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CEREBRAL GLYCOGENOLYSIS AND GLYCOLYSIS IN MALATHION-TREATED HYPERGLYCAEMIC ANIMALS

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Abstract—Treatment with malathion resulted in an increase in the level of blood glucose and lactate and reduced cerebral glycogen, 2 hr after its administration. The blood pyruvate level was not changed. The activities of glycogenolytic enzymes (glycogen phosphorylase and phosphoglucomutase) were increased significantly in the brain, whereas that of glucose-6-phosphatase remained unchanged. The activity of the glycolytic enzyme-hexokinase was increased significantly in malathion-treated animals, whereas those of the glucose-6-phosphate and lactate dehydrogenases were not significantly changed. The changes in enzyme activities may be a compensatory mechanism to provide energy in the form of glucose to cerebral tissue on account of stimulatory effects in malathion-treated animals.

Malathion is an organophosphorous compound which inhibits cholinesterase leading to stimulatory effects, tremors and convulsions [1–3], and also induces hyperglycaemia [4]. It was also reported that hyperglycaemia induced by malathion is accompanied by depletion of glycogen in certain brain regions of rats [4]. Since the cerebral glycogen content is regulated by various factors and enzymes [5], we determined changes in the level of glycolytic and glycogenolytic enzymes in the brain of malathion-treated hyperglycaemic animals.

METHODS

Adult female albino rats, 150 ± 10 g, were used. The female rats were kept separate from the males 10 weeks before experiments. The animals had food and water *ad lib.* except for 18 hr before experiments; this resulted in more uniform results. The animals were injected with malathion (500 mg/kg, i.p.) and observed for tremors or convulsions, if any. They were decapitated 2 hr after treatment. Controls received normal saline. The blood glucose level was determined by the method of Nelson [6]. The glycogen was extracted according to the method of Lebaron [7] and estimated colorimetrically as described by Montgomery [8]. For enzyme estimations, brain was homogenized in ice-cold distilled water to give a 10% homogenate (w/v); the homogenate was diluted to 1% (w/v) which was used for the assay of glycogen phosphorylase [9] (EC 2.4.1.1), glucose-6-phosphatase (EC 3.1.3.9) and phosphoglucomutase [10] (EC 2.7.5.1). Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity was assayed according to the method of Kornberg and Horacker [11] and lactate dehydrogenase (EC 1.1.1.27) by the method of Kornberg [12]. Cerebral cholinesterase activity (EC 3.1.1.7) was determined by the method of Ellman *et al.* [13]. For the assay of hexokinase [14] (EC 2.7.1.1), a 10% homogenate in a medium containing 0.15 M KCl, 0.005 M EDTA and 0.005 M $MgCl_2$ (pH 7) was made and centrifuged at 500 *g* for 10 min in a refrigerated centrifuge at 0°; the supernatant fraction was used for enzyme estimation. The sample of the enzyme being assayed also gave a correct assay when added to the assay system of the tissue sample. Protein was estimated according to the method of Lowry *et al.* [15]. Lactic acid and pyruvic acid in the blood were measured by the method of Barker and Summerson [16] and Theodore *et al.* [17] respectively.

The data were analysed statistically using Student's *t*-test, and significant differences between the means were determined.

RESULTS

Changes in the levels of blood glucose, lactate and pyruvate and the glycogen content of brain in malathion-treated animals are given in Table 1. The results indicate that malathion increased the levels of blood glucose and lactate, whereas that of pyruvate was not changed significantly. The glycogen content of the brain was reduced in malathion-treated animals. The activities of glycogen phosphorylase and phosphoglucomutase were increased significantly, whereas the activity of glucose-6-phosphatase was not changed (Table 2). The activity of hexokinase was increased, whereas the activities of glucose-6-phosphate dehydrogenase and lactate dehydrogenase were not altered significantly in malathion-treated animals (Table 3). The cerebral cholinesterase activity was reduced significantly in malathion-treated animals which also developed tremors and mild convulsions (Table 1).

