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Effects of K^+ Channel Blockers on Inwardly and Outwardly Rectifying Whole-Cell K^+ Currents in Sheep Parotid Secretory Cells

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Summary. We have used whole-cell patch-clamp techniques to examine the sensitivities of the inwardly and the outwardly rectifying K^+ currents in sheep parotid cells to K^+ channel blockers. Extracellular tetraethylammonium ($ID_{50} \approx 200$ μmol/liter), quinine ($ID_{50} \approx 100$ μmol/liter), verapamil ($ID_{50} \approx 30$ μmol/liter) and charybdotoxin ($ID_{50} < 0.1$ μmol/liter) reduced the outwardly rectifying current but had no effect on the inwardly rectifying current. Quinidine inhibited the outwardly rectifying current ($ID_{50} \approx 200$ μmol/liter) and, at a concentration of 1 mmol/liter, reduced the inwardly rectifying current by 35%. Extracellular Ba^{2+} inhibited both the inwardly and outwardly rectifying K^+ currents but the inwardly rectifying K^+ current was more sensitive to it ($ID_{50} \approx 1$ μmol/liter) than was the outwardly rectifying K^+ current ($ID_{50} \approx 2$ mmol/liter). Extracellular Cs^+ reduced the inwardly rectifying K^+ current ($ID_{50} \approx 100$ μmol/liter) without affecting the outwardly rectifying current; 4-aminopyridine (1 or 10 mmol/liter), lidocaine (0.1 or 1 mmol/liter) and flecainide (0.01 or 0.1 mmol/liter) affected neither current. In excised outside-out patches, the addition to the bath of quinine (100 μmol/liter), quinidine (100 μmol/liter), verapamil (100 μmol/liter) or charybdotoxin (100 nmol/liter) inhibited Ca^{2+}- and voltage-sensitive 250 pS K^+ channels (BK channels), but 4-aminopyridine (1 mmol/liter) and lidocaine (0.1 mmol/liter) did not. The pattern of blocker sensitivities is thus consistent with the hypothesis that BK channels are responsible for the outwardly rectifying whole-cell current seen in resting sheep parotid cells.

Key Words inwardly and outwardly rectifying K^+ currents · BK channels · TEA · Ba^{2+} · Cs^+ · sheep parotid

Introduction

In previous patch-clamp studies we have shown that sheep parotid secretory cells have at least four types of K^+ channel in their basolateral membranes [33]. Of these channel types, two were common enough to be considered as playing a role in the normal physiological function of sheep parotid secretory cells. One was a 250 pS voltage- and Ca^{2+}-activated K^+ (BK) channel that was blocked by the addition of 10 mmol/liter tetraethylammonium (TEA) to the extracellular solution [6]. The other was a 30 pS K^+ channel that was activated by hyperpolarization of the cell potential, was not blocked by 10 mmol/liter TEA [6] and functioned as an inward rectifier [19]. Since the BK channel was activated by the addition of acetylcholine to the bath [33], it seems likely to be involved in supporting agonist-stimulated secretion as it is in other exocrine cells [29]. The activity of the 30 pS K^+ channel was not affected by acetylcholine, however, and it was highly active even in unstimulated cells [33]. Its function remains unclear.

Recently, we have used whole-cell patch-clamp techniques to show that sheep parotid cells contain an outwardly rectifying and an inwardly rectifying conductance [19]. The inwardly rectifying conductance is selective for K^+, and by comparing the dependence on extracellular K^+ of the conductance of the inwardly rectifying current to that of the 30 pS K^+ channel, we were able to demonstrate that the 30 pS K^+ channel probably carries it [19]. The ion selectivity of the outwardly rectifying current has not been established, and in the present paper we demonstrate that it is a K^+ current.

In epithelial transport studies several different specific K^+ channel blockers have been used to determine the role of K^+ conductances in supporting cell function [7, 15, 27, 36]. The present study investigates the action of various K^+ channel blockers on the inwardly and outwardly rectifying K^+ currents using the whole-cell patch-clamp technique so as to provide the detailed knowledge of blocker sensitivities needed for investigation of the role of these conductances in the normal function of sheep parotid cells.

Materials and Methods

Cross-bred sheep fed on a lucerne-oaten chaff mix (30%:70%) with water *ad libitum* were fasted for 24 hr before the start of the experiment. They were killed with a captive-bolt pistol and

the parotid glands were excised, placed in a physiological saline solution, diced, and incubated in standard NaCl bathing solution (*see below*) containing collagenase (0.3 mg/ml, Worthington Type II, Freehold, NJ) for 10 min at 37°C in a shaking water bath. Excess medium was then replaced with fresh solution and the tissue incubated once more for 25–30 min. In some experiments, cells were incubated for 10 min in standard NaCl bathing solution containing trypsin (1 mg/ml Difco, Detroit, MI) to facilitate single cell preparation before a second incubation in collagenase-containing solution. The tissue was then dissociated by trituration, centrifuged and washed with the standard NaCl bathing solution. Finally, the parotid cells were filtered through 200 and 75 μm mesh Nitex screen (Allied Screen Fabrics, Sydney, Australia) and suspended in the standard NaCl bath solution. The filtrate, which contained isolated cells and small clumps of cells, was plated out onto petri dishes. Only single cells were used for whole-cell experiments.

Patch-Clamp Methods

Standard patch-clamp techniques were used [14]. Patch-clamp pipettes were pulled from borosilicate microhematocrit tubes (Modulohm, Hevik, Denmark) so as to have resistances (when filled with the standard KCl solution) of 1–3 MΩ for whole-cell recordings and 3–10 MΩ for single channel recordings. The reference electrode was a Ag/AgCl electrode which was connected to the bath via an agar bridge (10 mg/ml) filled with the 150 mmol/liter KCl solution.

An Axopatch 1C patch-clamp amplifier (Axon Instruments, Foster City, CA) was used to measure whole-cell and single channel currents. Single channel currents were recorded on tape with a video cassette recorder and a Sony 501-ES PCM unit modified as described by Bezanilla [3]. Single channel recordings were replayed, filtered at 500 Hz by an 8-pole Bessel low-pass filter, and digitized at 1 kHz with a 12-bit AD-converter before being analyzed on a microVAX II computer. In whole-cell voltage-clamp studies, the command voltage was generated and the whole-cell current was sampled with a MacLab4 interface (ADInstruments, Sydney, Australia) attached to a Mac IIci computer. Tracings for illustrative purposes were printed out by the computer with a laser printer. Outward current, defined as positive charge leaving the pipette, has been indicated as an upward deflection in all traces, and potential differences are reported as pipette potential with respect to bath potential. All experiments were performed at about 20°C.

Characteristics of Sheep Parotid Secretory Cells in the Whole-Cell Configuration

The access resistance in these studies was 9.62 ± 0.64 MΩ (n = 30) and was not electronically compensated during the experiments. Experiments in which access resistance was not stable were discarded. The cell potential with the standard solutions in the pipette and the bath was −66.09 ± 1.23 mV (n = 53) and the cell capacitance was 42.60 ± 1.60 pF (n = 30).

Solutions and Chemicals

The standard pipette solution (pH 7.4) contained (in mmol/liter): KCl (140), $MgCl_2$ (1.0), HHEPES (10), EGTA (0.5 or 5) and glucose (10). The bathing solution (pH 7.4) contained (in mmol/liter): NaCl (145), KCl (5), $CaCl_2$ (1), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), NaHEPES (7.5), HHEPES (7.5) and glucose (10). Chemicals employed were of AR grade or higher. NaHEPES, HEPES, EGTA, and tetraethylammonium (TEA) chloride, barium chloride, cesium chloride, quinine hydrochloride, quinidine hydrochloride, 4-aminopyridine, verapamil and lidocaine were from Sigma (St. Louis, MO). Pure charybdotoxin was from Latoxan (Rosans, France).

