Effect of pH on Metabolism and Ultrastructure of Guinea Pig Cerebral Cortex Slices

BY KUSUM K. PATEL, Ph.D., J. FRANCIS HARTMANN, Ph.D., AND MAYNARD M. COHEN, M.D., Ph.D.

Abstract:
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Lactic acidosis is a prominent biochemical alteration which follows cerebral ischemia and is of sufficient degree to result in pH decrease in involved and adjacent tissue. Because of the significance of acidosis in the cerebral ischemic process, we have investigated ultrastructural changes and some biochemical parameters under varying pH conditions using guinea pig cerebral cortex slices.

Metabolic rates decreased markedly during acidic incubations, and increased only minimally during basic incubations. Acidic pH affected oxygen and glucose utilization rates and fine structure to a greater degree than did an equal change in pH from 7.4 toward the alkaline. Glucose consumption was affected by pH deviations from 7.4 to a greater degree than oxygen uptake.

Additional Key Words: acidosis (in vitro) alkalosis (in vitro) O2 uptake glucose consumption aerobic incubation brain slices electron microscopy brain (in vitro)

Concentrations of lactic acid in both brain and cerebrospinal fluid rise during and following cerebral ischemia. In humans, meeting electroencephalographical and clinical criteria of brain death, there is a marked increase in CSF lactic acid over normal values. Zupping et al. noted a significant increase in the CSF lactate and pyruvate in patients with cerebral infarcts. Lactic acid increases in tissue and a rise in the lactate-to-pyruvate ratio is the earliest observable biochemical alteration following inadequate oxygen supply. Hypotensive dogs showed progressive increase in lactate in the cerebral tissue correlating with the duration of shock. A pH drop of 0.6 (from 7.1 to 6.5) has been calculated to occur in tissue as a result of oxygen deprivation which may be sufficient to produce deleterious effects on neural metabolism and activity. The acidosis may thus compound the damaging effect of oxygen deprivation. pH has an additional biological importance related to determination of net electric charge of the amino acids, which affects cell membrane permeability.

The advantages of the in vitro experimental methods are their ease of control, speed, and relative reproducibility. The results of in vitro studies on brain metabolism have generally been confirmed by results of work in vivo. The present work was undertaken to study the effect of various pH values on aerobic metabolism of cerebral slices incubating at 37°C in glucose phosphate medium. Data were gathered on respiratory and glucose utilization rates and correlated with changes in the ultrastructure.

Methods

BIOCHEMICAL

Brain slices were prepared from young and mature guinea pigs and floated into preoxygenated medium as described by Patel et al. After draining a slice gently on a glass surface it was weighed to the nearest milligram. Three slices from one hemisphere, incubated at pH 7.4, served as controls for three slices from the other hemisphere incubated under the experimental condition selected. In this manner both control and experimental tissues were subjected to the same preparative conditions to obviate the effects of swelling, ionic shifts, and other changes that occur during preparation. Required basic or acidic pH values were obtained with NaOH and HCl, respectively.

The main compartment of the Warburg flask contained 3 ml of oxygenated medium fortified with 10 mM D-glucose and 1 μCi (U-14C) glucose when radioactivity was studied. A filter paper wick was placed in 0.2 ml of 0.3 N-NaOH in the center well of the flask to trap the evolved carbon dioxide.

Slices were incubated aerobically in a Warburg bath at 37°C. Incubation was begun within 25 minutes of sacrifice of the animal. Oxygen was supplied at the rate of three liters per minute for five minutes. After an equilibration for five minutes, manometric readings were taken at ten-minute intervals and corrected for
TABLE 1

Rates of Oxygen and Glucose Consumption by Brain Slices in Media at Various pH

<table>
<thead>
<tr>
<th>pH of incubation medium</th>
<th>Oxygen consumption</th>
<th>Glucose consumption</th>
<th>Relative specific activity of CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/g wet wt/h</td>
<td>µmol/g wet wt/h</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>92.8 ± 6.4</td>
<td>69.0 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>6.2</td>
<td>70.4 ± 6.5</td>
<td>29.8 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>94.7 ± 4.7</td>
<td>67.6 ± 2.9</td>
<td>0.72 ± 0.014</td>
</tr>
<tr>
<td>6.4</td>
<td>81.5 ± 2.0</td>
<td>31.0 ± 4.5</td>
<td>0.62 ± 0.016*</td>
</tr>
<tr>
<td>7.4</td>
<td>106.8 ± 2.5</td>
<td>72.8 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>94.5 ± 2.4</td>
<td>61.1 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>102.8 ± 5.0</td>
<td>70.4 ± 2.9</td>
<td>0.63 ± 0.026</td>
</tr>
<tr>
<td>8.4</td>
<td>107.6 ± 3.0</td>
<td>87.8 ± 4.8</td>
<td>0.62 ± 0.014</td>
</tr>
<tr>
<td>7.4</td>
<td>93.8 ± 2.7</td>
<td>72.0 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>9.0</td>
<td>95.6 ± 2.5</td>
<td>79.6 ± 4.0</td>
<td></td>
</tr>
</tbody>
</table>

As the rates of oxygen and glucose consumption were shown statistically not to be affected by the three slice levels or by the incubation times of 20, 30 or 40 minutes, each value is a mean ± SE of nine observations, except in case of pH 9.0 and its control, where 18 observations were made considering inconsistent effect of this basic pH.

