J. Membrane Biol. 131, 193–202 (1993)

The Journal of
Membrane Biology

An Inwardly Rectifying Potassium Channel in the Basolateral Membrane of Sheep Parotid Secretory Cells

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Summary. Using whole-cell patch-clamp techniques, we demonstrate that sheep parotid secretory cells have both inwardly and outwardly rectifying currents. The outwardly rectifying current, which is blocked by 10 mmol/liter tetraethylammonium (TEA) applied extracellularly, is probably carried by the 250 pS Ca^{2+}- and voltage-activated K^+ (BK) channel which has been described in previous studies. In contrast, the inwardly rectifying current, which is also carried by K^+ ions, is not sensitive to TEA. It is similar to the inwardly rectifying currents observed in many excitable tissues in that (i) its conductance is dependent on the square root of the extracellular K^+, (ii) the voltage range over which it is activated is influenced by the extracellular K^+ concentration and (iii) it is blocked by the addition of Cs^+ ions (670 μmol/liter) to the bathing solution. Our previously published cell-attached patch studies have shown that the channel type most commonly observed in the basolateral membrane of unstimulated sheep parotid secretory cells is a K^+ channel with a conductance of 30 pS and, in this study, we find that its conductance also depends on the square root of the extracellular K^+ concentration. It thus seems likely that it carries the inwardly rectifying K^+ current seen in the whole-cell studies.

Key Words sheep parotid · K^+ channel · inward rectifier · whole-cell currents

Introduction

The sheep parotid is of particular interest to us for two reasons. (i) Among those salivary glands that have been well studied, it is one of the few that secretes in the absence of nerve stimulation, i.e., it exhibits so-called spontaneous secretion: [6, 37], and the secretory process is not blocked by pharmacological agents known to block evoked secretion by other salivary glands [35], suggesting that the mechanism of spontaneous secretion is different from that of evoked secretion. (ii) Unlike the primary fluids of most other salivary glands, which are plasma-like in electrolyte composition [37], the primary fluid of the sheep parotid has a Cl^- concentration approximately half and a HCO_3^- concentration approximately twice that of plasma [7].

In previous patch-clamp studies [11] we have shown that the secretory cells of the sheep parotid contain at least four types of K^+ channel in the basolateral plasma membrane. Of these, two are common enough to be considered as having a role to play in normal secretory function. One of these is a 250 pS voltage- and Ca^{2+}-activated K^+ (BK) channel that is blocked by the addition of 10 mmol/liter tetraethylammonium (TEA) to the extracellular solution. The other is a 30 pS K^+ channel that is activated by hyperpolarization of the plasma membrane potential and is not blocked by 10 mmol/liter TEA [12]. As in other exocrine cells, the 250 pS K^+ channel is activated by the addition of acetylcholine to the bathing solution and thus seems likely to be involved in supporting muscarinically evoked secretion. The activity of the 30 pS K^+ channel, however, is not influenced by acetylcholine and is highly active even in resting cells. Although it is tempting to hypothesize that it may have a role to play in spontaneous secretion, this has not been demonstrated experimentally.

In this paper, we examine the whole-cell current-voltage relation of sheep parotid secretory cells in the hope of elucidating the role of the 30 pS K^+ channel in the electrophysiology of these cells [11].

Materials and Methods

Cell Preparation

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 their parotid glands were then excised, placed in a physiological salt solution, diced, and incubated in the standard NaCl-rich bathing solution containing collagenase (0.3 mg/ml, Worthington

