pulses to −120, −80 and 0 mV were applied in a cycle lasting 260 msec. The pipette contained the standard KCl solution and the bath contained the standard NaCl solution. (*B*) Representative recording of the membrane current response to 1 μmol/l ACh when the pipette EGTA concentration was increased to 10 mmol/l EGTA. (*C*) Representative recording of the membrane current response to 1 μmol/l ACh under low Cl^- conditions. Both the pipette and bath solutions contained 4 mmol/l Cl^- with the remainder of the Cl^- replaced with glutamate.

the outwardly rectifying K^+ current seen in unstimulated cells has been attributed to BK channels on the basis of blocker sensitivity [15] and cell-attached patch studies [36]. Cell-attached patch studies have also shown that the BK channels can be activated by the addition of ACh to the bath [36]. Nevertheless, tetraethylammonium (TEA), a known blocker of BK channels, fails to block the K^+ efflux and fluid secretion seen in sheep parotid glands stimulated by ACh [37].

In the present study, we have used whole-cell patch clamp techniques to investigate the blocker sensitivity of the ACh activated K^+ current in sheep parotid secretory cells and have compared it to the blocker sensitivity profile of the K^+ currents seen in the resting cells.

Materials and Methods

The cell preparation method used in the present experiments was the same as previously described [16]. Cross-bred sheep were killed with a captive-bolt pistol and the parotid glands were excised, placed in a physiological salt solution, finely chopped, and the pieces incubated for 20 min in our standard NaCl-rich bathing solution to which collagenase (0.5 mg/ml, Worthington Type 4, Freehold, NJ) had been added. The tissue was then incubated for 5 min in a similar salt solution containing trypsin (0.5 mg/ml) and once again incubated in a collagenase-containing solution for 15 min. The fluid containing the disaggregated tissue was then passed through 200 μm and 75 μm nylon mesh filters and the dispersed single cells so obtained were suspended once more in the standard NaCl-rich bath solution.

Membrane currents were recorded in the whole-cell patch clamp configuration using an Axopatch-1C amplifier (Axon Instruments, Foster City, CA) [16] from single cells in a continuously perfused chamber (volume 0.3 ml). The method we used to study the ACh-stimulated currents was similar to that used in other studies of this type [4, 31]. The membrane potential was held at −60 mV and a command pulse sequence consisting of a hyperpolarization to −120 mV (20 msec duration), followed by a hyperpolarization to −80 mV (20 msec duration) and then a depolarization to 0 mV (30 msec duration) with intervening periods held at −60 mV was repetitively applied at 260 msec intervals. The current was low-pass filtered, sampled and digitized at 0.2 kHz with a MacLab8 data aquisition interface (AD Instruments, Sydney,

