ORGANIC ANION TRANSPORT BY CHOROID PLEXUS OF *SQUALUS ACANTHIAS*

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Xenobiotics, such as the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and penicillin, are among the many organic anions that are absorbed from cerebrospinal fluid (CSF) to the blood by the choroid plexus. The cellular mechanisms that mediate and modulate transepithelial absorption of organic anions, or cations, are only now coming to light. Recently, Pritchard et al. (J. Biol. Chem. 274:33382-33387, 1999) demonstrated the presence of a Na^+-dependent dicarboxylate/organic anion exchanger at the apical pole of mammalian choroid plexus.

The dogfish shark (*S. acanthias*) provides a uniquely accessible choroid plexus that permits characterization of transport in intact tissue. In this preliminary investigation, cellular organic anion (OA^-) transport by the IVth choroid plexus was determined. The model substrate was fluorescein, which is transported by the choroid plexus from CSF to blood (Bresler et al. BBA 550:110-119, 1979) and transported by the renal dicarboxylate/OA^- exchanger (Miller, D.S. and Pritchard, J.B. Am. J. Physiol. 261:R1470-R1477, 1991).

Initially, fluorescein uptake by segments of the IVth choroid plexus isolated from a single shark was examined by confocal fluorescence microscopy; tissue was imaged after 30-min incubation at 24°C with 5 μM fluorescein in elasmobranch Ringers. Epithelial cell fluorescence exceeded extracellular fluorescence and was markedly reduced by 0.5 mM KCN, indicating metabolically-dependent uptake of fluorescein across the plasma membrane. Furthermore, capillary lumen fluorescence markedly exceeded epithelial fluorescence, indicating absorption of substrate from the CSF compartment into the blood compartment. Epithelial fluorescence was also reduced by 5 mM LiCl. At 10 μM, glutarate markedly stimulated uptake; however, at 25 and 50 μM, uptake was reduced.

In subsequent studies, 30-min uptake of 5 μM fluorescein by segments of isolated IVth choroid plexus was examined by fluorimetric assay of tissue lysate (excitation/emission ratio:

494 nm/518 nm). The mean 30-min total tissue:medium(T:M) fluorescein concentration ratio for control tissues was 10.8 ± 1.4 (SEM n = 7 sharks). At 0.5 mM, KCN reduced T:M ratios by 53 ± 3.0% (n = 4); higher concentrations were tested but the tissue literally fell apart. The T:M ratio was reduced approximately 30% by 1 mM ouabain (n = 1) and an average of 27.0% by 5 mM LiCl (n = 2). At 100 μM, 2,4-D reduced uptake 37.9 ±4.7% (n = 4); the prototypic OA^- *p*-aminohippurate (PAH, 1 mM) reduced uptake 24.5% (n = 2).

In the last set of studies, transepithelial unidirectional fluxes of 1 μM fluorescein were determined and the transepithelial electrical properties of IVth choroid plexus, paired segments of the anterior plexus, were measured in Ussing chambers attached to voltage clamps (22°C). The mean transepithelial resistance was 62.6 ± 4.9 $\Omega \times cm^2$; the transepithelial potential ranged from 0.53 -2.12 mV (CSF side positive; n = 3, *i.e.*, paired segments from tissue isolated from 3 sharks). Phloridzin, an inhibitor of electrogenic Na^+-glucose cotransport, had a marked effect in this tissue. However, in contrast to the renal proximal tubule, a strong response was observed when the agent was added to the blood-side, rather than the CSF-side. The mean phloridzin-sensitive glucose current was 2.8 ± 0.8 μA x cm^{-2}. These observations suggest the presence of a Na^+-glucose cotransporter in shark choroid plexus. Ninety-minute unidirectional fluxes of 1 μM fluorescein were monitored under open-circuit conditions. The blood-to-CSF flux was 1.0 pmol x cm^{-2} x 1.5 h^{-1}; the CSF-to-blood flux was 88.6 pmol x cm^{-2} x 1.5 h^{-1}; thus the net absorptive flux of fluorescein was some 87-fold greater than the secretory flux. This demonstrates the feasibility of using this technique for measuring transepithelial fluxes of OA^-. It should be noted that, because of the elaborate convolutions at the apical pole of the IVth plexus, the actual surface area of conducting and transporting tissue is considerably underestimated.

Collectively, these preliminary data suggest that active fluorescein uptake by choroid plexus is coupled to cellular metabolism and energetically coupled to Na^+,K^+-ATPase. The observed inhibition by LiCl suggests Na^+ dependence. Whether this reflects a direct or indirect coupling of fluorescein uptake to the Na^+ gradient remains to be determined. The biphasic effect of glutarate on epithelial fluorescein uptake is reminiscent of the energetic coupling of dicarboxylate/OA^- exchange to Na^+/dicarboxylate cotransport previously described for mammalian choroid plexus. These data indicate that shark choroid plexus is a useful experimental model for further examination of the cellular mechanisms that mediate and modulate transport of xenobiotic organic anions across the CSF-blood barrier.

This work was funded in part by NSF-IBN9808616; NINDS-NS10475; NSF-IBN9604070. Ms. Brooks was supported by NSF DBI-9820400; Ms. Zeien was supported by a University of Florida Summer Fellowship.