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Dynamics of interstitial and intracellular pH in evolving brain infarct

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Abstract

We examined the relationships between intracellular pH (pH_i) and interstitial pH (pH_e) in a rat model of focal ischemia. Interstitial pH was measured with pH-sensitive microelectrodes, and the average tissue pH was measured with the [^{14}C]dimethadione method in rats subjected to occlusion of the right middle cerebral and common carotid arteries (MCA-CCAO). In normal cortex, pH_e and pH_i were 7.24 ± 0.07 and 7.01 ± 0.13 (means $\pm$ SD, $n = 6$), respectively. In the ischemic cortex, pH_e fell to 6.43 ± 0.13 , whereas pH_i decreased only to 6.86 ± 0.11 ($n = 5$) 1 h after MCA-CCAO. After 4 h of ischemia, the pH_e was 6.61 ± 0.09 and pH_i was 6.62 ± 0.20 ($n = 4$). Treatment with glucose before ischemia markedly lowered the pH_e (5.88 ± 0.17) but not pH_i (6.83 ± 0.03 , $n = 4$) measured 1 h after ischemia. In the ischemic cortex of animals made hypoglycemic by pretreatment with insulin, neither pH_e (7.25 ± 0.06) nor pH_i (6.99 ± 0.13 , $n = 4$) decreased. The demonstrated difference in pH_i and pH_e indicates that some cells remained sufficiently functional to maintain a plasma membrane gradient of protons within the evolving infarct. If the calculated pH_i values accurately reflect the true pH_i of cells within zones of severe focal ischemia, then cerebral infarction can proceed at pH_i levels not greatly altered from normal.

Keywords

ischemia; lactic acidosis; neuronal cell death; microelectrodes; direct current potential; interstitial space

Small changes in the intracellular pH (pH_i) may greatly affect flux through ion channels, membrane excitability, and cellular metabolism (5,8). Therefore, pH_i is tightly regulated in normal brain at ~ 7.04 , whereas interstitial pH (pH_e) normally is ~ 7.3 (29). The accumulation of lactic acid in ischemic brain may greatly disturb the normal H^+ homeostasis, and it has been suggested that excessive lactic acidosis facilitates ischemic mechanisms leading to cerebral infarction (26).

In animal models of severe global forebrain ischemia where blood flow is reduced to $<90\%$ of normal values, the accumulation of lactate in brain is proportional to the tissues stores of glycogen and glucose (27,31). Microelectrode measurements indicate that pH_e falls during global ischemia from the normal 7.3–6.8 in animals that are normoglycemic and to as low as

6.2 in hyperglycemic animals subjected to an identical ischemic insult (11,19). Because energy-dependent ion pumping is lost during severe ischemia, protons have traditionally been considered to distribute equally between intra- and extracellular water (31). Kraig et al. (19) proposed, however, that the glial cell compartment might acidify more intensively than the remaining brain tissue during severe ischemia in hyperglycemic animals. In support of this hypothesis, astrocytic pH_i was shown to decline considerably below pH_e during such conditions (18).

In contrast to the severe depression of blood flow in global ischemia, considerable perfusion remains in areas of focal ischemia where a cerebral infarct is evolving (3,24). Focal ischemia, unlike global ischemia, is less of a closed system, and protons plus lactate generated in the tissue may be washed out by the remaining blood flow. In addition continued interactions between CO_2 and the buffer ion HCO_3^- are more likely to transpire. Accordingly, the distribution and equilibrium concentrations of H^+ among extracellular and various intracellular compartments during focal ischemia may be even more complex than in global ischemia.

In this study, the pH_e was measured directly by microelectrodes and the average pH_i was calculated from the distribution of the weak acid dimethadione (DMO) in rats subjected to focal neocortical ischemia. The rat model of focal ischemia used in this study has been well characterized (3), and cerebral infarction evolves to completion in the core ischemic zone over a period of 1–3 h (13). During the first hour of focal ischemia, the average pH_i in the ischemic core was only mildly decreased and was consistently higher than pH_e in the ischemic core as well as in the surrounding border zones of less severe ischemia. The results suggest that pH_i regulation is at least partially preserved in some cells during the early stages of cerebral infarction and that such injury may proceed in tissue with pH_i values less severely disturbed than in brain injury associated with hyperglycemia and global ischemia.

