Kv1.1 and Kv1.3 channels contribute to the delayed-rectifying K⁺ conductance in rat choroid plexus epithelial cells

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Two types of K⁺ conductance have been identified in whole cell recordings from rat choroid plexus (22): a delayed-rectifying K⁺ (Kv) conductance and an inward-rectifying K⁺ (Kir) conductance. The Kir conductance is carried by Kir7.1 channel proteins, which when expressed in Xenopus oocytes exhibit properties that are almost identical to those observed in choroid plexus (10, 22). Immunocytochemical studies also showed that Kir7.1 channels are expressed in the apical membrane of the choroid plexus epithelium (27). By contrast, less is known about the molecular identity and the location of the channels carrying the Kv conductance. This conductance exhibits time-dependent activation at depolarizing potentials > 0 mV and time-dependent inactivation at potentials more positive than 40 mV (22). It therefore resembles conductances observed in cells in which Kv1 proteins are heterogeneously expressed (7, 8).

The aim of the present study was to determine whether two well-characterized members of the Kv family (Kv1.1 and Kv1.3) contribute to the Kv currents in choroid plexus and to identify the membrane in which these proteins are expressed.

Serotonin (5-HT), acting at 5-HT₂C receptors, reduces the rate of CSF secretion (for review, see Ref. 29). This inhibition of CSF secretion may involve a reduction in K⁺ channel activity, given that the channels have an important role in the secretory process. Indeed, the activity of Kv1.1 and Kv1.3 channels in some cells is also known to be inhibited by serotonin acting at 5-HT₂C receptors (1, 3, 15, 31). Furthermore, single-channel recording studies previously showed that the open probability of K⁺ channels in the apical membranes of mouse choroid plexus epithelial cells is reduced by 5-HT (16). A second aim of this study was to investigate the regulation of the whole cell Kv conductance in rat choroid plexus by 5-HT. The potential role of protein kinase C (PKC) in channel inhibition was also determined.

The data obtained suggest that Kv1.1 and Kv1.3 channels are expressed in the choroid plexus epithelium and make a significant contribution to the whole cell K⁺ conductance. Furthermore, the Kv conductance is inhibited by 5-HT through the actions of PKC. Preliminary accounts of some of these data have been published as abstracts (36, 38).

METHODS

Tissue samples. Adult Sprague-Dawley rats were killed by an overdose of halothane (Zeneca Laboratories), and the choroid plexus was removed from the fourth ventricle of the brain. Tissue samples were kept in ice-cold, control bath solution (in mM: 140 NaCl, 5 KCl, 1 CaCl₂, 5 MgCl₂, 5 HEPES, 10 mannitol, 5 glucose; pH 7.3 with NaOH; osmolality = 298 ± 4 mosmol/kgH₂O, n = 6) and used in patch-clamp experiments within 3 h of isolation; 2) snap-frozen in glycerol and phospholipid 12-myristate 13-acetate, both of which activate protein kinase C. These data suggest that 5-HT acts at 5-HT₂C receptors to activate protein kinase C, which inhibits the Kv channels. In conclusion, Kv1.1 and Kv1.3 channels make a significant contribution to K⁺ efflux from the cell by the Na⁺/K⁺-ATPase and therefore help control cell volume; 2) they maintain the membrane potential of the cell (Vₘ), which is important in establishing the electrochemical gradient for anion efflux needed to drive secretion; and 3) they contribute to the process of K⁺ absorption from the CSF to the blood. This last role is particularly important because K⁺ absorption is vital in maintaining CSF K⁺ concentration ([K⁺]), which in turn is essential for the normal activity of the central nervous system (17, 44). K⁺ absorption is a carefully regulated process, so that the [K⁺] within the CSF is maintained between 2 and 3 mM even when plasma [K⁺] is varied over a range of 4–8 mM (17, 19). The mechanism by which K⁺ absorption is controlled is not known; however, K⁺ channels are potential sites of regulation.

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liquid N₂ for use in Western blot analysis; or 3) fixed in 4% paraformaldehyde-PBS for 30 min for immunocytochemistry.

All procedures were in accordance with Schedule One methods of UK Home Office regulations.