Statistics

Results are reported as mean ± SEM (n).

Results

As shown in Fig. 1, the steady-state whole-cell current-voltage relation of sheep parotid secretory cells has both outwardly and inwardly rectifying components. The inwardly rectifying component is evident at potentials more negative than −80 mV, is characteristically noise-free, activates rapidly and then, at strongly hyperpolarizing potentials, slowly inactivates. We have previously shown this inwardly rectifying current to be carried by K^+ ions [19]. The outwardly rectifying current becomes evident at potentials more positive than −20 mV, has a characteristically noisy time course and activates over 10 to 20 ms following depolarization. Since the ion selectivity of this current has not previously been determined, we now present evidence that it too is carried by K^+ ions. Thus, replacement of the K^+ in the pipette solution by Cs^+ (Fig. 2) reduced the current at +80 mV from 2.4 ± 0.2 nA (n = 14) to 0.13 ± 0.01 nA (n = 7), that is, to 5.4% (n = 7) of the control level. It was also abolished by replacement of pipette K^+ with Na^+ (*data not shown*). The characteristics of this current (*see above*) are consistent with the hypothesis that it is carried by 250 pS voltage- and Ca^{2+}-activated K^+ (BK) channels, which are commonly observed in cell-attached patches on these cells [33] and have been described in many other exocrine tissues [5, 29].

Effect of TEA

TEA blocks BK channels in a variety of tissues including exocrine glands [5, 20]. As shown in Figs. 3*A* and *B*, TEA, when added to the bath in concentrations ranging from 0.01 to 1 mmol/liter, caused a dose-related reduction in the outward current. The dose-response relation for the effect of TEA on the outward current at a holding potential of 80 mV showed that 10 mmol/liter TEA inhibited it by 90.4 ± 3.8% (n = 3) and that the ID_{50} is approximately 100 μmol/liter. These findings are similar to

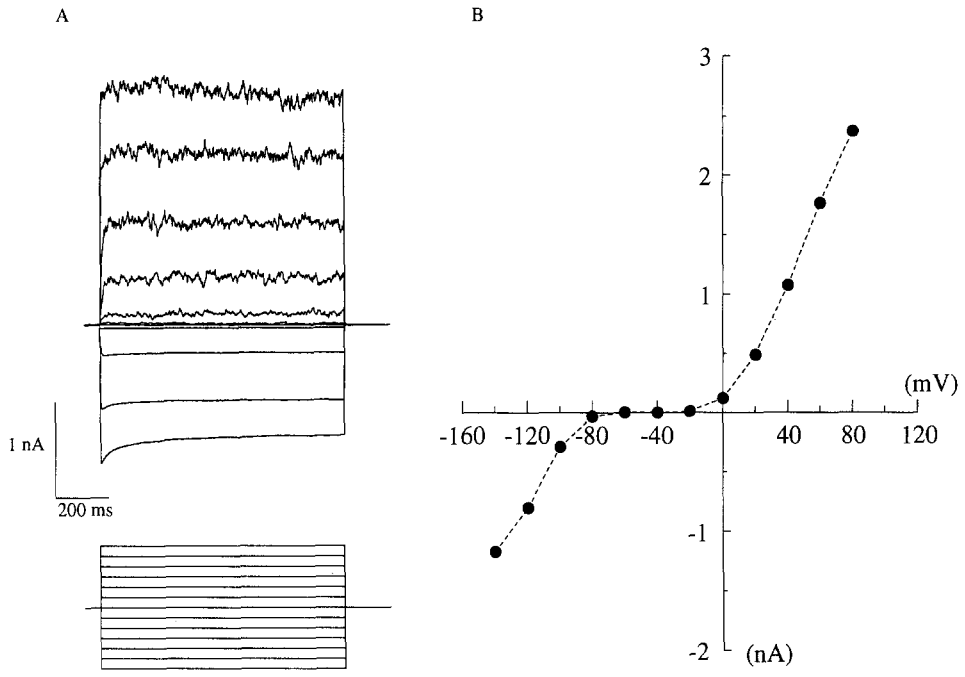

Fig. 1. Whole-cell patch-clamp study of a single sheep parotid cell. The cell was held at a pipette potential of −60 mV, and test voltage pulses ranged between −140 and +80 mV. The pipette contained the control KCl solution and the bath the control NaCl solution.

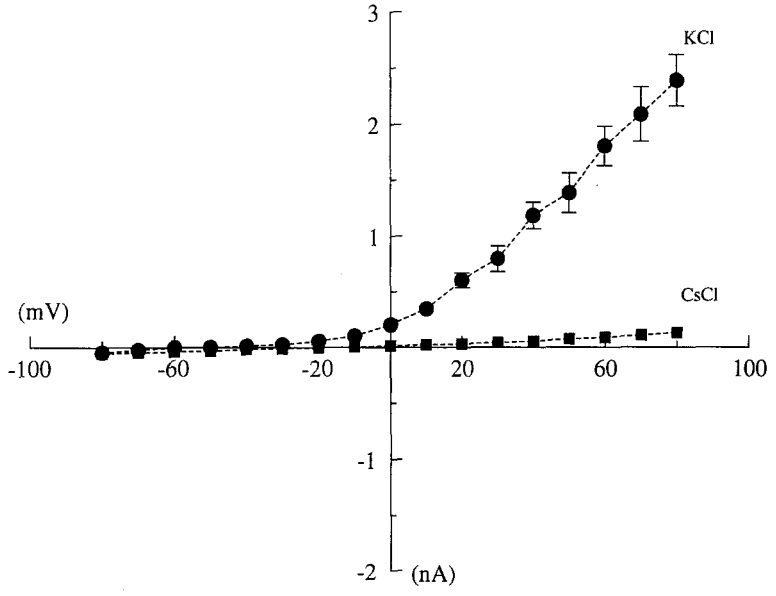

Fig. 2. Steady-state whole-cell current-voltage relation of sheep cells with the control KCl solution (filled circles) in the pipette and with a pipette solution in which the KCl had been replaced by CsCl (filled squares). The control curve is the mean of 14 separate experiments and the CsCl curve is the mean of seven experiments. SEMs are represented by the bars except where these lay within the symbol.

those for the BK channels in pig pancreatic acinar cells [20], insulin-secreting HIT cells [8], and dog tracheal smooth muscle [26]. The maximal inhibition produced by TEA is not significantly different from the reduction in current produced by replacing the pipette K^+ with Cs^+ which indicates that there is little or no outward K^+ current that is insensitive to extracellular TEA. We have previously demonstrated in outside-out patches that the BK channel in sheep parotid secretory cells is blocked by TEA [6].