MATERIALS AND METHODS

Surgical preparations

Male spontaneous hypertensive rats (Taconic Breeding Laboratory), weighing 250–270 g, were fasted overnight but allowed free access to water. Some animals were made hyperglycemic by administering 50% glucose (1.5 ml ip) 0.5 h before surgery. Hypoglycemia was achieved in other animals by injecting insulin (2 international units/kg sc) 2–3 h before surgery. The animals were anesthetized with halothane (5%) and, after placement of an endotracheal tube, they were mechanically ventilated with a rodent respirator using a 30% oxygen-70% nitrogen mixture and halothane anesthesia (3% during surgery, 1.25% during electrophysiological and pH measurements). Catheters were inserted into a femoral vein and artery, and suxamethonium (75 mg/kg) was given. The animals were placed in a stereotaxic headholder, which was fitted with a water jacket to maintain rectal temperature at 37°C. Focal ischemia of the right neocortex was produced by occluding first the right common carotid artery (CCA) and then the right middle cerebral artery (MCA) distal to the rhinal fissure (3). During the experiment, mean arterial blood pressure was monitored continuously (Beckman R511 polygraph), whereas arterial pH, PCO_2 , PO_2 (Corning 158 pH/blood gas analyzer), and glucose (Beckman glucose analyzer) were measured every 20 min. Brain temperature was measured in selected animals and remained within a range of 36.5–37.2°C in both ischemic and nonischemic brain tissue.

Measurement of pH_e

Double-barreled pH electrodes were constructed using the H^+ ionophore tridodecylamine (1) as previously described (19). The electrodes were calibrated at the beginning and end of

each experiment in 50 mM phosphate buffer (pH 6.0, 7.0, 7.4) in a cylinder glued to neck muscles such that the phosphate buffer was in electrical continuity with the animal. Such electrodes responded linearly between pH 4.5 and 7.6 (19); however, we did not calibrate the electrodes in these experiments below pH 6.0 and can therefore not exclude deviations from actual pH in measurements between pH 5.5 and 6.0. A craniotomy was made over the right parietal cortex starting at bregma and extending 5–6 mm laterally. A second 2-mm-diameter craniotomy was made over the left hemisphere, 4 mm lateral to the bregma. Dura was carefully removed, and both craniotomies were covered by mineral oil. The microelectrode was lowered 400 μm into the interstitial space of frontoparietal neocortex using a MM-3 micromanipulator (Narishige Instruments, Tokyo). pH_e was measured repeatedly at loci 1, 2, 3, 4, and 5 mm lateral from midline in the right hemisphere and 4 mm lateral from the midline in the nonischemic left hemisphere (Fig. 1). Mean pH_e values from each loci were recorded. In selected animals, the electrode position was verified by injection of 0.5 μl saturated Alcian blue from the reference barrel of the electrode immediately before decapitation.

Measurement of pH_i

[^{14}C]DMO (New England Nuclear, 55 $\mu\text{Ci}/\text{mmol}$) was analyzed for radiochemical purity by paper chromatography using a hexane-methanolacetic acid carrier. Radiochemical purity was always >98%. Autoradiographic experiments were carried out by intravenous injection of 50 μCi [^{14}C]DMO either 15 min (1-h group) or 3 h (4-h group) after MCA-CCA occlusion. The animals were decapitated 1 h after tracer injection. Final plasma [^{14}C]DMO concentrations were measured using a Searle Mark III liquid scintillation counter. Coronal brain sections (20- μm thick) were cut serially at -25°C , mounted on glass cover slips, and exposed to Kodak SB-5 film for 5 days.

The optical densities of the autoradiograms were measured at the same loci where the pH_e values were recorded 1–5 mm lateral to bregma in the right hemisphere and 4 mm laterally in the left hemisphere. A Quantimet 970 image analyzer was used to determine the optical density, using [^{14}C]methyl methacrylate standards (New England Nuclear) on the same film.

The average tissue pH (pH_i) was calculated as described by Waddel and Butler (33), where it is assumed that, at equilibrium, the uncharged form of DMO (HDMO) is distributed equally in all compartments, whereas the concentration of the impermeable charged form of DMO (DMO^-) is determined by the Henderson-Hasselbach equation

$$\text{pH} = \text{pK}_a + \log (\text{DMO}^- / \text{HDMO}) \quad (1)$$

where pK_a is the apparent ionization constant of DMO (6.13).

