A Relation between [NAD\(^+\)]/[NADH] Potentials and Glucose Utilization in Rat Brain Slices\(^*\)

(Received for publication, December 23, 1975)

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Changes in several parameters involved in the control of metabolism were correlated with changes in glucose utilization in rat brain slices incubated under conditions which reduced glucose oxidation by 40 to 70%. The parameters included: the concentrations of ATP, ADP, AMP, and the adenylate energy charge; the cytoplasmic oxidation-reduction state ([NAD\(^+\)]/[NADH]), determined from the [pyruvate]/[lactate] equilibrium; the mitochondrial oxidation-reduction state, determined from the [NH\(_4\)]/[2-oxoglutarate]/[glutamate] equilibrium; the cytoplasmic and mitochondrial oxidation-reduction potentials (in volts), calculated from the respective [NAD\(^+\)]/[NADH] ratios using the Nernst equation; and the difference between the cytoplasmic and mitochondrial [NAD\(^+\)]/[NADH] potentials. The conversion of [3,4-\(^14\)C]glucose to \(^14\)CO\(_2\) and of [\(^13\)C\(^6\)]glucose to acetylcholine and to lipids, proteins, and nucleic acids by the brain slices were also determined.

The values obtained by subtracting the mitochondrial from the cytoplasmic [NAD\(^+\)]/[NADH] potentials correlated more closely with glucose utilization than did other parameters, under the conditions studied. For the synthesis of acetylcholine, the correlation coefficient was 0.96, and for the production of \(^14\)CO\(_2\) from [3,4-\(^14\)C]glucose it was 0.82.

*These studies were supported in part by Grants HD-06576, HD-34504, HD-461205, and MH-171691 from the United States Public Health Service and by the California State Department of Mental Hygiene.
isolated by the method of Glazer and Weber (8-10). Acetylcholine was precipitated as the roineketate salt and converted to the chloride form by column chromatography (8, 9). "Blanks" for all radioactive measurements were obtained by adding HClO4 to the media before adding tissue. Under these experimental conditions, the conversion of [U-14C]glucose to CO2 and to acetylcholine, lipids, proteins, and nucleic acids was proportional to the amount of tissue incubated and to the length of the incubations (9).

When tissues other than acetylcholine were determined, the incubations were terminated by pouring the incubation mixture through cheese cloth. The tissue was retained on the cheese cloth and the whole was immediately immersed in liquid N2. The total time for transfer and freezing was less than 1 s. The frozen tissue was ground in liquid N2, processed, and assayed for ATP, ADP, AMP, 2-oxoglutaric acid, glutamic acid, and pyruvic acid by the methods of Lowry and Passonneau (11). Lactic acid (12) and NH3 (13) were also measured in these extracts by published enzymatic methods. Protein was determined by the biuret reaction (14).

Calculations—All results were expressed as nanomoles per mg of protein. Nanomoles of [U-14C]glucose incorporated into each fraction were calculated by dividing the disintegrations per min in the sample by the specific activity of the [U-14C]glucose in the incubation medium. This value was multiplied by 3 to calculate nanomoles of medium. This value was multiplied by 3 to calculate nanomoles of incorporated [U-14C]glucose into biosynthetic products, even though the flux into acetylcholine, lipids, proteins, or nucleic acids was less than 2% of that to 14CO2 (8, 9). None of the biosynthetic paths seemed consistently more sensitive to inhibition than any other.

Reducing the concentration of glucose or oxygen in the incubation flask or inhibiting the utilization of O2 with KCN or amobarbital decreased ATP and 2-oxoglutaric acid and increased AMP and NH3 in the brain slices (Table II). Lactate levels fell with reduced glucose and rose with reductions in O2 or inhibition of O2 utilization (Table II).

For both mitochondria and cytoplasma, incubation with less glucose led to increases in the [NAD+]/[NADH] ratio while incubation with N2 or under other conditions that impaired O2 utilization led to decreased [NAD+]/[NADH] ratios (Table III). The adenylate energy charge and the difference between the cytoplasmic and the mitochondrial [NAD+]/[NADH] potentials fell under both sets of conditions (Table III).