**Patch-clamp recording.** K⁺ channel activity in choroid plexus cells was monitored by whole cell patch-clamp methods as previously described (22). Small pieces of choroid plexus tissue, bathed in the control bath solution, were secured with a stainless steel wire to the base of a small perfusion chamber (bath volume = 600 µl) mounted on the stage of an inverted microscope (Olympus IMT-2). Patch pipettes were made from hematocrit capillary tubes (Oxford Labware) with a two-stage vertical puller (PB-7; Narishige). The tip resistances of the patch pipettes were 2–4 MΩ. Conventional whole cell recordings were made after gigahm seals had been obtained on the exposed apical membrane of the epithelial cells. Whole cell currents (voltage-clamp mode) were measured with an Axopatch-1D amplifier (Axon Instruments). Step-voltage pulses were generated by computer with pCLAMP software (Axon Instruments) and a Digidata 1200 interface (Axon Instruments), and the resultant currents were stored on the computer hard disk. Series resistance compensation was not applied in this study. Vₘ was measured in current-clamp experiments in which data were recorded on a digital tape recorder (BioLogic Science Instruments). In the current-clamp study, data were included for analysis only if the resting Vₘ for the cells was more negative than −35 mV.

In the majority of experiments the pipette solution contained (in mM) 120 K aspartate, 20 KCl, 0.5 EGTA, 5 HEPES, and 35 manniot with the pH adjusted to 7.2 with KOH (osmolality 285 ± 6 mosmol/kgH₂O; n = 4). In a series of experiments, 5 mM BAFTA replaced the 0.5 mM EGTA (292 mosmol/kgH₂O). Channel inhibition was investigated with margatoxin (MgTx; Alomone Laboratories), dendrotoxin-K (DTx-K; Alomone), tetrodotoxin (Sigma), and CsCl (Sigma). The effects of the following channel regulators were also examined: 5-HT (Sigma), mesulergine (Sigma), 1,2-dioctanoyl-sn-glycerol (DOG; Sigma), phorbol 12-myristate 13-acetate (PMA; Sigma) 4°C and diluted to the appropriate concentration in the bath solution. The maximum concentration of dimethyl sulfoxide in any experiment was 0.1% (v/vol), which has no affect on K⁺ channel activity.

**Western blot analysis.** A protein fraction enriched for plasma membrane proteins was prepared from choroid plexus tissue as previously described (37). Aliquots of the fraction containing 30 µg of protein solubilized in Laemmli buffer were separated on 7% SDS-polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membrane, and, after being blocked with Tris-buffered salmine-Tween containing 1% (vol/vol) nonfat milk for 1 h at room temperature, blots were incubated overnight at 4°C with rabbit polyclonal antibodies for the α-subunits of Kv1.1 and Kv1.3 (provided by Dr. H. G. Knaus, University of Innsbruck, Innsbruck, Austria). The antibodies were raised to the following peptide sequences: Kv1.1, amino acids 458–475 and Kv1.3, amino acids 456–474 (21). Both antibodies were affinity purified and used at a dilution of 1:1,000. A horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:10,000; Amersham Pharmacia Biotech) was used as the secondary antibody. The signal was developed with ECL-Plus chemiluminescence (Amersham Pharmacia Biotech) and visualized on X-ray film. Nonspecific immunoreactivity was assessed by preadsorbing the antibody for 1 h with a 2:1 excess of the corresponding antigen.

