Bull. Environ. Contam. Toxicol. (1994) 53:655–662

Environmental Contamination and Toxicology

Alteration in the Tranquilizing Potency of Chlorpromazine in Rats Exposed Chronically to the Insecticide, Endosulfan

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Received: 5 September 1993/Accepted: 2 May 1994

An awareness about the influence of organochlorine insecticide on drug action has been growing ever since clinical (Kolmodin et at. 1969; Poland et al. 1970) and experimental (Conney and Burn 1972) evidence documented that environmental chemicals can alter the effects of some therapeutic agents. Since alteration of the therapeutic actions of drugs is of major clinical concern, it is rational to investigate the interaction of the therapeutic agents with the widely used insecticides. A major tranquilizer, chlorpromazine (CPZ) and the cyclodiene organochlorine insecticide, endosulfan have been chosen for the present study. Endosulfan is a well known neurotoxic agent capable of producing central excitatory effects in man (Aleksandrowicz 1979; Environmental Health Criteria, 1984; Shemesh et al.1988) and in animals (Gupta 1976). On the other hand, the central depressive action of CPZ is known to result in tranquilizing effects (Fielding and Lal 1978). A pharmacodynamic interaction can occur between these agents, if endosulfan by virtue of its central stimulant action blocks the tranquilizing action of CPZ. Both endosulfan and CPZ are associated with the microsomal drug metabolizing enzyme system. Endosulfan is a multifunction oxidase inducer (Tyagi et al. 1984; Singh and Pandey 1989) and CPZ is a substrate (Coccia and Westerfield 1967)of these enzymes. As a result, endosulfan may alter the tranquilizing potency of CPZ through an enzyme induction. The present study tested the behavioral responses, Spontaneous Motor Activity (SMA), Conditioned Avoidance Response (CAR), motor coordination and pentobarbital sleeping time, after injecting a tranquilizing dose of CPZ in rats exposed repeatedly to endosulfan.

MATERIALS AND METHODS

One hundred and sixty inbred immature (2-3 weeks after

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weaning) male Wistar rats weighing 60-70 g were used. The experimental and test animals were chosen randomly from a healthy stock and were divided into 4 batches for the 4 behavioral tests. Each batch was then divided into 4 groups (n=10), to have 2 test and 2 control groups. Thus each group was likely to have sibling and nonsibling animals. The rats were housed 5 per cage and were maintained at room temperature (30-34 C) to which they were accustomed. The animals had free access to a balanced diet (Gold Mohur, Bangalore, India) and drinking water. Experimental procedures were started 3 days after grouping and caging were done.

A dose of endosulfan that produced no lethality after administering for 90 days, as in a previous study of the present investigators (Paul et al. 1993) was used. A fine suspension of technical grade endosulfan (Bharat Pulverising Mills, Bombay, India; 95% pure, containing alpha and beta isomers in a 2:1 ratio) was prepared in distilled water with an equivalent amount of an inert suspending agent,tragacanth powder. It was administered to test animals by gavage (PVC tube, 1.0 mm diameter) at 2 mg/kg per day for 90 days, in a volume of 0.2 ml/ 100 g body weight. Control animals received a suspension of tragacanth powder in a similar manner. Twenty four hr after the last administration, the test and control groups were injected intraperitoneally with CPZ(4 mg/kg) or distilled water. Thus, each batch had the following groups :- 1. Endosulfan + CPZ (test); 2. Endosulfan + distilled water (endosulfan control); 3. Tragacanth + CPZ (CPZ control); and 4. Tragacanth + distilled water (Control).

Motor activity was measured in the first batch of animals using a vibration sensor cage (Paul et al. 1993). Each animal was given 2 habituation sessions in the chamber for 10 min with 10 min intervals. Activity was monitored for a duration of 10 min before and 15, 60, 120 and 180 min after CPZ or distilled water injection in test and control animals. The animals were taken out of the chamber while not recording activity and were replaced at the applicable time.

