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Hydrogen Ion Buffering During Complete Brain Ischemia

RICHARD P. KRAIG, **WILLIAM A. PULSINELLI**, and **FRED PLUM**
Cerebrovascular Disease Research Center, Department of Neurology, Cornell University Medical College, New York, NY 10021 (U.S.A.)

Abstract

As a first step to quantify $[H^+]$ changes in brain during ischemia we used H^+-selective microelectrodes and enzyme fluorometric techniques to describe the relationship between interstitial $[H^+]$ ($[H^+]_o$) and peak tissue lactate after cardiac arrest. We found a step function relationship between $[H^+]_o$ and tissue lactate rather than the linear titration expected in a homogeneous protein solution. Within a blood glucose range from 3–7 mM, brain lactate rose from 8–13 mmol/kg along with a rise in $[H^+]_o$ of 99 ± 6 nM (0.44 ± 0.02 pH). At higher blood glucose levels (17–80 mM), brain lactate accumulated to levels of 16–31 mmol/kg; concurrently $[H^+]_o$ rose by 608 ± 16 nM (1.07 ± 0.02 pH). The unchanging level of $[H^+]_o$ between 8–13 and 16–31 mmol/kg lactate implies that $[H^+]_o$ is at a steady-state, but not equilibrium with respect to $[H^+]$ in other brain compartments. We propose that ion-transport characteristics of astroglia account for the observed relationship of $[H^+]_o$ to tissue lactate during complete ischemia and suggest that brain infarction develops after plasma membranes in brain cells can no longer transport ions to regulate $[H^+]$.

Keywords

buffer; glial cell; hydrogen ion; pH; brain ischemia; lactate; hyperglycemia; hydrogen ion microelectrode

INTRODUCTION

Complete brain ischemia imposed during normoglycemia results in selective neuronal destruction; other tissue elements are spared and tissue lactate accumulates to a level of up to 13 mmol/kg[39]. By contrast, equivalent degrees and duration of ischemia delivered during hyperglycemia produce brain infarction with necrosis of all tissue elements[40]. Concomitantly, lactate levels rise to greater than 16 mmol/kg[40]. It has been postulated that lactic acidosis (hydrogen ion concentration ($[H^+]$) greater than normal) may cause severe ischemic brain injury[33]. If so, the micro-physiological mechanisms and degree of this putative damage remain undefined. Furthermore, the relationship of brain lactate content to $[H^+]$ in cellular or interstitial compartments is unknown and difficult to predict because of the dynamic and interactive mechanisms for $[H^+]$ homeostasis between brain cells and their microenvironment. An understanding of the molecular processes for $[H^+]$ homeostasis in brain is needed to provide a more quantitative interpretation of interrelated biochemical reactions and physiological processes during ischemia and subsequent ischemic brain injury.

In the present study we used H^+-selective, liquid-membrane, double-barrelled microelectrodes and enzyme fluorometric techniques to examine the relationship between

Correspondence: R. P. Kraig, Cerebrovascular Disease Research Center, Department of Neurology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021, U.S.A.

interstitial $[H^+]$ ($[H^+]_o$) and peak tissue lactate content during complete ischemia. As will be shown, a step function relationship was found between $[H^+]_o$ and peak tissue lactate levels rather than the linear titration expected in a homogeneous protein solution[31]. The result suggests that net H^+ movement out of cells reflects steady-state but not equilibrium conditions for at least the first 3–5 minutes of complete ischemia. The changes in $[H^+]_o$ can be accounted for by alterations in the carbonic acid-buffer system of the brain. A preliminary report of some aspects of this work has appeared[26].

METHOD

Twenty-one male Wistar rats (250–400 g) were anesthetized with halothane (5% induction; 3% maintenance during surgery; 1.5–2.0% during recording) and spontaneously ventilated with a 30% oxygen-nitrogen mixture via a modified inhalation mask[29]. The tail artery and femoral vein were cannulated and a 1–2 mm craniotomy was made 1 mm caudal to bregma and 2–3 mm lateral to the sagittal suture. The animals were then mounted in a head holder and warmed to 37 °C with a water jacket. Warmed normal (37 °C) Ringer solution flowed through a superfusion cup placed around the craniotomy site[24]. H^+ ion selective micropipettes (ISMs) were positioned 800 μm down in the paramedian neocortex. Arterial pH, oxygen tension (pO_2) and carbon dioxide tension (pCO_2) were stabilized and measured with a Corning 158 blood gas analyzer. Selected animals were given an intraperitoneal injection of 0.93 M D-glucose (8.35 g/kg) or an intravenous injection of regular insulin (2.0–6.0 units/kg of U-100 Iletin). Blood glucose was measured with a Glucometer (Miles Laboratories). When animals achieved the desired blood glucose concentration, complete ischemia (cardiac arrest) was created by intravenous injection of 1 ml of 1 M KCl. When brain $[H^+]_o$ reached a plateau, animals were decapitated and their heads were quickly dropped into liquid nitrogen. Samples of the neocortex from the recording zone were dissected from whole brains at −25 °C. The brain sections were powdered under liquid nitrogen and the frozen powders were weighed (20–30 mg) on a precision balance (Roller–Smith). The weighed powders were layered over 75 μl of frozen 3.0 M perchloric acid and further processed as previously described[39]. Enzyme fluorometric techniques were used to measure tissue lactate[53], adenosine triphosphate (ATP), adenosine diphosphate (ADP), phosphocreatine (PCr) and creatine (Cr)[30].

