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MODIFICATION OF DIAZINON-INDUCED CHANGES IN CARBOHYDRATE METABOLISM BY ADRENALECTOMY IN RATS

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Abstract—Treatment with diazinon (40 mg/kg, i.p.) resulted in hyperglycemia and depletion of glycogen from cerebral and peripheral tissues 2 hr after its administration in rats. The activities of the glycogenolytic enzymes glycogen phosphorylase and phosphoglucomutase were increased significantly in brain and liver, whereas that of glucose-6-phosphatase was not altered. The activities of the glycolytic enzymes hexokinase and lactate dehydrogenase were increased only in the brain. The cholinesterase activity of the brain was reduced by treatment with diazinon. The activities of the hepatic gluconeogenic enzymes fructose 1,6-diphosphatase and phosphoenolpyruvate carboxykinase were also increased significantly in diazinon-treated animals. The level of lactate was increased in brain and blood, whereas that of pyruvate was not changed. The activity of glucose-6-phosphate dehydrogenase was not changed significantly. The cholesterol and ascorbic acid contents of adrenals were depleted in diazinon-treated animals. The hyperglycemia and changes in carbodyhdrate metabolism were abolished by adrenalectomy, suggesting the possible involvement of the adrenals in the induced changes in diazinon-treated animals.

Diazinon, an organophosphorous compound, is commonly used in the control of agricultural and household pests. It inhibits cholinesterase activity producing central stimulatory effects—hyperexcitability, tremors and convulsions owing to accumulation of acetylcholine in the brain [1, 2]. The compound also produces hyperglycemia [3, 4] via an unknown mechanism. The hormones of the adrenal glands—catecholamines and corticosterids—are often involved in the production of hyperglycemia or changes in carbohydrate metabolism. The aim of the present study was to investigate the role of the adrenals in the production of certain changes in carbohydrate metabolism in diazinon-treated animals.

MATERIALS AND METHODS

Adult female albino rats, 160–180 g, were maintained on a 12-hr light–dark cycle and had food and water *ad lib*. The female animals were separated from the males 10 weeks before experiments. They were fasted for 18 hr before use since this produced more uniform results. The animals were divided into three groups. Animals of group one served as controls and were given normal saline. Those of group two were injected with diazinon (40 mg/kg, i.p.). The animals of group three consisted of adrenalectomized animals; bilateral adrenalectomy was performed under light ether anesthesia. The adrenalectomized animals were given 1% sodium chloride in drinking water. They were treated with diazinon (40 mg/kg, i.p.) 10 days after adrenalectomy. The animals showed hyperactivity and convulsions. The animals were decapitated 2 hr after the last treatment. Blood was collected in heparinized tubes for the estimation of glucose by the method of Nelson [5]. Brain, liver and adrenals were dissected and weighed quickly. Glycogen content of brain and liver was extracted according to the method of LeBaron [6] and estimated colorimetrically as described by Montgomery [7]. Glycogenolytic enzymes were assayed in 1% homogenate prepared in ice-cold 0.25 M sucrose. Glycogen phosphorylase (EC 2.4.1.1) and glucose-6-phosphatase (EC 3.1.3.9) were assayed by the method of Hers and Hoof [8] and phosphoglucomutase (EC 2.7.5.1) by the method of Najjar [9]. For assaying hexokinase (EC 2.7.1.1) activity, tissue was homogenized in a medium containing 0.15 M KCl, 0.005 M EDTA and 0.04 M $MgCl_2$. For lactate dehydrogeanse (EC 1.1.1.27), phosphate buffer (pH 7.4) was used. Both the enzymes were assayed according to the procedures of Crane and Sols [10] and Kornberg [11] respectively. Fructose 1,6-diphosphatase (EC 3.1.1.3.11) and phosphoenolpyruvate carboxykinase (EC 4.1.1.49) were assayed in 1% homogenate prepared in 0.25 M sucrose by the methods of Mendicino and Vasornely [12] and Phillips and Berry [13] respectively. Cerebral cholinesterase activity (EC 3.1.1.7) was measured according to the method of Ellman *et al.* [14] using acetylthiocholine as the substrate. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity was assayed according to the method of Kornberg and Horacker [15]. Levels of lactate and pyruvate were measured by the method of Barker and Summerson [16] and Theodore *et al.* [17] respectively. Adrenal ascorbic acid and cholesterol contents were estimated according to the methods of Roe and Kuether [18] and Chiamori and Henry [19] respect-

