FREE AMINO ACIDS AND VOLUME REGULATION IN THE BRAIN OF THE LITTLE SKATE, *Raja erinacea*

Roy P. Forster, JoAnn Hannafin, Jeffrey S. Shiffrin and Leon Goldstein, Department of Biological Sciences, Dartmouth Collete, Hanover, NH 03755 and Division of Biology and Medicine, Brown University, Providence, RI 02912

Recent studies in mammals suggest that retention of osmotically active solutes by brain tissue may be the cause of the cerebral edema seen in the dialysis dis-equilibrium syndrome that occurs during rapid hemodialysis of uremic subjects. Although several solutes have been ruled out as possibilities, the idea that intracellular amino acids may be the causative agents has not been tested heretofore.

It is technically difficult to measure free amino acids in the mammalian brain due to their naturally low level and the problem of contamination by proteolysis during preparation of the sample for analysis. Forster and Goldstein have shown that the skate, *Raja erinacea*, has high levels of intracellular free amino acids and is able to regulate these amino acids in response to osmotic stress (Am. J. Physiol., 230:925-931, 1976). This osmotically tolerant elasmobranch is also in a natural state of uremia (as defined by mammalian standards) due to high levels of urea that are used to maintain osmotic equilibrium with the environment. "Hemodialysis" of these skates can be mimicked by acclimating them to 50% seawater. In this study, we measured the concentration of free amino acids and water in brain and plasma, and plasma osmolarity, of skates acclimated both rapidly and slowly to half strength seawater (½ SW).

Specimens of the little skate, *Raja erinacea*, of mixed sex, with weights ranging from 1 to 2 kg were taken by otter trawl off Mt. Desert Island in the Gulf of Maine. During experiments they were kept in a large aquarium supplied with running seawater, and were fed up to the period of experimentation. Gradual dilution of the pool from SW to ½ SW was accomplished by reducing the salinity by 10% per day down to ½ SW and skates were then allowed to equilibrate with the external medium for two days. Rapid dilution was accomplished by transferring the skates directly from SW to ½ SW. Salinity was followed by measuring chloride concentration of the aquarium water.

After the fish were bled, they were killed by transection of the spinal cord and the whole brain was excised immediately. The brain was sliced longitudinally; one half was frozen between blocks of dry ice, the other half was taken for analysis of water content. Approximately 0.5 gm samples of freshly sliced brain were blotted lightly on filter paper, weighed in tared vials and dried for twenty-four hours at 100°C. Water content of the plasma was determined on two ml samples using the same procedure. For analysis of ninhydrin positive substances (NPS) frozen pieces of brain were homogenized in eight volumes of cold water and one volume of cold 50% trichloroacetic acid (TCA). After standing on ice for 30 minutes the mixture was centrifuged for fifteen minutes and the TCA extracted three times with three volumes of hydrated ether. Plasma extract was prepared in a similar way using equal volumes of plasma and 10% TCA. NPS (mainly free amino acids) were determined by a photometric method (Moore and Stein, J. Biol. Chem., 176:367-388, 1948). Correction was made for the NPS due to urea. Profiles of individual amino acid concentrations were obtained by analysis of sulfosalicylic acid extracts of the brain with a Durrum D-500 automatic amino acid analyzer. Plasma osmolarity was determined on freshly collected samples by cryoscopic osmometry (Fiske).

For the in vitro experiments, whole brain was excised, lightly blotted and transferred to a dish containing balanced isotonic elasmobranch medium on ice. Slices were cut free hand with a thin razor blade. Individual slices were weighed and placed in Erlenmeyer flasks containing three to

six ml of medium. Slices were incubated in an atmosphere of 99% O_2 and 1% CO_2 for one hour at 15°C. Metabolic inhibitors were added to the incubation medium prior to the addition of slices. Following incubation the slices were blotted lightly, reweighed and extracted with TCA. Percent water changes were derived from differences in weights, before and after incubation. K^+ levels were determined by flame photometry (Instrumentation Laboratory Spectrophotometer Model 343), and total amino acids were measured, by the ninhydrin method, on the TCA extracts.

