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CYCLODIENE INSECTICIDES-INDUCED CHANGES IN THE CENTRAL DEPRESSIVE EFFECT OF CHLORPROMAZINE IN RATS

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Abstract: Spontaneous motor activity (SMA), conditioned avoidance response (CAR), muscle coordination (MC) and pentobarbital sleep were tested in rats treated orally for 90 days with tolerated doses of the cyclodiene insecticides, aldrin (1 mg/kg) and endosulfan (2 mg/kg). The same tests were repeated in similarly treated animals after injecting chlorpromazine (4 mg/kg, i.p.). Both the insecticides shortened pentobarbital sleeping time indicating their microsomal enzyme inducing property. Aldrin suppressed SMA, CAR and MC, whereas endosulfan stimulated SMA, inhibited CAR and unaltered MC. However, their concurrent action with CPZ did not result in change in the central depressive effects of the latter, but its potency during the course of its action was altered. Its potency 15 min after injection was greater and 60-180 min later was lesser in these animals than that observed in control animals. This finding was interpreted to suggest that aldrin and endosulfan has quickened the biotransformation of CPZ and thereby shortened its duration of action. A temporary promotion of its potency was accounted to its active metabolites, since prior to inactivation, CPZ is known to be metabolized by the microsomal enzymes to active compounds.

Key words: chlorpromazine aldrin endosulfan spontaneous motor activity conditioned avoidance response muscle coordination

INTRODUCTION

Workers who had chronic occupational exposure to the organochlorine insecticides were reported to respond differently to therapeutic agents (1, 2). Since it is a clinical concern, experimental approach to test an interaction between therapeutic agents and the environmental chemicals is rational. The major tranquilizer, chlorpromazine (CPZ) and the organochlorine cyclodiene insecticides, aldrin and endosulfan have been chosen for the present interaction study. Aldrin is known to undergo epoxidation in the biological system to an equipotent metabolite, dieldrin (3). Therefore, the toxicities of the parent compound and the metabolite are indistinguishable. Endosulfan, aldrin and its epoxide, dieldrin are well known neurotoxic insecticides and have been reported to produce central excitatory toxicity in man (4, 5, 6, 7) and in experimental animals (8, 9, 10, 11). CPZ is a central depressant and is know to produce tranquilizing effect (12). These insecticides and CPZ are associated with the microsomal drug metabolizing enzyme system, the former as inducers (13, 14, 15, 16) and the latter as a substrate (17, 18). If, on account of these factors, a pharmacodynamic or a

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pharmacokinetic or both interactions occur between CPZ and the insecticides, then the pharmacological action of the former is likely to be altered by the latter. In order to investigate this, the effect of CPZ was tested on spontaneous motor activity (SMA), conditioned avoidance response (CAR), muscle coordination (MC) and pentobarbital sleep of rats treated repeatedly with tolerated doses of aldrin and endosulfan which induced microsomal enzyme activity and produced significant but not toxic behavioural changes.

METHODS

Colony bred 240 immature (2-3 weeks after weaning) male Wistar rats weighing 60-70 g were used. The animals were divided randomly into 4 batches for the 4 behavioural tests. Each batch was then divided into 6 groups (n=10) to have 3 test and 3 control groups. The rats were housed 5 per cage and were maintained at room temperature (30-34°C). The animals had free access to a balanced pellet diet (Gold Mohur, Calcutta, India) and tap water. Three days after acclimatization, experimental procedures were started.

A fine suspension of technical grade aldrin (NOCIL, India; 96% pure) and endosulfan (Bharat Pulverising Mills, Bombay, India; 95% pure containing α and β isomers in a 2:1 ratio) were prepared in distilled water with an equivalent amount of tragacanth powder and were administered by gavage in a volume of 0.2 ml/100 g body weight at 1 mg/kg/day and 2 mg/kg/day dose levels, respectively for 90 days. These dose levels of aldrin and endosulfan were tolerated by immature rats without any obvious toxic effects in our previous chronic study (19), hence the same doses were used in this study. Control animals received a suspension of tragacanth powder in a similar manner. Twenty four h after the last administration, the control and test animals were injected intraperitoneally with CPZ (4 mg/kg) or distilled water so that each batch had the following groups : aldrin + CPZ, endosulfan + CPZ (test), aldrin + distilled water (aldrin control), endosulfan + distilled water (endosulfan control), tragacanth + CPZ (CPZ control) and tragacanth + distilled water (untreated control).