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ively. Data were analyzed statistically using Student's *t*-test.

RESULTS

The effects of diazinon on the levels of blood glucose and glycogen in liver and brain, and cerebral cholinesterase activity are given in Table 1. Diazinon significantly increased the level of blood glucose and reduced the glycogen content of the brain and liver 2 hr after treatment. The cholinesterase activity was reduced significantly in brain (Table 1). The activities of the glycogenolytic enzymes glycogen phosphorylase and phosphoglucomutase were increased significantly in both liver and brain, whereas that of glucose-6-phosphatase was not altered in either of the tissues (Table 2). The activities of the glycolytic enzymes hexokinase and lactate dehydrogenase were also increased in brain. Glucose-6-phosphate dehydrogenase activity was not changed significantly by treatment with diazinon (Table 2). Lactate content of brain and blood was increased whereas that of pyruvate was not changed significantly (Table 3). The activities of the gluconeogenic enzymes fructose 1,6-diphosphatase and phosphoenolpyruvate carboxykinase were increased significantly in liver; fructose 1,6-diphosphatase activity was also increased in brain (Table 4). Ascorbic acid and cholesterol contents of adrenals were depleted by treatment with diazinon (Table 5). The induced changes in the level of blood glucose, cerebral and hepatic glycogen (Table 1), lactate (Table 3), and other enzymes (Tables 3–4) were abolished by adrenalectomy.

DISCUSSION

The results indicate that hyperglycemia induced by diazinon was accompanied by depletion of glycogen in liver and brain, changes in the activities of glycolytic and glycogenolytic enzymes and depletion of ascorbic acid and cholesterol from the adrenals. It was reported previously that cerebral glycogen content depends on the state of activity of brain; glycogen levels are reduced during activation or stimulatory states and increased after treatment with barbiturates or sedatives [20, 21]. Thus, the depletion of glycogen (Table 1) in the brain may be related to stimulatory effects in diazinon-treated animals which also had significantly low levels of cerebral cholinesterase activity (Table 1). Further, treatment with certain organophosphorous compounds resulted in the accumulation of cyclic AMP which has stimulatory effects and is involved in the storage of glycogen [22]. An increase in the level of acetylcholine at the neuroeffector sites induces the release of catecholamines [23]. These changes may activate glycogen phosphorylase [24] which, according to our results, was increased significantly in brain and liver of diazinon-treated animals (Table 2). The activity of phosphoglucomutase, another glycogenolytic enzyme, was also increased in diazinon-treated animals (Table 2), suggesting greater formation of glucose-6-phosphate from glucose-1-phosphate. Glucose-6-phosphatase activity, the enzyme which catalyzes the final step of glycogenolysis, was not changed significantly (Table 2). The activity of hexokinase, an important enzyme of the glycolytic pathway, was also increased (Table 2), tending toward greater formation of glucose-6-phosphate from glucose which is the main source of energy for the brain [25] and is actively metabolized during stimulatory states, tremors and convulsions [26]. In liver, hexokinase activity was not changed significantly, suggesting that the demand of glucose was not increased in hepatic tissue in diazinon-treated animals. The activity of cerebral lactate dehydrogenase was increased slightly but significantly (Table 2). It was reported previously that the organophosphorous compounds inhibit the respiratory enzymes and reduce the oxygen uptake *in vitro* as well as *in vivo* [1]. These changes favor the anaerobic glycolysis in diazinon-treated animals. This is in agreement with our finding that the level of lactate was increased in the brain and blood, whereas that of pyruvate was not changed significantly (Table 3). Since the activity of glucose-6-phosphate dehydrogenase (Table 2) was not changed significantly in diazinon-treated animals, it seems that the direct oxidation of glucose through the hexose monophosphate pathway was not altered in these animals.

