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# THE EFFECT OF CERTAIN DRUGS ON GLYCOGEN AND ACETYLCHOLINE LEVELS IN CEREBRAL AND PERIPHERAL TISSUES IN RATS WITH MALATHION INDUCED HYPERGLYCAEMIA

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The hyperglycaemia induced by malathion (500 mg/kg, i.p.) in rats was accompanied by depletion of glycogen in certain brain regions and peripheral tissues and an increase in the level of cortical and striatal acetylcholine. Thn induced changes were abolished by pralidoxime (100 mg/kg, i.p.) given immediately after malathion, but persisted when oxime was given 30 min after malathion treatment. Atropine (25 mg/kg, i.p.) prevented the malathion induced hyperglycaemia and changes in the level of glycogen. The cortical and striatal acetylcholine levels in these animals were significantly higher than in controls. Pretreatment with reserpine (1.0 mg/kg, i.p.) daily for three days did not modify the malathion induced changes in the levels of blood glucose, glycogen and acetylcholine. The increased acetylcholine level in the brain may have been related to changes in the level of glycogen and blood glucose in malathion treated animals.

The organophosphorous compounds, besides producing the acute toxic effects (1) have been reported to produce hyperglycaemia (2, 3, 4). The acute toxic effects induced by these compounds are abolished or modified by atropine and oximes (5, 6). The effect of these drugs on hyperglycaemia induced by organophosphorous compounds is not clear. The present study has been undertaken to investigate the effect of atropine and pralidoxime on the level of blood glucose and glycogen in certain brain structures and peripheral tissues in malathion treated animals. The level of acetylcholine in certain brain regions was also determined.

## MATERIAL AND METHODS

Adult male albino rats of Wistar strain 175 ± 10 g were used. The animals were maintained on a 12 h light dark schedule at a temperature of 30 ± 1 °C and had food and water *ad libitum*. The animals were fasted for 18 h before use since preliminary experiments indicated that more uniform results were obtained in this manner. The animals were divided into several groups. The animals in group 1. were injected with malathion (500 mg/kg, i.p.). Those from group 2. received pralidoxime (100 mg/kg, i.p.), immediately or 30 minutes after an injection of malathion. The animals in group 3. received malathion (500 mg/kg, i.p.) followed immediately by atropine (25 mg/kg, i.p.). The animals in group 4. received reserpine (1.0 mg/kg, i.p.) daily for three days, followed by malathion (500 mg/kg, i.p.). Reserpine was used to investigate the possible role of catecholamines in malathion induced hyperglycaemia. Control animals received physiological saline. The animals were killed by immersion in liquid air according to the near freezing technique of *Takahashi and Aprison* (7) one hour after malathion administration.

The brains were rapidly removed. The cortex and the striatum were dissected out and weighed rapidly. The cortex and the striatum of right and left sides were used for estimating the level of total acetylcholine and glycogen content respectively. The liver and diaphragm were also removed for glycogen estimation.

Acetylcholine was extracted according to the method of *Crossland* (8) and assayed on guinea pig illeum. The specificity of the assay was confirmed as described previously (9).

Glycogen was extracted according to the method of *Lebaron* (10) and estimated spectrophotometrically (11). Blood sampling and estimation of glucose level were done according to the method of *Nelson* (12).

The drugs used in the study include pralidoxime (2-formyl 1-methyl pyridinium oxime chloride), atropine and malathion (0,0-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate).

## RESULTS

Changes in the level of blood glucose and glycogen content in brain structures and peripheral tissues in malathion treated animals are presented in Table 1. The level of blood glucose was raised and glycogen content reduced in the brain (cortex and striatum) as well as in the peripheral tissues (liver and diaphragm). Changes in the level of blood glucose and glycogen content were prevented by pralidoxime or atropine given immediately after malathion (Table 1); pralidoxime had no effect when given 30 minutes after the administration of ma-

lathion (Table 1). Pretreatment with reserpine did not modify the induced changes in the level of blood glucose or glycogen content in malathion treated animals (Table 1).