The average pH_i was calculated as described by Arnold et al. (2)

$$\text{pH}_i = \text{pK}_a + \log \left\{ \left[\frac{C_t}{C_e} \times \left(1 + \frac{V_e}{V_i} \right) - \frac{V_e}{V_i} \right] \times (10^{\text{pH}_e - \text{pH}_a} + 1) - 1 \right\} \quad (2)$$

In the operational equation for calculation of the average pH_i , pK_a is the ionization constant of DMO, C_t is the concentration of DMO in the tissue water, C_e is the concentration of DMO in the extracellular space, V_e and V_i are the volumes of extracellular and intracellular space, respectively, and pH_e is the pH of the interstitial fluid. C_t was obtained from measurements of autoradiographic image density and translated into ^{14}C radioactivity concentration with knowledge of the brain water and plasma water content. Brain water

content was measured (see below) in normal and ischemic neocortex at 1 and 4 h, and the plasma water was assumed to be 93%, C_e can be calculated from Eq. 3, where C_p is the concentration of DMO in the plasma measured by scintillation counting; the ratio of V_e to V_i was determined by direct measurements of the sucrose distribution space (see below); pH_e was measured with extracellular H^+ -sensitive microelectrodes; pH_p is the H^+ concentration of plasma measured on a Corning 158 blood gas analyzer

$$C_e = \frac{C_p (10^{pH_e - pK_a} + 1)}{10^{pH_p - pK_a} + 1} \quad (3)$$

Brain tissue water content

Animals were prepared as described above and decapitated either 75 min or 4 h after MCA-CCA occlusion. The brains were frozen in Freon-12 cooled over dry ice. Tissue samples were taken from neocortex 1.5 and 4.5 mm laterally from bregma in a cryostat at -25°C using a 1.5-mm hollow tissue punch. Each tissue sample (weight $\sim 3\text{--}4$ mg) was placed directly onto a preweighed aluminium tray and weighed within the cryostat (Cahn electrobalance 26) for the determination of wet tissue weight (W). Dry tissue weight (D) was determined for each sample after removal from the cryostat and dessication in an oven at 100°C for 24 h. All measurements were recorded to nearest 0.01 mg. Tissue water content, expressed as a percentage of wet tissue weight, was calculated as $(W - D)/W \times 100$.

Measurement of interstitial volume

Two methods were used to measure interstitial volume in neocortex during focal ischemia. Changes within the first minutes of MCA-CCAO were detected with the interstitial space marker, tetramethylammonium chloride (TMA), as described by Nicholson and Phillips (25). TMA-sensitive electrodes (25) were filled with K^+ exchanger (Corning 477317), and the ion-selective barrel was backfilled with 150 mM TMA. The electrodes were allowed to equilibrate with 150 mM TMA for at least 4 h before the experiment. The electrodes were calibrated with 150 mM NaCl, in which 3.3, 12.5, and 50 mM Na^+ was replaced by TMA^+ in a plastic cup glued to the neck muscles; thus the plastic cup was in electrical continuity with the animal. The resultant electrodes were essentially insensitive to K^+ (slope < 3 mV for a 10-fold change in $[K^+]$).

A 4-mm opening was drilled through the skull, with the center positioned 5–6 mm to right of bregma. The dura was removed, and a plastic cylinder with an inner diameter of 15 mm was placed over the craniotomy site. Leaks between cup and skull were filled with petroleum jelly. A mixture of agar (1.5%) and artificial cerebrospinal fluid (CSF) (see below) containing 50 mM TMA was then heated to 95°C . As the temperature fell below 39°C , the stirred mixture was poured slowly into the cup over the craniotomy site. Direct contact between the agar and underlying neocortex allowed diffusion of TMA into neocortex. After 30 min of equilibration, the agar was removed and the TMA electrode was lowered $400\text{ }\mu\text{m}$ into the frontoparietal cortex using a MM-3 micromanipulator (Narishige Instruments, Tokyo). Baseline measurements of the extracellular TMA concentration were made for a period of 5–10 min. The ipsilateral MCA-CCA was then occluded, and the TMA concentration was measured for an additional period of 5 min. The artificial CSF solution consisted of 143 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$, 0.7 mM $MgCl_2$, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, and 50 mM TMA titrated to a pH 7.3.

Measurements of the distribution space of $[^{14}\text{C}]$ sucrose were used to determine the interstitial volume during prolonged focal ischemia. A 4-mm craniotomy hole was drilled 5–6 mm lateral to bregma over the right or left hemispheres. The dura was carefully removed,

and a 15-mm diameter plastic cylinder was sealed to the cranium with petroleum jelly. A mixture of agar (1.5%) and artificial cerebrospinal fluid (see above) was prepared at 95°C. In this case, as the temperature of the solution fell, 100 $\mu\text{Ci/ml}$ of [^{14}C]sucrose (New England Nuclear, 540 mCi/mmol) were added during constant stirring. When the mixture reached 39°C, it was poured slowly into the cups overlying the craniotomy sites. After 1 h of exposure to the [^{14}C]sucrose, the agar cups were removed from the cortical surface, the animals were decapitated, and their brains and the agar cups frozen in Freon-12 cooled over dry ice. Serial sections (20- μm thick) of the frozen brains and the [^{14}C]sucrose-agar mixtures were then cut in a cryostat at -25°C and exposed to Kodak SB-5 films for 5 days together with ^{14}C standards.