Percent water content of the brain (which was assumed to be an index of tissue volume) and plasma increased following gradual acclimation of skates to ½ SW; brain water increased by 6% and plasma water by 3%. Simultaneously, NPS fell 52% in the brain, but negligible change occurred in the plasma (Table 1). Plasma osmolarity dropped from 957±47 mOsm to 595±17 mOsm, a 35% decrease. Determination of the concentration profiles of free amino acids in the brain showed that taurine composed

TABLE 1

Water, Ninhydrin Positive Substances (NPS) and Osmolarity in Brain and Plasma of *Raja erinacea*, Following Gradual Environmental Dilution

Seawater	Percent Water		NPS (µmoles/ml)		Osmolarity
	Brain	Plasma	Brain	Plasma	Plasma
100%	78±2 (9)	91±1 (9)	173±20 (9)	12±2 (9)	957±46 (11)
50%	82±3 (6)	93±1 (6)	82±14 (6)	11±1 (6)	595±17 (11)
P value	$p < .001$	$p < .001$	$p < .001$	$p > .05$	$p < .001$

Values are means ± standard deviation. Number of fish per group are shown in parentheses.

50% of the total free amino acids and fell 52% during gradual environmental dilution. The other amino acids measured showed inconsistent changes. Similar amino acid patterns were observed in the brains of the stingrays, *D. sabina*, acclimated to SW and ½ SW (Boyd et al., J. Exp. Zool., in press).

During rapid acclimation of skates to ½ SW, percent water content of the brain and plasma, plasma osmolarity and NPS were measured at 4, 24, 48, and 72 hours following environmental dilution (Table 2). NPS in brain decreased dramatically (25%) within 4 hours after transfer; a more gradual decrease continued until 72 hours when the levels stabilized (52% decrease). Brain and plasma hydration reached their maximum at 48 hours, then returned to levels comparable to those found in skates acclimated gradually to ½ SW.

An attempt was made to develop an in vitro model to obtain information on the mechanism of regulation of brain free amino acid concentrations in skates undergoing environmental dilution. Brain K^+ concentration was used as an indicator of the physiological integrity of the incubated brain slices. The effects of medium dilution on amino acid efflux were tested using balanced elasmobranch solution with the urea and NaCl content diluted to one-third. Ouabain and lithium chloride substitution were used to test the dependency of net amino acid efflux on the Na^+ - K^+ exchange pump. Changes in percent water, total amino acids (NPS) and K^+ are summarized in Table 3.

TABLE 2

Water, NPS, and Osmolarity in Brain and Plasma of *Raja erinacea* Following Rapid Acclimation to 50% Seawater

Time	Percent Water		NPS (μmoles/ml)		Osmolarity
	Brain	Plasma	Brain	Plasma	Plasma
Control (9)	78±2	91±2	173±20	12±2	957±47
4 hours (6)	80±1**	94±1*	129±20**	16±5	869±22*
24 hours (6)	82±4***	96±1*	114±6*	14±2	684±52*
48 hours (6)	87±5*	98±2*	90±11*	13±3	595±30*
72 hours	82	97	83	11	571

Values in parentheses indicate the number of fish per group. Values significantly different from controls are marked $p < .001$*, $p < .01$**, $p < .05$***. Values are means ± standard deviation.

TABLE 3

Water Content, Nimhydrin Positive Substances (NPS) and K^+ Concentration Changes in Brain Slices of *Raja erinacea*, Incubated in Vitro

Medium	% Water	% Change NPS	% Change K^+
Control (8)	77±3	(-) 11 ± 4	(-) 18 ± 27
LiCl* (14)	90±7 $p < .001$	(-) 35 ± 7 $p < .001$	(-) 58 ± 23 $p < .001$
Ouabain, 10^{-4} M (12)	93±5 $p < .001$	(-) 46 ± 5 $p < .001$	(-) 76 ± 18 $p < .001$
Dilute medium** (10)	90±6 $p < .001$	(-) 35 ± 10 $p < .05$	(-) 37 ± 15 $p < .05$

Numbers of experiments per group shown in parentheses. *LiCl was substituted for NaCl in the incubation medium. **Dilute medium was prepared by decreasing NaCl from 300 mM to 200 mM and urea from 360 mM to 280 mM.