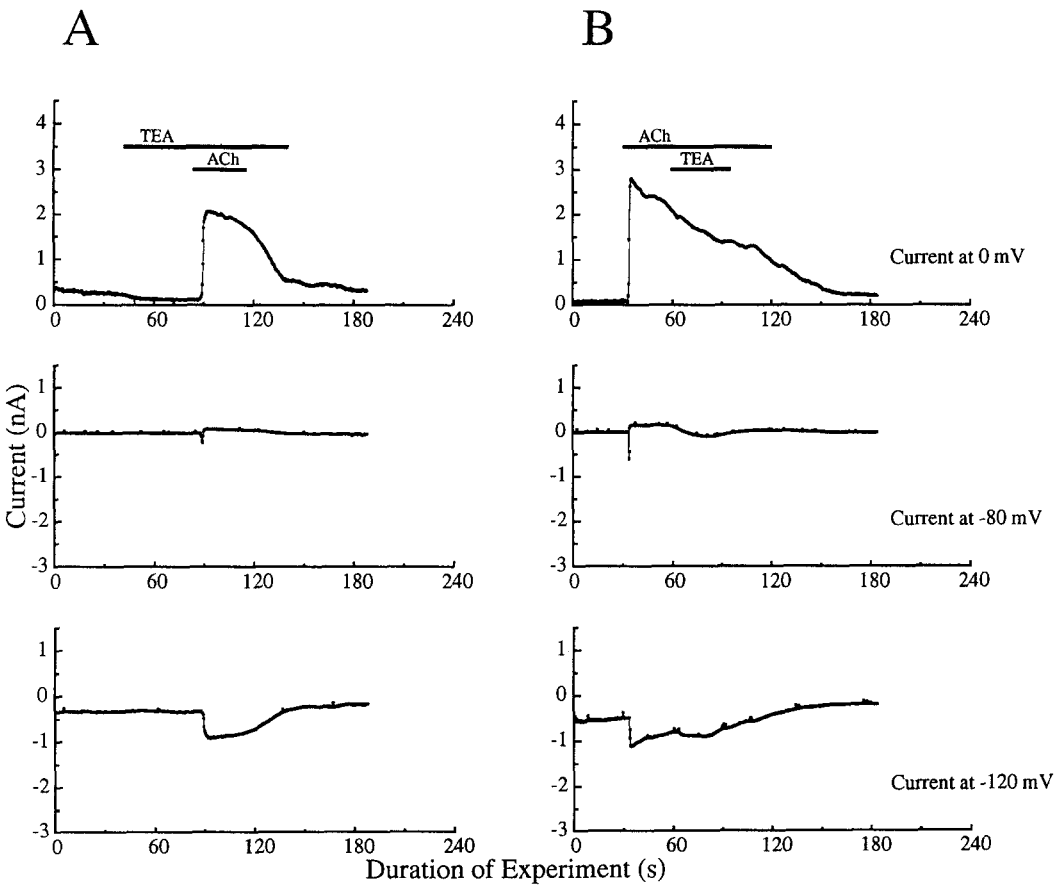

Fig. 2. Effects of tetraethylammonium (TEA) on ACh-evoked membrane currents. In both pipette and bath solutions all but 4 mmol/l Cl^- was replaced with glutamate. (*A*) 10 mmol/l TEA was applied prior to 1 μmol/l ACh as indicated. (*B*) 10 mmol/l TEA applied during the sustained phase of the 1 μmol/l ACh response. (*A*) and (*B*) are representative records obtained from different cells.

Australia) attached to a Macintosh IIvx computer. The reference electrode was a Ag/AgCl pellet placed in the bath. To avoid the effects of varying liquid junction potentials, the Cl^- concentration in the bath solution was not altered during the course of an experiment. All experiments were performed at room temperature (20–22°C).

The standard NaCl-rich bathing solution contained (in mM): 145 NaCl, 5 KCl, 1 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 10 D-glucose, 7.5 HEPES, 7.5 NaHEPES (pH 7.4 adjusted with NaOH). For the Cl^- replacement experiments, the bathing solution contained (in mM): 145 Na-glutamate, 5 K-glutamate, 1 $CaCl_2$, 1 $MgCl_2$, 1.2 NaH_2PO_4, 10 D-glucose, 7.5 HEPES, 7.5 NaHEPES (pH 7.4 adjusted with NaOH). The standard KCl-rich pipette solution contained (in mM): 140 KCl, 1 $MgCl_2$, 10 D-glucose, 0.5 EGTA, 10 H-HEPES (pH 7.4 adjusted with KOH). For the Cl^- replacement experiments, the pipette solution contained (in mM): 138 K-glutamate, 2 KCl, 1 $MgCl_2$, 10 D-glucose, 0.5 EGTA, 10 H-HEPES (pH adjusted to 7.4 with KOH). All chemicals were obtained from Sigma Chemical (St. Louis, MO). Results are given as means ± SEM with the number of cells examined (n) in parentheses.

Results

Effects of ACh on Whole-Cell Currents

Under the conditions of the present experiments, 0 mV is close to the zero current potential for Cl^- conductances and −80 mV is close to the zero current potential for K^+ conductances [4, 31]. The whole-cell current at 0 mV should thus measure the current carried by the outwardly rectifying K^+ conductance and the whole-cell current at −80 mV should measure the current carried by the Cl^- conductance, as has previously been reported in studies on these cells [14, 16]. In addition to the outwardly rectifying K^+ conductance, sheep parotid secretory cells also contain an inwardly rectifying K^+ conductance [16] so that the whole-cell current at −120 mV should reflect the currents carried by it and by the Cl^- conductance.

When we clamped single sheep parotid secretory cells repetitively at these three voltages (*see* Materials and Methods) using the standard KCl-rich pipette solution and the standard NaCl-rich bath solution and exposed the cell to a 30 sec pulse of ACh (1 μmol/l), the outward current at 0 mV and the inward currents at −80 and at −120 mV were increased (Fig. 1*A*). The responses consisted of an initial transient peak, followed by a sustained phase of lower magnitude. The outward current at 0 mV reached a peak of 1.56 ± 0.21 nA (n = 13) at 2.24 ± 0.35 sec (n = 13) after the start of the ACh pulse and the inward currents at −80 and −120 mV reached peaks,

Table. Effects of K^+ channel blockers on ACh-induced currents

Blocker	Dose (mmol/l)	Peak current at 0 mV (nA)	*P*	Peak current at −120 mV (nA)	*P*	Inhibition of resting current at 0 mV (%)	Current at 0 mV in sustained phase (nA)[a]
TEA	10.0	1.64 ± 0.28 (n = 10)	0.300 (NS)	−0.57 ± 0.15 (n = 10)	0.373 (NS)	75.5 ± 7.3 (n = 10)	1.04 ± 0.29 (n = 12)
Control		1.94 ± 0.29 (n = 7)		−0.45 ± 0.10 (n = 7)			
Verapamil	0.1	1.60 ± 0.33 (n = 4)	0.900 (NS)	−0.40 ± 0.15 (n = 4)	0.864 (NS)	60.7 ± 13.0 (n = 4)	0.60 ± 0.24 (n = 17)
Control		1.66 ± 0.36 (n = 4)		−0.45 ± 0.23 (n = 4)			
Quinine	1.0	0.57 ± 0.18 (n = 10)	0.001	−0.18 ± 0.06 (n = 10)	0.013	57.5 ± 8.9 (n = 10)	0.06 ± 0.03 (n = 4)
Control		1.76 ± 0.39 (n = 7)		−0.39 ± 0.11 (n = 7)			
Quinine	0.1	1.15 ± 0.13 (n = 4)	0.045	−0.38 ± 0.04 (n = 4)	0.035		
Control		1.76 ± 0.02 (n = 2)		−0.62 ± 0.05 (n = 2)			
Quinidine	1.0	0.40 ± 0.14 (n = 4)	0.001	−0.15 ± 0.05 (n = 4)	0.009	14.0 ± 3.1 (n = 4)	0.09 ± 0.03 (n = 4)
Control		1.54 ± 0.12 (n = 4)		−0.39 ± 0.04 (n = 4)			
4-Aminopyridine	10.0	0.77 ± 0.11 (n = 6)	0.009	−0.22 ± 0.08 (n = 6)	0.036	[b]	[b]
Control		1.51 ± 0.24 (n = 7)		−0.41 ± 0.05 (n = 7)			
Ba^{2+}	0.1	1.37 ± 0.18 (n = 5)	0.334 (NS)	−0.47 ± 0.14 (n = 5)	0.255 (NS)		
Control		1.66 ± 0.26 (n = 5)		−0.31 ± 0.04 (n = 5)			
Control for sustained phase							1.37 ± 0.22 (n = 5)

