

Decrease of Glucose Utilization Rate in the Spinal Cord of Experimental 2,5-Hexanedione Poisoning Rats: Application of Microdetermination Technique

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Abstract: To examine the effects of 2,5-hexanedione (HD) on the glucose metabolism in the spinal cord, glucose utilization rate (GUR) and distribution volume of glucose (DV) were measured in the white matter and anterior horn of the spinal cord in 8 rats exposed to HD for 4 weeks and in 10 control rats. The GUR and DV were determined by the quantitative microdetermination method using non-tracer 2-deoxyglucose based on the three-compartments model of Sokoloff. GURs in the white matter and anterior horn and DV in the anterior horn in the HD-exposed rats were significantly lower than those in the control rats. In the multiple regression analysis, GUR in the white matter of HD-exposed rats was significantly related to blood HD concentration. It is suggested that the decrease of GUR in the white matter is a major effect of HD in the spinal cord.

Key words: Glucose utilization rate, Distribution volume, 2,5-Hexanedione, Microdetermination, 2-Deoxyglucose, Spinal cord, White matter, Anterior horn

Introduction

n-Hexane and methyl n-butyl ketone (MnBK) induce changes in the nervous system characterized by swelling accompanied with accumulation of neurofilaments in distal portion of axons in the peripheral nerves and central nervous tissue¹⁻⁶. 2,5-Hexanedione (2,5-HD) is considered to be the common neurotoxic metabolite of n-hexane and MnBK^{1,6}. It has been observed that this compound also affects the central nervous system function such as delay in the latencies of auditory brainstem response and somatosensory-evoked potentials⁷.

Although pyrrole-mediated crosslinking of neurofilament proteins by 2,5-HD probably initiates the accumulation of neurofilaments^{1,8-14}, a bulk of investigations¹⁵⁻²⁰ suggested that a direct effect of 2,5-HD on the nervous tissue is the inhibition of enzymes in glycolysis, leading to studies on glucose utilization rate (GUR, the rate of glucose utilization in the tissue) in experimental 2,5-HD poisoning. Griffiths *et al.*²¹ observed reduction in GUR in the superior colliculus of rats exposed to 0.5% 2,5-HD in drinking water for 3 weeks, preceding the morphological changes in the optic pathways to the superior colliculus which were seen after 5 weeks of exposure. Planas and Cunningham²² examined GURs in various regions of brain in rats received 2,5-HD of 500 mg/kg/day for 15 days and of 250 mg/kg/day for 21 days; they

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found significant decrease in GUR in the inferior colliculus in the former group of rats. This group also examined the effects of single administration of 500 mg/kg of 2,5-HD, and found that GUR decreased in the brain cortex, inferior colliculus and hippocampus only 3 hours after dosing. Thus, the decrease in GUR is an early effect of 2,5-HD on the central nervous system.

Recently, two of the authors established a fluorometric microassay technique for determination of glucose and glucose 6-phosphate using an enzymatic amplification method²³. Using this technique, they developed a quantitative microdetermination method of GUR^{24,25}. This method allows direct and quantitative measurement of GUR using non-radioactive (non-tracer) deoxyglucose whereas in the previous studies^{21, 22} radioactive deoxyglucose was used. By this method, distribution volume of glucose (DV, ratio of glucose concentration in the nervous tissue to that in plasma), as a marker of uptake of glucose from plasma into the nervous tissue, can be also measured^{24, 25}.

It is believed that glucose is the obligatory energy substrate of the brain²⁶. The above microdetermination technique revealed that the GUR and DV in the spinal cord as well as the dorsal root ganglion are almost same as those in the brain tissue^{24, 25}. Also, it was observed that blocking of glycolysis affects the peripheral nerve function such as axonal transport²⁷ and membrane depolarization after impulse activity²⁸. Thus, glucose utilization should play key roles in both the central and peripheral nervous systems.

In the present study, to examine the effects of 2,5-HD on glucose metabolism in the spinal cord, we measure GUR in the white matter (posterior column) and anterior horn of the spinal cord, respectively, which has not been examined in the previous studies. Also, DV in these tissues is measured. Dose-effect relationships between 2,5-HD and GUR and DV are assessed using blood 2,5-HD concentration. As 2,5-HD (and n-hexane) affects mainly nerve fibers in the white matter in the spinal cord²⁻⁵, it is expected that more profound change in the GUR or DV is observed in the white matter than in the anterior horn.

Materials and Methods

Animals

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 2,5-HD (HD-exposed rats); this dosing was expected to cause definite neurological changes within a period shorter than those reported using lower concentrations of 2,5-HD in drinking water, i.e. 0.4% for

78 days²⁹, 0.5% for 5 weeks²¹, 6 weeks³⁰, 2 months¹⁵ and 10–12 weeks¹⁷, and 1% for 70 days¹¹. The remaining rats were fed for the same period without administration of 2,5-HD (control rats).

