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On the Neurotoxicity of Chlordecone: A Role for γ-Aminobutyric Acid and Serotonin

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Rats receiving the chlorinated insecticide, chlordecone (80 mg/kg i.p.), exhibit hyperexcitability, exaggerated startle response and tremors within a few hours after the injection. Since the chlordecone-elicited tremors are relieved by injections of muscarinic receptor blockers, acetylcholine has been implicated indirectly in the mechanism of chlordecone toxicity. Our studies on acetylcholine steady-state levels and turnover in several brain structures failed to detect evidence for an involvement of cholinergic presynaptic mechanisms in the chlordecone toxicity. We then investigated whether GABA is involved by measuring the rate of its accumulation following intraventricular injection of gabaculine. Chlordecone reduced the rate of GABA accumulation in striatum, but not in cerebellum, brainstem or hippocampus. Such inhibition appeared 4 h after drug injection thereby preceding the onset of tremors. Since tremors elicited by chlordecone are attenuated by the administration of serotonin receptor blockers and since chlordecone increases serotonin (5-HT) turnover[7,10], we studied whether 5-HT recognition sites are modified following chlordecone administration. We have found a reduction of the B_{max} of 5-HT_1 receptors in striatum and hippocampus without any modification in the kinetic characteristics of 5-HT_2 receptors. We have also shown that the temporal relationship between down regulation of 5-HT_1 recognition sites, reduction of striatal GABA turnover and tremors caused by chlordecone allows one to consider these 3 phenomena as reciprocally dependent. In conclusion our results favor the possibility that tremors may result from a decrease in the striatal GABergic tone which probably is elicited by an increase of serotonergic activity caused by chlordecone by a yet unknown mechanism.

INTRODUCTION

Only limited information is available regarding the biochemical mechanisms specifically affected by chlordecone (Kepone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one), a chlorinated insecticide. Acute or chronic exposure of rats to chlordecone causes hyperexcitability, exaggerated startle response and tremor[9]. Several hypotheses could account for the neurotoxic manifestations caused by chlordecone[3,9,17]. Among them, one suggests that chlordecone inhibits mitochondrial and synaptosomal membrane bound Na^+, K^+-ATPases both in vivo and in vitro[9] thereby blocking uptake and storage of various neurotransmitters[4,10]. Chlordecone decreases γ-aminobutyric acid (GABA), dopamine and norepinephrine uptake by a crude synaptosomal fraction prepared from mouse brain[2]. These changes appear to be due to a decrease in the number of specific uptake sites for these 3 compounds, thereby corroborating the view that chlordecone affects membrane transport[9,10].

However, the profile of chlordecone toxicity suggests that this insecticide may cause neurotoxicity in the central nervous system by action on a specific neurotransmitter pathway[3]. Chlordecone significantly reduces the steady-state concentration of dopamine in mouse[10] but not in rat[11] striatum. Brain concentration of norepinephrine[10], GABA, glutamate, glycine, aspartate, taurine, or neuropeptides such as enkephalin or substance P[11] are not altered 24 h after injection of chlordecone when the rats exhibit tremors.

Recently some studies have focussed on a possible contributory role of serotonin (5-HT) in the chlordecone-induced neurotoxicity[10]. Chlordecone fails to change the 5-HT content of mouse brain[6] and rat striatum, hypothalamus, cerebellum and spinal cord[11].

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However, 5-hydroxyindoleacetic acid content of mouse brain[6] and rat brain structures[11] is significantly increased suggesting that chlordecone increases 5-HT turnover presumably by increasing the firing rate of 5-HT neurons. Moreover, administration of pizotifen, a serotonergic receptor blocker[16], significantly attenuates chlordecone-induced tremor[7], suggesting that serotonin receptors participate in the neurotoxic effects of chlordecone.

In an attempt to clarify the molecular events operative in mediating chlordecone tremors we have examined the effects of this insecticide on the cholinergic, GABAergic and serotonergic neuronal systems. We have measured the turnover rate of acetylcholine and GABA in several rat brain areas at various times following chlordecone administration. Moreover we have performed radioligand binding studies using specific ligands for 5-HT_1 and 5-HT_2 recognition sites[14] located in synaptic plasma membranes prepared from brains of rats receiving chlordecone (80 mg/kg i.p.).