The results indicate an increase in the activities of the gluconeogenic enzymes, fructose 1,6-diphosphatase and phosphoenolpyruvate carboxykinase in liver (Table 4) which is the main site of gluconeogenesis in the body [27]. It was reported previously that organophosphorous compounds by

Table 1. Effect of diazinon (40 mg/kg, i.p.) on the level of blood glucose, cerebral and hepatic glycogen, and cerebral cholinesterase activity in rats

Treatment	Blood glucose (mg/100 mL)	Glycogen (mg/100 g)		Cerebral cholinesterase activity (μmol acetylthiocholine hydrolyzed/min/g)
		Liver	Brain	
Control	95.24 ± 3.42	312.85 ± 12.56	97.13 ± 3.95	21.48 ± 0.79
Diazinon	196.47 ± 6.78*	201.56 ± 10.95†	65.22 ± 3.11†	10.06 ± 0.42*
Diazinon in adrenalectomized animals	105.42 ± 3.09	303.90 ± 11.36	94.25 ± 4.05	9.48 ± 0.41

Animals were killed 2 hr after treatment. Values are means ± SE; each group consisted of eight animals.
* Significantly different from control value ($P < 0.01$).
† Significantly different from control value ($P < 0.02$).

Table 2. Effect of diazinon (40 mg/kg, i.p.) on glycogenolytic and glucolytic enzymes and on glucose-6-phosphate dehydrogenase activity in normal and adrenalectomized animals

Treatment	Glycogenolytic enzymes			Glycolytic enzymes		Glucose-6-phosphate dehydrogenase (nmol NADP reduced/min/mg protein)
	Glycogen phosphorylase (μmol P_i formed/min/g tissue)	Phospho glucomutase (μmol acid-stable P_i formed/min/g tissue)	Glucose-6-phosphatase (μmol P_i liberated/min/g tissue)	Hexokinase (μmol glucose phosphorylated/min/mg protein)	Lactate dehydrogenase (nmol NADH oxidized/min/mg protein)	
			Brain			
Control	28.57 ± 1.83	8.12 ± 1.88	2.03 ± 0.25	3.89 ± 0.48	245.40 ± 10.00	13.08 ± 2.42
Diazinon	39.10 ± 1.79*	12.05 ± 1.89†	2.09 ± 0.28	4.91 ± 0.43†	285.69 ± 12.80*	12.79 ± 2.28
Diazinon in adrenalectomized animals	27.76 ± 1.68	9.01 ± 1.78	2.00 ± 0.21	3.81 ± 0.40	248.39 ± 11.33	13.21 ± 2.33
			Liver			
Control	23.54 ± 1.88	24.33 ± 1.05	5.71 ± 1.25	1.37 ± 0.22	603.80 ± 14.08	58.29 ± 4.93
Diazinon	29.89 ± 1.76†	31.89 ± 1.01*	5.99 ± 1.23	1.38 ± 0.24	589.60 ± 13.50	53.59 ± 3.89
Diazinon in adrenalectomized animals	22.48 ± 1.80	23.97 ± 1.00	5.65 ± 1.02	1.35 ± 0.26	601.73 ± 15.61	56.68 ± 3.98

Animals were killed 2 hr after treatment. Each value is the mean ± SE of eight animals.
* $P < 0.05$ compared with control.
† $P < 0.01$ compared with control.