**Immunofluorescence.** Choroid plexus tissue was fixed in 4% paraformaldehyde-PBS for 30 min and subsequently cryoprotected in 30% sucrose buffer for a further 30 min. The tissue was embedded in optimum cutting tissue compound (R. A. Lamb) and snap-frozen in N-methylbutane cooled on dry ice. Serial 4-µm frozen sections were then cut with a Leica CM3050 cryostat (Leica Instruments) and mounted on gelatin-coated glass slides. Sections were rehydrated in PBS for 5 min. To permeabilize and block the sections, slides were incubated with PBS containing 1% (wt/vol) bovine serum albumin (BSA) and 0.1% (vol/vol) Triton X-100 for 1 h at room temperature. Slides were washed several times with 1% BSA-PBS to remove residual Triton X-100 and subsequently incubated overnight at 4°C with the affinity-purified Kv1.1 and Kv1.3 antibodies (1:100 dilution), a rabbit polyclonal aquaporin 1 antibody (1:500; Vector Laboratories), and a rabbit polyclonal AE2 antibody (1:1,000; a gift from Dr. Seth Alper, Harvard University, Boston, MA), all diluted in 1% BSA-PBS. The slides were washed three times with PBS to remove unbound primary antibody and then incubated with a goat anti-rabbit secondary antibody conjugated to fluorescent Cy3 (1:400; Jackson ImmunoResearch Laboratories) for 1 h at room temperature in the dark. Slides were then washed several times in PBS and mounted with Vectashield (Vector Laboratories). Nonspecific immunoreactivity was assessed by omission of the primary antibody from the protocol. Immunofluorescence was visualized with a Zeiss Axio-plan 2 microscope. Images were acquired with a Hamamatsu digital camera and processed with the KS300 version 3.0 software package (Carl Zeiss). Final images were prepared with Adobe Photoshop software.

**Data presentation and statistics.** Current-voltage (I/V) relationships are for the maximum current measured at each Vₘ. Currents were normalized for whole cell capacitance and are expressed as picoamperes per picofarad. Data are expressed as means ± SE of observations from n cells. Statistical comparison of current amplitudes was usually by one-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc test. Where stated, however, Student’s t-tests for paired and unpaired data were used.

**RESULTS**

**Whole cell K⁺ currents in choroid plexus cells.** Figure 1A shows a typical profile for whole cell currents recorded from a choroid plexus epithelial cell. The holding potential was −40 mV, and 1-s step-voltage pulses were applied from −120 to 60 mV in 20-mV increments. Two types of K⁺ current are evident in this trace, as previously reported (22). At hyperpolarizing potentials from −120 to −50 mV, a delayed-rectifying K⁺ (Kv) current and an inward-rectifying K⁺ (Kir) conductance in choroid plexus epithelial cells are evident. A: current profile recorded from a holding potential of −40 mV by applying 1-s voltage pulses from membrane potential (Vₘ) = −120 to 60 mV at 20-mV increments. B: mean ± SE current-voltage (I/V) relationship for peak current densities (pA/pF) from 28 cells.
potentials (−120 to −60 mV), time-independent, inward-rectifying currents were observed. Depolarizing potentials (more positive than −20 mV) evoked delayed-rectifying currents, which exhibit time-dependent activation and slower time-dependent inactivation at $V_m$ more positive than 20 mV.

Both conductances were observed immediately on attaining the whole-cell configuration, as shown in the $I/V$ relationship in Fig. 1B, which was recorded from 28 cells within 2 min of attaining the whole cell configuration. Under the recording conditions used in this study, however, the inward-rectifying currents quickly inactivated. Inactivation of these channels was previously reported (22) and is probably due to loss of intracellular factors from the cytoplasm during the whole cell recording. The delayed-rectifying $K^+$ conductance, which does not inactivate, is the subject of the remainder of this article.

Effect of Kv1.1 and Kv1.3 inhibitors on $K^+$ currents. The contribution of Kv1.1 and Kv1.3 channels to $K^+$ currents ($I_K$) was examined with MgTx, a selective blocker of Kv1.3 channels at a concentration of 1 nM (13), and DTx-K, an inhibitor of the Kv1.1 channel subtype at 10 nM (30, 33). Figure 2A shows that 1 nM MgTx inhibited currents measured at $V_m = 60$ mV. $I_K$ was reduced significantly to 81 ± 6% of control in six experiments (see Fig. 4; Student’s $t$-test for paired data, $P < 0.05$). Figure 2B shows that increasing the concentration of MgTx to 10 nM produced a greater inhibition of $I_K$ (64 ± 4% of control, see Fig. 4; $n = 6$; $P < 0.05$), and Fig. 2C illustrates that $I_K$ measured at all $V_m$ were inhibited by MgTx. Addition of 10 nM DTx-K to the bath solution also reduced $I_K$ (Fig. 3A). In four experiments the mean inhibition was to 66 ± 2% of control (Fig. 4; $P < 0.05$). Figure 3B shows that the currents at all $V_m$ were inhibited by 10 nM DTx-K. In five cells, a combination of MgTx and DTx-K inhibited $I_K$ to 67 ± 7% of control (Fig. 4), an effect that is not significantly different from that observed with either toxin alone ($P > 0.1$).

