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Increase in Rat Pup Ultrasonic Isolation Calls Induced by Lindane

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Abstract: S. RIVERA, C. SANFELIU, M. GARCÍA, F. COMELLAS AND E. RODRÍGUEZ-FARRÉ. Increase in Rat Pup Ultrasonic Isolation Calls Induced by Lindane. *Neurotoxicology* 13(1):235-240, 1992. *The neurotoxic agent lindane was tested for its ability to alter the rate of ultrasonic isolation calls of suckling rats. Doses that did not produce any sign of convulsant activity significantly increased the number of calls and the cumulative time of calling in male pups. At days 10 - 13 of age after a single dose of 20 mg/kg lindane, animals showed more than twice the control call values. After daily dosing with 10 mg/kg during the first week of age call increases also appeared. It is suggested that lindane has an anxiogenic effect mediated through its action on the benzodiazepine-GABA$_A$ receptor-chloride channel complex.* © 1992 Intox Press, Inc.

Key Words: Ultrasonic Isolation Calls, Distress Calls, Suckling Rats, γ-Hexachlorocyclohexane, Lindane, Anxiety Testing

INTRODUCTION

Mammalian vocalizations are a behavioral measure of emotional expression. One category of vocalization is the isolation call, given by infants when separated from their littermates or parents (Newman, 1988). Young rodents emit isolation calls in the ultrasonic range (Sales and Pye, 1974) which are a potent stimulus for maternal retrieval and care (Smotherman *et al.*, 1974). Calls increase in number and intensity along the 1st week of life to peak during the 2nd week, and abruptly decrease after the eye opening of the rat pup around day 15. This distress response is highly sensitive to environmental stimuli and pharmacological manipulations (Oswalt and Meier, 1975; Winslow and Insel, 1990).

Benzodiazepines (BDZ) are highly effective in decreasing vocalizations, probably via their anxiolytic action (Gardner and Budhram, 1987). Indeed, anxiogenic agents such as pentylenetetrazole (PTZ) selectively increase distress call emission (Insel *et al.*, 1986). Therefore, the GABA$_A$ receptor complex may play a physiological role in this basic emotional behavior.

Lindane (γ-hexachlorocyclohexane) is an hyperstimulant neurotoxic compound that binds to the picrotoxinin site on the chloride channel of the GABA-A receptor (Fishman and Gianutsos, 1988). It has been suggested that this agent induces an anxiogenic response (Hijzen and Slangen, 1989; Llorens *et al.*, 1990). To test whether lindane would mimic anxiogenic agents in their effects on pup distress call behavior the present study was carried out.

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MATERIAL AND METHODS

Animals

Wistar rats (IFFA Credo, France) were used. One day after birth (day 1 of age) pups were randomized among the several litters. Each dam and its litter were individually housed under standard conditions.

Treatment

Animals were administered vehicle (olive oil) or lindane (99.5% pure, Merck) by gavage (0.03ml/10 g body wt). A Silastic-R silicone elastic tubing was used for animals up to 8 days of age and conventional cannulae for older ones. Non-convulsant doses that were reported to produce some effects on motor behavior and cerebral 2-deoxyglucose uptake were used (Rivera *et al.*, 1990; submitted).

Single Dosing. 30 male rat pups aged 10 - 13 days (17 - 25 g body wt) were randomly assigned to the following treatments: vehicle, 10 mg/kg or 20 mg/kg lindane (10 animals/group). Testing was performed 1 hr later. Baseline values obtained in a pretrial performed immediately before administration showed no statistical differences between groups. Animals that failed to emit ultrasounds in this preliminary testing were discarded from the study and replaced.

Repeated Dosing. 12 litters were randomly distributed among 4 daily treatments: vehicle or 10 mg/kg lindane, either during the 1st or the 2nd postnatal week. Only 2 males and 2 females from each litter were used in this study to avoid the litter effect (6 male and 6 female pups/group). Testing was performed on alternate days from the 2nd to the 20th day of age. Body weight was recorded twice a week.

Apparatus and Procedure

Rat pups were individually tested. Litters were previously separated from their mothers and maintained in a cage with clean bedding warmed by infrared light. Each pup was gently put in a glass beaker inside a sound attenuated chamber. Recording of ultrasonic vocalizations started after 0.5 min of habituation time and lasted 2 min for the animals tested once and 3 min for those daily tested.