A graphical procedure, described by Rail et al. (28), was used to estimate the tissue equilibrium concentration of [^{14}C]sucrose. Because sucrose has a high diffusion coefficient in water ($2.1 \times 10^{-6} \text{ cm}^2/\text{s}$), the concentrations of sucrose in agar and in the interstitial fluid at the surface of the cortex (C_o) should be equal (21). An estimate of C_o can be made by plotting the concentration of sucrose at several distances from the cortical surface and extrapolating to the ordinate intercept. The sucrose distribution space (SDS) is then determined as

$$\text{SDS} = (C_o / C_{\text{agar}}) \times 100$$

The sucrose concentration at various distances from the cortical-agar interface were measured densitometrically (Quantinet 970 image analyzer) from the ^{14}C autoradiographs.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare mean $\text{pH}_e - \text{pH}_i$ differences across the four groups at each of the six loci. The Newman-Keuls multiple-comparison test was used to determine which groups significantly differed from one another. ANOVA was also used to compare mean physiological variables across the groups, and Tukey's multiple range test was used to determine which groups significantly differed from one another. Differences in the mean values for brain water content between ischemic and nonischemic hemisphere were compared with the paired Student's *t* test.

RESULTS

Physiological variables

Table 1 presents the mean arterial blood pressure, arterial P_{O_2} , P_{CO_2} , pH, and blood glucose concentrations for all experimental animals during the measurements of pH_e , pH_i , and the extracellular volume. Except for plasma glucose concentrations, the blood physiological values differed little between the groups studied. Plasma glucose was 300 ± 73 and 51 ± 2 mg/dl in the animals made hyperglycemic and hypoglycemic, respectively. All other groups had plasma glucose concentrations in the range of 120–150 mg/dl.

Water content

The average water content of the evolving infarcts 1 and 4 h after MCA-CCA occlusion was ~1–2% higher than that of the contralateral control hemispheres (Table 2). However, this difference only achieved statistical significance ($P < 0.01$) for the 4-h animals at *loci* 1.

TMA measurements

The calibration curves for TMA electrodes showed an increase in potential of 59 ± 6 mV for a 10-fold change in concentration. A typical ischemic depolarization identified as a steep negative deflection of the direct current potential (19 ± 1 mV, $n = 4$) was recorded 1–3 min after MCA-CCA occlusion. The simultaneous increase of TMA potential was 23 ± 6 mV corresponding to an increase of TMA concentration to a level $120 \pm 41\%$ (mean $\pm$ SD, $n = 4$; range 73–150%) above the preischemic level (Fig. 2A). The TMA concentration after the initial ischemia-related rise showed a gradual decline of ~ 1 mV/min compared with the preischemic baseline. Although the TMA concentration was not measured for more than 5 min, the rate of decline suggested that the TMA concentration would return to preischemic values in ~ 20 min. In one control animal, TMA was not applied to the cortical surface, and in this case, the TMA potential did not change during the ischemic depolarization (Fig. 2B). The average increase in TMA concentration during ischemic depolarization represents an $\sim 60\%$ decrease in the volume of the interstitial space.

Sucrose distribution volume

The distribution volume of [^{14}C]sucrose in both the ischemic and nonischemic neocortex was in the range of 15–18% at 1 and 4 h after MCA-CCA occlusion (Table 3).

Direct current potential

The direct current (DC) potentials measured at *loci* 1 and 2 were stable at -5 to -8 mV and those at *loci* 4 and 5 were stable at -20 to -25 mV. There was no obvious correlation between the DC potential and pH_e (data not shown). Recurrent depolarizations similar to spreading depression-like waves were often detected at *loci* 1 or 2 (Fig. 3). There was an initial alkalinization during the course of these spreading depression-like signals followed by a pH_e fall to ~ 6.7 . In hypoglycemic animals, only the initial alkalinization was recorded during the course of the recurrent depolarizations (Fig. 3). Spreading depression-like signals were never detected in hyperglycemic animals.

