Effects of Oxygen Saturation and PCO₂ on Brain Uptake of Glucose Analogues in Rabbits

BY FRANK G. BERSON, M.D., MARIA SPATZ, M.D., AND IGOR KLATZO, M.D.

Abstract:
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Intraperitoneal injection of 6% sodium pentobarbital (30 mg per kilogram) and maintained with small intravenous supplements of 2% pentobarbital, as required. With the animal in a supine position, the first surgical incision was made in the midline of the neck for the purpose of exposing the trachea, as well as the right common carotid artery for subsequent cannulation. A tracheotomy was then immediately performed and ventilation controlled with a small animal respirator (Harvard Apparatus Co, Millis, Massachusetts). Intravenous tubocurarine (Lilly) was also administered (1.2 mg in 1 cc) to keep each animal in phase with the respirator. A second incision was made in the groin and the femoral artery cannulated in order to monitor blood pressure (Statham pressure gauge; Brush 440 Recorder by Gould, Inc, Cleveland, Ohio) and withdraw arterial samples for blood gas analysis (Instrumentation Laboratory 313 pH/Blood Gas Analyzer). Once the tracheotomy was completed, the experiments consisted of two 15-minute controlled ventilation periods. For the first 15 minutes all animals were respirated to stabilize them in a normal range of blood gas values. By varying the flow rates of gases through a modified Kreiselman resuscitator (Ohio Chemical and Surgical Equipment Co, Madison, Wisconsin) connected to the ventilator, the concentrations of inspired O₂, CO₂, and N₂ were quickly changed to a particular combination, depending upon the desired blood gas levels, or left unchanged in the case of control animals. After ten minutes of this second ventilation period, the arterial blood pressure was again checked and then a second sample withdrawn for blood gas analysis (the Po₂ and PCO₂ were not shown to change significantly after the first ten minutes of a ventilat-
TABLE 1

Effect of P0₂ on Brain Uptake of 14C 2-Deoxy-D-Glucose

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Range of P₀₂ (mm Hg)</th>
<th>Mean P₀₂/se (mm Hg)</th>
<th>Mean pH ± SE</th>
<th>Mean arterial BP (mm Hg) ± SE</th>
<th>Mean BUI ± SE</th>
<th>Significance level compared with control BUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td>5</td>
<td>357-498</td>
<td>35.2 ± 1.7</td>
<td>7.51 ± 0.02</td>
<td>86.0 ± 2.4</td>
<td>70.83 ± 3.43</td>
<td>NS†</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>71-100</td>
<td>32.4 ± 1.0</td>
<td>7.52 ± 0.01</td>
<td>89.6 ± 3.1</td>
<td>68.27 ± 1.99</td>
<td>—</td>
</tr>
<tr>
<td>Mild-moderate hypoxia</td>
<td>5</td>
<td>33-55</td>
<td>29.8 ± 1.9</td>
<td>7.52 ± 0.02</td>
<td>87.0 ± 4.6</td>
<td>65.48 ± 2.99</td>
<td>NS</td>
</tr>
<tr>
<td>Severe hypoxia</td>
<td>9</td>
<td>12-15</td>
<td>28.8 ± 1.8</td>
<td>7.50 ± 0.01</td>
<td>78.3 ± 4.1</td>
<td>39.40 ± 0.88</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

*Arterial oxygen saturation.
†Brain uptake index = standard error.
‡Not significant.

The brain uptake index is the index of unidirectional fractional extraction of test tracers.

Mean arterial blood pressures were obtained by electric integration in the Brush amplifier-recorder. Values for oxygen saturation (S₀₂) were derived from a rabbit hemoglobin dissociation curve and corrected for variations in pH.

Results

There was a significant decrease in the brain uptake of both glucose analogues in severe hypoxia (tables 1 and 2). The control BUI values for 2-deoxy-D-glucose and 3-0-methyl-D-glucose were 68.27 ± 1.99 SE and 37.35 ± 1.68 SE, respectively, as compared with 39.40 ± 0.88 SE and 19.30 ± 0.66 SE when the P₀₂ was lowered to 10 to 15 mm Hg (S₀₂ = 10% to 15%). In mild-moderate hypoxia and hyperoxia, however, there was no significant change in BUI for either 2-deoxy-D-glucose or 3-0-methyl-D-glucose when compared to the controls. Although mean Pco₂ and pH were approximately the same for all groups, the mean arterial blood pressures in severe hypoxia were slightly lower in the 2-deoxy-D-glucose experiments (78 mm Hg) and markedly lower in the 3-0-methyl-D-glucose experiments (63 mm Hg). Figure 1, however, shows that despite a wide range of blood pressures from 55 to 95 mm Hg, there was no related variation in BUI values for 2-deoxy-D-glucose.

