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Acid-induced injury in elasmobranch brain

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Abstract

Sodium lactate injection into rat brain produces coagulation necrosis consistent with infarction when pH_o is held at ≤ 5.30 for 20 min. Such injury may result from excessive astroglial acidification. If true, then brain damage from acidosis in elasmobranchs might evolve differently since glial reaction there to another necrotizing injury, exposure to extreme cold, is dissimilar from that seen in mammals. Accordingly, pH_o was monitored and sodium lactate (pH 4.00–7.00) injected into skate (*Raja erinacea*) cerebella. Necrosis was seen only when pH_o was ≤ 4.86 for 20 min; and pH_o rise after injections was unaffected by those which destroyed brain, and not slowed as in rat. Thus elasmobranchs are less susceptible to irreversible brain injury from acidosis, a capacity which may result from their lower body temperature compared to mammals.

Keywords

Acidosis; Infarction; Ischemia; Ion electrode; Hydrogen ion; Lactic acid; Elasmobranch; Skate

Acid–base regulation of brain is non-homogeneous among different cell types, with most variations occurring within astroglia [10]. Initially, these cells shift more alkaline when brain activity rises [4]. However, under the pathological condition of hyperglycemic and complete ischemia [9,12,14] these cells become preferentially markedly more acidic than the remainder of brain. Irreversible injury of astrocytes, because of their excessive acidosis [9,12,14] may be a key event in the evolution of ischemic infarction [16].

Nonetheless, little is known about how excessively low levels of astroglial pH are associated with brain necrosis or if such injury is a generalized response seen in other species. Sodium lactate, when microinjected into rat brain produces coagulation necrosis in a pattern consistent with infarction when interstitial pH (pH_o) is held at 5.30 or less for 20 min [13]. This previous work is compared here to similar injections done in elasmobranch brain. The elasmobranch was chosen for comparison because of physiologic differences its phylogenetically more primitive brain has compared to mammals [5]. Furthermore, the elasmobranch brain is already known to respond differently than the mammalian counterpart to another necrotizing injury – exposure to excessive cold [8,11]. Thus differences may also exist in the manner by which elasmobranch brain responds to acid-induced necrosis and such differences may lead to information which bears on the pathogenesis of infarction.

Skates (*Raja erinacea*; 1–2 kg) of either sex were anesthetized with i.v. sodium pentobarbital (30 mg/kg) and transferred to a recording chamber where they were mounted on a soft plastic platform and partially immersed in sea water to prevent cutaneous injury. Animals were respiration by passing sea water (16°C) through their spiracles at a rate of 1–2 liters/min. Cerebella were exposed and superfused with aerated (99% O_2 –5% CO_2) Ringer (in mM: NaCl

288, KCl 6, CaCl₂ 5, MgCl₂ 3, NaHCO₃ 8, urea 350, pH 7.35–7.40) [5]. Tridodecylamine-based [1] and double barrel pH sensitive microelectrodes were fabricated and microinjections performed as previously described [13]. Briefly stated, microelectrodes were positioned 200 μ m behind the tip and adjacent to the wall of a fused silica micro- (170 μ m) injection needle (no. 2-1361; Supelco). Needle–electrode arrays were advanced 500–1000 μ m into exposed cerebella and solutions injected at 0.5 μ l/min for 20 min while monitoring pH_o. Injection solutions consisted of 296 mM sodium lactate (to approximate ionic strength of interstitial fluid) and 390 mM of urea (so that injectant molality equalled that of skate interstitial fluid [5]) that were adjusted to 4.0, 4.5, 5.0, 5.5, 6.0 and 7.0 pH with HCl. At the conclusion of pH_o recordings (i.e. 60–90 min after injections) pH microelectrodes were calibrated in situ by immersing them in phosphate buffer solutions which bracketed the experimentally observed pH range. Electrical continuity with the animal was maintained by attaching a small bottomless cup for the buffers to the animal's moist skin with cyanoacrylate. Next craniotomies were closed with a rubber patch attached to the skull by cyanoacrylate. Animals were placed in a recovery pond (13–16°C) with continued artificial ventilation for 24 h before being re-anesthetized and killed by intracardiac perfusion with fixative (4% paraformaldehyde–1% glutaraldehyde in 288 mM NaCl, 350 mM urea, and 100 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 for light microscopic examination of injection zones.