At a holding potential of 0 mV, 1 mmol/liter TEA reduced the current by 80.7 ± 7.4% (n = 3). This is not significantly different (P = 0.914, un-

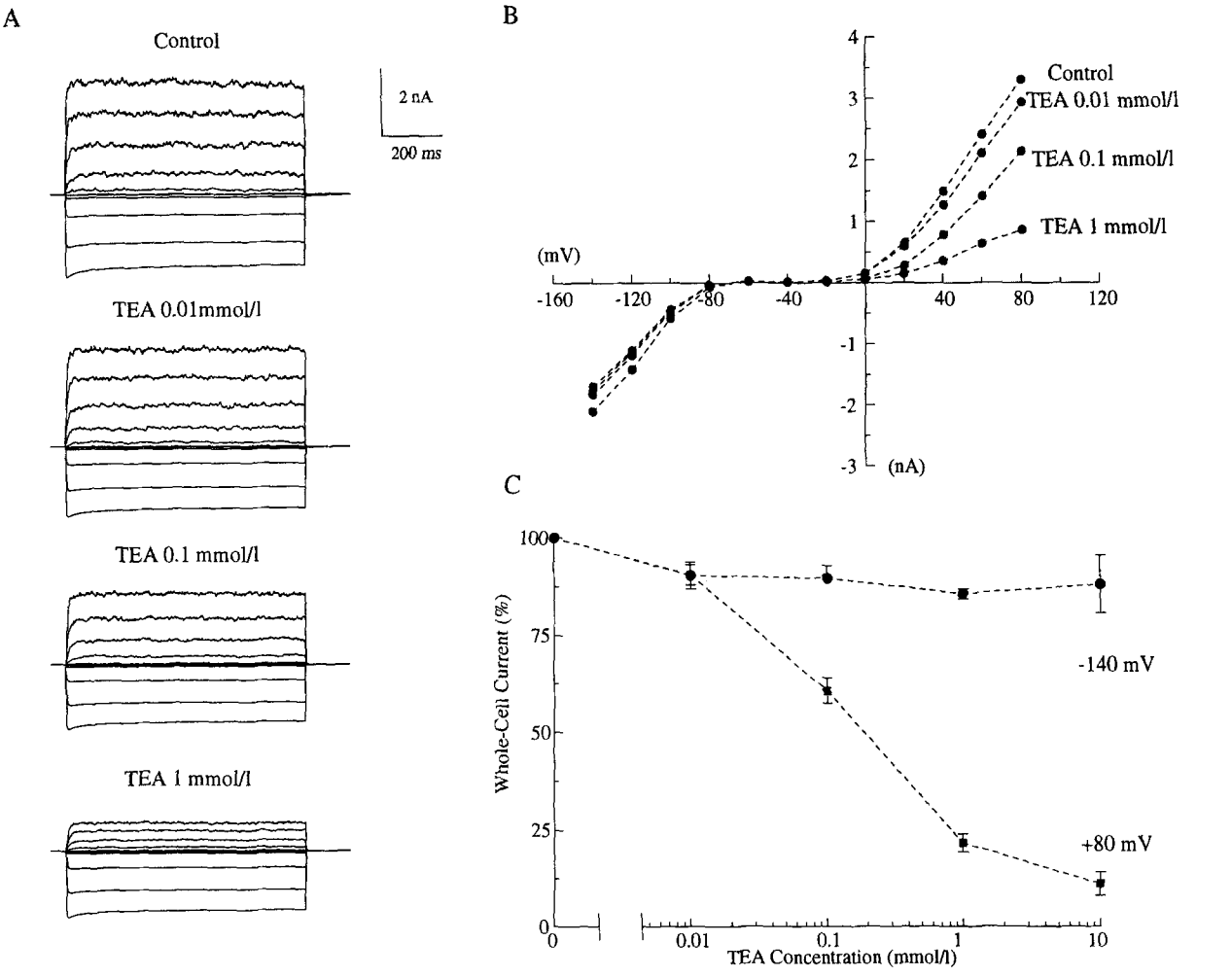

Fig. 3. Whole-cell patch-clamp study on the effect of extracellular tetraethylammonium (TEA) on the whole-cell current-voltage relation of single sheep parotid cells. (*A*) Representative whole-cell recordings from a single cell exposed to concentrations of TEA ranging between 0 and 1 mmol/liter. The cell was held at −60 mV and stepped for intervals of 800 ms, to potentials ranging between −140 and +80 mV. (*B*) Steady-state current-voltage relations obtained from the experiment shown in panel *A*. (*C*) Dose-response relations for the effect of TEA on the inward current at −140 mV (filled circles) and the outward current at +80 mV (filled squares). Each point is the mean of 3–4 experiments.

paired *t*-test) from the reduction at 10 mmol/liter TEA (81.7 ± 4.5%, $n = 3$) indicating that 1 mmol/liter TEA produces a maximal inhibition at this holding potential.

Even at the highest dose tested, TEA did not inhibit the inward current. Similar insensitivity to TEA has been reported for the inwardly rectifying K^+ channel in other tissues [18].

Effect of Quinine

Quinine and its D-stereoisomer quinidine have been shown to block K^+ channels in a number of tissues [9, 12, 21], although these agents may not be specific for K^+ channels [11]. Figure 4*A* and *B* show representative whole-cell current recordings before and after the addition of quinine (100 μmol/liter) to the bath. Quinine (100 μmol/liter) markedly reduced the outward whole-cell K^+ current evoked by membrane depolarization but had little effect on the inwardly rectifying K^+ current. The outward current at +80 mV was reduced by 1 mmol/liter quinine extracellularly to 16.7 ± 4.2% ($n = 5$) of its control level. This inhibitory effect of quinine was dose dependent over the range 1 μmol/liter to 1 mmol/liter, and the concentration that reduced the outward current by 50% was approximately 100 μmol/liter (Fig. 4*C*). Extracellular quinine appeared to reduce the inward current, although even at 1 mmol/liter this