Motor activity was measured in the first batch of animals using an activity meter (20). The apparatus consisted of an acrylic chamber (40 x 40 x 40 cm) with a vibrations sensing tray as a floor on which the animal was placed. The vibrations caused by the movements of the animal were picked up by the sensors and were converted into electrical signals which activated the counter. Each animal was given habituation session (10 min) in the chamber and 10 min later the activity was measured for 10 min, before and 15, 60, 120 and 180 min after CPZ or distilled water injection. The animals were taken out of the chamber while not recording activity and replaced at the applicable time.

The activity chamber was then converted into a conventional poleclimbing apparatus by replacing the vibration sensing tray with one having a grid floor. As described previously (21), electric shock (unconditioned stimulation, 500 volt with a duration of 50 ms at interval of 1 s) was delivered through the grid floor. A buzzer (conditioned stimulation, significantly audible but not loud 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 apparaus was used to test CAR in the second batch of animals. Twentyfour h prior to the test (90th day of the treatment), the animals were allowed to get acclimatized in the chamber for 10 min and then buzzer and electric shock were delivered simultaneously for 15 s with 30 s interval, in order to train them for the pole-climbing performance. The animals learnt to climb the pole in order to escapes from shock, and latter they learnt to avoid shock by climbing the pole, when exposed to buzzer alone for 15 s. The latter was their CAR. The animals had sufficient trials (buzzer alone) until they responded in 3 consecutive trials. Twentyfour h 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 between the starting of buzzer

and the moment the rat climbed the pole within the allowed 15 s. The non-responding animals were given the permitted time, 15 s as their CAR-time.

Muscle coordination was tested in the third batch using a rota-rod apparatus as described previously (22). The rational of this test was that animals whose motor coordination was deteriorated dropped off from the moving rod (a horizontal iron rod 2.5 cm diameter and 57 cm long with toughened surface, moving on its axis at 10 r.p.m.) into a tray 10 cm below and 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, and 2 min later, the endurance time was determined by measuring the time between the placing of the animals on the moving rod and the time 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 sodium (40 mg/kg) 15 min after CPZ or distilled water. The former 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) and the latter was the elapsed time from loss of righting reflex to its return.

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

RESULTS

The data presented in Fig. 1 A, B and C illustrate SMA, CAR and MC of rats treated with aldrin, endosulfan and CPZ and the concurrent action of the former two compounds with the latter. The occurance of habituation to the activity chamber was evident from the SMA data of the tragacanth control group. On account of this phenomenon, the data of test animals

