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Evidence for two types of potassium current in rat choroid plexus epithelial cells

Tohru Kotera*, Peter D. Brown

Department of Physiological Sciences, University of Manchester, Manchester M13 9PT, UK

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Abstract. The whole-cell patch-clamp technique was applied to rat choroid plexus epithelial cells. The resting membrane potential was −53 mV. The whole-cell conductance was mainly K^+ selective, and the K^+ current observed appeared to contain two distinct components. Depolarizing voltage pulses (more positive than 0 mV) evoked time-dependent outward currents which resembled delayed-rectifying K^+ currents in other tissues. The current exhibited time-dependent activation and, at potentials more positive than 40 mV, slower time-dependent inactivation. The reversal potential measured by tail current analysis showed a shift of 43 mV for a tenfold increase in extracellular K^+ concentration ($[K^+]_o$). The current was reduced by extracellular 5 mM Ba^{2+}, 5 mM tetraethylammonium (TEA^+), 5 mM Cs^+ and 1 mM 4-aminopyridine (4-AP). In contrast, hyperpolarizing voltage pulses evoked time-independent, inward-rectifying currents. The reversal potential measured by voltage-ramp commands showed a shift of 42 mV for a tenfold increase in $[K^+]_o$. The chord conductance did not appear to increase with increasing $[K^+]_o$. The current was reduced by extracellular 5 mM Ba^{2+} and 0.5 mM Cs^+, but not by 5 mM TEA^+ or 1 mM 4-AP. These data suggest that two populations of K^+ channel contribute to the conductance of choroid plexus epithelial cells.

Key words: Potassium current – Choroid plexus – Cerebrospinal fluid – Outward-rectification – Inward-rectification – Epithelium

Introduction

The epithelial cells of the choroid plexuses secrete most of the cerebrospinal fluid (CSF) into the ventricles of the brain [1, 26]. Na^+ is thought to be pumped directly into the CSF by Na^+-K^+ATPase [26]. Recent patch-clamp experiments have identified anion channels in the apical membrane of the mammalian choroid plexus which may have a significant role in Cl^- and HCO_3^- secretion into the CSF [1, 7, 10]. These observations have led to the proposal of a model for CSF secretion by the choroid plexus which explains the movements of Na^+, Cl^- and HCO_3^- into the CSF [1]. The transport of K^+ ions across the choroid plexus epithelium, however, is only poorly understood.

Unidirectional flux studies of the amphibian choroid plexus have shown that there is a net absorption of K^+ (CSF to plasma) across the epithelium [26]. Zeuthen and Wright [27] suggested, from data obtained in amphibian choroid plexus, that more than 90% of K^+ which is pumped into the cells across the apical membrane by Na^+-K^+ ATPase is recycled through ion channels in the same membrane. However, some K^+ leaves the cells via ion channels in the basolateral membrane, and this accounts for the net absorptive flux. Regulating the activity of either population of channel (apical or basolateral) will cause changes in the rate of K^+ absorption, and this may help explain how the choroid plexus controls the K^+ concentration in the CSF [12]. Single-channel patch-clamp studies have identified Ca^{2+}-activated K^+ channels in the apical membrane of the *Necturus* choroid plexus epithelial cells [2, 4]. Whole-cell studies of the same cells, however, suggested that these channels may not make a significant contribution to the total K^+ conductance [20].

In the present study the whole-cell patch-clamp technique has been used to characterize K^+ conductances in rat choroid plexus epithelial cells. Two distinct K^+ currents are described: a time-dependent, outward-rectifying current, and a time-independent, inward-rectifying current. A preliminary account of some of this work has been published in abstract form [16].

Materials and methods

Cell and tissue preparation. Adult Sprague-Dawley rats were killed by an overdose of diethyl ether and the choroid plexus

* *Present address:* The First Division, Department of Internal Medicine, Kyoto University Faculty of Medicine, 54 Syogoin-Kawaramachi, Sakyo, Kyoto 606-01, Japan
Correspondence to: P. D. Brown

was removed from the fourth ventricle. The tissue was kept in an ice-cold 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES)-buffered saline and used within 2–3 h. Most experiments were performed on intact tissue, but isolated epithelial cells were used in some experiments. The isolated cells were prepared using the pronase digestion method previously described [24]. The tissue was incubated in Ca^{2+}- and Mg^{2+}-free Hank's buffer (Gibco), containing 0.3 mg/ml pronase (Boehringer Mannheim, Germany) and 0.5 mg/ml DNase I (Sigma) for 10 min at 37°C. The pronase solution was removed and the tissue was gently agitated in fresh buffer to liberate epithelial cells. The supernatant was collected and the sediment was washed with fresh buffer. The pronase digestion procedure was repeated twice, before the cells (single cells and small clusters) were collected and resuspended in the ice-cold HEPES-buffered saline. Cells were used within 2 h of isolation.