The production of 14CO2 from [3,4-14C]glucose and the synthesis of acetylcholine were most closely correlated with the difference between the cytoplasmic and the mitochondrial [NAD+]/[NADH] potentials, i.e. to the [NAD+]/[NADH] potential across mitochondrial membranes (Table IV). The correlation coefficients were 0.92 for 14CO2 production and 0.96 for acetylcholine synthesis. The difference between the cytoplasmic and the mitochondrial [NAD+]/[NADH] potentials also correlated more closely than did the other parameters studied with the incorporation of [U-14C]glucose into proteins (r = 0.90), lipids (r = 0.82), and nucleic acids (r = 0.78).

Inhibition of one or another of the biosynthetic pathways can occur without any alterations in glucose to CO2. High concentrations (1 mM) of cycloheximide or puromycin inhibited protein synthesis by 33 ± 8% (p < 0.01). Actinomycin D, 0.25 μM, inhibited nucleic acid synthesis by 25 ± 3% (p < 0.01). Acetylcholine synthesis was stimulated by 0.1 mM norepinephrine, 0.1 mM epinephrine, or 1 mM dibutyryl cyclic AMP.

### RESULTS

Reducing the concentration of glucose, replacing the O2 with N2, or inhibiting the utilization of O2 with 0.5 mM KCN or 0.5 mM amobarbital reduced the oxidation of [3,4-14C]glucose to 14CO2 by 40 to 73% (Table I). These conditions also reduced the incorporation of [U-14C]glucose into biosynthetic products, even though the flux into acetylcholine, lipids, proteins, or nucleic acids was less than 2% of that to 14CO2 (8, 9). None of the biosynthetic paths seemed consistently more sensitive to inhibition than any other.

### DISCUSSION

The difference between the [NAD+]/[NADH] potentials in the cytoplasm and in the mitochondria correlated better with the biosynthetic activities in rat brain slices incubated under conditions which impair glucose oxidation.

### Table I

Biosynthetic activities in rat brain slices incubated under conditions which impair glucose oxidation

<table>
<thead>
<tr>
<th></th>
<th>Control (14)</th>
<th>Glucose, 0.5 mM (6)</th>
<th>Nitrogen (6)</th>
<th>KCN, 0.5 mM (6)</th>
<th>Amobarbital, 0.5 mM (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14CO2</td>
<td>134.6 ± 3.6</td>
<td>81.2 ± 4.0a</td>
<td>65.7 ± 5.7a</td>
<td>45.5 ± 3.6a</td>
<td>77.1 ± 2.0a</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>1.21 ± 0.08</td>
<td>0.81 ± 0.04a</td>
<td>0.80 ± 0.05a</td>
<td>0.55 ± 0.11a</td>
<td>1.05 ± 0.04a</td>
</tr>
<tr>
<td>Lipids</td>
<td>2.12 ± 0.04</td>
<td>1.25 ± 0.08b</td>
<td>1.04 ± 0.04a</td>
<td>0.93 ± 0.10a</td>
<td>1.31 ± 0.05a</td>
</tr>
<tr>
<td>Proteins</td>
<td>0.87 ± 0.04</td>
<td>0.22 ± 0.08c</td>
<td>0.52 ± 0.06a</td>
<td>0.23 ± 0.08c</td>
<td>0.61 ± 0.06c</td>
</tr>
<tr>
<td>Nucleic acids</td>
<td>0.54 ± 0.01</td>
<td>0.18 ± 0.02d</td>
<td>0.23 ± 0.04a</td>
<td>0.05 ± 0.01c</td>
<td>0.18 ± 0.05e</td>
</tr>
</tbody>
</table>

* Significant difference from control, p < 0.001.
* Significant difference from control, p < 0.01.
[**NAD**\(^+\)/[**NADH**]] Potentials and Glucose Utilization