Composition of incubation medium: NaCl, 98 mM; KCl, 27 mM; MgSO₄, 1.2 mM; KH₂PO₄, 4.0 mM; Na₂HPO₄, 17.5 mM; and glucose, 10 mM.

*(P = 0.001) significance of difference from preceding figure.

changes in the room pressure or bath temperature as indicated by thermobarometric readings.

Respiration was terminated at the end of 20, 30, or 40 minutes by immediate removal of flasks from the manometers, placing them in an ice-filled tray and quickly adding 0.3 ml 14% perchloric acid to the main compartment. The contents of the main compartment and two rinses with 1% perchloric acid, 1 ml each, were transferred to a cold homogenizing tube. Homogenization was carried out in the cold, and the homogenizing tube was rinsed with 1 ml 1% perchloric acid. The homogenates, including those from the thermobarometers, were centrifuged for 30 minutes at 3,000 g in the refrigerated Sorvall RC-2. The supernatants were analyzed for glucose on the Technicon Dual Channel AutoAnalyzer. The difference in values obtained for glucose concentration in the homogenates from those of the controls gave glucose consumption by brain slices during the incubation. The total CO₂ evolved and the amount of glucose carbon incorporated into carbon dioxide were measured to obtain relative specific activity (RSA) of CO₂. Total carbon dioxide was measured by titrating the center well contents with HCl using a Metrohm automatic titrator. Labeled carbon dioxide and the initial activity of radioactive glucose were counted in a Packard Tri-carb Model 3003 scintillation spectrometer with PPO-POP-Toluene mixture. The RSA of CO₂ was calculated by dividing the specific activity of CO₂ with the specific activity of glucose and multiplying the quotient by 6.

TABLE 2

Occurrence of Decline and Increase in the Rates of Oxygen and Glucose Uptake by Brain Slices, due to Change in pH of the Incubation Medium from pH 7.4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH</th>
<th>No. of instances with uptake change</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Decline</td>
<td>Increase</td>
</tr>
<tr>
<td>Oxygen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidity</td>
<td>6.2</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Basic</td>
<td></td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidity</td>
<td>6.4</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>Basic</td>
<td></td>
<td>6</td>
<td>21</td>
</tr>
</tbody>
</table>

The number of instances was obtained from individual data on each slice at each incubation time and at each pH.
ULTRASTRUCTURAL

The top two cortical slices were employed for studying the effect of acidic and basic incubations on the fine structure. The procedures for incubation, and for preparation of tissue and micrographs were as described by Patel et al. The osmolality of the glucose-phosphate medium at pH 7.4 was 293 mOsm. It was almost unchanged in the basic media, pH 8.4 and 9.0, was increased by 5 units in pH 7.0 medium, and by 11 units in more acidic media, pH 6.4 and 6.2. The osmolality of the fixative, 1% OsO₄, in glucose-free phosphate medium, was 310 mOsm. Tonicity of the medium as a whole can be increased or decreased some 30% without great effect on respiration or respiratory response.

RESULTS

BIOCHEMICAL

Incubation time and pH of the incubation medium were the two variable factors under study. However, variations in animal and slice-level were additional factors introduced by the method of tissue preparation. Animal variation was eliminated by using one cerebral hemisphere for the experimental pH and the contralateral hemisphere for control pH 7.4. Statistical analysis indicated no significant difference attributable to slice levels or to incubation times; and the mean uptake values using the top two or all three slices were very comparable. This permitted us to combine the results at each pH irrespective of slice level or incubation duration.

Table 1 shows the rates of oxygen uptake and glucose consumption by brain slices incubated at five different pH values as compared to control rates at pH 7.4. Oxygen and glucose consumption rates decline in acidic incubations and increase in basic incubations. In figure 1 the effect of three incubation times is illustrated separately. At pH 9.0, occasional decline was obtained. The effect of acidic or basic pH on each slice was therefore examined (table 2). It was evident from nine observations for each of the

FIGURE 2

Neuropil from slice incubated under optimum conditions for 30 minutes at pH 7.4. Except for some swelling of an astrocyte process (A) and a dendritic spine (D), the appearance approaches that of normal well-fixed cerebral cortex, x 46,500.