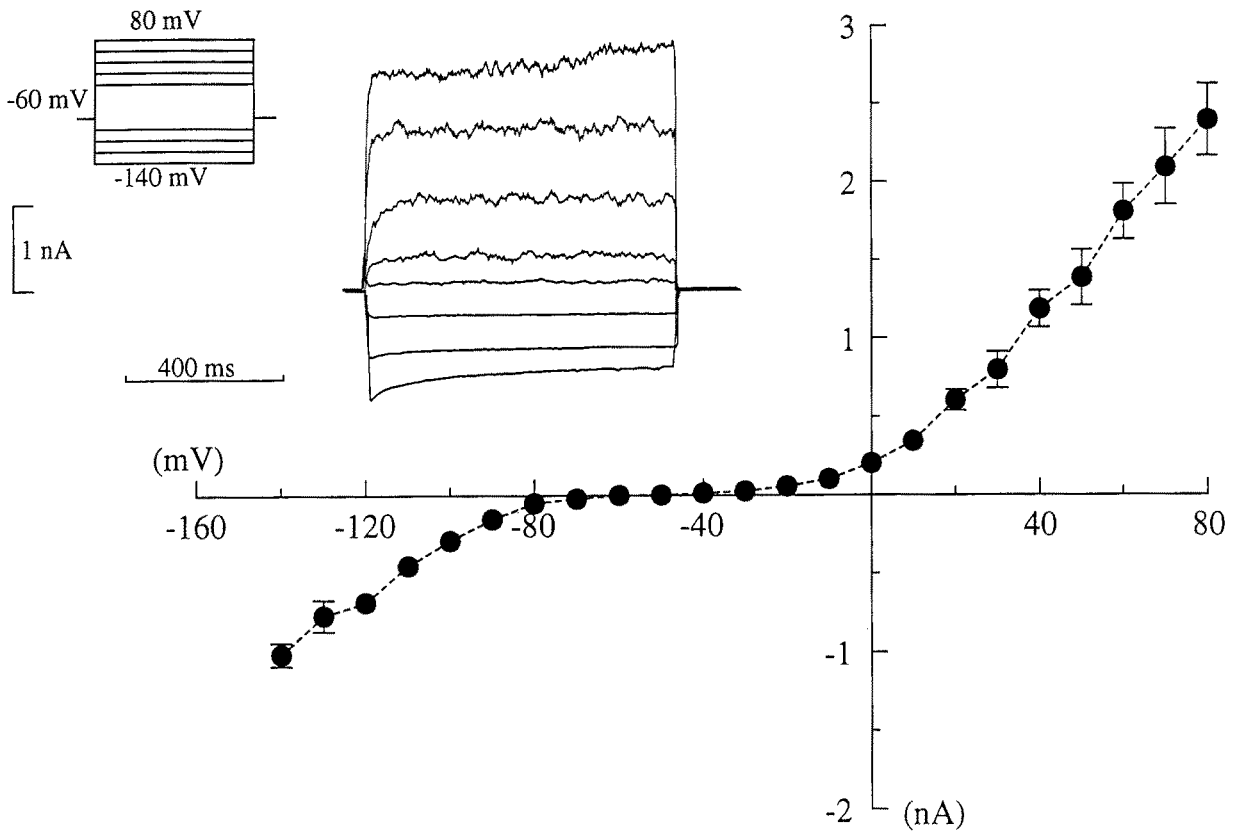

Fig. 1. Steady-state current-voltage relation of single sheep parotid secretory cells held in the whole-cell patch-clamp configuration. The cells were held at a potential of −60 mV between test voltage pulses. The curve is the mean of 14 separate experiments. The bars represent SEM but when these were so small as to lie within the symbols they have been omitted. Also shown are representative records of the responses of the whole-cell current to 800 ms voltage steps from the resting potential of −60 mV. Outward currents are shown as upward deflections.

Type II, Freehold, NJ), for 10 min at 37°C in a shaking water bath. Excess medium was then replaced with a fresh solution and the tissue incubated once more for 25–30 min. The tissue was then dissociated by trituration, centrifuged and washed once more with the standard NaCl-rich bathing solution. Finally, the parotid cells were filtered through 200 μm and 75 μm mesh Nitex screens (Allied Screen Fabrics, Sydney, Australia) and resuspended in the standard NaCl-rich bathing 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 [16]. 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, connected to the bathing solution via an agar bridge (100 mg/10 ml) filled with a 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 which were then recorded on video 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 with 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. Tracings for illustrative purposes were printed out by the computer with a laser printer. The series resistance was not electronically compensated during the experiments, nor have the potentials reported in this paper been corrected for the series resistance. Since the average series resistance in these experiments was 12.44 MΩ ± 0.42 (SEM, n = 142), the conductances of currents in the nanoampere range will be underestimated as a result of the voltage drop across the series resistance. The pipette potential was corrected for the liquid junction potential between the pipette solution and the external solution when necessary. 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.

Solutions and Chemicals

The composition (in mmol/liter) of the standard solutions was as follows: (i) the pipette (KCl-rich) solution: pH 7.4, KCl (140), $MgCl_2$ (1), HHEPES (10), EGTA (0.5 or 5) and glucose (10); (ii)