Slice water content increased by 17% following incubation in the dilute medium, while increases of 21% and 17% were seen in the ouabain and lithium chloride media. K^+ concentrations showed no significant change in control slices indicating that the brain cells remained physiologically intact during the one-hour incubation period. However, the concentration of this cation fell significantly in slices incubated in LiCl, ouabain and dilute medium, probably as a result of decreased activity of the Na^+ - K^+ exchange pump. NPS decreased 24% following incubation in both the low sodium (dilute) medium and the sodium free (LiCl) medium. These results suggest that part of the effect of

environmental dilution on net amino acid efflux may be due to lowered concentrations of Na^+ in the extracellular fluid. A decrease in amino acid concentration of 35% was seen following incubation of brain slices in the ouabain medium. It seems possible that net amino acid efflux may exhibit some sodium pump dependency.

In the elasmobranch model used in this study, amino acids leave the brain rapidly in response to a rapid decrease in plasma osmolarity and at least on this basis do not appear to be solid candidates for the unidentified intracellular osmolytes retained by mammalian brain during rapid hemodialysis. However, even though the skate brain is able to rapidly modulate its intracellular amino acid concentration, species differences in the blood-brain barrier may cloud any conclusion with respect to the mammalian dialysis disequilibrium syndrome.

This work was sponsored by NIH Grant HL 04457-18 and NSF Grant PCM 75-14322.

DISTRIBUTION OF 1-^{14}C-HEXADECANE IN THE LOBSTER, *Homarus americanus*, AT VARIOUS TIMES AFTER INJECTION INTO THE PERICARDIAL SINUS

Gary L. Foureman, Peter Hall, and John R. Bend, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

The United States is highly dependent upon foreign sources of petroleum for energy production. The increasing demand for these hydrocarbons has resulted in drilling for oil on the outer continental shelf and the transportation of large quantities of crude and refined oil in coastal waters. These developments threaten local marine and estuarine ecosystems in the event of catastrophic spills. The effect of chronic exposure of marine animals to low levels of hydrocarbon pollutants should not be totally discounted, particularly in relation to human health. Many marine invertebrates accumulate lipophilic compounds, such as the carcinogenic polycyclic aromatic hydrocarbon benzo[a]pyrene (Dunn and Stich, Bull. Envir. Cont. Toxicol., 15:398, 1976). Significant quantities of foreign organic chemicals can thus be introduced into our food via this mechanism.

In this report we describe the distribution of hexadecane, a relatively nonvolatile alkane crude oil constituent, in lobster following intracardial administration. The chemical nature of the radioactivity remaining in the hepatopancreas 7 days after dosing was also determined.

Male and female hard-shelled lobsters (360-490 g) were purchased locally (Thurstons, Bernard, Me.). 1-^{14}C-Hexadecane (54 mCi/mmole) was obtained from the Radiochemical Center, Amersham, England. It was diluted with hexadecane (Aldrich Chemical Co.) prior to use and was administered by injection into the pericardial sinus dissolved in Emulphor:ethanol:water (3:3:4, by volume). The lobsters received a dose of 10 mg (4 µCi) ^{14}C-hexadecane/kg. After treatment lobsters were placed in 40 liter aquaria equipped with flowing sea water (12-15°C). The lobsters were fed pieces of herring during the experiment.

Plasma was removed from the pericardial sinus just before sacrifice. Subsequently, the tissues were dissected from the carcass and weighed. Triplicate aliquots of plasma (0.1 ml) and duplicate tissue samples (80-200 mg) were solubilized by digestion in 2 ml NCS (Amersham-Searle) by incubation at 50-60° overnight. Scintillation solvent 18 ml (5 g PPO and 0.25 g POPOP per liter toluene) was added and the radioactivity determined in a Packard Tri-Carb counter. Counting efficiency was determined using the external standard technique and varied from 50-90% for almost all samples.