THE EFFECT OF HYPERCAPNIA ON CSF ELECTROLYTES IN *S. acanthias*

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Hypercapnia elicits a prompt and large rise in CSF HCO_3^- concentration in all vertebrates. The effect is particularly striking in *S. acanthias* (Am. J. Physiol., in press, 1972 and Bull. MDIBL, 10:49, 1970). In the following experiments we show the effect of hypercapnia on Na^+ and Cl^- concentration in CSF of *S. acanthias.*

TABLE 1. The Effect of Hypercapnia cn CO_2 Equilibria in Plasma and CSF of S. *acanthias*

	PLASMA 0	PLASMA 3 hrs	PLASMA Δ	CSF 0	CSF 3 hrs	CSF Δ	
A. NORMAL FISH pCO_2 6 → 18 mm Hg							
pH	7.21	7.08	– 0.13 ± .05	7.47	7.41	– .06	
HCO_3^-, mM	4.0	8.2	+ 4.2 ± .6	7.0	17.0	+ 10	± 0.7
B. METHAZOLAMIDE 50 mg/kg at -40 min pCO_2 11 → 25 mm Hg							
pH	7.22	6.86	– 0.36 ± .06	7.43	7.26	– .17	± .05
HCO_3^-, mM	6.2	6.6	+ 0.4	9.9	16.5	+ 6.6	± 1.0

5% CO_2 was introduced into the gill perfusate at 0 time, in both series A and B.

In Series B, the fish swam freely for about 30 minutes after the injection of the carbonic anhydrase inhibitor

n = 6-8. . S.E. given where changes are significant.

Table 1A shows that when pCO_2 is raised to 18 mm Hg by admitting 5% CO_2 gas to sea water perfusing the gills, CSF CHO_3^- rises 10 mM in 3 hours. As a result, pH homeostasis is very nearly preserved. In the blood, HCO_3^- rises only 4.2 mM, with resulting drop in pH.

Table 1B shows the same type experiment, except that carbonic anhydrase is inhibited. Note that inhibition itself causes a rise in pCO_2, which also elicits a rise in CSF HCO_3^-. When 5% CO_2 is added during inhibition, the rise in CSF HCO_3^- is less than in the normal, 6.6 mM. The rise in plasma HCO_3^- is essentially abolished.

These results are explained by the hydroxylation of CO_2 at sites of formation of CSF, catalyzed by carbonic anhydrase. The result is HCO_3^- formation in CSF and probably in brain interstitial fluid. The plasma data are due to catalyzed hydroxylation of CO_2 in the red cells, with subsequent shifts into plasma. The process in cells forming CSF is much faster, because secretory cells can remove product more rapidly than can red cells.

TABLE 2. The Effect of 3 Hours Hypercapnia on CSF Electrolytes in *S. acanthias*

mM	PLASMA Initial	PLASMA Δ	CSF Initial	CSF Δ
A. NORMAL FISH pCO_2 6 → 18 mm Hg				
Na^+	257	+ 5	269	+ 13 ± 3.5
Cl^-	263	+ 2	278	+ 9 ± 2.3
HCO_3^-	4.0	+ 4.2 ± 0.6	7.0	+ 10 ± 0.7
B. METHAZOLAMIDE PRETREATED pCO_2 11 → 25 mm Hg				
Na^+	255	+ 4	269	+ 3 ± 2.1
Cl^-	254	- 3	263	+ 1 ± 3.3
HCO_3^-	6.2	+ 0.4	9.9	+ 6.6 ± 1.0

Δ represents the change after 3 hours of hypercapnia, from the initial measurement. In Series B methazolamide was given about 40 minutes before the initial sampling.

n = 6-8. S.E. given where changes are significant.

Table 2A shows that the rise in CSF HCO_3^- during hypercapnia is accompanied by a rise in CSF Na^+. Conceivably HCO_3^- rise could elicit Cl^- exit or exchange; clearly this is not the case, since Na^+ rises and Cl^- does also. The cation rise (Na^+ = 13 mM) is accompanied by anion rise (Cl^- + HCO_3^- = 19 mM). Lack of balance here is due either to experimental error or failure to measure all charged particles.

Table 2B shows that carbonic anhydrase inhibition decreases Na^+ and Cl^- entry to CSF during hypercapnia. The small cation rise (Na^+ = 3 mM) is now accompanied by a small anion rise (Cl^- + HCO_3^- = 7.7 mM).

These data support the idea (Am. J. Physiol., in press 1972) that a significant amount of Na^+ transport into CSF is linked to HCO_3^- formation. The precise nature of the Cl^- effect is not yet known; either it is secondary to the transfer of Na^+ and HCO_3^-, or it represents a function of carbonic anhydrase (or closely related protein also inhibited by sulfonamides) in Cl^- transport as such.

In this model utilizing hypercapnia, carbonic anhydrase inhibition decreases the transport of Na^+, Cl^-, and HCO_3^- into the CSF. It is interesting to observe that in micro-puncture studies of the proximal tubule, inhibition also decreases the reabsorption of all three ions (Kunau, J. Clin. Invest., in press 1972).

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MEASUREMENT OF CEREBROSPINAL FLUID VOLUME IN *S. acanthias* DURING CARBONIC ANHYDRASE INHIBITION

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In studying the relations between ion movements and fluid production of CSF (Maren, Bull. Mt. Desert Island Biological Lab., 9:33, 1969, and 10:44, 1970) knowledge of the volume of CSF of *S. acanthias* became important. Oppelt *et al.* (Comp. Biochem. Physiol., 17:857, 1966) showed that intravascular or intraventricular administration of a carbonic anhydrase inhibitor reduced CSF production rate by 28%. The effect of inhibition on CSF volume was not determined. We have now measured the volume of CSF before and after carbonic anhydrase inhibition. The method was the dilution of Blue Dextran 2000, a carbohydrate polymer of molecular weight about 2×10^6, which appears to have no affinity for tissue.

Fourteen male fish of 1-2 kg were removed from live cars, and a portion of the chondrocranium removed so that a thin layer of cartilage remained above the brain. The cerebellum was located visibly, 0.25 ml of CSF withdrawn and 0.25 ml of Blue Dextran (3000 μg/ml in shark Ringer) injected into the cerebellar ventricle. Half of the fish had been given 50 mg/kg i.v. of methazolamide, a powerful carbonic anhydrase inhibitor with a half life of 1.5 days, 7-8 hours before the dye was injected. The fish were returned to the live cars where they swam freely for 15 minutes. They were then killed, and all CSF was withdrawn. Following centrifugation, aliquots of the clear blue solution were measured into 3-4 volumes of shark Ringer solution and absorbance measured at 650 nm against a standard curve of Blue Dextran.

From dye dilution the volume (V) of the CSF in controls was (mean $\pm$ S.E.) 1.67 $\pm$ 0.16 ml. From fish pretreated with methazolamide V = 1.22 $\pm$ 0.04 ml. In each series, withdrawal of all CSF from the cavities yielded a volume about 0.3 ml less than measured with dye. Both the dye and