H^+ ISMs were constructed as previously described[24, 34] using a H^+-selective ion exchanger based on tridodecylamine (Fluka Chemical)[3] and connected to unity-gain, high-performance buffer amplifiers. Ion and reference signals were processed through 5-Hz active filters (Burr Brown) and subtracted via an instrumentation differential amplifier (Analog Devices 521K) to yield the pure electrochemical signal for $[H^+]$. $[H^+]_o$, DC slow potentials and blood pressure (measured with a Statham P-50 pressure transducer) were recorded on a Gould 2600S chart recorder. An indifferent macroelectrode containing Ag–AgCl–1 M KCl/3.5% agar was positioned on muscle adjacent to the craniotomy site. Electrodes and $[H^+]$ recordings were calibrated as previously described[24]; pH calibrating solutions were referenced to Fisher pH standards measured with a glass pH electrode (Thomas 4094-L15) on an Altex/Beckman pH meter (PHI-71). H^+ ISM responses were corrected for temperature through the Nicholsky equation[10]. Sodium bicarbonate-based standards were also corrected for animal superfusion temperature (37 °C) by estimating changes in carbonic acid equilibrium constants and carbon dioxide solubility[32, 46] from pH measurements made with the Thomas pH electrode at 25 °C. Calibration data were fitted to a least squares best fit of mV vs pH and animal experimental data were referenced to this calculated line.

Brains from halothane-anesthetized and spontaneously breathing rats were frozen with liquid nitrogen by the method of Pontén[37] for titration as homogenates. Samples of cerebral cortex (150–200 mg) were dissected from whole brains at −25 °C and then homogenized in

100 mM sodium fluoride (3 ml) under mineral oil at 25 °C. Homogenate pH was measured with a glass pH electrode (Thomas 4094-L15) on an Altex/Beckman pH meter (PHI-71) while 50 μl aliquots of 0.01 M lactic acid were added with stirring. Sodium fluoride pH had previously been adjusted to 7.00 with sodium hydroxide.

RESULT

Rats were divided into two groups, one with blood glucose levels of 3–7 mM (normoglycemic) and the other with blood glucose levels of 17–80 mM (hyperglycemic). Blood (Table I) and brain (Table II) physiologic variables were similar in all animals. Spontaneously breathing, normothermic rats under normoxic conditions had a mild respiratory acidosis as a result of halothane anesthesia (Table I). Control animals that did not experience ischemia had levels of brain high-energy phosphate reserves and lactate content that are normal for the state of mild hypercarbia[22,39] (Table II) After cardiac arrest when $[H^+]_o$ reached a plateau, high-energy phosphates declined to similar levels in all rats (Table II). Brain lactate rose to 7.80–12.80 mmol/kg in normoglycemic animals and to 16.30–31.30 mmol/kg in hyperglycemic animals. High-energy phosphate values from animals whose brain lactate rose to 13–16 mmol/kg were not included in the averages shown in Table II but were similar.

$[H^+]_o$ in brain rose as blood pressure fell (Fig. 1). The rate of $[H^+]_o$ change was insignificantly slower (0.15 vs 0.22 pH/min) in normoglycemic animals compared to hyperglycemic ones. A characteristic[24] brief alkaline-going transient coincided with the development of a slow DC potential after cardiac arrest. Similar DC changes have been correlated with neuronal depolarization during ischemia[5,13,14]. Brain $[H^+]_o$ rose to a new plateau in about 3 min in normoglycemic animals (Fig. 1A) and 5 min in hyperglycemic animals (Fig. 1B). With blood glucoses at 3–7 mM and ischemic brain lactates of 8–13 mmol/kg, $[H^+]_o$ increased by 99 ± 6 nM (0.44 ± 0.02 pH; Fig. 1A; Table II). With blood glucoses of 17–80 mM, ischemic brain lactates rose to 16–31 mmol/kg and $[H^+]_o$ increased by 608 ± 16 nM (1.07 ± 0.02 pH) (Fig. 1B; Table II). With brain lactate values between 13–16 mmol/kg, $[H^+]_o$ underwent an intermediate change.

H^+ is generated in a 1:1 stoichiometric relationship with lactate in completely ischemic brain[1,27]. Therefore, if lactic acid produced in cells mixes with the interstitial space, $[H^+]_o$ should rise in direct proportion to the total brain lactate content (see below). Instead, peak $[H^+]_o$ levels in normoglycemic and hyperglycemic animals were bimodally distributed according to tissue lactate content (Fig. 2). In spite of the fact that preischemic blood glucoses ranged from 3–7 mM and brain lactates ranged from 8–13 mmol/kg in normoglycemic animals, $[H^+]_o$ always rose by a constant amount of 99 ± 6 nM (0.44 ± 0.02 pH). Similarly, although preischemic blood glucoses ranged from 17–80 mM and brain lactates ranged from 16–31 mmol/kg in hyperglycemic animals, $[H^+]_o$ always rose by a constant, but larger, amount of 608 ± 16 nM (1.07 ± 0.02 pH).