Patch-clamp recording. A small piece of intact tissue was secured to the base of a small chamber (bath volume approx. 0.4 ml) with a stainless steel wire on the stage of an inverted microscope (Olympus IMT-2, Japan) as previously described [7]. Isolated cells adhered to the glass bottom of the chamber. The bath was perfused with solution (gassed with 100% O_2) at a rate of about 2.5 ml/min.

Conventional whole-cell recording methods were employed throughout, after gigaohm seals had been obtained on the exposed apical (ventricular) membrane of cells in intact choroid plexus epithelium, or on the surface of isolated cells. The patch pipettes were made from haematocrit capillary tubes (Oxford Labware, USA) using a two-stage vertical puller (PB-7, Narishige, Japan). The tip resistance of the patch electrodes was 5–10 MΩ when filled with pipette solution (see below). Ag-AgCl pellets were used both for the patch electrode and for the reference electrode.

Membrane currents (voltage-clamp mode) and membrane potentials (current-clamp mode) were measured with an Axopatch-1D amplifier (Axon Instruments, USA). Data were stored on videotape with a VHS tape recorder (Akai, Japan) via a digital pulse code modulator (Sony PCM-701ES, Japan), and on the hard disk of a personal computer. Step-voltage pulses and voltage-ramps were generated by the computer using pClamp software (Axon Instruments) and a TL-1 interface (Axon Instruments). Membrane capacitance was estimated and compensated under voltage-clamp mode using the capacitance compensation circuit of the amplifier. Uncompensated series resistance was 6–15 MΩ. The leak current component was not subtracted.

Solutions. The composition of all the bath and pipette solutions used in this study is given in Table 1. Chemicals, such as potassium aspartate (K aspartate), sodium aspartate (Na aspartate), adenosine 5′-triphosphate (Na_2ATP), HEPES, ethylene glycol-bis(β-aminoethyl ether)*N,N,N′,N′*-tetraacetic acid (EGTA) and *N*-hydroxyethylethylenediaminetriacetic acid (HEDTA), were purchased from either Sigma or Merck. Intracellular Ca^{2+} activity ($[Ca^{2+}]_i$) was estimated to be no more than 10 nM [21] in the control, KCl and K^+-free pipette solutions. The high-Ca^{2+} pipette solution ($[Ca^{2+}]_i$ = 500 nM [6]) was used to study the effect of $[Ca^{2+}]_i$ on channel activity. In some experiments the following K^+ channel blockers were used: barium chloride (Ba^{2+}) (Merck), tetraethylammonium chloride (TEA^+) (Sigma), caesium chloride (Cs^+) (Merck) and 4-aminopyridine (4-AP) (Sigma). These were all dissolved in the control bath solution.

All experiments were performed at room temperature (19–23°C). The data are expressed as the mean ± SEM of *n* observations. Significance (*P*) was determined by Student's *t*-test for unpaired data.

Table 1. Composition of the bath and the pipette solutions

Constituent	Bath solutions (mM)			
	Control	50 K^+	145 K^+	K^+ free
NaCl	140	95		145
KCl	5	50	145	
$CaCl_2$	1	1	1	1
$MgCl_2$	1	1	1	1
HEPES	5	5	5	5

pH was adjusted to 7.3 with NaOH or KOH (for the 145 K^+ solution)

	Pipette solutions (mM)			
	Control	KCl	High Ca	K free
K aspartate	120			
KCl	20	140	46[a]	
Na aspartate				120
NaCl				20
$CaCl_2$			7	
$MgCl_2$	2	2		2
Na_2ATP[b]	2	2		2
EGTA	0.5	0.5		0.5
HEDTA			42	
HEPES	5	5	5	5

pH was adjusted to 7.2 with KOH or NaOH (for the K^+-free solution)

[a] Total K^+ concentration was approximately 150 mM with the added KOH

[b] Na_2ATP was omitted from the pipette solution in some experiments

HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Na_2ATP, adenosine 5′-triphosphate, disodium salt; EGTA, ethylene glycol-bis-(β-aminoethyl ether(*N,N,N′,N′*-tetraacetic acid; HEDTA, *N*-hydroxyethylethylenediaminetraacetic acid

Results

Membrane capacitance

The membrane capacitance of choroid plexus epithelial cells was measured in intact tissue and isolated cells. The value obtained from intact tissue was 54.5 ± 1.2 pF (n = 45). In isolated cells, the capacitance was 51.6 ± 3.8 pF (n = 7) for single isolated cells, and 54.7 ± 1.1 pF (n = 49) for cells which were part of small clusters. There was no significant difference between these values ($P > 0.5$). These results suggest that there is little cell-to-cell electrical coupling between adjacent epithelial cells of rat choroid plexus. This hypothesis is supported by the electrophysiological study which showed that there is a low coupling coefficient in amphibian choroid plexus [22], and by the fact that gap junctions cannot be observed in rat choroid plexus [25]. If there is little cell-to-cell coupling, whole-cell currents can be measured in intact tissue. In this study, therefore, most experiments were performed on intact tissue.

