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Cholinesterase Activity in Rats Treated with Propoxur

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Propoxur (2-isopropoxyphenyl N-methylcarbamate) as others carbamate insecticides involves a cholinesterase (ChE) inhibition which is directly related to the cholinergic effects of these compounds. Exposure to lower amounts of carbamates cause a determinable but symptomless depression of the enzyme. Therefore determination of ChE activity in blood is used as an indicator of exposure to these compounds.

VANDEKAR et al. (1971) ascertained a dose related dependence between brain and plasma ChE activity at different doses of propoxur infused into the juglar vein of rats. Also good correspondence between the degree of enzyme depression and the intensity of symptoms were found.

This paper reports the influence of propoxur administered to rats in single 0.5 LD_{50} doses and in a subacute study on the activity of ChE in blood and brain in relation to propoxur levels in these materials. Also effects of different doses of propoxur on the activity of ChE in blood were investigated.

MATERIALS AND METHODS

Chemicals: Propoxur of high purity was obtained from the Institute of Organic Industry, Warsaw.

Animals and administration: Male albino rats weighing 180-210 g were given propoxur as a solution in diethylene glycol-ethanol (7:3 v/v). The insecticide was administered: a) as a single i.v. dose of 5 mg/kg, b) as a single oral dose of 50 mg/kg, c) daily for 14 days 30 mg/kg, for the next 28 days the dose was elevated to 50 mg/kg, d) in the dose-response study as single oral doses of 70.0, 20.9, 7.0 or 2.1 mg/kg. Control animals received the corresponding amount of the solvent.

Methods: Blood and brain were obtained from animals sacrificed by decapitation. Only in the dose-response study blood samples were collected from the tail vein. Cholinesterase activity in blood and brain was determined by the method of ELLMAN et al. (1961). Student's t-test was used to test for difference between control and treatment means. The 0.05 level of probability was used as the criterion of significance. Propoxur in blood and brain was determined by a gas chromatographic method (KRECHNIAK & FOSS 1979).

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RESULTS

The results of ChE activity are expressed as percentages of mean control or pretreatment values.

In animals treated with propoxur, typical symptoms of stimulation of the parasympatic system were noticed: salivation, involuntary defecation and urination, secretion from the nose, tremors. Also paralysis of posterior extremities were observed. The intensity of symptoms was very differentiated in individual animals. Such symptoms were not observed in controls. Seven animals died during injection of propoxur.

Single dose studies.

The effects of single intravenous and oral doses of propoxur on the activity of cholinesterase and the level of propoxur in blood and brain are presented in figure 1.

The lowest activity of ChE following intravenous administration of propoxur was found after 5 min. The activity of the enzyme was significantly decreased for 60 min. Then a significant increase in the enzyme activity was found in blood 2 h and in brain 4 h after dosing.

The animals given propoxur orally showed the greatest decline in ChE activity in blood 15 min and in brain 30 min after dosing. A significant decrease of ChE activity was ascertained in blood for 12 h and in brain for 1 h. The period of enzyme inhibition was followed by a significant increase of ChE activity in blood and brain.

Symptoms of stimulation of the parasympatic system occurred immediately after intravenous injection and disappeared within 15 min. After oral dosage these symptoms occurred roughly 5 to 10 min later and disappeared after 20 to 40 min.

The highest concentration of propoxur after intravenous injection was found both in blood and brain at the 5-min determination. The insecticide is rapidly eliminated from tissues so that after 6 h only traces of propoxur could be estimated.

Following oral administration the highest concentration of propoxur in blood was found after 15 min. At that time only traces of the compound could be found in brain. Propoxur reached its highest level in brain at 1 h after administration and subsequently declined.

Subacute study.

In the subacute study (figure 2) the animals received propoxur orally during the first two weeks in a daily dose of 30 mg/kg, then the dose was elevated to 50 mg/kg. However, despite the elevation of the dose a significant decrease in ChE activity was found in brain only for 14 and in blood for 28 days. At the end of the experiment a slight increase of enzyme activity in brain was ascertained.

