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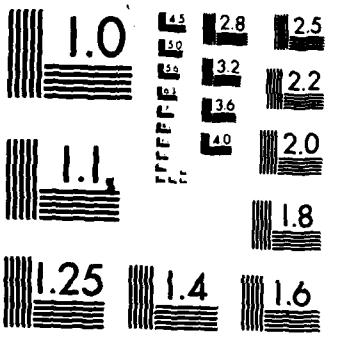

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THE EFFECTS OF ORGANOPHOSPHORUS ANTICHOLINESTERASE COMPOUNDS ON BRAIN GLUCOSE AND ENERGY METABOLISM

ANNUAL SUMMARY REPORT

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The purpose of these experiments was to determine the effect of an organophosphorus cholinesterase inhibitor, paraoxon, on glucose utilization and the levels of intermediary metabolites in brain. The 24 hr. I.V. LD_{50} of paraoxon in rats was 0.764 ± 0.032 μmol/kg. A dose of 0.8 of the LD_{50} did not result in any evidence of hypoxia in rats up to 2 hr. post-injection. The levels of glucose, pyruvate, glutamate, lactate, ATP, and phosphocreatine were determined in the following brain areas: cortex, midbrain, thalamus-basal ganglia, cerebellum and brain stem at 2, 10, 32 or 128 min. after administration of 0.8

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or 0.5 of the LD_{50} of paraoxon. The only consistent change observed was an elevation of tissue glucose. Injection of 0.8 of the LD_{50} of paraoxon resulted in a significant depression in glucose utilization in the cortex for up to 30 min. However this decrease was not observed at 128 min. when the peripheral cholinergic effects were also absent. The diminished glucose utilization may be the cause of the increase in brain glucose observed. The decrease in glucose utilization may be due to an increase in inhibitory cholinergic neuronal activity.

SUMMARY

The purpose of these experiments was to determine the effect of an organophosphorus cholinesterase inhibitor, paraoxon, on glucose utilization and the levels of intermediary metabolites in rat brain. The 24 hr. I.V. LD_{50} of paraoxon in rats was found to be 0.764 ± 0.032 μmol/kg. A dose of 0.8 of the LD_{50} did not produce hypoxia in the animals up to 2 hr. post-injection as reflected by the blood pH, pCO_2 or pO_2: The levels of glucose, pyruvate, glutamate, lactate, ATP and phosphocreatine were fluorometrically determined in the following brain areas: cortex, midbrain, thalamus-basal ganglia, cerebellum and brain stem at 2, 10, 32 or 128 min after administration of 0.8 or 0.5 of the LD_{50} dose of paraoxon. Although several of the metabolite levels were altered the only consistent pattern observed was an elevation in tissue glucose. This elevation may be due to an increased blood glucose or the depression of brain glucose utilization by paraoxon. Brain glucose utilization was measured using the uptake of [^{14}C]glucose and [^{3}H]fluorodeoxyglucose in rat brain cortex at the same time periods indicated above. Injection of 0.8 of the LD_{50} of paraoxon resulted in a significant depression in glucose utilization for up to 30 min. However this decrease was not observed at 128 min. when the peripheral cholinergic effects were also absent. The diminished glucose utilization may be due to an increase in the activity of inhibitory cholinergic neurons in the brain. Future work will examine the effects of paraoxon on glucose utilization in the brain regions listed above and the effects of Soman on brain metabolites and glucose utilization in the same brain regions.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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INTRODUCTION

Organophosphorus anticholinesterase compounds (OPACs) are known to produce a variety of toxic neurophysiological effects. It has been demonstrated that these compounds affect both the peripheral and central nervous system. Since the brain is critically dependent on the oxidation of glucose for energy to function, the effect of OPACs on glucose utilization and energy metabolism in the brain could be involved in its toxic effects.