Application of 5 mM TEA+, the nonspecific $K^+$ channel blocker, inhibited $I_K$ to 21 ± 3% of the control value (Fig. 4; $n = 4$).

Fig. 4. Inhibition of $K^+$ currents by 1 nM MgTx, 10 nM MgTx, 10 nM DTx-K, a combination of 10 nM MgTx + 10 nM DTx-K, or 5 mM tetraethylammonium chloride (TEA+). The mean current at $V_m = 60$ mV in the presence of the inhibitor is expressed as a fraction of the control current ($I/I_0$). Differences compared with the control current were determined by Student’s $t$-test for paired data ($*P < 0.05$, $**P < 0.001$). The number of cells in each group is indicated in parentheses.

Contribution of Kv1.1 and Kv1.3 to $V_m$ in choroid plexus epithelial cells. To determine the contribution of Kv1.1 and Kv1.3 to the $V_m$ of the epithelial cells, current-clamp experi-

Fig. 2. Inhibition of $K^+$ currents by margatoxin (MgTx). Current traces measured during 1-s voltage steps from $V_m = −40$ mV to 60 mV in the absence and presence of 1 (A) and 10 (B) nM MgTx. C: $I/V$ relationships in the absence (○) and presence (●) of 10 nM MgTx ($n = 6$).

Fig. 3. Dendrotoxin-K (DTx-K) inhibits $K^+$ currents in choroid plexus. A: current traces measured during 1-s depolarizing voltage steps to 60 mV in the absence and presence of 10 nM DTx-K. B: mean $I/V$ relationship in the absence (○) and presence (●) of 10 nM DTx-K ($n = 4$).
ments were performed in the presence of MgTx and DTx-K. In seven cells, \(V_m\) in control conditions was \(-41.6 \pm 2.7\) mV, which is similar to the value previously obtained in current-clamp experiments (22). Figure 5A shows that superfusing a cell with nonspecific K\(^+\) channel blockers (5 mM TEA\(^+\) and 1 mM CsCl) caused a depolarization of \(V_m\), which reached a maximum within 60 s (Fig. 5A). In four cells, \(V_m\) was depolarized by these blockers to \(-33.7 \pm 3.5\) mV (Fig. 5B; \(P < 0.05\) by Student’s paired \(t\)-test). Addition of 10 nM MgTx and 10 nM DTx-K to the bath solution also caused \(V_m\) to depolarize (Fig. 5C). Figure 5D summarizes these data, showing that \(V_m\) was depolarized from \(-42.3 \pm 6.0\) mV to \(-35.7 \pm 7.2\) mV in the presence of the toxins \((n = 3; P < 0.05\) by Student’s paired \(t\)-test).

The contribution of the Kv channels to the whole cell conductance was further examined in experiments in which the time-dependent currents at depolarizing potentials \((i_{Kv})\) were electrically isolated. This was achieved by recording currents evoked by voltage steps from a holding potential of \(-60\) mV (at which the Kv channels are active; see Fig. 6A) and from a holding potential of \(+60\) mV (at which the Kv channels are inactive; see Fig. 6B). To prevent reactivation of Kv channels at the holding potential of \(+60\) mV, the voltage steps were reduced from 1 s to 100 ms and the interpulse interval was increased from 2 s to 10 s (Fig. 6B). The \(I/V\) relationships for the two sets of currents are shown in Fig. 6C. The \(I/V\) relationship in Fig. 6D is for \(i_{Kv}\), obtained by subtraction of the currents recorded with the holding potential of \(+60\) mV from those recorded with the holding potential of \(-60\) mV. A similar method was previously used to isolate time-dependent from time-independent currents in smooth muscle cells (11). The mean \(I/V\) relationship recorded in five choroid plexus cells is shown in Fig. 6E. The \(I/V\) reversed at \(V_m = -73 \pm 4\) mV \([K^+\) equilibrium potential \((E_K) = -84\) mV], with negative currents observed at potentials more negative than \(-80\) mV. When the bath solution was exchanged for one containing 45 mM K\(^+\), the \(I/V\) relationship was shifted to the right (Fig. 6E). The new reversal potential was \(-33 \pm 6\) mV \((n = 5)\), which is close to the \(E_K\) of \(-29\) mV.