Sodium lactate, when injected into skate brain (Fig. 1), induced pH_o changes which, in most respects, were similar to those seen in rat [13]. Resting pH_o previously has not been reported in skate. In 19 animals baseline pH_o was 7.29 $\pm$ 0.01 (mean $\pm$ S.E.M.). Skate pH_o changed rapidly to reach a new steady-state shortly after the onset of microinjections. Furthermore, plateau pH_o was always slightly higher than injectant pH (see Table I). This effect was also seen in the rat and thought to reflect the influence of progressive titration of non-volatile brain physicochemical H⁺-buffers which could be expected to be most abundant near 4.50 pH [15]. The latter conclusion stems from the observation that mammalian brain proteins have H⁺-buffer moieties which are normally exposed to aqueous media (and thus available for titration) and other buffer moieties which are normally sequestered but become available for titration through acid-induced protein denaturation [15]. A similar maximal disparity between injectant pH and pH_o was seen in skate when injecting 4.50 pH sodium lactate which suggests that skate brain proteins react to acidosis in a manner and to an extent analogous to proteins of mammalian brain. In addition though, skates differed from mammals in showing a second maximal disparity during injection of solution at 6.0 pH when pH_o reached only 6.41 $\pm$ 0.05 (Table I). Protein denaturation seems an unlikely cause for this latter buffering effect because the degree of acidosis (i.e. 6.41 pH) is mild compared to that typically needed (i.e. 3–5 pH) to denature proteins [15]. Instead, an increase in the ability of skate brain to remove acid loads near 6.0 pH seems a more likely possibility especially in light of the manner in which pH_o returned to baseline after injections.

Return of pH_o to baseline after injections did not correlate with the ultimate tissue effect. In rat [13] the effect of lethal (i.e. those which produced coagulation necrosis) injectants was apparent immediately after injections since pH_o took more time (i.e. greater than 10–20 min) to return to baseline after lethal as compared to non-lethal (less than 10 min) injections. In skate pH_o always took less than 10 min to return to baseline in spite of the lethal effect of the most acidic (4.00 and 4.50 pH) injectants.

The histological result of lethal injections is shown in Fig. 2. Skate cerebellar cortex, like that of other vertebrates, consists of a distinct molecular, Purkinje cell, and granule layer (Fig. 2A) [7]. Lethal injections produced a circumscribed but irregular zone of coagulation necrosis. The central area of necrotic zones showed homogenation of the neuropil and neurons (i.e. Purkinje and granule cells shown in Fig. 2B) which appeared as eosinophilic ghosts. Other neurons

closer to the edge of necrotic zones were shrunken with eosinophilic cytoplasm and pyknotic nuclei. Nuclear debris and inflammatory cells were present throughout necrotic zones. On the other hand, inflammatory cells were confined to needle tract areas after non-lethal injections (not shown).

These results show that lactic acid when injected into skate brain requires almost 3 times the concentration needed in rat (4.86 vs 5.30 pH) to produce a similar pattern of necrosis. Furthermore, and perhaps most importantly, pH_0 returned to normal after lethal injections in skate more promptly than in rat. Thus the key observation of this study is that skate is more capable than rat of dissipating extreme acid loads within brain in spite of the ultimately lethal effect of these injectants.

