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Biochemical Changes in the Brain Consequent to Dietary Exposure of Developing and Mature Rats to Chlordecone (Kepone)

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Biochemical Changes in the Brain Consequent to Dietary Exposure of Developing and Mature Rats to Chlordecone (Kepone). SETH, P. K., AGRAWAL, A. K., AND BONDY, S. C. (1981). *Toxicol. Appl. Pharmacol.* **59**, 262-267. Adult male rats receiving 10 or 30 ppm chlordecone (Kepone) in the diet for 90 days exhibited decreased binding of [^{3}H]spiroperidol in membranes prepared from the striatum. [^{3}H]Muscimol and [^{3}H]quinuclidinyl benzilate binding in the cerebellum were also depressed. The binding of [^{3}H]diazepam and [^{3}H]serotonin to cortical membranes was unaltered in treated animals. The areas of brain of exposed animals which exhibited a reduced ability to bind several ligands for specific neurotransmitter-receptor sites also possessed an increased amount of membrane protein. The frontal cortex of chlordecone-dosed rats where ligand binding was not altered, showed no significant change in membrane protein content. Thus chlordecone-induced alterations in receptor properties could be accounted for in terms of a region-specific hyperplastic increase in nonreceptor proteins. Thirty days after cessation of dosing ligand-binding properties and membrane protein from regions of treated animals did not differ significantly from controls, suggesting that these effects were reversible at the dose levels used. Male and female rats exposed indirectly throughout gestation and lactation showed no abnormal concentrations of membrane protein at 30 days of age after a maternal diet of 1 or 6 ppm chlordecone. No decrease in cerebellar binding of muscimol or quinuclidinyl benzilate, in frontal cortical binding of serotonin, or in striatal binding of spiroperidol was observed. At the 6-ppm dose level, male rats had an elevation of striatal dopamine binding. These data illustrate that gestational exposure to chlordecone can have effects that are in an opposite direction than those observed after exposure of adults to a higher dose level.

Chlordecone (Kepone, decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta(*cd*)pentalen-2-one) has been used as an insecticide and is also found as a contaminant of the insecticide mirex (Carlson *et al.*, 1976). Chlordecone has been found to cause signs of neurotoxicity in man. These include tremor, headaches, abnormal elevation of cerebrospinal fluid pressure, mental symptoms, and disturbances of vision (Cohn *et al.*, 1978; Cannon *et al.*, 1978; Sanborn *et al.*, 1979; Taylor *et al.*, 1978). Adverse effects on CNS function have also been demonstrated experimentally in rodents by chemical (Chang-Tsui and Ho, 1979, 1980) and behavioral means (Reiter *et al.*, 1977; Dietz and McMillan, 1979; Tilson *et al.*, 1980; Huang *et al.*, 1980), and in fish by histological means (Couch *et al.*, 1977). Hyperexcitability has been reported to occur in both man and mouse (Lanson *et al.*, 1979; Huang *et al.*, 1980).

The present study was performed to determine whether such neurotoxic effects could be mediated by effects on neurotransmission. A survey of high-affinity binding sites of various neurotransmitters was used as an objective means of detecting damage to a specific neuronal circuit (Bondy, 1980). While the binding capacities of some brain

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regions for certain transmitter species were altered in adult rats exposed to chlordecone, these changes could best be accounted for in terms of a hyperplasia leading to increased amounts of nonreceptor protein. These effects appeared largely reversed a month after the cessation of dosing. Rats exposed throughout gestation showed an increase of striatal dopamine binding which could not be attributed to a regional change in membrane protein content.

METHODS

(a) Chlordecone administration. Eight-week-old male albino rats (Fischer 344/N strain, Charles River Breeding Lab, Inc., six to eight animals/group) were fed 10 or 30 ppm chlordecone in their diet for 90 days. Food and water were freely available. Animals were killed immediately after 90 days of dosing or 30 days after cessation of dosing.