The surface area of a choroid plexus epithelial cell was estimated from the capacitance measurements to be about 5400 μm², using the generally accepted value of 1 μF/cm². This value is nine times larger than the value of 600 μm² which can be calculated for a cuboid cell with a side length of 10 μm [14]. This difference in val-

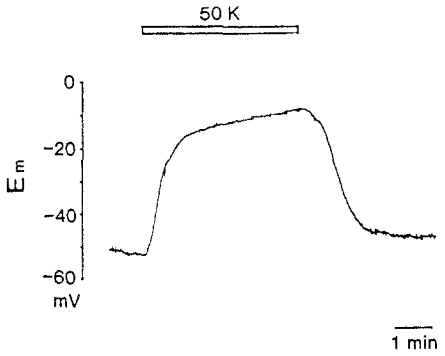

Fig. 1. The effect of a change in extracellular K^+ concentration ($[K^+]_o$) on the membrane potential (E_m). E_m was monitored in current-clamp mode. The pipette contained the control (K aspartate) solution. Extracellular K^+ concentration ($[K^+]_o$) was increased from 5 mM to 50 mM as indicated by the *bar*. The *trace* is representative of four similar observations

ues suggests that the microvilli on the apical membrane and infoldings at the basolateral membrane make a substantial contribution to the surface area of the cell.

Resting membrane potential

Estimates of the membrane potential were made in the current-clamp mode 1 min after establishing the whole-cell configuration. The membrane potential was in the range of −45 to −64 mV with an average value of −53 ± 1 mV (n = 28) with the control (K aspartate) pipette solution. A similar value of −54 ± 1 mV (n = 12) was obtained using the KCl pipette solution. The effect of the K^+ gradient on the membrane potential was studied by changing extracellular K^+ concentration ($[K^+]_o$) (Fig. 1). A tenfold increase in $[K^+]_o$ (from 5 to 50 mM) caused a 45 mV depolarization (from −53 to −8 mV). This change was reversed upon returning to the control bath solution (Fig. 1). These results suggest that the plasma membrane of choroid plexus epithelial cells is highly permeable to K^+ at rest.

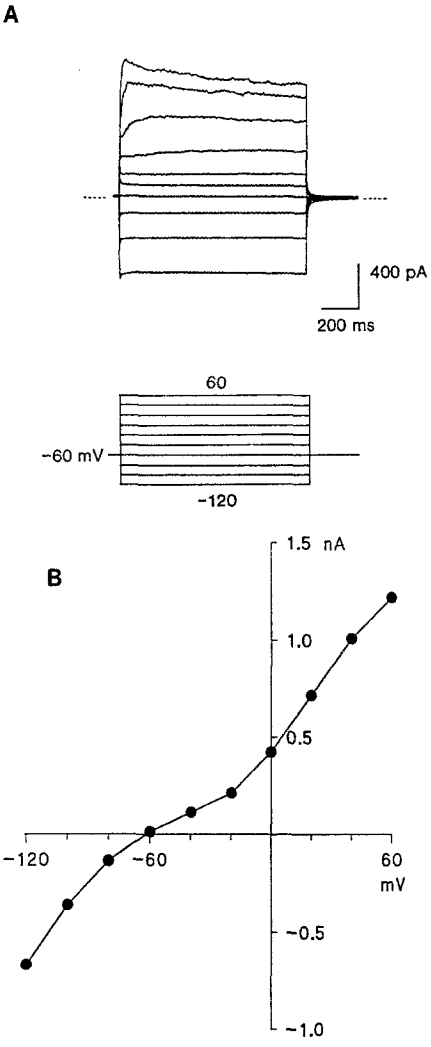

Fig. 2 A, B. Current profiles and current/voltage (*I/V*) relationship of the whole-cell current. **A** Current profiles. The holding potential (V_h) was −60 mV, from which 1-s step-voltage pulses were applied over the range from −120 to 60 mV at 20-mV increments. The zero-current level is indicated by the *dashed line*. **B** *I/V* relationship for the currents in **A**. Peak current amplitudes are plotted as a function of membrane potential

Whole-cell currents

Figure 2 A shows typical traces of whole-cell currents recorded in a choroid plexus epithelial cell. The holding potential (V_h) was −60 mV (close to the resting membrane potential) and 1-s step-voltage pluses were applied over the range −120 to 60 mV in 20-mV increments. Two types of currents were apparent. In the voltage range from −120 to −20 mV, the currents were time independent. In contrast, depolarizing voltage pulses (more positive than 0 mV) evoked time-dependent outward currents. The peak amplitude of these currents is plotted as a function of voltage in Fig. 2 B. This current/voltage (*I/V*) relationship exhibits both outward- and inward-rectifying components. The removal of ATP from the control pipette solution did not effect either component. Outward- and inward-rectifying currents were observed in most cells, and both displayed a small amount of "run-down" during whole-cell recording (e. g. an approximate decrease of 10% of the maximum outward current over 10 min). The inward-rectifying current appeared more sensitive to "run-down", and a few cells displayed only the outward-rectifying component, with hyperpolarizing pulses evoking small inward currents (data not shown).