It has been reported that subseizure doses of OPACs lower regional deoxyglucose utilization by rat brain[1] [2]. However this effect was not noted with physostigmine or oxotremorine while conflicting results have been reported with the anticholinergic, scopolamine. Evidence has been presented that some OPACs produce uncoupling of oxidation and phosphorylation in rat brain synaptosomes[3]. Two publications reported that dichlorvos and trichlorphan produced a small increase in rat brain ATP[4] [5]. There are no reports in the literature regarding the effects of the OPACs on the levels of glycolytic, Krebs cycle or high energy intermediates in brain. Thus, information is lacking which would correlate the effect of OPACs on glucose utilization and the levels of intermediary metabolites in discrete brain areas. This would be of interest since the neurophysiological effects of the OPACs could be due to their production of a metabolic deficit in brain. This report presents some preliminary results on the effects of paraoxon (diethyl-p-nitrophenyl phosphate) on brain glucose utilization and metabolites in rats.

METHODS

Rats weighing 250-300 g were used in all experiments. They were allowed food and water ad lib.

Glucose utilization was determined using a method which combines features from a number of different techniques. Rats were injected in the tail vein with a mixture of D-[6-^{14}C]glucose and D-[^{3}H]fluorodeoxyglucose and sacrificed 2 or 5 min. later by subjecting them to 0.75 s of microwave radiation. The microwave instrument used was a 25 kW oven operating at 915 MHz and its characteristics have previously been described[6]. The brains were removed and dissected into regions. Each area was homogenized in the following volumes and concentrations of $HClO_4$: cortex, 9 vol of 1 N $HClO_4$; brainstem and midbrain, 0.75 ml of 2 N $HClO_4$; cerebellum and thalamus-basal ganglia, 0.75 ml of 4 N $HClO_4$. The homogenates were spun at 15,000 xg for 10 min and the supernatants neutralized with 1 N KOH and allowed to remain at 0°C for 30 min. The supernatant was respun and separated from the precipitated $KClO_4$. The extract was then chromatographically separated to identify the amounts of radioactive label in free glucose and flurodeoxyglucose and in metabolites of these two compounds. The details of this technique have been published[7] [8] [9] [10].

Metabolite concentrations in the neutralized extracts were determined either spectrophotometrically or fluorometrically by standard enzymatic techniques[11]. The following metabolites were measured: glucose, glutamate, pyruvate, lactate, ATP and phosphocreatine.

For the determination of blood gases and pH, the rats were anesthetized with sodium pentobarbital and the left femoral artery cannulated one day prior to experimentation. Arterial blood samples were taken and the pCO_2, pO_2 and

pH were determined with a Model 213 pH Blood Gas Analyzer (Instrumentation Lab., Inc.). Rectal temperature was taken using a YSI Model 47 Tele-Thermometer (Yellow Springs Inst., Co.). Paraoxon (Aldrich Chemical Co.) was dissolved in 30% ethanol-water (v/v) and further diluted with saline for injection. The compound was given i.v. via the tail vein.

All chemicals used for the metabolite assays were reagent grade.

Protein content was determined by the method of Lowry et al.[12]. The results were statistically analyzed using Student's 2-tailed t-test. A value of 0.05 or less was considered to be significant.

RESULTS

The intravenous LD_{50} for paraoxon in rats has not been reported. We therefore determined this value in rats with the method described by Weil[13]. Trial doses revealed that rats survived an injection of 0.70 μmol/kg of paraoxon for 24 hr. but not a dose of 0.91 μmol/kg. Using these preliminary findings we examined the 24 hr. lethal effect of four doses (Table 1). Based on these results the 24 hr. LD_{50} of paraoxon was calculated to be 0.764 ± 0.032 μmol/kg with a range of 0.732 - 0.796 μmol/kg. In order to minimize the possibility of any deaths during our procedures we chose to investigate the effect of 0.8 and 0.5 of the LD_{50} dose for our experiments.

Paraoxon produces dyspnea and seizure activity which could result in hypoxia. Such an effect would produce alterations in brain levels of several labile metabolites. Under these conditions we would not be able to distinguish between those effects which directly result with paraoxon from any secondary changes due to hypoxia. In order to determine whether paraoxon would produce hypoxia a series of rats was injected i.v. with 0.8 of the LD_{50} dose of this compound and their arterial pO_2, pCO_2, pH and body temperature measured over a period of 2 hr (Table 2). All of the animals exhibited excessive salivation and tremors for at least 60 min. after injection. The blood gas values obtained at any of the time periods were no different from those measured prior to administration of paraoxon (0 time). The only exception was the 120 min pO_2 value which was significantly higher than that found at 0 times. A decreased body temperature was also observed at 128 min. Since these results did not indicate any evidence of hypoxia we were confident that any alterations in brain metabolites would be due to a direct effect of paraoxon.