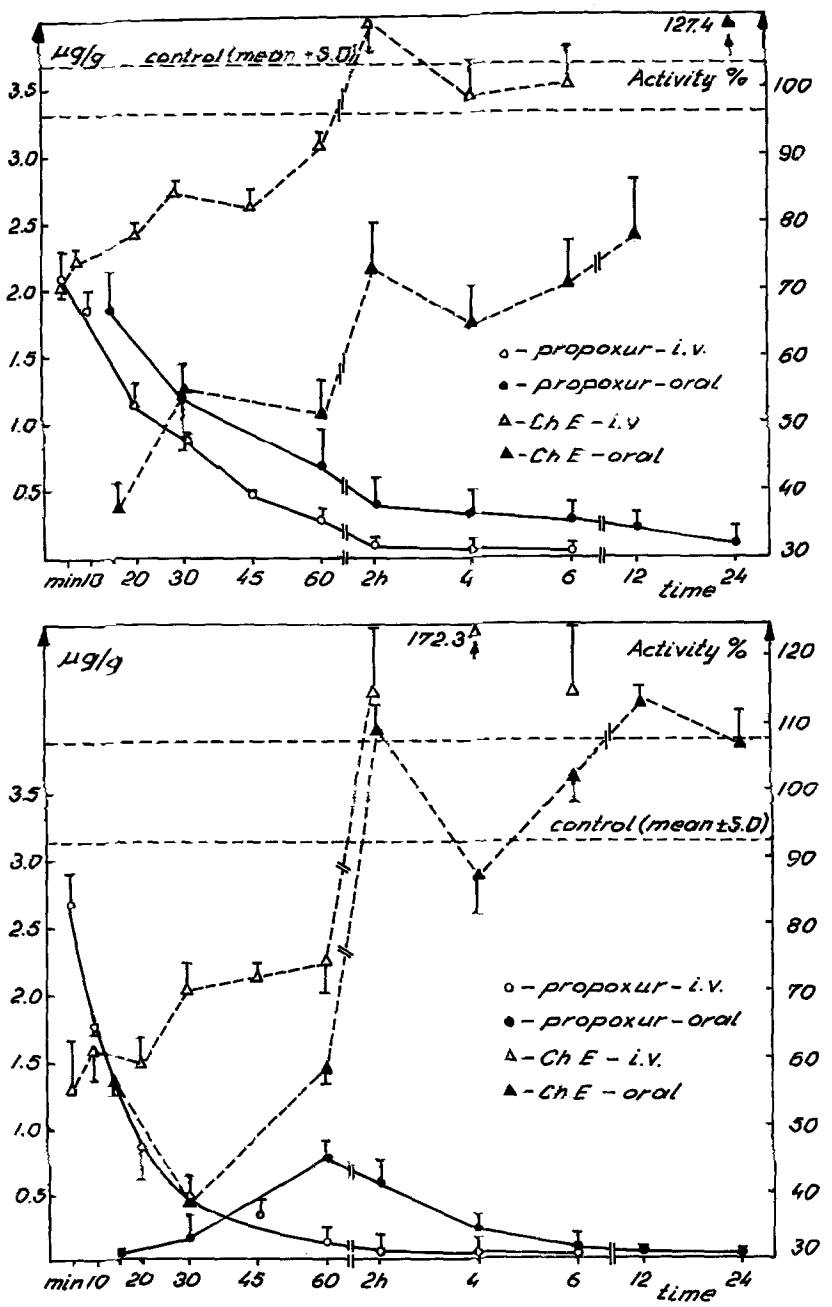

Figure 1. ChE activity and propoxur level in blood (above) and brain (below) of rats following single 0.5 LD_{50} doses of propoxur. Each point represents the mean ± S.E. in 6 animals.

