Clamping to -40 mV, which inhibited J_H, resulted in an I-V relationship which was no longer a series of straight lines, but rather a single convex-upward curve. Consequently, no rational breakpoint analysis could be performed. The intercept V_{ms}, however, could be determined as -16.2 + 1.3 mV (N=16).

From these results, it is clear that voltage clamping away from the spontaneous potential (zero) has two effects. Negative clamps inhibit J_H, presumably by increasing the energy requirement H^+ transport, but also change the electrical characteristics of the tissue, as is seen in the change in intercept voltage. Among the possible changes expected from the passage of current are changes in the ionic composition of the cell cytoplasm. These changes clearly take some time, as they are not manifest during or following the 1 sec pulse used to measure resistance or the I-V plot.

The time course of the change can be followed by measuring the long time-constant transient (LTCT) response of V_{ms} to a step change in I_{ms}. Using 4 min pulses of 38 $\mu A/cm^2$, the LTCT was recorded, sampling the voltage every second. An immediate response (V_0 = IR at 1 sec) was observed, followed by a gradual change to a new steady state value of V_{ms} (V_f). A plot of ($V_{ms} - V_f$) vs. time is found to be a single exponential from 10 seconds onward. The current pulse was applied and removed in both polarities; the four transients had a magnitude $[(V_0-V_f)/V_0]$ between 0.51 and 0.87, while the exponential time constant ranged from 29 to 80 seconds. These results on a single tissue are similar to the data from frog gastric mucosa (Kidder & Rehm, Biophys. J. 10:215, 1970), but in marked contrast to the dogfish gastric mucosa, which even under sufficient oxygenation has a magnitude of zero and thus an indeterminant time constant.

SODIUM INFLUX ACROSS THE BLOOD-BRAIN BARRIER (BBB) CONTRIBUTES TO BRAIN INTERSTITIAL FLUID (ISF) VOLUME REGULATION IN THE LITTLE SKATE (Raja erinacea)

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Brain ISF volume depends on the total tissue content of extracellular electrolytes, chiefly Na and Cl. Previously we have shown in response to hypernatremic dehydration the brain of the little skate loads NaCl in sufficient quantity to restore its ISF volume to normal (Bulletin, MDIBL 21:4, 1981). In this abstract we present evidence indicating that the volume regulatory NaCl flux requires inwardly directed (toward brain from plasma) [Na] and [Cl] gradients, occurs at the BBB and is partially inhibited by "loop" diuretics.

There are three possible routes of NaCl entry into brain - via the fluid around the brain (the extradural fluid or EDF), via the cerebrospinal fluid (CSF) or across the BBB. Using ^{125}I-RISA and ^{22}Na to trace EDF penetration into brain, Melton and Cserr (Bulletin, MDIBL 22:45, 1982) have shown that the EDF is not the source of the volume-regulatory NaCl.

In this study, Na influx from CSF to brain was evaluated both by direct observation of CSF volume (judged by the upward distension of the transparent meningeal roof of the third ventricle) and by measurement of the ratio of concentrations, ^{22}Na-CSF/^{22}Na-plasma, 30 min after IV ^{22}Na injection (an estimate of CSF turnover). The quantity of Na taken up by the brain during volume regulation is about the same as that in the entire volume of skate CSF (30 μl). During hypernatremia there was no obvious decrease in CSF volume. Also there was no increase in CSF turnover; the CSF plasma ^{22}Na ratios being .21±0.8 (N=3) and .24±.07 (N=6), control and hypernatremic, respectively. Thus, CSF may be excluded as a major source of volume regulatory NaCl during hypernatremia.

Na influx across the BBB was assessed by determining vascular permeability to ^{22}Na using the technique of Ohno et al. (AJP 235: H299, 1978) as modified by Cserr and DePasquale (Bulletin, MDIBL 22: 44, 1982) for use in the skate. This method yields the average unidirectional influx coefficient, k. of ^{22}Na across the BBB for the

30 minutes following IV tracer injection. Three regions of brain were analyzed, cerebellum, medulla oblongata and telencephalon. k_i was measured in isosmotic control skates; in skates rendered hyperosmotic with NaCl (56 mosmoles/kg 1M) or fructose (56 mosmoles/kg 1M); and, to test if NaCl influx across the BBB is carrier mediated, in control and hyperosmotic skates pretreated with methazolamide (100 mg/kg IV), furosemide (10 mg/kg IV) or bumetinide (1 mg/kg IV). There were five skates in each experimental group and measurements of k_i in the hyperosmotic skates were made during the first 5 to 35 min of hypertonicity.

Value of k_i (in units of 10^{-4} min^{-1}; mean $\pm$ SE) for ^{22}Na in isosmotic control skates were 14.0 $\pm$ 3.8, 7.0 $\pm$ 1.0 and 6.6 $\pm$ 0.8 in cerebellum, medulla and telencephalon, respectively. In skates rendered hyperosmotic with NaCl or fructose, k_i increased linearly with plasma osmolality in all three brain regions (four-fold per 100 mOsm), to a degree sufficient to account for the Na loaded during hypernatremia. Furosemide and bumetinide inhibited the increase in k_i by an average of 41%. Neither drug affected the isosmotic k_i. Methazolamide did not affect the increase in k_i.

The NaCl influx into brain in hypernatremic skates is associated with an inwardly directed NaCl concentration gradient (i.e., from plasma towards interstitium). The results summarized in Table 1 suggest this gradient is needed for

TABLE 1

LOSS OF EXTRACELLULAR ELECTROLYTES FROM BRAIN DURING FRUCTOSE HYPERTONICITY

Condition	Telencephalon		Medulla		Cerebellum	
	Na	Cl	Na	Cl	Na	Cl
Control	470 $\pm$ 15	432 $\pm$ 7	350 $\pm$ 10	298 $\pm$ 21	453 $\pm$ 21	428 $\pm$ 19
Hypertonic 35 min	470 $\pm$ 16	437 $\pm$ 12	332 $\pm$ 14	267 $\pm$ 11	405 $\pm$ 6*	382 $\pm$ 5*
Hypertonic 2 hr	455 $\pm$ 23	386 $\pm$ 14*	316 $\pm$ 12	243 $\pm$ 14	402 $\pm$ 13*	345 $\pm$ 12*

Brain electrolyte concentrations in mEq/kg dry weight. Plasma osmolality increased by 55$\pm$7 mOsm and 67$\pm$5 mOsm after 35 min and 2 hr, respectively, of fructose-induced hypertonicity. Values are means $\pm$SE and N=5 in each experimental group.

NaCl loading. With fructose-induced hypertonicity, plasma [Na] and [Cl] fall and brain ISF [Na] and [Cl] rise together setting up outwardly directed [Na] and [Cl] gradients. Associated with this outwardly directed gradient, there is a loss of brain NaCl, increasing with time and most marked in the cerebellum.

The necessity for inwardly directed [Na] and [Cl] gradients, localization to the BBB, and partial inhibition by "loop" diuretics suggest that ISF volume regulation in the skate brain during hypertonicity is due to a passive increase in BBB permeability, in part carrier mediated. Supported by PHS NS 11050.

SOMATOSTATIN INHIBITS THE STIMULATION OF CHLORIDE SECRETION IN THE SHARK RECTAL GLAND AT A STEP BEYOND ADENYLATE CYCLASE

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Previous reports have shown that somatostatin blocks the stimulatory effect of VIP or veratrine on chloride