

Research Article

Endosulfan and its toxicity on GABA, 5-HT, and Dopamine in the Albino Rat Brain

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Abstract

Objectives: The objective of this study was to investigate the effects of endosulfan administration on gamma-aminobutyric acid (GABA), 5-HT, and dopamine.

Methods: Ex vivo magnetic resonance spectroscopy along with high performance liquid chromatography was used to analyze forebrain tissue from rats that were administered oral endosulfan for 4 hours, 18 hours, and 18 days.

Results: In the endosulfan-treated rats, a significant dose-dependent increase in the binding of [3H]-5 HT in the frontal cortex was seen after 18 hours in the endosulfan-treated rat. However, no such effect was observed after 4 hours of treatment and no significant changes were detected in GABA receptor binding following exposure to endosulfan. Monoamine oxidase activity was significantly decreased in the corpus striatum of the animals exposed to endosulfan at a dose of 5 mg and 15 mg/kg body weight for 18 days.

Conclusion: The maximum number of binding sites for dopamine and an increase in serotonin receptor sites without any change in the binding affinity was observed after administration of endosulfan in albino rats.

Keywords: Dopamine, endosulfan, gamma-amino-butyric acid, 5-HT

Endosulfan and hexachlorocyclohexane both belonging to the chlorinated group of pesticides, are widely used for crop protection. Endosulfan has been reported to cause central nervous system disorders in industrial workers and farmers. The individual and farmers exposed to high levels of Endosulfan are reported to exhibit epilepsy, hyperactivity irritability, tremors, convulsions and paralysis. Experimental studies show that Endosulfan affects the levels of some neurotransmitters and their receptors.^[1]

Endosulfan is a broad-spectrum organochlorine insecticide and acaricide for control of agriculture pests on a variety of field, fruit, and vegetable crops. Endosulfan active in-

redient is mixture of two isomers α and β , in the ratio of approximately 70% and 30% respectively.^[2] Endosulfan has been reported to cause central nervous system disorders in industrial workers and farmers. The individual and farmers exposed to high levels of Endosulfan are reported to exhibit epilepsy, hyperactivity irritability, tremors, convulsions and paralysis. Experimental studies show that Endosulfan affects the levels of some neurotransmitters and their receptors.^[3]

Major neurotransmitter receptors including acetylcholine (muscarinic), dopamine, serotonin (5HT) and GABA are known to transducer their signals to the cell interiors

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through the phosphoinositide derived messenger system. Endosulfan residue has been identified in a variety of environmental media (air, surface water, ground water, soil and sediment) and its metabolites have been reported in human and domestic animals milk^[4,5], fruit and vegetable.^[6,7] The most likely way for people to be exposed to endosulfan is eating the contaminated food with it. Exposure to endosulfan may occur by breathing, eating, or drinking the substance, or by skin contact.^[8] In mammals, commercial endosulfan is transformed into more water soluble metabolites, mostly endosulfan sulfate, followed by ether and diol metabolites. All of these metabolites are bio accumulated in the adipose tissue, depending on their lipophilicity.^[9] Hence, information on the mechanism of neurotoxicity of these pesticides is needed to develop suitable therapeutic measures against them.

Methods

All procedures were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, as well as the guidelines of the Animal Welfare Act.

Methods

Adult male wistar albino rats of industrial toxicology Research Centre, breeding colony, Lucknow were used in this study. Adult male wistar albino rats were used. Chemical Endosulfan obtained from Hoechst pharmaceuticals limited. All the chemicals used were analar or guaranteed grade.

Treatment of Animals:

Endosulfan was dissolved in corn oil and administered orally with the help of cannula. Endosulfan (5mg and 15 mg/kg body weight) was administered for four hours and daily up to 18 hours. The control rats received corn oil (2mg/kg body weight) in an identical manner for both the groups.

Biochemical Assays

Preparation of crude synaptic membrane and neurotransmitter receptor binding assay.

A crude membrane fraction was prepared from brain regions by homogenisation of tissue in 19 volumes of 0.32 M sucrose followed by centrifugation for 14 minutes.^[10]

Determination of Glutamate Dehydrogenase Activity

The enzyme activity was measured by method of Rajlaxmi et al.^[11]

Protein Estimation

The protein was estimated by lowry method^[12]

Determination of Monoamine Oxidase Activity

The enzyme activity was measured by the method of tabor et al.^[13]

Table 1. Effect of Endosulfan on the activity of Monoamine Oxidase (MAO) and Glutamate Dehydrogenase (GDH)

Treatment	Activity of striatal monoamine oxidase	Activity of cortical monoamine oxidase	Activity of Glutamate dehydrogenase
Control	1.64±0.12	2.0±0.40	16.8±0.6
5 mg/kg b.w	0.50±0.09***	1.6±0.2	14.2±1.9
15 mg/kg b.w	0.69±0.12**	0.7±0.3*	15.1±0.8

Activity of GDH is expressed in U/mg of protein
Activity of MAO is expressed in terms of n moles of benzaldehyde formed min⁻¹ mg⁻¹ of protein. Data are mean±S.E from six animals
*p<0.05, **p<0.01, ***p<0.001

Results

The binding of spiperone was found to be decrease in a dose dependent manner on exposure to Endosulfan at a dose of 5 mg and 15 mg/kg body weight. The decrease in the binding of [³H]-spiperone was more pronounced, when the animals were exposed to 15 mg/kg body weight Endosulfan, for 18 days.