The activity chamber was then converted into a conventional pole-climbing apparatus, by replacing the vibration sensing tray with one having a grid floor. As described previously (Jacobsen 1964), electric shocks (unconditioned stimulation, 500 volts with a duration of 50 msec at intervals of 1 sec) was delivered through the grid floor. A buzzer (conditioned stimulation, significantly audible but not loud enough to interfere with the response of the animal) was fixed at the bottom of the grid floor. The shock and buzzer stimulations were operated manually. A pole was suspended from the lid. The pole-climbing apparatus was used to test CAR

in the second batch of animals. Twenty four hr prior to the test (90th day of treatment), the animals were allowed to get acclimatized in the chamber for 10 min. Then buzzer and electric shocks were delivered simultaneously for 15 sec with 30 sec intervals. The animals learned to climb the pole in order to escape from shocks. Later, they learned to avoid shocks by climbing the pole, when exposed to buzzer alone for 15 sec. This was their CAR. The animals had sufficient trials (buzzer alone) until they responded in 3 consecutive trials. Twenty four hr after the training session, CAR-time was measured, prior to and 15, 60, 120 and 180 min after injecting CPZ or distilled water. CAR-time was the time in sec between the starting of buzzer and the moment the rat climbed the pole, within the allowed 15 sec.

Muscle coordination was tested in the third batch using a rota-rod apparatus,as described previously (Dunham and Miya 1957). The rational of this test was that animals whose motor coordination deteriorated dropped off from the moving rod (horizontal iron rod 2.5 cm diameter and 57 cm long with roughened surface, moving on its axis at 10 r.p.m) into a tray 10 cm below, while the unaffected ones were able to stand as long as 2 min or more. The animals were allowed to acclimatize on the moving rod for 2 min. After 2 min, the endurance time was determined by measuring the time between the placing of the rat on the moving rod and the moment it fell down during an allowed test period of 2 min. The test was repeated 15, 60, 120 and 180 min after CPZ or distilled water injection.

Sleep latency and duration of sleep were determined in the fourth batch of animals which received intraperitoneally a hypnotic dose of pentobarbital (40 mg/Kg) 15 min after CPZ or distilled water. Sleep latency was measured as the time between the injection of pentobarbital and the loss of righting reflex (failure to return to an upright posture after being placed on its back). Duration of sleep was the elapsed time from loss of righting reflex to its return.

All behavioral tests were performed between 10.00 and 14.00 hr under the same light and temperature conditions as the housing. The observer was unaware of the groupings. The data of each behavioral element were analyzed using one way analysis of variance.

RESULTS AND DISCUSSION

The individual and combined effects of endosulfan and CPZ on SMA, CAR and motor coordination are illustrated in figure 1 A, B and C, respectively. Endosulfan, as shown previously, stimulated SMA (Paul et al. 1993),