Vocalizations emitted were detected using a Knowles Electronic (BT1759-146FZ) microphone hanging 10 cm over the animal. Signals were fed through an amplifier (gain 120 dB) with a band-pass filter between18 KHz (6th order) and 150 KHz (2nd order). A level detector digitalized the signals according as they were upper or lower than 2.2 V. This

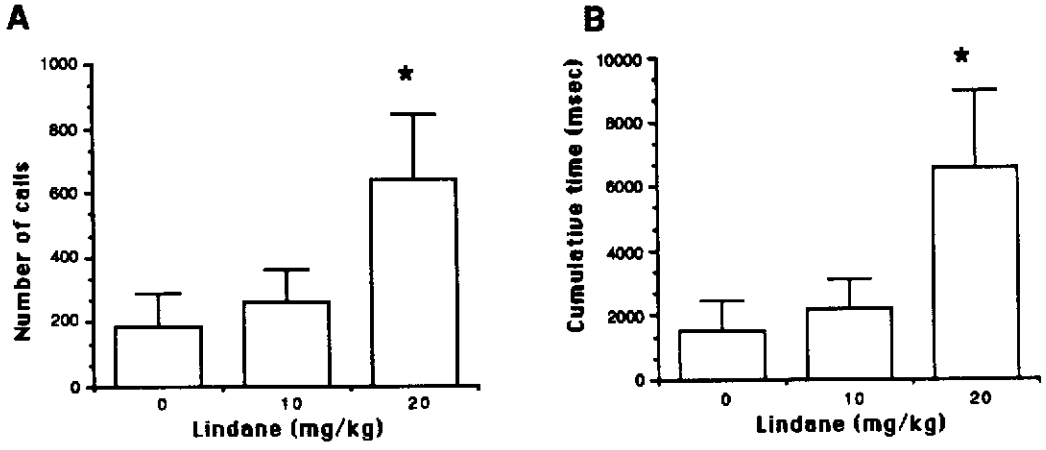

FIG. 1. Changes in ultrasonic distress vocalizations after single administration of lindane. Bars represent the mean ± SE of 10 rat pups of 10 - 13 days of age. A: Total number of calls during 2 min testing; B: Cumulative time of all calling emitted. (*$p < 0.05$).

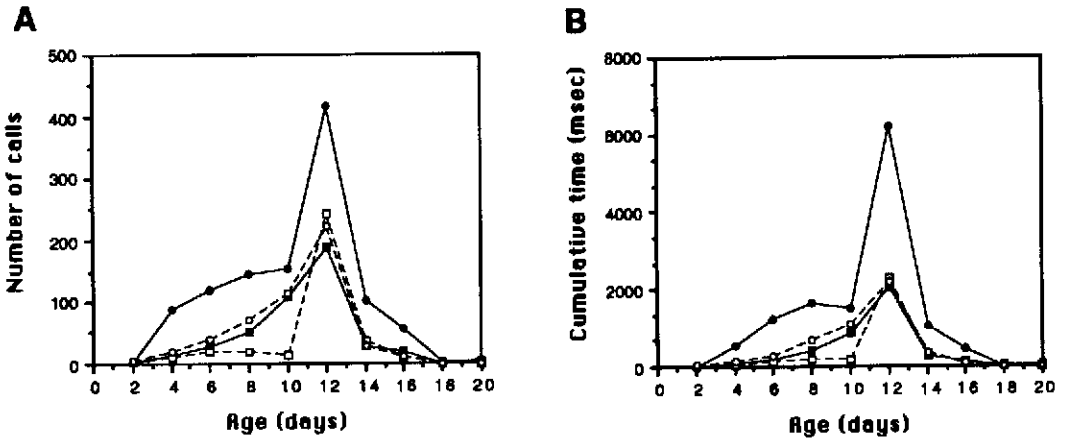

FIG. 2. Changes in ultrasonic distress vocalizations after daily administration of 10 mg/kg lindane during the 1st postnatal week. A: Total number of calls during 3 min testing. B: Cumulative time of calling. Groups are as follows: control males (—■—), lindane-dosed males (—●—), control females (- - -□- - -), and lindane-dosed female rats (- - -○- - -). Points represent the mean of 6 animals. For statistical evaluation, see Results.

voltage corresponded to a sound pressure level of 75 dB. Digital signals were sent to a PC computer through a port and recorded each msec during the whole testing time. Records were processed to obtain the number of calls and the cumulative duration of all calling emitted.

