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Toxicokinetics of Ro 5-4864, Lindane and Picrotoxin Compared[1]

H. LYNN DRUMMER AND DOROTHY E. WOOLLEY[2]

Department of Animal Physiology, University of California, Davis, CA 95616

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DRUMMER, H L AND D E WOOLLEY *Toxicokinetics of Ro 5-4864, lindane and picrotoxin compared* PHARMACOL BIOCHEM BEHAV **38**(2) 235–242, 1991 —The effects produced by IP administration of these three agents in the rat were compared because of in vitro evidence that each modulates the picrotoxinin site of the $GABA_A$ receptor For each, hypothermia had the lowest threshold and convulsions the next, with hypophagia produced only by the highest dose of either Ro 5-4864 or lindane Convulsant effects had a shorter latency and a shorter duration than did hypothermia. Hypophagia, when present, lasted the longest. Myoclonus was the seizure type with the lowest threshold for all three agents At the highest dose, lindane produced a high incidence of maximal clonic (hopping) seizures, whereas Ro 5-4864 and picrotoxin produced a high incidence of maximal tonic seizures instead On a mole/kg basis, picrotoxin was 40 times more effective than the other two agents and produced seizures which started later, peaked later, and persisted longest Ro 5-4864 and lindane were effective at equimolar concentrations and, in combination, produced effects which suggested either dose-addition or synergism The data are consistent with the hypothesis that the toxic effects of both Ro 5-4864 and lindane may be attributable, at least in part, to an action at a subpopulation of $GABA_A$ receptors

Food intake $GABA_A$ receptor Hypothermia Insecticide Lindane Myoclonus Peripheral benzodiazepine Picrotoxin Ro 5-4864 Temperature regulation Toxicokinetics Seizures

THE $GABA_A$ receptor is a hetero-oligomer, ligand-gated chloride channel with binding sites for GABA, benzodiazepines (BDs), barbiturates, and picrotoxinin (PTX) (30). These sites allow complex allosteric interactions by a wide variety of therapeutic and toxic agents. In vitro evidence suggests that the PTX site, which is a channel gating site of the $GABA_A$ receptor, may play a key role in the modulation of GABAergic mechanisms by a wide variety of agents, including the atypical benzodiazepine Ro 5-4864 and some convulsant insecticides. Insecticides that bind to the PTX receptor (1, 22, 24) and inhibit GABA-stimulated chloride flux (2, 5, 14, 18, 29) include lindane (the gamma isomer of hexachlorocyclohexane), pyrethroids, and convulsant cyclodienes. Ro 5-4864, at micromolar levels, modulates binding at the PTX site (16, 43, 46) and inhibits GABA-stimulated chloride flux (28) The BD site of the $GABA_A$ receptor binds clonazepam with high affinity and is termed the central BD (cBD) receptor

This laboratory has examined evidence in vivo that suggests that several of the toxic effects of lindane may be attributed to anti-GABAergic effects Lindane produces hypophagia and hypothermia in addition to convulsions (20, 48–50). Agents that enhance GABAergic activity, such as clonazepam and diazepam, benzodiazepines which act at the cBD site, and phenobarbital, which may act at the barbiturate site, effectively antagonized all of these effects of lindane (20, 49, 50). By contrast, Ro 5-4864 exacerbated lindane-induced hypophagia, hypothermia, seizures and death, even though, by itself, the dose of Ro 5-4864 had no effect on the endpoints measured (20)

The convulsant effect of Ro 5-4864 has been attributed to an action at the PTX receptor by some authors (37,46), and Ro 5-4864-induced seizures, like those induced by lindane, are antagonized by diazepam, clonazepam, and barbiturates (37). However, at low nanomolar levels, Ro 5-4864 binds to a site on the mitochondrial membrane, i.e., the "peripheral" BD (pBD) receptor (45). Since a dose of Ro 5-4864 which binds at the PTX site will presumably have saturated pBD sites, the proconvulsant effect of Ro 5-4864 upon lindane-induced seizures (20) may have been due to an effect at the pBD receptor, rather than through an action at the PTX receptor. In fact, others have attributed the convulsant and proconvulsant effects of Ro 5-4864 to an action at the pBD site (4, 9, 10)

If Ro 5-4864 exacerbated the effects of lindane (20) because both bind to the PTX site, then Ro 5-4864 should have effects similar to those of lindane and picrotoxin However, if Ro 5-4864 exacerbated the toxic effects of lindane through an action at the pBD site, then there may be differences in the profiles of the toxic effects of lindane and Ro 5-4864 compared with those of picrotoxin, since picrotoxin does not bind to the pBD site (36,40) Picrotoxin, in addition to its well-known property of initiating convulsions, reduces food intake (21) and produces hypothermia (23,38). Ro 5-4864 produces seizures [e.g., (37,46)] and reduces food intake (8). However, prior to our preliminary studies (47), Ro 5-4864 had not been reported to exert an effect upon temperature regulation.