MATERIALS AND METHODS

Treatment of animals

Male Sprague–Dawley rats (170–200 g) were obtained from Zivic-Miller (Allison Park, PA). The animals were housed 5 per cage with constant access to food and water. Chlordecone suspended in corn oil was injected intraperitoneally (i.p.) (80 mg/kg in 2 ml/kg vehicle). Control rats were injected with an equal volume of corn oil. At various times from chlordecone injections rats were killed and their brains were removed and rapidly dissected over ice according to Glowinski and Iversen[8]. The rats that were used in transmitter turnover studies were killed by microwave irradiation (3.5 kW, 2.45 GHz, 75 W/cm^2; Cober Electronics, Stamford, Conn.) focussed on the head for 1.8 s.

Chemicals

Phosphoryl[$^2H_9$]choline, [$^2H_9$]choline (Ch) and [$^2H_9$]ACh were purchased from KOR Isotopes (Cambridge, MA). [$^2H_6$]Ch and [$^2H_6$]ACh were purchased from Merck, Sharpe and Dohme (Montreal, Canada).

[^{3}H]5-HT (27.06 Ci/mmol), [^{3}H]spiroperidol (26.7 Ci/mmol), [^{3}H]mianserin (56.5 Ci/mmol) were purchased from New England Nuclear (Boston, MA). [^{3}H]Ketanserin and ketanserin were donated by Janssen Pharmaceutica (Beerse, Belgium). Lysergic acid diethylamide-25 was obtained from Sandoz (Basel, Switzerland) and mianserin was obtained from Organon (Amsterdam, Holland). 5-HT and 5,7-dihydroxytryptamine (5,7-DHT) were obtained from Sigma Chemical Co. (St. Louis, MO). Mepyramine was obtained from Robinson Laboratories (San Francisco, CA). All other chemicals were obtained in the purest form commercially available.

Measurement of transmitter turnover rate

To determine the turnover rate of acetylcholine, phosphoryl[$^2H_9$]choline (15 μmol/kg/min) was infused through the lateral tail vein at a rate of 0.1 ml/min. After 9 min the animals were killed by focused microwave irradiation and the brains quickly dissected. The stable isotopic enrichment of acetylcholine and choline was measured mass fragmentographically[18]. The fractional rate constant for acetylcholine efflux (k_{ACh}) was calculated from equations described elsewhere[15] and the turnover rate for acetylcholine was obtained by multiplying the fractional rate constant by the steady-state concentration of acetylcholine.

For comparative purposes, the turnover rate of GABA was estimated from the rate of GABA accumulation after the inhibition of its catabolism with gabaculine (100 μg/5 μl i.c.v.) and evaluating the GABA accumulation at different times after the inhibition of GABA transaminase. The tissue content of GABA was measured mass-fragmentographically[1].

[^{3}H]Mianserin and [^{3}H]ketanserin specific binding

Tissue samples from cortex, hippocampus and striatum were homogenized in 20 vols. of ice-cold Tris-HCl (50 mM; pH 7.4). The homogenates were centrifuged at 17,000 *g* for 15 min and the pellets were washed twice with the same volume of buffer. Aliquots of the crude synaptic membrane suspension were incubated with different concentrations of specific ligands. For [^{3}H]mianserin binding studies[5] 0.2 ml aliquots of the crude synaptic membrane suspension were incubated with various concentrations of [^{3}H]mianserin (0.12–8.0 nM) for 30 min at 37 °C in the presence of the histamine$_1$ receptor blocker, mepyramine (300 nM). For [^{3}H]ketanserin binding[12]

studies, aliquots of the crude synaptic membrane suspension were incubated with various concentrations of [^{3}H]ketanserin (0.25–4.0 nM) for 30 min at 37 °C. After incubation the mixture was rapidly filtered under reduced pressure through GF/B glass fiber filters. The filters were washed 3 times with 5 ml of ice-cold buffer, placed in scintillation vials containing 10 ml Aquassure (New England Nuclear, Boston, MA), and the radioactivity counted by scintillation spectrometry.