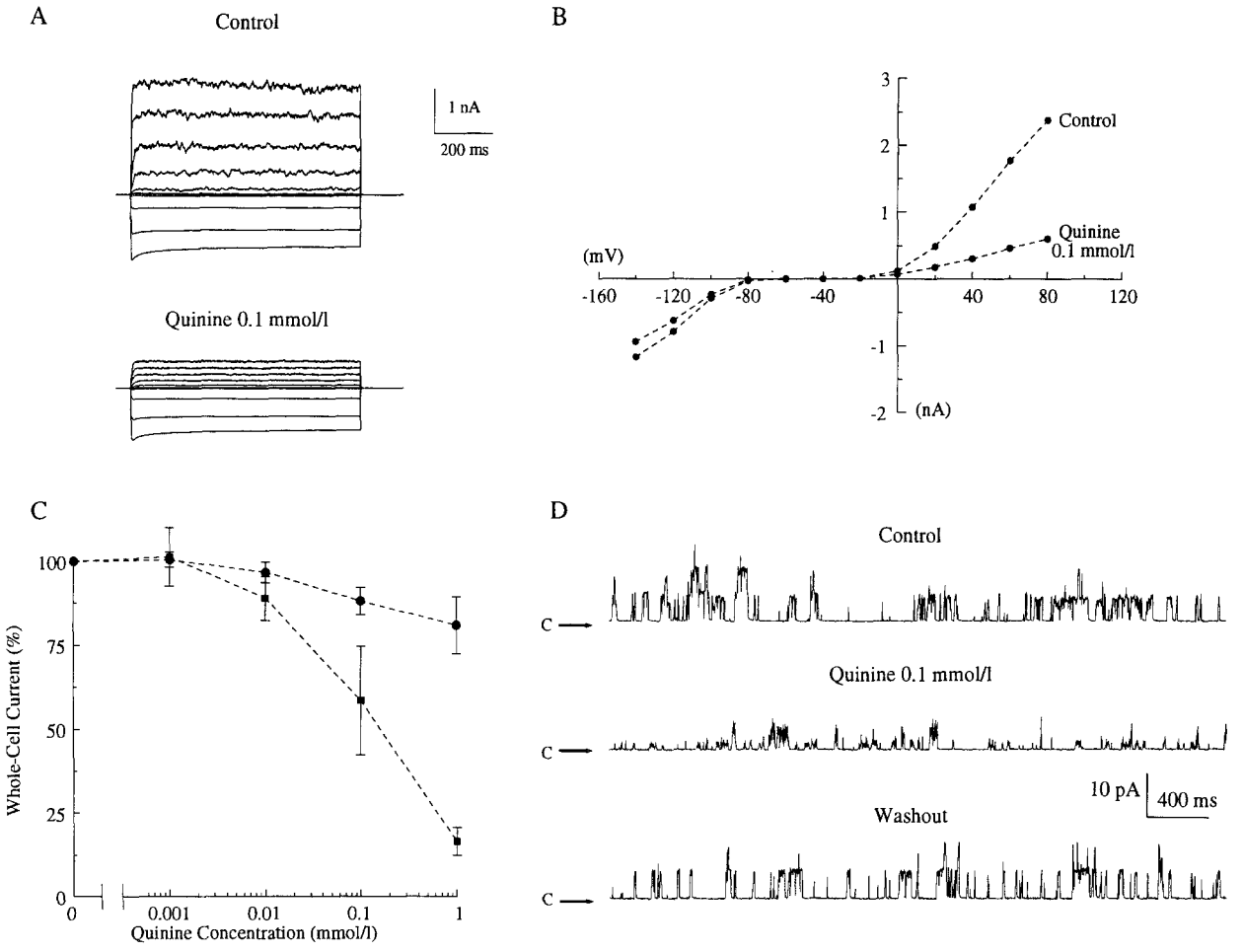

Fig. 4. Whole-cell patch-clamp study on the effect of extracellular quinine on the whole-cell current-voltage relation of single sheep parotid cells. (*A*) Representative whole-cell recordings from a single cell before and after the addition of quinine to the bath. The cell was held at −60 mV and stepped for intervals of 800 ms to potentials ranging between −140 and +80 mV. (*B*) Steady-state current-voltage relations obtained from the experiment shown in panel *A*. (*C*) Dose-response relations for the effect of quinine on the inward current at −140 mV (filled circles) and the outward current at +80 mV (filled squares). Each point is the mean of 3–6 experiments. (*D*) Outside-out excised patch study on the effect of quinine added to the bath. The pipette contained the control KCl solution and the bath contained the control NaCl solution. The pipette potential was held at 0 mV. Current leaving the pipette is shown as an upward deflection.

apparent reduction did not reach statistical significance (5 experiments, $P > 0.05$, paired *t*-test). Figure 4*D* shows an outside-out excised patch experiment in which we investigated the effect of quinine on the BK channel. At a clamp voltage of 0 mV, outward K^+ currents due to BK channels were observed when the pipette contained the standard KCl solution and the bath contained the standard NaCl solution. The addition of 100 μmol/liter quinine to the bath made the channels flicker rapidly between open and closed states and reduced the total charge carried by the BK channels in outside-out patches (that is, the current flowing through the BK channels in the patch over a 30-s period) by 40.7 ± 13.3% (n = 3) at 0 mV. Removal of the quinine from the bath largely reversed this blockade. The magnitude of the blockade observed in the excised patch studies was not significantly different from that observed in whole-cell studies using the same quinine concentration and holding potential (Table). These results agree with previous findings on BK channels in epithelial tissues, such as pig pancreas [21] and the thick ascending limb of the nephron [13], and in nonepithelial tissues, such as rat pancreatic β cells [25] and insulin-secreting HIT cells [8].

Effect of Quinidine

We also tested the effect of quinidine on whole-cell currents in sheep parotid secretory cells. Figure 5*A*

Table. Effects of extracellular inhibitors on K^+ currents in sheep parotid secretory cells*

Extracellular Inhibitors	Whole-cell currents				Concentration of inhibitor (μmol/liter)	Inhibition in whole-cell curent at 0 mV (%)	Inhibition of total charge movement through BK channels in o.o. patches at 0 mV (%)	*P*
	At +80 mV		At −140 mV					
	Inhibition	ID_{50} (μmol/liter)	Inhibition	ID_{50} (μmol/liter)				
TEA	Yes	100	No		1,000	80.7 ± 7.4 (n = 3)		
Quinine	Yes	100	No		100	22.8 ± 7.2 (n = 4)	40.7 ± 13.3 (n = 3)	NS
Quinidine	Yes	200	Yes	>1000	100	21.8 ± 12.1 (n = 3)	52 (n = 2)	NS
Verapamil	Yes	30	No		100	72.0 ± 11.7 (n = 4)	53.3 ± 14.8 (n = 3)	NS
Charybdotoxin	Yes	<0.1	No		0.1	71.3 ± 1.8 (n = 3)	81.3 ± 16.8 (n = 3)	NS
Ba^{2+}	Yes	2000	Yes	1	100	29.2 ± 5.0 (n = 4)		
Cs^+	No		Yes	100	100	−0.5 ± 7.9 (n = 5)		

* The outside-out (o.o.) patch studies were conducted with the same bath and pipette solutions as used in the whole-cell experiments. The significances of the differences between inhibition observed in the whole-cell studies and those observed in the outside-out patch studies were assessed from unpaired t-tests.