Simple titrations of a strong acid against a H^+-buffered solution produce at least a slightly graded increase in $[H^+]$ to progressively greater acid concentrations. To examine how brain physicochemical H^+ buffers might limit $[H^+]$ at lactate concentrations approximating those encountered in these experiments, we titrated diluted rat cerebral cortex homogenates with lactic acid (Fig. 3). The pH fell steadily without inflection from 7.00 to 6.00 as we added lactic acid up to about 40 mM/kg of brain homogenate. Such a linear relationship would be expected for H^+ titration of a protein solution where H^+ buffer equilibrium constants could be expected to be evenly distributed[31] over physiologic pH (6.6–7.7)[47]. The result (Fig. 3) confirmed that physicochemical processes alone cannot explain the step function relationship found between $[H^+]_o$ and peak tissue lactate during complete ischemia (Fig. 2).

Instead, the progressive rise in tissue lactate unaccompanied by a change in peak $[H^+]_o$ between 8–13 and 16–31 mmol/kg lactate implies that brain $[H^+]$ must be partitioned unequally between cellular and interstitial compartments during the early minutes of complete ischemia.

To determine if unknown anions[2] released to the interstitial space during ischemia [24,34] could have influenced the response of the H^+ ISM to changes in $[H^+]_o$ we examined the H^+ ISM response in rat brain homogenates exposed to several different $[H^+]$. A linear H^+ ISM response occurred with addition of homogenate to potassium hypophosphate solutions buffered with sodium hydroxide over a $[H^+]$ range between pH 4.4 to 7.6 (Fig. 4). It is known that H^+ ISMs such as those used in this study do not suffer from biologically important cation interferences[3]. Therefore, the result makes it unlikely that the constant peak levels of $[H^+]_o$ observed during complete ischemia in vivo reflected an artifact of interfering ions.

DISCUSSION

Excess H^+ is produced according to a 1:1 stoichiometric relationship with lactate during ischemia[1,27]. However, the $[H^+]$ in any brain compartment (intracellular or interstitial) cannot be predicted on simple physical-chemical grounds from net tissue lactate concentrations because of the different H^+ regulatory mechanisms in a multicompartment system. Brain-cell H^+ buffering includes at least 3 physiologic and chemical mechanisms: 1) metabolic production and consumption of acids and bases; 2) ion transport across membranes; 3) physicochemical H^+ buffers[47]. All 3 processes begin to change simultaneously when $[H^+]$ is perturbed but they adjust $[H^+]$ to a new steady-state at different rates in various brain compartments[43]. H^+ ISMs can accurately detect rapid changes in $[H^+]$ in a single brain compartment such as the interstitial space but thus far have not been used in a detailed study of $[H^+]$ homeostasis during ischemia.

This study reports brain $[H^+]_o$ changes during complete ischemia as a first step in trying to quantify $[H^+]$ homeostasis in mammalian brain ischemia. In the cardiac arrest model studied, the absence of blood flow prevented escape of excess H^+ or CO_2 from brain. It is known that about 106 mM Na^+- and 57 mM Cl^- are lost from the interstitial space within a minute or two of the onset of ischemia[15]. After that only residual ion gradients, which exist across intact plasma membranes, or cell physicochemical H^+ buffers are likely to influence $[H^+]$ in any brain compartment. Furthermore, the examination of $[H^+]$ homeostasis in the interstitial space is simplified because the compartment contains only a single H^+ buffer, carbonic acid. During the first minutes of complete ischemia, if cell membranes remain intact, other H^+ buffers are unlikely to enter the interstitial space.

The present results imply that $[H^+]_o$ is not at electrochemical equilibrium with $[H^+]$ in other brain compartments after 3–5 min of complete ischemia. If this is correct, energy in the form of membrane barriers or the potential energy of ion gradients across membranes must be present to explain the steady-state constancy of $[H^+]_o$ despite the progressively increasing intracellular H^+ content that the rising brain lactate level implies, H^+ permeability of intact mammalian brain cell plasma membranes during conditions of ischemia has not been determined[43]. It is known, however, that negatively charged membranes can decrease their permeability to ions in the presence of increasing $[H^+]$ because of surface charge titration[51]. If intact brain-cell membranes behave similarly, their permeability to H^+ may diminish as their adjacent microenvironment becomes more acidotic. Furthermore, since the effective hydrated ionic radius of H^+ is 0.9 nm while the primary hydrate radii of other ions (Na^+, K^+, Cl^-, HCO_3^-) lies between 0.3 and 0.4 nm[8], excess H^+ may be unable to diffuse passively from brain cells until the structural integrity of the cell membranes is disrupted.

H^+ as such need not cross membrane-bound compartments to alter the $[H^+]$ in a second compartment. In biological fluids $[H^+]$ is determined by the tissue pCO_2, the total weak acid concentration and the strong ion difference ([SID]) in any compartment[24,34,48]. The [SID] represents the sum of the strong base cations minus the sum of the strong acid anions. Important strong ions include sodium (Na^+), potassium (K^+), calcium, magnesium, chloride (Cl^-) and lactate. Therefore, membrane impermeability to H^+ could also mean that net changes in pCO_2, total weak acid, or the [SID], are such that no net change in $[H^+]$ occurs in the interstitial space despite considerable change of H^+ content in the intracellular space.