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Table 1. Effect of malathion (500 mg/kg, i.p.) on the levels of blood glucose, lactate and pyruvate, and on cerebral glycogen and cholinesterase activities of rats

Treatment	Blood			Brain	
	Glucose (mg/100 ml)	Lactate (mg/100 ml)	Pyruvate (mg/100 ml)	Glycogen (mg/100 g)	Cholinesterase*
Control	94.60 ± 3.34	12.67 ± 1.36	2.82 ± 0.68	94.65 ± 1.88	22.68 ± 0.83
Malathion	200.50 ± 4.06†	22.67 ± 1.58†	3.01 ± 0.67	60.05 ± 1.73†	9.74 ± 0.76†

Animals were killed 2 hr after treatment. Each group consisted of eight animals. Values are means ± SE.
* Cholinesterase activity is expressed as moles of acetylthiocholine hydrolysed/min/g × 10^{-6}.
† Significantly different from control value ($P < 0.01$)

Table 2. Changes in the level of glycogenolytic enzymes in cerebral tissues of malathion (500 mg/kg, i.p.) treated rats

Treatment	Glycogen phosphorylase (μmoles of P_i formed/min/g tissue)	Phosphoglucomutase (μmoles of acid stable P_i formed/min/g tissue)	Glucose-6-phosphatase (μmoles of P_i liberated/min/g tissue)
Control	28.57 ± 1.83	8.12 ± 1.88	2.03 ± 0.25
Malathion	38.16 ± 1.81*	12.86 ± 1.89*	2.07 ± 0.24

Animals were killed 2 hr after treatment. Each group consisted of eight animals. Values are means ± SE.
* Significantly different from the control value ($P < 0.01$).

Table 3. Effect of malathion (500 mg/kg, i.p.) on the levels of hexokinase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase activities in cerebral tissue of rats

Treatment	Brain enzymes		
	Hexokinase (μmoles of glucose phosphorylated/min/mg protein)	Lactate dehydrogenase (nmoles of NADH oxidized/min/mg protein)	Glucose-6-phosphate dehydrogenase (nmoles of NADP reduced/min/mg protein)
Control	3.89 ± 0.48	245.40 ± 8.05	13.08 ± 2.35
Malathion	5.38 ± 0.45*	290.30 ± 9.01	14.98 ± 2.34

Animals were killed 2 hr after treatment. Each group consisted of eight animals. Values are means ± SE.
* Significantly different from the control value ($P < 0.01$).

DISCUSSION

It was reported previously that the activity of hexokinase [18], an enzyme of the glycolytic pathway, is fairly high in cerebral tissue. According to our results, treatment with malathion resulted in a significant increase in the cerebral hexokinase activity (Table 3) which causes phosphorylation of glucose to glucose-6-phosphate. An increase in cerebral hexokinase activity during convulsions was also found by Bertram *et al.* [19]. The depletion of cerebral glycogen in malathion-treated animals (Table 1) was accompanied by an increase in the activity of glycogen phosphorylase (Table 2) which decomposes glycogen to glucose-1-phosphate. Further, according to our results (Table 2), the activity of phosphoglucomutase was also enhanced, tending formation of glucose-6-phosphate from glucose-1-phosphate. Thus, both the glycolytic and glycogenolytic enzymes were activated, causing greater formation of glucose-6-phosphate from glucose which is the main source of energy for the brain [20]. However, the activity of glucose-6-phosphatase was not altered (Table 2), nor was that of lactate dehydrogenase (Table 3), which is mainly involved in anaerobic glycolysis. The level of pyruvic acid was also not altered significantly after treatment with malathion (Table 1). According to Huckabee [21], lactic acid concentration depends on changes in pyruvate production as well as changes in cellular respiration. Thus, an increase in the concentration of lactic acid in malathion-treated animals (Table 1), without any significant change in pyruvate, may be related to changes in cellular respiration. It was reported previously that organophosphorous compounds interfere with oxygen uptake [22] and depress the respiration of brain *in vitro* as well as *in vivo* [1, 23]. These changes may favour the metabolism of glucose to lactic acid which, according to our results, was increased significantly in malathion-treated animals (Table 1). Lastly, treatment with malathion did not

change significantly the glucose-6-phosphate dehydrogenase activity (Table 3), which suggests that oxidation of glucose through the hexose monophosphate pathway was not altered in malathion-treated animals. The induced changes in the activity of various enzymes may provide extra energy by mobilizing glycogen or glucose under anaerobic conditions to account for the hyperexcitability or stimulatory effects in malathion-treated animals.

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