Neurotoxic effects of DDT: protection by cycloheximide

Recently, Hrdina, Singhal & others (1971) reported that the principal neurotoxic symptoms (hyperexcitability, tremors, convulsions and hyperpyrexia) seen after acute administration of *pp'*-DDT to male and female rats were associated with a significant decrease in the concentration of acetylcholine in the striatum. The alterations in acetylcholine were time-dependent and correlated well with the occurrence and the severity of tremors. Administration of *pp'*-DDT also results in a marked stimulation of the quartet of key rate-limiting gluconeogenic enzymes in both liver and kidney cortex (Kacew, Singhal & Ling, 1971). Whilst examining the influence of inhibitors of RNA and protein synthesis on the DDT-induced increases in gluconeogenic enzyme activities, we noted that pretreatment with cycloheximide protected the animals against the neurotoxic effects of DDT. This prompted us to examine whether the decrease in striatal acetylcholine observed after DDT treatment could also be prevented by cycloheximide and by another inhibitor of protein synthesis such as actinomycin D. We now report that cycloheximide, but not actinomycin D, prevents the insecticide-induced decrease in striatal acetylcholine as well as the development of tremors and hyperpyrexia.

Male Wistar rats, 180–220 g, were divided in the following six groups with at least four rats in each group: (*a*) control rats, (*b*) animals treated with *pp'*-DDT, (*c*) rats injected with cycloheximide or actinomycin D and (*d*) *pp'*-DDT-treated animals given either cycloheximide or actinomycin D. *pp'*-DDT was dissolved in corn oil and administered orally in a dose of 60 mg/100 g of body weight. Cycloheximide was injected in a single dose (70 μg/100 g, i.p.) 30 min before *pp'*-DDT, whereas actinomycin D (40 μg/100 g, i.p.) was administered in two divided doses 30 min before and 2½ h after treatment with the insecticide. Control animals received an equal volume of the corresponding vehicle. Rats were carefully observed for the development of neurotoxic symptoms and their colonic temperatures were measured at hourly intervals, with a rectal probe. Since the effects of DDT on the central nervous system were most pronounced at approximately 5 h (Henderson & Woolley, 1970; Hrdina & others, 1971) after its administration, animals were killed at this time using the "near-freezing" technique of Takahashi & Aprison

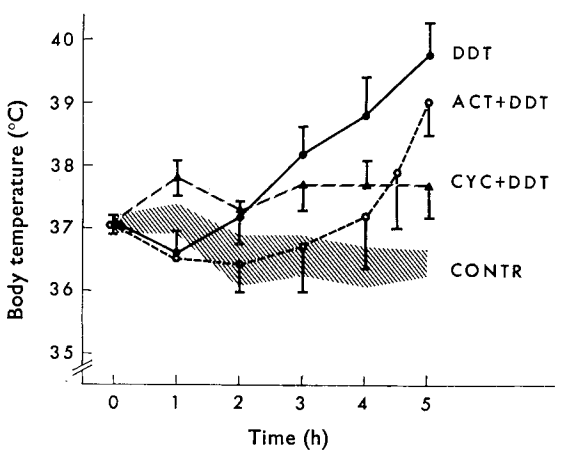

FIG. 1. Effects of actinomycin D and cycloheximide on *pp'*-DDT-induced hyperpyrexia. Experimental conditions are reported in the text. Each point represents the mean value ± s.e. of at least four animals. DDT: *pp'*-DDT, 60 mg/100 g, orally; ACT + DDT: actinomycin D, 40 μg/100 g, i.p. + *pp'*-DDT; CYC + DDT: cycloheximide, 70 μg/100 g, i.p. + *pp'*-DDT; CONTR: range of temperature changes in control animals. On the abscissa: time (in h) after administration of *pp'*-DDT.

Table 1. *Effects of cycloheximide and actinomycin D on* pp'*-DDT-induced changes in striatal acetylcholine.*

Treatment	Acetylcholine (nmol/g wet wt)	% of controls	Tremors
Controls	50·2 ± 1·4[a] (22)	100	
pp'-DDT, 60 mg/100 g orally	31·3 ± 1·8* (8)	62	+++
Cycloheximide 70 μg/100 g, i.p. ..	54·5 ± 9·0 (4)	109	—
Cycloheximide + *pp'*-DDT	50·5 ± 4·1† (4)	101	—
Actinomycin D 40 μg/100 g, i.p. ..	49·2 ± 4·0 (4)	98	—
Actinomycin D + *pp'*-DDT	29·1 ± 1·8* (4)	58	+++

[a] Means ± s.e. In brackets: number of rats in each group. +++ marked tremors; — no tremors.