If plasma membranes remain intact during complete ischemia, the brain could be regarded as a 3-compartment system consisting of neurons, glia and interstitial space, each entirely bounded by plasma membranes. In mammalian brain, H^+ ions are likely to cross plasma membranes via some combination of exchange transport (antiport) systems for Na^+/H^+ and Cl^-/HCO_3^-. Electrically neutral Na^+/H^+ and Cl^-/HCO_3^- antiport systems help to regulate intracellular $[H^+]$ ($[H^+]_i$) in several different kinds of animal cells including mammalian astroglia[17,18,19, 20] and they may be important in H^+ regulation in mammalian neurons as well, The transmembrane Na^+ gradient sustained by a selective impermeability to Na^+ across plasma membranes plus the action of energy-requiring Na^+ pumps are thought to provide sufficient driving force to power both antiport systems[43,50]. Evidence suggests that in some cell types Cl^-/HCO_3^- antiport may be an active process that requires ATP[4]. Experimental data indicate that two equivalents of intracellular H^+ can be neutralized for each equivalent of Na^+ entering and Cl^- leaving a cell[43]. More than 20 years ago it was suggested that HCO_3^- secreted from brain cells buffered $[H^+]_o$ [49]. Interference with this mechanism has been invoked as a possible mechanism of tissue damage during brain ischemia[36].

Studies of ion transport mechanisms by Kimelberg and his colleagues indicate that astroglial cells provide a major source of HCO_3^- in brain[18] and may serve as important regulators of the organ's $[H^+]$ homeostasis[19,20]. We have extended their theoretical model[19] to include neurons (Fig. 5A) so as to postulate a mechanism (Fig. 5B and C) which may account for the observed relationship of $[H^+]_o$ to tissue lactate described in Fig. 2. Na^+/H^+ and Cl^-/HCO_3^- antiport systems for H^+ regulation have not been demonstrated in mammalian neurons[43]. However, their ubiquity in other animal cell types[43], including mammalian astroglia[17,18,19,20] and vertebrate neurons[6] suggests the likelihood of their presence in mammalian nerve cells as well.

Fig. 5A describes schematically a proposal for the movement of H^+ from brain cells to blood without specific restrictions to stoichiometry. The theoretical basis for the diagram is as follows: under normal physiologic conditions excess H^+ can leave neurons either by combining with intracellular HCO_3^- to form CO_2 and water or by countertransport to the interstitial space by Na^+/H^+ antiport (Fig. 5A). In the interstitial space, excess H^+ also combines with HCO_3^- to form CO_2 and water. Excess CO_2 which diffuses readily through cell compartments [12,16,28] quickly enters blood. CO_2 also diffuses into astroglial cells to be rapidly hydrated to carbonic acid by carbonic anhydrase[11,17,23,44,45]. The speed by which carbonic anhydrase hydrates aqueous CO_2 to carbonic acid and the subsequent ionization of that compound to H^+ and HCO_3^- establishes a concentration gradient of H^+ toward blood and HCO_3^- toward the interstitial space by Cl^-/HCO_3^- antiport so as to maintain a constant total brain HCO_3^- pool of about 12 mmol/kg at normal arterial pCO_2[38]. In anesthetized rats where arterial pCO_2 ranged from 59 to 67 mm Hg (Table I), predicted total brain HCO_3^- is about 14 mmol/kg[22,42] (Kraig, unpublished observations).

Fig. 5B describes the hypothetical events during normoglycemic ischemia. During complete ischemia it is proposed that brain cells have a diminished ability to remove excess H^+

because of a lowered transmembrane Na^+ gradient. Large amounts of NaCl leave the interstitial space[15,52] and are believed to enter dendrites[52]. Also neurons are likely to be depolarized in complete ischemia[13,14]. The constellation of a lowered transmembrane Na^+ gradient and loss of membrane polarization should minimize efflux of H^+ out of neurons via Na^+/H^+ antiport. Astroglia maintain a high degree of membrane diffusional impermeability to Na^+ (and Cl^-) when extracellular K^+ is elevated to 54 mM[54] in tissue culture and might also maintain an impermeability to Na^+ in vivo during complete ischemia when extracellular K^+ is known to rise to similar levels[15]. Since $[Na^+]_o$ falls to 48 mM in complete ischemia[15] while astroglial $[Na^+]$ is estimated to be around 20 mM under normal conditions[20], the Na^+ gradient across glial cell membranes may be too low to drive H^+_i out. If so, Cl^-/HCO_3^- antiport could be the sole mechanism remaining for brain cells to remove excess H^+ across intact plasma membranes during ischemia.