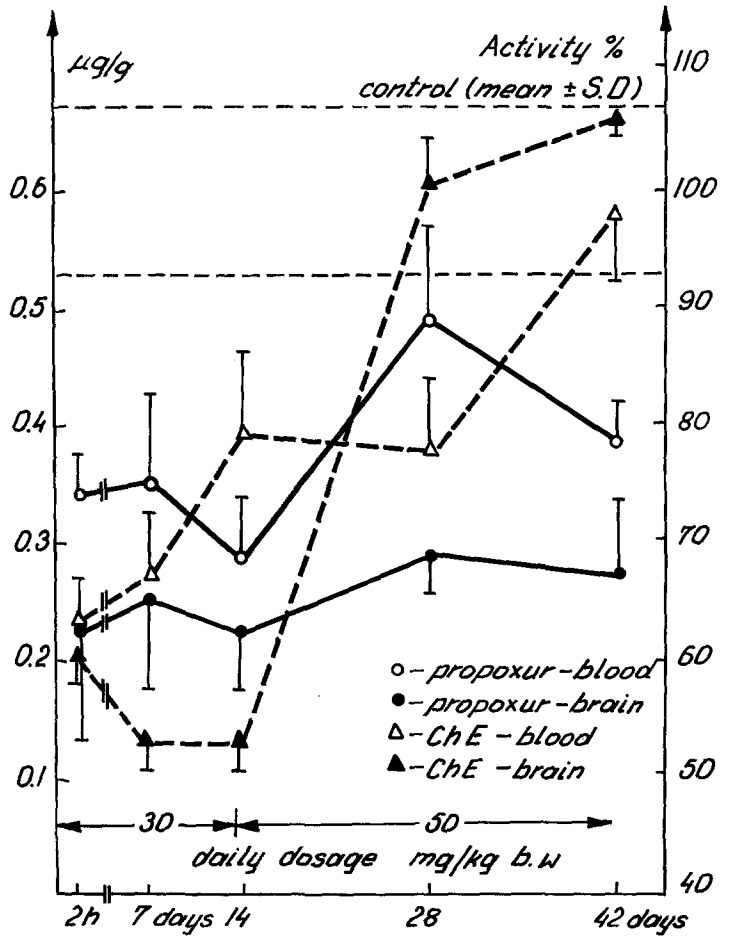

Figure 2. ChE activity and propoxur level in blood and brain of rats exposed to propoxur for 42 days. Each point represents the mean ± S.E. in 6 animals.

Cholinergic symptoms (salivation, tremors) were noticed during the first five days of the experiment. After the dose was elevated these symptoms were observed again for three days but were less intense.

No significant changes of the levels of propoxur were noticed during the first two weeks of dosing. After the elevation of the dose a slight increase was found in blood but not in brain.

Dose response study.

Figure 3 presents the effects of different single oral doses of propoxur on ChE activity.

The oral LD_{50} in male rats was estimated by GAINES (1969) at 83 mg/kg, by VANDEKAR et al. (1971) at 100 mg/kg.

The highest dose in this study (70 mg/kg) was selected as evoking a clearly toxic but not lethal effect.

The greatest decrease in enzyme activity was ascertained 10 min after dosing. Only in rats given the smallest dose (2.1 mg/kg) the lowest activity was found after 20 min. The decrease in ChE activity is dose dependent. The lowest values ranged from 83.7 to 52.2% of preexposure values.

Also the period of time when the activity of the enzyme is decreased is dose dependent. It ranged from 30 min at the smallest dose to 24 h at the dose of 70 mg/kg.

