Bull. Environm. Contam. Toxicol. 29, 605–610 (1982)

Changes in Toxicity of DDVP, DFP, and Parathion in Rats Under Cold Environment

D. P. Chattopadhyay, S. K. Dighe, D. K. Dube, and Purnanand

Division of Biochemistry, Defence Research and Development Establishment, Gwalior-474002, India

Introduction of cholinesterase (ChE) inhibitors into our environment, mainly in the form of pesticides, caused great concern regarding their accidental exposure. It is known that among the various environmental conditions, temperature is an important factor which can influence the concentration of a specific toxic agent at the biological receptor. Cold-induced alteration in several drug metabolizing enzymes has been reported (DEWHURST 1963; INSCOE & AXELROD 1960; INSCOE et al. 1965; KALSER & KUNIG 1969; FULLER et al. 1972). It is also known that the capacity to tolerate and metabolize a toxicant often reflects the titers of various biotransformation enzymes. Influence of ambient temperature on the toxicity of several organophosphorus (OP) compounds has been reported earlier (STREICHER 1951; BAETJER & SMITH 1956; MARTON et al. 1962; MEETER & WOLTHUIS 1968, 1969; MEETER 1970, 1973; AHDAYA et al. 1976). These studies were mainly concerned about the changes either in body temperature or mortality or both; information on the inhibition and reactivation of ChE activity were very fragmentary. The present paper describes our efforts to relate the differences in the toxicity among the three structurally as well as metabolically different OP-compounds such as dimethyl 2,2-dichlorovinyl phosphate (DDVP), diisopropyl phosphorofluoridate (DFP) and O,O-diethyl O-p-nitrophenyl phosphorothionate (parathion) with their effects on the inhibition of whole blood ChE activity (EC 3.1.1.7 and EC 3.1.1.8) and its subsequent regeneration to free enzyme, and body temperature in the rats under cold environment.

MATERIALS AND METHODS

Parathion and DFP were prepared according to the method of TOY & BECK (1950), and SAUNDERS & STACEY (1948), respectively. DDVP (technical grade, 95% purity) was a gift of Ciba-Geigy of India. Identity and purity of the OP-compounds were confirmed by TLC, GLC, IR, UV and NMR-spectroscopy. Acetylcholine chloride was purchased from Sigma Chemical Co., USA. All reagents were analytical grade.

Male albino rats (140±10g) from DRDE animal breeding colony, raised on commercial pellet diet (Hind Lever, India) and water ad libitum were used. One group of animal was kept at room temperature (28±2C) and the other group was exposed to cold (5±1C) for 3 days prior to treatment with OP-compounds.

Blood samples of the animals were collected from orbital sinus using

0007-4861/82/0029-0605 $01.20

heparinized tubes under mild ether anesthesia, and ChE activity was determined immediately to avoid the possibility of any in vitro reactivation. ChE activity was assayed by the method HESTRIN (1949), as described by AUGUSTINSSON (1954). Rectal temperature was determined using a temperature recorder (Electrolaboratoriet, Type Z8, Copenhagen).

The animals at room temperature and those pre-exposed to cold for 3 days were injected i.p. with a single dose of one of the three OP-compounds (DDVP: 12.5 and 6.25 mg/kg; DFP: 4.0 and 2.0 mg/kg; parathion: 3.5 and 1.75 mg/kg; each dose correspond to either 50% or 25% of LD_{50}). OP-compounds were dissolved in safflower oil half an hour before injection. The volume of injection was 5 mL/kg. In the case of cold exposed animals all measurements were made under cold environment.

Rectal temperature and whole blood ChE activity were determined prior to treatment and at different time interval (0.0, 0.25, 0.5, 1.0, 3.0, 24.0, 48.0 and 72.0 h) following the administration of OP-compounds. The values are expressed as percent inhibition of ChE activity using the pretreatment sample as 0% inhibition.

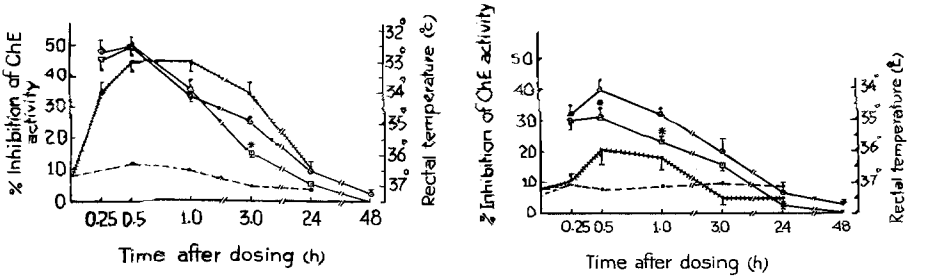
RESULTS AND DISCUSSION

No change was observed in the level of whole blood ChE activity and body temperature of the animals after 3 days of cold exposure. A slight fall in body temperature was noticed during the early period (between 1 and 3 h) of cold exposure without any change in ChE activity. Administration of safflower oil had no effect either on body temperature or ChE activity of the animals with and without cold exposure.