Malathion increased the level of cortical and striatal acetylcholine which was not modified by pretreatment with reserpine (Table 2). In animals given pralidoxime immediately after malathion, the level of acetylcholine was within the normal range. Administration of pralidoxime 30 minutes after malathion, did not significantly change the increase in the level of acetylcholine induced by malathion. The level of acetylcholine in atropine malathion treated animals was significantly higher than in controls but was lower than that recorded following malathion alone (Table 2).

The malathion treated animals developed tremors or convulsions, and respiratory embarassment which were abolished by atropine or pralidoxime given immediately after malathion. These effects were not modified by the oxime given 30 minutes after malathion.

## DISCUSSION

As was expected, malathion induced an increase in the level of cortical and striatal acetylcholine (Table 2). Corpus striatum was included in the present study because it has a high concentration of acetylcholine and even small changes in the level of acetylcholine can be detected in this brain region (13, 14). According to our results, changes in the level of acetylcholine were accompanied by hyperglycaemia and depletion of glycogen in certain brain structures and peripheral tissues of malathion treated animals (Table 1). It is of interest that changes in the level of glycogen followed the pattern similar to acetylcholine which is reduced during excitatory states and convulsions (15, 16) and increased after anaesthesia and sedatives (17, 18). It was previously reported that certain organophosphorous compounds (e. g. soman) increased the concentration of cyclic AMP which is involved in the storage of glycogen (19, 20) and is reduced with the rise in blood glucose level owing to glycogenolysis (21). Thus the stimulatory effects induced by organophosphorus compounds, accompanied by an increase in the level of acetylcholine and cyclic AMP, may be linked in some way with the depletion of glycogen content in malathion treated animals (Table 1). The changes induced by malathion are similar to those induced by tremorine which has also been reported to increase the level of acetylcholine (16, 22) and reduce the glycogen content in certain brain structures of rats (23).

The treatment with pralidoxime immediately after malathion might prevent sufficient inhibition (phosphorylation) of cholinesterase and the increase in brain acetylcholine which may be related to induced changes in malathion treated animals (Tables 1 and 2). Pralidoxime

Table 1.

*The effect of pralidoxime, atropine and reserpine on the level of blood glucose and glycogen content in the cortex, striatum, liver and diaphragm of malathion treated rats. All the animals were killed 1 h after treatment with malathion. Each figure represents the mean ±S.E. of 10 animals.*

| Group | Pretreatment | Treatment | | Blood glucose (mg/100 ml) | Glycogen (mg/100 g) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Brain | | Liver | Diaphragm |
| | | | | | Cortex | Striatum | | |
| 1 | None | None | | 98.41 ±3.11 | 44.21 ±2.71 | 74.12 ±3.12 | 312.23 ±13.57 | 172.87 ±6.69 |
| 2 | Malathion (500 mg/kg, i.p.) followed by | None | | 225.71[a] ±12.31 | 34.12[a] ±1.98 | 54.71[a] ±2.01 | 83.37[a] ±4.58 | 53.13[a] ±3.58 |
| 3 | | Pralidoxime (100 mg/kg, i.p.) | immediately | 114.12[c] ±4.17 | 43.71[d] ±1.56 | 75.51[c] ±2.42 | 298.14[c] ±11.34 | 164.63[c] ±5.15 |
| 4 | | | after 30 min | 205.11[a] ±9.17 | 36.21[b] ±1.61[b] | 52.52[a] ±2.81 | 90.11[a] ±4.88 | 59.95[a] ±4.03 |
| 5 | | Atropine (25 mg/kg, i.p.) | immediately | 106.12[c] ±3.71 | 42.51[d] ±1.92 | 71.24[c] ±3.41 | 301.15[c] ±12.14 | 189.63[c] ±7.25 |
| 6 | Reserpine* (1 mg/kg, i.p.) | Malathion (500 mg/kg, i.p.) | | 212.71[a] ±14.51 | 34.61[a] ±1.52 | 50.7[a] ±3.1 | 91.45[a] ±4.01 | 59.12[a] ±2.98 |

* Reserpine (1 mg/kg, i.p.) was administered daily for 3 days before treatment with malathion.
[a] Significantly different from the corresponding values in controls (group 1), $p < 0.01$.
[b] Significantly different from the values of group 1, $p < 0.05$.
[c] Significantly different from the values in malathion treated animals (group 2), $p < 0.01$.
[d] Significantly different from the values of group 2, $p < 0.05$.