Mechanisms which account for the return of pH_0 to baseline after lethal injections are unknown. However, two general inferences can be drawn from existing literature. First, if skate brain H^+ -buffer capacity is similar to that of neural tissue from other organisms, it will closely approximate the buffer capacity of mammalian brain [18]. Thus, enhanced physicochemical buffering of proton equivalents would be an unlikely mechanism to account for the speedier return of pH_0 to baseline after lethal injections in skate. Second, the principal mechanism for movement of proton equivalents out of the interstitial space and into cells (and perhaps to the vasculature) is through Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$ antiport [18]. Such exchange transport is typically powered by the transmembrane sodium gradient [18]. If intracellular sodium concentrations are similar in elasmobranchs and mammals, then the transmembrane sodium gradient (and the consequent ability to antiport proton equivalents) would be greater in the former species than the latter. This conclusion stems from the fact that interstitial sodium concentration is approximately 288 mM in skates and 154 mM in rats. Therefore, even in the face of extreme interstitial acidosis, a condition thought to retard Na^+/H^+ antiport and $\text{Cl}^-/\text{HCO}_3^-$ antiport of brain cells [14], these transport mechanisms might still be operational in skate. Accordingly, after the most acidic injections skate brain cells (and especially their glia since these cells are thought to be the principal source of bicarbonate buffer to the interstitial space [6]) might be more acidotic than those of rat because of continued antiport activity in the former compared to the latter animal. Yet skate brain required almost 3 times more acid than was needed in rat to produce coagulation necrosis.

The lower brain temperature (16°C) of elasmobranchs may be a key variable which accounts for both the increased acid concentration needed to destroy skate brain and the continued normalcy of pH regulatory behavior seen there shortly after exposure to extreme levels of acidity. Support for this contention comes from the fact that brain necrosis from ischemia can be reduced by hypothermia in mammals [2,3]. Thus, if brain infarction in mammals stems from excessive astroglial acidosis, hypothermia might increase the organ's threshold susceptibility to acid and reduce the deterioration in pH regulatory mechanisms seen shortly after exposure to acid. Finally, evidence that acid-induced necrosis (or infarction) is highly temperature dependent would be consistent with the hypothesis that such injury results from free radical mediated processes [17].

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References

1. Ammann D, Lanter F, Stevens RA, Schulthess P, Shijo Y, Simon W. Neutral carrier based hydrogen ion selective microelectrode for extra- and intracellular studies. *Anal Chem* 1981;53:2267–2268. [PubMed: 7316213]
2. Berntman L, Welsh FA, Harp JR. Cerebral protective effect of low grade hypothermia. *Anesthesiology* 1981;55:495–498. [PubMed: 7294402]
3. Busto R, Dietrich WD, Globus MY-T, Valdes I, Scheinberg P, Ginsberg MD. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury, *J. Cereb. Blood Flow Metab* 1987;7:729–738.
4. Chesler M, Kraig RP. Intracellular pH of astrocytes increases rapidly with cortical stimulation. *Am J Physiol* 1987;253:R666–R670. [PubMed: 3116863]
5. Cserr HF, Bradbury MWB, Mackie K, Moody EJ. Control of extracellular ions in skate brain during osmotic disturbances. *Am J Physiol* 1983;245:R853–859. [PubMed: 6660332]
6. Kimelberg, HK.; Bourke, RS. Anion transport in the nervous system. In: Lajtha, A., editor. *Hand-book of Neurochemistry*, Vol. 1, Chemical and Cellular Architecture. 2. Plenum; New York: 1982. p. 31-67.
7. Klatzo, I. Cellular morphology of the lemon shark brain. In: Gilbert, PW.; Mathewson, RF.; Rall, DP., editors. *Sharks, Skates, and Rays*. Johns Hopkins Press; Baltimore: 1967. p. 341-359.
8. Klatzo I, Steinwall O. Observation on cerebrospinal fluid pathways and behavior of the blood-brain barrier in sharks. *Acta Neuropathol* 1965;5:161–175. [PubMed: 5886199]
9. Kraig RP, Chesler M. Glial acid-base behavior in brain ischemia. *J Cereb Blood Flow Metab* 1987;7:S126.
10. Kraig, RP.; Chesler, M. Dynamics of volatile buffers in brain cells during spreading depression. In: Somjen, G., editor. *Cerebral Hypoxia and Stroke: Reversible and Irreversible Effects and their Prevention*. Plenum; New York: in press
11. Kraig RP, Jaeger CB. Absence of fibrillary acidic protein response to cryogenic injury in skate (*Raja erinacea*). *Mount Desert Island Biol Bull.* in press.
12. Kraig, RP.; Nicholson, C. Profound glial acidosis in presumed gila during ischemia. In: Raichle, ME.; Powers, WJ., editors. *Cerebrovascular Diseases*, 15th Princeton-Williamsburg Conference; Raven, New York. 1987. p. 97-102.
13. Kraig RP, Petito CK, Plum F, Pulsinelli WA. Hydrogen ions kill brain at concentrations reached in ischemia. *J Cereb Blood Flow Metab* 1987;7:379–386. [PubMed: 3611202]
14. Kraig RP, Pulsinelli WA, Plum F. Carbonic acid buffer changes during complete brain ischemia. *Am J Physiol* 1986;250:R348–357. [PubMed: 3082219]
15. Kraig RP, Wagner RJ. Acid-induced changes of brain protein buffering. *Brain Res* 1987;410:390–394. [PubMed: 3594248]
16. Plum F. What causes brain infarction? *Neurology* 1983;33:222–233. [PubMed: 6681661]
17. Pulsinelli, WA.; Kraig, RP.; Plum, F. Hyperglycemia, cerebral acidosis, and ischemic brain damage. In: Plum, F.; Pulsinelli, WA., editors. *Cerebrovascular Diseases*, 14th Princeton-Williamsburg Conference; Raven, New York. 1985. p. 201-205.
18. Roos A, Boron WF. Intracellular pH. *Physiol Rev* 1981;61:296–434. [PubMed: 7012859]