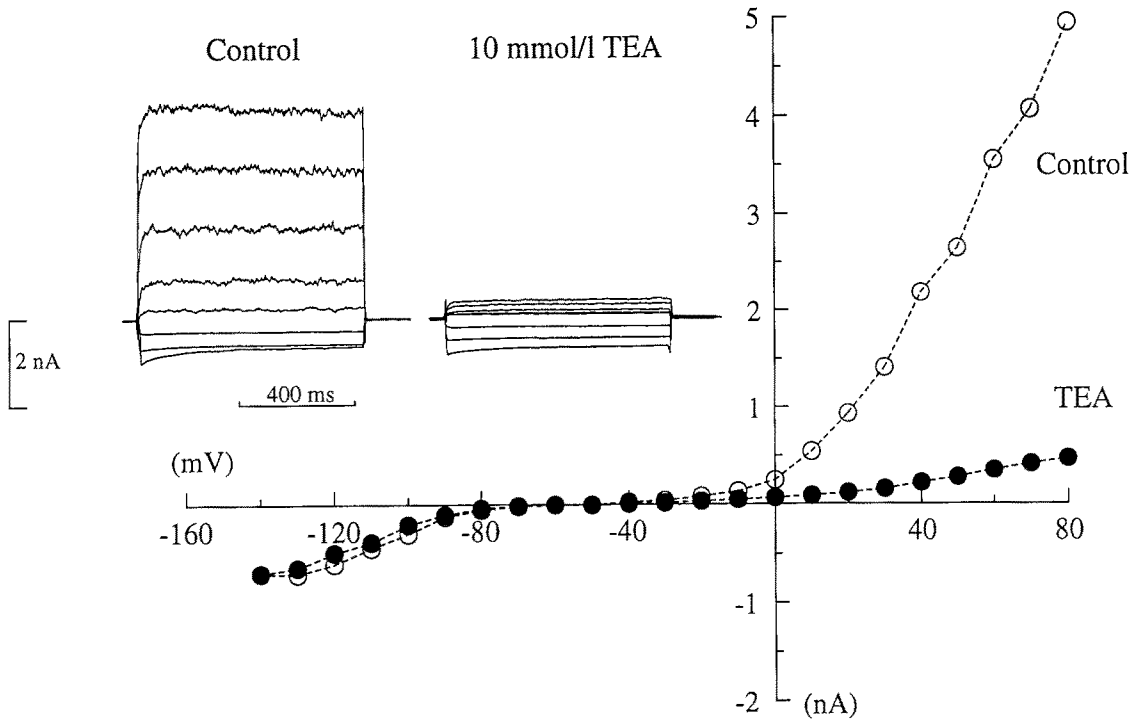

Fig. 2. Steady-state whole-cell current-voltage relation of single sheep parotid secretory cells before (open circles) and after (solid circles) addition of 10 mmol/liter TEA to the bathing solution. Also shown are the whole-cell current responses to voltage steps from the resting potential of −60 mV to test potentials of −140, −120, −100, 0, +20, +40, +60, and +80 mV, before and after addition of 10 mmol/liter TEA to the bathing solution.

the bathing (NaCl-rich) solution: pH 7.4, 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). When we needed to change the concentration of K^+ in the pipette solution, we used equimolar substitution of NaCl for KCl. Chemicals employed were of AR grade or higher. NaHEPES, HEPES, EGTA, and tetraethylammonium chloride were obtained from Sigma (St Louis, MO).

Results

When the steady-state whole-cell current-voltage relation was determined for single cells with the standard KCl-rich ($[Ca^{2+}] < 1$ nmol/liter) solution in the pipette, it was found to exhibit both outwardly and inwardly rectifying components (Fig. 1). The outwardly rectifying component, which was observed when the cell potential was stepped to potentials more positive than −40 mV (Fig. 1), was activated over a period of approximately 50 ms (Fig. 1), had a characteristically noisy time course (Fig. 1) and was blocked by the addition of 10 mmol/liter TEA to the bathing solution (Fig. 2). This current was probably carried by the 250 pS voltage- and Ca^{2+}-activated K^+ channels that are found in the secretory cells of the sheep parotid [12] and have been implicated in the TEA-sensitive, outwardly rectifying currents seen in many other secretory epithelia [10, 21]. The inwardly rectifying component of the steady-state whole-cell current-voltage relation, which was seen at cell potentials more negative than −80 mV (Fig. 1), was almost instantaneous in onset (Fig. 1), had a relatively noise-free time course (Fig. 1), and was not blocked by the addition of 10 mmol/liter TEA to the bathing solution (Fig. 2).

We first attempted to determine which ion carried the inwardly rectifying current. In a single experiment, replacement of all but 2 mmol/liter of the Cl^- in the pipette solution with glutamate had no effect on the magnitude of the inward current (Fig. 3), indicating that it was not attributable to the efflux of Cl^- ions from the cell. Similarly, in two experiments, replacement of Na^+ in the bathing solution with N-methyl-D-glucamine did not reduce the steady-state inward current (Fig. 3), indicating that it was not attributable to the influx of Na^+ ions. Finally, in two experiments, replacement of extracellular K^+ with Cs^+ abolished the inwardly rectifying current (Fig. 3), suggesting that it was due to K^+ influx. This conclusion is strengthened by results of experiments in which the extracellular K^+ concentration was increased (Fig. 4). Increasing the ex-