Specific binding was determined from the difference between the total binding and the binding remaining after displacement with large concentrations of specific displacers: 10 μM cold mianserin for [^{3}H]mianserin; and 10 μM cold ketanserin for [^{3}H]ketanserin.

Binding assays for [^{3}H]5-HT and [^{3}H]spiroperidol

Crude synaptic membranes from cortex, hippocampus and striatum were prepared as described above. The final pellet was suspended in 20 vols. of cold Tris-HCl buffer pH 7.4 and preincubated at 37 °C for 10 min to remove endogenous 5-HT[13]. After centrifugation the pellet was resuspended in Tris buffer containing 0.1% ascorbic acid, 10 μM pargyline and 4 mM $CaCl_2$. 0.2 ml Aliquots of the tissue suspension were incubated at 37 °C for 15 min with different concentrations of [^{3}H]5-HT (0.5–15 nM) or [^{3}H]spiroperidol (0.5–2.0 nM). Non-specific binding was determined in assay tubes containing LSD-25 (10 μM).

5,7-Dihydroxytryptamine (5,7-DHT) lesions

Rats weighing 160–180 g were anesthetized with pentobarbital and lesions were produced by injecting 5,7-dihydroxytryptamine (5,7-DHT) stereotaxically into the lateral ventricle (200 μg of free base dissolved in 10 μl of saline containing 0.01% ascorbic acid) at a rate of 2.5 μl/min. Sham-lesioned rats were given injections of the vehicle. Rats from both groups were pretreated with desmethylimipramine (25 mg/kg i.p.) 40 min before the neurotoxin injection to minimize 5,7-DHT uptake into noradrenergic neurons[13]. Sham and lesioned rats were injected with chlordecone or vehicle 30 days after surgery.

RESULTS

Acetylcholine turnover

The data shown in Table I indicate that at various times (from 1 to 48 h) following chlordecone doses eliciting tremors the acetylcholine content and turnover of hippocampus and striatum remains unchanged. Moreover, in chlordecone-treated rats neither the content or the turnover rate of acetylcholine changed in the frontal cortex or cerebellum (data not shown).

GABA turnover

Fig. 1 shows that following administration of gabaculine doses that inhibit GABA catabolism there is about a 4-fold increase in the concentration of GABA in the hippocampus (60 nmol/mg protein/h),

TABLE I

Concentration and turnover rate of acetylcholine at various times following administration of chlordecone (80 mg/kg i.p.)

Data were analyzed with the multiple comparison test of Dunnett. Groups consisted of 8 animals and results were determined as mean ± S.E.M.

Hours after chlordecone	*Hippocampus*			*Striatum*		
	ACh (nmol/mg prot)	K_{ACh} *(h^{-1})*	TR_{ACh} *(nmol/mg prot/h)*	*ACh (nmol/mg prot)*	k_{ACh} *(h^{-1})*	TR_{ACh} *(nmol/mg prot/h)*
Control	0.29 ± 0.05	6.9 ± 0.90	2.0 ± 0.14	1.00 ± 0.06	10.0 ± 1.10	10 ± 0.93
1	0.28 ± 0.01	5.7 ± 0.32	1.7 ± 0.13	0.95 ± 0.04	9.7 ± 0.98	9.2 ± 0.61
2	0.26 ± 0.03	6.4 ± 0.39	1.7 ± 0.16	1.14 ± 0.09	8.9 ± 0.92	10.1 ± 1.16
4	0.33 ± 0.02	6.0 ± 1.3	1.9 ± 0.42	0.95 ± 0.07	10 ± 1.21	9.5 ± 0.91
6	0.27 ± 0.01	5.8 ± 0.91	1.8 ± 0.45	1.00 ± 0.08	10.3 ± 1.15	10.3 ± 0.93
12	0.29 ± 0.01	6.1 ± 0.33	1.8 ± 0.14	0.95 ± 0.05	9.2 ± 0.96	8.7 ± 0.75
24	0.26 ± 0.01	5.2 ± 0.75	1.4 ± 0.24	0.90 ± 0.10	10.8 ± 1.8	9.8 ± 0.80
48	0.28 ± 0.01	6.2 ± 0.71	1.5 ± 0.18	1.14 ± 0.09	10.5 ± 1.16	11.9 ± 1.32

