Modification of Malathion Induced Neurochemical Changes by Adrenalectomy in Rats

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Received February 27, 1990; Accepted July 5, 1990

ABSTRACT

The neurochemical changes induced by malathion, an organophosphate compound, were determined in rats. Maximal changes were found in the brain 2 h after the administration of malathion in a dose of 500 mg/kg ip. The activities of cholinesterase and succinic dehydrogenase were reduced whereas those of glycogen phosphorylase, phosphoglucomutase, and hexokinase were increased; the lactate content of brain was also increased. In malathion treated adrenalectomized animals, changes in the activities of cerebral cholinesterase and succinic dehydrogenase were still present; other changes were, however, abolished by adrenalectomy. Activities of certain enzymes, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase were not significantly altered by malathion in normal or adrenalectomized animals. The results indicate that cerebral cholinergic mechanism in malathion treated animals was not modified by adrenalectomy which, however, abolished or reduced changes in the activities of certain glycolytic and glycogenolytic enzymes that are involved in the utilization or metabolism of glucose. The brain lactate content in malathion treated adrenalectomized animals was, also, not significantly different from the control values, suggesting the modification of induced changes by adrenalectomy.

Index Entries: Glycogen; glycogen phosphorylase; phosphoglucomutase; adrenals; cholinesterase; hexokinase; adrenalectomy; glucose-6-phosphate; pyruvate; lactate; lactate dehydrogenase; glucose-6-phosphate dehydrogenase.

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INTRODUCTION

Malathion (*O, O*-dimethyl, *S*-(1,2-dicarbethoxy ethyl) phosphorodithioate) has been reported to induce toxic effects in rats (Holmstedt, 1959), man (Hayes 1963; Namba et al., 1970) and other experimental animals (Durham et al., 1966). These effects are associated with neurochemical changes, inhibition of cerebral cholinesterase activity (Dubois et al., 1953), and other enzymes (Murphy and Cheever 1968), accumulation of acetylcholine (Matin and Husain 1984), and depletion of glycogen (Agarwal and Matin 1981) in the brain. The cerebral glycogen content is regulated by various factors and enzymes (French, 1964), as well as activity of adrenals that also modify or influence central effects (Rothballer, 1959; Russell, 1960). The neurochemical changes induced by malathion in normal and adrenalectomized rats have been determined in the present study.

EXPERIMENTAL PROCEDURES

Materials

Adult female albino rats, 150 ± 10 g, were used. The animals were maintained on a 12 h light dark cycle, and had food and water *ad libitum*. They were kept separate from the males. The animals (controls and experimentals) were fasted for 18 h before use, since this resulted in more uniform results. They were divided into three groups. Animals of group one served as controls and were given normal physiological saline (0.9% NaCl); those of group two were injected with malathion (125, 250 or 500 mg/kg ip) and 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 malathion 10 d after adrenalectomy.

Enzyme Assays

The animals were killed by immersion in liquid nitrogen. After decapitation, the brain was removed quickly and cholinesterase activity (E.C. 3.1.1.7.) measured, using acetylthiocholine as the substrate (Ellman et al., 1961). The glycogen content of brain was extracted, according to the method of LeBaron (1955), and estimated colorimetrically as described by Montgomery (1957). Glycogenolytic enzymes were assayed in 1% homogenate prepared in ice-cold, 0.25*M* sucrose. 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 (1966), and phosphoglucomutase (E.C. 2.7.5.1.) by the method of Najjar (1955). For assaying hexokinase (E.C. 2.7.1.1.) activity, tissue was homogenized in media

containing 0.15*M* KCl, 0.005*M* EDTA, and 0.04*M* $MgCl_2$. For lactate dehydrogenase (E.C. 1.1.1.27), phosphate buffer (pH 7.4) was used. The enzymes were assayed according to the procedures of Crane and Sols (1955) and Kornberg (1955), respectively. Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49.) activity was assayed according to the method of Kornberg and Horecker (1955). Succinic dehydrogenase was determined in mitochondrial suspension according to the method of Slater and Bonner (1952).