Interstitial pH

All pH_e electrodes used had a slope >50 mV for a 10-fold increase in the concentration of protons. In the nonischemic neocortex, the mean pH_e ranged between 7.2 and 7.3 in the four groups (Table 4). pH_e at *loci* 1 differed little from nonischemic tissue. Extracellular acidity tended to increase towards the ischemic core, and occasional pockets of high acidity (6.1–6.2) were found. In three animals, pH_e was measured continuously for 2–4 h at the same spot and was stable during the recording. Repeated measurements of pH_e at the same point varied only 0.1 to 0.2 pH units during the 1- and 4-h recording times. In the ischemic core (*loci* 3–5), mean pH_e was between 6.43 and 6.64 at 1 and 4 h after MCA-CCA occlusion in normoglycemic animals but decreased to as low as 5.5 (mean 5.88) in the ischemic core of hyperglycemic animals. In contrast, pH_e changed little from control values in the ischemic core of hypoglycemic animals (Table 4).

Average intracellular pH

The distribution of [^{14}C]-DMO was homogeneous throughout the nonischemic hemisphere (Fig. 4) and yielded a range of mean pH_i of 7.07–7.11. The calculated pH_i in the nonischemic neocortex was between 7.01 and 7.03 in all groups. In the ischemic core (*loci* 3–5), the calculated pH_i was 6.76–6.86 after 1 h in normoglycemic animals but fell to 6.62–6.71 after 4 h. The transition from tissue with normal pH_i to tissue with decreased pH_i occurred between *points* 1 and 3 in all animals, with a sharp transition in 50% of the animals after 1 h and in all animals after 4 h (Fig. 4). The transition was extremely sharp in all hyperglycemic animals at 1 h (Fig. 4). However, the calculated pH_i was still only decreased

to 6.83 ± 0.03 . Surprisingly, the DMO autoradiograms from hypoglycemic rats showed a homogeneous isotope distribution (Fig. 4) and no decline of pH_i in the ischemic core (Table 4).

The usual relationship of higher intracellular vs. extracellular acidity in normal brain tissue was reversed within the ischemic zone of normoglycemic and hyperglycemic animals at 1 h. The usual relationship of pH_i to pH_e was little altered (Fig. 5) in the ischemic cortex of hypoglycemic rats.

DISCUSSION

In this study, pH_i and pH_e were measured simultaneously in rats undergoing focal ischemia and infarction. In contrast to the more acidic pH_i vs. pH_e in nonischemic tissue, the average calculated pH_i was found to be 0.4 pH units higher than pH_e after 1 h ischemia in normoglycemic animals. This difference was even more pronounced in hyperglycemic animals, in which pH_i was 1.0 pH units higher than pH_e . pH_i and pH_e remained relatively unaltered in the ischemic core of hypoglycemic rats. After 4 h of focal ischemia, pH_i and pH_e had nearly equalized, indicating the failure of pH homeostatic mechanisms that regulate intracellular pH in an acidic environment. Before considering the implications of these observations, it is important to consider the validity and assumptions of the DMO technique for pH_i determination in ischemic tissue.

DMO is a weak acid with a pK_R of 6.13 (2). The weak acid technique for measuring pH_i is based on the assumptions that the undissociated species, HDMO, equilibrates freely across membranes but that the anion, DMO^- , does not, that at equilibrium the concentration of HDMO is equal in all compartments, and finally that the ratio of the ionized form of DMO to the protonated form can be calculated with the Henderson-Hasselbach equation (33). In fact, HDMO has a plasma membrane permeability of 1.9×10^{-4} cm/s, whereas DMO^- has a permeability coefficient of only 1.5×10^{-7} cm/s (14).

Hakim et al. (9) demonstrated that a steady-state distribution of DMO is reached within 10 min in both normal and ischemic human brain tissue and within 60 min in normal rat brain (15). DMO has been widely used autoradiographically to study pH_i in experimental stroke (15,30), and the results of these studies are similar to our observations. Measurements of pH_i in barnacle muscle using the DMO technique were in excellent agreement with values obtained using pH-sensitive microelectrodes (4). Nonetheless, the application of the DMO technique to ischemic brain tissue has not been cross validated using other measures of pH_i .

If the permeability coefficient of the charged form, DMO^- , were to increase during ischemia, the DMO method would become invalid. Existence of facilitated transport of the charged form of DMO^- is highly unlikely in view of its extremely low permeability (1.5×10^{-7} cm/s in normal tissue). Furthermore, electrical impedance of the astrocytic plasma membrane remains relatively intact during early ischemia (18). Evidence for the behavior of neuronal membrane impedance during ischemia is lacking. Nevertheless, some resistance to the movement of ions across neuronal membranes is likely since impedance in these cells does not fall to zero during ischemia (18). Thus formation of a pore in the plasma membrane rendering ischemic cells permeable to DMO^- seems unlikely. Last, carrier-mediated transport of the neutral HDMO, if it existed, would not affect steady-state distribution of the tracer and thereby not change the pH measurement.

