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Alterations in Acetylcholinesterase Activity in Plasma and Synaptosomal Fractions from C.N.S. of Rats Acutely Intoxicated with Lindane. Effect of Succinylcholine

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Acute intoxication in vertebrates with the insecticide lindane (hexachlorocyclohexane) has been found to produce an exaggerated responsiveness to sensory stimuli, hypersalivation, fasciculation of voluntary muscles, convulsions and death as a result of its neurotoxic effects (Joy et al. 1982). These effects most probably reflect a peripheral site of action of lindane, although there may also be a central increase in parasympathetic tone. It has also been suggested that the neurotoxic of lindane is due to its capacity to induce a hyperactivity of the central and peripheral cholinergic system, causing an increase in acetylcholine release from the cholinergic synaptic endings (Shankland 1978). Moreover, variations in cerebral levels of tryptophan (Aldegunde et al. 1980) and other amino acid neurotransmitter such glutamate, aspartate and γ-aminobutyrate (GABA) in response to lindane intoxication have also been reported (Muñoz-Blanco et al. 1982).

In the present study acetylcholinesterase activity (AChE) is used as a biochemical marker and the test of toxic action. AChE activity in plasma and synaptosomal fractions from the cervical cord, pons-medulla, cerebellum, midbrain, diencephalon and telencephalon of rats treated with lindane is determined.

Succinylcholine (SCh) is a depolarizing and desensitizing relaxant drug which has been used in the treatment of this intoxication (Jaeger et al. 1984). Its effect on the neurotoxic symptoms and on acetylcholinesterase in the aforementioned areas was also studied in order to determine its therapeutic value.

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MATERIALS AND METHODS

Twenty eigth male Winstar rats weighing between 285-290 g. were divided into four groups. Each group was fasted overnight before the experiments. Group I (ten rats) was used to determine the average time of death after they were injected (i.p.) with 225 mg/kg lindane in corn oil. This group was not used in the analysis itself. Group II (six rats) was used as the control and was injected (i.p.) 6 ml/kg corn oil. Group III (six rats) received the same treatment as Group I. Group IV (six rats) also received the same treatment as I and III but when the first symptoms of intoxication appeared, succinylcholine was administered (i.p.) at a dose of 1 mg/kg.

Thirty minutes after treatment the animals in Groups II,III and IV were decapitated and blood samples taken. The brains and cervical cords were then removed and placed in Tris-HCl 0.01M at a pH of 7.4, 0.32 sucrose, at 0-4 °C and gently stirred.

The brains were dissected and the pons-medulla, cerebellum, midbrain, diencephalon and telencephalon taken out.

Crude synaptosomal fractions were prepared as previously described (Muñoz-Blanco et al. 1982). Blood samples were centrifuged at 3000 x g for 10 min at 0-4 °C. AChE activity was measured in plasma aliquots. This activity and protein concentration were measured in separate aliquots.

Protein was measured by the Lowry method (Lowry et al. 1951) and the enzyme activity determined at 540 nm following acetylcholine iodine hydrolysis as in the method of Booth and Clark, 1978. The unit of enzyme activity is defined as the amount of enzyme that hydrolyzes one mol of substrate per minute. Specific activity is expressed in the synaptosomal fractions as mU/mg protein and as mU/ml in plasma.

RESULTS AND DISCUSSION

The animal intoxicated with lindane showed symptoms of hyperexcitability with tonic-clonic convulsive crises after a 10-15 min lag. Death ocurred by generalized spastic paralysis 38±3 minutes after injection (Group I). The first signs of lindane intoxication appeared 10-15 minutes after it was administered, succinylcholine was them injected to group IV and

Table I. AChE activity in different CNS areas and plasma in rats treated with lindane. Effect of succinylcholine. The activity values are expresed in mU/mg protein or mU/ml of plasma. The dates are X ± S.E.M. In all cases n = 6.

	SC	PM	CB	MB
Control(II)	205.8±5.0	270.6±5.6	85.4±3.2	380.1±8.4
Lindane(III)	345.1±4.4	370.0±5.8	149.6±5.6	850.9±23
Succinylcholine and lindane(IV)	586.7±18	467.3±17	209.9±8.0	441.5±15

	DC	TL	PLASMA
Control(II)	240.9±5.9	289.2±5.9	406.1±6.7
Lindane(III)	620.1±20	603.3±20	640.8±13
Succinylcholine and lindane(IV)	367.8±8.0	584.4±17	823.0±19

SC= Cervical cord; PM= pons-medulla; CB= cerebellum; MB= mid-brain; DC= diencephalon; TL= telencephalon.

hyperactivity and convulsion significantly diminished until the animals were killed 30 minutes later.

Table I shows AChE activity in plasma and in the synaptosomal fractions of the different C.N.S. areas studied in the three experimental groups. Figure I gives the percentage variations of AChE activity in animals treated with lindane, and lindane plus succinylcholine with respect to controls (Group II).

From the results, we can see that lindane intoxication produces a significant increase ($p < 0.001$) in AChE activity in both plasma and synaptosomal fractions. This ranging between 36.7% (pons-medulla) and 150 % (diencephalon). Administration of succinylcholine to rats intoxicated with lindane increased AChE activity in the cervical cord (70 %, $p < 0.001$), pons-medulla (26 %, $p < 0.001$), cerebellum, (40 %, $p < 0.001$) and in plasma (28 %, $p < 0.001$). On the other hand in the midbrain and diencephalon there was a converse decrease of this activity (48 %, $p < 0.001$) and (41 %, $p < 0.001$), respectively. However, AChE activity in these two areas was still significantly higher with respect to the control group (16 %, $p < 0.01$, for the midbrain and 53 %, $p < 0.001$, for the diencephalon).