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three acidic pH values corresponding to nine control pH observations that oxygen consumption declined in every instance and that glucose consumption declined in 26 out of 27 instances. Nine observations for pH 8.4 and 18 observations for pH 9.0 were gathered. Basic pH caused an increase in the rate of oxygen consumption in 18 out of 27 instances and in that of glucose consumption in 21 out of 27 instances. To find if the single rise in the rate of glucose uptake due to acidic pH and the occasional declines in the oxygen and glucose consumption rates due to basic pH were by chance alone, two by two contingency tests were run. The probability of occurrence of decline and increase in the consumption rates independent of direction of pH change was found to be $0.1 \times 10^{-6}$ in case of oxygen consumption and $0.2 \times 10^{-7}$ in case of glucose consumption.

The fall expressed as percent deviation (fig. 1), in oxygen and glucose consumption rates from respective control rates, was greater with greater acidity, e.g., the rate of oxygen uptake was diminished by 10% to 15% at pH 7.0 and 21% to 26% at pH 6.2. Secondly, acidosis as well as alkalosis affected glucose consumption rates more than oxygen consumption rates. The oxygen uptake rate at pH 6.2 following 30 minutes of incubation (fig. 1) was 78.7% of that obtained at pH 7.4, while the glucose consumption rate under the same conditions was only 37.3% of that at pH 7.4. This results in an increased ratio of oxygen uptake to glucose consumption, a situation occurring in hypoglycemic conditions.8

The data in table 1 reveal that changes in pH toward the acidic produced greater metabolic alterations than those toward the basic. A pH diminution of 1.0 below pH 7.4 produced deviations in oxygen and glucose utilization rates greater than...
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twice that of those resulting from a pH increase of 1.0 above the same value.

Incorporation of radioactive carbon into evolved carbon dioxide was significantly lower at pH 6.4 and was almost unchanged at pH 8.4 compared to that at pH 7.4 (table 1).

ULTRASTRUCTURAL

Figure 2 is a micrograph prepared from a slice incubated under conditions considered optimal for biochemical studies. The ultrastructure does not illustrate ideally fixed normal tissue, but is employed as a baseline for alterations resulting from experimental incubation. At the end of 30 minutes of incubation at pH 7.4, the fine structure is well preserved except for some astrocytic swelling attributable principally to slicing. When the incubation was extended to 40 minutes, a few swollen mitochondria and some increase in the volume of interstitial space were found.

Incubation of brain slices at pH values above or below 7.4 results in ultrastructural changes that are much more severe in acidic than in basic media. This is in conformity with the report on rabbit brain slices incubated in glycylglycine medium at pH 6.4 and in bicarbonate medium at pH 8.4. The changes principally involve swelling of astrocytic and neuronal cell bodies and processes, with the exception of the smallest neuronal processes near their terminations, where they appear compressed and exhibit increased electron density that is accentuated by the staining technique used (figs. 2 and 3). Mitochondria also exhibit swelling which is of two types not correlated with the conditions of incubation or limited to any particular cell type. In some mitochondria, only the space within the cristae is enlarged with little increase in overall size, while the mitochondrial matrix shows nearly normal

FIGURE 4

The mitochondrion in the swollen dendrite (D) exhibits swelling that involves primarily the intracristal spaces while the matrix shows nearly normal density. Incubation at pH 7.0 for 20 minutes. × 48,500.
density (fig. 4). Other mitochondria show generalized swelling up to two or three times normal size with a greatly decreased density of the matrix (fig. 5). From pH 7.0 to pH 6.2, swelling of cells and processes is progressively more severe, and at the latter value the tissue is so disrupted as to be all but unrecognizable (figs. 6 and 7).

Throughout the pH range studied it was noted that the extracellular space was enlarged and resembled the "pseudoextracellular space" found by Long et al. in the gray matter in cerebral edema.

For a given deviation from the baseline value of pH 7.4 the changes were much more severe on the acid side. For instance, while the tissue incubated at pH 6.4 for 20 minutes is severely damaged (fig. 6), that incubated at pH 8.4 appears much better preserved (figs. 8 and 9). It seems worth noting that while the cellular swelling at low pH is accompanied by a general loss of cytoplasmic density, that occurring in basic media involves mostly mitochondria and cisterns of endoplasmic reticulum without dissolution of the intervening cytoplasmic matrix (fig. 9).

**Discussion**

Studies simulating alkalosis and acidosis in patients have been conducted on various isolated tissues incubated in media at pH values above or below 7.4. Earlier investigations on cerebral respiratory rate under unfavorable pH conditions concluded that both acidosis and alkalosis alter the chemistry of the tissue. Our in vitro experiments demonstrated that distinct changes in both metabolism and ultrastructure of the tissue occurred upon incubating the brain slices in acidic or basic media.