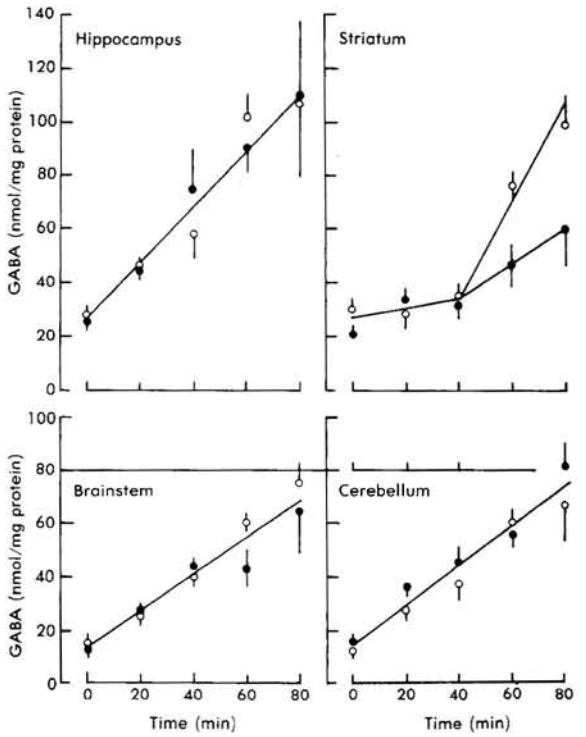

Fig. 1. GABA accumulation following intraventricular injection of D,L-gabaculine (100 μg) and/or chlordecone (80 mg/kg, 24 h, i.p.) in hippocampus, striatum, brainstem, and cerebellum. Open circles indicate animals receiving only gabaculine. Closed circles refer to rats receiving both gabaculine and chlordecone. Data are means ± S.E.M. Each group consists of 8–10 measurements.

brainstem (40 nmol/mg protein/h) and cerebellum (45 nmol/mg protein/h). This accumulation proceeds unimpaired during the 80 min of our studies. Chlordecone administration 24 h before gabaculine fails to modify the GABA content in hippocampus, brainstem or cerebellum or the rate of GABA accumulation elicited by gabaculine. In striatum, however, where the GABA accumulation appears to be biphasic, changing slope 40 min following gabaculine administration, the rate of GABA accumulation between 40 and 80 min is greater in rats receiving only gabaculine (70 nmol/mg protein/h). During this same time period, in chlordecone-treated rats, the rate of GABA accumulation is 20 nmol/mg protein/h; a reduction of more than three-fold when compared to rats receiving only gabaculine. A time-course study of the chlordecone induced modifications of GABA turnover revealed that the inhibition of GABA accumulation is already present 4 h after the drug injection and in a time-dependent manner reaching a maximum at 24 h after chlordecone administration (Table II).

TABLE II

Time dependent inhibition of GABA accumulation and of [^{3}H]5-HT specific binding in striatum of rats receiving chlordecone

A: the striatal content of GABA was measured by mass-spectrometry (see Materials and Methods). The rate of GABA accumulation was calculated on the bases of GABA accumulation between 40 and 80 min after inhibition of GABA-T by gabaculine (100 μg/5 μl i.c.v.) in striatum of rats receiving vehicle or chlordecone (80 mg/kg i.p.) 4, 6, 12 and 24 h before microwave irradiation.
B: the value of [^{3}H]5-HT specific binding in striatal membranes prepared form control rats was 98.3 ± 4.3 fmol/mg protein.

	A. Rate of GABA accumulation (nmol/mg prot/h)	*B. [^{3}H]5-HT specific binding (% of control)*
Control	77	100
4 h	56*	74*
6 h	37	54
12 h	33	45
24 h	26	28

* $P < 0.05$ (Student's t-test) when compared to control.