All results were obtained by 1 μmol/l ACh stimulation in low Cl^- conditions. The significances of the differences between ACh-induced peak currents with and without blocker were determined using the unpaired *t*-test.

[a] The ACh-induced sustained outward current was measured 40–45 sec after the start of the ACh pulse, i.e., 10–15 sec after the addition of the blocker.

[b] Not calculated in 4-aminopyridine experiments because it caused oscillations in membrane currents.

respectively, of −3.61 ± 0.73 nA (n = 13) and −4.70 ± 0.82 nA (n = 13), at 1.04 ± 0.73 sec (n = 13) after the start of the ACh pulse. When Cl^--rich pipette solutions were used, the time courses of the inward currents at −80 and −120 mV were similar to each other but were quite distinct from the time course of the current at 0 mV (Fig. 1*A*). When the Ca^{2+}-chelating capacity of the pipette solution was increased by raising the EGTA concentration from 0.5 to 10 mmol/l, the currents induced by 1 μmol/l ACh were completely abolished (n = 3, Fig. 1*B*).

To examine the contribution of the Cl^- conductance to the ACh-evoked current, we studied the effects of ACh (1 μmol/l) when all but 4 mmol/l Cl^- in the pipette and bath solutions had been replaced by glutamate (Fig. 1*C*). Under these conditions, the current induced by 1 μmol/l ACh at 0 mV (2.08 ± 0.08 nA, n = 105) was not significantly different from the current observed with normal Cl^--rich solutions in the pipette and bath (1.56 ± 0.21 nA, n = 13), whereas the current induced by 1 μmol/l ACh at −80 mV was reduced from −3.61 ± 0.73 nA (n = 13) to −0.23 ± 0.02 nA (n = 105). The current induced by 1 μmol/l ACh at −120 mV was decreased from −4.70 ± 0.82 nA (n = 13) to −0.57 ± 0.03 nA (n = 105) and its time course closely paralleled the time course of the current at 0 mV (Fig. 1*C*).

Effects of K^+ Channel Blockers on ACh-induced Currents

To study the effects of K^+ channel blockers on the ACh response, we used low-Cl^- solutions to eliminate the contribution of the Cl^- conductance to the current at −120 mV. We have shown previously that TEA blocks