The metabolites were determined enzymatically in extracts of rapidly frozen slices, as described in detail in the text. The conditions of incubation are described in the heading of Table I and in detail in the text. Values, in nanomoles per mg of protein, are means ± S.E. The number of samples is given in the parentheses.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (14)</th>
<th>Glucose, 0.5 mM (6)</th>
<th>Nitrogen (6)</th>
<th>KCN, 0.5 mM (6)</th>
<th>Amobarbital, 0.5 mM (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>10.23 ± 0.31</td>
<td>2.78 ± 0.37*</td>
<td>6.34 ± 0.35*</td>
<td>4.72 ± 0.30*</td>
<td>7.87 ± 0.22*</td>
</tr>
<tr>
<td>ADP</td>
<td>2.01 ± 0.05</td>
<td>2.15 ± 0.21</td>
<td>2.32 ± 0.13*</td>
<td>1.85 ± 0.08</td>
<td>2.09 ± 0.08</td>
</tr>
<tr>
<td>AMP</td>
<td>1.17 ± 0.05</td>
<td>2.28 ± 0.11*</td>
<td>1.66 ± 0.12*</td>
<td>1.90 ± 0.13*</td>
<td>1.62 ± 0.08*</td>
</tr>
<tr>
<td>Ammonium</td>
<td>4.38 ± 0.22</td>
<td>11.49 ± 0.70*</td>
<td>5.52 ± 0.10*</td>
<td>5.42 ± 0.37*</td>
<td>6.75 ± 0.84*</td>
</tr>
<tr>
<td>2-Oxoglutarate</td>
<td>0.491 ± 0.016</td>
<td>0.289 ± 0.055*</td>
<td>0.366 ± 0.054*</td>
<td>0.308 ± 0.050*</td>
<td>0.246 ± 0.024*</td>
</tr>
<tr>
<td>Glutamate</td>
<td>48.5 ± 1.0</td>
<td>19.5 ± 1.02*</td>
<td>54.7 ± 3.7</td>
<td>52.2 ± 4.3</td>
<td>65.9 ± 4.7*</td>
</tr>
<tr>
<td>Lactate</td>
<td>47.83 ± 1.62</td>
<td>9.14 ± 0.73*</td>
<td>118.4 ± 6.75*</td>
<td>126.0 ± 7.1*</td>
<td>132.7 ± 11.8*</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>1.25 ± 0.04</td>
<td>0.40 ± 0.04*</td>
<td>1.09 ± 0.10</td>
<td>0.71 ± 0.10*</td>
<td>1.29 ± 0.08</td>
</tr>
</tbody>
</table>

*Significant difference from control, p < 0.001.

**Table III**

Derived metabolic parameters in brain slices with impaired glucose oxidation

The values are calculated from those in Table II. The cytoplasmic oxidation-reduction state was calculated from the [pyruvate]/[lactate] equilibrium. The mitochondrial oxidation-reduction state was calculated from the [**NAD**\(^+\)]/[**NADH**] equilibrium. The potentials (E) were calculated from the Nernst equation. See “Experimental Procedures” for details.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Low glucose</th>
<th>Nitrogen</th>
<th>KCN</th>
<th>Amobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP/ATP</td>
<td>0.683 ± 0.006</td>
<td>0.620 ± 0.027*</td>
<td>0.727 ± 0.011*</td>
<td>0.665 ± 0.017*</td>
<td>0.769 ± 0.009*</td>
</tr>
<tr>
<td>AMP/ADP</td>
<td>2.37 ± 0.68</td>
<td>4.015 ± 0.066*</td>
<td>0.901 ± 0.012*</td>
<td>0.912 ± 0.017*</td>
<td>0.652 ± 0.008*</td>
</tr>
<tr>
<td>[<strong>NAD</strong>(^+)]/[<strong>NADH</strong>] (volts)</td>
<td>-0.195 ± 0.001</td>
<td>-0.181 ± 0.002*</td>
<td>-0.224 ± 0.003*</td>
<td>-0.236 ± 0.005*</td>
<td>-0.213 ± 0.003*</td>
</tr>
<tr>
<td>[<strong>NAD</strong>(^+)]/[<strong>NADH</strong>] (volts)</td>
<td>-0.304 ± 0.002</td>
<td>-0.303 ± 0.005*</td>
<td>-0.341 ± 0.003*</td>
<td>-0.339 ± 0.004</td>
<td>-0.349 ± 0.004*</td>
</tr>
<tr>
<td>[<strong>NAD</strong>(^+)]/[<strong>NADH</strong>] (volts)</td>
<td>0.189 ± 0.002</td>
<td>0.118 ± 0.004*</td>
<td>0.116 ± 0.005*</td>
<td>0.104 ± 0.004*</td>
<td>0.136 ± 0.004*</td>
</tr>
</tbody>
</table>

*Significant difference from control, p < 0.001.