Determination of Lactate, Pyruvate, and Other Substances

Levels of lactate and pyruvate were measured by the method of Barker and Summerson (1941) and Theodore et al. (1943), respectively. Ascorbic acid content of adrenals was measured according to the method of Roe and Koether (1943), and that of cholesterol by the method of Chiamori and Henry (1959).

Statistical Analysis

The data were examined by analysis of variance followed by Student's *t*-test between means. Significant differences between the means calculated as *p* values are given in Tables.

RESULTS

The effect of malathion (125, 250, and 500 mg/kg ip) on cerebral glycogen content and cholinesterase activity is given in Table 1. The cholinesterase activity was significantly lowered, and glycogen content depleted in the brain of malathion treated animals. Maximal changes were found after the administration of malathion in a dose of 500 mg/kg ip (Table 1).

The effect of malathion (500 mg/kg ip) on cerebral glycogen content and cholinesterase activity, 0.5, 1, and 2 h after treatment, was also determined (Table 2). The induced changes were maximum (Tables 1 and 2) 2 h after treatment with malathion (500 mg/kg ip). The changes were slight but significant 0.5 and 1 h after the administration of malathion (Tables 1 and 2). In adrenalectomized animals, malathion reduced the level of cholinesterase activity but had no significant effect on glycogen content of brain (Tables 1 and 2).

The activities of glycogen phosphorylase and phosphoglucomutase were significantly increased whereas that of glucose-6-phosphatase was not altered by the administration of malathion (Table 3); These changes were, however, absent in adrenalectomized animals. Hexokinase activity was also increased by malathion in normal rats, but not in adrenalectomized animals (Table 4). Activities of glucose-6-phosphate dehy-

Table 1
Effect of Malathion on Cerebral Glycogen Content and Cholinesterase Activity of Rats

	Glycogen (mg/100 g) (mean ± SEM)	Cholinesterase Activity (μ mol of acetylthiocholine hydrolyzed/min/g) (mean ± SEM)
Controls	98.75 ± 4.02	21.95 ± 0.71
Malathion		
125 mg/kg, ip	92.44 ± 2.42**	18.23 ± 0.32**
250 mg/kg, ip	83.26 ± 2.95*	14.14 ± 0.43*
500 mg/kg, ip	64.23 ± 2.11*	10.18 ± 0.52*

*Significantly different from control values, $p < 0.01$. The animals were killed 2 h after treatment. Each group consisted of eight animals.
**Significantly different from control values, $p < 0.05$.

Table 2
Effect of Malathion (500 mg/kg ip) on Cerebral Glycogen Content and Cholinesterase Activity at Different Time Intervals in Normal and Adrenalectomized Rats

	Glycogen (mg/100 g) (mean ± SEM)	Cholinesterase activity (μ mol of acetylthiocholine hydrolyzed/min/g) (mean ± SEM)
Controls	97.12 ± 2.83	22.16 ± 0.63
Malathion (500 mg/kg, ip)		
0.5 h	90.43 ± 2.14**	17.32 ± 0.24**
1.0 h	81.01 ± 2.72*	13.81 ± 0.41*
2.0 h	65.92 ± 3.01*	9.46 ± 0.31*
Adrenalectomized animals (controls)	95.74 ± 2.62	21.08 ± 0.72
***Malathion (500 mg/kg, ip) in adrenalectomized animals)	93.91 ± 3.23	11.05 ± 0.51*

*Significantly different from controls as well as adrenalectomized controls, $p < 0.01$. Each group consisted of eight animals.
**Significantly different from controls as well as adrenalectomized controls, $p < 0.05$.
***Adrenalectomized animals were killed 2 h after the administration of malathion (500 mg/kg, ip).

drogenase and lactate dehydrogenase were not significantly changed by treatment with malathion in normal or adrenalectomized animals (Table 4). Succinic dehydrogenase activity was significantly reduced in malathion treated normal as well as adrenalectomized animals (Table 4). Lactate content of brain was increased only in malathion treated normal animals whereas that of pyrunate was not significantly changed (Table

Table 3
Effect of Malathion (500 mg/kg, ip) on the Level of Cerebral Glycogenolytic Enzymes in Normal and Adrenalectomized Animals