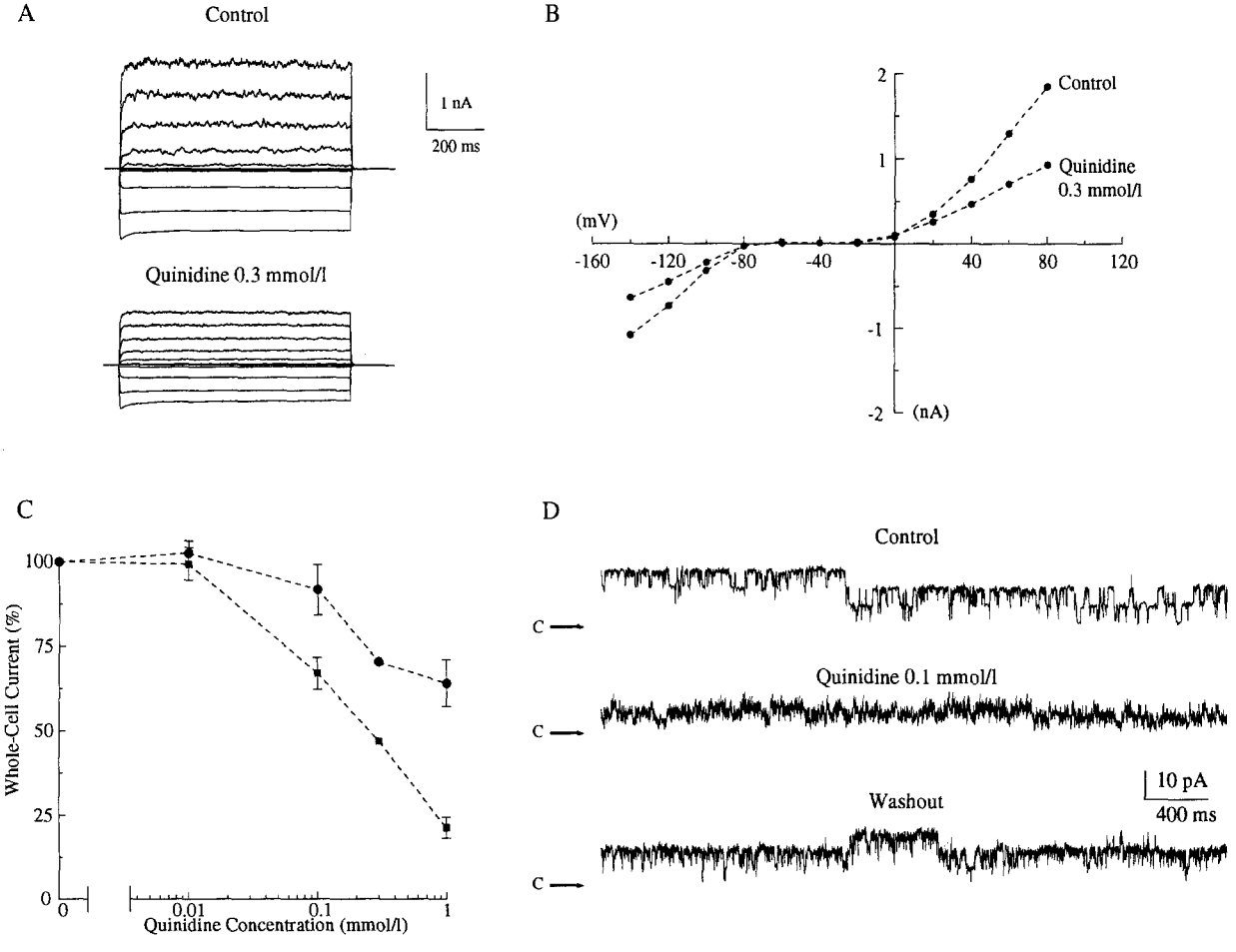

Fig. 5. Whole-cell patch-clamp study on the effect of extracellular quinidine on the whole-cell current-voltage relation of single sheep parotid cells. (*A*) Representative whole-cell recordings from a single cell before and after the addition of quinidine to the bath. The cell was held at −60 mV and stepped for intervals of 800 ms to potentials ranging between −140 and +80 mV. (*B*) Steady-state current-voltage relations obtained from the experiment shown in panel *A*. (*C*) Dose-response relations for the effect of quinidine on the inward current at −140 mV (filled circles) and the outward current at +80 mV (filled squares). Each point is the mean of 2–3 experiments. (*D*) Outside-out excised patch study on the effect of quinidine added to the bath. The pipette contained the control KCl solution and the bath contained the control NaCl solution. The pipette potential was held at 0 mV. Current leaving the pipette is shown as an upward deflection.

shows representative whole-cell current records before and after the addition of quinidine (300 μmol/liter) to the bath. Quinidine inhibited the outward K^+ current associated with membrane depolarization, and in a concentration of 1 mmol/liter, reduced the outward current at 80 mV to 21.4 ± 3.2% (n = 3) of its control value. The inhibitory effect of quinidine on the outward current was dose-dependent over the range 10 μmol/liter to 1 mmol/liter and 50% inhibition occurred at about 200 μmol/liter (Fig. 5*C*). Quinidine (100 μmol/liter) in the bath reduced the total K^+ charge flowing through the BK channels in excised outside-out patches at 0 mV to 52% (n = 2) of the control level and made them flicker rapidly between open and closed states (Fig. 5*D*). Quinidine thus had an effect similar to that of quinine (*cf.* Fig. 4*D*). The magnitude of the blockade observed in the excised patch studies was not significantly different from that observed in whole-cell studies using the same quinidine concentration and holding potential (Table). These results agree with previous findings on BK channels in epithelial tissues such as pig pancreas [21], and in nonepithelial tissues such as gastric smooth muscle cells [34].

In the present study, quinine did not significantly inhibit the inwardly rectifying K^+ current (Fig. 4), but quinidine (1 mmol/liter) significantly ($P < 0.05$ by a paired t-test) reduced the current at −140 mV to 63.2 ± 6.0% (n = 3) of its control value (Fig. 5*C*). This has also been reported in ventricular myocytes from guinea pig [1, 17] and dog [31].

Effect of Verapamil

Verapamil has been found to block a K^+ conductance in proximal kidney tubule [32], and K^+ channels in the thick ascending limb of Henle's loop [4] and in respiratory epithelial cells [22]. Typical whole-cell recordings before and after the addition of verapamil (100 μmol/liter) to the bath are shown in Fig. 6*A*. Verapamil inhibited the outward currents, but did not affect the inward currents. Figure 6*B* shows that the inhibitory effect of verapamil on the outward current evoked by depolarization to +80 mV is dose dependent. The highest dose tested inhibited the outward current by 75.3 ± 5.7% (n = 4). The outward current was 50% inhibited at about 30 μmol/liter. Figure 6*D* shows that 100 μmol/liter verapamil when added to the bath induced rapid flickering of BK channel currents in outside-out patches. In three patches, 100 μmol/liter verapamil reduced the total charge flowing through the BK channels in outside-out patches by 72.0 ± 11.7% at 0 mV. This inhibitory effect was completely reversible. Its magnitude was similar (Table) to that observed for this concentration of verapamil in whole-cell studies at a holding potential of 0 mV. On a molar basis, verapamil (ID_{50} ~ 30 μmol/liter) was a more potent blocker of the outwardly rectifying K^+ current than TEA, quinine, quinidine or Ba^{2+}.

Effect of Charybdotoxin

Charybdotoxin (CTX), a protein from the venom of the scorpion *Leiurus quinquestriatus,* is a specific and potent slow blocker of Ca^{2+}-activated large conductance K^+ channels [28]. In sheep parotid secretory cells it caused a marked reduction in the outwardly rectifying K^+ current, but did not affect the inwardly rectifying K^+ current (Fig. 7*A*). The outward current in the presence of CTX was 35.9 ± 2.6% (n = 3) of the control value. Figure 7*B* shows representative recordings before and after addition of 100 nmol/liter CTX to the bath. CTX (100 nmol/liter) reduced the total charge flowing through BK channels in outside-out patches by 81.3 ± 16.8% at 0 mV (n = 3) and the inhibitory effect was completely reversible (Fig. 7*C*). Its magnitude was similar (Table) to that observed in the whole-cell studies.

Effect of Ba^{2+}

Perhaps the most commonly used K^+ channel blocker is Ba^{2+}. This divalent cation has been shown to block various K^+ conductances and K^+ channels including inwardly rectifying K^+ channels and the BK channel [2, 21, 24]. Figure 8*A* shows the effect of Ba^{2+} on the whole-cell currents. In a low concentration (1 μmol/liter) Ba^{2+} reduced the inwardly rectifying K^+ current. This inhibition developed over several hundred milliseconds following a hyperpolarizing voltage step. A higher concentration of Ba^{2+} (10 μmol/liter) was associated with a more pronounced and more rapidly developing inhibitory effect. Concentrations of Ba^{2+} less than 1 mmol/liter had little effect on the outwardly rectifying K^+ current, but 10 mmol/liter Ba^{2+} reduced the outward current at 80 mV to 14.2 ± 2.3% (n = 5) of the control level. The inhibitory effects of Ba^{2+} on the inward and outward currents were dose dependent over the range 1 μmol/liter to 10 mmol/liter (Fig. 8*C*). In contrast to the high sensitivity of the inwardly rectifying K^+ current (ID_{50} = 1 μmol/liter) to Ba^{2+}, the outwardly rectifying K^+ current was much less sensitive (ID_{50} = 2 mmol/liter). The sensitivity of the inwardly rectifying K^+ current is similar to that reported in other tissues [24, 30]. The relative insensitivity of the outward current agrees with find-