**Western blot analysis and immunofluorescence for Kv1.1 and Kv1.3.** Western blot analysis with affinity-purified antibodies specific for Kv1.1 and Kv1.3 determined protein expression in membrane protein samples from choroid plexus. The anti-Kv1.1 antibody identified two bands of \(-55\) and 66 kDa in the choroid plexus membrane sample (Fig. 7A). Figure 7B shows that the anti-Kv1.3 antibody identified a single band of \(-75\) kDa in choroid plexus. The interactions of the antibodies with the choroid plexus membranes were specific, because the bands were not observed when the primary antibodies were preadsorbed with an excess of the respective antigen.

Immunocytochemistry was used to determine the site of channel expression, i.e., apical or basolateral membrane. Figure 7C illustrates the localization of Kv1.3 channel protein in choroid plexus with a secondary antibody labeled with Cy3 (red emitted light). There is distinct staining in the apical membrane of the epithelium. The antibody also detected proteins in cells of the villus core but not in the basolateral membrane of the epithelium. The antibody also detected proteins in a small area of the epithelial cells, and again there was no staining at the basolateral membrane.

To help demonstrate that the Kv1 proteins are expressed in the apical membrane of the choroid plexus cells, the expressions of aquaporin 1 and AE2 were determined. As previously reported, aquaporin 1 is confined to the apical membrane of the epithelial cells (28, 37) and shows a pattern of staining similar to both Kv1.1 and Kv1.3 (Fig. 7Cii). By contrast, the distribution of AE2 (a marker for the basolateral membrane; Refs. 37, 39) was very different from that of the Kv1 proteins (Fig. 7Civ). The same secondary antibody was used with each of the
primary antibodies. Figure 7Cv shows that there is virtually no nonspecific immunofluorescence with this antibody in the absence of primary antibodies.

5-HT inhibits Kv conductance by acting at 5-HT₂C receptors. Figure 8A shows current profiles recorded from a cell under control conditions and from the same cell after a 5-min superfusion with 1 μM 5-HT. The outward currents observed at depolarizing \( V_m \) were greatly reduced in the presence of 5-HT. Figure 8B shows the \( I/V \) relationships recorded from four cells before and after superfusion with 1 μM 5-HT. \( I_K \) was reduced at all \( V_m \) by the exposure to 5-HT. Figure 8C summarizes the changes in \( I_K \) at \( V_m \) = 60 mV. In four cells exposed for 8 min to 1 μM 5-HT, \( I_K \) was reduced to 48 ± 5% (n = 4) of the initial value recorded in the absence of 5-HT (1 min). Maximum inhibition was observed after 7.3 ± 1.2 min. In time-matched control experiments performed in the absence of 5-HT, \( I_K \) was not reduced significantly over 8 min of whole cell recording (91 ± 3% of control; n = 8; \( P > 0.1 \) by paired t-test). Figure 8D summarizes data obtained in experiments with the BAPTA pipette solution. The mean initial current density (1 min) was not significantly different from that measured with the control pipette solution (\( P > 0.1 \) by Student’s unpaired t-test; \( n = 4 \)). The effect of 5-HT on \( I_K \), however, was greatly attenuated in the presence of BAPTA. Thus at 8 min the current density was not significantly reduced compared with 1 min (87 ± 3%; \( P > 0.1 \) by Student’s paired t-test).

