HYDROGEN ION-INDUCED BRAIN DAMAGE IN SKATE (RAJA ERINACEA)

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Brain infarction from ischemia is a leading cause of death and disability in the United States (Kurtzke, In: Cerebrovascular Survey Report, F.H. McDowell & L.R. Caplan, eds., N.I.H., pp. 1-34, 1985). The present treatment of acute stroke is empiric and largely ineffective. Effective therapy to prevent or ameliorate ischemic brain damage is likely to come from an improved understanding of fundamental physiologic processes whose dysfunction accompanies infarction. One such process is pH regulation in ischemic brain.

Hyperglycemia worsens outcome from nearly complete ischemia (of 20 minutes duration) to include infarction in animals and man (Pulsinelli et al., Neurology 32:1239-1246, 1982). This is presumed to result from augmented brain acidosis created by enhanced anaerobic glycolysis. Glia may be the site and source of this accentuated acidosis. Indirect measurements (Kraig et al., Am. J. Physiol. 250: R348-R357, 1986) suggest that failure of glial membrane antiport mechanisms for pH regulation and retained plasma membrane impermeability to hydrogen ions (or pH-related ionic species) can result in a total loss of glial bicarbonate and fall in intracellular pH to between 4.2-5.2 under conditions which could evolve to infarction. Recently, we succeeded in making direct microelectrode measurements of intracellular pH which confirm this very extreme level of intracellular acidosis (Kraig and Nicholson Soc. Neurosci. 12:65, 1986). In other work (Kraig and Chesler, J. Cereb. Blood Flow & Met. 7(suppl. 1):S126, 1987) we used staining with horse radish peroxidase to identify the source of this extreme acidosis as glial cells. Finally, by injecting sodium lactate solutions (adjusted to various pH levels) directly into brain (Kraig et al., J. Cereb. Blood Flow & Met. 7:379-386, 1987) we discovered that mammalian brain could be destroyed in a pattern consistent with infarction when interstitial pH was held at less than 5.3 for 20 minutes. Taken together these results provide strong evidence that severe glial acidosis is a neccessary concomitant of brain infarction.

The molecular mechanisms by which severe acidosis destroys brain are unknown. Irreversible and generalized denaturation of brain proteins does not appear to be a likely cause of acid-induced ischemic injury since levels of acidity below 4.5 pH are needed to permanently denature lumped rat brain proteins (Kraig and Wagner, Brain Res. 410:390-394, 1987). Of course this does not preclude the possibility that specific vital brain proteins can be denatured at a less extreme level of acidity. However, an intriguing and potentially more important possible mechanism is that of acid-induced, and free radical mediated brain injury (Pulsinelli et al., In: Cerebrovascular Diseases, 14th Princeton-Williamsburg Conference, F. Plum & W.A. Pulsinelli, eds., Raven, N.Y., pp. 201-205, 1985). Temperature and endogeneous brain buffer capacity are two variables which can influence the capacity of biological systems to support the generation of free radicals induced by acidosis. Elasmobranchs (at Mount Desert Island) live in sea water which is approximately 13^{o} C and since their internal carbon dioxide tension is only around 8 torr, they have considerably less bicarbonate stores than mammalian species. Accordingly, examination of acid-induced injury in the elasmobranch may begin to reveal important clues as to how acidosis can destroy the mammalian brain.

Skates (1-2 kg) of either sex were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and mounted on a soft plastic surface beneath sea water so as to prevent cutaneous injuries. Animals were artificially ventilated by passing fresh sea water (16° C) through their spiracles at a rate of approximately 1-2 l/min. The cerebellum was exposed and superfused with aerated (99% oxygen; 1% carbon dioxide) Ringer (288 mM NaCl, 6 mM KCl, 5 mM $CaCl_2$, 3 mM $MgCl_2$, 8 mM $NaHCO_3$, and 350 mM urea). Hydrogen ion-selective microelectrodes were fabricated using tridodecylamine (Ammann et al., Anal. Chem. 53:2267-2269, 1981). The micro-injection solution consisted of 150 mM sodium lactate with sufficient urea so that solution molality equalled that of skate plasma (pH was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, and 7.0 with HCl). Solutions were injected into cerebellar cortex at a rate of 0.5 ul/min. for 20 minutes via a 170 um fused silica needle and interstitial pH monitored as previously described (Kraig et al., J. Cereb. Blood Flow & Met. 7:379-386, 1987). Electrodes were positioned 200 um behind the tip and adjacent to the wall of the injection needles. Needle-electrode arrays were advanced 500-1,000 um into the cerebellar cortex. After a micro-injection, the craniotomy was closed with a rubber patch that was attached to the skull with cyanoacrylate. Artificial ventilation was continued for 24 hours (13-16° C) at which time animals were re-anesthetized and killed by intracardiac perfusion-fixation with Karnovsky (4% paraformaldehyde, 1% glutaraldehyde in 388 mM NaCl, 350 mM urea, and 200 mM phosphate buffer at 7.2 pH). Brains were removed, stored in fixative and subsequently embedded in paraffin, sectioned and stained with hematoxylin and eosin.

With the onset of injections, interstitial pH fell quickly to reach a new steady-state peak that was near that of the injection solution pH. After injections interstitial pH returned to baseline within minutes and at essentially the same rate in all experiments in spite of the fact that the most acidic injectants (4.0 & 4.5 pH) produced brain necrosis. The return of interstitial pH toward baseline was dramatically different in mammalian brain after lethal (i.e. those which produced coagulation necrosis) injections. In rat, interstitial pH returned much more slowly to baseline after lethal (compared to nonlethal) injections (Kraig et al., J. Cereb. Blood Flow & Met. 7:379-386, 1987) so that lethal injury could be identified immediately after micro-injections. Brain necrosis was seen in skate only after injections which held interstitial pH at $\leq$ 4.86 for 20 minutes.

These results show that lethal injury of elasmobranch brain by acid injection requires more than three times as much acid (4.86 compared to 5.30 interstitial pH) and more time to become manifest than comparable injections in mammalian brain. Since skate brain possesses considerably less bicarbonate stores than mammalian brain, one might have expected skate brain to be more vulnerable to acid injury than mammalian brain. Instead the results of this investigation suggest skate brain is less vulnerable to severe acidosis. Such protection may relate to the lower brain temperature of the skate compared to the rat. If pH-related physiologic processes in both species are similar, these results suggest that a reduction in brain temperature may lessen necrosis of mammalian brain from severe acidosis.

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