These comparisons of the in vivo effects support the in vitro

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[2]Requests for reprints should be addressed to Dr Dorothy E Woolley

evidence and suggest that Ro 5-4864, lindane and picrotoxin may produce some of their toxic effects through a common mode of action Further, if the primary action is upon the same receptor site within the same target tissue, e.g., certain neurons within the CNS, then these agents should also produce a similar order of thresholds and temporal sequence for the toxic effects. However, it is important to note that, unlike the case of lindane (20,48), there are no dose-response studies in the literature for either picrotoxin or Ro 5-4864 which compare thresholds and time courses for hypothermia, convulsions and hypophagia.

The purpose of the present study, therefore, was two-fold 1) to compare the toxicokinetics of the effects of Ro 5-4864, lindane and picrotoxin in vivo, using seizures, food intake and core temperature as endpoints, and 2) to determine if the effects of Ro 5-4864 and lindane are additive, as would be expected if they act at the same site

METHOD

Adult female Sprague-Dawley rats (Charles River, Portage, MI) were housed individually in stainless steel hanging cages in a temperature- (22–23°C) and light- (14.10 h light:dark cycle) controlled room Rats were allowed free access to water and ground rodent chow (Purina 5001; St. Louis, MO), except when food was removed the evening prior to administration of drugs.

Lindane, picrotoxin (Sigma Chemical Co., St. Louis, MO) and Ro 5-4864 (Hoffmann-La Roche Inc., Nutley, NJ) were dissolved in dimethylsulfoxide (DMSO; Crown Zellerbach Corp , Camas, WA) and administered IP in a volume of 0.5 ml/kg body weight When rats received both Ro 5-4864 and lindane, the injection volume was 0 25 ml/kg to keep the total volume of vehicle per unit body weight the same. Controls received the vehicle only.

Prior to testing, food intake, body weight, and colonic temperatures were measured daily for several days to assure that these were stable, according to procedures described previously (20, 48, 50). After overnight fasting, colonic temperatures and body weights were measured prior to dosing. Following administration of drugs or vehicle, rats were observed for the appearance of abnormal behaviors and convulsions. Food was returned at 2 h and collection of food intake data was begun 4 h after dosing, was collected at 2-h intervals through the first 2 h following lights out at 10 h after dosing, and then for the balance of the overnight period

For the dose-response studies of lindane and Ro 5-4864, 30 rats weighing 330 ± 5 g (mean $\pm$ S E.M) and 326 ± 5 g, respectively, were divided into 4 groups of 7–8 rats each After the initial measurements of colonic temperature and body weight on the morning after the overnight fast, rats were injected with 0, 5, 10, or 20 mg/kg of either lindane or Ro 5-4864. For the dose-response study of picrotoxin, 22 Sprague-Dawley rats weighing 356 ± 4 g were divided into 4 groups of 5 or 6 rats each, and were injected with 0, 0.5, 1, or 2 mg/kg picrotoxin For the study of the interactions between lindane and Ro 5-4864, 31 rats weighing 286 ± 3 g were divided into 4 groups of 7 or 8 rats 2 groups received 10 mg/kg of either Ro 5-4864 or lindane, one group received 10 mg/kg of each of the 2 drugs for a combined dose of 20 mg/kg, and one group (controls) received only the vehicle Ro 5-4864 was administered 20 min before lindane, and all animals not receiving one or both drugs received the vehicle at the appropriate times.

The significance of differences between means of treated and control groups was determined after analysis of variance (ANOVA) when the overall F ratio was significant at the $p<0.05$ level Fisher's Least Significant Difference (LSD) test was used as the post hoc test and the level of significance was corrected for the numbers of comparisons by the Bonferroni method (27)

RESULTS

Behavioral Effects (Table 1, Figs. 1 and 2)

All three agents produced a variety of signs of toxicity, such as vacuous chewing, periods of rapid respiration, piloerection, vocalization (both spontaneous and upon handling), and periods of prolonged quiet standing and staring. No attempt was made to quantitate these minimal signs of toxicity. In addition, all three agents produced excessive salivation in those rats with severe seizures. Ro 5-4864, lindane and picrotoxin produced an abnormal prone posture characterized by the rat lying immobile with chin resting on the cage bottom and limbs frequently protruding through the wire cage bottom (Table 1) This posture was easily differentiated from postseizure depression during which a rat lies on its side. Upon handling, the rats were found to be hypotonic or even flaccid

All 3 agents produced a similar spectrum of seizures, including myoclonus and clonic and tonic seizures (Table 1) Myoclonus, i e., head or whole body jerks, usually single, was the seizure type with the lowest threshold Progressively more severe seizures included, in order minimal clonus with rhythmic movements of the forelimbs, head or jaw, minimal tonic seizures consisting of tonic contractions of head, neck and trunk muscles, torsion of the trunk and usually brief loss of righting reflex [also see (20) for more complete description], hopping seizures (maximal clonus) with bilateral, synchronous fore- and hindlimb clonus which propelled the animal upward; and maximal tonic seizures, characterized by the presence of tonic limb flexion or extension.