Increase in arterial Pco₂ resulted in a decrease in

TABLE 2

Effect of P0₂ on Brain Uptake of 14C 3-0-Methyl-D-Glucose

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Range of P₀₂ (mm Hg)</th>
<th>Mean P₀₂/se (mm Hg)</th>
<th>Mean pH ± SE</th>
<th>Mean arterial BP (mm Hg) ± SE</th>
<th>Mean BUI ± SE</th>
<th>Significance level compared with control BUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td>4</td>
<td>369-494</td>
<td>31.0 ± 1.5</td>
<td>7.51 ± 0.03</td>
<td>83.8 ± 1.3</td>
<td>34.79 ± 0.44</td>
<td>NS†</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>68-99</td>
<td>30.1 ± 0.9</td>
<td>7.54 ± 0.01</td>
<td>82.9 ± 4.7</td>
<td>37.35 ± 1.68</td>
<td>—</td>
</tr>
<tr>
<td>Mild-moderate hypoxia</td>
<td>3</td>
<td>33-44</td>
<td>30.3 ± 1.5</td>
<td>7.59 ± 0.04</td>
<td>80.0 ± 10.4</td>
<td>37.55 ± 2.22</td>
<td>NS</td>
</tr>
<tr>
<td>Severe hypoxia</td>
<td>4</td>
<td>10-12</td>
<td>27.5 ± 1.0</td>
<td>7.52 ± 0.03</td>
<td>62.5 ± 4.3</td>
<td>19.30 ± 0.66</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

*Arterial oxygen saturation.
| Brain uptake index = standard error.
‡Not significant.

692

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EFFECTS OF OXYGEN SATURATION AND Pco,

Effect of mean arterial blood pressure on brain uptake of \( ^{14} \)C 2-deoxy-D-glucose in severe hypoxia. These points represent the same nine experiments described in table I, severe hypoxia group.

BUI for both glucose analogues (fig. 2). The BUI for 2-deoxy-D-glucose decreased to approximately 37 when the Pco, was increased to 100 mm Hg. If expressed in terms of pH (fig. 3), there was an equivalent fall in BUI with a decrease in pH to 7.10.

Similarly, the BUI for 3-0-methyl-D-glucose decreased to approximately 20 at a Pco, of 100 mm Hg or a pH of 7.10. When severe hypercapnia was superimposed on severe hypoxia (fig. 4), there was no additional change in the BUI of either glucose analogue. Also, the data in figures 2 and 3 show an increase in the BUI of 3-0-methyl-D-glucose with hypocapnia and resulting respiratory alkalosis: at a Pco, of 15 mm Hg and a pH of 7.75, the BUI increases to approximately 50. It should be noted that the points in figure 3 for pH versus BUI correlate well with the calculated linear regression, whereas curves could only be approximated for Pco, versus BUI in figure 2.

Two additional series of experiments were performed using 3-0-methyl-D-glucose. First, 10 mM cold (unlabeled) phlorizin, a competitive inhibitor with 3-0-methyl-D-glucose,\(^{12}\) was injected with the isotope at various levels of arterial Pco,. The results, seen in figure 2, show that the BUI of 3-0-methyl-D-glucose with phlorizin plotted against Pco, forms a curve which runs parallel to and significantly below the curve for the uninhibited analogues. Second, inhibition of 3-0-methyl-D-glucose uptake with varying concentrations of cold 3-0-methyl-D-glucose\(^{7}\) (self-inhibition) was studied in both hypocapnic and control animals (fig. 5). In the hypocapnic animals the BUI with 0.5 mM inhibitor was 25.01 ± 4.28 SE (n = 5), a decrease of 51\% from the noninhibited value of 51.23 ± 2.61 SE (n = 5). In the control animals, however, a comparable decrease (49\%) from the noninhibited value of 37.25 ± 1.27 SE (n = 6) was seen not with 0.5 mM but with 5 mM inhibitor, which resulted in a BUI of 19.11 ± 1.35 SE (n = 6). Max-
Effect of Pco₂ on brain uptake of ¹⁴C-glucose analogues in severe hypoxia. Oxygen saturation was 18% or less in all experiments. Same symbols as in figures 2 and 3 for experiments without inhibition.