The effect of 0.8 of the LD_{50} dose of paraoxon on metabolite levels is presented in Tables 3 through 7. The most consistent finding was a significant elevation in glucose levels at the 8 min period in all brain areas, a depression of pyruvate in the thalamus-basal ganglia and brainstem and a decrease in lactate in the cerebellum at all time periods. Although some of the other metabolites were also affected, no consistent pattern was observed.

Similarly, after 0.5 of the paraoxon LD_{50}, glucose was significantly elevated in all brain areas at 8 or 128 min except for the cortex (Tables 8-11). Glutamate was depressed at all time periods in the brain stem. No definite pattern of changes was apparent with the other metabolites.

The most consistent result observed at these 2 doses was the elevation of glucose. In view of these findings we plan to measure blood glucose and brain glucose-6-phosphate levels in our future experiments with paraoxon and Soman.

The results given in Table 13 indicate that the 0.8 LD_{50} dose of paraoxon produced a decrease in the utilization of glucose in rat brain cortex. This effect was observed as early as 2-5 minutes following administration of the compound and the depression was still evident at 32 minutes. However at 128 minutes the glucose utilization rate was returning to control values. It should also be noted that the peripheral cholinergic effects had also disappeared at this time. These results are similar to those reported for other organophosphorus compounds.

DISCUSSION

The use of a subconvulsive dose of paraoxon in these experiments did not result in the production of hypoxia. Thus, any effects observed at these doses are not due to changes in blood pCO_2, pO_2 or pH. One study has also reported that these same parameters are not affected up to 10 min after administration of a convulsive dose of Soman[14].

The elevation of brain glucose by anticholinesterase drugs has not been reported. However, it is known that low doses of organophosphate compounds result in nicotinic effects upon the adrenal medulla. Thus the elevation of brain glucose may reflect an increased blood glucose produced by a release of catecholamines. Another possibility is that the decreased brain glucose metabolism results in a lower demand for glucose with its subsequent increase in tissue. It has been shown that depressant drugs which decrease brain glucose metabolism also result in an elevated blood glucose[15 16]. The stress of the injection of the drug and isotopes is probably not involved since groups of control animals are injected with saline and sacrificed at the same time periods as the treated groups.

Paraoxon induced a severe depression of glucose utilization in rat brain cortex at the earliest time period examined. This depression persisted for at least 30 minutes but was not observed at 128 minutes. At the latter time the peripheral cholinergic effects were also no longer apparent. It will be interesting to see if a similar pattern is found in the other brain areas. The depressed glucose utilization observed in brain cortex is similar to that reported with subconvulsive doses of organophosphate compounds but not with physostigmine or neostigmine[1]. Convulsive doses of Soman are reported to increase brain glucose utilization but this effect was not seen with subconvulsive doses[2]. The elevation of acetylcholine (ACh) by OPACs is usually associated with an increased activity in the CNS such as convulsions. Thus upon first observation it would appear difficult to reconcile the decreased glucose utilization observed with an increased ACh. However, it has been demonstrated that approximately 13-18% of the cholinergic neurons in cerebral cortex are inhibitory[17]. It could be postulated that the increased ACh produced by the OPACs results in an inhibition of synaptic firing and consequently a decreased demand for energy. This in turn would diminish glucose metabolism. The possibility that the OPACs have a direct effect upon neuronal excitability or glucose metabolism must also be considered.

The elevation in brain glucose and decreased glucose utilization without alteration in the levels of ATP and phosphocreatine found with paraoxon has also been reported to occur with depressant drugs[15 16 18]. Maintenance of these levels may be due to changes in turnover rates which would not be apparent when only steady-state concentrations are determined. The lack of response may be a protective mechanism by the brain to retain its energy stores in face of a declining energy source.

Our future work will examine the effect of paraoxon on glucose utilization in the brain areas used for metabolite assays. We also plan to determine the action of Soman on brain metabolites and glucose utilization in the same areas and at the varying time periods described in this paper.

