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Effect of Chronic Endosulfan Treatment on Pharmacological Actions of Diazepam in Rats

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Repeated administration of the cyclodiene insecticide, endosulfan has been reported to induce drug metabolizing microsomal enzymes in rats resulting in changes in the pharmacological and toxicological properties of drugs that are metabolized by microsomal enzymes (Agarwal et al. 1978; Tyagi et al. 1984; Singh and Pandey 1989). Alterations of the therapeutic actions of drugs is of major clinical concern requiring further investigation.

The purpose of this study was to demonstrate changes in the hypnotic, sedative and muscle relaxant actions of diazepam, resulting from the enzyme inducing properties of endosulfan.

MATERIALS & METHODS

Colony bred male Wistar strain rats weighing 60-70 g were used. They were divided randomly for test (n=10) and control (n=10) groups, caged 5 in each and were maintained under standard laboratory condition. They were allowed free access to a balanced diet and drinking water.

Rats were administered by gavage Technical grade endosulfan (Excel Industries, Bombay; 95% pure, containing alpha and beta isomer in 2:1 ratio) at 2mg/kg/day for 90 days in volume of 0.2 ml/100 g of body weight. The dosing solution was prepared by suspending endosulfan in water with an equivalent amount of tragacanth powder. Control animals were administered a suspension of tragacanth powder.

Twenty four hours after the last administration of endosulfan, the test and control animals were injected i.p. with 12 mg/kg of diazepam or 35 mg/kg of pentobarbitone. Sleep latency and duration of sleep were determined. Sleep latency was measured as the time

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between the injections and the loss of righting reflex, while duration of sleep was measured from the loss of righting reflex to its return. Pentobarbitone was used as a positive control for hypnotic effects.

The sedative action of diazepam (4 mg/kg) was tested by measuring spontaneous motor activity (SMA) 15, 30 and 60 min after its administration to test and control animals. A vibration sensor cage devised by the authors was used. The apparatus consists of an acrylic (black) cabinet (40 x 40 x 40 cm) with a transparent perforated detachable lid. The floor is a detachable tray consisting of a laminate plate fitted with an array of vibration sensors made up of piezo-electric crystals. The vibrations produced by the movements of the animal placed on the laminate plate are picked up by the sensors and are converted to electrical signals which are amplified. The amplified signals activate the counter which records adding one digit for every activity pulse received. A two digit thumb wheel switch is provided to set the time, as required from 1-99 min. While recording animal activity, the instrument was placed on a cushion pad in a quiet room in order to eliminate externally - induced vibrations. Motor activity was tested in the morning between 10 and 11.30 The instrument was subjected initially, to vigorous testing with untreated rats of same sex and weight. The animal was placed in the chamber for 1 min to get accustomed and the activity was then recorded for 10 min. The mean activity counts recorded for 10 rats (2023 ± 177) was much greater than that (400-700 counts/10-15 min) measured previously using photocell cage (Butcher et al. 1972). It appears, therefore, that the vibration sensing device is highly sensitive to the movements of rats, since it records locomotion from one spot to another, as well as other activities such as scratching, preening and gnawing. On account of its satisfactory performance in recording every movement of rat, this instrument was used for measuring SMA.

Muscle relaxation by diazepam was tested in test and control animals as described by Mason (1964) using a rota-rod apparatus designed by Dunham and Miya (1957). It is a horizontal iron rod 2.5 cm diameter and 57 cm long with roughened surface, moving on its axis 10 r.p.m. Metal discs divide the rod into 3 sections so that multiple tests can be done. Animals whose motor co-ordination is impaired drop off from the rod into a tray 10 cm below within a test period of 2 min. Prior to endosulfan treatment, rats were placed on the moving rod and those which stayed for 2 min were chosen and they were randomly divided for test and control groups. Prior to the test they were placed on the moving rod for 2 min to get accustomed. In order to measure motor

co-ordination, they were placed on the moving rod and the time elapsing until they fell down (endurance time) during the allowed 2 min test period was measured prior to (0 min) and 15, 30 and 60 min after diazepam (8 mg/kg) injection.

RESULTS AND DISCUSSION

The data presented here (Table 1) and that reported previously (Gupta and Gupta 1977) show that sleep latency of pentobarbitone sodium is prolonged and duration of sleep is shortened in rats treated repeatedly with endosulfan, suggesting that microsomal enzyme activity has been induced in them by the latter. The hypnotic effect of diazepam was, on the other hand, prolonged in similarly treated rats.