Animal inhibition was achieved with 20 mM cold 3-O-methyl-D-glucose, 18.13 ± 2.18 SE (n = 3) for hypocapnic animals and 12.17 ± 2.16 SE (n = 4) for control animals, a decrease of 65% and 67%, respectively, from experiments using no inhibitor.

A comparison of tritiated H₂O uptake with BUI is depicted in figure 6 for 3-O-methyl-D-glucose under various experimental conditions. Within a range of 0.18% to 0.45% ³H₂O uptake, in which 50% to 75% of animals in each group are found, there is no correlation whatsoever between BUI and water uptake. With higher ³H₂O uptake, however, all BUI values are low.

Table 3 shows the results of the effect of Pco₂ and Po₂ on the uptake of ¹⁴C-labeled sucrose with decapitation 15 seconds following injection of the isotope mixture. The control group had a BUI of 4.13 ± 0.12 SE compared with a BUI of 2.68 ± 0.21 SE in severe hypoxia, 2.62 ± 0.36 SE in severe hypoxia with hypercapnia, and 2.15 ± 0.20 SE in severe hypercapnia.

The mean serum glucose (mg %) was 135.7 ± 7.1 SE (n = 7) for controls, 141.8 ± 6.5 SE (n = 9) for hypoxia, 152.7 ± 21.7 SE (n = 6) for severe hypoxia and 158.2 ± 10.7 SE (n = 5) for severe hypercapnia. None of the experimental values, however, differed significantly from the control value (P > 0.05).

Discussion

The results of this investigation show that lowering the oxygen saturation and/or increasing the carbon dioxide tension in arterial blood can decrease the brain uptake of glucose analogues in the rabbit. The effect on both glucose analogues studied was approximately the same. Severe hypoxia (SaO₂ < 18%) resulted in about a 40% decrease in the BUI of 2-deoxy-D-glucose and a 45% decrease in the BUI of 3-O-methyl-D-glucose. Similarly, severe hypercapnia and respiratory acidosis, with a Pco₂ of 100 mm Hg and pH of 7.10, resulted in about a 45% decrease in the BUI of both 2-deoxy-D-glucose and 3-O-methyl-D-glucose.
from control values. A respiratory alkalosis of pH 7.75 induced by a PCO2 of approximately 15 mm Hg increased the BUI of 3-O-methyl-D-glucose by 35%. Inhibition studies on these hypocapnic animals revealed, however, that they were more sensitive to inhibition than control animals; that is, the Km, or concentration of inhibitor (unlabeled 3-O-methyl-D-glucose) necessary to decrease the BUI by 50% was 0.5 mM in hypocapnic animals as compared with 5 mM in control animals. Neither glucose metabolism nor serum glucose levels can explain the effects of oxygen saturation and PCO2 on brain uptake of glucose analogues. Even though 2-deoxy-D-glucose is partially metabolized and 3-O-methyl-D-glucose is not metabolized, the BUI of each was affected similarly by hypoxia and hypercapnia. Serum glucose did not differ significantly from control levels in either hypoxia, hypoxia or hypercapnia.

Due to the fact that PO2 and PCO2 have been shown to have a profound effect on cerebral blood flow, a full interpretation of these results would be aided by a knowledge of the changes in blood flow in these rabbits and the effect of such changes on BUI. Although quantitative determinations of blood flow have not as yet been done in rabbits under these experimental conditions, there is sufficient collateral information in our data to support the contention that these changes in BUI can be explained only partially by changes in blood flow. There must be significant changes, therefore, in permeability.

The data on uptake of 14C-labeled sucrose support the conclusion that PO2 and increased PCO2 result in an increase in cerebral blood flow, since sucrose does not cross a functional blood-brain barrier. The significant decrease in the BUI of sucrose under these conditions simply confirms that less intravascular sucrose will be found in the brain if it is cleared more rapidly from the cerebral circulation by increased blood flow. Although brain uptake of tritiated water has been thought to be only flow limited, recent work by Raichle et al. suggests that water uptake is partly diffusion or permeability limited in the brain. Now, if BUI were only a function of blood flow in these experiments, one would expect the BUI to be a function of 3H2O uptake (ratio of injected to recovered 3H2O), regardless of the effect of flow on brain permeability of water. When one plots BUI against the uptake of 3H2O, however, as was done in figure 6 for 3-O-methyl-D-glucose, one notes that over 62% of animals under various experimental conditions were within a range of 3H2O uptake in which there was no correlation whatsoever between BUI and percent of 3H2O uptake. In other words, the ratio of injected to recovered water in the experimental brain (in 62% of the rabbits) did not differ significantly from the ratio of injected to recovered water in the controls. Similar observations were made with 2-deoxy-D-glucose. It is clear, though, that a high 3H2O uptake (greater than 0.45%) almost always correlated with a low BUI under experimental conditions which were associated with marked increases of blood flow. A decreased BUI but due to both the increased 3H2O (3H2O brain/injectate) and 14C 3-O-methyl-D-glucose (14C 3-O-methyl-D-glucose brain/injectate) distribution in the regions with increased blood flow was described by Pardridge and Oldendorf recently.