Changes in the carbonic acid buffer system of the brain could account for the observed relationship (Fig. 2) of $[H^+]_o$ to tissue lactate during complete ischemia. Since peak $[H^+]_o$ remained constant as tissue lactate climbed from 8 to 13 mmol/kg (Fig. 2) and brain pCO_2 rose in a linear manner[25], either $[HCO_3^-]_o$ increased proportionally or some new H^+ buffer became available to the interstitial space to keep $[H^+]_o$ constant. The abrupt rise in $[H^+]_o$ between 13 to 16 mmol/kg lactate (Fig. 2) reflects a change from net secretion to absorption of interstitial HCO_3^- into increasingly acidotic cells. Note that this change is equivalent to a decrease in $[SID]_o$ (see Eqn. 1 below). The driving force for glial secretion of HCO_3^- between 8 to 13 mmol/kg lactate might be residual transmembrane ion gradients while the driving force for cellular uptake of interstitial HCO_3^- above 13 mmol/kg lactate might reflect a steep drop of cellular HCO_3^- stores producing a strong concentration gradient from the interstitial space towards acid-producing brain cells. Note that interstitial HCO_3^- could enter either neurons or glia. However, it is unlikely that all HCO_3^- leaves the interstitial space because $[H^+]_o$ never goes below an average pH of 6.18. Why uptake of interstitial HCO_3^- should ultimately stop above ca. 16 mmol/kg lactate is unknown.

Fig. 5C describes the hypothetical acid–base events during complete hyperglycemic ischemia when lactate content exceeds ca. 16 mmol/kg (to 19 mmol/kg; see ref. [25]). If the decline in interstitial HCO_3^- stopped when the effective aqueous concentration of CO_2 approximated $[HCO_3^-]_o$, $[H^+]_o$ would reach a plateau near the first ionization constant of carbonic acid which is 7.352×10^{-7} eq/l at 37 °C and 6.18 pH[32] (Kraig, unpublished observations). This can be seen from Eqn. (1) [34]:

$$[H^+]_o = K'_1 \times \frac{S' pCO_2}{[SID]_o} \quad (1)$$

where K'_1 is the first ionization constant for carbonic acid corrected for $[H^+]$, temperature and osmolality[32]; S' is the solubility constant for CO_2 in cerebrospinal fluid corrected for temperature[32] (recall that $S'pCO_2$ = aqueous $[CO_2]$; and $[SID]_o$ is essentially equal to $[HCO_3^-]_o$ (refs. [24], [34], [48]), (See ref. [34] for details of derivation of Eqn. 1.)

As Fig, 2 shows, during hyperglycemia, when lactate rose to between 16 to 31 mmol/kg, $[H^+]_o$ reached a plateau of 6.607×10^{-7} eq/l, a value closely similar to that expected for the first ionization constant of carbonic acid, 7.352×10^{-7} eq/l. Under such circumstances $[H^+]_o$ during complete ischemia might never fall below a pH of about 6.18 despite high tissue lactate content because of a loss of HCO_3^- from acid producing brain cells and the consequent absence of transmembrane HCO_3^- flux or rise in tissue pCO_2 (Fig. 5C). If HCO_3^- stores are exhausted at this point in brain cells which continue to produce lactic acid, only the possible existence of other intracellular physicochemical H^+ buffers could protect

against development of excessive cellular acidosis followed by alterations of the charge state, configuration and, therefore, the function of vital cell proteins[41]. Infarction of the brain would be the expected result.

H^+ is a major product of metabolism and its homeostasis is of profound biological importance to all organisms. Maintenance of the structure and functional integrity of vital cell proteins may be the key reason that cells and organisms have evolved complicated mechanisms to maintain the homeostasis of $[H^+]$[41]. Throughout evolution carbonic acid, the histidine–imidazole complex and to a lesser extent, the N-terminal α-amino groups of proteins[31] seem to have arisen as the primary physicochemical H^+ buffering mechanisms of animal cells[7]. Pulmonary ventilation, renal excretion of H^+ and chemical regulation of the vascular resistance of brain arterioles are additional physiologic mechanisms to control brain $[H^+]$. Furthermore, Na^+/H^+ and Cl^-/HCO_3- antiport across plasma membranes are ion transport systems for the removal of excess cell H^+ which appear to have been conserved throughout the animal world[43] These exchange ion transport systems may have evolved to circumvent an impermeability of plasma membranes to H^+ during severe acidosis.

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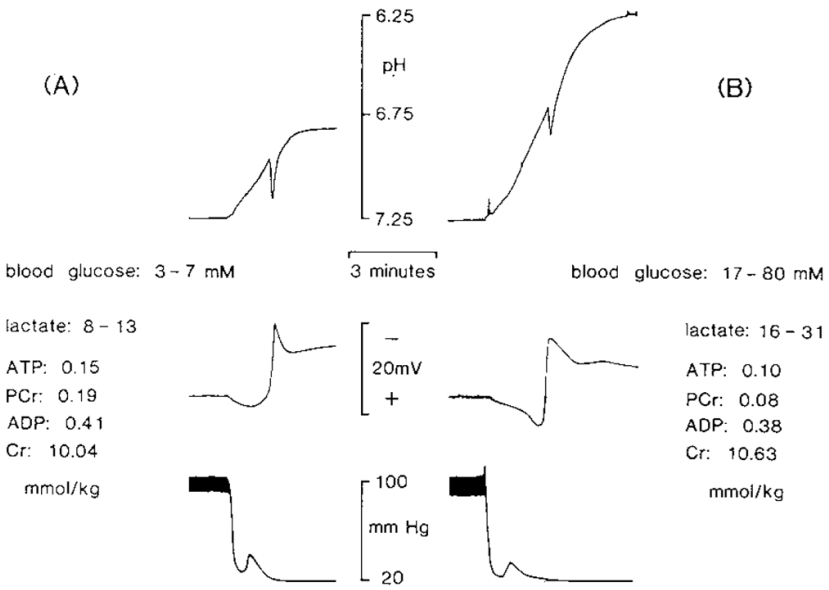

Fig. 1.