At the start of experiment, body weight of rats was 120–140 g and not significantly different between the two groups ($p > 0.05$). During the period of 2,5-HD administration, two of the HD-exposed rats died. At the time of GUR measurement, the HD-exposed rats exhibited neurological signs such as paralysis of limbs; body weight in the HD-exposed rats (90–130, mean 106) was significantly smaller than that in the control rats (250–280 g, mean 270) ($p < 0.05$).

Measurement of GUR and DV

GUR and DV were measured by the quantitative microdetermination method previously reported^{24, 25}. The rats were loosely fitted to a plastic holder and 0.25 mmol/kg of 2-deoxyglucose in 0.2 ml of physiological saline was injected within 5 sec into their tail vein. The rats were then immersed in liquid nitrogen 4 min later to fix the nervous tissue in a short period. According to the method of Lowry and Passonneau³¹, freeze-dried sections (14–25 μm) were prepared from the lumbar spinal cord; small pieces (0.7–2.3 μg dry weight) of the white matter and anterior horn were dissected out from the sections. Frozen blood clots were also collected from the left ventricles and used for analyses.

2-Deoxyglucose, 2-deoxyglucose 6-phosphate, glucose and glucose 6-phosphate in the tissue and blood clot samples were measured by the fluorometric microassay after they were amplified using an enzymatic amplification method²³. Using concentrations of these substances in the tissue and blood clot samples, 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^{32, 33}. Similarly, DV was calculated as the ratio of glucose concentration in the nervous tissue to that in plasma.

Measurement of 2,5-HD in blood

2,5-HD was extracted from the blood clot samples by dichloromethane after the samples were hydrolyzed using hydrochloric acid; concentration of 2,5-HD in the extract was measured by gas chromatography-mass spectrometry (Shimadzu GC14 QP1000), using a OV-1 capillary column (Nihon Chromato)³⁴.

Statistical analysis

Differences in blood 2,5-HD concentration, GUR and body weight between the HD-exposed and control rats were analyzed by Student's *t*-test. Relationships of GUR and DV to blood 2,5-HD concentration and body weight were analyzed by multiple regression analysis (fixed variable method). The analysis was performed by SPSS ver 6.1 (SPSS Japan) using a microcomputer.

Results

GUR, DV and blood 2,5-HD concentrations in the HD-exposed and controls rats are shown in Table 1. GUR in the white matter and anterior horn and DV in the anterior horn in the HD-exposed rats were significantly lower than those in control rats, respectively. Blood 2,5-HD concentration was significantly increased in the HD-exposed rats as compared with the controls.

Table 1. Glucose utilization rate (GUR)^a, distribution volume (DV)^b and blood 2,5-hexanedione (HD)^c concentrations in 8 HD-exposed and 10 control rats: means with ranges in parentheses

	HD-exposed rats	Control rats
GUR:		
White matter	1.46 (0.97–1.84)**	2.25 (0.82–3.99)
Anterior horn	3.48 (2.75–4.13)*	6.45 (4.19–9.16)
DV:		
White matter	0.12 (0.09–0.16)	0.12 (0.11–0.14)
Anterior horn	0.13 (0.11–0.14)***	0.19 (0.17–0.24)
Blood HD	2103.8 (105.8–6631.4)*	1.46 (1.0–1.9)

^a μmol/min/g dry weight tissue. ^b ml/g dry weight tissue. ^c μg/dl. * ** *** Significantly different from controls at p<0.05, p<0.01, and p<0.001, respectively.

Table 2. Regressions of GUR and DV on HD and body weight in 8 HD-exposed rats^a: multiple regression analysis

Dependent variable	R ^b	Standardized regression coefficients for independent variables	
		HD	Body weight
GUR:			
White matter	0.955*	- 0.977**	- 0.342
Anterior horn	0.509	- 0.522	- 0.160
DV:			
White matter	0.250	- 0.198	- 0.207
Anterior horn	0.509	- 0.522	- 0.160

^a GUR, DV and HD as in Table 1. ^b Multiple regression coefficient. *p<0.05; **p<0.01.

In the multiple regression analysis, GUR in the white matter was significantly related to blood 2,5-HD in the HD-exposed rats (Table 2). Figure 1 illustrates the relationship of GUR in the white matter to blood 2,5-HD concentration in these rats.

Discussion

GUR in the white matter was significantly decreased in rats exposed to 2,5-HD, with its mean blood concentration of 2103.8 (range 105.8–6631.4) μg/dl. It has been observed in rats that, 6 hours after the end of 6-hr exposure to 1000 ppm of n-hexane, blood 2,5-HD reached a peak of approximately 150 μg/100 g³⁵. Similarly, 4 hours after the end of exposure to 5000 ppm of n-hexane for 12 and 24 hours, blood 2,5-HD reached a peak of 2950 and 8890 μg/dl on average, respectively, which correspond to chronic exposure to 10–140 ppm of n-hexane in humans³⁶. Thus, it seems that exposure levels of the 2,5-HD rats in the present study are equivalent to the level between the previous two studies. However, extent of chronic exposure which corresponds to the blood 2,5-HD levels in the present study remains to be investigated further.