Fig. 1.

Composite of pH_o changes from sodium lactate injections. Typical records are shown of pH_o changes produced by intracerebellar injections of 296 mM sodium lactate made isotonic with added urea and adjusted to 4.00 (a), 4.50 (b), 5.00 (c), 5.50 (d), 6.00 (e), and 7.00 (f) pH.

Microinfusions ($0.5 \mu\text{l}/\text{min}$ for 20 min beginning at upward arrow and stopping at downward arrow) caused pH_o to shift to a new steady-state during all but the most alkaline (records e and f) injections. After injections all pH_o variations returned to resting conditions at approximately the same rate even though the most acidic injectants (records a and b) produced brain necrosis. These results suggest that H^+ -homeostatic mechanisms of the elasmobranch brain, unlike those of its mammalian counterpart, do not reveal any impairment immediately after exposure to a $[H^+]$ which ultimately proves to be lethal.

Fig. 2. Brain necrosis associated with sodium lactate injections. Typical laminar cerebellar architecture, consisting of the molecular layer (ML), Purkinje cell layer (PCL), and granule cell layer (GCL), is shown under normal circumstances (A). Arrowheads point to Purkinje cell bodies. After lethal injections an irregular but circumscribed zone of coagulation necrosis was evident. Within necrotic zones (B) eosinophilic neuronal ghosts could be seen (arrowheads pointing toward degenerating Purkinje cells with ML above and GCL below). Semi-lucent, degenerating granule cells (open arrow) are evident. Inflammatory cells (arrow) were present throughout necrotic zones. Bar = 100 μ m.

TABLE IMAXIMUM pH_o FROM ACID INJECTIONS AND ASSOCIATED HISTOLOGICAL DAMAGE

Injectant pH	pH_o *	Necrosis?
7.00	7.13 ± 0.04 ($n=3$)	no
6.00	6.41 ± 0.05 ($n=5$)	no
5.50	5.67 ± 0.02 ($n=2$)	no
5.00	5.09 ± 0.01 ($n=3$)	no
4.50	4.86 ± 0.05 ($n=3$)	yes
4.00	4.13 ± 0.09 ($n=3$)	yes

* Mean $\pm$ S.E.M.