Effect of Pharmacological Doses of 3-0-methyl-D-glucose and 2-Deoxy-D-glucose on Rat Brain Glucose and Lactate

LEONARD P. MILLER, PH.D.,* JOHN B. VILLENEUVE, B.A., LEON D. BRAUN, B.A., AND W.H. OLDENDORF, M.D.*

SUMMARY The present investigation examined the effects of two glucose analogues, 3-0-methyl-D-glucose (30MG) and 2-deoxy-D-glucose (2DOG) on basal levels of rat brain glucose and lactate. The results showed that pretreatment (iv) with 30MG up to 2 g/kg caused a transient drop in brain glucose levels to 42% of control value within 2.5 min and a drop in lactate levels to 75% of control value by 5 min. 2DOG administration (2 g/kg) affected glucose in a biphasic response with an initial drop to 46% of control value seen by 2.5 min, followed by a progressive increase to 290% of the control value by 40 min. This elevated level of glucose was sustained for approximately 40 min. Lactate levels responded to 2DOG administration by a decrease to 37% of control value within 10 min post-injection and returned to near basal levels by 160 min. A dose response was also examined for both compounds. Behaviorally 30MG had no apparent effects. However, the response to 2DOG was a reduction in voluntary movements, piloerection, irregular clonic jerks, splayed limbs and fits of wild running. These experiments were designed to evaluate the potential of 30MG or 2DOG for attenuating the well-documented rise in brain lactate levels following an ischemic insult. Our results suggest that under certain experimental conditions either 30MG or 2DOG could prevent brain lactate rise and might have beneficial effects in minimizing the neuropathological consequences of ischemic damage that could be related to increases in brain lactate.

Methods

Materials

Dowex AG1-X8 resin was purchased from Bio-Rad, Richmond, CA 94804. Glutamic-Pyruvic transaminase, 2-deoxy-D-glucose and 3-0-methyl-D-glucose were obtained from Sigma, St. Louis, MO 63178. Glucose-6-phosphate dehydrogenase and nicotinamide-adenine dinucleotide phosphate were from Calbiochem-Behring Corp., San Diego, CA 92112. Polyethylene (PE-10) Intramedic™ tubing was supplied by Curtin Matheson Scientific, Inc. Brea, CA 92621. Sprague-Dawley rats weighing 225–300 g were from Simonsen labs, Gilroy, CA and were maintained on a 12 hr light and dark cycle with free access to food and water.

Cannulation

On the day prior to experimentation, a catheter filled with Heparin solution (PE-10 tubing 15 cm in length) was inserted into the tail vein of each animal while under ether anaesthesia. A 15 cm length of aluminum sleeve completely covering the rat’s tail prevented removal of the catheter by the rats. Animals were then kept overnight in individual cages. On the experimental day each rat was lightly restrained in a plastic cylindrical open ended tube. Following injection of either 30MG or 2DOG over 30 sec through the catheter, each animal was killed by high intensity microwave fixation (2.1 sec exposure, 3.8 kW; Metabostat 4094, Gerling Moore, Palo Alto, CA) at the indicated times. The entire brain rostral to the midbrain (minus olfactory bulbs) was removed, weighed and homogenized in 2 parts 0.9N HClO₄. Samples were centrifuged, neutralized with 1 part 1.8N KHC₆O₄, and supernatant frozen at −20°C until analysis of glucose or lactate levels within 1 week.

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Metabolite Determination

The determination of BUI values for 14C-glucose in conscious animals was exactly as described by Braun et al.19

The column eluates containing glucose were then analyzed directly for glucose using the spectrophotometric procedure as described by Lowry and Passonneau.20 For determination of lactate, the supernatant homogenates were analyzed directly using the procedure of Lowry and Passonneau.20

Results

Behavioral Effects

For all doses of administered 30MG, there were no observable behavioral effects. The results with 2DOG were quite different. At the highest dose (3 g/kg) approximately 1/3 of the animals died within 30-40 min. At 1 g/kg none of the animals died but they did display a series of observable behavioral responses over an approximately 4 hr time period. Ten minutes following 2DOG administration (1 g/kg) rats became ataxic with limbs splayed. Voluntary movement was reduced but the animals were able at all times to right themselves. Irregular clonic jerks of the limbs were infrequently observed with occasional fits of wild running. Pilocerection was observed over an approximately 2 hr time period.