[^{3}H]5-HT specific binding

Table III shows the kinetic characteristics of [^{3}H]5-HT specific binding to crude synaptic membranes prepared from cerebral cortex, hippocampus and striatum of rats receiving either corn oil or chlordecone. There is a 70% reduction in the number of 5-HT_1 binding sites in striatum and a 30% reduction in hippocampal membranes. In both cases the apparent affinity (K_d) remains unchanged. In contrast, no differences are detected in either the B_{max}or the K_d of 5-HT_1 recognition sites to cortical membranes from the same rats. A time-course study (Table II) revealed that the down-regulation of 5-HT_1 recognition sites in striatal membranes is already present 4 h after the injection of the insecticide. This inhibition lasts longer than 24 h.

[^{3}H]Spiroperidol or [^{3}H]ketanserin specific binding

To elucidate whether chlordecone modifies 5-HT_2 recognition sites, we studied the binding characteristics of [^{3}H]spiroperidol and [^{3}H]ketanserin, two ligands which bind 5-HT_2 receptors. Table IV shows that administration of chlordecone fails to alter the

TABLE III

Kinetic characteristics of [^{3}H]serotonin (5-HT$_1$) specific binding to crude synaptic membranes from cortex, hippocampus and striatum of rats treated with chlordecone (80 mg/kg i.p.)

	Cortical membranes		*Hippocampal membranes*		*Striatal membranes*	
	K_d *(nM)*	B_{max} *(fmol/mg prot)*	K_d *(nM)*	B_{max} *(fmol/mg prot)*	K_d *(nM)*	B_{max} *(fmol/mg prot)*
Control	9.5 ± 0.6	170 ± 12	10 ± 0.3	340 ± 9.4	8.4 ± 0.5	90.6 ± 1.5
Chlordecone	12 ± 0.8	170 ± 13	9.1 ± 0.4	250 ± 13*	8.9 ± 0.6	25.5 ± 1.1*

* $P < 0.01$ (Student's t-test) when compared to values obtained in controls.

TABLE IV

Kinetic characteristics of [^{3}H]spiroperidol and [^{3}H]ketanserin (5-HT$_2$) specific binding to crude synaptic membranes prepared from brains of rats treated with chlordecone (80 mg/kg i.p.)

	Cortical membranes		*Hippocampal membranes*		*Striatal membranes*	
	K_d *(nM)*	B_{max} *(fmol/mg prot)*	K_d *(nM)*	B_{max} *(fmol/mg prot)*	K_d *(nM)*	B_{max} *(fmol/mg prot)*
[^{3}H]Spiroperidol specific binding						
Control	1.5 ± 0.1	190 ± 11	0.6 ± 0.1	83 ± 6	—	—
Chlordecone	1.6 ± 0.1	203 ± 12	0.71 ± 0.1	78 ± 6	—	—
[^{3}H]Ketanserin specific binding						
Control	0.8 ± 0.08	105 ± 9	0.6 ± 0.08	45 ± 5	0.6 ± 0.08	33 ± 4
Chlordecone	0.9 ± 0.08	100 ± 7	0.6 ± 0.06	51 ± 3	0.5 ± 0.09	29 ± 3

kinetic characteristics of [^{3}H]spiroperidol or [^{3}H]ketanserin specific binding to crude synaptic membranes prepared from cerebral cortex, hippocampus or striatum.

