Distribution of Lindane in Brains of Control and Phenobarbital Pretreated Dogs at the Onset of Lindane-Induced Convulsions[1]

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Hexachlorocyclohexane (HCH) is a chlorinated hydrocarbon pesticide composed of five structurally similar isomers. The pharmacological properties of the individual isomers were worked out as early as 1948 (MCNAMARA & KROP, 1948), and both the oral and parenteral toxicities of most isomers were investigated later (VAN ASPEREN, 1954). The γ isomer of HCH is a widely used insecticide marketed under the trade name of Lindane. This chemical exhibits much of the same pharmacological and toxicological properties as does DDT, dieldrin and other members of the organochlorine group of insecticides. One of these toxic properties is the ability of high doses to produce signs of central nervous system (CNS) hyperexcitability in animals (ST. OMER, 1971). This CNS stimulation is characteristic and predictable enough with Lindane so that it has been used experimentally to induce convulsions in a manner similar to the use of pentylenetetrazol and electroshock. Little information however is actually available about the manner in which Lindane brings about the CNS excitation or the series of events triggering the convulsion. Although the distribution of Lindane in the CNS has been determined 24 hours after administration (KORANSKY & ULLBERG, 1964), little is known about the distribution at the time of the convulsion. It is known, however, that barbiturates can be used to successfully combat Lindane-induced convulsions. Chronic exposure to an agent like phenobarbital prevents the convulsion from occurring and acute exposure to an agent like amobarbital will stop a convulsion that has already started. The present investigation was undertaken in an attempt to determine the uptake and distribution of Lindane in the brain of dogs at the onset of convulsions elicited by Lindane and to ascertain if chronic pretreatment with phenobarbital altered this distribution.

Methods

A total of 8 male purebred beagle dogs weighing 8-11 kg were used in this study. Four dogs received 35 mg/kg/day of phenobarbital-sodium (Pb) in their chow for 60 days. Four other dogs received normal chow. At the end of the 60 day pretreatment period all dogs received an infusion through the saphenous vein of a Lindane emulsion (2 gm lecithin, 1.5 gm lindane, 15 cc peanut oil, 85 cc saline) at a rate of 7.5 mg of lindane per minute. Blood for analysis of Lindane was drawn from the jugular vein approximately every 5 minutes during the infusion. Control dogs were infused until they convulsed and phenobarbital treated dogs were infused for 60-70 minutes before the infusion was terminated due to lack of signs of CNS excitation. At the end of the infusion period,

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blood was drawn from the jugular vein and the animal killed by electroshock. The brain was immediately removed and dissected into 12 discrete regions which were frozen (-20°) until analysis. The amount of Lindane present in brain samples was determined by homogenizing the sample in two volumes of 25% methyl alcohol-water (MeOH) and extracting the homogenate three times, each with 25 ml of hexane. Blood samples were diluted with 7 volumes of 50% MeOH-water prior to hexane extraction. The amount of Lindane present in the hexane extract was determined using a gas chromatograph equipped with an electron capture detector and a 2 m glass column packed with 3% DC 200 on 100/120 Gas Chrom Q. The chromatograph was operated at 200° with a nitrogen flow rate of 125 ml/minute. Retention time of an analytical standard of Lindane under these conditions was 2-1/2 minutes and recovery of Lindane added to brain tissue was in excess of 95%. Differences between control and phenobarbital-treated dogs were analyzed using a standard two-tailed Student's t test.

Results

Table 1 shows convulsion parameters from control and phenobarbital-treated dogs. Control dogs convulsed with a mean time of 27 minutes while phenobarbital dogs had not convulsed even after 60-70 minutes of Lindane infusion. Phenobarbital animals had a slightly, but significantly, greater concentration of Lindane in their blood, even though the blood levels of Lindane were equal in the two groups throughout the first 24 minutes of the infusion.