$[H^+]_o$ changes during complete ischemia. A typical recording of $[H^+]_o$ (upper trace) and simultaneous DC signal (middle trace) is shown from a double-barrelled H^+ ISM positioned 800 μm deep in the paramedian neocortex prior to cardiac arrest induced by intravenous KCl injection (bottom trace). Animals were pretreated with insulin or glucose to alter brain glucose and brain lactate production during ischemia. In the presence of low brain carbohydrate stores (A), lactate production rose to 8–13 mmol/kg during ischemia and $[H^+]_o$ changed by an average of 0.44 pH (99 nM). By contrast, in the presence of high brain carbohydrate stores (B), lactate rose to 16–31 mmol/kg associated with an average $[H^+]_o$ change of 1.07 pH (608 nM). High-energy phosphate reserves fell to similar levels in the above two experiments.

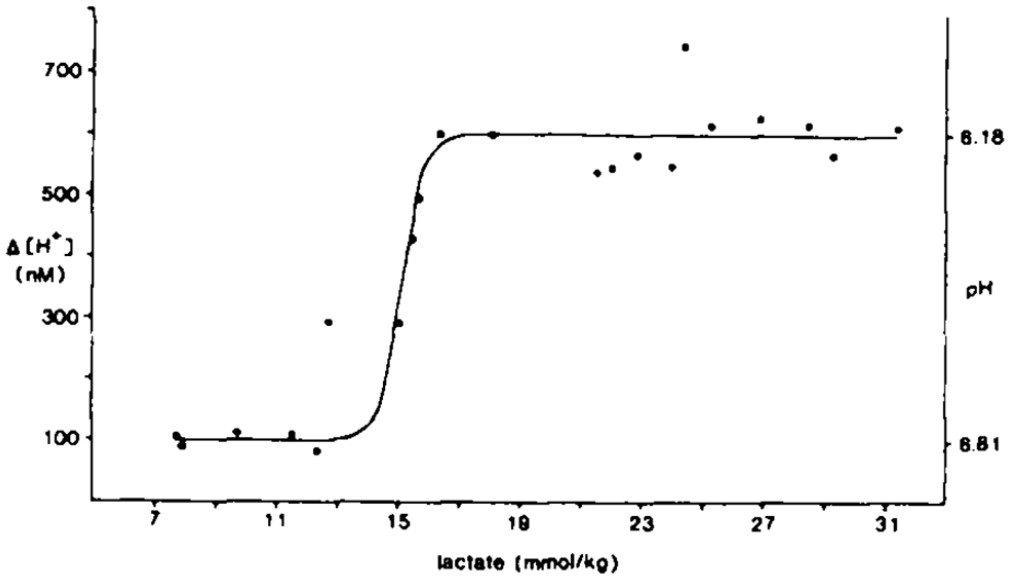

Fig. 2.

Comparison of peak changes in $[H^+]_o$ with lactate production during complete ischemia. Change in $[H^+]_o$ measured with an ISM is shown compared to lactate assessed by enzyme fluorometric techniques from tissue frozen in liquid nitrogen at the peak $[H^+]_o$ change (black dots). $[H^+]_o$ rose by 99 ± 6 nM (0.44 ± 0.02 pH; n = 5) until lactate reached 13 mmol/kg at which point $[H^+]_o$ abruptly rose 608 ± 16 nM (1.07 ± 0.02 pH; n = 12) by 16 mmol/kg lactate and remained constant through 31 mmol/kg lactate. $[H^+]_o$ values are listed as the change in $[H^+]_o$ from baseline (preischemia) to peak $[H^+]_o$ so as to discount interanimal baseline variability. To the right in the figure average pH values are shown which correspond to the measured $[H^+]_o$ changes compared to an average baseline pH of 7.25 (Table II).