Both components of the whole-cell current were abolished when K^+ in the pipette and the bath solutions was replaced with Na^+. Under these conditions, only small currents were observed in response to depolarizing or hyperpolarizing voltage pulses (Fig. 3). The inward-rectifying component was still apparent when only K^+ in the pipette solution was replaced with Na^+ (data not shown). These results indicate that most of the whole-cell current is carried by K^+ channels in choroid plexus epithelial cells {much of the remaining current is carried by Cl^- channels which can be stimulated transiently by guanosine-5′-*O*-(3-thiotriphosphate) (GTP[γ-S]), cyclic

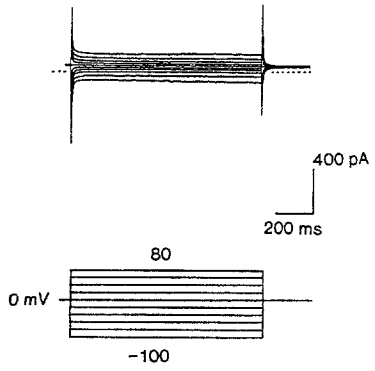

Fig. 3. Whole-cell current in the absence of K^+. Both the bath and the pipette contained K^+-free solutions in which K^+ had been replaced with Na^+ (see Table 1). V_h was 0 mV, and 1-s voltage-step pulses were applied over the range of −100 mV to 80 mV at 20-mV increments. The zero-current level is indicated by the *dashed line*

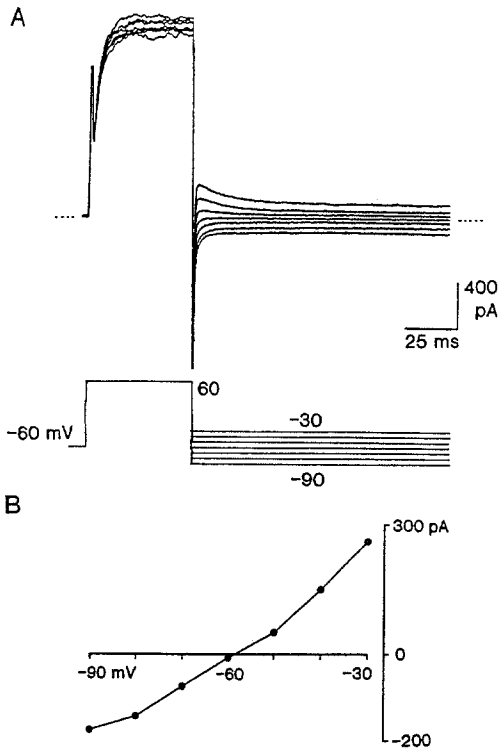

Fig. 4 A, B. Tail current analysis of the outward-rectifying current. **A** Tail currents were measured using a double-pulse protocol. V_h was −60 mV, and the outward current was activated by 50-ms voltage pulses to 60 mV at intervals of 2 s. Tail currents were initiated by stepping to various negative potentials in the range of −90 to −30 mV and were measured at 10-mV increments for 125 ms. The bath and the pipette contained control solutions. **B** Instantaneous *I/V* relationship for the tail currents in **A**. The results are representative of seven observations.

adenosine monophosphate (cAMP) and protein kinase A [17]}. These observations also suggest that at least two distinct populations of K^+ channel contribute to the whole-cell conductance. In the remainder of this paper the outward-rectifying and the inward-rectifying component of the K^+ current are characterized in greater detail.

The time-dependent, outward-rectifying current

In cells dialysed with the control pipette solution, time-dependent outward currents were observed in response to depolarizing voltage pulses (more positive than 0 mV) (Fig. 2 A). The slope conductance increased progressively as the voltage pulses became more positive (5.1 nS at −20 mV; 8.7 nS at 20 mV; 10.0 nS at 60 mV; Fig. 2 B). The currents also activated more quickly with larger depolarizations. However, at potentials more positive than 40 mV, time-dependent inactivation of the current was observed after the initial period of activation (Fig. 2 A).