Female rats of the same strain and age were given two dose levels of chlordecone in the diet (1 and 6 ppm) for 60 days prior to mating. Exposure to the chlordecone diet continued throughout gestation and lactation until pups were 12 days old. Offspring were then raised to 30 days of age using a chlordecone-free diet, at which time they were killed by decapitation. Brain regions from animals exposed as adults or throughout gestation and lactation were dissected into regions (Iversen and Glowinski, 1966) and frozen at −20°C.

(b) Membrane preparation and incubation. A crude membrane fraction was prepared from brain regions by homogenization of tissue in 19 vol of 0.32 M sucrose followed by centrifugation (50,000*g*, 10 min). The precipitate from this step was then homogenized in distilled water, pH 7.4, and recentrifuged. The final pellet was suspended in 40 mM Tris–HCl buffer, pH 7.4, at a concentration representing 50 mg original tissue/ml.

Binding incubations were carried out in triplicate in a final volume of 1 ml containing 40 mM Tris–HCl, pH 7.4, together with appropriate labeled and unlabeled pharmacological agents. The incubation mixture used in the assay of serotonin also included 10^{-5} M pargyline, 4×10^{-3} M $CaCl_2$, and 5.7×10^{-3} M ascorbic acid. The amount of tissue used per tube corresponded to 5–10 mg original wet weight and contained 300–400 μg membrane protein as determined by the method of Lowry *et al.* (1951). At the end of a 15-min incubation at 37°C samples were filtered on glass fiber disks (25-mm diameter, 0.3-μm pore size, Gelman Inc., Ann Arbor, Mich.) and washed twice rapidly with 5 ml Tris buffer. Filter disks were then dried and counted in 5 ml of a scintillation mixture using a Packard Tri-Carb 2660 scintillation counter at an efficiency of 38–43% in order to determine membrane bound radioactivity. Control incubations were carried out simultaneously with the experimental series containing no unlabeled competing ligand in order to determine the extent of nonspecific binding. The final concentration of unlabeled competing compounds in control incubations was 10^{-6} M. Specific binding was taken to be that binding that was displaced in the presence of this large excess of the competing compound. The assay of the dopamine receptor was performed using 10^{-9} M [1-*phenyl*-4-^{3}H]spiroperidol (23 Ci/mmol) as the binding ligand and haloperidol as the competing compound in control tubes. In a parallel manner 10^{-9} M DL-[*benzilic*-4, 4′-^{3}H]quinuclidinyl benzilate (QNB) (29 Ci/mmol) was used to measure muscarinic sites with atropine as a competitor. Benzodiazepine sites were estimated with 0.7×10^{-9} M [*methyl*-^{3}H]diazepam (73 Ci/mmol) and the unlabeled compound as competitor. [*methylene*-^{3}H(N)]Muscimol (8.0×10^{-9} M, 7.3 Ci/mmol) and unlabeled GABA were used in GABA binding-site assays. [1,2-^{3}H(N)]Serotonin (3.1×10^{-9} M, 30 Ci/mmol) was used together with unlabeled serotonin in the assay of this receptor. The method used was thus essentially similar to other filtration binding methods (Yamamura *et al.*, 1978). However, we felt it necessary to establish basic binding characteristics prior to studies on animals treated with chlordecone. These included delineation of saturability, specificity, reversibility, and regional distribution (Bondy, 1980).

(c) Analysis of plasma-testosterone levels. Serum was drawn from rats after decapitation and, after coagulation and sedimentation of erythrocytes, testosterone levels were determined by radioimmunoassay using a kit supplied by Serono Laboratories, Braintree, Massachusetts (Midgely, 1966). Purified testosterone was used as a standard. Antiserum to testosterone was raised in rabbits and the assay performed using ^{125}I-labeled 3-*O*-carboxymethyloxinetyrosine methyl ester as a radioactive tracer. Serum samples extracted with anhydrous diethyl ether were incubated at 20°C for 3 hr and then with the second antibody (sheep antirabbit gamma globulin) at 20°C for 18–20 hr in order to precipitate the antibody complex. Radioactivity was measured in a gamma counter.

(d) Statistical treatment. Differences between groups were assessed using Fisher's least significant difference test after a one-way analysis of variance. The accepted level of significance in all cases was $p < 0.05$ using a two-tailed distribution. Each data point represents values derived from six to eight individual animals.