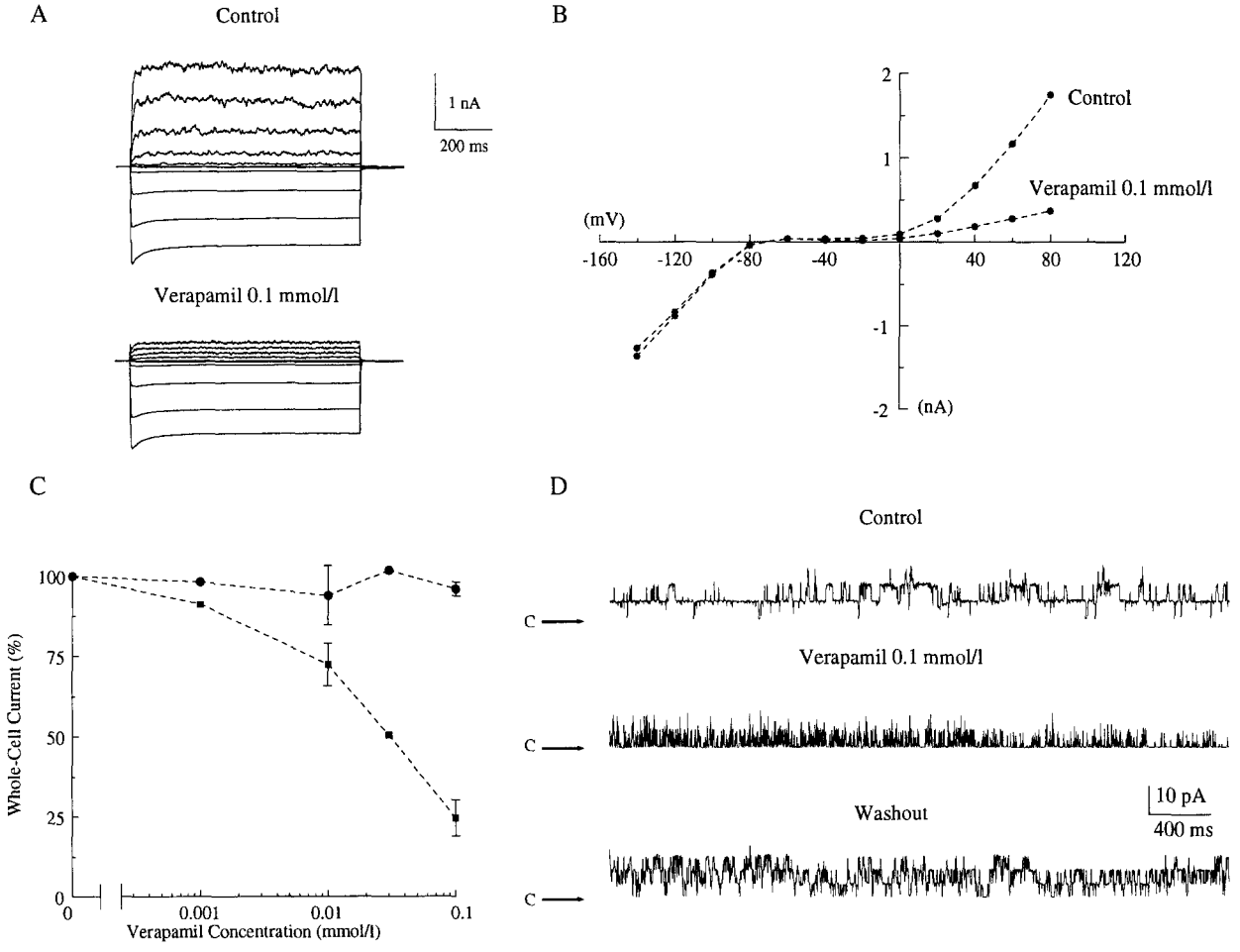

Fig. 6. Whole-cell patch-clamp study on the effect of extracellular verapamil on the whole-cell current-voltage relation of single sheep parotid cells. (*A*) Representative whole-cell recordings from a single cell before and after the addition of verapamil to the bath. The cell was held at −60 mV and stepped for intervals of 800 ms to potentials ranging between −140 and +80 mV. (*B*) Steady-state current-voltage relations obtained from the experiment shown in panel *A*. (*C*) Dose-response relations for the effect of verapamil on the inward current at −140 mV (filled circles) and the outward current at +80 mV (filled squares). Each point is the mean of 2–4 experiments. (*D*) Outside-out excised patch study on the effect of verapamil added to the bath. The pipette contained the control KCl solution and the bath contained the control NaCl solution. The pipette potential was held at 0 mV. Current leaving the pipette is shown as an upward deflection.

ings for BK channels in pig pancreatic acinar cells [21] and the cells of the thick ascending limb of the loop of Henle [13].

Effect of Cs^+

Cs^+ is known to block inwardly rectifying K^+ currents in a variety of tissues [24, 30]. Representative whole-cell current records before and after the addition of Cs^+ to the bath are shown in Fig. 9*A*. Cs^+ in a low concentration (10 μmol/liter) reduced the inward current at −140 mV slightly but did not alter the outward current (Fig. 9*B*). The blocking effect of Cs^+ on the inward current became more pronounced with increasing hyperpolarization of the cell membrane potential (Fig. 9*B*) indicating that the block by Cs^+ is voltage dependent. Higher concentrations (100 μmol/liter and 1 mmol/liter) of Cs^+ were associated with more pronounced inhibitory effects. The inhibitory effect of Cs^+ on the inward current evoked by membrane hyperpolarization to −140 mV was dose dependent over the range 10 μmol/liter to 10 mmol/liter, and was half maximal at about 100 μmol/liter (Fig. 9*C*). Cs^+ did not inhibit the outward current produced by membrane depolarization. The sensitivity of the inwardly rectifying K^+ current to Cs^+ (ID_{50} = 100 μmol/liter) is similar to that reported in other tissues [24, 30].