There is further evidence to support the contention that BUI changes do not simply reflect blood flow changes. First, hypercapnia (PCO2 = 100 mm Hg) has been shown in dogs to have a much stronger dilating effect on cerebral vessels than severe hypoxia (S,O2 = 20%). Although the O2 saturations in these rabbit experiments were slightly lower (around 15%), one would still expect a greater blood flow in severe hypercapnia. However, the BUI values for both glucose analogues under these two conditions were essentially the same. Second, hypoxia (S,O2 less than 60%) has been shown in dogs to abolish autoregulation and result in a passive pressure-flow relationship. Figure 1 shows, however, that the BUI of 2-deoxy-D-glucose in severe hypoxia is essentially independent of a wide range of arterial blood pressures which, in the absence of autoregulation, would be expected to result in a correspondingly wide range of cerebral blood flow. Finally, single step hyperxia to levels above 300 mm Hg has been shown to decrease cerebral blood flow by at least 20% in man. In these rabbit experiments there is no significant change in BUI in a similar single step hyperxia.

**Table 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Range of PO2 (mm Hg)</th>
<th>Range of S,O2* (%)</th>
<th>Mean PO2 (mm Hg) ± SE</th>
<th>Mean pH ± SE</th>
<th>Mean arterial BP (mm Hg) ± SE</th>
<th>Mean BUI ± SE</th>
<th>Significance level compared with control BUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>76-  89</td>
<td>97-98</td>
<td>30.3 ± 0.6</td>
<td>7.47 ± 0.02</td>
<td>88.8 ± 6.3</td>
<td>4.13 ± 0.12</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>3</td>
<td>12-  16</td>
<td>17-19</td>
<td>30.3 ± 1.8</td>
<td>7.49 ± 0.06</td>
<td>66.7 ± 6.0</td>
<td>2.68 ± 0.21</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Hypoxia-hypercapnia</td>
<td>3</td>
<td>15-  19</td>
<td>6- 8</td>
<td>128.0 ± 8.5</td>
<td>6.98 ± 0.02</td>
<td>7.33 ± 8.3</td>
<td>2.62 ± 0.36</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>3</td>
<td>89- 111</td>
<td>91-94</td>
<td>107.3 ± 5.2</td>
<td>7.07 ± 0.03</td>
<td>91.7 ± 4.4</td>
<td>2.15 ± 0.20</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* Arterial oxygen saturation.
† Brain uptake index ± standard error.
The mechanisms by which glucose uptake is reduced by low arterial oxygen saturation and either increased or decreased by arterial Pco₂, are not known and require further study. Blood flow determinations need to be made in order to clarify the relationship of flow with the brain uptake of glucose analogues. Also, since glucose is transferred into the brain by facilitated diffusion and is not energy dependent, it would be important to compare these results with the effects of oxygen saturation and carbon dioxide tension on brain uptake of actively transported substances, such as amino acids. Certain essential and non-essential amino acids have been tested, and the results do not always parallel those found with glucose analogues. For example, the BUI of L-leucine is increased rather than decreased by severe hypercapnia, whereas hypoxia does not appear to affect the BUI of L-alanine and D-leucine. Recently Betz and colleagues reported that, unlike glucose, ten minutes of anoxia had no effect on leucine transport in the brain.

Finally, the demonstration of a decrease in the BUI of glucose analogues in hypoxia and/or hypercapnia in these experiments must be reconciled with the finding that ischemic gerbils show an increase in facilitated diffusion of glucose from blood to brain. It is true that the gerbils were injected with the isotope five minutes after circulation had been re-established to an ischemic hemisphere, and the increase in BUI, therefore, might represent a rebound phenomenon in a postischemic brain. In fact, preliminary experiments on recovery, in which blood gases are returned to normal before the isotope is injected, have suggested an increase in the BUI of 3-O-methyl-D-glucose after severe hypercapnia.

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References
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