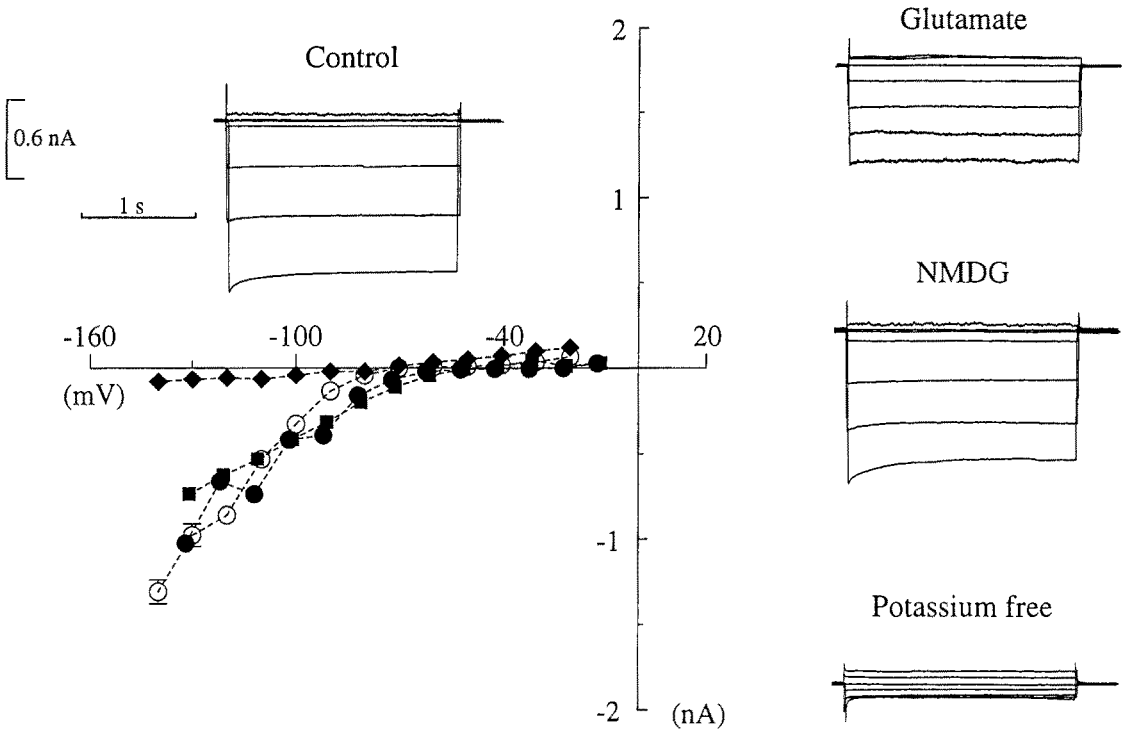

Fig. 3. Steady-state whole-cell current-voltage relations of single sheep parotid cells in the voltage range −140 to −20 mV with (i) glutamate substituted for Cl^- in the pipette solution (1 experiment; filled squares); (ii) N-methylglucamine substituted for Na^+ in the bathing solution (2 experiments; filled circles) and (iii) Cs^+ substituted for K^+ in the bathing solution (2 experiments; filled diamonds). The curves are superimposed on the control curve (14 experiments; open circles). Also shown are representative records of the responses of the whole-cell current from these experiments. In each case, the cell potential was held at −60 mV and stepped to potentials ranging between −140 mV and −20 mV at intervals of 20 mV.

tracellular K^+ concentration from 5 to 30 mmol/liter was accompanied by an increase in the whole-cell conductance (measured at a cell potential of −100 mV, when the current-voltage relation is almost rectilinear) from 24.0 nS ± 1.4 (SEM, n = 14) to 40.8 nS ± 6.3 (SEM, n = 7). The reversal potential of the inward current, as estimated by extrapolation from the data lying in the range in which its current-voltage relation was rectilinear, shifted from −86.6 mV ± 8.1 (SEM, n = 14) to −36.1 mV ± 6.9 (SEM, n = 6). Following a smaller increase in K^+ concentration from 5 to 12 mmol/liter, the conductance calculated for the inward current was 26.8 nS ± 3.8 (SEM, n = 6) and the reversal potential was −68.6 mV ± 5.3 (SEM, n = 6).

A plot of the logarithm of the conductance calculated from the inward current as a function of the logarithm of the extracellular K^+ concentration reveals a rectilinear relation with a slope of 0.30 (Fig. 4*B*); in other words, the conductance of the inward rectifier has a power dependency on the extracellular K^+ concentration. The power dependency we have observed is less than the square root dependency reported for inward rectifiers in other tissues [30], but this can be explained by the errors of clamp voltages caused by the series resistance. In our experiments, the mean series resistance was 12.44 MΩ ± 0.42 (SEM, n = 142), and once the command voltages are corrected for this, the slope of the relation between the logarithm of the conductance calculated from the inwardly rectifying current and the logarithm of the extracellular K^+ concentration increases to 0.51. When the reversal potential of the inward current is plotted as a function of the logarithm of bathing solution K^+ concentration (Fig. 4*C*), a rectilinear relation is obtained with a slope of +65 mV/decade, indicating that the inwardly rectifying conductance is highly K^+ selective.

One interesting effect of increasing the bathing solution K^+ concentration is that not only did the magnitude of the inward current increase, but the voltage range over which it is activated was altered (Fig. 4*A*). As mentioned previously, with 5 mmol/liter K^+ in the bathing solution, the inward current only became obvious at cell potentials more negative than −70 mV, but when the bathing solution contained 30 mmol/liter K^+, the inward current was