Convulsions were not observed in the groups receiving the lowest doses of Ro 5-4864 and picrotoxin, and only 1 rat had a single myoclonic jerk in the group receiving the lowest dose of lindane. At the highest doses, multiple episodes of myoclonus were a prominent feature of the seizures produced by all three drugs. Additionally, at the highest dose, lindane produced a high incidence of hopping seizures (75%), whereas Ro 5-4864 produced a higher incidence of maximal tonic seizures (57%) than of hopping seizures (29%) Maximal tonic seizures were produced by both the middle and high doses (40% and 60%, respectively) of picrotoxin, but only the middle dose produced hopping seizures (20%) (Table 1). Thus, at the highest doses, lindane was more effective in producing severe hopping seizures than in producing maximal tonic seizures, whereas the reverse was true for picrotoxin and Ro 5-4864 Only one death occurred in all three dose-response studies, and this was at 11 min in the group receiving the highest dose of lindane

The time course for seizures was remarkably similar for Ro 5-4864 and lindane compared with that for picrotoxin (Fig 1) Peak convulsant effects of all three doses of lindane and of the two highest doses of Ro 5-4864 occurred within the first 15 min and no seizures were recorded for either drug after 30 min. By contrast, the incidence of seizures produced by picrotoxin peaked between 16–45 min and persisted throughout the first h after administration. Thus seizures produced by picrotoxin had a longer latency, peaked later, and persisted longer than those produced by the other two drugs

Seizures and other behavioral signs of toxicity produced by the combination of 10 mg/kg each of lindane and Ro 5-4864 were similar to those produced by 20 mg/kg of either drug given alone (Table 1 and Figs 1 and 2). With the combination of the two agents, the incidence of myoclonus was slightly greater and of minimal tonic seizures was approximately equal to that seen with

TABLE 1

PERCENT OF RATS EXHIBITING SELECTED ABNORMAL BEHAVIORS*

Drug (mg/kg)	Prone	Seizures (Any Type)	Myoclonus	Min Clonic	Min Tonic	Hopping (Max Clon)	Max Tonic
Picrotoxin							
0 5	17						
1	40	80	60	60	40	20	40
2	40	100	100	80	100		60
Ro 5-4864							
5							
10	13	26	26	13	13		
20	43	71	71	57	57	29	57
Lindane							
5	29	14	14				
10	29	29	29	29	14	14	
20	63	88	63	38	50	75	13
Combination†							
Ro 5-4864 (R)	50	13	13				
Lindane (L)	29	29	14	14	29		
R + L	63	100	88	63	50	75	50

*Each rat was scored once for each category with the result expressed as a % of the group See the text for explanation of prone and definition of seizure types

†Ro 5-4864 and lindane were administered in a dose of 10 mg/kg singly or in combination (R + L) for a total dose of 20 mg/kg

20 mg/kg of either drug administered alone. On the other hand, the incidence of hopping seizures was equal to that produced by 20 mg/kg of lindane, and the incidence of maximal tonic seizures was similar to that induced by 20 mg/kg of Ro 5-4864, whereas the combination should have produced approximately 52% hopping [(75 + 29)/2] and 35% tonic [(57 + 13)/2] seizures if the effects were additive, rather than synergistic (Table 1) Thus the combination appeared to produce additive effects on myoclonus and minimal tonic seizures and synergistic effects on maximal seizures.

Peak convulsant activity for the drug combination occurred during the first 15 min, like that produced by the equivalent dose of 20 mg/kg of either drug administered alone (Figs. 1 and 2) The combination, however, produced seizures which persisted longer than did those produced by any dose of either drug alone, i.e , throughout the 1-h period of observation rather than for only 30 min as in the dose-response studies (Figs. 1 and 2). The drug combination produced two deaths, one at 16 min and another at 48 min (Fig 2), compared with only one death produced by 20 mg/kg of either drug (lindane) alone (Fig. 1).

Hypothermic Effects (Figs. 3 and 4)

All three doses of either lindane or picrotoxin and the two highest doses of Ro 5-4864 produced significant hypothermia. The lowest dose of picrotoxin produced hypothermia only after a 15-min delay For all three agents, the maximal decrease occurred at 60 min after administration of the highest dose, whereas the maximal decrease occurred earlier at lower doses. At the highest doses, seizures attenuated the drop in temperature at 15 and 30 min, thereby obtunding a dose-response effect at these times and delaying the maximal decrease in temperature to 60 min from 30 min.