To determine the reversal potential (E_{rev}) of the time-dependent outward-rectifying current, tail current analysis was performed uisng a double-pulse protocol. At 5 mM $[K^+]_o$, the membrane potential was depolarized by the first step pulse (from a V_h of −60 mV to 60 mV for 50 ms), and then tail currents were measured at various second step pulses (in the range from −90 to −30 mV in 10-mV increments for 125 ms; Fig. 4 A). The *I/V* relationship for the tail currents reversed (E_{rev}) at −60.6 ± 0.8 mV ($n = 7$; Fig. 4 B). E_{rev} was −17.8 ± 1.3 mV ($n = 4$) when measured at 50 mM $[K^+]_o$ using a similar pulse protocol (first step from a V_h of −20 mV to 90 mV, second steps in the range of −60 to 0 mV). Thus, there was a 43 mV shift for a tenfold increase in $[K^+]_o$. These results suggest that the time-dependent, outward-rectifying current is largely K^+ selective.

To study the pharmacology of the outward-rectifying current, the effects of several K^+ channel blockers were investigated. Figure 5 shows superimposed current traces evoked by steps to 60 mV before (control), during (Ba, TEA, Cs) and after (recovery) the application of the blocker to the bath solution. Ba^{2+} (5 mM) reduced the outward current by 59.2 ± 4.3% ($n = 5$; Fig. 5 A). TEA^+ (5 mM) also reduced the current by 51.8 ± 3.3% ($n = 5$; Fig. 5 B). Two other known K^+ channel blockers, 5 mM Cs^+ (21.4 ± 2.9% reduction, $n = 5$; Fig. 5 C) and 1 mM 4-AP (35.0 ± 4.1% reduction, $n = 4$; not illustrated), caused a slight reduction of the outward-rectifying current. The apparent incomplete recovery following block of both outward-rectifying and inward-rectifying currents may be in part due to channel "run-down".

The Ca^{2+} dependence of the outward-rectifying current was studied using pipette solutions with different $[Ca^{2+}]_i$. The maximum current amplitude at 60 mV was 1160 ± 56 pA ($n = 17$) when the pipette contained the control solution ($[Ca^{2+}]_i \leq 10$ nM). When the high Ca^{2+} pipette solution ($[Ca^{2+}]_i = 500$ nM) was used, no significant change was observed in either the current amplitude (1012 ± 42 pA, $n = 4$, $P > 0.06$), or the current profile. These results suggest that the time-dependent, outward-rectifying K^+ current may be Ca^{2+} independent.

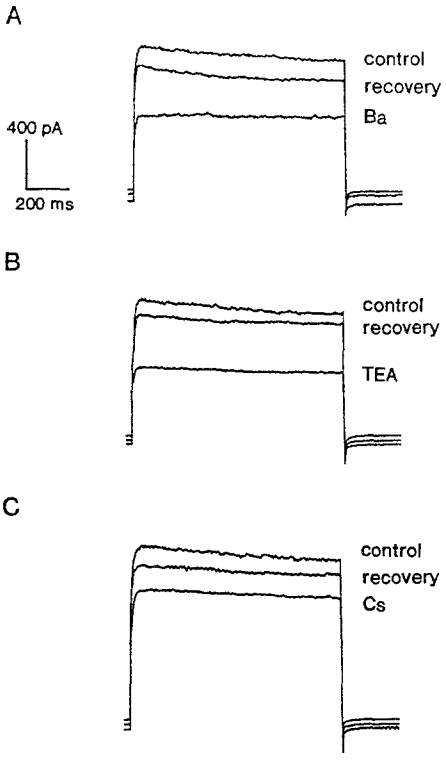

Fig. 5 A–C. The effects of K^+ channel blockers on the outward-rectifying current. **A** Ba^{2+} (5 mM); **B** tetraethylammonium (TEA, 5 mM); **C** Cs^+ (5 mM). V_h was −60 mV. Outward currents were measured during 1-s depolarizing voltage steps to 60 mV: before (*control*), during (*Ba, TEA, Cs*) and after (*recovery*) the application of the blocker to the bath. The results are representative of five similar observations for each blocker