RESULTS

Adult Exposure

Ninety days after commencement of a chlordecone-containing diet, the specific binding of several labeled ligands to striatum and cerebellum was reduced when expressed on a protein basis (Table 1). These reductions may have been dose dependent since the changes caused by the 10-ppm dose tended to be intermediate between untreated and 30-ppm-treated rats. However, 10- and 30-ppm data did not differ significantly. No changes were seen in the binding of serotonin or diazepam to frontal cortical membranes of treated rats. This was in contrast to the 14–31% reductions of striatal binding of spiroperidol and cerebellar binding of QNB or muscimol.

Regional analysis of membrane protein content revealed that those areas showing reduced receptor binding (striatum and cerebellum) as a result of chlordecone exposure also contained an abnormally high concentration of membrane protein (Table 2). This effect showed more dose relatedness than the altered binding characteristics and was thus more pronounced (34–36% increase) in rats receiving the higher chlordecone dose (30 ppm). Frontal cortical membrane content was not significantly altered (less than 9%). Therefore, the apparent reduction in striatal and cerebellar receptor binding of treated rats could in part be attributed to an increased level of nonreceptor membrane components. When this elevation of protein was taken into account, the overall binding capacities of these brain areas were similar in treated and untreated rats.

Thirty days after restoration of rats to a normal chlordecone-free diet, changes in cerebellar and striatal protein content were restored to control values (within 5% of untreated animals). The reductions in receptor binding in treated rats were also restored to control values. Behavioral reversal of chlordecone toxicity has also been reported (Huang *et al.*, 1980).

TABLE 1

EFFECTS OF DIETARY CHLORDECONE ON THE HIGH-AFFINITY RECEPTOR SITES IN BRAIN REGIONS OF ADULT MALE RATS

Putative receptor species	Chlordecone treatment (ppm)		
	0	10	30
Dopamine (striatum)	395 ± 28	339 ± 19	301* ± 22
Muscarinic (cerebellum)	90 ± 8	71 ± 7	65* ± 4
GABA (cerebellum)	496 ± 64	352* ± 32	344* ± 48
Serotonin (frontal cortex)	71 ± 9	62 ± 7	75 ± 5
Benzodiazepene (frontal cortex)	35 ± 4	40 ± 2	39 ± 1

Note. Binding is expressed as pmol/g protein ± SE.
* Value differs from zero-dose value ($p < 0.05$, Fisher's least significant difference test). Six to eight animals were used in each group. For details, see text.

Serum testosterone levels were 2.0 ± 0.3 and 2.2 ± 0.6 ng/ml from 10-ppm- and 30-ppm-exposed rats, respectively. Neither of these values differed significantly from the corresponding control values (2.5 ± 0.3 ng/ml).

Neonatal Exposure

Rats were exposed indirectly throughout gestation and lactation to chlordecone in the maternal diet (see Methods). When these rats were 30 days old both males and females were tested for changes in regional transmitter binding sites.

No significant changes in regional membrane protein content were apparent relative to control values for male and female rats. However, striatal binding of [^{3}H]spiroperidol was increased in animals whose mother received the higher (6 ppm) dose. Significance was achieved only for male offspring (Table 3). Frontal cortical sero-

TABLE 2

REGIONAL BRAIN WET WEIGHTS AND MEMBRANE PROTEIN CONTENT OF RATS RECEIVING CHLORDECONE

Region	Chlordecone treatment (ppm)		
	0	10	30
Striatum			
Weight	76 ± 2	76 ± 6	82 ± 4
Protein	4.3 ± 0.2	4.5 ± 0.1	6.4* ± 1
Cerebellum			
Weight	259 ± 7	268 ± 6	257 ± 7
Protein	14.0 ± 6.0	17.4* ± 0.8	18.6* ± 2.2
Frontal cortex			
Weight	417 ± 18	421 ± 24	432 ± 17
Protein	34.0 ± 1.6	37.4 ± 2.0	37.3 ± 1.0

Note. Protein content and weight are expressed as mg per region ± SE.

