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INVOLVEMENT OF SEROTONERGIC RECEPTORS IN ENDOSULFAN NEUROTOXICITY

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Abstract—Single dose of 1 and 3 mg/kg endosulfan did not show any significant effect on binding of ^{3}H-serotonin to frontal cortical membranes as well as on foot-shock-induced fighting behaviour in rats, where as repeated exposure of endosulfan (3 mg/kg for 30 days) caused a significant increase in ^{3}H-serotonin binding. Scatchard analysis showed an increase in the affinity of the receptor (K_D) in the treated animals while number of receptor sites (B_{max}) remained unaltered. Long term endosulfan exposure caused aggressive behaviour (foot-shock-induced fighting behaviour) which was blocked by methysergide, a 5-HT blocker. These results indicate the involvement of serotonergic receptors in endosulfan neurobehavioral toxicity.

Endosulfan a chlorinated hydrocarbon is widely used as an important insecticide in crop protection [1,2]. Farmers exposed to endosulfan exhibit epilepsy, hyperactivity, irritability, tremor, convulsions and paralysis [3,4]. Involvement of central nervous system in the neurotoxicity of endosulfan has been shown by several experimental studies conducted on small rodents and cats [5]. In our recent studies endosulfan was also found to induce aggressive behavior in rats (unpublished work).

Inspite of the above information little is known about the mechanism by which endosulfan produces its neurotoxic effects. Biogenic amines are known to play significant role in the abnormal behavior. Involvement of serotonin in aggressive behaviour has been described by several workers [6, 7]. To investigate whether endosulfan induced aggressive behavior is caused by alterations in the function of the serotonergic system, we have studied the sensitivity of 5-HT receptor in endosulfan exposed animals using high affinity receptor binding assay.

MATERIALS AND METHODS

Treatment of animals. Eight week old male albino rats of ITRC breeding colony kept on *ad libitum* pellet diet (Hind Levers, Bombay) and water under standard laboratory conditions were used in this study. In the single exposure study rats were administered endosulfan dissolved in 40% propylene glycol (i.p., 2 ml/kg) at a dose of 1 and 3 mg/kg. The animals of control group received an equivalent amount of vehicle in identical manner. In the repeated exposure study, animals of experimental group were administered 3 mg/kg endosulfan up to 15 and 30 days while that of control group received vehicle in an identical manner. Eight animals were sacrificed 24 hr after the last dose by decapitation from each group. The brains were removed and frontal cortices dissected out immediately (8) and frozen at −20° till biochemical analysis.

Biochemical procedures. Crude membranes were prepared from the frontal cortices [9] by homogenizing the tissue in 19 vol. of 0.32 M sucrose followed by centrifugation (50,000 *g*) for 10 min. The sediment was re-homogenized in deionized water, centrifuged and finally suspended in 40 mM Tris–HCl pH 7.4 buffer at a concentration corresponding to 50 mg/ml of the original wet wt of tissue.

Binding incubations were carried out as described earlier [10]. The reaction was run in triplicate in 1 ml final volume containing 40 mM Tris–HCl pH 7.4, 10^{-5} M pargyline, 4×10^{-3} M $CaCl_2$ and 5×10^{-3} M ascorbic acid along with 3×10^{-9} M ^{3}H-serotonin (33 ci/mmoles NEN). The amount of membrane used per tube corresponded to 10 mg wet wt of tissue equivalent to 500–600 μg protein [11]. At the end of 10 min incubation at 37°, the contents were filtered on glass fibre discs (GelmanInc, Ann Arbor, MI) and washed twice with 5 ml cold Tris buffer. Filters were dried and counted in 5 ml of scintillation mixture (PPO + POPOP + Methanol + Dioxan, Toluene and naphthalene) in LKB scintillation counter at an efficiency of 38–40%. In order to assess the degree of non-specific binding, unlabelled serotonin (10^{-6} M) was used in the incubation mixture. The difference between binding of ^{3}H-serotonin in absence and presence of 10^{-6} M cold 5-HT was recorded as specific binding. Basic binding characteristics including delineation of saturability, specificity, reversibility and regional distribution were established prior to experiment.