The role of 5-HT₂C receptors in \( K^+ \) channel inhibition was examined with the specific 5-HT₂C antagonist mesulergine (14, 18). Cells were exposed to either 1 μM 5-HT or 1 μM 5-HT plus 300 nM mesulergine for 10 min. The currents carried by the Kv channels (\( I_{Kv} \)) were then measured with the subtraction protocol (see Fig. 6). Figure 9A shows that the whole cell currents recorded with 5-HT alone were much smaller than those in the presence of 5-HT and mesulergine. Data from a number of experiments are summarized in Fig. 9B. 5-HT (1 μM) caused a significant reduction of \( I_{Kv} \) (obtained by the subtraction method) compared with controls (\( P < 0.001 \)). The effect of 5-HT on \( I_{Kv} \), however, was almost completely abolished by mesulergine (\( P > 0.1 \) compared with control). \( I_{Kv} \) was unaffected by 300 nM mesulergine in the absence of 5-HT.
fore investigated. The effects of 5-HT were examined in the presence of calphostin C (a PKC inhibitor). In six cells exposed to 100 nM calphostin C for 10 min, 1 μM 5-HT had no significant effect on I

PKC activity (41) and the activation of diacylglycerol (Ref. 24) or 500 nM PMA (a phorbol ester that activates PKC; Ref. 4). The mean I/V relationship for I

Kv from cells in the absence or presence of 30 μM DOG is shown in Fig. 10A. I

Kv was reduced by DOG at each Vm. Figure 10B shows that I

Kv (at Vm = 60 mV) was significantly reduced compared with control by preincubating cells with 30 μM DOG and with 500 nM PMA. In control experiments 500 nM 4α-PDD (a phorbol ester that does not activate PKC) did not significantly inhibit I

Kv (Fig. 10B). The additive effects of 500

(13.8 ± 0.8 pA/pF; n = 4; P > 0.1 compared with control). Furthermore, 5-HT did not affect the time-independent currents measured from a holding potential of 60 mV [control current = 6.1 ± 0.4 pA/pF (n = 12); + 1 μM 5-HT = 7.0 ± 1.4 pA/pF (n = 9); P > 0.1 by unpaired t-test].

Activation of PKC by 5-HT causes inhibition of I

Kv. Activation of 5-HT2c stimulates phospholipase C, resulting in an increase in intracellular Ca2+ activity (41) and the activation of PKC (12). The increase in intracellular Ca2+ activity is unlikely to be responsible for channel inhibition, because changes in Ca2+ are without effect on the K
v channels in choroid plexus (22). The role of PKC in channel activation was there-
Although Kv1.1 makes a significant contribution to the K\(^+\) current observed in choroid plexus epithelial cells (23). Furthermore, immunoprecipitation studies showed that Kv1.1 and Kv1.3 interact as heterotetramers with Kv1.2 and Kv1.4 in the brain (9).

Thus it is possible that the two Kv1 subunits expressed in the choroid plexus interact with each other, and possibly with other subunits, e.g., Kv1.2 and Kv1.6. This conclusion will be difficult to confirm by immunoprecipitation, because only very small quantities of choroid plexus tissue can be collected from rat brain. The interaction of different subunits may, however, explain the rather surprising effects of 5-HT observed in the choroid plexus epithelial cells.

Expression of Kv1 channels in choroid plexus epithelial cells. A voltage-dependent K\(^+\) conductance was observed in whole cell recordings from rat choroid plexus epithelial cells. The properties of this conductance are similar to those observed in cells expressing members of the Kv1 family of channels. The conductance was partially blocked by DTx-K, which is a potent and specific inhibitor of Kv1.1 channels (30), MgTx, a specific inhibitor of Kv1.3 at low concentrations (13), and, most recently, alveolar epithelial cells (23). Furthermore, immunoprecipitation studies showed that Kv1.1 and Kv1.3 interact as heterotetramers with Kv1.2 and Kv1.4 in the brain (9).

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observation that the effects of MgTx and DTx-K were not additive in the choroid plexus. Both toxins are quite large peptides (i.e., MgTx = 39 amino acids and DTx-K = 57 amino acids), so that only a single molecule of toxin (either DTx-K or MgTx) may be able to interact with a single heterotetramer containing Kv1.1 and 1.3 subunits.