* Value differs from zero-dose value ($p < 0.05$, Fisher's least significant difference test). Adult male rats receiving dietary chlordecone as described under Methods. Six to eight animals were used in each group.

tonin and diazepam binding and cerebellar muscimol and QNB binding values were similar in control and experimental animals, and no significant differences were noted.

DISCUSSION

The increased concentration of membrane protein in adult rats exposed chronically to chlordecone was region specific. The finding that the rate of DNA synthesis in rat striatum is selectively increased (Messing *et al.*, 1978) by morphine administration suggests that localized glial proliferation can be induced in this region. The increased protein content that we are reporting may be related to the anabolic activity of this compound which has been demonstrated in the chick oviduct (Palmiter and Mulvihill, 1978). This latter result was attributed both directly to the estrogenic activity of chlordecone and to a secondary effect, mediated by increased levels of progesterone. The amygdala have been shown to bind labeled corticosterone and estradiol to a greater extent than does the cortex (McEwen, 1980). Chlordecone also caused significant receptor changes within the cerebellum. This brain region has estradiol and testosterone binding capacity (Fox, 1980) but little corticosterone binding is found in the cerebellum (McEwen, 1980). Since steroids bind heterogeneously to brain regions, this may in fact account for our finding of effects in the striatum and cerebellum but not cortex. The reversibility of effects could be explained in terms of loss of anabolic stimulation after removal of chlordecone from the diet, either directly or by way of restoration of normal levels of circulating hormones. The lack of an anabolic effect of chlordecone in neonatal animals might be due to absence of a critical hormone binding site or to a rapid reversal of excess protein synthesis after removal of the toxicant from the diet at the end of lactation. However, since no alteration of testosterone levels was found in treated rats, the effect is unlikely to be mediated by changes in the level of this anabolic hormone.

Chronic exposure of adult rats to chlordecone produced pronounced, but apparently reversible, effects of membrane protein content and transmitter–receptor sites in several brain regions. Chang-Tsui and Ho (1980) have also suggested that one of the actions of chlordecone in the central nervous system is on neurotransmitter and receptor interaction. Gestational and lactational dosing with this agent caused modifications in dopamine receptor binding intensity that were detectable 18 days after cessation of maternal dosing. These changes were in the opposite direction to those caused by dosing of adult rats. A tendency of a toxic agent to cause opposite receptor changes in the brain of gestationally or neonatally exposed animals relative to adult exposure has also been observed in the case of acrylamide (Agrawal, *et al.*, 1981, Agrawal and Squibb, 1980). These data confirm the general concept of the distinctive susceptibility of young animals to toxicants and the possible tendency for early toxic insult to be less

TABLE 3

EFFECT OF GESTATIONAL AND NEONATAL KEPONE EXPOSURE ON REGIONAL HIGH-AFFINITY BINDING SITES IN THE BRAIN

		Chlordecone dose to mothers (ppm)					
		Male			Female		
Region	Receptor	0	1	6	0	1	6
Cerebellum	GABA	229 ± 21	254 ± 8	267 ± 14	270 ± 10	258 ± 18	286 ± 27
Cerebellum	Muscarinic	71 ± 2	80 ± 2	76 ± 4	77 ± 3	82 ± 3	75 ± 1
Frontal cortex	Serotonin	137 ± 12	135 ± 9	151 ± 11	146 ± 12	151 ± 11	139 ± 17
Frontal cortex	Diazepam	70 ± 14	63 ± 14	90 ± 15	66 ± 10	79 ± 15	72 ± 15
Striatum	Dopamine	172 ± 13	168 ± 2	219 ± 8*	203 ± 14	192 ± 18	231 ± 15

Note. Binding is expressed as pmol/g protein ± SE.

* Value differs from zero-dose value ($p < 0.05$, Fisher's least significant difference test). Six to eight animals were used in each group. For details, see text.

reversible than the corresponding phenomenon in the adult. This is in contrast to the plasticity of the young brain as evidenced by its adaptibility to major neurological trauma such as infarction of the dominant cerebral hemisphere. The sensitivity of the immature brain to environmental agents acting over a long period of time may be greater than its susceptibility to acute damage by stroke or other anoxic episodes.

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