Our measurements of pH_i in hypoglycemic rats subjected to focal ischemia also supports the accuracy of the DMO technique under ischemic conditions. Hypoglycemic animals maintained normal pH_i and near normal pH_j in fully depolarized tissue of the ischemic core. If factors in the evolving infarct, other than pH, tended to alter DMO concentration in

ischemic tissue, one would expect an increased accumulation of DMO in the ischemic core of hypoglycemic animals relative to control. In fact, DMO content remained either normal or was slightly lower than in the control hemisphere. Thus, we believe that the DMO technique provides a reliable measure of average pH_i during focal ischemia.

Conflicting results were obtained using the concentration changes of TMA and the distribution space of [^{14}C]sucrose to estimate the interstitial volume. TMA concentration increased ~120% during the ischemic depolarization, indicating an acute shrinkage of the interstitial volume to ~40% of its initial volume. The acute contraction of the interstitial space is consistent with other reports using TMA measurements (11) during ischemia. In contrast, measurements of the sucrose distribution space at 1 and 4 h indicated little or no change in the interstitial volume. Interstitial volume has, to our knowledge, not previously been measured during prolonged brain ischemia. Results from the present study suggest that the interstitial space initially shrinks to 40% of normal but then returns to and remains normal for up to 4 h of ischemia. The fall of the TMA concentration, which followed the rapid increase, might reflect increased cellular permeability to TMA (11) or, alternatively, that the interstitial space after the acute shrinkage slowly returned towards the normal volume. The latter explanation is favored by the observation that the sucrose distribution space in the ischemic core after 1 and 4 h of MCA-CCA occlusion did not differ significantly from the control hemisphere. The interstitial volumes measured at 1 and 4 h were used in the calculation of pH_i at these times. If a 60% smaller volume was used in loci with acidic interstitial space, it would change the calculated pH_i value by <0.1 pH unit.

The magnitude of the fall in pH_i during focal ischemia will depend on the rate and magnitude of acid production, its washout from the tissue, and on the buffering capacity of the tissue. Several reactions generate H^+ in ischemia, but all are of minor importance in comparison with the production of lactic acid (10,20). The lactate concentration rises to $8.8 \pm 1.1 \mu\text{mol/g}$ in the ischemic core of this model during 4 h of MCA-CCA occlusion (12). In view of this relatively small increase in brain lactate and the continued availability of $\text{HCO}_3^- - \text{CO}_2$ buffering from the blood it is not surprising that the average pH_i of normoglycemic animals decreased only a few tenths pH units.

Neocortical pH_i values in the range of 6.2–6.9 have been found in the ischemic core of normoglycemic rats subjected to MCA occlusion and DMO autoradiography (15,30). Thus the observations in the present series of experiments are consistent with and confirm earlier reports. Importantly, Nakai et al. (23) found that after 3 h of ischemia tissue pH had fallen to 6.41–6.60 in ischemic cortex of hyperglycemic animals but only to 6.87 in normoglycemic animals. Application of other techniques for measuring average pH_i in focal stroke, including neutral red (17) and umbelliferone (6,22), revealed pH changes down to 5.8–6.0. These values are considerably below those reported using DMO, and we have no adequate explanation for the differences in results. With the use of nuclear magnetic resonance spectroscopy, Thulborn and Crockard (32) reported pH_i values of 6.5–6.6 in gerbils subjected to unilateral CCA occlusion, whereas Komatsumoto et al. (16) found in nonfasted MCA-occluded cats an average pH_i decline to 6.2–6.3.

Controversies exist with regard to the proton distribution in brain during ischemia. Traditionally, ischemia is believed to upset pH regulation, with the excess of protons generated by the ischemic tissue being distributed diffusely and homogeneously throughout all brain water (31). However, under conditions of hyperglycemia and complete or total ischemia, Kraig et al. (19) suggested that brain possessed cellular compartments with pH_i levels 1–2 units lower than the ambient interstitial pH. Intracellular horseradish peroxidase injections into such highly acid regions suggested that this compartment was comprised of astrocytes (18).