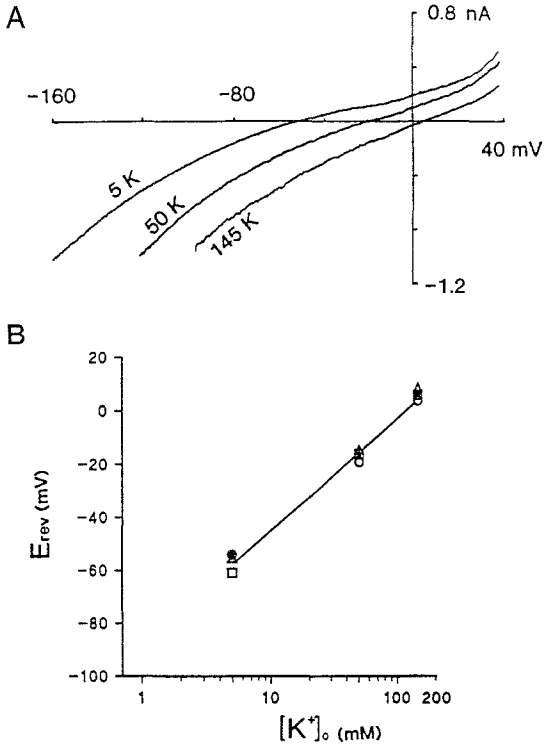

Fig. 6 A, B. The effect of changes in $[K^+]_o$ on the inward-rectifying current. **A** Instantaneous *I/V* relationships, at three different values of $[K^+]_o$, obtained by voltage-ramp commands in one cell. The cell was dialysed with a control pipette solution. The bath solution was initially a control solution (5 mM $[K^+]_o$). $[K^+]_o$ was subsequently increased to 50 mM and then to 145 mM. V_h was −60 mV, −20 mV and 0 mV for 5 mM, 50 mM and 145 mM $[K^+]_o$ respectively. Voltage-ramp commands (from V_h −100 mV to V_h + 100 mV in 0.8 s) were applied at each $[K^+]_o$. Similar results were obtained in three other cells. **B** The relationship between $[K^+]_o$ and reversal potential (E_{rev}) of the inward-rectifying current. Values of E_{rev} were measured from the instantaneous *I/V* relationships (as shown in **A**) and plotted against $[K^+]_o$ on a semilogarithmic scale. Four *symbols* (○, □, △, ★) indicate results obtained from four cells. The straight line has a slope of 42 mV/tenfold change in $[K^+]_o$

Time-independent, inward-rectifying current

Time-independent currents were observed in most cells when the membrane potential was stepped to voltages more negative than −20 mV (Fig. 2 A). These currents appeared to be almost instantaneous in onset. The slope conductance for the *I/V* relationship increased at voltages more negative than −80 mV (approx. the equilibrium potential of K^+, E_K). The average slope conductance was 4.7 ± 0.2 nS at −40 mV and 10.5 ± 0.5 nS at −100 mV (n = 10), which indicates inward rectification (Fig. 2 B).

To investigate the selectivity of the current for K^+, the dependence of E_{rev} on $[K^+]_o$ was studied. Figure 6 A shows the instantaneous *I/V* relationships obtained at three different $[K^+]_o$ in one cell, using voltage-ramp commands. V_h was −60 mV, −20 mV and 0 mV for 5 mM, 50 mM and 145 mM $[K^+]_o$ respectively, and voltage-ramp commands (from −100 to +100 mV in 0.8 s) were applied. The *I/V* relationship shifted in the positive direction with increasing $[K^+]_o$, as would be expected for K^+ currents (i. e. E_{rev} becomes more positive as does E_K). The chord conductance did not become larger with increasing $[K^+]_o$. Values for E_{rev} obtained in four series of experiments were plotted as a function of $[K^+]_o$ on a semilogarithmic scale (Fig. 6 B). The straight line fitted by linear regression analysis to these data gave a slope of 42 mV per decade change in $[K^+]_o$. These results suggest that the time-independent current is carried mainly by K^+-selective channels.

The effects of several K^+ channel blockers on the inward-rectifying current were investigated. Membrane currents at a hyperpolarizing voltage (−120 mV) were measured before (control), during (Ba, Cs) and after (recovery) the application of the blocker to the bath solution (Fig. 7 A, B). Ba^{2+} (5 mM) inhibited the inward current by 80.2 ± 3.4% (n = 5), while 0.1 mM Ba^{2+} reduced the current by 39.8% (n = 3; data not shown). The inward-rectifying current was more sensitive to extracellular Cs^+ than the outward-rectifying current. Cs^+ (5 mM) caused a 60.0 ± 3.6% (n = 3) reduction in the inward-rectifying current (Fig. 7 B). The inward-rectifying current was reduced by 34.0 ± 3.2% (n = 3) at 0.5 mM Cs^+ (lower traces in Fig. 7 C), while the outward-rectifying current (at 60 mV) was completely unaffected by 0.5 mM Cs^+ (upper traces in Fig. 7 C). TEA^+ (5 mM) and 4-AP (1 mM) had little effect on the inward-rectifying current (data not shown).