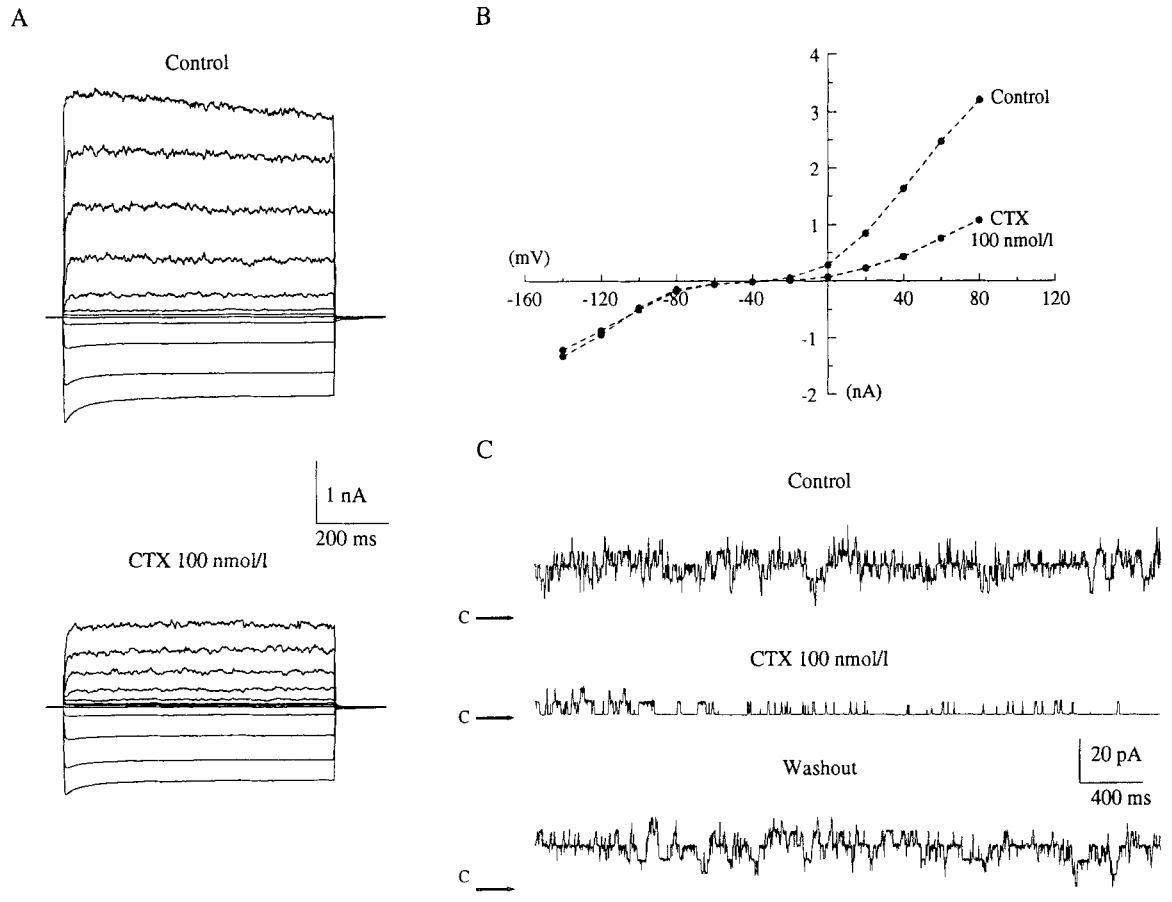

Fig. 7. Whole-cell patch-clamp study on the effect of extracellular charybdotoxin (CTX) on the whole-cell current-voltage relation of single sheep parotid cells. (*A*) Representative whole-cell recordings from a single cell before and after the addition of CTX to the bath. The cell was held at −60 mV and stepped for intervals of 800 ms to potentials ranging between −140 and +80 mV. (*B*) Steady-state current-voltage relations obtained from the experiment shown in panel *A*. (*C*) Outside-out excised patch study on the effect of CTX added to the bath. The pipette contained the control KCl solution and the bath contained the control NaCl solution. The pipette potential was held at 0 mV. Current leaving the pipette is shown as an upward deflection.

Effects of Lidocaine, 4-Aminopyridine and Flecainide

We also tested lidocaine, a substance that has been shown to block basolateral K^+ channels in the rectal gland of *Squalus acanthias* [12]. Lidocaine (100 μmol/liter and 1 mmol/liter) added to the bath inhibited neither the outwardly rectifying nor the inwardly rectifying K^+ currents. The outward (at +80 mV) and inward (at −140 mV) currents in the presence of lidocaine (100 μmol/liter) extracellularly were 97.7 ± 3.2% (n = 4) and 99.4 ± 3.4% (n = 4) of their control values, respectively. In the presence of 1 mmol/liter lidocaine these currents were 88.27 ± 14.65% (n = 2) and 95.22 ± 1.81% (n = 2), respectively. In one experiment on an outside-out patch, we did not observe any inhibitory effect of lidocaine (100 μmol/liter) on the activity of BK channels when the pipette contained standard KCl solution and the bath contained standard NaCl solution.

The antiarrhythmic agent, flecainide, has been reported to block the delayed rectifier K^+ current in cat ventricular myocytes [10]. We examined its effect (10 μmol/liter and 100 μmol/liter) on the whole-cell currents in sheep parotid cells, but we observed no inhibition. The outward (at +80 mV) and the inward (at −140 mV) currents in the presence of flecainide (100 μmol/liter) were 103.6 ± 4.8% (n = 3) and 101.7 ± 12.6% (n = 3) of their respective control values.

We also tested the effect of 4-aminopyridine (1 and 10 mmol/liter) on the whole-cell currents in sheep parotid cells but observed no inhibitory effect at either concentration.

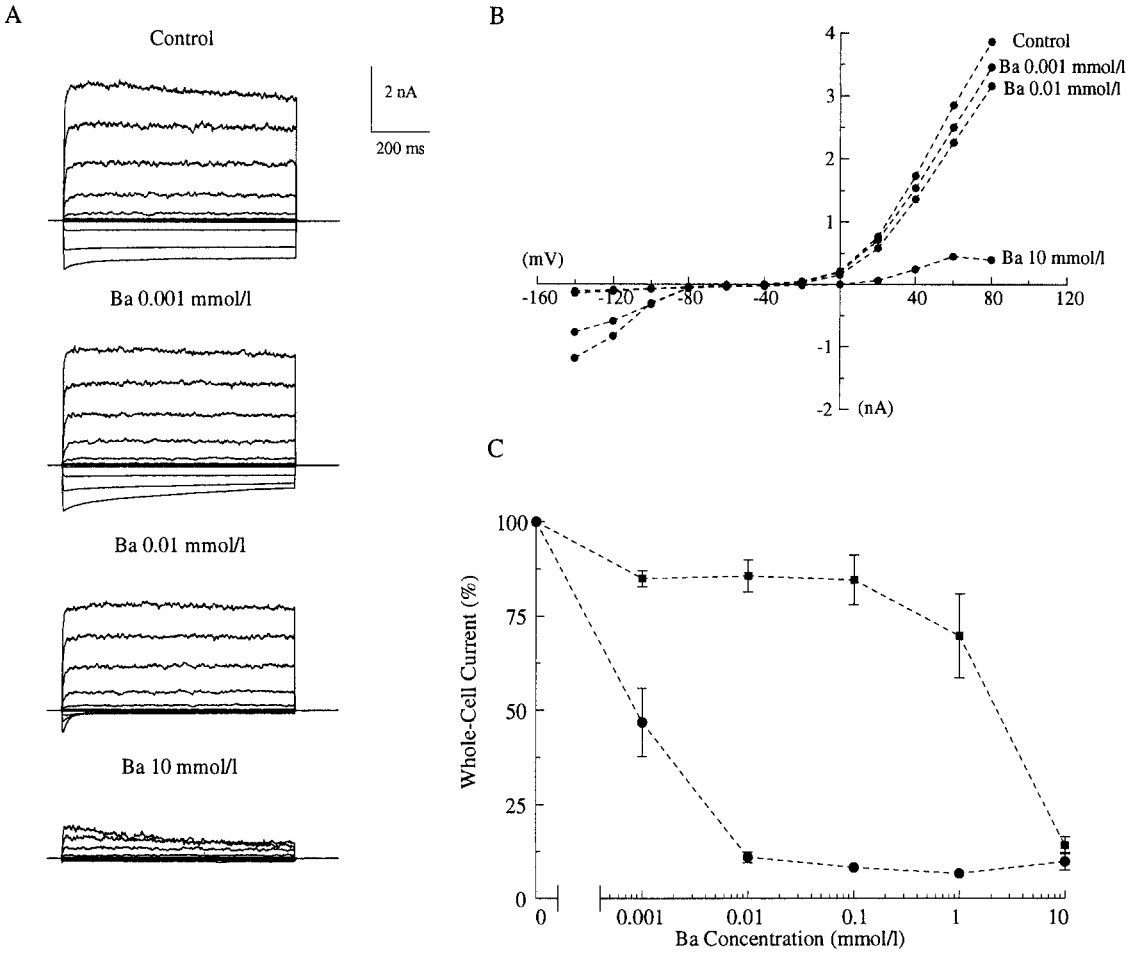

Fig. 8. Whole-cell patch-clamp study on the effect of extracellular Ba^{2+} on the whole-cell current-voltage relation of single sheep parotid cells. (*A*) Representative whole-cell recordings from a single cell before and after the addition of Ba^{2+} to the bath. The cell was held at −60 mV and stepped for intervals of 800 ms to potentials ranging between −140 and +80 mV. (*B*) Steady-state current-voltage relations obtained from the experiment shown in panel *A*. (*C*) Dose-response relations for the effect of Ba^{2+} on the inward current at −140 mV (filled circles) and the outward current at +80 mV (filled squares). Each point is the mean of 3–5 experiments.