	A Glycogen phosphorylase (mean ± SEM)	B Phosphoglucomutase (mean ± SEM)	C Glucose-6-phosphatase (mean ± SEM)
Controls	27.32 ± 1.72	7.91 ± 0.95	2.12 ± 0.19
Malathion (500 mg/kg, ip)	39.01 ± 1.68*	11.89 ± 0.36*	2.51 ± 0.15
Adrenalectomized controls	28.14 ± 1.41	8.03 ± 0.65	2.32 ± 0.16
Malathion (500 mg/kg, ip) in adrenalectomized animals	30.16 ± 1.23	8.24 ± 0.41	2.43 ± 0.13

*Significantly different from controls as well as adrenalectomized controls, $p < 0.01$. The animals were killed 2 h after treatment. Each group consisted of eight animals.
A = μmol Pi formed/min/g tissue.
B = μmol acid stable Pi formed/min/g tissue.
C = μmol of Pi liberated/min/g tissue.

5). Ascorbic acid and cholesterol contents of adrenals were depleted by treatment with malathion (Table 6).

DISCUSSION

The results indicate that inhibition of cholinesterase activity was accompanied by depletion of glycogen in the brain (Tables 1 and 2), changes in the activities of glycolytic and glycogenolytic enzymes, and succinic dehydrogenase activity (Tables 3–5). The cerebral cholinesterase activity in normal controls (Table 1) was not significantly different from that of adrenalectomized animals (Table 2), suggesting that adrenalectomy *per se* did not influence the level of cerebral cholinesterase activity. Furthermore, the values of cholinesterase actvitiy in malathion treated adrenalectomized animals were not significantly different from the corresponding values in malathion treated normal animals (Table 2), indicating that inhibition of cholinesterase was not modified by adrenalectomy. Cholinesterase is widely distributed in brain, R. B. C., and at various nerve terminals, including those of adrenal medulla that are also cholinergic (Augustinsson, 1963). Thus, the inhibition of cholinesterase in malathion treated animals may stimulate neuroeffector sites of adrenal medulla, resulting in the secretion of catecholamines. These changes may activate glycogen phosphorylase (Sutherland and Rall, 1960), which according to our results, was significantly increased in the brain of

Table 4
Effect of Malathion (500 mg/kg, ip) on the Levels of Hexokinase, Lactate Dehydrogenase, Glucose-6-Phosphate Dehydrogenase, and Succinic Dehydrogenase Activities in the Cerebral Tissue of Normal and Adrenalectomized Rats

	A Hexokinase	B Lactate dehydrogenase	C Glucose-6-phosphate dehydrogenase	D Succinic dehydrogenase
Controls	3.95 ± 0.31	250.16 ± 7.15	12.95 ± 2.23	23.67 ± 1.85
Malathion (500 mg/kg, ip)	5.43 ± 0.38*	275.23 ± 6.24	13.42 ± 1.95	14.21 ± 1.76*
Adrenalectomized controls	4.01 ± 0.29	266.2 ± 5.13	14.04 ± 2.71	24.21 ± 1.52
Malathion (500 mg/kg, ip) in adrenalectomized animals	3.84 ± 0.25	271.15 ± 8.04	13.76 ± 2.24	15.16 ± 1.23*

*Significantly different from the values of controls as well as adrenalectomized controls, $p < 0.01$. The animals were killed 2 h after treatment. Each group consisted of eight animals.

A = μmol glucose phosphorylated/min/mg protein.
B = nmol NADH oxidized/min/mg protein.
C = nmol NADP reduced/min/mg protein.
D = nmol of $K_3Fe(CN)_6$ reduced/min/mg protein.

Table 5
Effect of Malathion (500 mg/kg, ip) on the Brain Content of Pyruvate and Lactate in Normal and Adrenalectomized Rats

	Pyruvic acid (m mol/kg) (mean ± SEM)	Lactic acid (m mol/kg) (mean ± SEM)
Controls	0.22 ± 0.05	2.34 ± 0.18
Malathion	0.25 ± 0.03	3.95 ± 0.16*
Adrenalectomized controls	0.23 ± 0.02	2.41 ± 0.14
Malathion in adrenalectomized animals	0.21 ± 0.04	2.39 ± 0.11

*Significantly different from the controls as well as adrenalectomized controls, $p < 0.01$. The animals were killed 2 h after treatment. Each group consisted of eight animals.