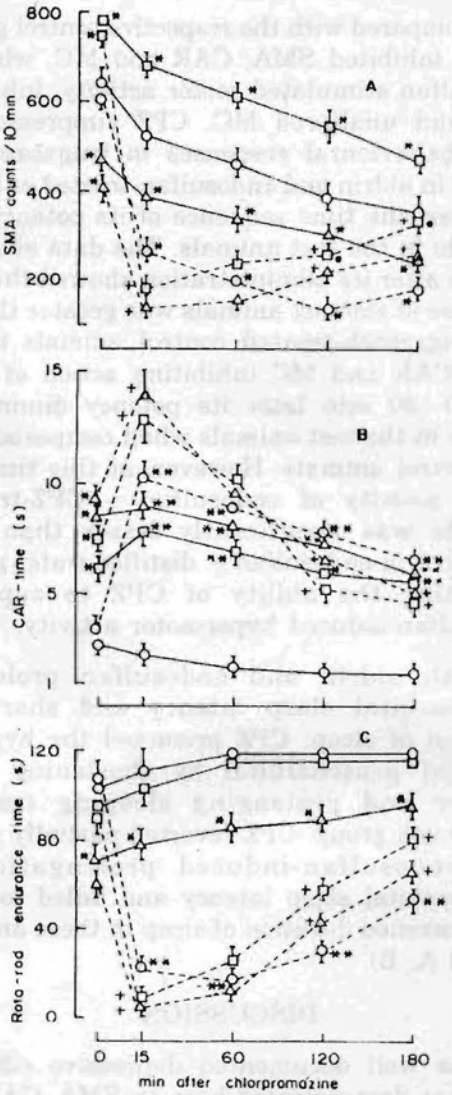

Fig. 1 : SMA counts (A), CAR-time (B) and rota-rod endurance time (C) of rats treated with aldrin (Δ), endosulfan (□) and tragacanth (O). Straight and broken lines indicate the data recorded after distilled water and CPZ, respectively. Rats received aldrin (1 mg/kg/day), endosulfan (2 mg/kg/day) of tragacanth for 90 days and 24 h after the last administration, distilled water or CPZ (4 mg/kg) was injected intraperitoneally. Each point represents mean ± SEM of 10 animals.

*P<0.05,
**P<0.01 compared with tragacanth + distilled water group.
•P<0.05 compared with tragacanth + CPZ group.
°P<0.05 compared with endosulfan + distilled water group. (One way analysis of variance).

were compared with the respective control group. Aldrin inhibited SMA, CAR and MC, whereas endosulfan stimulated motor activity, inhibited CAR and unaltered MC. CPZ suppressed all these behavioural responses in tragacanth as well as in aldrin and endosulfan-treated animals. However, the time sequence of its potency was variable in the test animals. The data obtained 15 min after its administration showed that the response of the test animals was greater than of the tragacanth-treated control animals to the SMA, CAR and MC inhibiting action of CPZ. But 60-180 min later its potency diminished greatly in the test animals when compared with the control animals. However, at this time the motor activity of endosulfan + CPZ-treated animals was significantly lesser than that measured in endosulfan + distilled water group, indicating the ability of CPZ to suppress endosulfan-induced hypermotor activity.

Both aldrin and endosulfan prolonged pentobarbital sleep latency and shortened duration of sleep. CPZ promoted the hypnotic effect of pentobarbital by shortening sleep latency and prolonging sleeping time in tragacanth group. CPZ reverted partially aldrin and endosulfan-induced prolongation of pentobarbital sleep latency and failed to alter the shortened duration of sleep in these animals (Fig. 2 A, B).

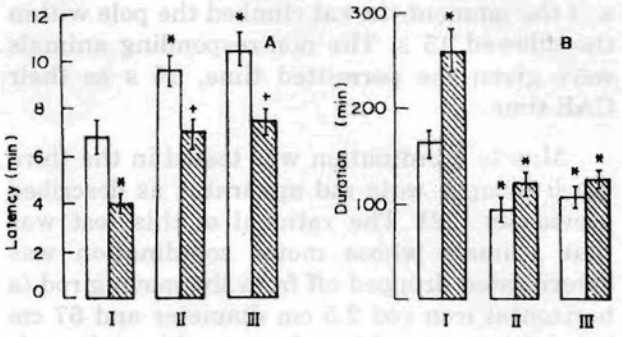

Fig. 2 : Pentobarbital sleep latency (A) and duration of sleep (B) in rats treated with tragacanth (I), endosulfan (II) and aldrin (III). Open and stripped bars indicate distilled water and CPZ groups respectively. Rats received aldrin (1 mg/kg/day), endosulfan (2 mg/kg/day) or tragacanth for 90 days and 24 h after the last administration CPZ (4 mg/kg) or distilled water and 15 min later pentobarbital sodium (40 mg/kg) were injected intraperitoneally. Each bar represents mean ± SEM of 10 animals.