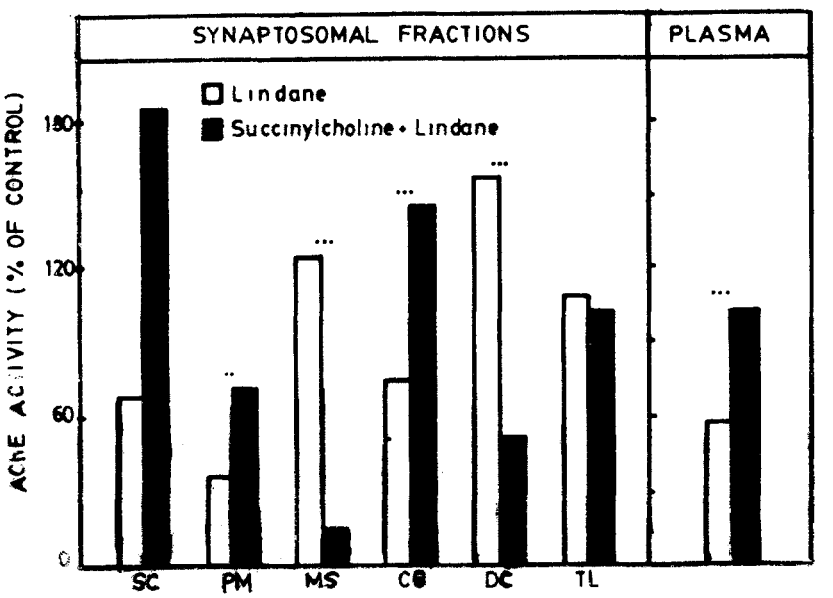

Figure 1. Percentage variations of AChE activity
*** $p < 0.001$
** $p < 0.01$
* $p > 0.05$

Activity was observed (48 %, $p < 0.001$ and 41 %, $p < 0.001$, respectively). However, the AChE activity in these areas persisted, significantly increased with respect to the control group (16 %, $p < 0.01$, for midbrain and 53 %, $p < 0.001$, for diencephalon).
No differences were observed in telencephalon of rats subjected to the same succinylcholine treatment (Fig. 1).

There were no significant differences in the telencephalon of rats subjected to the succinylcholine treatment (Fig. 1).

Acute intoxication with lindane produced a marked increase in AChE activity in both plasma and crude synaptosomal fractions of the different C.N.S. areas studied. This enhacement is probably due to a direct or indirect activation by lindane of the central or peripheric cholinergic neurotransmitter system which, in turn, triggered off tonic-clonic convulsions and death in the intoxicated animals. These results are in agreement with those found by Nordberg (Nordberg et al.1982) who reported an increase of acetylcholine (ACh) release in convulsions, or those by Uchida (Uchida et al. 1975a,1975b) who also found that lindane increased ACh release on the cockroach abdominal ganglion. This suggests that poisoning by lindane may be attributed to a direct action on presynaptic cholinergic receptors in C.N.S. which is exerted through the enhaced release of the transmitter(Shankland 1978). Furthermore, an increase in AChE activity correlated to an increase on ACh turnover in C.N.S. has long been sufficiently demostrated (Cheney and Costa 1977). The variations on AChE activity in the C.N.S. areas studied could be due to differences between them in cholinergic innervation and/or in the accumulation of this organochlorine insecticide. The generalized increase in AChE activity in both plasma and C.N.S. areas strongly suggests the involvement of cholinergic systems in the lindane poisoning symptoms. However, the possibility that the triggering of the convulsive crises (the most prominent feature of lindane toxicity) (Joy 1982), was initially caused by an alteration of other neurotransmitters that may regulate cholinergic systems, probably the GABAergic systems (Booth and Clark, Muñoz-Blanco et al. 1984) cannot ruled out.

The administration of succinylcholine, a drug used in the treatment of lindane poisoning, produces an

attenuation in the clinical signs of the intoxication. Although the effects of this drug on the neuromuscular end-plate are well known, the direct or indirect action of this cholinergic antagonist on C.N.S. has hardly been studied. Nevertheless, it has been widely demostrated that muscarinic antagonist in C.N.S. increase ACh (Cheney and Costa 1977, Kilbiger 1984). Our results on the cervical cord, pons-medulla and cerebellum might be explained by suggesting that succinylcholine behaves in these areas as a muscarinic antagonist which would provoke an increase in the number of ACh molecules available for degradation by AChE with a corresponding increase in its activity. Intracarotid infusion of hexamethomium, a nicotinic antagonist, used to anaesthetized dogs have been shown to decrease ACh released in C.N.S. (Rao et al. 1970). Therefore, we suggest the possibility of the midbrain and diencephalon being C.N.S. areas with a high density of nicotinic receptors. Hence, in these areas, succinylcholine could decrease ACh release and consequently AChE activity.

Succinylcholine administered to subjects intoxicated by lindane (Jaeger et al. 1984) could block the nicotinic receptors in the neuromuscular end-plate, thus impeding convulsions and any other signs of acute intoxication. Such blocking increases the number of molecules available for degradation by AChE, increasing its activity in the plasma.

In short, our results suggest a non-specific action of succinylcholine on the central levels similar to that of atropine on muscarinic and nicotinic receptors (Minota and Kuba 1984). They also suggest that the determination of acetylcholinesterase activity in plasma may be used as a biochemical test to differentiate diagnosis between acute intoxication by organophosphate compound or by lindane.

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