Biochemical Effects

Previous results from this laboratory21 showed that iv administered 30MG resulted in time-dependent effects on brain glucose levels. Because the focus of the present investigation was on lactate, it was decided to examine the effects of 30MG administration not only on brain glucose but also lactate levels. The results in table 1 show that 2.5 min following 30MG (2 gr/kg) administration brain glucose levels decreased to 42% of control value. Glucose levels then returned to control value within 20 min. These glucose results correspond with the previous investigation.21 Lactate levels also showed a small decrease at 5 min to 75% of control value. Table 2 shows the results of a dose response analysis of 30MG from 1 to 4 g/kg. At the highest dose administered (4 gr/kg) glucose levels decreased to 32% of control value with a corresponding but smaller decrease in lactate levels to 59% of control value. The results in table 2 also show that the injection vehicle itself (2 ml Ringer’s Solution) caused a small increase at 2.5 min in brain lactate (1.73 /imoles/g brain) compared to uninjected control value (1.44 /imoles/g brain, table 1) with no effect on brain glucose.

In the next series of experiments, we examined the effect of 2DOG administration on brain glucose and lactate. At 2.5 min following the injection of 2DOG brain glucose levels decreased to 46% of control value (table 3). This was followed by a progressive increase with a maximal level attained at 40 min which was sustained for 40 min before decreasing. Brain lactate levels responded to 2DOG administration with a maximal decrease at 10-20 min post-injection. These present results are similar to the observations of Horton et al22 using mice and injecting 2DOG ip at 3 g/kg. Differences between our results and that study probably reflect their decapitation and freezing of the head vs. our two-second microwave fixation.

To adequately examine a dose response for 2DOG, it was necessary to sacrifice animals at a number of different time points (2.5, 20 and 40 min post-injection). The results of this analysis are shown in table 4 where 2DOG administration was varied from 0.25 to 3.0 g/kg. At 2.5 and 20 min following injection of

| TABLE 1 Effect of 3-0-Methyl-D-Glucose Administration (2 g/kg, iv) on Rat Whole Brain Glucose and Lactate Levels Versus Time (U moles/g brain) |
|---|---|---|
| Time (min) | Glucose | Lactate |
| 0 (control, un.injected) | 1.57 ± 0.17 | 1.44 ± 0.12 |
| 2.5 min post-injection | 0.66 ± 0.08‡ | 1.15 ± 0.31 |
| 5 min post-injection | 0.81 ± 0.06‡ | 1.08 ± 0.07† |
| 10 min post-injection | 1.14 ± 0.16° | 1.14 ± 0.10° |
| 20 min post-injection | 1.56 ± 0.49 | 1.27 ± 0.27 |

3-0-methyl-D-glucose dissolved in 2 ml of Ringer’s solution was administered slowly through a tail vein catheter over 30 sec. The animals (220–240 g) were sacrificed at the indicated times by focused microwave for 2.5 sec. Brains were allowed to chill in situ, removed, weighed and homogenized in 2 volumes of 0.9N perchloric acid. Following centrifugation each supernatant was neutralized with 1 volume of 1.8N KHCO3, recentrifuged and supernatants frozen until chemical analyses were performed generally within 1 week. Control animals were injected with 2 ml of Ringer’s Solution.