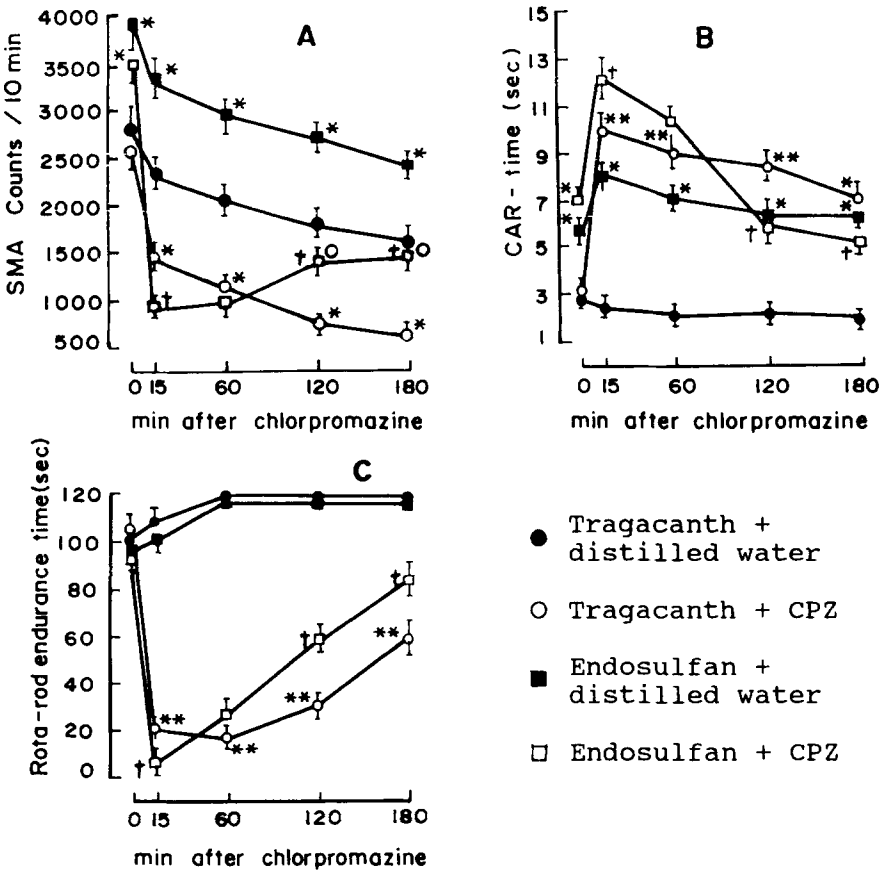
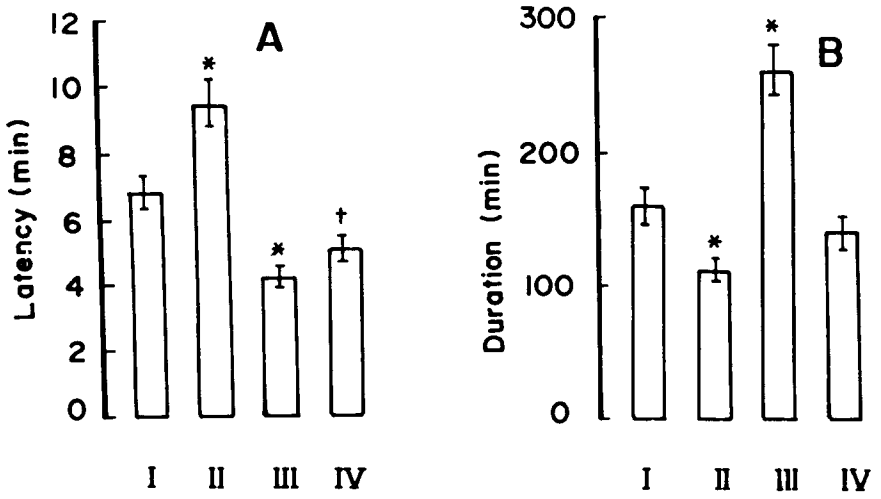


Figure 1. SMA counts (A), CAR-time (B) and Rota-rod endurance time (C) of test and control animals. CPZ (4 mg/kg) was injected 24 hr after the last administration of endosulfan. Each point represents mean ± S.E.M. of 10 animals. * P < 0.05, ** P < 0.01 compared to tragacanth + distilled water group. † P < 0.05 compared to tragacanth + CPZ group. o P < 0.05 compared to endosulfan + distilled water group. (ANOVA, Snedecor and Cochran 1967).

inhibited CAR (Paul et al. 1992) and unaltered motor coordination (Paul et al.1993). CPZ, as shown here, is known to suppress SMA, CAR and motor coordination (Fielding and Lal 1978). Its concurrent action, 15 min after injection, with endosulfan resulted in a powerful inhibition of all these behaviorial responses. The CAR and motor coordination inhibiting action of CPZ gradually diminished in these animals. Thus, its potency 120-180 min later was significantly lesser than that measured in tragacanth + CPZ groups. Its SMA inhibiting action also decreased. But activity counts recorded 120-180 min later were significantly lesser than that recorded in endosulfan + distilled water



I Tragacanth + distilled water
II Endosulfan + distilled water
III Tragacanth + CPZ
IV Endosulfan + CPZ