TABLE 1

Convulsion Parameters from Phenobarbital Treated and Control Dogs Infused with Lindane[a]

	CONTROL	PHENOBARBITAL
Time to Convulse (Min.)	27.0 ± 5.7	65.0 ± 5.0 [b]
Total Lindane Administered (Mg)	202.0 ±35.0	488.0 ±31.0 [b]
Total Dose (Mg/Kg)	2.4 ± 0.2	4.3 ± 0.5 [b]
Lindane Blood Level (μgm/ml)	0.49± 0.02	0.62± 0.03 [b]

[a] Data are the Mean ± S.D. of 4 dogs.

[b] Values from phenobarbital treated animals differed significantly from control values at $P \leq 0.05$.

Table 2 shows the distribution of recovered Lindane in the 12 brain structures. Predominantly grey matter structures contain 3-5% of the total recovered Lindane whereas mixed white plus grey structures contain 15-20% of the total. Medulla and occipital cortex from phenobarbital-treated dogs both have more recovered Lindane than the same structures from control dogs and the pons from treated dogs has less Lindane than the pons from control animals.

TABLE 2

Distribution of Lindane in Brain Structures from Phenobarbital and Control Dogs[a]

	CONTROL	PHENOBARBITAL
Parietal Cortex	6.9±0.2	7.3±0.2
Occipital Cortex	2.5±0.4	5.1±1.2[b]
Motor Cortex	4.2±0.9	5.2±1.1
Prefrontal Cortex	4.6±0.6	4.4±0.1
Superior Colliculus	3.1±0.8	2.9±0.8
Inferior Colliculus	2.8±0.4	2.9±0.7
Caudate Nucleus	6.3±1.2	5.3±0.5
Hippocampus	3.5±0.8	4.3±0.4
Pons	18.5±0.8	15.5±1.0[b]
Medulla	12.3±1.9	20.1±4.9[b]
Cerebellum	14.3±2.3	12.3±1.2
Vermis	17.7±6.0	16.2±3.9

[a]Data are reported as percent of recovered Lindane and represent the Mean ± S.D. of 4 dogs. The weight of each brain structure was equal for phenobarbital and control dogs except for motor cortex and medulla, where the tissue from phenobarbital dogs was significantly heavier ($P \leq 0.05$) than from control dogs. Drug treatment described in Methods.

[b]Phenobarbital-treated dogs differed significanlty from control dogs at $P \leq 0.05$.

Table 3 shows the tissue-to-blood (T:B) ratio for Lindane in the 12 brains structures. Control dogs had 5-12 times more Lindane in brain tissue than in blood at the time of convulsion. Phenobarbital-treated dogs also had considerably more Lindane in brain than in blood at the termination of the infusion. However, the T:B ratio for phenobarbital-treated dogs was less than that for control dogs in half of the areas studied. If the amount of Lindane recovered in each of the 12 brain structures is expressed as a ratio of the total Lindane administered to the animal rather than as a ratio of the total Lindane recovered, then significanlty less Lindane is recovered in all brain areas from phenobarbital-treated dogs than from controls. This fact is evident by the greatly increased amount of Lindane administered to the phenobarbital group (Table 1) and the nearly equal concentration of Lindane which were seen in brain structures from both phenobarbital and control dogs.

Although not presented in tabular form, the concentration of Lindane was approximately equal in all brain structures and no difference in concentration was seen between control and phenobarbital treated dogs.

TABLE 3

Tissue:Blood Ratio of Lindane in Brain Structures from Phenobarbital and Control Dogs[a]

	CONTROL	PHENOBARBITAL
Parietal Cortex	7.21± .80	4.86±.59[b]
Occipital Cortex	7.05± .46	5.88±.23[b]
Motor Cortex	6.97± .33	5.29±.20[b]
Prefrontal Cortex	6.35± .69	5.37±.57
Superior Colliculus	9.97± .64	8.09±.64[b]
Inferior Colliculus	9.54± .69	9.36±.97
Caudate Nucleus	5.13± .73	4.73±.69
Hippocampus	5.64± .35	6.52±.57
Pons	12.61±1.16	9.00±.76[b]
Medulla	6.22± .68	6.82±.24
Cerebellum	6.82± .35	5.52±.34[b]
Vermis	5.74± .77	5.31±.45

[a] Data are expressed as the ratio of the concentration of Lindane in tissue to the concentration of Lindane in blood x 100 and represent the Mean ± S.D. of 4 dogs. Drug treatment as described in Methods.