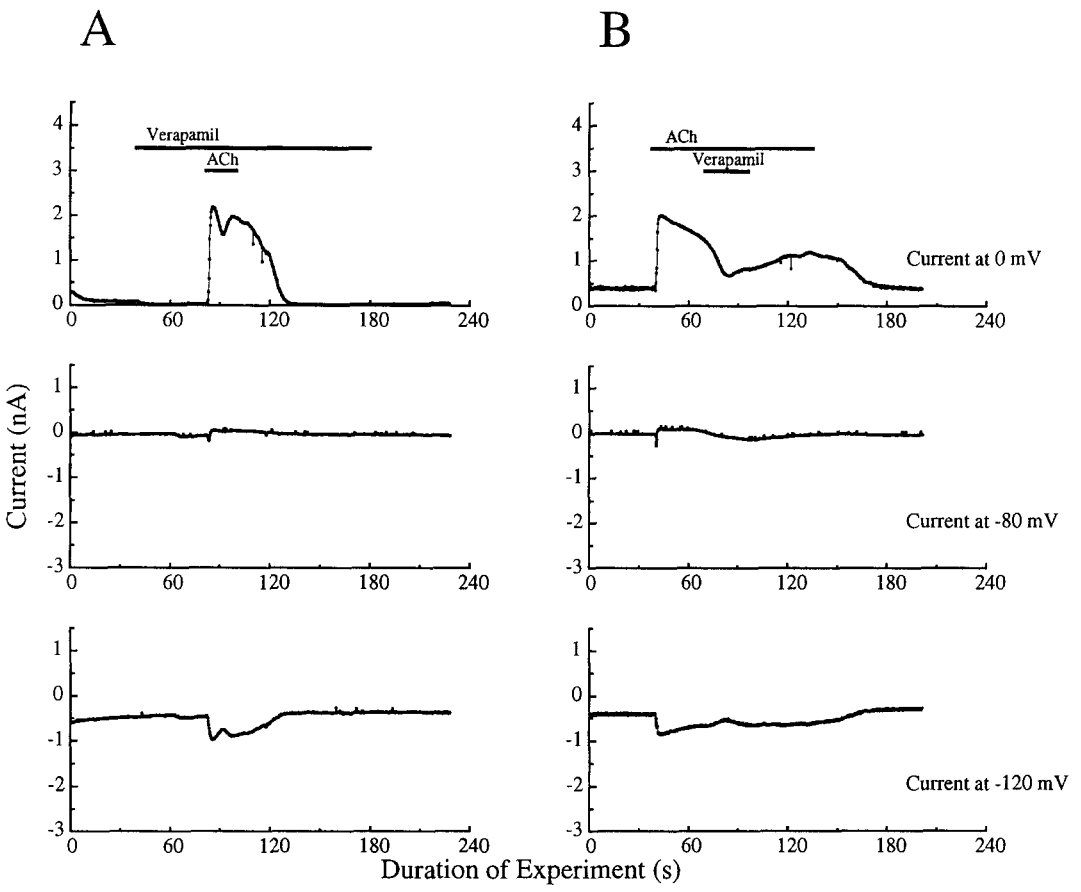

Fig. 3. Effects of verapamil on ACh-induced membrane currents. In both the pipette and bath solutions all but 4 mmol/l Cl^- were replaced with glutamate. (*A*) 100 μmol/l verapamil was applied prior to 1 μmol/l ACh as indicated. (*B*) 100 μmol/l verapamil applied during the sustained phase of the 1 μmol/l ACh response. (*A*) and (*B*) are representative records obtained from different cells.

the outwardly rectifying K^+ current and the BK channels in resting sheep parotid cells [5, 16] without affecting the inwardly rectifying K^+ current or the 30 pS K^+ channel which is believed to carry it [5, 16]. In the present study, as shown in Fig. 2*A* and the Table, we found that the resting current at 0 mV was inhibited by 75.5% ± 7.3 (n = 10) during the extracellular application of TEA (10 mmol/l). This agrees with our earlier studies which showed that TEA inhibited the resting current at 0 mV by 80% [16]. Surprisingly, however, when 1 μmol/l ACh was then added to the bath, the ACh-induced currents at both 0 mV and −120 mV were not significantly different from those observed in the absence of TEA (Fig. 2, Table).

We also investigated whether TEA had any effect on the sustained phase of the ACh response (Fig. 2*B*). TEA application did not significantly (P = 0.489) reduce the sustained phase of the outward current. This was calculated by comparing the ACh-evoked outward current recorded between 40 and 45 sec after the start of the ACh pulse (i.e., between 10 and 15 sec after addition of the blocker) in the presence of TEA with the current recorded during the comparable period during ACh exposure under control conditions (Table).

Verapamil has also been found to block outwardly rectifying K^+ currents and BK channels in unstimulated sheep parotid secretory cells [15] without affecting the inwardly rectifying K^+ current [15]. In our previous studies, verapamil (100 μmol/l) inhibited the whole-cell current at 0 mV by 72% in unstimulated cells [15]. In the present study, we found that 100 μmol/l verapamil reduced the current at 0 mV in unstimulated sheep parotid cells by 60.7% ± 13.0 (n = 4), but it failed to suppress the ACh-induced transient currents (Fig. 3*A*, Table). Verapamil did, however, significantly (P = 0.005) reduce the sustained current induced by ACh in low-Cl^- conditions (Fig. 3*B*, Table). Then when we used the standard Cl^--containing pipette and bath solutions, we found that verapamil significantly (P = 0.048) reduced the ACh-induced sustained outward current at 0 mV from 1.20 ± 1.36 nA (n = 3) to 0.32 ± 0.08 nA (n = 6) and the sustained inward current at −80 mV from −0.57 ± 0.14 nA (n = 3) to −0.25 ± 0.06 nA (n = 6). Since it affected both the K^+ and the Cl^- currents, this