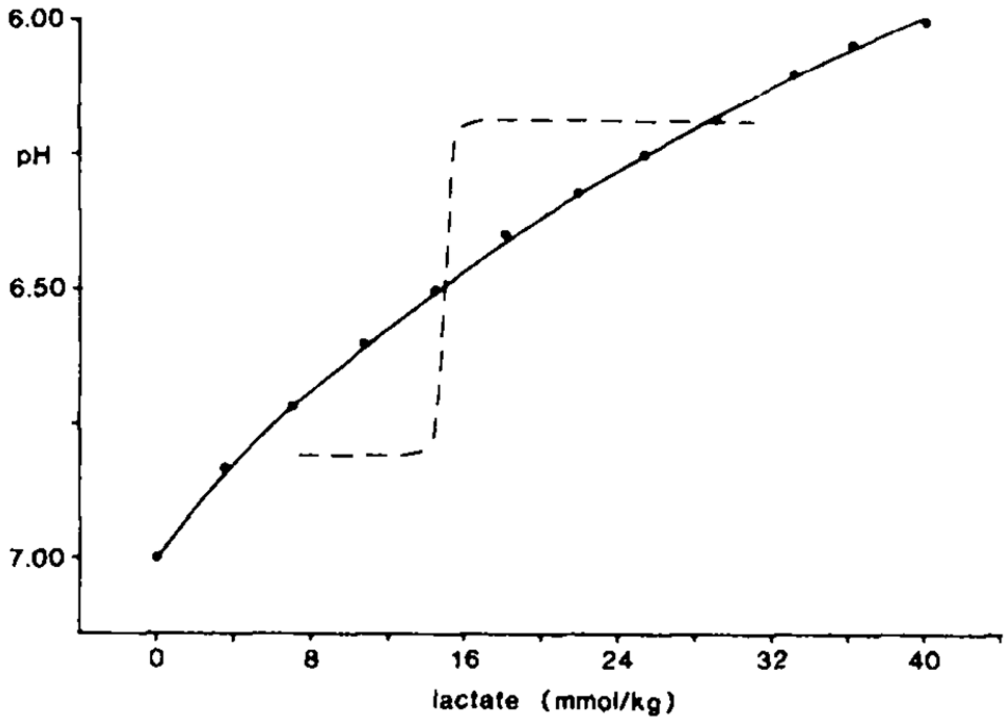

Fig. 3.

Titration of rat cerebral cortex homogenate. Solid line shows the change in pH produced when lactic acid (mmol/kg brain) was added to a homogenate of rat cerebral cortex in 100 mM sodium fluoride while under mineral oil. Note that $[H^+]$ rose steadily without an inflection. For comparison the data from Fig. 2 are superimposed here as a dotted line.

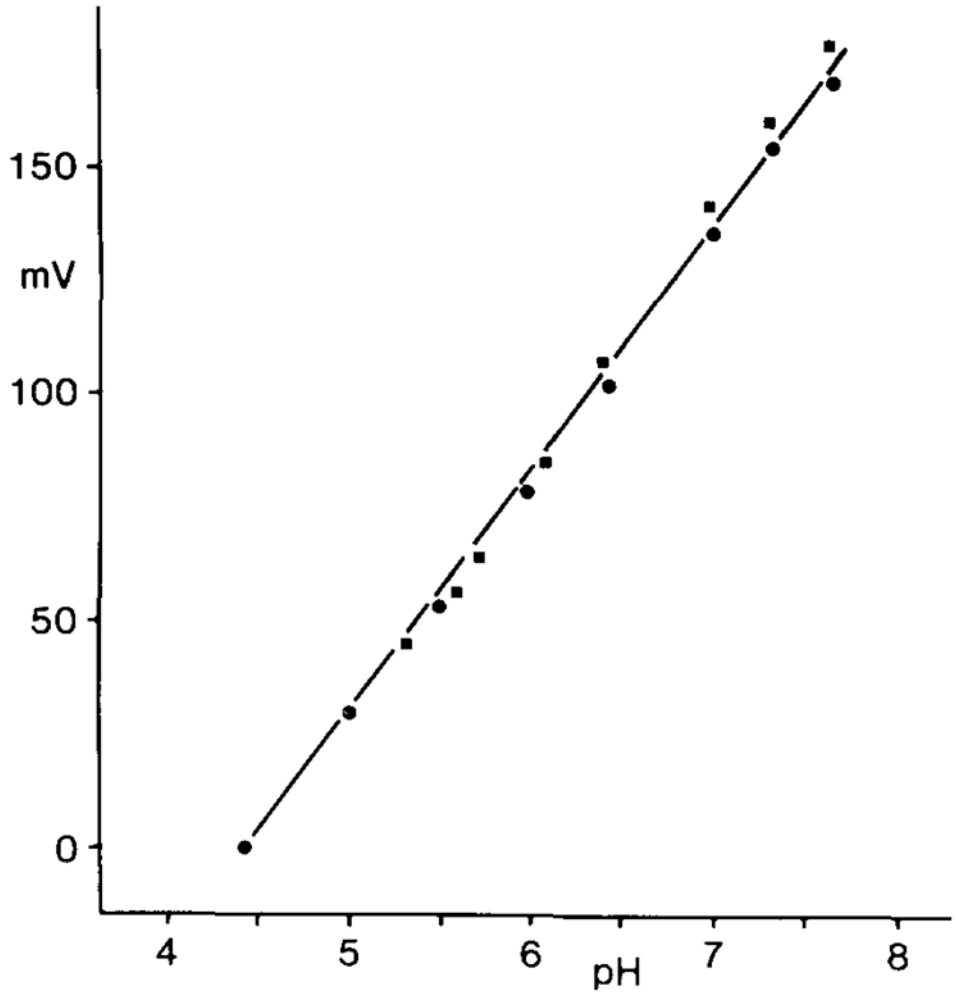

Fig. 4.

Response of an H^+ ISM to changes in $[H^+]$. Potassium hypophosphate solutions were buffered to different $[H^+]$ with sodium hydroxide (solid dots) to show the linearity (r = 0.999) and sensitivity (53 mV/decade change in $[H^+]$) of liquid membrane H^+ ISMs based on the neutral H^+ ionophore tridodecylamine. Potential electrode interference by brain macromolecules and their breakdown products during ischemia was assessed by adding aliquots of rat cerebral cortex homogenates to the original buffer solutions (solid squares). The addition of homogenates of cerebral cortex to the original buffer solutions did not degrade the linearity (r = 0.999) or sensitivity (57 mV/decade change in $[H^+]$) of the H^+ ISM.

