

# Alteration of Glucose Metabolism in the Spinal Cord of Experimental Lead Poisoning Rats: Microdetermination of Glucose Utilization Rate and Distribution Volume

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**Abstract:** To examine the effects of lead on glucose metabolism in the spinal cord, glucose utilization rate (GUR) and distribution volume of glucose (DV) in the anterior horn and white matter were determined in 9 rats exposed to lead for 4 weeks and 10 control rats. The GUR and DV were determined by the quantitative microdetermination method using non-tracer amount of 2-deoxyglucose based on the three-compartments model of Sokoloff. The GUR and DV in the anterior horn in the lead-exposed rats were significantly lower than those in the controls. It is thus suggested that glucose metabolism, as measured by the GUR and DV, in the anterior horn is inhibited by lead; the anterior horn cells seem sensitive to lead neurotoxicity.

**Key words:** Glucose utilization rate, Distribution volume, 2,5-Hexanedione, Microdetermination, 2-Deoxyglucose, Spinal cord

Evidence has been given that inhibition of glucose metabolism is one of major effects of lead on the nervous system. The decrease in conversion of [<sup>3</sup>H] and [<sup>14</sup>C] glucose into lactate, citrate and malate was observed in cerebral cortex slices obtained from two groups of rats with mean blood lead (BPb) concentrations of 31.8 and 54.2  $\mu\text{g}/\text{dl}$ , respectively, which had been given drinking water containing 200 or 600 ppm of lead for 20 days<sup>1</sup>. The conversion rate of [<sup>14</sup>C] glucose to <sup>14</sup>CO<sub>2</sub> was reduced in brain capillaries isolated from cerebral cortex of calves with BPb of 118  $\pm$  0.19  $\mu\text{g}/\text{dl}$ , which were given 15 mg/kg bw of lead acetate

orally for 7 to 8 days, showing no histopathological changes<sup>2</sup>. Uptake of glucose analog (3-O-methylglucose) by the MEly cells (a cell line of mouse cerebral microvessel endothelium) was inhibited after the cells were incubated with a medium containing 10<sup>-4</sup> M of lead for 2 days<sup>3</sup>. As a more acute effect, Bertoni and Sprenkle (1988)<sup>4</sup> demonstrated the decrease of glucose utilization rate (GUR) in the medial geniculate bodies, inferior colliculus and sensory cortex of the brain of rats 45 min after intravenous injection of lead acetate, when the animals showed BPb concentrations of about 160  $\mu\text{g}/\text{dl}$ , using the 2-deoxyglucose autoradiographic method.

Recently, two of the authors developed a

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microdetermination method of GUR using an enzymatic amplification technique<sup>5,6</sup>. This method allows direct and quantitative measurement of GUR using non-radioactive deoxyglucose. In the present study, the method is applied to rats exposed to lead, to measure GURs in the white matter (posterior column) and anterior horn of the spinal cord, respectively, which have not been examined in the previous studies. Also, distribution volume of glucose (DV, ratio of glucose concentration in plasma to that in nervous tissue) is measured.

Twenty male Sprague-Dawley rats, aged 6 weeks, were used. Ten rats were fed for 4 weeks with drinking water containing 2.5% (w/v) of lead acetate (lead-exposed rats). The remaining rats were fed for the same period without administration of lead (control rats). At the start of experiment, their body weight was 120–140 g and were not significantly different between the two groups ( $p>0.05$ ). At the time of GUR measurement, none of the rats exhibited neurological signs such as paralysis of limbs. BPb concentrations, measured by flameless atomic absorption spectrophotometry (Hitachi Z-8000), in the lead-exposed rats were 125–244 (mean 209.8)  $\mu\text{g}/\text{dl}$ , which were significantly higher than those in the control rats (2–10  $\mu\text{g}/\text{dl}$  with a mean of 5.4) ( $p<0.01$ ). Body weight in the former group (190–210 g, mean 202) was significantly smaller than that in the latter group (250–280 g, mean 270) ( $p<0.05$ ).