Both oxygen and glucose consumption in vitro are augmented at the alkaline pH values but are diminished when the pH is acidic. Table 1 and figure
EFFECT OF pH ON METABOLISM AND ULTRASTRUCTURE

FIGURE 6

Incubation for 20 minutes at pH 6.4. Severe swelling with general loss of background density is apparent throughout. The dendrite (D) is identifiable only by the presence of a few randomly oriented microtubules, while the remaining cellular processes are not identifiable with certainty. X 46,500.

I reveal that this effect is more pronounced at acidic than basic pH. In clinical studies, patients with alveolar hypoventilation showed no significant abnormalities in mental state if the arterial CO₂ tension was less than 90 mm Hg and the pH above 7.25, while a semicomatose or comatose state was observed when the arterial CO₂ was above 130 mm Hg and the pH below 7.14.19

Swanson18 demonstrated significantly lower concentrations of creatine phosphate and adenosine triphosphate in acidotic brain slices (pH 6.5). He interpreted this change to be due to decreased synthesis rather than increased utilization of these compounds.

The generally better-preserved appearance of slices incubated at high pH is contrasted with those incubated in acid media. Palade20 showed that with fixation in unbuffered OsO₄, the penetration of the fixative was preceded by a wave of tissue acidification that resulted in loss of cytoplasmic densities in the electron micrographs, as well as to coarse precipitation of nucleoplasm. Since the present experiments involved buffered fixatives throughout, it seems likely that the slices incubated below pH 7.4 were damaged by H⁺ concentrations before the start of fixation.

The altered capacity to utilize oxygen and glucose at an unfavorable pH would be followed by change in the capacity to maintain the respiratory chain and hence the high energy phosphate concentrations. Alterations in the mitochondrial fine structure under unfavorable pH conditions (figs. 4 and 5) are conceivable since cerebral mitochondria carry carbohydrate utilizing enzyme systems21 and play an active role in oxidative phosphorylation.22 Chordikian et al.23 reported rat brain mitochondrial
fraction in isotonic phosphate buffer in the pH range 5 to 9 to show progressively increased swelling and to give a straight line relationship upon plotting pH against ΔOD.

At an acidic pH the oxygen uptake is reduced from values obtained at pH 7.4, to a lesser extent than is glucose consumption. The relative specific activity of CO₂ at pH 7.4 was significantly different (P = 0.001) from that at pH 6.4 (table 1), indicating increased endogenous substrate utilization at pH 6.4. Since brain has little freely available endogenous substrate (small amounts of glycogen and glucose and amino acids related to glutamate), some ultrastructural changes observed following the incubations in acidic media may be secondary to combustion of endogenous substrates essential to the integrity of cells. The alkaline pH 8.4 did not produce a significant effect on the relative specific activity of CO₂ (table 1).

Enerson and Berman noted cellular swelling under hypoxic conditions, but the fall in respiration secondary to acidosis was greater than that which resulted from swelling alone. The ionic and fluid movements as controlled by internal and/or external milieu appear to determine the volume of the interstitial and astrocytic spaces. The swelling and resulting increased volume of astrocytic processes may be reducing the interstitial space volume by the compressing effect on the adjoining areas or may be found with accompanying enlargement of interstitial space volume. Both combinations were encountered in the micrographs prepared from our pH treatments. An enlarged interstitial space in the presence of astrocytic swelling suggests a phenomenon comparable to formation of pseudoextracellular space in intact animals with cerebral edema.

In vitro studies of the effect of graded hypoxia showed that a smaller degree of hypoxia produced...
more severe alterations in rat kidney cortical slices in comparison to brain cortex and liver slices. With 90% N₂-10% O₂, the kidney slices respired at one-third the rate and the brain and liver slices at one-half the rate of that with 100% O₂.

It is evident that pH exerts an effect different from anoxia. Whereas oxygen uptake falls and glucose consumption rises in anoxia in order to meet the increased energy demands, both glucose and oxygen utilization rates decline during acidosis and increase during alkalosis. Under anaerobic conditions, the utilization of glucose was diminished with a rise in pH. At alkaline pH values, no more carbohydrate appeared to be oxidized aerobically than that at pH 7.4 since the increase in glucose utilized could be accounted for by an increase in lactate produced. The acceleration of glycolysis under alkaline conditions has been reported for brain and other tissues. Our work demonstrates that acidic pH causes derangement in the glycolytic pathway in the sense that glucose degradation was suppressed. In unreported current work decreased formation of pyruvate and lactate from glucose occurred under acidosis. Investigations are in progress to determine whether inhibition of oxidative metabolism by acidic pH also contributes to the observed reduction in oxygen consumption.

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References
FIGURE 9

Part of a neuron from a slice incubated at pH 8.4 for 20 minutes. Many swollen profiles of endoplasmic reticulum, the largest of which is labeled ER, are present in the cytoplasm which contains clusters of RNP particles which stain darkly against a moderately dense background. X 34,800.


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