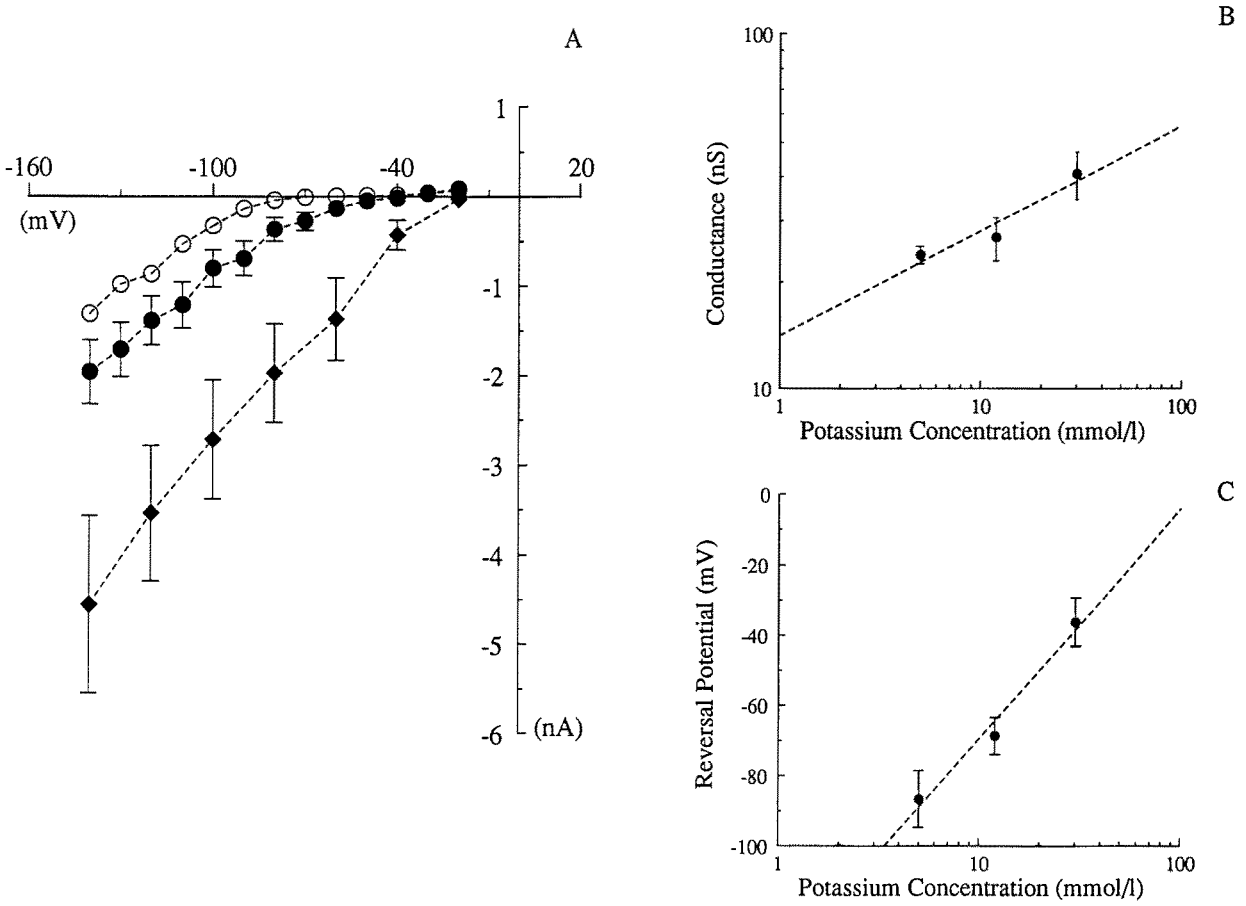

Fig. 4. (*A*) Steady-state whole-cell current-voltage relations of single sheep parotid cells in the voltage range −140 to −20 mV with 12 mmol/liter (filled circles) and 30 mmol/liter K^+ (filled diamonds) in the bathing solution. Each curve is the mean of 4–5 experiments and is superimposed on the control curve from Fig. 1 (open circles). (*B*) Log-log plot of the conductance of the inward current as a function of the extracellular K^+ concentration. The data are from *panel A*. (*C*) Semi-logarithmic plot of the reversal potential of the inward current as a function of extracellular K^+ concentration. The data are from *panel A*.

activated at potentials more negative than −40 mV. Dependency of the voltage sensitivity of a current on the extracellular K^+ concentration is an unusual phenomenon, but it is a characteristic feature of the inward rectifiers of excitable tissues such as that seen in guinea pig ventricular myocytes [18, 30]. The extremely rapid activation of the current following a voltage step seen in the sheep parotid (Fig. 1) is also a feature of the inwardly rectifying current of guinea pig ventricular myocytes.

It has been reported that the inward rectifier in many excitable cells is inhibited by Cs^+ applied extracellularly [30]. Figure 5 shows the effect of Cs^+ on the inwardly rectifying K^+ currents in sheep parotid cells. The addition of 670 μmol/liter Cs^+ to the bathing solution caused marked reduction of the inward current.

The channel responsible for the inwardly rectifying current in guinea pig ventricular myocytes has a single channel conductance of 28 pS in symmetrical isotonic KCl solutions [30] and its characteristic feature is the dependency of its single channel conductance on the extracellular K^+ concentration. In particular, as K^+ concentration in the pipette solution is varied, the single channel current-voltage relation of the inward rectifier seen in cell-attached patches remains rectilinear and its slope conductance is proportional to the square root of the pipette solution K^+ concentration [30].

Figure 6 shows the current-voltage relations of the 30 pS K^+ channel in cell-attached patches on sheep parotid secretory cells for pipette solutions in which the K^+ concentration was varied between 140 mmol/liter and 5 mmol/liter by the substitution of Na^+ for K^+. At each pipette K^+ concentration, the single-channel current-voltage relation was rectilinear over the range we could study. As the pipette K^+ concentration decreased, the single channel conductance decreased, reaching 4 pS at 5 mmol/liter K^+, and the reversal potential as estimated by ex-

