ORGANIC ANION TRANSPORT BY CHOROID PLEXUS OF *SQUALUS ACANTHIAS*

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Xenobiotics, such as 2,4-dichlorophenoxyacetic acid (2,4-D; Pritchard *J. Pharmacol. Exp. Ther.* 212:354-359, 1980) and neurotransmitter metabolites, such as 5-hydroxyindoleacetic acid (Cserr and Van Dyke *Am. J. Physiol.* 1971 220:718-23), are among the many organic anions removed from brain extracellular fluid across the cerebrospinal fluid (CSF)-blood barrier by the choroid plexus, as demonstrated for mammals. The molecular mechanisms for organic anion and cation transport across this barrier are only now being elucidated. In the experiments reported here, organic anion transport across the intact CSF-blood barrier was directly examined in the IVth choroid plexus of the spiny dogfish shark (*S. acanthias*). Transepithelial unidirectional fluxes of 10 μM [^{14}C]2,4-D were determined in paired halves of IVth plexus mounted in Ussing chambers attached to voltage clamps (22°C). Electrical, biochemical, and morphological properties were also evaluated to further characterize this experimental model of the CSF-blood barrier. The morphology and ultrastructure of the IVth plexus were examined by scanning and transmission electron microscopy. Localization of the multidrug resistance transporter p-glycoprotein (P-gp) was examined by fluorescence immunostaining and confocal microscopy.

Electron micrographs showed that shark IVth plexus, like mammalian choroid plexus, consists of a polarized epithelium with fenestrated capillaries. Tight junctions separate the microvillous apical membrane from the basolateral (blood-facing) membrane and thereby separate the CSF from the blood. P-gp was localized to the apical membrane that interfaces with CSF, as in mammalian choroid plexus (Vallabhaheni et al. *Proc. Natl. Acad. Sci.* 96:3900-3905, 1999). Importantly, unlike mammalian choroid plexus in which the epithelium covers both sides of the organ, shark IVth plexus is essentially a single sheet of epithelium that covers the IVth ventricle. Therefore, when the isolated intact IVth plexus is mounted in Ussing chambers, the epithelial tissue discretely separates the CSF and blood compartments.

For tissue mounted in flux chambers, the mean transepithelial resistance was 59.5 ± 9.9 (SE) $\Omega \cdot cm^2$; the mean transepithelial potential was +1.4 ± 0.5 mV (CSF-side positive; paired segments from 17 sharks). Sixty-min unidirectional fluxes of 10 μM [^{14}C]2,4-D were determined under open-circuit conditions (n = 13). The mean CSF-to-blood flux was 2.0 ± 0.5

nmol·cm^{-2}·h^{-1}, and the mean blood-to-CSF flux was 0.4 ± 0.1 nmol·cm^{-2}·h^{-1}. The net CSF-to-blood flux was 1.6 ± 0.5 nmol·cm^{-2}·h^{-1}, with a flux ratio of 4. Probenecid (1 mM), a prototypic inhibitor of organic anion transport, reduced the CSF-to-blood 2,4-D flux by more than 50% ($P < 0.01$) without significantly changing the blood-to-CSF flux ($P = 0.3$); net flux decreased 77.0% ± 9.3% ($P < 0.001$, $n = 5$). Pritchard et al. (*J. Biol. Chem.* 274:33382-33387, 1999) demonstrated lithium-sensitive Na^+-dependent dicarboxylate/organic anion exchange at the apical (CSF-facing) pole of mammalian choroid plexus. In shark plexus, 5 mM LiCl reduced the net CSF-to-blood flux of 2,4-D by 50.3% ($n = 2$). These data indicate that 2,4-D is actively transported from CSF to the blood by an organic anion pathway and may be energetically coupled to sodium transport, as it is in mammals. This work was funded in part by NSF-IBN980616; NINDS-NS39452S; NIEHS-ES10439; NSF-IBN9604070. A.R.A. Villalobos was supported by a 2000 NIA. Ms. Jerman was supported by NSF-DBI-9820400.