At one h, the maximal mean decreases from pretreatment values were 3 2, 2.3 and 1 8 degrees and the percent recovery from peak effect between 1 and 2 h was 47%, 13%, and 33% for the highest dose of picrotoxin, Ro 5-4864, and lindane, respectively Thus, of the 3 agents, the highest dose of picrotoxin produced the greatest decrease in colonic temperature, followed by the most rapid rate of recovery, whereas the rate of recovery was slowest for Ro 5-4864 Colonic temperatures were back to normal by 4 h for all three drugs. At convulsant doses of all three agents, hypothermia peaked later and had a longer duration than did the convulsant effects. The time course and degree of hypothermia were markedly similar for lindane and Ro 5-4864, although, at the lowest dose, lindane was the more effective and, at the highest dose, Ro 5-4864 was the more effective in producing hypothermia The combination of Ro 5-4864 and lindane (Fig. 4) produced hypothermia with a maximal mean decrease from pretreatment values of 1 6 degrees at 1 h, i e., similar to the hypothermia produced by 20 mg/kg of either agent when given alone, and a 19% recovery from peak effect by 2 h, i e , a slope of recovery between those produced by the highest dose of either lindane or Ro 5-4864 alone (Fig. 3).

Effects on Food Intake (Figs. 5–8)

In the dose-response studies (Figs 5–7), only the highest dose of either lindane or Ro 5-4864 decreased food intake, whereas picrotoxin did not produce a significant effect on food intake at any dose. Food intake was significantly reduced at two time periods after drug administration, i.e., the 2–4-h interval and one of the nocturnal periods, i e., either the 10–12- or the 12–24-h interval after drug administration With this food intake protocol, control rats eat maximally during the 2–4-h interval when food is first returned and during the first 2-h interval after lights are turned off which is 10–12 h after drug administration, and eat minimally during the intervening intervals. Thus, unless it is particularly severe, a drug-induced hypophagia will not necessarily be different from control values during the 4–10-h period, but may be reflected in a depression of cumulative food intake. Indeed, the highest dose of both Ro 5-4864 and lindane produced significant depression of food intake at all cumulative intervals examined Thus hypophagia had a higher threshold but, when present, per-

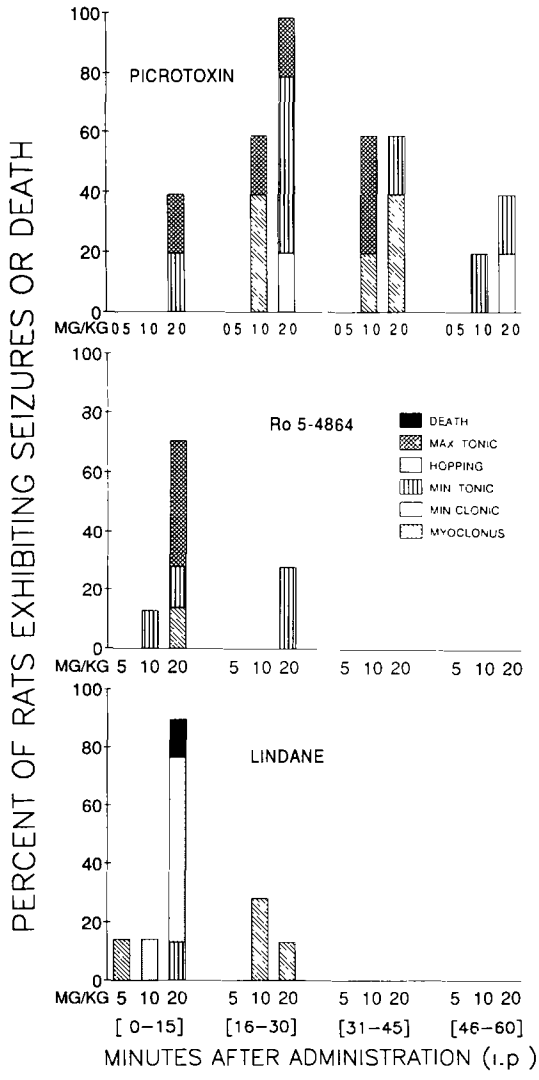

FIG 1 A comparison of the dose-response effects produced by picrotoxin, Ro 5-4864 or lindane on the incidence of seizures or death with time after administration in the female rat Each rat was scored once per 15-min interval for worst behavior and the results expressed as a percent of the group Severity of behavioral effect ranged from myoclonus (least severe) to death

sisted for a longer time than did hypothermia and seizures

The combination of 10 mg/kg each of lindane and Ro 5-4864 resulted in significant depression of food intake in the 2–4-, 10–12-, and 12–24-h intervals following administration of lindane, and in a depression of cumulative food intake at the 2–4-, 2–12-, and 2–24-h periods, with a trend toward reduced food intake at all other cumulative intervals (Fig 8). A comparison of these results with the hypophagic effect of 20 mg/kg of either lindane or Ro 5-4864 reveals the dose-addition effects on this endpoint. In this experiment, the subconvulsant dose of Ro 5-4864 (10 mg/kg) depressed food intake, but only during the 2-h interval after lights went out. This may be related to the high concentration of Ro 5-4864 binding sites in the pineal gland (3,6), an organ involved in circadian regulation