Maximum inhibition of whole blood ChE activity was noticed at 0.5 h after DDVP treatment to the animals with and without cold exposure (Fig. 1a and 1b). Lesser inhibition and faster recovery of ChE activity in the cold exposed animals treated with a lower dose of DDVP compared to the animals at room temperature (Fig. 1b) might have been due to increased detoxification of DDVP as a result of induction of drug metabolizing enzymes under cold stress; however, the same mechanism could not explain the effect of a higher dose of DDVP on the inhibition and reactivation of the ChE activity which were almost similar in both the groups of animals excepting that at 3 h after DDVP treatment the reactivation of ChE activity was significantly higher in the cold exposed animals than those at room temperature (Fig. 1a).

A slower absorption of DFP under cold temperature could be one of the possible reasons for the slower rise in the rate of inhibition of ChE activity after administration of DFP in the cold exposed animals (Fig. 2a and 2b).

Unlike DDVP and DFP a longer time (3 h) was required by parathion to produce maximum inhibition of ChE activity (Fig. 3a and 3b); this delay in achieving the maximum inhibition was due to the time



(a) Dose: 12.5 mg/kg (b) Dose: 6.25 mg/kg

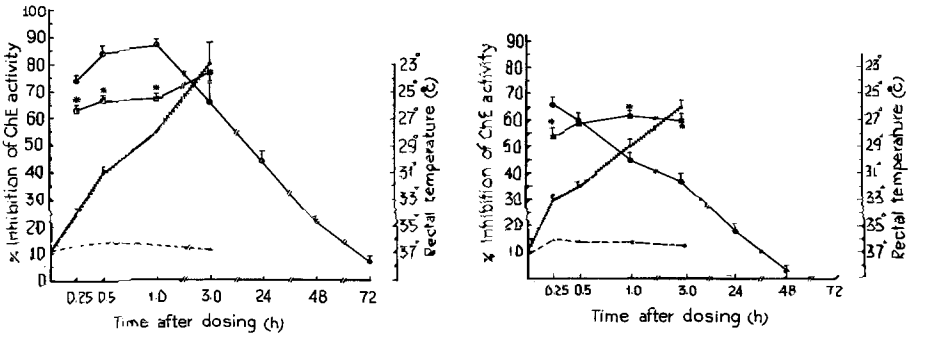
Figure 1. Effect of DDVP on the inhibition of whole blood ChE activity and body temperature.

Each point is the mean and the bars indicate ± S.E. The asterisks (*) indicate significant difference ($P<0.01$. o——o: ChE activity at 28C.

□——□ : ChE activity at 5C.

●---● : Rectal temperature at 28C.

●++++● : Rectal temperature at 5C.



(a) Dose: 4.0 mg/kg (b) Dose: 2.0 mg/kg

Figure 2. Effect of DFP on the inhibition of whole blood ChE activity and body temperature.

Each point is the mean and the bars indicate ± S.E. The asterisks (*) indicate significant difference ($P<0.01$). o——o: ChE activity at 28C.

□——□: ChE activity at 5C.

●---●: Rectal temperature at 28C.

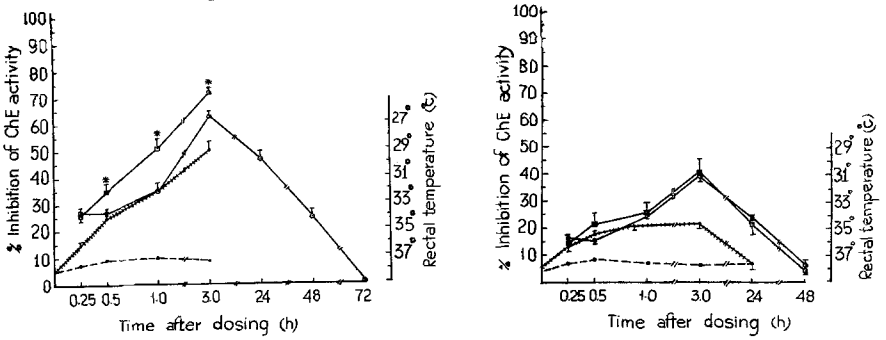
●++++●: Rectal temperature at 5C.

taken for the biotransformation of parathion to paraoxon since parathion has no anti-ChE activity (GAGE 1953). The higher inhibition of ChE activity in the cold exposed animals than those at room temperature after administration of parathion at a higher dose (Fig. 3a) would suggest an increase in the hepatic microsomal oxidative desulfuration of parathion to paraoxon induced by cold stress. However, at a lower dose of parathion the activation process might have been well coupled to secondary metabolism of the active metabolite

(deactivation), hence there was no difference in the inhibition of ChE activity and its reactivation between the animals with and without cold exposure.

