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Cl^- Channel Expression in Choroid Plexus Epithelial Cells

Peter D. Brown, Hidetoshi Kajita, Aneela Majid, Tracey Speake

School of Biological Sciences, University of Manchester, Manchester, U.K.

The choroid plexuses are found one in each ventricle of the brain. They consist of a rich vascular core which is covered with a simple cuboidal epithelium. These epithelial cells form a selective barrier between the blood and the central nervous system, and are responsible for the secretion of the cerebrospinal fluid (CSF). The process of CSF secretion is carefully regulated, so that the composition of the CSF is maintained within well defined limits e.g. the concentrations of HCO_3^-, K^+ and Ca^{2+} remain constant when plasma concentrations are varied (1, 2).

The mechanism of CSF secretion differs from the secretory process in most other epithelia in three important respects: i) Na^+-K^+ ATPase is located on the apical membrane of the choroid plexus epithelium; ii) secretion is dependent on HCO_3^-, and iii) secretion is stimulated by cAMP but does not involve CFTR (3). A combination of electrophysiological and molecular biological techniques have been employed to investigate the expression of anion transporters in the mammalian choroid plexus.

Cl^--HCO_3^- exchange (AE2)

As a result of radioisotope studies it was proposed that the exchanger could contribute to HCO_3^- secretion across the apical membrane of the choroid plexus epithelium (4). Immunocytochemical studies, however, showed that expression of the AE2 protein is restricted to the basolateral membrane of the epithelium (5). Here it may contribute to the regulation of intracellular pH (5), and is probably the main route for Cl^- uptake into the epithelial cells (1). AE2 cannot, however, directly contribute to HCO_3^- movement into the CSF.

Key Words: *Choroid Plexus; Cl^- Channels; ClC-2; HCO_3^-*

Address for correspondence: Dr. Peter Brown
G.38 Stopford Building, School of Biological Sciences, University of Manchester, Manchester M13 9PT, U.K., Tel: +44.161-275-5463, Fax: +44.161-275-5600, E-mail: peter.d.brown@man.ac.uk

Na^+-HCO_3^- cotransport (NBC)

NBC is expressed in HCO_3^--transporting epithelia, e.g. pancreatic ducts (6). RT-PCR methods have therefore been used to examine possible expression of NBC in the choroid plexus. Primers were designed to amplify the 5' region of the rat pancreatic or kidney isoforms of NBC. PCR products were produced by neither primer pair from choroid plexus cDNA, although products were produced from the positive controls (i.e. pancreas or kidney). These data suggest that neither of the major epithelial isoforms of NBC are expressed in the choroid plexus.

Anion channels

The basolateral localisation of AE2 and the lack of NBC expression, suggest that anion channels must provide the main route for HCO_3^- efflux across the apical membrane of the choroid plexus. Two anion conductances have been identified in whole-cell patch clamp experiments. An outward-rectifying conductance is activated by cell swelling. This conductance is similar to volume-sensitive anion channels found in most cells, and is unlikely to make a significant contribution to anion efflux from the choroid plexus. The other anion conductance in the choroid plexus, has a high HCO_3^- permeability and is activated by cAMP/protein kinase A. It therefore has the properties which are consistent with a role in CSF secretion (3).

The channel shows time-dependent activation at hyperpolarizing potentials, has an inward-rectifying I-V and is blocked by Zn^{2+} or Cd^{2+}. These properties are similar to those of ClC-2 (Table 1), mRNA for which is expressed in the choroid plexus (7). Other properties, however, were found to differ from those of ClC-2 (e.g. halide selectivity, H^+-sensitivity and ATP-dependence; Table 1), so that the molecular identity of the inward-rectifying channel remained in some doubt. Anti-sense oligonucleotides to ClC-2 were therefore used to investigate the effects of reducing ClC-2 expression in choroid plexus cells. This manoeuvre caused a significant reduction in the inward-rectifying conductance, strongly suggesting that ClC-2 is responsible (8). ClC-2 protein expression has also been determined in Western analysis. Immunocytochemical studies have shown that ClC-2 expression is predominantly in the apical membrane of the

Table 1. Comparison of the properties of ClC-2 expressed in different cells

	Xenopus oocytes	Neurons	Choroid plexus
Inward-rectifying	Yes	Yes	Yes
Time-dependent	Yes	Yes	Yes
Zn^{2+}/Cd^{2+} block	Blocked	Blocked	Blocked
PKA	No effect	Activated	Activated
PKC	No effect	Inhibited	Inhibited
Halide selectivity	$Cl^->I^-$	$Cl^->I^-$	$I^->Cl^-$
H^+-sensitivity	Activated	Activated	Blocked
ATP-dependence	?	No	Yes
P_{HCO3}: P_{Cl}	?	?	1.5

choroid plexus. Thus the ClC-2 channel in the choroid plexus appears to be the main route for HCO_3^- efflux into the CSF.

In conclusion, ClC-2 expressed in the choroid plexus is a cAMP-activated, HCO_3^- permeable channel, which may play an important role in CSF secretion. The reason for the differences in properties between ClC-2 in the choroid plexus and other expression systems remains to be determined

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