Behavioral study. Ten animals each from control and endosulfan treated groups were randomly selected for aggressive behavior study. Fighting behavior was induced in the randomly selected pairs of adult rats by subjecting them to foot-shock. The method employed was essentially similar to that of [27]. Shocks were given employing an aggressometer (Techno, India). The pairs of rats were placed in perspex enclosure ($8 \times 8 \times 7\frac{1}{2}$ in.) and a current of 2 mA and 100 V was employed. The frequency of pulses was 5/sec. Pairs of rats which would exhibit at least one fighting episode in one minute were

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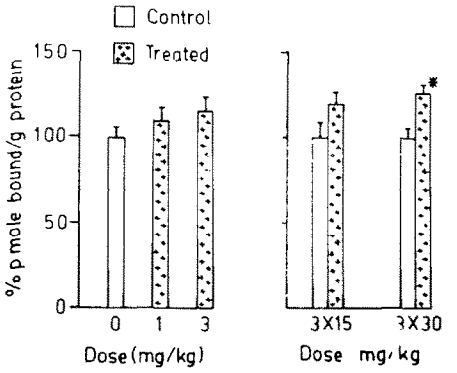

Fig. 1. Effect of single and repeated administration of endosulfan on ^{3}H-serotonin binding in frontal cortical membrane. Each value represents the mean ± S.E. from 8 animals.
*Differs significantly from respective controls ($P < 0.05$).

selected for the aggressive behavior study. This preliminary screening to exclude "non-responders" was done in all the pairs of rats selected for the present study. The time lapse between the commencing of foot-shock and onset of actual fighting was designated as latency. The frequency of fighting episode was observed during one minute period of foot-shock. A fighting episode was considered positive when the rats converged abruptly to close quarters and stood face to face on their hind legs and struck and bit savagely at each other. The return of the rats to quadrupedal posture was considered as the demarcation between fighting episode.

RESULTS

Specific binding of ^{3}H-serotonin to the frontal cortical membrane of control and endosulfan treated animals are shown in Fig. 1. As compared to the controls single administration of endosulfan (1 and 3 mg/kg) caused no significant change in the binding of ^{3}H-serotonin. On repeated exposure to endosulfan the binding of ^{3}H-serotonin remained unaltered up to 15 days. However, administration of endosulfan

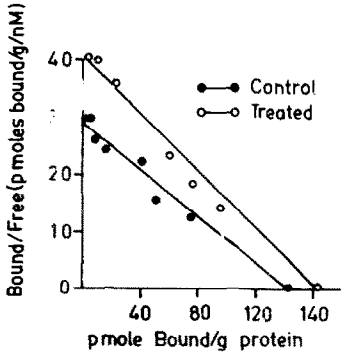

Fig. 2. Scatchard analysis of binding of ^{3}H-serotonin to frontal cortical membranes after repeated exposure of endosulfan. Each data point of saturation curve in mean of three separate experiments carried out in triplicate. B_{max} for control = 132.8 ± 9.3 pmoles/g protein, and for treated = 142.4 ± 8.7 pmoles/g protein; K_D for control = 4.6 ± 0.21 nM and for treated = 3.5 ± 0.19 nM.

Table 1. Foot-shock-induced fighting behavior after repeated administration of endosulfan (3 mg/kg) in adult rats

Treatment days	Fighting score	
	Control	Treated
15	10.5 ± 1.1	20 ± 1.5*
30	12.0 ± 1.0	25 1.6*

Each value is the mean ± S.E. from 10 animals.
* $P < 0.01$ when compared to respective controls.

(3 mg/kg) for 30 consecutive days, caused a significant ($P < 0.05$) increase in the binding of ^{3}H-serotonin. The increase in binding may be due to alterations in number of binding sites (B_{max}) or affinity (K_D) of the receptor. Kinetic analysis of the binding of ^{3}H-serotonin was performed to study the same and the Scatchard plots [13] are presented in Fig. 2. The exposure to endosulfan caused no significant change in the maximum number of high affinity binding sites as the B_{max} value of control (132.7 ± 9.3 pmoles/g) and the treated (142.4 ± 8.7 pmoles/g protein) animals were not different significantly. However, the affinity of the receptor seems to be affected as dissociation constant of control (K_D = 4.6 ± 0.21 nM) differed significantly from treated (K_D = 3.5 ± 0.19 nM). Endosulfan (1×10^{-7} M to 10×10^{-7} M) *in vitro* produced no significant change in the binding of ^{3}H-serotonin when directly added into the incubation mixture (data not shown).

The aggressive behavior of animals was measured in terms of fighting score using electric foot-shock-induced technique. Results summarized in Table 1 show that 30 consecutive doses of endosulfan (3 mg/kg) resulted in a significant ($P < 0.01$) increase in fighting behavior. Propylene glycol (40%) alone cause no significant effect on fighting behavior as compared to saline treated animals.

The results in Fig. 3 show the effect of methysergide (10 mg/kg), a blocker of serotonin, on endosulfan-induced aggressive behavior. Administration of methysergide 1 hr before electric foot-

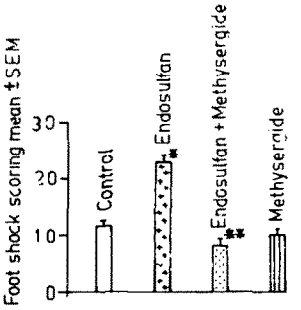

Fig. 3. Effect of methysergide on foot-shock-induced fighting behavior of endosulfan pretreated rats. Each value represents mean ± S.E. from 10 animals.
*Differ significantly ($P < 0.01$) from vehicle treated animals.
**Differ significantly ($P < 0.01$) from endosulfan pretreated animals.

shock treatment completely blocked the endosulfan (3 mg/kg, 30 days) induced fighting behavior. However, methysergide alone caused no significant effect on foot-shock-induced fighting behavior (Fig. 3).