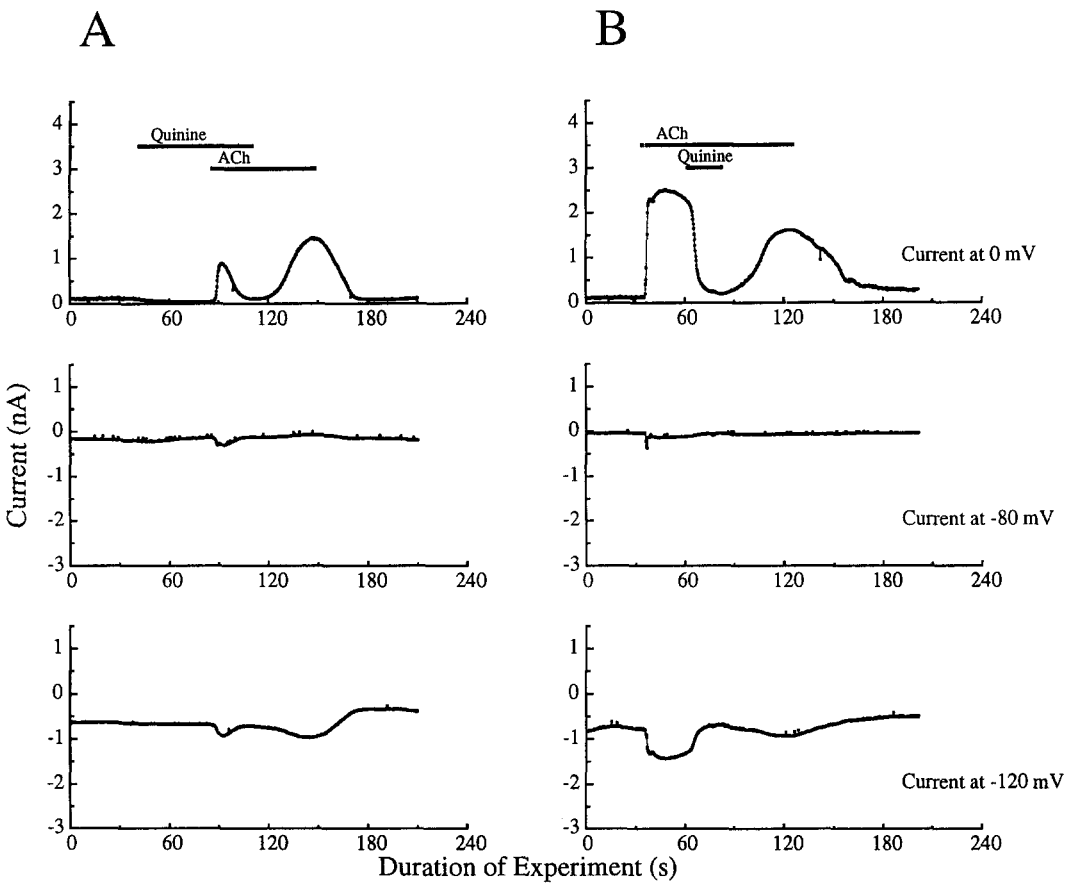

Fig. 4. Effects of quinine on ACh-induced membrane currents. Both the pipette and bath solutions had all but 4 mmol/l Cl^- replaced with glutamate. (*A*) 1 mmol/l quinine was applied prior to 1 μmol/l ACh as indicated. (*B*) 1 mmol/l quinine applied during the sustained phase of the 1 μmol/l ACh response. (*A*) and (*B*) are representative records obtained from different cells.

suggests that verapamil acted, not as a channel blocker, but as a blocker at some point prior to channel activation, perhaps at the receptor as an antagonist (*see* Discussion).

Quinine blocks the outwardly rectifying whole-cell K^+ current and the BK channels in unstimulated sheep parotid secretory cells [16] but in our previous studies it failed to produce a statistically significant reduction in the inwardly rectifying K^+ current [16]. In the present study, we find that quinine (1 mmol/l) reduces the whole-cell current at 0 mV by 57.5% ± 8.9 (n = 10) and the inward current at −120 mV by 28.7% ± 3.4 (n = 10). As shown in Fig. 4*A*, subsequent addition of 1 μmol/l ACh causes only a small, transient increase in the currents at 0 mV and −120 mV (Table). After removal of quinine, the ACh-induced currents gradually increased (Fig. 4*A*). In a concentration of 100 μmol/l, quinine had a smaller inhibitory effect (Table).

We also observed the effects of quinine on the sustained phase of the ACh response. At a concentration of 1 mmol/l, quinine strongly reduced the sustained currents induced by 1 μmol/l ACh, both at 0 mV and at −120 mV, and this effect was reversible (Fig. 4*B*, Table). Because we were concerned that quinine might have been acting as a muscarinic blocker [2, 13], we also examined whether it inhibited the ACh-evoked Cl^- current observed at −80 mV with the standard KCl-rich pipette solution. Under these conditions, we found that 1 mmol/l quinine reduced the ACh-evoked K^+ current (at 0 mV) from 1.37 ± 0.33 nA (n = 9) to 0.41 ± 0.29 nA (n = 6), but failed to inhibit (P = 0.318) the ACh-evoked Cl^- current (at −80 mV) (control: −4.0 ± 1.47 nA, n = 9, quinine −6.26 ± 2.98 nA, n = 6).

Quinidine, the D-stereoisomer of quinine, inhibits the outwardly rectifying K^+ current in unstimulated sheep parotid cells and produces a small, but statistically significant reduction in the inwardly rectifying K^+ current [15]. In the present study, as shown in Fig. 5*A*, we found that quinidine (1 mmol/l) reduced the outward current at 0 mV by 14.0% ± 3.1 (n = 4) and inhibited (P = 0.013) the inward current at −120 mV by 34.5% ± 7.5 (n = 4). Despite its relatively weak effect on the resting currents, it inhibited both the initial transient and the sustained membrane currents evoked by ACh (Fig. 5, Table). Because we were concerned that quinidine