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TABLE 1. THE LD_{50} DOSE OF PARAOXON IN RATS

Group	Dose (μmoles/kg)	Dose (mg/kg)	Number Surv ng for 24 h
A	0.700	0.193	5/5
B	0.749	0.206	3/5
C	0.801	0.220	1/5
D	0.858	0.236	0/5

Each group contained 5 rats and all survivors were observed for 24 hours after tail vein injection of the drug.

TABLE 2. EFFECT OF PARAOXON ON BLOOD GASES, pH AND BODY TEMPERATURE IN RATS AT VARYING TIME INTERVALS

TIME (Min)	pCO_2	pO_2	pH	T (°C)
0	35.0 ± 7.8	86.0 ± 11	7.45 ± 0.07	37.6 ± 0.7
2	36.5 ± 3.8	81.1 ± 9.6	7.43 ± 0.07	37.4 ± 0.2
30	39.1 ± 4.3	88.6 ± 7.0	7.42 ± 0.05	37.2 ± 0.6
60	33.5 ± 4.0	96.6 ± 7.5	7.41 ± 0.06	36.3 ± 1.4
120	32.7 ± 5.6	104.5 ± 3.1*	7.41 ± 0.06	34.9 ± 2.1*

* $P < 0.02$

All animals were injected i.v. with 0.8 of the LD_{50} dose of Paraoxon (0.764 μmoles/kg). Each value is the mean ± SD of 5 rats.

TABLE 3. THE EFFECT OF PARAOXON ON METABOLITE LEVELS IN RAT BRAIN CORTEX

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	20.4 ± 2.3	123.2 ± 5.7	21.5 ± 4.3	22.5 ± 5.3	21.5 ± 3.8
2	17.8 ± 2.4	124.5 ± 8.3	23.7 ± 3.5	25.0 ± 6.5	23.5 ± 1.2
8	24.9 ± 3.2**	125.0 ± 6.2	23.2 ± 3.8	20.1 ± 8.3	24.2 ± 2.9
32	25.6 ± 5.9*	126.6 ± 4.7	24.2 ± 5.4	22.5 ± 8.7	24.7 ± 2.7
128	18.9 ± 3.5	116.4 ± 6.7*	17.6 ± 0.4*	21.7 ± 8.6	23.1 ± 3.8

Rats were injected with 0.8 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 4. EFFECT OF PARAOXON ON METABOLITE LEVELS IN RAT MIDBRAIN

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	21.25 ± 4.02	0.61 ± 0.12	105.13 ± 20.12	23.39 ± 2.58	34.30 ± 4.52	27.98 ± 3.42
2	19.47 ± 0.58	0.74 ± 0.13	100.20 ± 10.31	30.33 ± 5.26**	35.59 ± 5.26	24.91 ± 5.40
8	24.64 ± 0.23*	0.74 ± 0.09	109.11 ± 9.92	29.45 ± 5.49**	35.93 ± 2.40	31.60 ± 3.91
32	23.18 ± 2.26	0.80 ± 0.11**	108.53 ± 12.85	30.59 ± 6.25	34.31 ± 2.94	29.55 ± 4.66
128	23.01 ± 0.48	0.79 ± 0.11**	109.61 ± 12.68	27.99 ± 6.16	39.15 ± 1.72**	32.42 ± 4.78

Rats were injected with 0.8 of the LD_{50} for paraoxon. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 5. EFFECT OF PARAOXON ON METABOLITE LEVELS
IN RAT BRAIN THALAMUS-BASAL GANGLIA

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	25.51 ± 2.60	1.17 ± 0.15	133.92 ± 12.04	30.77 ± 3.74	33.87 ± 6.56	30.11 ± 4.68
2	22.61 ± 4.02	0.83 ± 0.25**	133.29 ± 14.13	30.30 ± 3.74	40.33 ± 4.85**	30.43 ± 4.84
8	29.42 ± 4.37*	0.916± 0.27*	139.64 ± 8.78	27.46 ± 3.23	41.48 ± 4.70*	32.60 ± 4.99
32	27.42 ± 2.41	0.864± 0.07**	141.11 ± 12.44	27.84 ± 2.69	42.07 ± 2.99**	29.66 ± 3.67
128	27.59 ± 4.69	0.605± 0.18**	124.40 ± 21.83*	23.62 ± 3.92**	45.03 ± 2.49**	27.05 ± 5.20