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Table 2.

*Changes in the level of cortical and striatal acetylcholine after the administration of malathion (500 mg/kg, i.p.), pralidoxime (100 mg/kg, i.p.), atropine (25 mg/kg, i.p.) and reserpine (1.0 mg/kg, i.p.). Animals were killed 1 h after the administration of malathion. Each group consisted of 10 animals.*

| | Acetylcholine (n mole/g, wet weight ± S.E.) | | | | | |
|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 | 6 |
| | Control | Malathion | Malathion + Pralidoxime*** | Malathion + Pralidoxime** | Malathion + Atropine*** | Reserpine* + Malathion |
| Cortex | 14.51 ±1.01 | 27.21[a] ± 1.94 | 15.32[b] ±1.78 | 25.87[a] ±1.75 | 25.82[a, c] ±1.24 | 26.52[a, d] ±1.46 |
| Striatum | 46.31 ±2.41 | 89.11[a] ±3.12 | 48.12[b] ±2.01 | 86.92[a] ±3.41 | 71.43[a, c] ±2.94 | 90.44[a, d] ±3.25 |

* Reserpine was administered daily for 3 days before treatment with malathion.
[a] Significantly different from the controls ($p < 0.01$).
[b] Significantly different from the corresponding values in malathion treated animals, group 2 ($p < 0.01$).
[c] Significantly different from the values in group 2 ($p < 0.05$).
[d] Significantly different from the corresponding values in group 3 ($p < 0.01$).
*** Administered immediately after malathion.
** Injected 30 min after malathion.

given 30 minutes after malathion, did not modify the induced changes since during this period, changes in the level of blood glucose and glycogen were fully established and were unaffected by the subsequent administration of pralidoxime which is a quarternary compound and hence does not cross the blood brain barrier in a sufficient amount (1). The finding that pretreatment with reserpine had no effect on the level of hyperglycaemia in malathion treated rats (Table 1) is consistent with those of other workers who reported that $\alpha$-adrenergic blockade prevented the catecholamine induced hyperglycaemia (24) but did not modify the organophosphate induced hyperglycaemia (4).

The depletion of glycogen from the peripheral tissues liver and diaphragm (Table 1) may also be related to the accumulation of acetylcholine resulting in the release of somatostatin which may be involved in the production of hyperglycaemia (25, 26). Support for the involvement of cholinergic mechanism is gained from the finding that atropine which blocks the cholinergic effects and the release of somatostatin (27), abolished the changes in the level of blood glucose and glycogen in the brain structures and peripheral tissues of malathion treated rats (Table 1).

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*Sažetak*

## UČINAK NEKIH LIJEKOVA NA GLIKOGEN I NIVO ACETILKOLINA U TKIVU MOZGA I PERIFERNIM TKIVIMA U ŠTAKORA S HIPERGLIKEMIJOM IZAZVANOM MALATIONOM

Ustanovljeno je da hiperglikemiju u štakora izazvanu malationom (500 mg/kg, i. p.) prati nestanak glikogena u nekim područjima mozga i perifernim tkivima i porast acetilkolina u kortikalnom i strijatalnom dijelu mozga. Ove promjene nestale su kad je odmah nakon malationa dan pralidoksim (100 mg/kg, i. p.), ali su se zadržale kad je oksim dan 30 minuta nakon malationa. Atropin (25 mg/kg, i. p.) je spriječio hiperglikemiju izazvanu malationom i promjene u nivou glikogena. Nivo acetilkolina u kortikalnom i strijatalnom dijelu mozga bio je značajno viši u tretiranih životinja nego u kontrolnih. Prethodni trodnevni tretman rezerpinom (1,0 mg/kg dnevno, i. p.) nije utjecao na promjene koje je izazvao malation u nivou glukoze u krvi, glikogena i acetilkolina. Povišene vrijednosti acetilkolina u mozgu mogu se dovesti u vezu s promjenama u nivou glikogena i glukoze u krvi životinja tretiranih malationom.

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