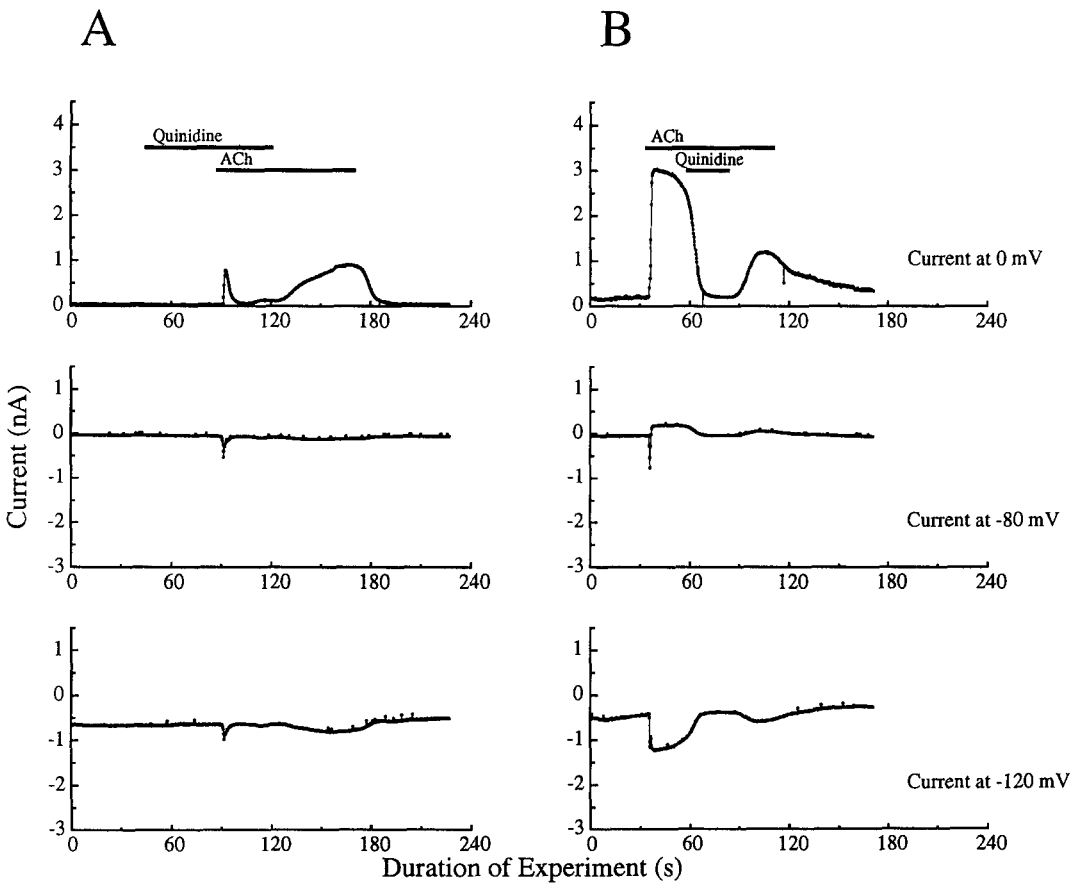

Fig. 5. Effects of quinidine on ACh-induced membrane currents. Both the pipette and bath solutions had all but 4 mmol/l Cl^- replaced with glutamate. (*A*) 1 mmol/l quinidine was applied prior to 1 μmol/l ACh as indicated. (*B*) 1 mmol/l quinidine applied during the sustained phase of the 1 μmol/l ACh response. (*A*) and (*B*) are representative records obtained from different cells.

might have been acting as a muscarinic blocker [2, 13], we examined whether it inhibited the ACh-evoked Cl^- current recorded at −80 mV with the standard KCl-rich pipette solution. Under these conditions, we found that 1 mmol/l quinidine reduced the ACh-evoked K^+ current (at 0 mV) from 1.37 ± 0.33 nA (n = 9) to 0.36 ± 0.20 nA (n = 5), but failed (P = 0.409) to inhibit the ACh-evoked Cl^- current (control: −4.0 ± 1.47 nA, n = 9; quinidine: −2.70 ± 1.02 nA, n = 5).

In our earlier study [15], 4-aminopyridine (4-AP) did not affect the resting whole-cell currents in unstimulated sheep parotid secretory cells. As shown in Fig. 6*A*, we now find that 4-AP caused the currents in resting cells to fluctuate in five of six cells studied. The magnitude of the fluctuations at 0 mV was 0.6 ± 0.23 nA (n = 5 cells) and their frequency was 0.7 ± 0.1 per 10 sec (n = 5 cells). 4-AP also inhibited the ACh-evoked K^+ current at 0 mV and at −120 mV (Fig. 6*A;* Table). We also studied the effects of 4-AP on the sustained phase of the ACh-evoked currents (Fig. 6*B*, Table). In a concentration of 10 mmol/l, in five cells, 4-AP reversibly decreased the ACh (1 μmol/l) response and the currents fluctuated in a manner similar to what we observed in the absence of ACh.

Ba^{2+} has been previously reported to block the inwardly rectifying K^+ current in unstimulated sheep parotid cells, producing complete blockade at a concentration of 100 μmol/l [15]. At this concentration, it has previously been shown to have no effect on the outwardly rectifying K^+ current [15]. In the present study, we found that Ba^{2+} (100 μmol/l), reduced the resting current at −120 mV by 80.4% ± 4.1 (n = 5) without affecting the resting current at 0 mV (−6.0% ± 11.2, n = 5). The presence of Ba^{2+} (100 μmol/l) in the bath had no effect on the magnitude of the ACh-induced currents at 0 mV or at −120 mV (Fig. 7, Table).