Rats were injected with 0.8 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 6. EFFECT OF PARAOXON ON METABOLITE LEVELS IN RAT BRAIN CEREBELLUM

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	30.20 ± 4.15	0.80 ± 0.11	151.54 ± 27.32	28.49 ± 3.31	29.50 ± 6.06	30.36 ± 3.19
2	31.00 ± 4.62	0.83 ± 0.20	151.04 ± 19.28	29.23 ± 5.89	33.90 ± 4.95	21.94 ± 3.78**
8	43.06 ± 7.37**	0.77 ± 0.16	153.16 ± 12.49	31.37 ± 4.44	28.41 ± 5.74	24.42 ± 3.85**
32	33.28 ± 4.10	0.74 ± 0.13	145.64 ± 7.84	28.46 ± 3.68	27.79 ± 5.96	24.39 ± 6.46*
128	33.54 ± 3.18	0.65 ± 0.14*	147.44 ± 22.29	25.86 ± 4.70	28.05 ± 5.00	23.83 ± 3.77**

Rats were injected with 0.8 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 7. EFFECT OF PARAOXON ON METABOLITE LEVELS IN RAT BRAIN STEM

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	23.27 ± 5.53	0.63 ± 0.11	104.27 ± 11.13	26.66 ± 3.69	34.45 ± 3.44	18.98 ± 3.35
2	22.83 ± 2.81	0.55 ± 0.08	99.38 ± 11.34	33.37 ± 2.29**	30.24 ± 2.12	19.27 ± 3.63
8	26.18 ± 4.26	0.50 ± 0.11*	98.62 ± 20.38	29.26 ± 5.21	30.94 ± 4.06	18.33 ± 2.56
32	25.04 ± 5.03	0.47 ± 0.15*	93.67 ± 14.57	32.14 ± 4.69*	31.66 ± 7.32	19.47 ± 2.65
128	22.92 ± 1.02	0.51 ± 0.11*	95.68 ± 10.71	33.10 ± 3.90**	27.74 ± 6.36*	18.37 ± 2.09

Rats were injected with 0.8 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 8. EFFECT OF PARAOXON ON METABOLITES LEVELS IN RAT BRAIN CORTEX

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	20.4 ± 4.1	1.39 ± 0.3	116.4 ± 17.3	19.7 ± 4.4	41.9 ± 17.2	26.9 ± 9.4
2	19.8 ± 4.0	1.40 ± 0.5	126.1 ± 9.2	22.6 ± 3.5	35.3 ± 2.7	23.6 ± 3.9
8	25.7 ± 5.8	0.98 ± 0.12	126.9 ± 16.5	22.3 ± 2.3	35.5 ± 6.5	22.8 ± 4.5
32	27.2 ± 10.6	1.20 ± 0.43	109.4 ± 22.3	18.9 ± 5.4	36.0 ± 1.9	24.1 ± 1.4
128	21.4 ± 3.6	1.55 ± 0.11	113.7 ± 8.8	34.7 ± 3.2**	22.3 ± 1.4**	22.3 ± 1.4

Rats were injected with 0.5 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 9. EFFECT OF PARAOXON ON METABOLITE LEVELS IN RAT MIDBRAIN

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	19.30 ± 3.23	0.70 ± 0.17	94.71 ± 21.04	37.95 ± 5.54	21.63 ± 1.16	21.43 ± 0.87
2	18.83 ± 5.48	0.68 ± 0.16	99.41 ± 29.67	37.82 ± 8.04	21.80 ± 0.85	23.50 ± 3.15
8	30.95 ± 3.07*	0.67 ± 0.12	82.83 ± 7.33	37.70 ± 7.67	20.70 ± 1.51	22.49 ± 0.25
32	34.28 ± 0.03*	0.72 ± 0.07	83.35 ± 5.32	37.37 ± 9.19	20.70 ± 0.08	23.79 ± 0.30
128	24.00 ± 7.73	0.64 ± 0.22	75.59 ± 15.05	23.22 ± 8.06*	20.28 ± 4.60	21.81 ± 7.14