In contrast to the conditions of global brain ischemia where blood flow is nearly or completely absent, considerable circulation remains in regions subjected to occlusion of single cerebral vessels. For example, in the MCA-CCA occlusion model used in this study, cerebral blood flow is reduced to ~25% of control values in the center of the ischemic core (3). In focal ischemia, the continuous delivery of substrate and glycolytic energy production makes it possible that some cells may continue to regulate pH_i and further that excess CO_2 may escape into the circulation. Thus, the divergent pathophysiological processes of focal vs. global ischemia make it likely that distinct pH changes occur with these two conditions. The importance of the present observations is that the simultaneous determination of pH_i and pH_e consistently showed that pH_i remains relatively normal during the first hour of ischemia, whereas pH_e decreased by ~0.4 pH unit in normoglycemic and by as much as ~1.0 pH units in hyperglycemic animals. Interestingly, Fievet et al. (7) demonstrated a similar uncoupling of pH_i from pH_e in trout erythrocytes at the onset of hypoxia. Hypoxia led to an extracellular acidosis of 0.6–0.8 pH units, whereas pH_i remained virtually constant throughout the period of hypoxia. Thus hypoxic erythrocytes responded in a qualitative similar manner as brain cells during focal ischemia, i.e., pH_i remained near normal concomitant with the development of excessive extracellular acidosis. A reasonable hypothesis is that the oxygen deprivation in both cases accelerates anaerobic glycolysis which provides sufficient energy to maintain H^+ homeostasis. Equilibration of pH_i and pH_e after 4 h of ischemia suggest damage to the regulatory system and is consistent with the observations that 3–4 h of focal ischemia in this model results in a near maximal infarct (13).

We conclude that some population of cells in the evolving brain infarct is able to maintain H^+ homeostasis despite marked interstitial acidosis. The distribution of protons in various compartments during focal ischemia is likely to be more complex than in global ischemia. Finally, if our observations accurately reflect the true average pH_i within zones of focal ischemia, then the process of cerebral infarction can proceed at pH_i values not greatly different from normal.

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FIG. 1.

Coronal section Of frozen rat brain stained with hematoxylin-eosin. Right middle cerebral and common carotid arteries were occluded 24 h before death. Neocortical infarct is demarcated by pale staining area. Loci of interstitial pH (pH_e) measurements are indicated by arrows, 1–5 mm from midline in ischemic hemisphere and 4 mm from midline in nonischemic hemisphere.

FIG. 2.

Direct current (DC) potential (*bottom trace*) and interstitial concentration of tetramethylammonium chloride (TMA) (*top trace*) in cortex after middle cerebral artery (MCA) occlusion (arrow). *A*: before MCA occlusion, brain was exposed to cerebrospinal fluid (CSF) in which 50 mM NaCl was replaced by 50 mM TMACl. Concentration of TMA was measured at a depth of 400 μ m by an ion-sensitive microelectrode with liquid exchanger (Corning 477317). Note rapid rise in TMA concentration at time of ischemic depolarization, indicating ~50% reduction in interstitial space. *B*: brain was exposed to CSF without TMA. No change in TMA concentration was detected at time of ischemic depolarization, indicating that rise in *A* is caused by an increase in TMA concentration ([TMA]) only.

FIG. 3.

Interstitial pH (*top trace*) and DC potential (*bottom trace*) in a hypoglycemic animal (54 mg/dl plasma glucose). Recording was made from 7 to 20 min after MCA-CCA occlusion 3 mm from midline. Repeated spontaneous depolarizations indicative of spreading depression-like waves were observed in DC potential recording. Initial alkalization, characteristic of spreading depression, occurred but following acidification usually seen with spreading depression was absent.

FIG. 4. Dimethadkme (DMO) autoradiograms from 1 h normogly-cemia (A), 4 h normoglycemia (B), 1 h hypoglycemia (C), and 1 h hyperglycemia (D).

FIG. 5.

Interstitial (pH_e) vs. intracellular (pH_i) pH in rats subjected to occlusion of right middle and common carotid arteries. Normal pH_e and pH_i values from nonischemic left cortex are indicated by arrows. Loci values are given millimeters lateral to midline. Most severely ischemic tissue lies between *loci* 3 and 5.