GUR was inversely related to blood 2,5-HD after controlling for body weight by the multiple regression analysis, indicating that its decrease in the white matter was

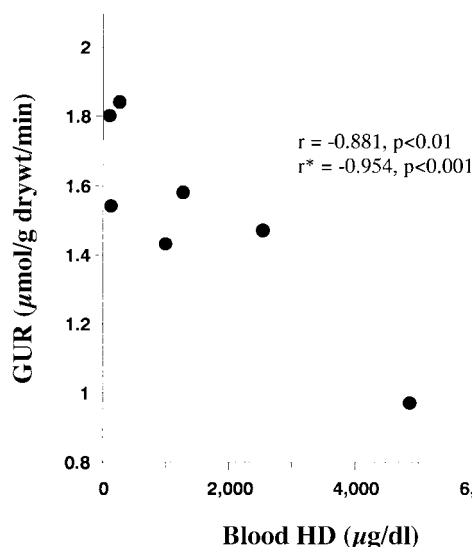


Fig. 1. Relationship between blood 2,5-hexanedione (HD) concentrations and glucose utilization rate (GUR) in the white matter of spinal cord in 8 HD-exposed rats. r=Pearson's correlation coefficient. r*=Partial correlation coefficient controlling for body weight.

not due to a debility (body weight loss). GUR in the anterior horn was also decreased in these rats, but did not significantly related to blood 2,5-HD. It is therefore suggested that GUR in the white matter was more sensitive to neurotoxicity of 2,5-HD than GUR in the anterior horn. Thus, the present study revealed a dose-dependent effect of 2,5-HD on the spinal cord, in addition to the previous observations of decreased GURs in the brain in rats exposed to 2,5-HD^{21, 22}.

DV of the anterior horn was also decreased in the present study, i.e. glucose uptake in the tissue was diminished in the HD-exposed rats. However, the change in DV was not significantly related to the blood 2,5-HD concentration. Therefore, it is still unclear whether the changes observed in the anterior horn were related directly to 2,5-HD neurotoxicity.

Degeneration of myelinated fibers in the white matter such as the ventromedial and ventrolateral tracts of the spinal cord has been observed in *n*-hexane poisoned rats²⁻⁵. The decreased GUR in the white matter related to blood 2,5-HD in the present study agrees with these histopathological observations. On the other hand, changes in the anterior horn, which is rich in motor neuron cell bodies and surrounding neuropils, were not related to blood 2,5-HD, suggesting that these neuronal components are not primary targets of 2,5-HD. Changes in glucose metabolism, as measured by GUR, thus reflected histopathological findings of 2,5-HD neuropathy. Further study is required to elucidate which of the effects of 2,5-HD on glycolytic enzymes¹⁵⁻²⁰ is most strongly related to the decrease of GUR.

Role of pyrrole-mediated crosslinking of neurofilament proteins or the changes in glucose metabolism in the pathogenesis of 2,5-HD (and *n*-hexane) neuropathy is still a matter of speculation. The crosslinking of neurofilaments is assumed to lead to impairment of their transport along the axon, resulting in the accumulation^{1, 8-14}. It is unclear whether such change is a single cause of axonal degeneration, however. The alterations in glucose metabolism might divert energy production away from the maintenance of structure and function of the axon, such as axonal transport and impulse conduction. Furthermore, as a variety of toxic effects of 2,5-HD have been observed, such as alteration in lipid metabolism in peripheral nerves³⁷, release of acetylcholinesterase from muscles³⁸, inhibition of mitochondrial respiration in brain³⁹ and infertility due to testis injury⁴⁰, it is likely that this compound has multiplicity in its neurotoxic actions.

Akabayashi and Kato²⁴ reported that GUR was $2.08 \pm 0.23 \mu\text{mol/g dry weight/min}$ and DV was $0.100 \pm 0.018 \text{ ml/g dry weight}$ in the white matter of the spinal cord in normal

rats. The corresponding measures in the control rats in the present study (mean 2.25 and 0.12) are close to these values. Also, GUR and DV in the anterior horn were reported to be $5.20 \pm 0.61 \mu\text{mol/g dry weight/min}$ and $0.147 \pm 0.013 \text{ ml/g dry weight}$, respectively, in normal rats²⁴. These values were somewhat lower than those in the present study (mean 6.45 and 0.19, respectively); this is probably due to spinal motor neuron cell bodies, which showed much higher GUR ($9.41 \pm 2.25 \mu\text{mol/g dry weight/min}$) and DV ($0.188 \pm 0.021 \text{ ml/g}$), were excluded from the anterior horn in the previous study²⁴. Thus, the technique used in the two studies have high reproducibility. As this technique applicable to single neuronal cell bodies and neuropils²⁵, it seems useful in understanding mechanisms underlying various neurotoxic disorders.

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