Values represent the mean ± SD for at least 3 rats. *p < 0.05, †p < 0.02, ‡p < 0.01 compared to appropriate control values.

| TABLE 2 Effect of Varying Doses of 3-O-Methyl-D-Glucose (iv) on Rat Whole Brain Glucose and Lactate at 2.5 Min Post-injection (U moles/g brain) |
|---|---|
| Dose (g/kg) | Glucose | Lactate |
| 0 (control, injected) | 1.57 ± 0.17 | 1.73 ± 0.05 |
| 1 | 1.18 ± 0.09† | 1.10 ± 0.25† |
| 2 | 0.66 ± 0.08§ | 1.15 ± 0.31* |
| 4 | 0.50 ± 0.12† | 1.02 ± 0.19† |

3-0-methyl-D-glucose dissolved in 2 ml of Ringer’s solution was administered slowly through a tail vein catheter over 30 sec. Following this injection the animals (220–240 g) were sacrificed at 2.5 min by focused microwave for 2.5 sec. For control animals only Ringer’s solution was injected. Brains were allowed to chill in situ, removed, weighed and homogenized in 2 volumes of 0.9N perchloric acid. Following centrifugation each supernatant was neutralized with 1 volume of 1.8N KHCO3, recentrifuged and supernatants frozen until chemical analyses were performed generally within 1 week. Control animals were injected with 2.0 ml of Ringer’s Solution.

Values represent the mean ± SD for at least 3 rats. *p < 0.05, †p < 0.02, ‡p < 0.01 compared to appropriate control values.

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TABLE 3  
Effect of 2-Deoxy-D-Glucose Administration (2 g/kg, iv) on Rat Whole Brain Glucose and Lactate Levels Versus Time  
(U moles/g brain)  

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control, un.injected)</td>
<td>1.57±0.17</td>
<td>1.44±0.12</td>
</tr>
<tr>
<td>2.5 min post-injection</td>
<td>0.72±0.14*</td>
<td>0.98±0.16*</td>
</tr>
<tr>
<td>5 min post-injection</td>
<td>0.96±0.02*</td>
<td>0.78±0.06*</td>
</tr>
<tr>
<td>10 min post-injection</td>
<td>1.42±0.16</td>
<td>0.53±0.06*</td>
</tr>
<tr>
<td>20 min post-injection</td>
<td>2.60±0.20*</td>
<td>0.52±0.05*</td>
</tr>
<tr>
<td>40 min post-injection</td>
<td>4.56±0.29*</td>
<td>0.75±0.06*</td>
</tr>
<tr>
<td>80 min post-injection</td>
<td>4.36±0.24*</td>
<td>0.71±0.08*</td>
</tr>
<tr>
<td>160 min post-injection</td>
<td>3.28±0.23*</td>
<td>0.92±0.11*</td>
</tr>
</tbody>
</table>

2-deoxy-D-glucose dissolved in 2 ml of Ringer’s solution was administered (2 g/kg) slowly through a tail vein catheter over 30 sec. The animals (220–240 g) were sacrificed at the indicated times by focused microwave for 2.5 sec. Brains were allowed to chill in situ, removed, weighed and homogenized in 2 volumes of 0.9N perchloric acid. Following centrifugation each supernatant was neutralized with 1 volume of 1.8N KHCO3, recentrifuged and supernatants frozen until chemical analyses were performed generally within 1 week.

Values represent the mean ± SD for at least 3 rats.
*Significantly different from appropriate control values with at least p < 0.05.

2DOG at 0.25 to 2.0 g/kg, the effects on brain glucose levels were similar. At 40 min, however, following injection of 1 to 3 g/kg of 2DOG, we observed a significantly greater increase in brain glucose levels compared to values from animals which received 0.25 g/kg of 2DOG. An analysis of lactate levels revealed similar effects at all time points examined following the administration of 1.0 to 3.0 g/kg 2DOG. At a 2DOG dose of 0.25 g/kg lactate levels had decreased compared to un.injected controls (1.44 µmole/g brain, table 1) but not to the same extent as shown with the higher doses of 2DOG (1 to 3 g/kg).