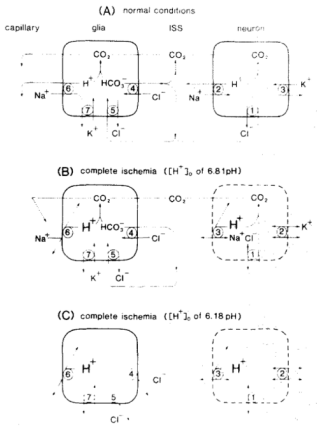

Fig. 5.

Schematic representations of possible plasma membrane antiport systems for the regulation of brain $[H^+]$. A: under normal conditions excess neuronal H^+ could be neutralized to CO_2 by HCO_3^- that entered through Cl_-/HCO_3^- antiport (1). CO_2 would rapidly move to blood because of its high diffusability through interstitial space (ISS) and cells. Alternatively, excess neuronal H^+ could be moved to ISS by Na^+/H^+ antiport (2) energized by the transmembrane Na^+ gradient created by Na^+/K^+ ATPase (3). Astroglia could manage excess H^+ by the same antiport systems though two additional features are required (adapted from ref. [19]). First. Na^+/H^+ (6) and Cl^-/HCO_3^- (4) antiport systems would be on opposite sides of the cell so as to create a directional flux of H^+ towards blood. Second. Cl^-/HCO_3^- antiport would be directed as needed to provide HCO_3^- to ISS and neurons (1) or to buffer excess intraglial H^+ (5). Since astroglia are likely to contain carbonic anhydrase, CO_2 would be most readily hydrated in glia, thus helping to create a gradient of H^+ toward blood (6) and HCO_3^- toward ISS and neurons (4). Na^+/K^+ ATPase (7) would return cellular Na^+ to ISS and ISS K^+ to astroglia. B: during ischemia $[Na^+]_o$ and $[Cl^-]_o$ fall by 106 mM and 57 nM, respectively (see ref. [51]). Without a driving force for Na^+/H^+ antiport (2 and 6), excess H^+ which could not passively leak from cells could be neutralized by Cl^-/HCO_3^- antiport (1 and 5) to maintain pH_o at 6.81. Once the total brain pool of HCO_3^- had been reduced so that Cl^-/HCO_3^- antiport would no longer be feasible, excess H^+ would be confined to acid-producing brain cells. C: during ischemia with lactate accumulation beyond 16 mmol/kg and no further change in $[HCO_3^-]_o$ only intracellular buffers could then limit further accumulation of H^+_i. If $[H^+]_i$ rose sufficiently to alter the charge state, and therefore the conformation, of vital cell proteins, membrane and enzyme dysfunction might disable or kill brain cells. Stoichiometry for the proposed antiport systems is not specified.

TABLE I

Blood physiologic variables

	Controls (n = 2)	Preischemia	
		Blood glucose 3–7 mM (n = 5)	Blood glucose 17–80 mM (n = 14]
Rectal temperature (°C)	37 ± 0.5	37 ± 0.5	37 ± 0.5
pH	7.31 ± 0.02	7.30 ± 0.02	7.23 ±0.03
pO_2(mm Hg)	110 ± 3	104 ± 2	122 ± 7
pCO_2 (mm Hg)	59 ± 3	59 ± 5	67 ± 7
Systolic pressure (mm Hg)	103 ± 0	119 ± 1	109 ± 3

Values are mean ± S.E.M.

TABLE II

Brain physiologic variables

	Preischemia	Ischemia	
		Blood glucose 3 - 7 rnM	Blood glucose 17–80 rn M
pH_o	7.25 ± 0.02 (n=21)*	6.81 ± 0.02 (n=5)	6.18 ± 0.02 (n=12)
$[H^+]_o$ (nM)	56 ± 2 (n = 21)*	155 ± 8 (n = 5)	664 ± 20 (n = 12)
ΔpH_o		0.44 ± 0.02 (n = 5)	1.07 ± 0.02 (n = 12)
Δ $[H^+]_o$ (nM)		99 ± 6 (n=5)	608 ± 16 (n= 12)
lactate (mmol/kg)	1.04 ± 0.03 (n = 5)	7.80 – 12.80 (n = 5)	16.30 – 31.30 (n = 12)
ATP (mmol/kg)	3.41 ± 0.13 (n=5)	0.09 ± 0.01 (n=5)	0.14 ± 0.01 (n=14)
ADP (mmol/kg)	0.39 ± 0.04 (n = 5)	0.37 ± 0.00 (n = 5)	0.41 ± 0.01 (n = 8)
PCr (mmol/kg)	4.92 ± 0.06 (n=5)	0.09 ± 0.01 (n=5)	0.11 ± 0.01 (n=14)
Cr (mmol/kg)	5.06 ± 0.15 (n = 5)	10.34 ± 0.36 (n = 5)	10.45 ± 0.17 (n = 8)

* Represents preischemic brain $[H^+]_O$

Values are mean ± S.E.M.