Discussion

In this study, we have shown that TEA, which blocks the outwardly rectifying K^+ current seen in resting sheep parotid cells [15], fails to block the ACh-evoked K^+ current. Verapamil, which also blocks the outwardly recti-

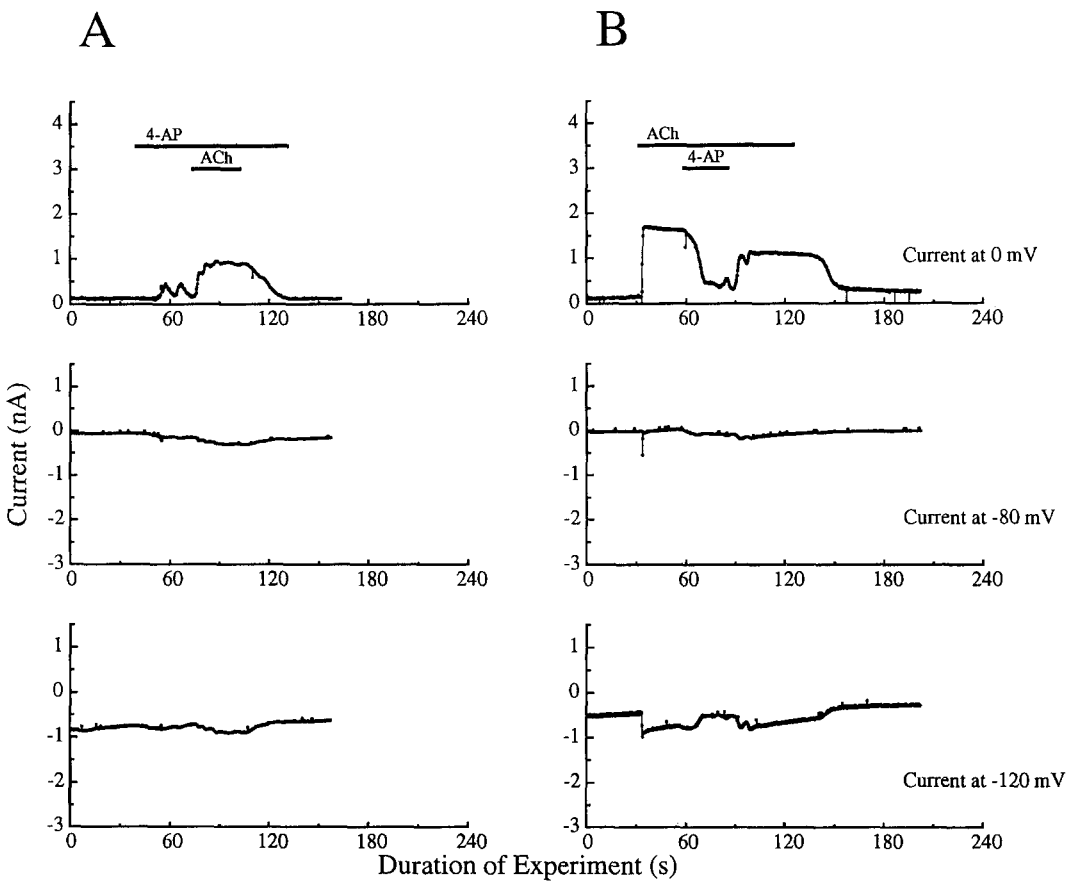

Fig. 6. Effects of 4-aminopyridine (4-AP) on ACh-induced membrane currents. Both the pipette and bath solutions had all but 4 mmol/l Cl^- replaced with glutamate. (*A*) 10 mmol/l 4-AP was applied prior to 1 μmol/l ACh. (*B*) 10 mmol/l 4-AP was applied during the sustained currents induced by 1 μmol/l ACh. (*A*) and (*B*) are representative records obtained from different cells.

fying K^+ current in resting cells [15], failed to inhibit the transient phase of the ACh-evoked K^+ current but did reduce the sustained phase significantly, perhaps by altering Ca^{2+} entry, as has been proposed in ciliary muscle [33], or by acting as a muscarinic antagonist [1], although why the sustained phase of the response would be more sensitive to muscarinic blockade than the transient phase is unclear. We have further shown that Ba^{2+}, which blocks the inwardly rectifying K^+ current in unstimulated sheep parotid cells, fails to block the ACh-evoked K^+ current. Thus, our blocker studies suggest that neither of the two conductances carrying K^+ currents in unstimulated sheep parotid cells [15, 36] is a major carrier of the ACh-evoked K^+ current.

The demonstration that the ACh-evoked K^+ current in sheep parotid cells is not sensitive to TEA provides an explanation for the failure of TEA to inhibit ACh-induced fluid secretion and ACh-induced K^+ secretion by sheep parotid glands [37]. The question immediately arises, of course, of whether ACh-activated K^+ conductances in other exocrine secretory tissues are also insensitive to TEA. In the rat parotid and rat mandibular glands, BK channels have been demonstrated by single channel patch clamp methods and, as in the sheep parotid, they appear to be responsible for the dominant resting K^+ conductance [4, 12, 22] and to be inhibitable by TEA [4, 24]. BK channels have also been shown to be activated by ACh in cell-attached patches in rat parotid [32], mouse mandibular [9, 10], and sheep parotid glands [36]. Nevertheless, as found here for the sheep parotid, when whole-cell methods were used to investigate the ACh-evoked K^+ currents in rat parotid, they were found not to be sensitive to TEA [17, 30]. Similarly, there is good evidence to show that TEA does not block muscarinically evoked K^+ efflux or fluid secretion in the rat parotid [6], the bovine parotid [18], or the rat mandibular gland [7], notwithstanding that some studies have shown that TEA can block responses to low doses of ACh and to less potent muscarinic agonists in some glands, such as the rat pancreas [8], the rat mandibular gland [8], the rat parotid [6, 32], and the sheep parotid [37]. It has become clear that these apparent contradictions arise because TEA can act as a weak muscarinic blocking agent in many glands [5, 30].