Both 30MG and 2DOG are known to compete with glucose for transport across the blood-brain barrier (BBB). This most likely accounts for the initial decrease in brain glucose levels. 2DOG is then phosphorylated to 2DOG6P by hexokinase. The results of Horton et al22 have shown that high levels of endogenous 2DOG6P will cause inhibition of hexose phosphate isomerase (HPI) resulting in the observed decrease in lactate. As a consequence of this inhibition of HPI, Horton et al22 also documented a rise in glucose uptake we obtained plasma from animals pretreated with either 2DOG or 30MG. These plasma were subsequently used to determine the brain uptake indices (BUI, %) for C14-glucose (table 5). Using plasmas from both 30MG and 2DOG injected animals, BUI values were significantly lower than control values.

Discussion
The present investigation examined the effects of two glucose analogues, 30MG and 2DOG, on basal levels of rat brain glucose and lactate in an effort to evaluate their possible potential for minimizing the well-documented ischemia-induced rise in brain lactate. Our results showed that pretreatment with 30MG (2 g/kg) caused a decrease in brain glucose levels to 42% of control value and a decrease in lactate levels to 75% of control value (table 1). The effects of 2DOG on both glucose and lactate basal levels were much more pronounced. Following 2DOG administration (table 3), the effects on glucose were biphasic with an initial drop to 46% of the control value at 2.5 min followed by an increase to 290% of control value at 40 min which was sustained for 40 min. Lactate levels decreased to 36% of the control value at 10 min but returned to near control levels by 160 min. These results thus suggest that under certain experimental procedures both 30MG and 2DOG could be effective in attenuating the rise in brain lactate levels following an ischemic insult.

While both 2DOG and 30MG compete with glucose for transport across the blood-brain barrier,30 30MG has no other apparent effects on cerebral glucose metabolism. This is most likely due to the fact that 30MG does not serve as substrate for hexokinase24 and washes out of brain unaltered. The biochemical and behavioral effects of 2DOG, however, are more pronounced and longer lasting. Initially 2DOG competes with glucose transport at the BBB. This most likely explains the initial decrease in brain glucose levels. 2DOG is then phosphorylated to 2DOG6P by hexokinase. The results of Horton et al22 have shown that high levels of endogenous 2DOG6P will cause inhibition of hexose phosphate isomerase (HPI) resulting in the observed decrease in lactate. As a consequence of this inhibition of HPI, Horton et al22 also documented a rise in glu-

TABLE 4  
Effect of Varying Doses of 2-Deoxy-D-Glucose (iv) on Rat Whole Brain Glucose and Lactate  
(U moles/g brain)  

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.8±0.2</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>0.7±0.2</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>2.0</td>
<td>0.7±0.1</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>3.0*</td>
<td>0.4</td>
<td>2.6±0.2</td>
</tr>
</tbody>
</table>

2-deoxy-D-glucose dissolved in 2 ml of Ringer’s solution was administered slowly through a tail vein catheter over 30 sec. Following this injection the animals (220–240 g) were sacrificed at 2.5, 20 or 40 min as indicated by focused microwave for 2.5 sec. Brains were allowed to chill in situ, removed, weighed and homogenized in 2 volumes of 0.9N perchloric acid. Following centrifugation each supernatant was neutralized with 1 volume of 1.8N KHCO3, recentrifuged and supernatants frozen until chemical analyses were performed generally within 1 week.

*This approximates an LD90 for two to three rats.
TABLE 5  Analysis of BUI of Glucose in Conscious Animals Using Rat Plasma from 3-0-Methyl-D-Glucose or 2-Deoxy-D-Glucose Treated Rats

<table>
<thead>
<tr>
<th>Composition of Bolus</th>
<th>BUI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringer's solution (control)</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Plasma from rat injected with Ringer’s solution</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>Plasma from rat injected with 3-0-methyl-D-glucose</td>
<td>11 ± 2*</td>
</tr>
<tr>
<td>Plasma from rat injected with 2-deoxy-D-glucose</td>
<td>12 ± 3*</td>
</tr>
</tbody>
</table>

3-0-methyl-D-glucose or 2-deoxy-D-glucose treated rats (2 g/kg) dissolved separately in 2 ml of Ringer's solution were administered slowly through a tail vein catheter over 30 sec. Following the injection the animals (220-240 g) were sacrificed at 2.5 min by decapitation and trunk blood was collected in a glass tube coated with heparin. Whole blood was centrifuged for 15 min using table top centrifuge to obtain plasma which was kept frozen until use in the single bolus injection experiments usually within 1 week. Values are means ± SD for 3 rats.