* $P<0.05$ compared with tragacanth + distilled water group.

\+ $P<0.05$ compared with tragacanth + CPZ group (one way analysis of variance).

DISCUSSION

The well documented depressive effect of CPZ was demonstrated here on SMA, CAR and MC of rats. Aldrin was proposed to impair motor function for its SMA, CAR and MC inhibiting effect, since its epoxide dieldrin was reported to produce muscle weakness in rats (23). Thus the effect resulted from the concurrent action of aldrin and CPZ was greater than their individual effects. A disruption of memory may also be proposed for the CAR inhibiting action of aldrin, since it has been reported to produce memory deficit in workers after chronic occupational exposure (5). Further evidence for its memory disrupting action emerged from a suppression of performance of dieldrin-treated rats in maze test (24). Endosulfan also appeared to disrupt memory since in the present and previous (19) studies it suppressed CAR without impairing motor coordination. Supporting this proposal, loss of memory was reported by workers who had chronic occupational exposure to endosulfan (6).

If the mechanism involved in the CAR inhibiting action of aldrin and endosulfan was additive with that of CPZ, then a pharmacodynamic interaction could be tentatively suggested for the result obtained 15 min after CPZ in test animals. Such an interaction was ruled out for the combined action of endosulfan and CPZ on SMA, and MC, since the former stimulated SMA and unaltered MC whereas CPZ was an inhibitor of both. In addition, endosulfan was proposed to stimulate motor activity by activating dopaminergic mechanism (25), whereas CPZ is a well established antidopaminergic agent (26). Thus, although CPZ was an antagonist of endosulfan at receptor level, its SMA inhibiting action was facilitated by endosulfan. However, 60 min later

its SMA, CAR and MC inhibiting action was weakened in both aldrin and endosulfan-treated animals. It was apparent from these findings that the time course of its action had been altered by these insecticides. If a variable bioavailability of CPZ was responsible for this result, then aldrin and endosulfan appeared to alter its pharmacokinetics.

Aldrin (13, 14) and endosulfan (15, 16) were reported to quicken the biotransformation of pentobarbital by activating microsomal enzymes, since a shortened sleeping time of the latter was accompanied by a decreased blood and brain concentrations of it in rats treated with aldrin (14) and endosulfan (15). The decreased hypnotic effect of pentobarbitone in the test animals of this study indicated that both these insecticides, at the doses used here, were able to activate liver microsomal enzyme system. Under this circumstance, the central depressive effect of CPZ is likely to be decreased in aldrin and endosulfan-treated animals, since it is known to be metabolized by the microsomal enzyme (17). If this proposal is acceptable, then a quickened biotransformation of CPZ could be accounted for the decreased SMA, CAR and MC inhibiting effect of CPZ 60 min after administration in aldrin and endosulfan-treated animals. A rapid inactivation of both pentobarbital and CPZ appeared to be responsible for the inability of the latter to revert the sleeping time of the former in these animals.

If, as proposed here, the biotransformation of CPZ was quickened by aldrin and endosulfan, then a promotion of its central depressive effects could result from this pharmacokinetic interaction, since prior to inactivation, CPZ was known to be metabolized into active compounds, demethylated CPZ, 7-hydroxy CPZ and 7, 8-dihydroxy CPZ (18) which readily penetrated into the brain (27). The contribution of these active metabolites accounted for the temporary increase in SMA, CAR and MC inhibiting effect of CPZ 15 min after its administration and for the partial reversal of the prolonged pentobarbital sleep in the test animals.

The interesting finding of this study was that CPZ inhibited endosulfan-induced hypermotor activity even after its SMA, CAR and MC inhibiting effect diminished considerably. Thus, the action of CPZ against the excitatory action of endosulfan did not seem to be influenced by its metabolic inactivation.

Concluded that aldrin and endosulfan by their enzyme inducing property decreased the duration of action of CPZ. However, CPZ was able to suppress effectively endosulfan-induced anxiety that resulted in a stimulation of locomotor activity.

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