Rats were injected with 0.5 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 10. EFFECT OF PARAOXON ON METABOLITE LEVELS
IN RAT BRAIN THALAMUS-BASAL GANGLIA

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	17.74 ± 2.38	0.40 ± 0.08	77.53 ± 6.22	21.36 ± 6.60	18.14 ± 2.61	20.11 ± 4.35
2	14.94 ± 0.95	0.42 ± 0.01	78.38 ± 0.57	24.60 ± 4.17	19.62 ± 0.75	21.17 ± 1.23
8	24.75 ± 2.96**	0.43 ± 0.08	74.86 ± 7.12	22.13 ± 1.89	19.57 ± 1.21	24.20 ± 0.01*
32	21.96 ± 2.51**	0.44 ± 0.05	68.28 ± 7.06*	24.66 ± 0.36	19.08 ± 0.46	23.48 ± 3.28
128	21.06 ± 0.70**	0.39 ± 0.06	70.19 ± 4.72*	18.68 ± 0.40	22.27 ± 3.25*	22.80 ± 2.60

Rats were injected with 0.5 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 11. EFFECT OF PARAOXON ON METABOLITE LEVELS IN RAT BRAIN CEREBELLUM

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	18.42 ± 2.18	0.46 ± 0.10	82.10 ± 7.09	22.34 ± 2.90	24.25 ± 3.96	17.66 ± 1.92
2	23.15 ± 0.73**	0.44 ± 0.04	86.42 ± 12.52	24.88 ± 3.73	21.97 ± 0.45	21.18 ± 1.44**
8	30.01 ± 1.26**	0.47 ± 0.03	73.40 ± 1.69**	23.90 ± 1.77	21.68 ± 3.73	19.58 ± 0.74*
32	28.42 ± 2.11**	0.58 ± 0.01	77.27 ± 1.19	28.32 ± 4.02**	21.33 ± 0.15	19.38 ± 0.85*
128	21.71 ± 5.08	0.44 ± 0.04	67.05 ± 4.59**	18.33 ± 1.13**	21.15 ± 4.36	19.77 ± 3.37

Rats were injected with 0.5 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 12. EFFECT OF PARAOXON ON METABOLITE LEVELS IN RAT BRAIN-BRAIN STEM

nmoles/mg protein

TIME AFTER INJECTION (Min)	GLUCOSE	PYRUVATE	GLUTAMATE	LACTATE	CREATINE-P	ATP
0	21.29 ± 3.76	0.58 ± 0.12	77.65 ± 5.73	32.45 ± 3.17	15.89 ± 1.83	22.24 ± 2.59
2	28.22 ± 1.08**	0.69 ± 0.10	90.81 ± 6.30**	33.94 ± 6.41	16.11 ± 2.15	20.48 ± 0.35
8	28.26 ± 5.60**	0.64 ± 0.08	58.43 ± 6.50**	26.33 ± 1.58**	14.43 ± 0.10*	19.34 ± 0.50
32	29.79 ± 3.44**	0.60 ± 0.08	65.46 ± 6.73**	36.49 ± 2.55*	13.15 ± 0.55**	20.10 ± 1.38
128	29.53 ± 3.16**	0.54 ± 0.07	56.67 ± 5.22**	28.95 ± 4.20	14.55 ± 0.30	16.61 ± 2.98**

Rats were injected with 0.5 of the paraoxon LD_{50}. Each value is the mean ± SD of 5-8 animals per group.

* $P < 0.05$
** $P < 0.01$

TABLE 13. THE EFFECT OF 0.8 LD_{50} DOSE OF PARAOXON ON GLUCOSE UTILIZATION IN RAT BRAIN CORTEX

TIME (Min)	H^3-FLUORODEOXYGLUCOSE	6-C^{14}-GLUCOSE	N
0	0.446 ± 0.119	0.791 ± 0.173	5
2	0.136 ± 0.156*	0.542 ± 0.398	5
10	0.283 ± 0.069	0.497 ± 0.181*	4
30	0.176 ± 0.107	0.433 ± 0.160**	5
128	0.383 ± 0.268	0.684 ± 0.411	5

All values are means (± SD) expressed as μmol/min per g. N is the number of pairs of animals used to calculate the rate of glucose utilization.
*$P < 0.05$, ** $P < 0.01$.

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