Figure 2.Pentobarbital sleep latency (A) and duration of sleep (B) in test and control animals. Pentobarbital (40 mg/Kg) was injected intraperitoneally 15 min after CPZ (40mg/kg) or distilled water to endosulfan or tragacanth treated animals. Each bar represents mean ± S.E.M. of 10 animals. * P < 0.05 compared to tragacanth + distilled water group. † P < 0.05 compared to endosulfan + distilled water group (Analysis of variance).

group. This finding indicated that although the SMA inhibiting action was diminishing,CPZ, at this dose, was able to suppress endosulfan-induced hypermotor activity. CPZ promoted the hypnotic effect of pentobarbital by shortening sleep latency and prolonging sleeping time. Endosulfan, on the other hand, prolonged pentobarbital sleep latency and shortened duration of sleep. CPZ reverted the prolonged sleep latency and not the shortened sleeping time of pentobarbital in endosulfan-treated animals (Fig. 2 A and B).

A suppression of pole-climbing avoidance response in endosulfan-treated animals was not accompanied by motor coordination impairment. Hence, endosulfan was suggested to disrupt memory process and not the somatic system for its CAR inhibiting action. In support of this hypothesis, workers who had exposure to unknown amount of endosulfan, reported memory deficit (Aleksandrowicz 1979). If this and the CAR inhibiting tranquilizing action of CPZ were additive, then a pharmacodynamic interaction might be suggested tentatively for the data recorded 15 min after CPZ in endosulfan-treated animals.

No such interaction appeared to be responsible for their combined SMA and motor coordination inhibiting action, since endosulfan stimulated motor activity and did not alter motor coordination; whereas CPZ inhibited both. Endosulfan was proposed to stimulate SMA by activating a dopaminergic mechanism (Anand et al. 1985). On the other hand, CPZ is a well established antidopaminergic agent (Chiodo and Bunney 1987).Interestingly, a powerful inhibition of motor activity resulted from their concurrent action. This finding indicated that endosulfan had facilitated the SMA inhibiting action of CPZ. But, its SMA, CAR and muscle coordination suppressing action decreased faster in these animals than in tragacanth-treated animals. Thus, endosulfan altered the time sequence of the central depressive action CPZ. If, a variable bioavailability of CPZ was responsible for this finding, then its pharmacokinetics were likely to be altered by endosulfan.

Endosulfan has been reported to inhibit the hypnotic effect of pentobarbital (Gupta and Gupta 1977). This finding was attributed to the microsomal enzyme inducing property of endosulfan (Tyagi et ai. 1984; Singh and Pandey 1989). This was suggested because a shortened sleeping time of pentobarbital in endosulfan-treated animals was accompanied by a decreased blood and brain concentrations of pentobarbital (Gupta and Gupta 1977). An indirect evidence for the enzyme inducing action of endosulfan was demonstrated here by the data that indicated a shortening of pentobarbital sleeping time in animals exposed to it. Since CPZ is known to be metabolized by the microsomal enzyme system (Coccia and Westerfield 1967), endosulfan is likely, as shown here, to shorten the duration of its central depressive actions. This factor and an increased metabolism of pentobarbital accounted for the shortened sleep measured in endosulfan + CPZ treated animals.

Before being metabolized into inactive compounds, CPZ has been reported to be biotransformed into active metabolites such as demethylated CPZ, 7-hydroxy CPZ and 7, 8 - dihydroxy CPZ (Morselli 1977). These metabolites are as active as CPZ and are able to penetrate into the brain (Alfredsson et al. 1977). If, as proposed here, the biotransformation of CPZ was quickened by endosulfan, then the action of the active metabolites that were formed initially were likely to contribute temporarily to its central effects. This factor was accounted to its increased SMA, CAR and motor coordination inhibiting potency 15 min after administration. Thus, CPZ was able to revert the prolonged pentobarbital sleep latency in endosulfan treated animals.

An interesting finding of this study was that CPZ was

able to exert its effect against endosulfan-induced hypermotor activity during the course of its action, even after its SMA, CAR and motor coordination depressing potency diminished considerably. If it resulted from its antidopaminergic property, (Chiodo and Bunney 1987), then this action of CPZ appeared to persist much longer than the mechanism that is involved in its central depressing action.