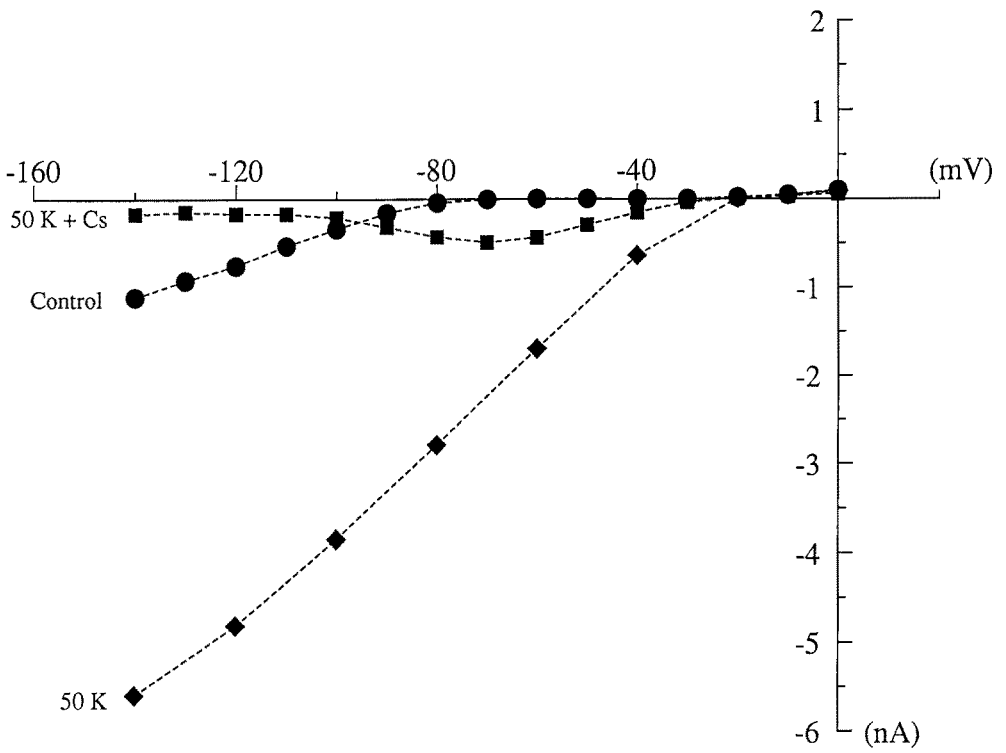

Fig. 5. Steady-state whole-cell current-voltage relations of single sheep parotid cells in the voltage range −140 to 0 mV, with 5 mmol/liter K^+ (filled circles), 50 mmol/liter K^+ (filled diamonds), or with Cs^+ (670 μmol/liter) plus 50 mmol/liter K^+ in the bath solution (filled squares).

trapolation became progressively more depolarized. The relation between single channel conductance and pipette K^+ concentration (Fig. 7*A*) shows a saturating relation that can be described by the Michaelis-Menten equation using a K_m of 36.8 mmol/liter and a maximum single channel conductance (V_{max}) of 33.4 pS. A plot of the logarithm of the single channel conductance as a function of the logarithm of pipette K^+ concentration (Fig. 7*B*) reveals a rectilinear relation with a slope of 0.55, close to the value of 0.48 reported by Sakmann and Trube [30] for the inward rectifier channel in guinea pig ventricular myocytes, and the value of 0.51 that we observed for the inwardly rectifying current in the whole-cell studies described above.

A plot of the estimated reversal potential as a function of the logarithm of pipette K^+ concentration (Fig. 7*D*) gives a rectilinear relation with a slope of 48 mV/decade. The dependency of the apparent reversal potential on pipette K^+ concentration is smaller than the theoretically expected 58 mV/decade. This discrepancy, which was also observed by Sakmann and Trube [30], may be due either to imperfect selectivity of the channel between K^+ and Na^+ ions or to imprecise estimation of the single channel reversal potential in cell-attached patches because the baseline patch conductance caused the cell potential to alter with changes in pipette potential [1]. Since our own studies, as well as those of others [14, 30], show that the inward rectifier is highly selective for K^+ over Na^+, it seems likely that the latter of the two explanations offered is the correct one.

Discussion

The evidence in the present study that the inward rectifier in sheep parotid cells is similar to that described in various excitable tissues is strong. We have demonstrated that the current satisfies each of the criteria for identifying an inward rectifier [18]: (i) the current-voltage relation is inwardly rectifying (*see* Fig. 1), (ii) the current is K^+ selective (Figs. 3 and 4), (iii) the activation curve depends on the electrochemical potential ($V - E_k$) rather than on the voltage (V) alone (Fig. 4), (iv) the current inactivates with kinetics having both fast and slow components at strong membrane hyperpolarization (Fig. 1) and (v) the current is inhibited in a voltage-dependent manner by ionic Cs^+ (Fig. 5) and Ba^{2+} (T. Ishikawa and D.I. Cook, *unpublished data*) applied