A significant dose dependant increase in the binding of [³H]-5 HT was observed in the frontal cortex after 18 hours. However, no such effect of the Endosulfan seen after 4 hours treatment. Scatchard analysis [³H] spiperone binding revealed that the decrease in the binding was due to the decrease in the maximum number of binding sites (Bmax) without any change in the affinity of the receptor. Similarly the increase in the 5-HT binding was due to increase in the maximum number of 5-HT binding sites.

No significant changes in the GABA receptors binding following exposure to Endosulfan were observed.

In vitro studies showed that Endosulfan at concentration of 10⁻⁴ M and 10⁻⁶ M decreased the binding of [³H] spiperone indicating a direct effect of Endosulfan on the rat dopamine receptors levels; the decrease was significant at the 10⁻⁴ M concentration of Endosulfan. When studied at the concentrations ranging from 10⁻⁴ M to 10⁻⁶ M of Endosulfan, no such change was observed in the binding of [³H]-5 HT.

Monoamine oxidase activity was significantly decreased in the corpus striatum of the animals exposed to Endosulfan at a dose of 5 mg and 15 mg/kg body weight for 18 days. The lower dose was found to have no such effect on frontal monoamine oxidase activity (Table 1).

Discussion

The present observations show that repeated administration of Endosulfan causes a marked alteration in the levels of dopamine and 5 HT receptors. The decrease in the

dopamine receptor binding suggests disturbance in the dopaminergic system, due to exposure of Endosulfan. Dopamine system plays a significant role in the regulation of locomotor activity. The decrease in the number of binding sites of dopamine receptors in the brain was also observed which may be indicative of increased dopamine levels, since sensitivity of dopamine receptor is often modified by a change in the availability of neurotransmitter within the synapse.^[14]

The present study shows that exposure to Endosulfan leads to increase in the levels of serotonin receptors in the frontal cortex region of the brain. Our study suggests that the receptor binding of [³H]-5HT was increased further confirming the previous observation, since the increase in binding may be due to decreased serotonin levels in the rat brain. Involvement of serotonergic system in the neurotoxicity induced by Endosulfan has been suggested by many investigators. We found the increase in [³H]-HT binding are in accordance with the previous observation.^[15]

It is significant to note that in comparison to other neurotransmitters, dopamine receptors appeared to be more sensitive to Endosulfan. The dopamine receptors have often been found to exhibit a greater sensitivity to neurotoxics. The decrease in the binding of [³H] spiperone after exposure to Endosulfan seems to be a direct effect as confirmed by in vitro studies, showing a significant alteration in the binding of [³H] spiperone.^[16]

There are several factors which could lead to the alterations in the neurotransmission of the nerve impulse including neurotransmitter synthesis, storage, release, interaction with receptors and termination of neurotransmitter action. Levels of brain monoamine neurotransmitters are however controlled by the availability of precursor amino acid or by the altered release of the transmitter from the presynaptic neuron or by the alteration in the activity of monoamine oxidase. Inhibition of monoamine oxidase activity has also been observed by Dubey et al^[17] after Endosulfan exposure in the rat liver.

Differences in the response in the monoamine oxidase activity of different brain regions in the presence of pesticides may be due to the specific nature of interaction between pesticide and membrane binding sites.

Involvement of muscarinic, cholinergic and serotonergic systems in the Endosulfan neurotoxicity has been suggested by previous findings.^[18, 19] Pigeons after a single exposure to Endosulfan showed characteristic neuronal hyperactivity indicating increased cholinergic muscarinic stimulation.^[19]

Batra et al in their study showed significant increase in Glutamate dehydrogenase & GABA levels and a signifi-

cant decrease in the binding of 3H – Mucimol to cerebellar membrane at the doses of 1 and 5 mg/kg body weight in GABAergic neurotransmission in rat brain on haloperidol treatment and suggest that other drugs of this group may also be involving GABAergic system in their action.^[20]

Conclusion

The present study showed that the decrease in the maximum number of binding sites of dopamine and increase in the serotonin receptors sites without any change in the binding affinity. The alterations in the binding of DA and 5 HT receptors are suggestive of the disturbances in these neurotransmitter systems. The study showed that the dopamine receptors are more sensitive to Endosulfan than other neurotransmitter receptors.

Disclosures

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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