[^{3}H]Mianserin specific binding

Since mianserin was proposed by Peroutka and Snyder[14] to bind to both 5-HT$_2$ and histamine$_1$ (H$_1$) binding sites, we performed kinetic studies of [^{3}H]mianserin specific binding (Fig. 2) in the presence of mepyramine (300 nM) to block H$_1$ receptors. Scatchard analysis of the specific binding of [^{3}H]mianserin to synaptic plasma membranes from control and chlordecone-treated rats indicates that in hippocampus, but not in cortex, there is a significant decrease (34%) in the B_{max} of [^{3}H]mianserin binding sites with no differences in K_d values. These results demonstrate that the specific binding of [^{3}H]mianserin to hippocampal membranes behaves similarly to the 5-HT$_1$ binding and differs from the specific binding of [^{3}H]spiroperidol and [^{3}H]ketanserin. Since striatal membranes contain a small number of [^{3}H]mianserin binding sites a study of the action of chlordecone on this brain structure was not feasible. However in order to clarify the nature of the [^{3}H]mianserin binding site, we studied the kinetic characteristics of the binding sites located in hippocampal membranes prepared from 5,7-DHT lesioned and sham-lesioned rats. We compared the binding characteristics of [^{3}H]mianserin and [^{3}H]5-HT to hippocampal membranes prepared from control and chlordecone-treated rats. Thirty-one days after intraventricular injection of 5,7-DHT there was a supersensitivity of 5-HT$_1$ and [^{3}H]mianserin binding. Furthermore the lesion prevented the chlordecone-induced down regulation of 5-HT$_1$ and [^{3}H]mianserin binding sites (Fig. 3).

DISCUSSION

Since chlordecone increases brain 5-HT turnover[11] to infer on the postsynaptic consequences of this increase we studied the binding characteristics of various ligands to 5-HT$_1$ and 5-HT$_2$ recognition sites located in crude synaptic membrane preparations. The kinetic characteristics of [^{3}H]spiroperidol or [^{3}H]ke-

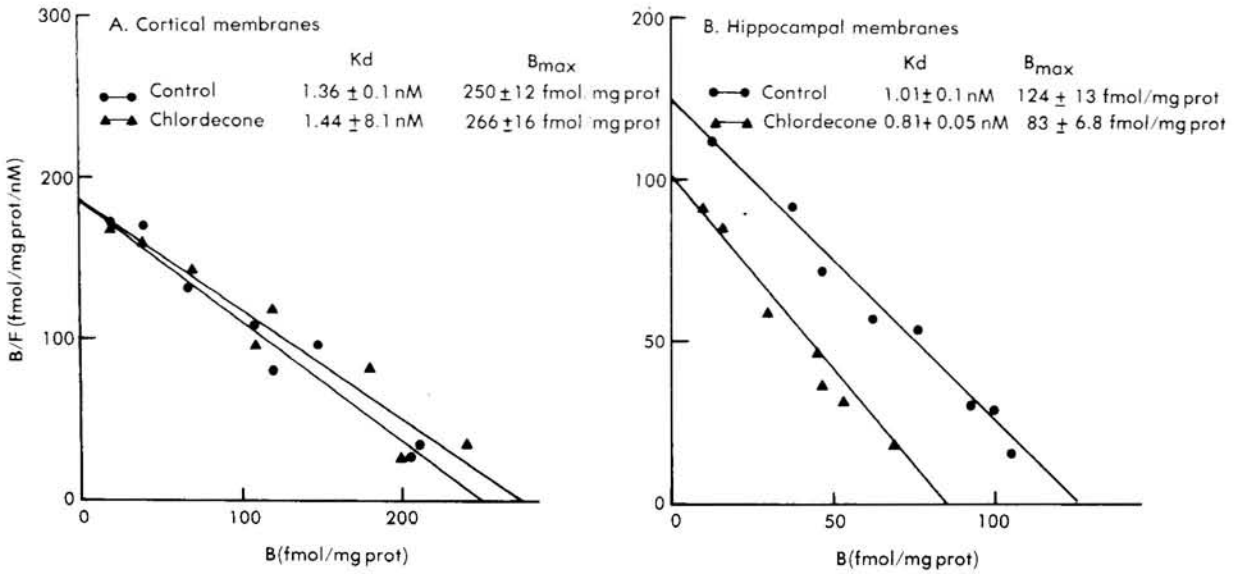

Fig. 2. Scatchard analysis for [^{3}H]5-HT specific binding to crude synaptic membranes from cerebral cortex and hippocampus of rats treated with ● vehicle or ▲ chlordecone. Each point represents the mean of 3 experiments run in triplicate. The S.E. values were always less than 5%.

