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

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Kv1.1 and Kv1.3 channels contribute to the delayed-rectifying $K^+$ conductance in rat choroid plexus epithelial cells. Am J Physiol Cell Physiol 286: C611–C620, 2004. First published November 5, 2003; 10.1152/ajpcell.00292.2003.—The choroid plexuses secrete, and maintain the concentration of, the cerebrospinal fluid. $K^+$ channels play an important role in these processes. In this study the molecular identity and properties of the delayed-rectifying $K^+$ (Kv) conductance in rat choroid plexus epithelial cells were investigated. Whole cell $K^+$ currents were significantly reduced by 10 nM dendrotoxin-K and 1 nM margatoxin, which are specific inhibitors of Kv1.1 and Kv1.3 channels, respectively. A combination of dendrotoxin-K and margatoxin caused a depolarization of the membrane potential in current-clamp experiments. Western blot analysis indicated the presence of Kv1.1 and Kv1.3 proteins in the choroid plexus. Furthermore, the Kv1.3 and Kv1.1 proteins appear to be expressed in the apical membrane of the epithelial cells in immunocytochemical studies. The $K^+$ conductance was inhibited by 1 µM serotonin (5-HT), with maximum inhibition to 48% of control occurring in 8 min ($P < 0.05$ by Student’s $t$-test for paired data). Channel inhibition by 5-HT was prevented by the 5-HT2C antagonist mesulergine (300 nM). It was also attenuated in the presence of calphostin C (a protein kinase C inhibitor). The conductance was partially inhibited by 1,2-dioctanoyl-sn-glycerol and phorbol 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 at the apical membrane of the choroid plexus.

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 main aim of the present study was to determine whether two well-characterized members of the Kv1 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 $K^+$ 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 then 1) kept in ice-cold, control bath solution (in mM: 140 NaCl, 5 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 5 HEPES, 10 mannitol, 5 glucose; pH 7.3 with NaOH; osmolality = 298 ± 4 mosmol/kgH$_2$O, $n$ = 6) and used in patch-clamp experiments within 3 h of isolation; or 2) snap-frozen in liquid nitrogen.

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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 tubing (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 gigahohm 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. \( V_m \) 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 \( V_m \) 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 360 mosmol/kg H₂O (120 K aspartate, 20 KCl, 0.5 EGTA, 5 HEPES, and 35 mM) 120 K aspartate, 20 KCl, 0.5 EGTA, 5 HEPES, and 360 mosmol/kg H₂O). Channel activity in choroid plexus cells was usually analyzed with the axon intracellular saline for whole cell currents recorded from a single cell. The holding potential was −35 mV. Currents were measured with an Axopatch-1D amplifier (Axon Instruments). To obtain cell-attached recordings, the pipette solution contained (in mM) 120 K aspartate, 20 KCl, 0.5 EGTA, 5 HEPES, and 360 mosmol/kg H₂O.

**Whole cell 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 saline containing 1% BSA and 0.1% (vol/vol) Triton X-100 for 1 h at room temperature, were incubated with the 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 \( V_m \). 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

*Fig. 1. Delayed-rectifying K⁺ (Kv) and inward-rectifying K⁺ (Kir) conductances in choroid plexus epithelial cells. A: current profile recorded from a holding potential of −40 mV by applying 1-s voltage pulses from membrane potential (\( V_m = −120 \) 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. The additive effects of 10 nM MgTx and 10 nM DTx-K were also examined. 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).

**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-
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 ($I_{K_{t}}$) 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 interepisode 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 $I_{K_{t}}$, 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 $\sim 55$ 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 $\sim 75$ 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. Figure 7Cii illustrates the localization of Kv1.3 channel expression, i.e., apical or basolateral membrane. Figure 7Cii illustrates the localization of Kv1.3 channel expression, i.e., apical or basolateral membrane. Figure 7Ciii illustrates the localization of Kv1.3 channel expression, i.e., apical or basolateral membrane. Figure 7Civ illustrates the localization of Kv1.3 channel expression, i.e., apical or 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. 7Ciii). 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

![Fig. 5.](https://example.com/fig5.png)

Fig. 5. Kv1.1 and Kv1.3 channels contribute to the $V_m$ in choroid plexus epithelial cell. $V_m$ was measured by current clamp during perfusion with TEA$^+$ (5 mM) and CsCl (1 mM) (A) and MgTx (10 mM) and DTx-K (10 mM) (C) as indicated by the arrows. Traces are representative of 4 (A) and 3 (C) observations. $B$ and $D$: $V_m$ in control conditions and in the presence of TEA$^+$ and CsCl (B) and MgTx and DTx-K (D). *Significantly different from control by Student’s paired $t$-test, $P < 0.05$. 