GUR and DV were measured by the microdetermination method previously reported<sup>5,6</sup>. After the injection of 0.25 mmol/kg of 2-deoxyglucose into the tail vein, concentrations of 2-Deoxyglucose, 2-deoxyglucose 6-phosphate, glucose and glucose 6-phosphate in the nervous tissue and blood were measured by the fluorometric microassay with an enzymatic amplification technique. From these values, GUR was calculated as the net rate of utilization of glucose, i.e. the net rate of glucose 6-phosphate formation, which equals the difference between the rate of glucose phosphorylation by hexokinase and glucose 6-phosphate dephosphorylation by phosphatase, in the nervous tissue, based on the three-compartments model of Sokoloff<sup>7</sup>. Similarly, DV was calculated as the ratio of glucose concentration in the nervous tissue to that in plasma.

GUR and DV in the anterior horn in the lead-exposed rats were significantly lower than those in the control rats, respectively (Table 1). It is thus suggested that utilization as well as uptake of glucose were deteriorated by lead in the anterior horn of the spinal cord. This is in line of observations of the adverse effects of lead on glucose metabolism<sup>1–4</sup>.

The anterior horn cell (spinal motor neuron) bodies have

**Table 1. Glucose utilization rate (GUR)<sup>a</sup> and distribution volume (DV)<sup>b</sup> in 10 control and 9 lead-exposed rats: means with ranges in parentheses**

	Controls	Lead-exposed
GUR:		
Anterior horn	6.45 (4.19–9.16)	4.52 (3.54–6.94)*
White matter	2.25 (0.82–3.99)	2.02 (1.24–2.72)
DV:		
Anterior horn	0.19 (0.17–0.24)	0.15 (0.12–0.17)**
White matter <sup>c</sup>	0.12 (0.11–0.14)	0.11 (0.09–0.15)

<sup>a</sup>  $\mu\text{mol}/\text{min}/\text{g}$  dry weight tissue. <sup>b</sup>  $\text{ml}/\text{g}$  dry weight tissue. <sup>c</sup> Data for one lead-exposed rat was not obtained because of technical reason. \*.\*\* Significantly different from controls at  $p<0.05$  and  $p<0.01$ , respectively (Student's *t*-test).

the highest values of GUR and DV in the spinal cord<sup>5</sup>). It is likely that they have a high energy demand for their physiological activities, such as axonal transport, a mechanism to supply materials required by axons for maintenance of their physical integrity. As we have observed the impairment of slow axonal transport in lead-exposed rats<sup>8</sup>), it may be hypothesized that the decreases of GUR and DV in the anterior horn observed in the present study reflected deterioration of glucose metabolism in the motor neuron cell bodies. Application of the single cell technique<sup>5,6</sup> would confirm this hypothesis.

Degeneration, reduction in diameter, and retardation of regrowth have been observed in peripheral nerve axons of lead-poisoned experimental animals<sup>9–11</sup>). Slowing of peripheral nerve conduction is usually minimal in lead neuropathy<sup>12</sup>), suggesting that changes in the nerve are mainly axonal degeneration with or without secondary demyelination. These findings also agree the hypothesis that the neuron cell bodies are affected by lead, resulting in a failure to supply materials required by axons. Beritic (1989)<sup>13</sup>) suggested in his review that lead neuropathy is of spinal origin, starting in the cell bodies of the anterior horn cells in the spinal cord.

By contrast, the white matter of the spinal cord did not show significant alterations in GUR or DV in the present study. This suggests that the axons and Schwann cells are not main targets of lead toxicity. On the other hand, the GUR in the white matter of the spinal cord was decreased and related to blood 2,5-hexanedione (HD) concentrations in 2,5-HD exposed rats<sup>14</sup>), in accordance with the findings that the white matter is affected greatly by 2,5-HD<sup>15</sup>). Thus, it is probable that the measurement of GUR and DV reflects the biochemical changes underlying neurotoxicity;

application of the method presented here could provide information for understanding the mechanisms of toxicity.

The present study did not demonstrate significant correlation between BPb concentrations and GUR or DV ( $p > 0.05$ ). This may be due to a small number of rats. Examination of GUR and DV in a larger number of lead-exposed rats especially at a lower BPb level will be necessary.

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