**Contribution of Kv1 channel proteins to choroid plexus epithelial cells.** The electrophysiological data in Figs. 1–3 show clearly that Kv channel activity can be observed at $V_m > 0$ mV (e.g., time-dependent activation of currents is observed at $20 \text{ mV}$ in Fig. 1A). The channels can only make a significant contribution to $K^+$ transport by the choroid plexus, however, if they are active at $V_m$ in the physiological range. Previous microelectrode studies reported that the $V_m$ of choroid plexus cells is anywhere between $-25$ and $-65$ mV, depending on species and cell stimulation by secretagogues (34, 42, 44). The activity of Kv1.1 and Kv1.3 at this range of $V_m$ was therefore assessed by examining the effects of 10 nM MgTx and 10 nM DTx-K on $V_m$ in current-clamp experiments. The toxins caused $V_m$ to depolarize by $-6$ mV. This depolarization, although small, is similar to that produced by the addition of a combination of the nonselective $K^+$ channel blockers Cs$^+$ and TEA$^+$. These data indicate that Kv1.1 and Kv1.3 are active in the physiological range of $V_m$. This conclusion is further supported by the results of experiments in which $I_{Kv}$ was studied in isolation by using a current subtraction protocol. Positive and negative currents attributable to the Kv channels were observed around the $E_K$ of $-84$ mV (and $-29$ mV; Fig. 6E). It should also be noted that both MgTx and DTx-K inhibited currents recorded at all $V_m$ from $-60$ to $+60$ mV in voltage-clamp experiments (see Figs. 2C and 3B). Thus, in conclusion, the Kv1.1 and 1.3 channels are active over the range of $V_m$ observed in choroid plexus epithelial cells.

A second important question concerning the contribution of the Kv channels to $K^+$ transport relates to the site of channel expression within the epithelium. The membrane fraction used in the Western blots is enriched for proteins expressed in both the apical and basolateral membranes of the cells (37), but it is vital to know in which of these membranes the Kv1 channels are expressed. Immunocytochemical studies were therefore performed with Kv1.1 and Kv1.3 antibodies. The clearest results were obtained with the Kv1.3 antibody, which bound to the apical brush-border membrane of the epithelial cells and to areas of core tissue, probably the capillary endothelial cells (e.g., see Ref. 6). The pattern of epithelial staining was almost identical to that obtained for aquaporin 1, which is known to be expressed exclusively in the apical membrane of the choroid plexus (28, 37).

The immunocytochemical data with the Kv1.1 antibody also indicate that this protein is expressed at the apical pole of the cell. It is not clear from the immunocytochemistry whether the Kv1.1 protein is actually expressed in the apical membrane. The fact that DTx-K-sensitive currents were observed in the electrophysiological experiments, however, suggests that at least some of the protein must be in this membrane. Furthermore, it seems very unlikely that Kv1.1 or Kv1.3 is expressed in the basolateral membrane, given the different pattern of staining observed with the AE2 antibody (AE2 is a marker for the basolateral membrane of the choroid plexus epithelium; Refs. 37, 39).

**Physiological role of apical membrane $K^+$ channels.** Immunocytochemical studies suggest that significant components of the two major $K^+$ conductances in the choroid plexus epithelium, Kir (i.e., Kir7.1; Ref. 27) and Kv (i.e., Kv1.1 and Kv1.3; this study), are expressed in the apical membrane. These observations are consistent with the data of Zeuthen and Wright (44), who demonstrated that $>90\%$ of the whole-cell $K^+$ conductance resides in this membrane. Apical $K^+$ channels have a specific role in the choroid plexus, because they permit the recycling of $K^+$, which is constantly pumped into choroid plexus cells from the CSF by Na$^+$-$K^+$-ATPases. Thus $~90\%$ of this $K^+$ is returned to the CSF via $K^+$ channels in the apical membrane, and the remaining $10\%$ of $K^+$ is transported via channels (or possibly transporters) in the basolateral membrane (44). [The identity of the basolateral pathway for $K^+$ efflux remains to be determined. Channels other than Kv1.1 and Kv1.3 that contribute to whole cell $Kv$ or Kir conductances may be involved. Another possibility is that in mammalian choroid plexus $K^+$ efflux is via transporters, e.g., KCC3, which is expressed in the basolateral membrane (32)]. This balance of $K^+$ efflux at the two poles of the cell is thought to result in the small, but significant, absorptive flux of $K^+$ (CSF to blood) across the epithelium. Modulation of any of the components in this model of $K^+$ transport will profoundly affect the rate of $K^+$ absorption, e.g., decreasing Kv channel activity will promote $K^+$ absorption, because less $K^+$ will be recycled at the apical membrane (39, 44). Modulating $K^+$ channel activity may also affect the rate of CSF secretion, because it will alter the $V_m$ of the cells. The $V_m$ is known to provide much of the driving force for anion efflux at the apical membrane, which is a rate-limiting step in CSF secretion (34, 39).