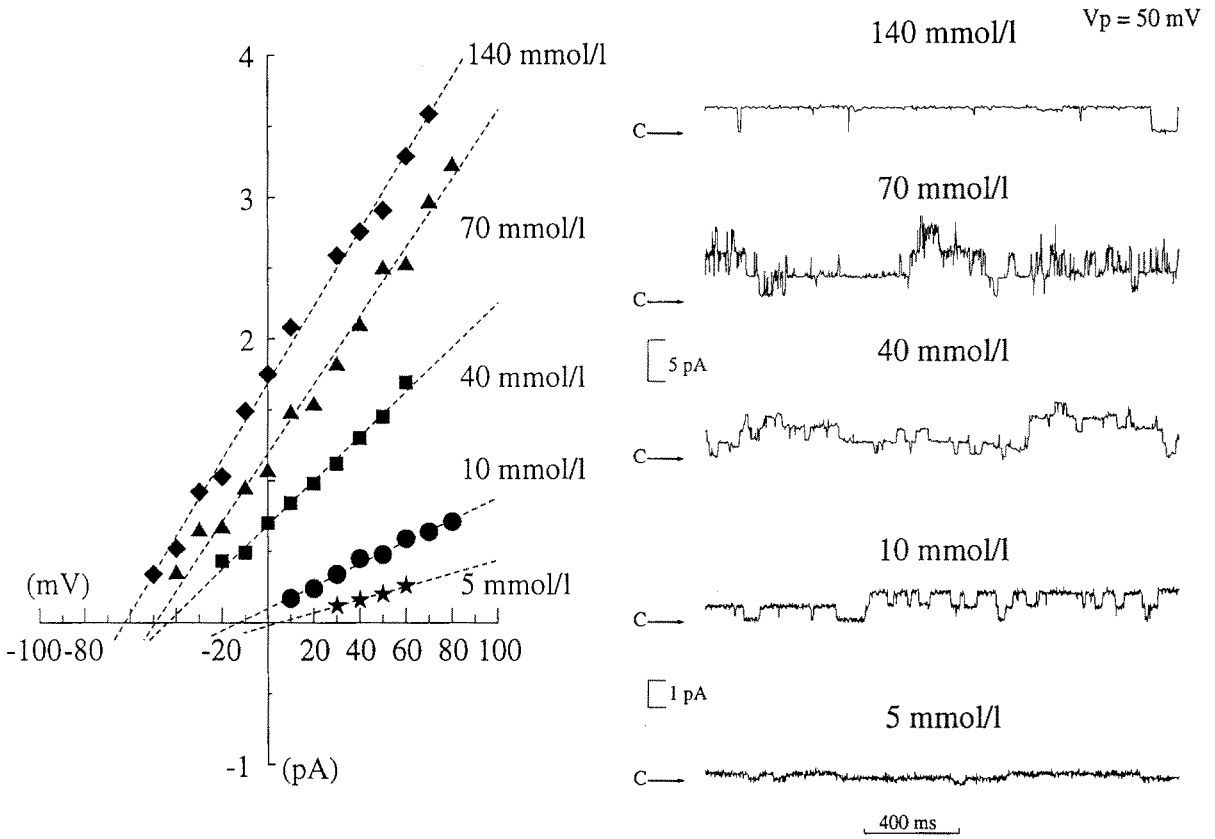

Fig. 6. Current-voltage relation of the 30 pS K^+ channel in cell-attached patches for pipette K^+ concentrations of 140 mmol/liter (10 experiments), 70 mmol/liter (3 experiments), 40 mmol/liter (3 experiments), 10 mmol/liter (4 experiments) and 5 mmol/liter (2 experiments). Also shown are representative recordings at pipette potentials of +50 mV for each pipette K^+ concentration.

extracellularly. These properties serve to distinguish the inwardly rectifying current from other types of current such as those described in sino-atrial node cells [36], in hippocampal neurons [15] and in neonatal rat spinal motor neurones [33], all of which have similar current-voltage relations although they are attributable to an increase in both Na^+ and K^+ permeability.

Although similar, the inward rectifier in sheep parotid cells differs in some significant respects from that reported in other tissues. In cardiac myocytes, inward rectification can be affected by removal of intracellular Mg^{2+}, and it has been suggested that this divalent cation causes rectification by acting as a blocking particle [24]. In three whole-cell experiments on the sheep parotid, 5 mmol/liter EDTA and 5 mmol/liter EGTA added to a nominally Mg^{2+}- and Ca^{2+}-free pipette solution failed to suppress the inward rectification. This suggests that the mechanism of inward rectification in sheep parotid cells differs from that in cardiac myocytes.

It has also been reported that the inwardly rectifying K^+ currents in kidney early distal tubule cells are regulated by intracellular pH [20]. We performed a series of experiments using pipette solutions with pH values of 6.8, 7.4 and 7.8, but found no marked difference in the size of the inward currents, suggesting that intracellular pH does not regulate the inwardly rectifying K^+ currents in sheep parotid cells. In guinea pig enterocytes, an inwardly rectifying K^+ current has been reported to be insensitive to changes in intracellular Mg^{2+} and pH [31] and in these respects resembles the inwardly rectifying K^+ current of sheep parotid cells.

Inwardly rectifying K^+ channels have been described in excitable cells including skeletal [27] and cardiac muscle [2, 19, 22, 30] as well as nonexcitable cells, including macrophages [25], glioma cells [4], osteoclasts [32], renal distal tubule cells [20], *Necturus* enterocytes [31], rabbit parietal cells [29], rat hepatocytes [17] and lens epithelial cells [13]. In these tissues, they have been reported as having several physiological roles. First, in many cells, both excitable and nonexcitable, the inward rectifier is involved in setting the resting membrane voltage [18]. Second, it has been suggested for bovine oligo-