Table 6
Effect of Malathion (500 mg/kg, ip) on Ascorbic Acid and Cholesterol Contents of Adrenals in Rats

Treatment	Cholesterol (mg/g tissue) (mean ± SEM)	Ascorbic acid (mg/g tissue) (mean ± SEM)
Controls	63.02 ± 3.56	4.15 ± 0.19
Malathion (500 mg/kg, ip)	41.51 ± 2.17*	2.57 ± 0.16*

*Significantly different from the values of controls, $p < 0.01$. Each group consisted of eight animals. The animals were killed 2 h after treatment with malathion.

malathion treated animals (Table 3). The activity of phosphoglucomutase, another glycogenolytic enzyme, was also increased in malathion treated animals (Table 3), suggesting greater formation of glucose-6-phosphate from glucose-1-phosphate. These changes favor the mobilization of glycogen that was significantly depleted in malathion treated animals (Tables 1 and 2). However, the activities of glucose-6-phosphatase that catalyzes glycogenolysis and lactate dehydrogenase that is involved in anaerobic glycolysis were not significantly changed in malathion treated normal or adrenalectomized animals (Tables 3 and 4). The activity of hexokinase was increased (Table 4), suggesting greater formation of glucose-6-phosphate from glucose, which is the main source of energy for the brain. (McIlwain and Bachelard, 1971). In adrenalectomized animals, malathion did not change the hexokinase activity, suggesting that phosphorylation of glucose to glucose-6-phosphate was not altered in malathion treated adrenalectomized animals (Table 4).

The results further indicate that pyruvate content of brain was not significantly changed after treatment with malathion whereas that of lactate was increased (Table 5). According to Huckabee (1958), 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 without any significant change in pyruvate may be related to changes in cellular respiration. It was reported previously that organophosphorous compounds interfered with oxygen uptake (Santolucito and Whitcomb, 1971) and depressed the respiration of brain in vitro, as well as in vivo (Andjelkovic and Milosevic 1968; Murtha and Harris, 1980). Our results also indicate reduced activity of succinic dehydrogenase in malathion treated animals (Table 4). These changes favor the metabolism of glucose to lactic acid that was significantly increased in malathion treated animals (Table 5). However, the brain lactate content in malathion treated adrenalectomized animals was not significantly different from the control values, indicating that the metabolism of glucose was modified by adrenalectomy (Table 5). Malathion, by its anticholinesterase action, might interfere with neuroregulatory pathways in the central nervous system that control the secretory activity of anterior pituitary (Anichkov et al., 1962), resulting in the release of ACTH (Pickford and Vogt 1951). Thus, it is likely that malathion may be inducing changes through more than one mechanism

1. Inducing release of ACTH which sets on adrenal cortex secreting corticosteroids (Haynes and Berthret, 1957);
2. Releasing catecholamines (Brizezinski and Ludwicki, 1973) by acting on neuroeffector sites of adrenal medulla; and
3. Acting directly on various cholinergic sites of the body.

Support for the possible involvement of adrenals is also suggested from the finding that the administration of malathion resulted in depletion of ascorbic acid and cholesterol from the adrenals (Table 6). Finally, treatment with malathion did not significantly change the glucose-6-phosphate dehydrogenase activity (Table 4), which suggests that oxidation of glucose through the hexose monophosphate pathway was not altered in malathion treated normal or adrenalectomized animals.

Since the inhibition of cholinesterase in malathion treated animals was not modified by adrenalectomy while changes in the level of glycogen, glycolytic, and glycogenolytic enzymes were abolished by adrenalectomy, it seems that cerebral glycolysis and glycogenolysis were influenced, at least in part, by adrenals and/or adrenergic mechanism triggered off by enhanced cholinergic activity in malathion treated animals.

ACKNOWLEDGMENTS

The authors are grateful to Indian Council of Medical Research (ICMR) for the fellowship sanctioned to K. Husain, and to American Cyanamid for the generous supply of malathion used in the above study. Technical assistance of Satguru Prasad and Ramesh Chandra is also acknowledged.

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