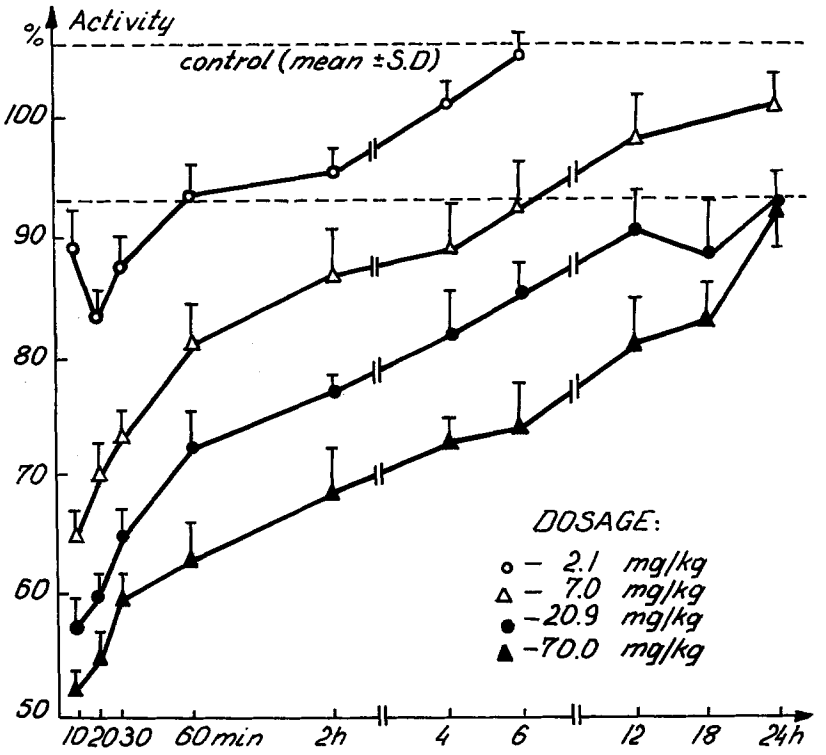

Figure 3. ChE activity in blood of rats following different single oral doses of propoxur. Each point represents the mean ± S.E. in 8 animals.

DISCUSSION

VANDEKAR et al. (1971) noticed in rats following intramuscular injection or intravenous infusion the onset of cholinergic symptoms when the ChE activity in brain and plasma dropped below 50% of normal. In our experiment (especially after intravenous injection) signs of poisoning occurred when considerably higher values of enzyme activity were estimated.

In rats given single 0.5 LD_{50} doses of propoxur a correlation between ChE activity and level of unchanged propoxur in blood and brain was found. However, the correlation occurred only during the

period when the enzyme activity was significantly decreased (that is 1 h after i.v. and 12 h after oral dosing). The correlation coefficient in blood for both routes was 0.92 ($P<0.001$), in brain after i.v. injection 0.88 ($P<0.005$). No correlation was found in brain after oral administration. This seems to be connected with the prevalence of the absorption phase in the pharmacokinetics of propoxur during the first hour after dosing (FOSS & KRECHNIAK 1980). Propoxur reached its peak level in brain at 60 min. However, at that time ends the inhibition period of the enzyme.

Also no correlation between ChE activity and propoxur level was found in the subacute study. The decreased enzyme activity despite the increase of the dose slowly recovered to normal values. We found a complete recovery of ChE activity within 28 days in brain and 42 days in blood.

In our experiments after the inhibition period a more or less pronounced increase of ChE activity was noticed. It may be connected with a kind of stimulation of enzyme synthesis.

The dose response study indicates that the degree of the decrease of ChE activity as well as the inhibition period caused by propoxur is dose dependent. Also the level of propoxur in blood assessed in parallel groups of animals was dose related. However, the latter assay is less sensitive as only at the 70 mg/kg dose measurable concentrations of propoxur were found during the whole period of investigation. At lower dosage levels often only trace amounts of propoxur were found, also the obtained results were less reproducible.

In general, determination of ChE activity though not specific is a sensitive and reliable test to assess acute exposure to propoxur. However, its use in chronic exposure is of little diagnostic value because of the rapid adaptation of the enzyme. In that case the level of propoxur in blood is a better index of exposure to this compound.

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