**Table IV**

Correlations between glucose utilization and several metabolic parameters

Values are correlation coefficients, calculated from data in Tables I and III by standard methods (21).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CO(_2)</th>
<th>Acetylcholine</th>
<th>Lipids</th>
<th>Proteins</th>
<th>Nucleic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy charge</td>
<td>0.509</td>
<td>0.599</td>
<td>0.580</td>
<td>0.840</td>
<td>0.639</td>
</tr>
<tr>
<td>ADP/ATP</td>
<td>0.310</td>
<td>0.403</td>
<td>0.407</td>
<td>0.687</td>
<td>0.453</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>0.085</td>
<td>0.062</td>
<td>0.017</td>
<td>0.373</td>
<td>0.003</td>
</tr>
<tr>
<td>[<strong>NAD</strong>(^+)]/[<strong>NADH</strong>]</td>
<td>0.483</td>
<td>0.312</td>
<td>0.388</td>
<td>0.017</td>
<td>0.312</td>
</tr>
</tbody>
</table>

Changes in glucose oxidation and in biosynthetic activities than did a number of other parameters in the brain slices described above. The values for these potentials were calculated from the concentrations of selected metabolites. The calculations involve several assumptions, notably that the glutamate dehydrogenase equilibrium is maintained in the mitochondria and the lactate dehydrogenase equilibrium in the cytoplasm. Krebs, Veech, Hawkins and their co-workers have presented experimental and theoretical justifications for these assumptions for several tissues including brain (17, 22, 23).

The correlation in vitro between "transmitochondrial" potentials and the rate of incorporation of labeled precursors into acetylcholine agrees with previous in vivo studies of mildly to moderately hypoxic brains (6). The correlations were similar even though the levels of several of the metabolites in brain slices differed from their concentrations in brain in vivo. The levels in brain slices reported in our experiments were, however, similar to values reported by other workers using similar preparations (16).

A number of factors can influence the rate of carbohydrate metabolism, and their quantitative importance can vary among different tissues and in the same tissue at different times (1). Among these factors are the pools of adenylate phosphates (2-4) and potentials (1, 4, 24-26) or transport (1, 4, 27, 28) across mitochondrial membranes. It has been suggested that the ratios of [**NAD**\(^+\)]/[**NADH**] are in equilibrium with the total pool of "energy-rich" compounds in both the cytoplasm and the mitochondria (22, 23). Changes in the adenylate phosphates frequently accompany changes in the rate of cerebral carbohydrate metabolism, but major changes in brain metabolism and function can occur without significant alterations in the adenylate energy charge or the levels of ATP (5, 6). Apparently, the brain can readjust its metabolism to maintain the adenylate energy charge and the level of ATP when carbohydrate metabolism is impaired relatively mildly. The
studies described in this paper do not imply that [NAD+]/[NADH] potentials are the only factors which regulate glucose metabolism in mildly hypoxic brain, and they provide no information on how potentials across mitochondrial membranes might be linked to biosynthetic activities in the cytoplasm.

These studies do confirm and extend previous observations that impairment of acetylcholine synthesis is an early and physiologically important consequence of mild to moderate impairment of the oxidation of glucose or pyruvate by the brain in vivo and in vitro (6–9, 29). Disorders in which the supply of glucose or oxygen to the brain becomes inadequate are a major cause of neurological disability. A more detailed understanding of the biochemical consequences of marginal reductions in cerebral glucose oxidation may have practical as well as theoretical value.

Acknowledgments—We thank Professor Donald J. Jenden and Professor George J. Popjak for helpful advice and support and Professor Paul D. Boyer for critical review of the manuscript. Ms. Ludmila Grauel gave expert technical assistance.

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