DISCUSSION

The several indices of toxicity produced by IP administration

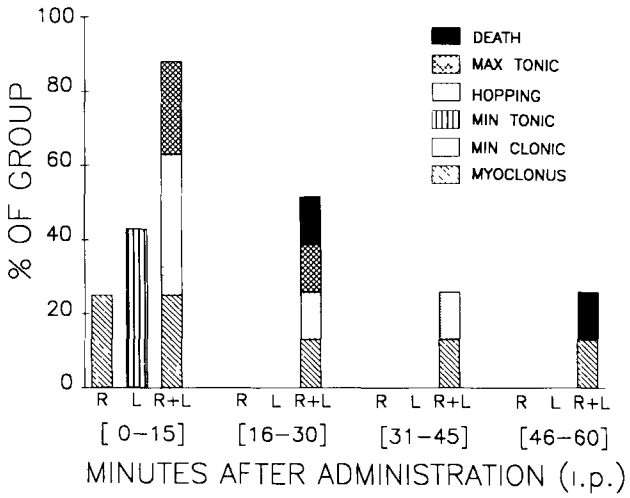

FIG 2 Incidence of seizures or death after administration of Ro 5-4864 (R), lindane (L) or after the combination (R + L) of Ro 5-4864 (− 20 min) and lindane Rats were scored once per 15-min interval as in Fig 1 Each drug was administered in a dose of 10 mg/kg

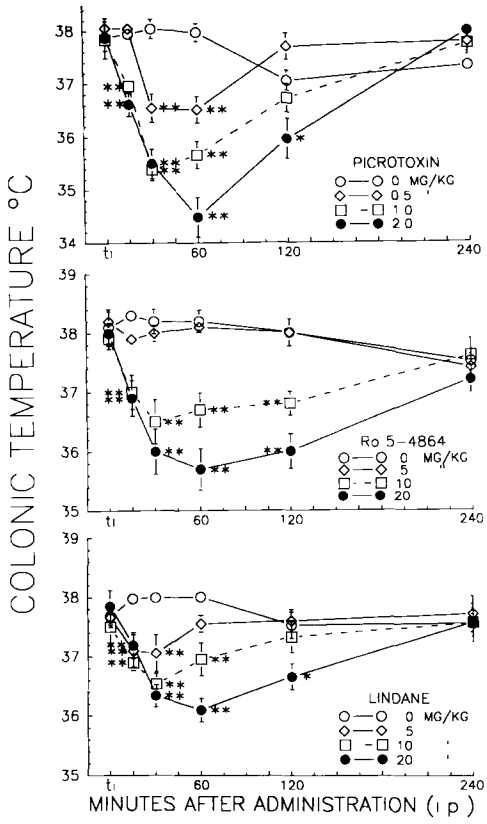

FIG 3 A comparison of the dose-dependent effects produced by picrotoxin, Ro 5-4864 or lindane on colonic temperature with time after administration Pretreatment values are indicated at the initial time (ti) In this and the following figures bracketed vertical lines represent SEMs and asterisks indicate the significance of the differences between the means of treated and control groups (*$p<0.05$, **$p<0.01$)

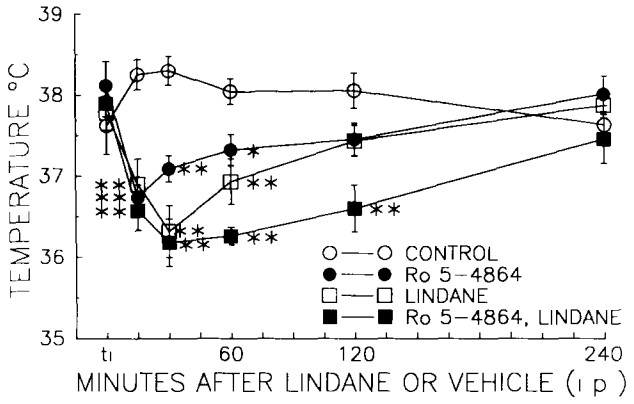

FIG 4 Effects of pretreatment with Ro 5-4864 on lindane-induced hypothermia Drugs were administered as in Fig 2

of Ro 5-4864, lindane, picrotoxin or the combination of lindane and Ro 5-4864 all had the same relative thresholds and time courses Hypothermia and the prone posture had the lowest threshold and convulsions the next, with hypophagia produced only by the highest doses of Ro 5-4864 and lindane in the dose-response study and by the combination of lindane and Ro 5-4864. A lower threshold for hypothermia than for seizures has also been reported for picrotoxin in mice (23) and in rabbits and dogs (38). Seizures began and ended sooner than did either hypothermia or hypophagia, while hypophagia was the most persistent effect seen The

