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CEREBRAL GLYCOLYSIS AND GLYCOGENOLYSIS IN DIAZINON TREATED ANIMALS

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Treatment with diazinon (40 mg/kg, i. p.) resulted in depletion of glycogen and increased activities of glycogen phosphorylase, phosphoglucomutase, hexokinase and lactate dehydrogenase in the brain of rats. The activity of glucose-6-phosphatase was not significantly changed. The level of lactic acid in the blood was raised, while that of pyruvic was not altered. The induced changes may be due to a compensatory mechanism providing extra energy on account of stimulatory effects in diazinon treated animals.

Diazinon (0,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate) is a widely used organophosphorus compound which has been reported to cause toxic effects in man (1, 2), rats (3) and other animals (4, 5). Organophosphorus compounds besides inhibiting cholinesterase and other enzymes (5) induce hyperglycaemia (6, 7) which has not been investigated in detail. The present report deals with changes in cerebral glycolysis and glycogenolysis in rats treated with diazinon.

MATERIAL AND METHODS

Adult female albino rats, 150 ± 10 g, which were kept separate from the males ten weeks before experiments, were used. The animals had food and water *ad libitum* except for 18 hours before experiments so as to obtain more uniform results. The experimental animals were injected with diazinon (40 mg/kg, i. p.), whereas controls received normal saline. The animals were killed 2 hours after receiving diazinon. The skull was opened and the brainstem was quickly removed. Glycogen was extracted

according to the method of *Lebaron* (8) and estimated colorimetrically as described by *Montgomery* (9). Cholinesterase activity (E.C. 3.1.1.7.) was measured by the method of *Ellman and co-workers* using acetylthiocholine as substrate (10). Levels of glycogen phosphorylase (E.C. 2.4.1.1.) and glucose-6-phosphatase (E.C. 3.1.3.9) were assayed by the method of *Hers and Hoof* (11). Phosphoglucomutase (E.C. 2.7.5.1) activity was determined according to the procedure described by *Najjar* (12). Cerebral hexokinase (E.C. 2.7.1.1.) activity was assayed by the procedure of *Crane and Sols* (13) and lactate dehydrogenase (E.C. 1.1.1.27) by the method of *Kornberg* (14). Lactic and pyruvic acids in the blood were measured by the method of *Barker and Summerson* (15) and of *Friedman and Hauger* (16), respectively.

The data were analysed by means of Student's t-test and significant differences between the means were determined.

RESULTS

The results indicate significantly low levels of cerebral glycogen in diazinon treated animals (Table 1). The level of lactic acid in the blood was increased while that of pyruvic acid was not significantly different from the control values.

Table 1.

Effect of diazinon (40 mg/kg i. p.) on the level of cerebral glycogen, blood lactic acid and pyruvic acid in rats. Animals were killed 2 hours after the administration of diazinon. The results are expressed as means of eight rats ± SEM.

	Cerebral glycogen (mmol/kg)	Lactic acid (mmol/L)	Pyruvic acid (mmol/L)
Controls	6.05 ± 0.33	1.41 ± 0.19	0.326 ± 0.077
Diazinon	4.22 ± 0.32*	2.23 ± 0.20*	0.340 ± 0.078

* $P < 0.02$

Brain acetylcholinesterase activity was also significantly reduced (Table 2) in diazinon treated animals which had tremor and mild convulsions.

The activities of glycogen phosphorylase, phosphoglucomutase and hexokinase were increased while that of glucose-6-phosphotase was not significantly altered in diazinon treated animals (Table 2). Lactate dehydrogenase activity was significantly higher than the control values (Table 2).

Table 2.

Effect of diazinon (40 mg/kg, i. p.) on the level of hexokinase, cholinesterase and glycogenolytic enzymes in the brain of rats. Animals were killed 2 hours after the administration of diazinon. Each figure represents the mean ± S. E. of eight rats.

Enzyme activities	Controls	Diazinon
Acetyl cholinesterase[a]	22.54 ± 0.82	10.21 ± 0.88*
Hexokinase[b]	3.89 ± 0.48	4.91 ± 0.43**
Lactate dehydrogenase[c]	245.41 ± 6.54	285.6 ± 8.81**
Glycogen phosphorylase[d]	28.57 ± 1.83	39.10 ± 1.79**
Phosphoglucomutase[e]	8.12 ± 0.88	12.05 ± 0.84**
Glucose-6-phosphatase[f]	2.03 ± 0.25	2.09 ± 0.28

* $p < 0.01$
** $p < 0.02$

a = μmoles of acetylthiocholine hydrolysed/min/g wet tissue.
b = μmoles of glucose phosphorylated/min/mg protein.
c = nmoles of NADH oxidized/min/mg protein.
d = μmoles of Pi formed/min/g wet tissue.
e = μmoles of Pi stable formed/min/g wet tissue.
f = μmoles of Pi liberated/min/g wet tissue.

DISCUSSION

The significantly low levels of cholinesterase activity, (Table 2) are consistent with the accumulation of acetylcholine in the brain of diazinon treated animals (3). The results further indicate a depletion of cerebral glycogen in diazinon treated animals (Table 1) which also showed tremors or convulsions. It was previously reported that the level of cerebral glycogen was influenced by the state of activity of the brain. The glycogen content of the brain was increased after anaesthesia and administration of certain barbiturates and sedatives (17, 18). Certain organophosphorus compounds have been reported to increase the level of cyclic AMP in the brain (19) which is believed to regulate the storage of glycogen (20). The results further indicate an increase in the activity of cerebral glycogen phosphorylase (Table 2) which converts glycogen to glucose-1-phosphate.

According to our results, the activity of phosphoglucomutase is also increased (Table 2) thus promoting the formation of glucose-6-phosphate. The increased cerebral hexokinase activity in diazinon treated animals (Table 2) further favours the formation of glucose-6-phosphate from glucose which is the main source of energy in the brain (21). It was previously reported that organophosphorus compounds are powerful inhibitors of dehydrogenase activity inducing anoxia or interfering in tissue respiration (22, 23). Thus the increased level of lactic acid (Table 1) is suggestive of glycogenolysis under anaerobic conditions. This is also consistent with the increased activity of lactate dehydrogenase in diazinon treated animals (Table 2). The changes in the activity of various enzymes may be a compensatory mechanism to provide extra energy by mobilizing glycogen or glucose on account of hyperexcitability and stimulatory effects in diazinon treated animals (Tables 1 and 2).

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Sažetak

GLIKOLIZA I GLIKOGENOLIZA U MOZGU ŽIVOTINJA TRETIRANIH DIAZINONOM

Davanje diazinona (40 mg/kg i. p.) imalo je za posljedicu iscrpljenje glikogena u mozgu štakora te povećanu aktivnost glikogen fosforilaze, fosfoglukomutaze, heksokinaze i laktat dehidrogenaze. Aktivnost glukoza-6-fosfataze nije bila značajnije promijenjena. Razina mliječne kiseline u krvi bila je povećana, dok se koncentracija pirogrožđane kiseline nije mijenjala. Ove inducirane promjene mogu biti posljedica kompenzacijskog mehanizma da bi se stvorila dodatna energija kao posljedica stimulatornih učinaka diazinona u trovanih životinja.

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