Table 3. Effect of diazinon (40 mg/kg, i.p.) on the levels of cerebral and blood pyruvic and lactic acid in rats

Treatment	Brain		Blood	
	Pyruvic acid (mmol/kg)	Lactic acid (mmol/kg)	Pyruvic acid (mmol/L)	Lactic acid (mmol/L)
Control	0.21 ± 0.06	2.36 ± 0.20	0.32 ± 0.07	1.41 ± 0.19
Diazinon	0.24 ± 0.08	3.88 ± 0.26*	0.34 ± 0.06	2.23 ± 0.20*
Diazinon in adrenalectomized animals	0.20 ± 0.05	2.60 ± 0.23	0.31 ± 0.06	1.43 ± 0.18

Animals were killed 2 hr after treatment. Values are means ± SE; each group consisted of eight animals.

* Significantly different from the control value ($P < 0.01$).

Table 4. Effect of diazinon (40 mg/kg, i.p.) on gluconeogenic enzyme activities in hepatic and cerebral tissues of normal and adrenalectomized rats

Treatment	Fructose 1,6-diphosphatase (μmol P_i formed/min/g tissue)		Phosphoenolpyruvate carboxykinase (μmol phosphoenolpyruvate formed/min/g tissue)	
	Liver	Brain	Liver	Brain
Control	7.92 ± 0.86	2.01 ± 0.25	16.89 ± 0.98	4.68 ± 0.85
Diazinon	11.06 ± 0.71*	2.80 ± 0.20*	20.56 ± 0.90†	4.89 ± 0.72
Diazinon in adrenalectomized animals	7.58 ± 0.74	2.00 ± 0.23	16.47 ± 0.82	4.59 ± 0.79

Animals were killed 2 hr after treatment. Values are means ± SE; each group consisted of eight animals.

* Significantly different from control value ($P < 0.01$).

† Significantly different from control value ($P < 0.05$).

Table 5. Effect of diazinon (40 mg/kg, i.p.) on ascorbic acid and cholesterol content of adrenals in rats

Treatment	Cholesterol (mg/g tissue)	Ascorbic acid (mg/g tissue)
Control	66.14 ± 3.54	4.51 ± 0.11
Diazinon	42.14 ± 2.96*	2.89 ± 0.16*

Animals were killed 2 hr after treatment with diazinon. Values are means ± SE; each group consisted of eight animals.

* Significantly different from the control value ($P < 0.02$).

their anticholinesterase action interfere with neuroregulatory pathways in the central nervous system which controls the secretory activity of the anterior pituitary [28], resulting in the release of ACTH [29]. Furthermore, ACTH has also been reported to stimulate the adrenals to secrete corticosteroids [30]. The increased activities of the hepatic gluconeogenic enzymes fructose 1,6-diphosphatase and phosphoenolpyruvate carboxykinase in diazinon-treated animals may be related to a central or peripheral effect of diazinon inhibiting cholinesterase at both the sites. Thus, the induced changes in diazinon-treated animals may be due to the release of catecholamines by a peripheral or central mechanism, release of corticosteroids promoting gluconeogenesis or a compensatory mechanism providing extra energy by mobilizing glucose owing to hyperactivity and convulsions induced by diazinon. Support for the involvement of adrenals is also gained from the finding that treatment with diazinon resulted in depletion of ascorbic acid and cholesterol from the adrenals (Table 5). Further, adrenalectomy prevented changes in the activities of gluconeogenic (Table 4) and other enzymes in diazinon-treated animals.

Since changes in the level of blood glucose, glycogen (Table 1) and related enzymes were accompanied by depletion of cholesterol and ascorbic acid (Table 5) from adrenals and since the induced changes in the activities of glycolytic, glycogenolytic and gluconeogenic enzymes were abolished by adrenalectomy (Tables 2–4), it seems that adrenal glands may be partly involved in the production of induced changes in carbohydrate metabolism mediated through the central or peripheral effect of diazinon.

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