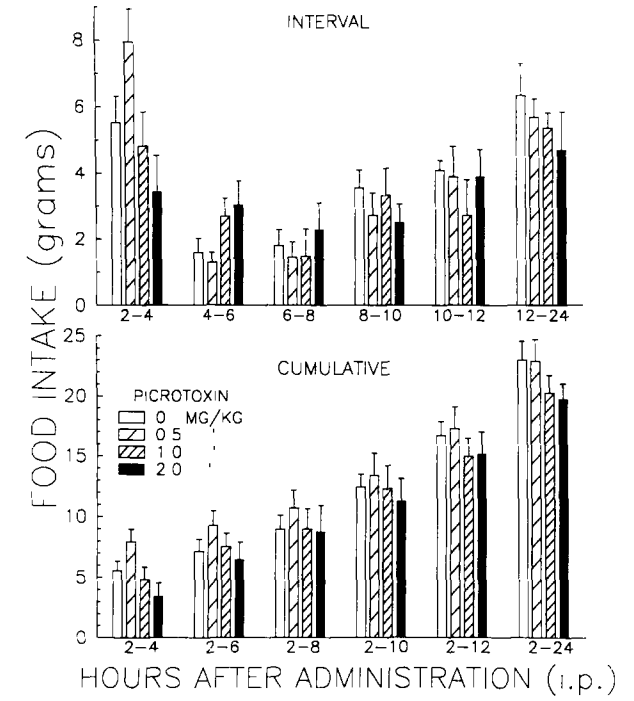

FIG 5 Changes in food intake with time after administration of 3 different doses of picrotoxin to the fasted rat Food was returned at 2 h after dosing and food intake was measured at 2-h intervals through 12 h postdosing and then for the balance of the 24-h period Food intake is presented for the collection intervals (top) and for cumulative periods (bottom)

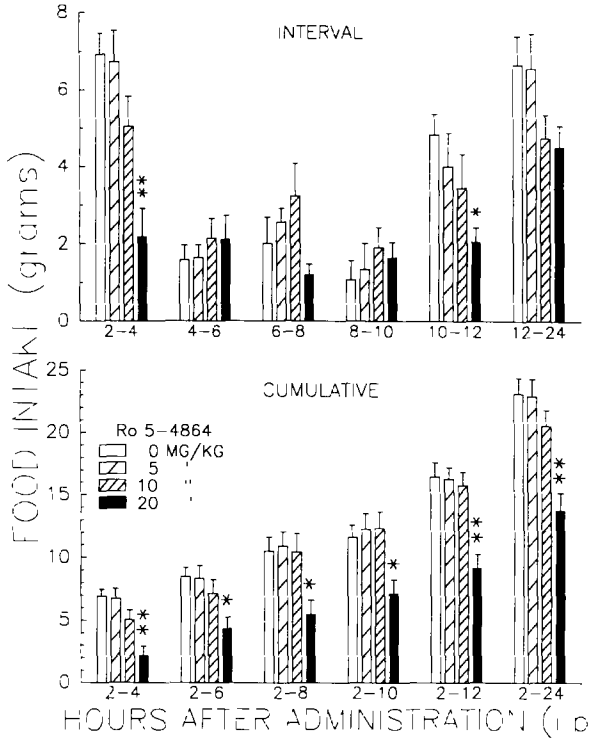

FIG 6 Changes in food intake with time after administration of 3 different doses of Ro 5-4864 Data were collected and are presented as in Fig 5

relative thresholds and temporal sequences reported here for the effects of lindane agree with our previous reports on the effects of IP administration (48) and contrast with our previous findings that hypophagia had a lower threshold than did hypothermia and seizures following PO administration (20, 48–50) This relatively greater effect on food intake when lindane was administered PO rather than IP was tentatively explained by a direct effect of lindane on the gastrointestinal tract, perhaps on GABAergic mechanisms, when it was given PO (48). These findings are consistent with the hypothesis that all three agents produce these effects by antagonism of GABAergic neurotransmission which is the only known pharmacological mechanism which they share in common. However, the fact that seizures, hypothermia, and hypophagia each have a different threshold and time course suggests that different target tissues and/or neuronal circuits are involved

The combination of lindane and Ro 5-4864 (10 mg/kg each) produced additive effects on hypothermia, hypophagia, myoclonus, and minimal tonic seizures In addition, evidence for a synergistic interaction was provided by the much longer duration of seizures and the greater incidence of severe seizures and death produced by the drug combination compared with the highest dose of either in the dose-response studies. In fact, the duration of seizures after administration of the combination of lindane and Ro 5-4864 was remarkably similar to that produced by the highest dose of picrotoxin alone. Similarly, in a previous study from this laboratory we observed that Ro 5-4864 appeared to produce synergistic effects when combined with lindane (20). A basis for such synergistic effects is not yet clear, but may be due to activation of a secondary mechanism.