Discussion

The present study has measured the dose-response relations of several K^+ channel blockers for both the inwardly rectifying and the outwardly rectifying K^+ currents in sheep parotid secretory cells. The results are summarized in the Table. We undertook this study for two reasons. First, K^+ channel blockers are generally not specific for particular K^+ channels, and so it is necessary to investigate a variety of K^+ blockers in order to characterize the channels in a tissue [5, 16]. Second, the interpretation of previously published work and the usefulness of K^+ channel blockers in future studies on this tissue depend on a clear definition of the pharmacology of these channels.

Physiological Implications of these Studies

The close similarity between the pattern of blocker sensitivities for the outwardly rectifying current and the sensitivities observed for BK channels in outside-out patches in this study (Table), suggests that the BK channels are the major channel type conducting the outwardly rectifying current. This conclusion is also supported by the similarity between the blocker sensitivities observed for the outward K^+ current and BK channels in other tissues [5, 23]. Our previous suggestion that the inwardly rectifying K^+ current is similar to the inwardly rectifying K^+ currents of excitable tissues [19] is also strengthened by the similarity of their blocker sensitivities.

The data in this study permit us to interpret the

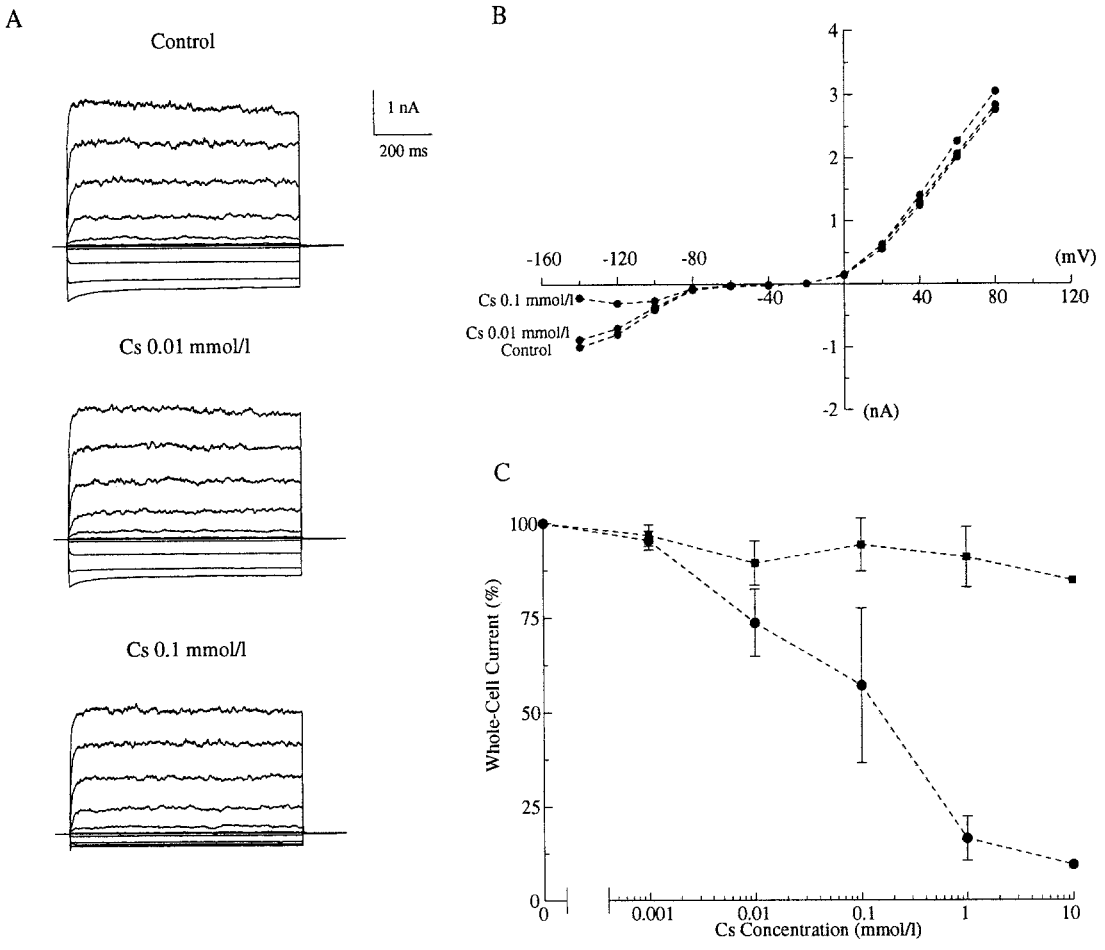

Fig. 9. Whole-cell patch-clamp study on the effect of extracellular Cs^+ on the whole-cell current-voltage relation of single sheep parotid cells. (*A*) Representative whole-cell recordings from a single cell before and after the addition of Cs^+ to the bath. The cell was held at −60 mV and stepped for intervals of 800 ms to potentials ranging between −140 and +80 mV. (*B*) Steady-state current-voltage relations obtained from the experiment shown in panel *A*. (*C*) Dose-response relations for the effect of Cs^+ on the inward current at −140 mV (filled circles) and the outward current at +80 mV (filled squares). Each point is the mean of 1–5 experiments.

studies by Wright and Blair-West on the effect of K^+ channel blockers on fluid secretion and K^+ efflux from the sheep parotid [35]. Wright and Blair-West found that TEA blocks both fluid secretion and K^+ efflux from this gland stimulated by bethanechol. Since in many epithelia, secretion is driven by K^+ efflux through BK channels that are activated by increased intracellular free Ca^{2+} evoked by muscarinic stimulation [5, 29], and the secretory cells of the sheep parotid contain BK channels that are activated by acetylcholine, the findings of Wright and Blair-West could be interpreted as demonstrating that also in sheep parotid cells, the BK channels support secretion by permitting K^+ efflux. Surprisingly, however, Wright and Blair-West also found that TEA failed to block fluid secretion and K^+ efflux in response to acetylcholine or nerve stimulation. The apparent contradiction between the bethanechol and acetylcholine results has been resolved by recent studies using Fura-2, in which it was demonstrated that TEA inhibits the increase in intracellular free Ca^{2+} produced by muscarinic agonists possibly by competing for the muscarinic receptor [6]. The concentration of acetylcholine used by Wright and Blair-West was sufficient to overcome the TEA blockade, whereas the concentration of bethanechol used by them was not. The present study shows that the concentrations of TEA used by Wright and Blair-West (1–3 mmol/liter) were adequate to block the outward current totally and presumably the BK channels that underlie it. We thus conclude that TEA does not inhibit fluid secretion or K^+ efflux from the sheep parotid gland and that BK channels may not be involved in stimulated secretion by this gland. The finding of Wright and Blair-West [35] that 4-aminopyridine has no effect on fluid secretion or on

K^+ efflux agrees with our finding in the present study that this agent has no effect on either the inwardly or the outwardly rectifying K^+ currents.

By using the other blockers described in this study to investigate secretion in perfused sheep parotid glands and K^+ efflux from sheep parotid cells, it will be possible to define the role of the inwardly and outwardly rectifying K^+ currents in the spontaneous and stimulated secretion by this gland.

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