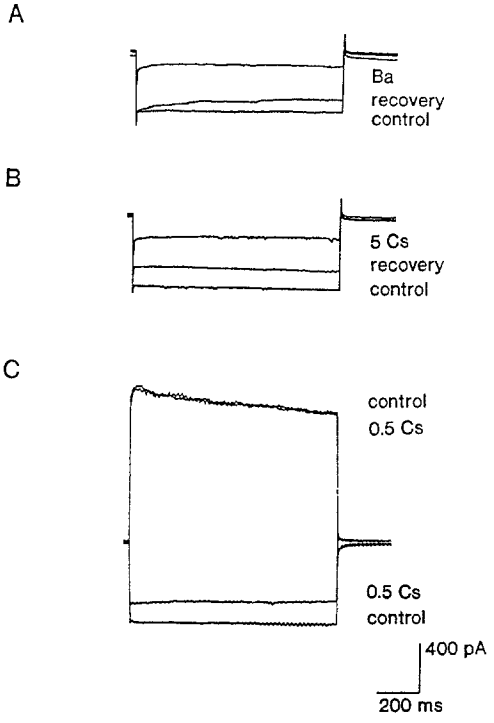

Fig. 7 A–C. The effects of K^+ channel blockers on the inward-rectifying current. **A** Ba^{2+} (5 mM); **B** Cs^+ (5 mM). V_h was −60 mV. Inward currents were measured during 1-s hyperpolarizing voltage steps to −120 mV before (*control*), during (*Ba, 5Cs*) and after (*recovery*) the application of the blocker to the bath. **C** Cs^+ (0.5 mM). V_h was −60 mV. Inward currents (*lower traces*) and outward currents (*upper traces*) were measured during 1 s hyperpolarizing (to −120 mV) and depolarizing (to 60 mV) voltage steps, before (*control*) and during (*0.5 Cs*) the application of the blocker to the bath. The results are representative of five (**A**) or three (**B, C**) observations

The Ca^{2+} dependence of the time-independent current was studied in the same way as for the outward-rectifying current. Current amplitude at −120 mV was unaffected by changes in $[Ca^{2+}]_i$ (≤ 10 nM; −473± 38 pA, $n = 17$, and at 500 nM; −447 ± 47 pA, $n = 3$, $P > 0.5$). These results suggest that the time-independent, inward-rectifying K^+ current may also be Ca^{2+} independent.

Discussion

K^+ conductance and membrane potential

The electrophysiological characteristics of epithelial cells from the mammalian choroid plexus have not been previously studied in any great detail. This is probably because of the small size of these cells and the very delicate nature of the tissue. The resting membrane potential measured in whole-cell current-clamp mode in the present study was −53 mV. This value is in good agreement with −59 mV obtained by the similar method in *Necturus* choroid plexus [20]. Similar values of about −50 mV have also been estimated from the *I/V* relationships for single K^+ channels in cell-attached patches in rat and mouse choroid plexus [1, 10]. It is also within the range of −45 mV [22, 27] to −88 mV [28], values which were obtained in amphibian tissues by conventional microelectrode methods. A tenfold increase in $[K^+]_o$ caused a large depolarization (Fig. 1), indicating that the epithelial membrane of the choroid plexus is predominantly K^+ permeable. This is in agreement with a previous microelectrode study of amphibian choroid plexus [27].

In voltage-clamp experiments the whole-cell conductance of the choroid plexus epithelial cells was also found to be predominantly K^+ selective. The whole-cell K^+ conductance appeared to be composed of two distinct components, i. e. time-dependent, outward-rectifying currents and time-independent, inward-rectifying currents. These distinct components of the conductance are possibly carried by two different populations of K^+ channel. Further evidence for this hypothesis is discussed below.

The time-dependent, outward-rectifying K^+ current

Depolarizing voltage pulses to potentials more positive than 0 mV evoked a voltage- and time-dependent outward current. The current appeared to activate more rapidly when the membrane potential was more strongly depolarized. The current also showed slow inactivation at potentials more positive than 40 mV. Three lines of evidence strongly suggest that this current is carried mainly by K^+: (1) the outward-rectifying current was not observed in the absence of K^+ in the pipette solution (Fig. 3); (2) E_{rev} was shifted by 43 mV for a tenfold increase in $[K^+]_o$; (3) the current was sensitive to K^+ channel blockers, such as Ba^{2+}, TEA^+ and 4-AP (Fig. 5).

In some respects the outward-rectifying current described resembles the Ca^{2+}-dependent K^+ currents which have been identified in *Necturus* choroid plexus [20]. These currents are thought to be carried by "maxi" Ca^{2+}-activated K^+ channels which have also been identified in amphibian choroid plexus [2, 4]. However, the outward-rectifying current in the present study appeared to be independent of $[Ca^{2+}]_i$, and Ca^{2+}-activated K^+ channels have not been observed in single-channel studies of the mammalian choroid plexus [1, 10]. Thus, the time-dependent outward current in the mammalian choroid plexus appears to be different from that in the amphibian choroid plexus. Instead it is more like the delayed rectifying K^+ currents which have been described in several non-excitable cells, e. g. human T lymphocytes [3], rat Leydig cells [5] and frog retinal pigment epithelial cells [9]. Each of these currents show voltage and time dependence of activation (and inactivation), and blocker specificity which are analogous to those shown in the present study.