Analysis of Data

One-factor ANOVA followed by Duncan's range test in the single dosing study and three-factor ANOVA (treatment, day and sex) in the repeated dosing experiments were performed.

RESULTS

Single Dose Experiment

Lindane 20 mg/kg increased ($p < 0.05$) the number of emitted isolation calls and their cumulative time, as compared to the control group (Fig. 1).

Repeated Dose Experiments

Animals dosed with lindane 10 mg/kg from day 1 to 7 of age showed a pattern of ultrasound emission different than the controls in the number of calls (ANOVA $F_{(1,228)} = 6.16$, $p = 0.014$) and in the total call time ($F_{(1,228)} = 4.75$, $p = 0.030$). The effect of sex was significant for the number of calls ($F_{(1,228)} = 4.10$, $p = 0.044$) but did not reach statistical significance for the total call time. If data were further analyzed after splitting them according to sex, ANOVA results for the treatment factor effect in males were $F_{(1,109)} = 6.47$, $p = 0.012$ and $F_{(1,109)} = 4.14$, $p = 0.044$, respectively, for the number of calls and calling time. No significant differences were obtained for females (Fig. 2).

In the experiment where rat pups were dosed from day 8 to 14 of age, no statistical significance for treatment or sex effects were obtained (not shown).

The effect of the day of age at testing was highly significant for both parameters in both experiments ($F_{(9,228)} = 9.38$, $p < 0.001$ and $F_{(9,228)} = 6.42$, $p < 0.001$, for the number of calls and total call time, respectively, in the 1st week administered animals; $F_{(8,205)} = 5.98$, $p < 0.001$ and $F_{(8,205)} = 5.43$, $p < 0.001$, for the same parameters in the 2nd week dosing experiment).

Evolution of body weight was similar in control and lindane-treated animals (not shown).

DISCUSSION

The age-related profile of ultrasonic isolation calls obtained in control pups was in gen-

eral agreement with that described in ontogenic studies (Sales and Pye, 1974).

Taken together, the results obtained indicate an increase of ultrasonic distress calls induced by lindane in isolated rat pups. Both parameters measured changed jointly and therefore the mean call duration was not modified by lindane treatment.

After a single dose of 20 mg/kg lindane calls were increased but 10 mg/kg had no effect. Insel *et al.* (1986) have found similar results with pentylenetetrazole (PTZ). Both compounds have been suggested to share common anxiogenic effects in adult rats in the plus-maze test (Llorens *et al.*, 1990). Hijzen and Slangen (1989) have also postulated an anxiogenic action of lindane in adult rats by startle response testing, after obtaining similar results with an inverse benzodiazepine (BDZ) agonist and opposite ones with the anxiolytic midazolam. Therefore, lindane-induced alterations of distress calling may be caused through a BDZ-modulated mechanism. This would be in agreement with the proposed mechanism of lindane action through the GABA system (Fishman and Gianutsos, 1988; Suñol *et al.*, 1989). Indeed, lindane antagonizes the $GABA_A$ receptor in the same way as PTZ does. Thus, the present results may provide further evidence for the role of the BDZ-GABA receptor-chloride channel complex in rodent attachment behavior.

Alterations of the age-related pattern of vocalizations were present at repeated doses of 10 mg/kg lindane when administered during the 1st week of age, but not during the 2nd one. Then, it may be postulated a higher sensitivity of the BDZ-binding site to a lindane-mediated effect during this early period. During the 1st postnatal week, for instance, the concentration of BDZ receptors increase from 20% to 60% of adult levels (Mallorga *et al.*, 1980). Distress isolation calls are the expression of an emotional behavior submitted to a multiple modulation which may include sex-related factors as observed in this study.

In conclusion, the present work supports an anxiogenic action of the neurotoxic agent lindane as suggested by previous experimental and clinical reports (Llorens *et al.*, 1990; Hijzen and Slangen, 1989; Kbare *et al.*, 1977).

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