TABLE 1

Physiological variables

Expt	n	MABP, mmHg	pH	Pco ₂ , mmHg	Po ₂ , mmHg	Hct, %	Glucose, mg/dl
<i>pH</i>							
1 h	6	133±12	7.34±0.03	40±7	101±8	46±3	139±27
4 h	5	122±18	7.37±0.06	37±2	96±8	49±4	136±31
Hyperglycemia	4	126±10	7.35±0.02	38±2	100±11	47±4	300±73
Hypoglycemia	4	120±11	7.36±0.04	36±3	108±17	45±2	51±2
<i>Sucrose</i>							
1 h	9	127±11	7.38±0.05	39±5	100±13	44±3	119±21
4 h	5	133±8	7.39±0.06	37±5	97±4	44±2	121±22
<i>TMA</i>							
1 min	5	119±15	7.36±0.02	41±2	99±8	45±2	110±22

Values are means ± SD; n, no. of animals. MABP, mean arterial blood pressure; Pco₂ and Po₂, partial pressure of CO₂ and O₂, respectively; Hct, hematocrit; TMA, tetramethylammonium chloride.

TABLE 2

Brain water content

	Control Hemisphere, %	Ischemic Hemisphere, %
1 h		
<i>Loci 1</i>	79.1±1.5	80.2±0.9
<i>Loci 5</i>	78.7±1.6	79.5±0.6
4 h		
<i>Loci 1</i>	78.2±1.8	80.6±0.7*
<i>Loci 5</i>	79.5±0.8	81.2±2.9

Values are means ± SD.

* $P < 0.01$ (paired t test, $n = 5$) compared with control hemisphere.

TABLE 3

Sucrose distribution space

	Distribution Volume, %	<i>n</i>
1 h		
Ischemic hemisphere	15±1	4
Control hemisphere	18±4	5
4 h		
Ischemic hemisphere	15±4	5
Control hemisphere	14±2	4

Values are means ± SD; *n*, no. of measurements.

TABLE 4

Average tissue, interstitial, and intracellular pH

<i>Loc</i>	<i>pH_i</i>	<i>pH_e</i>	<i>pH_i</i>	<i>pH_e - pH_i</i>	<i>n</i>
<i>1 h normoglycemia</i>					
1	6.99±0.10	7.02±0.19	6.95±0.09	0.06	6
2	6.85±0.13	6.65±0.39	6.91±0.14	-0.26	6
3	6.79±0.10	6.49±0.17	6.83±0.12	-0.34	6
4	6.80±0.09	6.43±0.13	6.86±0.11	-0.43*	5
5	6.77±0.05	6.55±0.13	6.76±0.06	-0.21	3
Opp	7.07±0.03	7.24±0.07	7.01±0.13	0.23	6
<i>4 h normoglycemia</i>					
1	7.06±0.05	7.23±0.11	7.00±0.05	0.23	5
2	6.90±0.19	6.97±0.30	6.88±0.16	0.09	5
3	6.70±0.20	6.57±0.17	6.71±0.24	-0.14	5
4	6.62±0.17	6.61±0.09	6.62±0.20	-0.01*	4
5	6.65±0.18	6.64±0.16	6.64±0.23	0.00	4
Opp	7.07±0.04	7.24±0.08	7.03±0.04	0.21	4
<i>1 h hyperglycemia</i>					
1	6.94±0.18	6.40±0.85	6.98±0.13	-0.58*	2
2	7.02±0.07	6.87±0.10	7.05±0.08	-0.18	3
3	6.86±0.13	6.14±0.54	6.92±0.12	-0.79*	4
4	6.75±0.13	5.88±0.17	6.83±0.03	-0.96*	4
5	6.77±0.12	5.98±0.13	6.85±0.02	-0.88*	4
Opp	7.10±0.18	7.29±0.09	7.02±0.04	0.27	4
<i>1 h hypoglycemia</i>					
1	7.11±0.10	7.33±0.12	7.04±0.06	0.29	3
2	7.10±0.01	7.27±0.11	7.04±0.04	0.22	3
3	7.11±0.02	7.20±0.00	7.07±0.03	0.13	4
4	7.06±0.08	7.25±0.06	6.99±0.13	0.26*	4
5	7.04±0.07	7.20±0.10	6.97±0.13	0.23	3

<i>Loci</i>	pH_t	pH_e	pH_i	$pH_e - pH_i$	<i>n</i>
OPP	7.11 ± 0.03	7.33 ± 0.10	7.03 ± 0.04	0.30	4

Values are means $\pm$ SD; *n*, no. of measurements. One-way analysis of variance was used to compare mean $pH_e - pH_i$ differences at each of 6 loci and among 4 groups. Newman-Keuls multiple-comparison test was used to determine which groups were significantly different from one another. pH_t , tissue pH; pH_e , interstitial pH; pH_i , intracellular pH; Opp, opposite.

* $P < 0.05$ compared with this loci of remaining groups.