The abnormal prone posture produced by all three drugs may be related to the periods of quiet standing or motor inactivity that

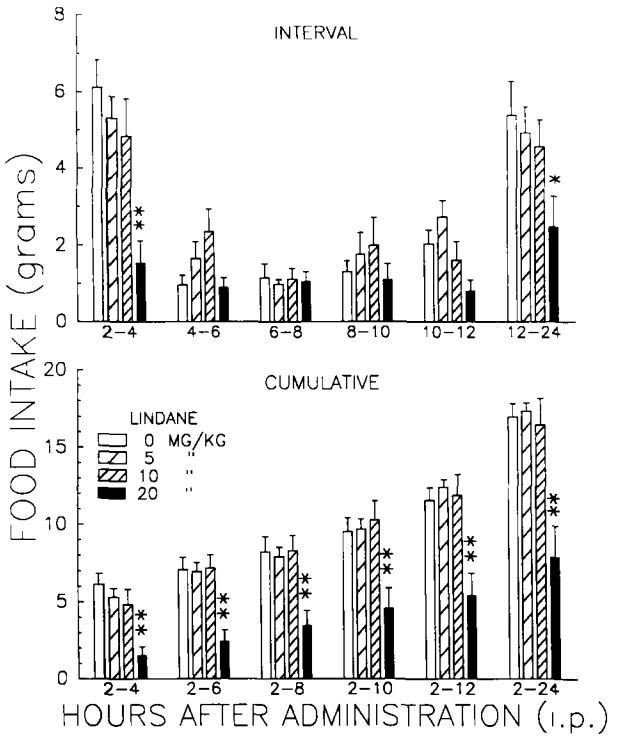

FIG 7 Changes in food intake with time after administration of 3 different doses of lindane Data were collected and are presented as in Fig 5

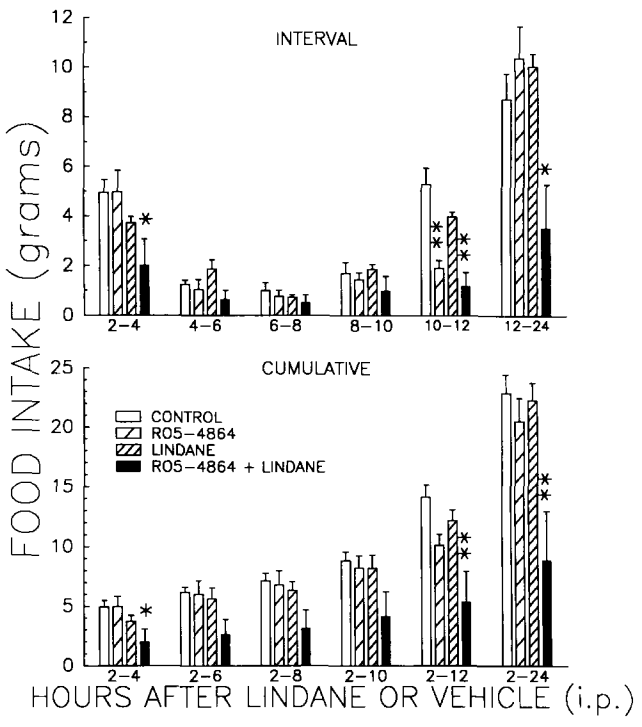

FIG 8 Effects of pretreatment with Ro 5-4864 on lindane-induced hypophagia Drugs were administered as in Fig 2, and data were collected and are presented as in Fig 5

have been reported previously for picrotoxin (31), lindane (20), and Ro 5-4864 (31). A relation is suggested because picrotoxin potentiated both the motor inactivity (31) and, at higher doses, the convulsions produced by Ro 5-4864 (31,37). For all three drugs, myoclonus, the hallmark of organochlorine insecticide poisoning (15,42), had a lower threshold than did clonic or tonic seizures. Myoclonus has been associated primarily with brainstem areas (41), although myoclonus has also been produced by limbic kindling (35) and by administration of the GABA antagonist bicuculline into the dorsal layers of the prepyriform cortex (32,44). The behavioral sequence of limbic automatisms and myoclonus followed by clonic-tonic seizures (32, 35, 44) observed with increasing doses of all three drugs suggests a primary site of action within the limbic forebrain with subsequent activation of the brainstem (13).

Although the most striking and important finding in this study was the marked similarity in the effects of lindane, Ro 5-4864 and picrotoxin, important differences were also observed, both in the absolute thresholds and in the kinetics of the dose-response effects produced by the three agents

At low doses picrotoxin was the most effective of the three agents in decreasing core temperature relative to effectiveness in producing seizures. For example, a 1 5°C decrease in temperature at 30 min was produced by 0 5 mg/kg of picrotoxin and by 10 mg/kg of either lindane or Ro 5-4864, yet, at these doses, picrotoxin was subconvulsant, whereas Ro 5-4864 and lindane produced an incidence of 26% and 29% convulsions, respectively. At one hour, a 2.2 ± 0.5°C decrease in temperature and a seizure incidence of 80 ± 9% was produced by 1 mg/kg of picrotoxin and by 20 mg/kg of either lindane or Ro 5-4864 Lindane and Ro 5-4864 also produced hypophagia at a dose of 20 mg/kg, whereas picrotoxin did not produce hypophagia even at the 2 mg/kg dose that produced a 3 5°C depression in core temperature and 100% seizures. Thus, on a weight/kg basis, picrotoxin was roughly 20 times more effective than lindane or Ro 5-4864 in producing hypothermia and seizures, but was relatively ineffective in producing hypophagia