* p < 0.05 compared to BUI values using plasma from rat injected with Ringer’s solution.

cose-6-P which affects hexokinase by end-product inhibition. This would explain the late increase in glucose above control levels as also shown in the present investigation. Despite these dramatic changes, both lactate and glucose time with return to basal levels. Thus the present investigation and the results of others show that 2DOG administration can result in considerable but transient derangement of cerebral glucose metabolism.

Many investigators have suggested that the rise in brain lactate following ischemia may be an important pathogenic factor rather than just symptomatic of the condition. The crucial event is not only the rise in lactate but also the concomitant increase in H+ ions with a subsequent decrease in intracellular pH. In a well designed series of experiments, Siesjo and his colleagues were able to control, somewhat, the level of lactic acid during incomplete brain ischemia (30 min). Under conditions of severe tissue lactic acidosis (30-40 μmol/gr), the brain showed persistent energy failure along with irreversible cell damage following 90 minutes of recirculation. When the tissue lactate rise was less severe (15-20 μmol/gr) they observed a recovery of the cortical energy state while histology studies revealed only discrete ribosomal changes. These studies suggest that there is a correlation between brain lactate levels and residual ischemic damage. Thus efforts at attenuating this observed rise could have beneficial effects.

The ability of a potentially therapeutic substance to penetrate the BBB is a necessary quality if this substance is to affect events within the brain. The use of 30MG or 2DOG has the advantage of utilizing the naturally existing glucose carrier protein at the BBB which is abundant throughout the brain. In addition, the development of any local burst firing might lead to a preferential accumulation of the glucose analogues compared to other unaffected brain regions due to the locally increased glycolytic demands.

The 'trickling blood supply' available to a focal ischemic area may, in the long run, be apparently deleterious to eventual tissue survival. Because of the relative state of anoxia in this focal ischemia area, glucose metabolism is accelerated with consequential buildup of lactate. It is possible that attenuation of this accelerated glucose metabolism can be accomplished by administering 30MG which would minimize glucose transport across the BBB. Alternatively, it might be necessary to cause a short term biochemical lesion of glucose metabolism. The present results suggest that, at the appropriately administered dose and time consideration, this could be accomplished using 2DOG.

Any long range potential for clinical application of either of these glucose analogues is hindered by a number of practical considerations. One, in particular, is that the solubility of these analogues is less than that of glucose and prohibitively large volumes of injectate could be required for human treatment if only one of these analogues were to be used. A combination of either of these analogues with phloretin, another competitor for BBB glucose transport, seems worthy of further study in animal models of ischemia. In addition, further investigations are needed to verify whether 2DOG loading on its own produces cellular damages similar to those observed in hypoglycemia. This is currently being explored.

It is suggested here that the proposed inhibition of glycolysis could ameliorate some undetermined portion of the deleterious effects of ischemia on brain. Although there undoubtedly are other etiologies of brain ischemic damage, it is probably worthwhile to correct any of these noxious influences that are recognized and can be clinically manipulated. There is a widespread therapeutic nihilism in the clinical management of ischemic attacks because it is felt that stroke represents a local effect of total ischemia and clinical care is not likely to be applied within the few minutes of total circulatory arrest required for irreversible damage. But angiographic studies support an incomplete ischemia in most infarcted areas and most ischemic dysfunction disappears or is markedly improved spontaneously with non-specific supportive data. Because of this reasoning, even small benefits biochemically resulting from practical therapy in the acute post-infarction period when the brain and its vasculature are most actively responding to the acute hypoperfusion could reap considerable benefits in long-term functional recovery.

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