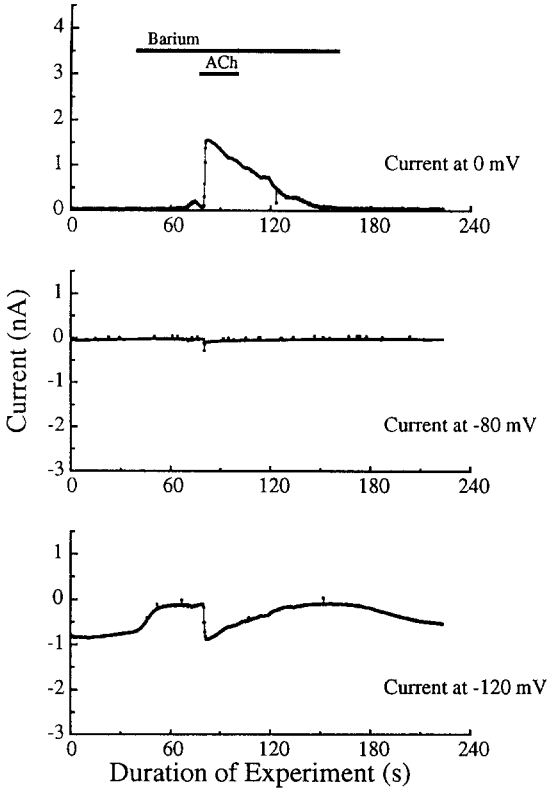

Fig. 7. Representative recording of the effects of Ba^{2+} (100 μmol/l) on ACh-induced membrane currents. Both the pipette and bath solutions had all but 4 mmol/l Cl^- replaced with glutamate.

Given the known sensitivity of BK channels to TEA [26], it seems safe to conclude that these channels do not make a major contribution to the ACh-evoked response of secretory cells in the bovine parotid [18], the sheep parotid [37], and the rat parotid [17, 30] and mandibular [7] glands. Nevertheless, there appear to be a few exocrine glands in which BK channels may have a role to play in supporting agonist-evoked K^+ currents. (i) Avian salt glands have been reported to have BK channels in cell-attached patches [28] and the K^+ current evoked by a Ca^{2+} ionophore can be blocked by TEA [19]. (ii) Similarly, in pig pancreatic acinar cells, BK channels are present in cell-attached patches and the whole-cell K^+ current evoked by CCK-5 is blocked by TEA [34]. (In this latter case, it cannot be excluded that TEA may have blocked the CCK receptors but, since TEA has been reported not to block pancreatic amylase secretion evoked by CCK in another mammalian species (guinea pig) [23], this possibility seems unlikely.) (iii) In lacrimal glands, BK channels, which dominate the resting conductance [35] and are activated by the addition of carbamylcholine [35] and ACh [20], have been studied by ensemble noise analysis and it has been claimed on the basis of the results that BK channels are responsible for the K^+ current evoked by carbamylcholine [35]. (iv) Finally, in mouse mandibular glands, it has been reported that TEA inhibits ACh-evoked K^+ currents studied by whole-cell techniques [31] although our own recent studies indicate that this is because TEA acts as a muscarinic antagonist (T. Hayashi, P. Poronnik, J.A. Young and D.I. Cook, *manuscript in preparation*).

The present data do not deny that ACh activates the BK channels, quite the contrary, we know that it does [36]: they simply indicate that BK channels are not the dominant ACh-activated K^+ conductances. The data do, however, raise the question of why channels corresponding to the major ACh-activated K^+ conductance have not been reported in cell-attached patch studies. A possible explanation is that cell-attached patch studies may have focused attention on the BK channels because they are easily observed. Alternatively, the cell-attached patch technique may have distorted the patch environment and altered the pattern of channel activation in favor of the BK channels. Yet another possibility is that the predominant ACh-activated K^+ conductance may actually be in the luminal membrane and hence inaccessible in conventional cell-attached patch studies. We are currently investigating these various possibilities. It is clear, however, that the blocker sensitivities observed in the whole-cell studies on sheep parotid and other salivary glands agree closely with those observed in studies on fluid secretion and K^+ efflux from intact glands [37], so the blocker sensitivities seem less likely to be artefactual than the findings of cell-attached patch studies, which predict quite different sensitivity patterns.

Our finding that 4-aminopyridine (4-AP) induced fluctuations in the resting current suggests that this agent may have actions on sheep parotid cells other than those of a K^+ channel blocker. Since it causes similar fluctuations in the currents during ACh exposure, it is not clear whether the inhibition of the ACh-induced current is a result of channel blockade or a consequence of a derangement in intracellular signalling. Since 4-AP has been previously reported to increase intracellular free Ca^{2+} [11] and to activate Ca^{2+}-activated K^+ channels in GH3 anterior pituitary cells by increasing intracellular Ca^{2+} [29], it would appear likely that it exerts at least part of its action in sheep parotid cells in the same way.

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