The molecular weights of Ro 5-4864 and lindane are 320 and 291, respectively. Picrotoxin has a dot structure and is a 1 1 mixture of two dilactones, i.e., picrotoxinin·picrotin. Picrotoxinin, which is the active agent, and picrotin, which is $\frac{1}{100}$th as toxic (33), have molecular weights of 292 and 310, respectively Because picrotoxinin has about the same molecular weight as do lindane and Ro 5-4864, and because it represents approximately one-half of the molecular weight of picrotoxin, picrotoxinin was approximately 40 times more potent on both a mg/kg and a mole/kg basis than were lindane and Ro 5-4864 in producing hypothermia and seizures. Lindane and Ro 5-4864 were about equally effective in producing hypothermia, seizures and hypophagia when administered either as mg/kg or mole/kg body weight Since lindane is considered a potent convulsant, Ro 5-4864 must also be, in agreement with others (46). This contradicts the assumption by some that Ro 5-4864 produces seizures only at high doses (45).

The relative in vivo effectiveness of the drugs was not predicted by in vitro studies. Picrotoxin was approximately as effective as lindane in inhibiting TBPS (t-butylbicyclophosphorothionate) binding at the PTX receptor with IC_{50}s of 0.2 μM and 0.15 μM, respectively, in tests reported by the same laboratory (1), and another laboratory reported that picrotoxinin was approximately as effective as lindane in inhibiting TBPS binding with IC_{50}s of 0.25 μM and 0.4 μM, respectively (7) Further, the similarity in molar effectiveness of lindane and Ro 5-4864 was unexpected since in vitro data suggested that lindane would be approximately 4–5 times more potent than would Ro 5-4864 The

IC_{50} for inhibiting TBPS binding was 0.15 μM for lindane (1) and 0.6 μM for Ro 5-4864 (46) The IC_{50} for inhibiting GABA-stimulated chloride uptake was 1 μM for lindane (2) and 5 μM for Ro 5-4864 (28). Because the in vitro data for the effects of lindane and Ro 5-4864 were collected in different laboratories the relative effectiveness of the drugs may be misleading.

After administration of picrotoxin, the onset of both hypothermia (at the lowest dose) and seizures occurred only after a latency when compared with the effects of lindane and Ro 5-4864. A latency for picrotoxin-induced seizures relative to other convulsant agents is well known (12,23); however, the mechanism for this phenomenon is unclear. Following administration of picrotoxin, the rates of both the decrease in temperature and the recovery from hypothermia were greater than for the other two drugs, although seizures persisted the longest A correspondence between liver concentration and convulsant activity (11) suggests that metabolic activation may be required to separate picrotoxinin and picrotin. Metabolism of picrotoxin could explain both the latency and greater rates of changes following picrotoxin administration. However, neither metabolism nor additional toxicokinetic factors for any of the three drugs can easily explain the difference in relative effectiveness for the various endpoints, i.e., the greater effectiveness of picrotoxin in producing hypothermia and seizures than in producing hypophagia compared with the other two drugs.

Since Ro 5-4864 binds at low nanomolar concentrations both to the pBD site (45) and at midnanomolar concentrations to calmodulin (26), these sites will be saturated before the PTX site is. The affinity of picrotoxin and lindane for calmodulin should be determined Indeed, in plants lindane inhibited calmodulin-dependent Ca^{2+}-ATPase activity (39). Picrotoxin has been reported to be inactive at the pBD site (36,40); if so, the pBD site cannot be the primary site of action of these three drugs. However, the compelling similarity in the effects of lindane and Ro 5-4864 warrants an examination of the affinity of lindane for this site. This is especially true since the pBD site may contribute to the toxicity of other insecticides, i.e , pyrethroids (9, 10, 36), some of which (Type II) also bind to the TBPS site (22), inhibit GABA-stimulated chloride flux (2,5) and cause hypothermia, clonic/tonic seizures and hypersalivation (19), just as lindane and Ro 5-4864 do.

Biochemical, electrophysiological, and molecular biological techniques have shown that there are multiple subtypes of the $GABA_A$ receptor [reviewed in (30)] Regional differences in the expression of the various subunits of the $GABA_A$ receptor (25) may explain the toxicokinetic differences between picrotoxin, lindane, and Ro 5-4864 Indeed, the ability of Ro 5-4864 to modulate GABA-stimulated Cl^- currents allosterically requires a subunit (34) with relatively restricted distribution in the rat brain (25) These findings complement earlier studies of TBPS binding (43,46) and of autoradiography of rat brain (17) which suggested that Ro 5-4864 acts at a subpopulation of PTX sites Electrophysiological studies of the action of lindane also led to the suggestion of a multiplicity of $GABA_A$ receptors (29) Thus the present data provide in vivo support for the hypothesis that the toxic effects of Ro 5-4864 and lindane may be attributable, at least in part, to an interaction at only a subpopulation of $GABA_A$ receptors, whereas picrotoxin may act at all $GABA_A$ receptor subtypes

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