tanserin binding to specific membranes prepared from cortex, hippocampus or striatum were unaffected, thereby ruling out the possibility that 5-HT$_2$ receptors are involved in the chlordecone-induced tremors. However, in striatal and hippocampal membranes prepared from chlordecone treated rats there is a significant decrease in [^{3}H]5-HT specific binding (Table III). Moreover in hippocampal membrane prepared from rats receiving chlordecone, the down-regulation of 5-HT$_1$ recognition sites is associated with a decrease in [^{3}H]mianserin binding. This association and the supersensitivity of [^{3}H]5-HT and [^{3}H]mianserin specific binding elicited by 5,7-DHT destruction of 5-HT neurons suggest that [^{3}H]mianserin recognition sites similarly to the 5-HT$_1$ recognition sites are under the control of 5-HTergic neurons. In 5,7-DHT lesioned rats the administration of chlordecone failed to down-regulate [^{3}H]5-HT and [^{3}H]mianserin binding sites in hippocampal membranes (Fig. 3) indicating that, at least in this structure, the integrity of the 5-HT axon terminals is a prerequisite for the down regulation of 5-HT$_1$ and mianserin binding sites elicited by chlordecone. Hence one can infer that the chlordecone induced down regulation reflects an activation of 5-HT neurons.

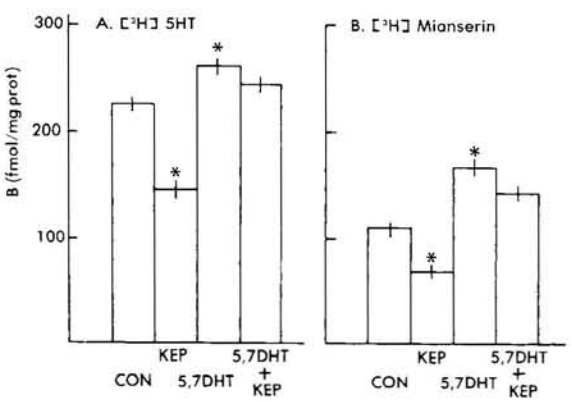

Fig. 3. [^{3}H]5-HT (15 nM) and [^{3}H$_3$]mianserin (5 nm) specific binding to membranes prepared from hippocampi of rats with a lesion of 5-HT axons by 5,7-DHT injection (see Materials and Methods). Each value represents the mean of 3 experiments. Asterisk indicates $P < 0.05$ (Student's t-test) when compared to values obtained in controls.

These effects, taken together with the observation that the tremors elicited by chlordecone are attenuated by injecting animals with pizotifen[7], a 5-HT receptor blocker, allowed us the inference that an activation of the serotonergic system could mediate the neurotoxic action of chlordecone. The two-fold increase in steady-state 5-HIAA concentrations in many brain areas of rats receiving chlordecone taken together with the down regulation of 5-HT$_1$ recognition sites allows one to infer that chlordecone activates 5-HT neurons. Moreover since the present

studies indicate that the decrease in 5-HT_1 recognition site is operative at 4 h following chlordecone when the tremors begin to appear, one can suggest that the two phenomena that are temporally related may also be causally related.

The chlordecone-elicited tremors are relieved also by injections of muscarinic receptor blockers, implicating acetylcholine indirectly in the mechanism of chlordecone toxicity. We failed to detect metabolic evidence for an involvement of cholinergic presynaptic mechanisms in the chlordecone toxicity. Since in several structures GABA and acetylcholine may innervate the same neuron and exert opposite effects (ACh may excite while GABA may inhibit firing), we have studied whether GABA turnover changes in structures that could be involved in the genesis of tremor. We measured GABA turnover in hippocampus, striatum, brainstem and cerebellum and found that chlordecone inhibits the turnover rate of GABA in striatum but not in the other structures we have studied. Since the inhibition of GABA turnover is temporally related to the down regulation of 5-HT_1 recognition sites (Table II) one might suggest that the tremors can now be studied in coincidence with two neurochemical defects. Hence chlordecone, through the activation of the 5-HT neurons could decrease GABAergic activity in striatum and thereby cause a relative increase in the cholinergic tone, causing tremors. Our studies fail to establish whether this activation of serotonergic tone is primary or secondary to another yet unknown mechanism. In view of the relative quantitative importance of dopamine in striatum, the primary role of this amine could be evoked; nevertheless other studies[11] indicate that the levels of DA and DOPAC in striatum of rats receiving chlordecone are unchanged ruling out the involvement of this amine in the tremorgenic effect of the insecticide.