DISCUSSION

Association of serotonergic system with aggressive behavior and increased motor activity has been suggested by several workers [14, 15]. The present study showed an increase in binding of ^{3}H-serotonin to cortical membrane as well as foot-shock-induced fighting behavior on repeated endosulfan exposure. These two phenomena exhibited a close relationship as the lower dose which caused no change in ^{3}H-serotonin binding also failed to produce any change in the behavioral response in the same animals. It has already been described that sensitivity of neurotransmitter receptor is modified by the changes in the availability of neurotransmitters with in the synapses [16]. The increase sensitivity of serotonin receptors caused by repeated exposure of endosulfan may similarly be related to low levels of serotonin at synapse.

The kinetic study (Scatchard analysis) showed that the increased sensitivity of serotonin receptor was due to the alterations in its affinity and not in the number of maximum binding sites. The decreased levels of serotonin are reported to cause aggressive behavior [17, 18]. Therefore the increase in the foot-shock-induced fighting behavior observed in our study may also be a reflection of low levels of serotonin due to endosulfan exposure. The involvement of serotonergic mechanism in endosulfan neurotoxicity was further suggested by our results with methysergide, a 5-HT blocker. The significant increase in foot-shock-induced fighting behavior caused by endosulfan was completely abolished by methysergide.

The absence of any significant effect of single exposure of endosulfan on behavior or sensitivity of 5-HT receptor suggest that perhaps a continuous exposure of pesticide at the receptor sites is needed for causing such type of hyperactivity in the animals.

The effect of endosulfan on serotonergic receptors appears to be indirect as it failed to produce any effect under *in vitro* conditions. The effect may have been caused by increase in the uptake of amine through presynaptic sites or decrease in its release in the cleft. It is also possible that endosulfan may have affected the other neuronal systems which regulate the activity of serotonergic systems.

The enhanced serotonergic receptor binding in frontal cortical membrane and increase in foot-shock-induced fighting behavioral response on long term endosulfan exposure suggests the involvement of serotonergic system in neurobehavioral toxicity of endosulfan. However, further studies with pharmacological agents known to modulate the sensitivity serotonergic receptors are needed to establish this.

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REFERENCES

1. H. Martin, *The Scientific Principle of Crop Protection* 5th edn. London (1964).
2. E. J. Miller, *Residue Rev.* **11**, 100 (1965).
3. G. E. thomas and J. S. Agr, *Vet. Ass.* **37**, 81 (1966).
4. R. C. Gupta, *Rev. Toxic.* **13**, 115 (1979).
5. M. Anand, R. N. Khanna, K. Gopal and R. N. Sur, *Vet. Human Toxic.* **22**, 385 (1980).
6. S. Garattini, E. Giacalone and L. Valzelli, in *Aggressive Behavior* (Eds. S. Garattini and E. B. Sigg), p. 285. John Wiley, New York (1969).
7. R. S. Sloviter, E. G. Drust and J. D. Conner, *J. Pharmac. exp. ther.* **206**, 339 (1978).
8. J. Glowinski and L. L. Iverson, *J. Neurochem.* **13**, 655 (1966).
9. A. K. Agrawal, P. K. Seth, R. E. Squibb, H. A. Tilson, L. L. Uphouse and S. C. Bondy, *Pharmac. Biochem. Behav.* **14**, 527 (1981).
10. P. K. Seth, J. S. Hong, C. D. Kilts and S. C. Bondy, *Toxicol. Lett.* **9**, 247 (1981).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
12. R. E. Tedeschi, D. H. Tedeschi, A. Mucha, L. Cook, P. A. Mattis and E. J. Fellows. *J. Pharmac. exp. ther.* **125**, 28 (1959).
13. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
14. J. A. Rosecrans and M. D. Adams, *Pharmac. Biochem. Behav.* **5**, 559 (1976).
15. W. L. Kostowski, E. Giacalone, S. Garattini and L. Valzelli, *Eur. J. Pharmac.* **4**, 371 (1968).
16. J. C. Schwartz, J. Costentin, M. P. Martres, P. Protais and M. Boudry, *Neuropharmacology* **17**, 655 (1978).
17. J. L. Gibbons, G. A. Barr, W. H. Bridger and S. F. Leibowits, *Pharmac. Biochem. Behav.* **9**, 91 (1978).
18. A. Weissman, D. K. Koe and S. S. Tennen, *J. Pharmac. exp. ther.* **151**, 339 (1966).