[b] Phenobarbital treated dogs differed significantly from control dogs at $P \leq 0.05$.

Discussion

Although both treatment groups have similar blood levels of Lindane (Table 1), the phenobarbital-pretreated group received twice as much Lindane as did the control group. This excess Lindane could perhaps be accounted for by a difference in quantitative rates of drug handling by the two experimental groups. Lindane is known to be metabolized by the same mixed function oxidases that are induced by phenobarbital treatment (KORANSKY et al., 1964). An increase in this enzyme activity brought about by phenobarbital could possibly act to remove some of the additional Lindane from the circulation of phenobarbital-treated dogs by transforming it into a more polar product that could be readily excreted into the urine. FREAL & CHADWICK (1973) have shown that less than 40% of an administer dose of Lindane is excreted in the urine 24 hours after treatment and it therefore appears unlikely that a significant portion of Lindane in the present study is passed into the urine during the 60 minutes of the infusion. Another possibility however, might be the induction by phenobarbital of the biliary transport mechanism (KLAASSEN, 1961). Lindane is known to be excreted to a significant degree by this mechanism, particularly following treatment with inducing agents (CHADWICK & FREAL, 1972). Although the phenobarbital-induced increase in the activity of these two routes of clearance of Lindane from the circulation could partially explain the similar blood levels after more than twice as much

Lindane had been administered to the phenobarbital dogs, it is most likely that the excess Lindane is merely being sequestered in fat stores. Even though NAKAJIMA et al. (1970) and DAVIDOW & FRAWLEY (1951) have shown little fat accumulation of Lindane under experimental conditions quite different from our own, a wealth of data exists to document the storage of chlorinated hydrocarbons in fat at early times after exposure.

The ability of phenobarbital pretreatment to prevent convulsions at a dose, blood level, and brain level of Lindane which convulses non-pretreated dogs suggests that phenobarbital treatment may have in some way altered the sensitivity of the brain to the convulsive effect of Lindane. Whether phenobarbital actually alters the sensitivity of a Lindane-sensitive receptor, stabilizes pools of chemicals released by Lindane (ST. OMER, 1971), competes for Lindane binding sites in the brain, or chemically antagonizes the effect of Lindane is unknown. The ability of phenobarbital to protect against human grand mal seizures is well known (GOODMAN & GILMAN, 1970) and the mechanisms of protection against Lindane-induced seizures may be similar. The lower T:B ratio, however, seen in several brain structures from the convulsing relative to non-convulsing dogs (Table 3) is of interest in this regard. The implication of this particular finding is that phenobarbital pretreatment decreases the uptake of Lindane into some brain structures. This could be accomplished by a phenobarbital-produced alteration in the permeability of capillaries at the brain:capillary interface, a mechanism reported to be operating in some instances of altered blood-brain barrier permeability (BECKER & AIRD, 1955). Alternatively, phenobarbital may be increasing the speed with which a more polar Lindane metabolite (FREAL & CHADWICK, 1973) is removed from the brain via the choroid plexus into the blood. This mechanism of drug action has been demonstrated for the effect of probenecid on brain levels of homovanillic acid (WERDINIUS, 1966) and 5-hydroxyindoleacetic acid (GULDBERG et al., 1966). Whether one or both of these mechanisms are working to alter the response of phenobarbital-treated dogs to the administration of Lindane is unknown and warrants further study.

The difference in distribution between structures composed of mixed white plus grey matter as opposed to exclusively grey matter (Table 3) is consistent with the findings of KORANSKY & ULLBERG (1964). These authors showed that 24 hours after a single dose of ^{14}C-lindane to rats the radioactivity was found almost exclusively in brain structures containing white matter. Our present finding may merely be an early indication of a differential distribution that may occur at later times. In addition, Lindane is very lipophilic and consequently would tend to localize in tissues with a high lipid content. Because white matter has 3-4 times more lipid than does grey matter (MORRISON, 1971), the distribution of Lindane in favor of white matter-containing structures is not surprising.

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