**Regulation of $I_{Kv}$ by 5-HT and PKC.** 5-HT acts at 5-HT$_{2C}$ receptors in the choroid plexus (12, 16) and has an inhibitory effect on CSF secretion (for review, see Ref. 29). Previous single-channel patch-clamp studies showed that the open probability of a 18-pS $K^+$ channel in the apical membrane of mouse choroid plexus was inhibited by 1 $\mu$M 5-HT (16). The mechanism of channel inhibition, however, was not determined in the single-channel experiments (16). In the present study, 1 $\mu$M 5-HT was found to inhibit $I_{Kv}$ in the choroid plexus. Maximum inhibition to 50% of control was observed in $\sim$8 min. This decrease in channel activity cannot be explained as channel “ rundown,” which accounts for a $<10\%$ decrease in activity over 8 min in time-matched controls. Channel inhibition by 1 $\mu$M 5-HT was not observed in the presence of 300 nM mesulergine, indicating the involvement of 5-HT$_{2C}$ receptors. The effect of 5-HT was specific to the Kv conductance, because the time-independent currents were not significantly different in the presence of 5-HT.

Kv1 channel inhibition by 5-HT acting at 5-HT$_{2C}$ was previously observed in a number of studies (1, 3, 15, 31). The mechanism of channel inhibition proposed in these studies, however, was quite varied, e.g., some studies indicate the involvement of PKC and/or Ca$^{2+}$ (3, 15), whereas others suggest that neither Ca$^{2+}$ nor PKC is involved (1, 5). In the choroid plexus, the effects of 5-HT were greatly attenuated when the pipette solution contained 5 mM BAPTA. The primary action of BAPTA is to buffer any 5-HT-induced changes in intracellular Ca$^{2+}$ activity (25). A direct effect of Ca$^{2+}$ on the Kv channels, however, cannot explain the inhibition because the channels in choroid plexus are Ca$^{2+}$ insensi-
tive (22). An alternative pathway must therefore be involved. The most likely explanation is that 5-HT inhibits the Kv channels by activating Ca$^{2+}$-dependent isoforms of PKC (i.e., α, β, or γ). This hypothesis is supported by data from a previous study demonstrating that 5 mM BAPTA inhibits these isoforms of PKC in choroid plexus epithelial cells (20).

Further evidence for the involvement of PKC in the 5-HT-induced inhibition of IK, was provided in two additional series of experiments. In the first of these, the effects of 5-HT were found to be attenuated by the presence of calphostin C, an inhibitor of PKC. In the second series, the effects of 5-HT were found to be mimicked by agents that directly stimulate the activity of PKC, i.e., DOG or PMA. Furthermore, the effects of 5-HT and PMA were not additive, suggesting that both compounds work on the same target, i.e., channels carrying the Kv conductance. These data are therefore consistent with previous observations that some Kv channels (including Kv1.1 and Kv1.3) are inhibited by PKC (1, 3, 5, 26). It cannot be concluded from these data are therefore consistent with previous observations that Kv1.1 and Kv1.3 channel proteins are involved. Indeed, the fact that the inhibition of Kv is greater than that observed with MgTx or DTX-K suggests that even if Kv1.1 and Kv1.3 are involved, other Kv channels must also be inhibited by PKC.

In conclusion, this study provides functional and immuno-
cytotoxic evidence for the expression of Kv1.1 and Kv1.3 in choroid plexus epithelial cells. These channel proteins are shown to make a significant contribution to K$^+$ transport across the apical membrane of the epithelial cell; however, other channel proteins must also be involved. Finally, 5-HT inhibits the Kv conductance by stimulating PKC. Channel regulation by 5-HT may be important in inhibiting the CSF secretion and/or increasing K$^+$ absorption by the choroid plexus epithelium.

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