REFERENCES

1 Bertilsson, L. and Costa, E., Mass fragmentographic quantitation of glutamic acid and γ-aminobutyric acid in cerebellar nuclei and sympathetic ganglia of rats, *J. Chromatogr.*, 118 (1976) 395–401.

2 Chang-Tsui, Y.-Y. H. and Ho, I. K., Effects of Kepone® (chlordecone) on synaptosomal γ-aminobutyric acid uptake in the mouse, *Neurotoxicology*, 1 (1979) 357–367.

3 Chang-Tsui, Y.-Y. H. and Ho, I. K., Effects of Kepone® (chlordecone) on synaptosomal catecholamine uptake in the mouse, *Neurotoxicology*, 1 (1980) 643–651.

4 Desaiah, D., Interaction of chlordecone with biological membranes, *J. Toxicol. Environ. Health*, 8 (1981) 719–730.

5 Dumbrille-Ross, A., Tang, S. W. and Seeman, P., High-affinity binding of [^{3}H]mianserin to rat cerebral cortex, *Europ. J. Pharmacol.*, 68 (1980) 395–396.

6 Fujimori, K., Nabeshima, T., Ho, I. K. and Mehendale, H. M., Effect of oral administration of chlordecone and mirex on brain biogenic amines in mice, *Neurotoxicology*, 3 (1982) 143–148.

7 Gerhart, J. M., Tilson, H. A., Hong, J. S. and Uphouse, L. L., Chlordecone-induced tremor: quantification and pharmacological analysis, *Tox. Appl. Pharmacol.*, in press.

8 Glowinski, J. and Iversen, L. L., Regional studies of catecholamines in the rat brain. I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine, and ^{3}H-DOPA in various regions of the brain, *J. Neurochem.*, 13 (1966) 655–669.

9 Guzelian, P. S., Comparative toxicology of chlordecone (kepone) in humans and experimental animals, *Ann. Rev. Pharmacol. Toxicol.*, 22 (1982) 89–113.

10 Ho, I. K., Fujimori, K., Huang, T. P. and Chang-Tsui, H., Neurochemical evaluation of chlordecone toxicity in the mouse, *J. Toxicol. Environ. Health*, 8 (1981) 701–706.

11 Hong, J. S., Tilson, H. A., Uphouse, L. L., Gerhart, J. and Wilson, W. E., Effects of chlordecone exposure on the brain neurotransmitters: possible involvement of the serotonin system in chlordecone-elicited tremor, *Tox. Appl. Pharmacol.*, submitted.

12 Leysen, J. E., Niemegeers, J. E., Van Neuten, J. M. and Laduron, P. M., ^{3}H-Ketanserin (R42468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding. Binding properties, brain distribution and functional role, *Molec. Pharmacol.*, 21 (1982) 301–314.

13 Nelson, D. L., Herbet, A., Bourgoin, S., Glowinski, J. and Hamon, M., Characteristics of central 5HT receptors and their adaptive changes following intracerebral 5,7-dihydroxytryptamine administration in the rat, *Molec. Pharmacol.*, 14 (1978) 983–995.

14 Peroutka, S. J. and Snyder, S. H., Two distinct serotonin receptors: regional variations in receptor binding in mammalian brain, *Brain Research*, 208 (1981) 339–347.

15 Racagni, G., Cheney, D. L., Trabucchi, M., Wang, C. and Costa, E., Measurement of acetylcholine turnover rate in discrete areas of rat brain, *Life Sci.*, 15 (1974) 1961–1975.

16 Rosecrans, J. A. and Glennon, R. A., Drug-induced cues in studying mechanisms of drug action, *Neuropharmacology*, 18 (1979) 981–989.

17 Wood, P. L. and Cheney, D. L., The effects of muscarinic receptor blockers on the turnover rate of acetylcholine in various regions of the rat brain, *Canad. J. Physiol. Pharmacol.*, 57 (1979) 404–411.