Acknowledgment. The authors are grateful to ICMR, New Delhi, India, for financial assistance.

REFERENCES

Aleksandrowicz R(1979) Endosulfan poisoning and chronic brain syndrome. Arch Toxicol 43: 65-68.

Alfredsson G, Weiesel F, Skett P (1977) Levels of chlorpromazine and its active metabolites in rat brain and the relationship to central monoamine metabolism and prolactin secretion. Psychopharmacology 53: 13-18

Anand, M, Mehrotra, S, Gopal, K, Sur RN, Chandra SV (1985) Role of neurotransmitters in endosulfan-induced aggressive behaviour in normal and lesioned rats. Toxicol Lett 24: 79- 84.

Chiodo LA, Bunney BS (1987) Population response of midbrain dopaminergic neurons to neuroleptics: further study on time course and nondopaminergic neuronal influences. J Neurosci 7: 629-633.

Coccia PF, Westerfield WW (1967) The metabolism of chlorpromazine by liver microsomal enzyme systems. J Pharmacol Exp Ther 157: 446-458.

Conney AH, Burns JJ (1972) Metabolic interactions among environmental chemicals and drugs. Science 178: 576-586.

Dunham NW, Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharmaceutical Assoc Sci Ed IXVI: 208-209.

Enviromental Health Criteria, 1984 Endosulfan, International Programme on Chemical Safety, WHO, Geneva, 40.

Fielding S, Lal H (1978) Behavioural actions of neuroleptics. In: Inversen LL, Iversen SD, Snyder SH (eds) Vol. 10, Handbook of psychopharmacology, Plenum Press, New York, P 91.

Gupta PK (1976) Endosulfan-induced neurotoxicity in rats and mice. Bull Environ Contam Toxicol 15: 708-713.

Gupta PK, Gupta RC (1977) Influence of endosulfan on pentobarbitone sleeping time and blood and brain concentrations in male rats. J Pharm Pharmacol 29:245-246.

Jacobsen E (1964) Tranquillizers and Sedatives. In: Laurence DR, Bacharach AL (eds) Vol 1, Evaluation of drug activities: Pharmacometrics, Academic Press, London, P 215.

Kolmodin B, Azarnoff DL, Sjoqvist F (1969) Effect of environmental factors on drug metabolism: decreased plasma half-life of antipyrine in workers exposed to chlorinated hydrocarbon insecticides. Clin Pharmacol Therapeut 10: 638-642.

Morselli PL (1977) Psychotropic drugs. In: Morselli PL (ed) Drug disposition during development, Spectrum Publications, Inc., New york, p 431.

Paul V, Balasubramaniam, E, Sheela S, Krishnamoorthy MS (1992) Effects of endosulfan and aldrin on muscle coordination and conditioned avoidance response in rats. Pharmacol Toxicol 71: 254-257.

Paul V, Kazi M, Balasubramaniam E, Sheela S (1993) Effect of chronic endosulfan treatment on pharmacological actions of diazepam in rats. Bull Environ Contam Toxicol 51: 18-23.

Poland AP, Smith D, Kuntzman R, Jacobsen M, Conney AH (1970) Effect of extensive occupational exposure to DDT on phenylbutazone and cortisol metabolism in human beings. Clin Pharmacol Therapeut 11: 724-732.

Shemesh Y, Bourvine A, Gold D, Bracha P (1988) Survival after acute endosulfan intoxication. J Toxicol Clin Toxicol 26: 265-268.

Singh SK, Pandey KS (1989) Differential effects of chronic endosulfan exposure to male rats in relation to hepatic drug metabolism and androgen metabolism. Ind J Biochem Biophys 26: 262-267.

Snedecor GW, Cochran WG (1967) Statistical Methods. Oxford & IBM Publishing Co.

Tyagi SR, Singh Y, Srivastava PK, Misra UK (1984) Induction of hepatic mixed function oxidase system in rats. Bull Environ Contam Toxicol 32: 550-556.