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primary antibodies. Figure 7C 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 Vm 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. IK was reduced at all Vm by the exposure to 5-HT. Figure 8C summarizes the changes in IK at Vm = 60 mV. In four cells exposed for 8 min to 1 μM 5-HT, IK 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, IK 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 IK, 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 (IKᵥ) 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 IKᵥ (obtained by the subtraction method) compared with controls (P < 0.001). The effect of 5-HT on IKᵥ, however, was almost completely abolished by mesulergine (P > 0.1 compared with control). IKᵥ was unaffected by 300 nM mesulergine in the absence of 5-HT.
1.4 pA/pF (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-HT_{2C} stimulates phospholipase C, resulting in an increase in intracellular Ca^{2+} activity (41) and the activation of PKC (12). The increase in intracellular Ca^{2+} activity is unlikely to be responsible for channel inhibition, because changes in Ca^{2+} are without effect on the Kv channels in choroid plexus (22). The role of PKC in channel activation was therefore 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_Kv (Fig. 9B). In control experiments, however, calphostin C did not affect the magnitude of I_Kv in the absence of 5-HT (12.2 ± 1.1 pA/pF; n = 3; P > 0.1). The effects of 5 μM bisindolylmaleimide (another PKC inhibitor) were also investigated, but this compound was a potent blocker of I_Kv in the absence of 5-HT (data not shown).

The role of PKC in channel inhibition was further investigated by preincubating cells for 10 min in bath solutions containing 30 μM DOG (a membrane-permeant analog 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 V_m. Figure 10B shows that I_Kv (at V_m = 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

A

B

C

D

Fig. 7. Expression of Kv1.1 and Kv1.3 in rat choroid plexus. A: in Western blot analysis the Kv1.1 antibody detected bands of ~55 and ~66 kDa. The positions of the molecular mass markers are indicated on the left. B: a band of ~75 kDa was detected by the Kv1.3 antibody. In A and B non-specific immunoreactivity was assessed by preabsorbing the antibody with the corresponding antigen (+Ag). C: immunocytochemical detection of Kv1.1 and Kv1.3. Sections of choroid plexus tissue were probed with primary antibodies for Kv1.3 (i), Kv1.1 (ii), aquaporin 1 (iii), and AE2 (iv). Primary antibody binding was detected by secondary antibodies conjugated to Cy3. In v, non-specific immunoreactivity was assessed in the absence of primary antibodies. The arrows indicate the apical membrane of the epithelium. Scale bars, 25 μm.

Fig. 8. Serotonin (5-HT; 1 μM) inhibits K^+ currents in rat choroid plexus cells. A: current profiles (V_m = -60 to +60 mV) from the same cell under control conditions and 5 min after perfusion with 1 μM 5-HT. B: I/V relationship in control conditions (○) and after maximum current inhibition with 1 μM 5-HT (●; n = 4). Effects of 5-HT on the K^+ currents with the control pipette solution (C) or with the 5 mM BAPTA pipette solution (D) are also shown. Mean current densities (n = 4) are shown in the absence (1 min) and presence (8 min) of 1 μM 5-HT. *Significantly different from 1 min by Student’s t-test for paired data (P < 0.05).
nM PMA and 1 μM 5-HT were also examined. $I_{K_v}$ was inhibited by this combination of compounds (Fig. 10B), but the inhibition observed was not significantly greater than that observed with each compound alone ($P > 0.1$).

**DISCUSSION**

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, 33). The 34% inhibition observed in the choroid plexus with 10 nM DTx-K, however, is much less than the block described for homomeric Kv1.1 channels expressed in Chinese hamster ovary (CHO) cells (80% inhibition; Ref. 30). This suggests that although Kv1.1 makes a significant contribution to $I_K$ in the choroid plexus, other Kv1 channels must also be involved. MgTx, a specific inhibitor of Kv1.3 at low concentrations (13), also blocked $I_K$ in the choroid plexus. At 1 nM, a concentration sufficient to block almost completely any Kv1.3 channels present (13), it caused a 19% inhibition of $I_K$. The additional inhibition of $I_{K_v}$ observed with 10 nM MgTx (36% inhibition) may therefore be due in part to the partial inhibition of other K$^+$ channels, e.g., Kv1.6 channels are blocked by higher concentrations of MgTx (13). In conclusion, it appears that Kv1.1 and Kv1.3 make a significant contribution to the Kv conductance in choroid plexus epithelial cells. Other channel proteins, however, must also contribute to this conductance.

Western blot analyses were performed to investigate Kv1 protein expression in the choroid plexus. Antibodies for Kv1.1 and Kv1.3 interacted specifically with proteins in a rat choroid plexus membrane fraction. The molecular mass of the single 75-kDa band determined with the Kv1.3 antibody is identical to that reported for Kv1.3 by Veh et al. (40) and Yuan et al. (43). The two bands of 55 and 66 kDa identified with the Kv1.1 antibody are also similar to the predicted molecular mass for Kv1.1 (60–64 kDa; Ref. 2). These data therefore support the conclusion that Kv1.1 and Kv1.3 are expressed in the choroid plexus epithelium.

The expression of two (or more) members of the Kv1 family of proteins in the choroid plexus is consistent with previous studies that have shown that Kv1 channels function as heterotetramers (8). Expression of combinations of Kv1.1, Kv1.2, Kv1.3, and Kv1.6 have been demonstrated in neurons (21), smooth muscle cells (43), 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). 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
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 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 $I_{Kv}$ 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 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 $Na^+\cdot K^+\cdot ATPases$. Thus $\sim 90\%$ 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 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-
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REFERENCES