The time-independent, inward-rectifying K^+ current

Time-independent, inward-rectifying currents were also observed in most cells (Fig. 2). The evidence for the K^+ selectivity of these currents was similar to that for the

outward-rectifying K^+ current: (1) the inward-rectifying current was not observed in the absence of extracellular K^+ (Fig. 3); (2) E_{rev} was shifted to 42 mV by a tenfold increase in $[K^+]_o$ (Fig. 6); (3) the current was sensitive to K^+ channel blockers (Ba^{2+} and Cs^+; Fig. 7). The inward-rectifying current was distinct, however, from the outward-rectifying current in a number of respects. The most noticeable difference was that the current was almost instantaneous in onset and did not exhibit time-dependent activation or inactivation (Fig. 2 A). It also exhibited inward-rectification with the slope conductance increasing at potentials more negative than E_K (Fig. 2 B). A third difference was that the pharmacological properties of the two currents differed. The inward-rectifying current was insensitive to TEA^+ and 4-AP, but was sensitive to 0.1 mM Ba^{2+} and to Cs^+ at concentrations as low as 0.5 mM (Fig. 7). Taken together these data strongly suggest that two separate populations of K^+ channel are present in the mammalian choroid plexus epithelial cell.

Inward-rectifying K^+ currents have recently been identified in several non-excitable cells, e. g. frog distal tubule cells [11], guinea-pig parietal cells [18], frog retinal pigment epithelial cells [9], and sheep parotid acinar cells [13]. The inward-rectifying K^+ currents in these cells and the choroid plexus share some of the characteristics of the classical inward rectifiers found in frog skeletal muscle [19] and starfish eggs [8]. There are, however, two distinct differences between the current described here and the classical inward rectifiers. First, the degree of inward rectification was not so steep as in the classical inward rectifiers, so that a significant outward current was observed near the resting membrane potential (Fig. 2 B).

Second, the chord conductance did not increase when $[K^+]_o$ was increased (Fig. 6 A). The chord conductance of the inward-rectifying K^+ current in frog retinal pigment epithelium [9] is also independent of $[K^+]_o$, but the reason for this independence remains. This phenomenon will probably not be understood in the choroid plexus without many additional studies, including experiments on the single-channel basis of the inward-rectifying K^+ current.

Possible physiological roles of K^+ currents

The location (apical or basolateral) of the two K^+ channel conductances cannot be determined by the whole-cell recording technique, and will not be known until the single-channel basis of these K^+ currents has been established. However, at least two lines of evidence suggest that they are likely to be located in the apical membrane. First, the previous microelectrode study in amphibian choroid plexus has suggested that 90% of K^+ conductance is located in the apical membrane [27]. Second, the membrane potential rapidly responded to changes in $[K^+]$ in the bath solution, which is in an immediate contact with the apical membrane of the intact tissue in our experiments (Fig. 1).

The outward-rectifying K^+ current is unlikely to have a significant physiological role, because the threshold of activation of the current was more positive than −20 mV (Fig. 2 A), which is considerably depolarized from the resting membrane potential. A similar conclusion has been reached for outward-rectifying K^+ currents in other non-excitable cells [15]. However, it is conceivable that the outward-rectifying K^+ current may be activated to prevent the membrane potential becoming too depolarized when the cell is depolarized with secretagogues, e. g. activation of anion conductances by cAMP may cause a depolarization of the apical membrane potential [17]. A simlar role was envisaged for the Ca^{2+}-dependent K^+ currents in amphibian choroid plexus [20].

The inward-rectifying K^+ currents, on the other hand, may have a more significant role in choroid plexus epithelial cells. The inward rectification is not complete, so that a substantial outward current can be observed at potentials around the resting membrane potential (approx. 50 mV) (Fig. 2 B). These currents could therefore make a significant contribution to the resting conductance of the cell, and may be fundamental in establishing the resting potential of the cell[1] (which is essential for the secretion of anions [1, 7]). Furthermore, Zeuthen and Wright [27] have suggested that as much as 90% of K^+ pumped into the cell by Na^+-K^+ ATPase in the apical membrane leaves the cell via ion channels in the same membrane. If the channels carrying the inward-rectifying K^+ current are located in the apical membrane, they might function as an important apical K^+ efflux pathway, and could contribute to K^+ homeostasis in the cell and CSF. Studying the regulation of the inward-rectifying K^+ current may therefore lead to a better understanding of K^+ homeostasis in CSF.

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[1] Inward-rectifying K^+ channels are also thought to make a significant contribution to the resting potential of other cells, e. g. cardiac myocytes [23].

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