* Statistically significant difference when compared with the values of control animals ($P < 0{\cdot}001$).

† Statistically significant difference when compared with the values of rats given *pp'*-DDT alone ($P < 0{\cdot}001$).

(1964). After decapitation of the animals, brains were rapidly weighed on a Roller-Smith torsion balance. Total acetylcholine was then extracted with acid-ethanol (Crossland, 1961), and assayed on the eserinized frog rectus preparation.

Prior administration of cycloheximide to DDT-treated rats completely prevented the development of hyperpyrexia (Fig. 1) and none of the animals displayed the typical neurotoxic signs (tremors and convulsions) observed with the insecticide. Data presented in Table 1 show that *pp'*-DDT produced a 38% decrease in the concentration of acetylcholine in the striatum. However, when DDT-fed rats were pretreated with cycloheximide, this decrease was prevented and the acetylcholine values remained close to those noted in the control animals. In contrast, actinomycin D failed to affect the DDT-induced alterations in striatal acetylcholine as well as the development of tremors and convulsions. In rats receiving both actinomycin D and DDT, the increase in body temperature was delayed and marked hyperpyrexia was seen only during the 5th h. Both cycloheximide and actinomycin D failed to exert any significant effects on the body temperature as well as on the concentration of striatal acetylcholine.

Cycloheximide may exert its action by interfering with the effects of DDT on enzymatic processes involved in the breakdown of brain acetylcholine. The reasons why cycloheximide, but not actinomycin D, is able to protect the animals against the development of tremors as well as the accompanying changes in the concentration of striatal acetylcholine are not immediately apparent. Whereas actinomycin D inhibits DNA-directed RNA synthesis by binding to the deoxyguanosine bases of DNA (Reich, 1963), cycloheximide suppresses protein synthesis by inhibiting either the transfer of aminoacyl-transfer RNA to ribosomes or the formation of peptide bonds (Siegel & Sisler, 1964). Whether the difference in the site of action of the two protein synthesis inhibitors can account for their differential effects on the DDT-induced changes in brain acetylcholine remains to be established. However, it is of interest that the marked increase in the concentration of 5-hydroxyindoleacetic acid in the brain stem of DDT-treated rats also remains unaffected by actinomycin D but is completely blocked by prior administration of cycloheximide (Peters, Hrdina & others, 1971).

This study was assisted by funds provided by the Public Health Research Grant

(Project No. 605–7–711) of the National Health Grants Program of the Government of Canada. The expert technical assistance of Mr. A. Maneckjee is gratefully acknowledged.

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October 28, 1971

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Does aspirin exist in polymorphic states?

Whether various polymorphic states of aspirin actually exist has been commented upon recently by Mitchell, Milare & others (1971) and by Pfeiffer (1971).

Mitchell & others (1971), using X-ray diffraction procedures and infrared spectra techniques (Nujol and attenuated total reflectance), were unable to demonstrate polymorphic differences in aspirin crystals as reported by Tawashi (1968). Based on Tawashi's evidence (1968 & 1969), Pfeiffer (1971) doubted whether true aspirin polymorphs had been prepared. He suggested the need for more explicit directions for preparation of aspirin polymorphs and additional evidence for identification of their presence.

Using the many analytical techniques available, confirmation of the formation of the polymorphs described by Tawashi (1968) was attempted. Samples of aspirin were recrystallized from 95% ethanol and n-hexane, following his procedure.

Aspirin (Merck U.S.P.) was saturated in hot 95% ethanol; upon cooling, large crystals were formed with a melting range of 139–142°. When dried *in vacuo* at room temperature and powdered, these crystals gave a broad melting point range of 126–137°. Following the same procedure, aspirin was likewise crystallized from n-hexane. Small needle-shaped crystals were obtained which gave a melting point of 127–133°. The melting points were determined by a hot stage microscope. Differential thermal absorption curves of these compounds showed melting points of 139° and 142°, respectively.

The crystals from ethanol were thick prisms tending to occur in clusters. Those from n-hexane were very thin blades, rods and needles. The original Merck aspirin material consisted of crystal fragments apparently produced by powdering longer crystals. They resembled the crystals from ethanol more than those from hexane and melted over the range 125–135°.

In spite of these differences in external habits and melting points, these samples of aspirin exhibited identical optical and spectral properties. The principal refractive indices were found to be α-1·505, β-1·645, and γ-1·655 for the original sample and for the crystals obtained from both solvents. The infrared spectra of both mulls and KBr discs were indistinguishable, in agreement with the findings of Mitchell &