From the rate of regeneration of inhibited ChE activity following the administration of different OP-compounds it is suggested that the reactivation of the enzyme activity could be attributed mainly to dephosphorylation of the phosphorylated (inhibited) enzyme with little contribution of newly synthesized enzyme.

In agreement with the earlier observations (AHDYAY et al. 1976; MARTON et al. 1962; MEETER & WOLTHUIS 1968, 1969; MEETER 1970, 1973a), we noticed hypothermic effect of the OP-compounds in the animals under cold exposure. In rats, most OP-compounds at sublethal doses cause hypothermia of central origin (MEETER & WOLTHUIS 1968; MEETER et al. 1971). It has been confirmed that good reactivating oximes which do pass the blood-brain barrier reduce the hypothermia (BENSCHOP et al. 1976; WOLTHUIS & KEPNER 1978, WOLTHUIS et al. 1981). Skin vasodilatation brought about by ChE-inhibitors cannot be the only factor responsible for the hypothermia (MEETER 1973b). The inability of the cold exposed rats to produce sufficient heat after administration of OP-compounds is not the reason for the hypothermia since we could not observe any change in the hepatic mitochondrial oxidative metabolism in the rats treated with DDVP (unpublished). However, the possibility of an impairment of thermogenesis in the brown adipose tissue, an important thermogenic organ in the cold defence in a low temperature environment, cannot be ruled out at present.



(a) Dose: 3.5 mg/kg

(b) Dose: 1.75 mg/kg

Figure 3. Effect of parathion on the inhibition of whole blood ChE activity and body temperature.

Each point is the mean and the bars indicate ± S.E. The asterisks (*) indicate significant difference ($P<0.01$). o——o: ChE activity at 28C.
□——□ : ChE activity at 5C.
●- - -● : Rectal temperature at 28C.
●——● : Rectal temperature at 5C.

Comparison among the doses used revealed that the magnitude of inhibition of ChE activity as well as the fall in body temperature

increased with the increase in the dose of the OP-compounds. The higher the anti-ChE activity of the OP-compounds the lower was the body temperature of the animals under cold exposure. The maximum fall in body temperature coincided in most cases with the time of maximum inhibition of ChE activity. However, the body temperature returned to normal level before the full recovery of the enzyme activity occurred.

The doses of DFP and parathion which were sublethal at room temperature appeared to be lethal under cold temperature (Table 1). An increased toxicity of OP-compounds under low ambient temperature was also demonstrated by others (BEATJER & SMITH 1956; STREITCHER 1951; MARTON et al. 1962; AHDAYA et al. 1976). Death of the animals treated with OP-compounds under cold environment might not have been due to hypothermia per se, the exact mechanism of increased toxicity at lower body temperature is not fully understood as yet.

Table 1. Mortality of rats under cold exposure at different time following the administration of OP-compound.

Treatment	Dose, mg/kg	Mortality (dead/total) at different time (h) after dosing			
		0.5	1.0	3.0	24.0
DDVP	6.25	0/9	0/9	0/9	0/9
DDVP	12.50	0/9	0/9	0/9	0/9
DFP	2.0	0/9	0/9	0/9	9/9
DFP	4.00	1/15	6/15	9/15	15/15
Parathion	1.75	0/8	0/8	0/8	1/8
Parathion	3.50	0/8	0/8	0/8	8/8

Inhibition of ChE activity, hypothermia and mortality of the animals following the administration of OP-compounds appeared to be in the following order of decreasing activity DFP > parathion > DDVP. The lowest activity of DDVP is attributed to its rapid detoxification to metabolites which did not inhibit ChE activity (HODGSON & CASIDA 1962).

Acknowledgment. We thank P. K. Ramachandran, Director, for useful discussion and keen interest in this study. We are also thankful to Ciba-Geigy of India, for the generous gift of DDVP, and to Miss Kalawati for secretarial assistance.

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Accepted August 31, 1982