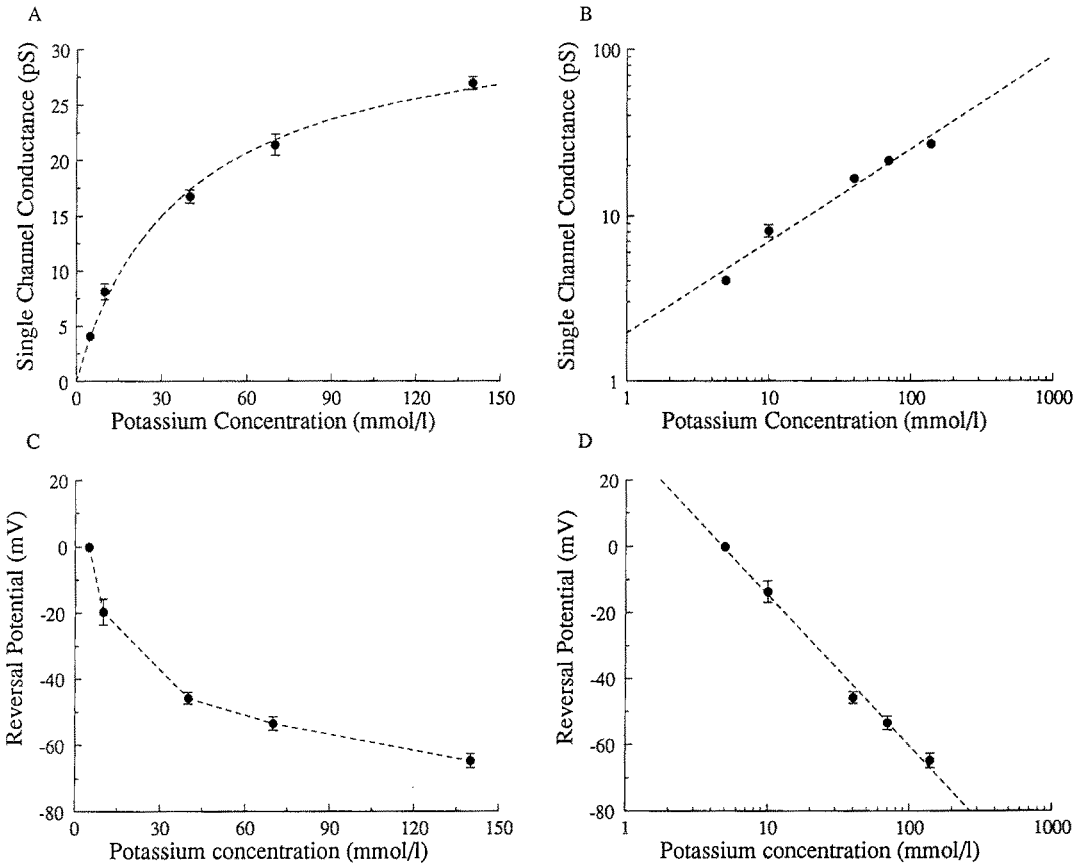

Fig. 7. (*A*) Plot of the conductances of the 30 pS channel in cell-attached patches (*see* Fig. 6) as a function of pipette K^+ concentration. The broken line is a nonlinear least-squares fit of the Michaelis-Menten equation. (*B*) Log-log plot of the single channel conductance of the 30 pS channel in cell-attached patches (*see* Fig. 6) as a function of pipette K^+ concentration. The broken line is the line of best fit. (*C*) Plot of the apparent reversal potential in cell-attached patches of the 30 pS K^+ channel as a function of pipette K^+ concentration. (*D*) Semi-logarithmic plot of the apparent reversal potential of the 30 pS K^+ channel as a function of pipette K^+ concentration in cell-attached patches. The broken line is the line of best fit.

dendrocytes that the function of the inward rectifier is to buffer transient increases in extracellular K^+ concentration resulting from neuronal activity [26].

The inward rectifier in the sheep parotid, however, is not involved in setting the cell membrane potential under the conditions used in the experiments described in this paper. This conclusion is based on our observation that the addition of 2 mmol/liter Cs^+ to the bathing solution to block the inward rectifier did not affect the reversal potential of the K^+ channels in cell-attached patches with KCl in the pipette; *viz., control*: -65.8 mV $\pm$ 1.8 (SEM, $n = 3$); *with* Cs^+: -63.3 mV $\pm$ 3.7 (SEM, $n = 3$).

The role of the inwardly rectifying K^+ channels in sheep parotid cells may be to buffer a rise in extracellular K^+, since salivary glands in many species including sheep parotid have been shown to lose K^+ to their surroundings when they are stimulated to secrete [5, 34]. This raises the question why, of the salivary glands so far studied, the sheep parotid is the only one to have an inward rectifier [10, 28]. A possible explanation for this is that in the other salivary glands that have so far been studied, the Na-K-2Cl cotransporter is present and acts as a K^+ uptake mechanism during secretion [9, 23], whereas in the sheep parotid, the Na-K-2Cl cotransporter appears to play no part in secretion [35].

The identification of the 30 pS K^+ channel in sheep parotid secretory cells as the inward rectifier, does not exclude the possibility that the channel supports spontaneous secretion. Current models of epithelial secretion are based on an electrogenic efflux of Cl^- across the apical membrane [9, 23] which is balanced by efflux of K^+ through K^+ channels in the basolateral [23] and possibly the apical membrane [8]. Inward rectifiers permit some outward current flow [18, 30] and, given the large number and high open probability of these channels in sheep parotid cells, this may be sufficient to drive anion

efflux across the apical membrane and hence to support spontaneous secretion. Our observation that block of the inward rectifier with Cs^+ does not depolarize the cells is not consistent, however, with the presence of an anion current flowing across the apical membrane. A possible solution to this dilemma may be that the anion being secreted by these cells is not Cl^- or PO_4^{3-}, but HCO_3^- which was not included in the solutions used in our experiments. The HCO_3^- concentration in the final saliva of the sheep parotid rises with increasing flow rate [6, 7], as would be expected if the gland secretes HCO_3^- rather than Cl^-. We are currently examining this possibility.

This project was supported by the Australian Research Council. We thank Professor J.A. Young for his critical reading of the manuscript and Drs. N. Sangster and J. Rothwell and Mr. R. Murphy for giving us access to their sheep.

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Received 21 April 1992; revised 8 July 1992