The data presented in Table 2 indicate that endosulfan treatment has enhanced the SMA of rats. The sedative effect of diazepam was more marked in test animals than in control rats, since a greater inhibition of SMA was found in the former group 15 min after its injection. The activity measured 30 and 60 min later also showed that the effect was more marked in test group than in control group.

The rota-rod endurance time data show that diazepam has produced a more powerful and prolonged muscle relaxation in test animals than in control group. (Table 3).

Table 1. The hypnotic effects of pentobarbitone (A) and diazepam (B) in endosulfan treated rats.

Treatment	A		B	
	Latency (min)	Duration (min)	Latency (min)	Duration (min)
Tragacanth	6.92 ±0.16	165.85 ± 9.56	5.50 ±0.42	70.55 ± 4.72
Endosulfan	9.10* ±0.75	129.50* ± 7.14	5.66 ±0.80	108.46* ± 8.52

Rats received sodium pentobarbitone (35 mg/kg) or diazepam (12 mg/kg) intraperitoneally 24 h after the last 90 days treatment with endosulfan (2 mg/kg/day, orally). Values are mean ± SEM of 10 animals. $P<0.05$ compared to respective control (Student's t-test).

Table 2. Effect of diazepam on SMA in endosulfan treated rats.

Treatment	Activity counts/10 min			
	min after diazepam			
	0	15	30	60
Untreated Control	2023 ±177	1402 ±204	1468 ±198	1735 ±148
Tragacanth	1938 ±102	563** ±66	740** ±78	913** ±85
Endosulfan	2580* ±212	167++ ±24	361++ ±24	636+ ±42

Rats received diazepam (4 mg/kg) intraperitoneally 24 h after the last 90 days treatment with endosulfan (2 mg/kg/day orally).
Values are mean ± SEM of 10 animals.
*P < 0.05, ** P < 0.01 compared to control.
+P < 0.05, ++P < 0.01 compared to tragacanth group (Student's t-test).

Table 3. Effect of diazepam on rota-rod endurance time in endosulfan treated rats

Treatment	Endurance time (Sec)					
	min		after			diazepam
	0	5	15	30	45	60
Untreated Control	120.00	120.00	120.00	120.00	120.00	120.00
Traga-canth	120.00	13.2 ±1.4	14.8 ±2.6	64.2 ±4.8	88.5 ±8.4	110.6 ± 2.2
Endo-sulfan	120.00	4.2* ±1.2	5.5* ±1.4	16.5** ±3.2	31.5** ±5.5	46.8** ±7.2

Rats received diazepam (8 mg/kg) intraperitoneally 24 h after the last 90 days treatment with endosulfan (2 mg/kg/day orally). Values are mean ± SEM of 10 animals.
*P < 0.05, ** P < 0.01 compared to control.
+P < 0.05, ++P < 0.01 compared to tragacanth group (Student's t-test).

The central stimulant action of endosulfan has been well documented with the clinical reports of convulsions in factory workers exposed to it (Ely et al. 1967; Aleksandrowicz 1979) and with its convulsant action in animals (Gupta 1976). Endosulfan did not produce convulsion in this study, but it increased SMA significantly. Diazepam is a well established anticonvulsant agent of clinical importance and it has been used successfully to control

convulsions caused by endosulfan poisoning too (Aleksandrowicz 1979). These well established facts totally rule out an interaction between their pharmacological actions for the present result showing an enhancement by endosulfan of the central depresant effect of diazepam.

The metabolic derivatives of endosulfan consisted of its sulfate, diol, α-hydroxy ether, lactone and ether. Although endosulfan sulfate appeared to be slightly more toxic than the parent compound, none of these metabolites have been accounted for the microsomal inducing or other toxicities, since they are readily excreted in the feces and urine (Dorough et al. 1978). The data showing a shortening of pentobarbitone sleeping time in test animals has been accounted to the microsomal enzyme inducing property of endosulfan. Diazepam is known to be biotransformed in the liver initially to a less active compound, N-desmethyl diazepam and subsequently to a more potent and long acting metabolite oxazepam (Schwartz et al. 1967; Randall 1973; Mennini et al. 1987). Taken together, endosulfan has been suggested to increase the formation of the active metabolite oxazepam, resulting in, as demonstrated here, a promotion of the potency and duration of the central depressant actions of diazepam. No untoward effect of diazepam was detected in this study, since the effect of a single dose of it was tested in endosulfan-treated animals. However, it may be predicted from the proposed pharmacokinetic interaction between them that it may be hazardous if the effects of diazepam persists longer during its repeated administration.

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