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Prenatal exposure to fipronil disturbs maternal aggressive behavior in rats

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ABSTRACT

Fipronil is a second-generation phenilpirazol insecticide that is used in agriculture and veterinary medicine for protection against fleas, ticks, ants, cockroaches and other pests. The insecticide blocks the chloride channels associated with the gamma-amino butyric acid (GABA) receptors in mammals and the chloride channels associated with the GABA and glutamate (Glu) receptors in insects. In this study, a commercial product that contain fipronil was administered orally to pregnant Wistar rats at dosages of 0.1, 1.0, or 10.0 mg/kg/day from the 6th to the 20th day of gestation (n = 10 pregnant rats/group) to assess the maternal aggressive behavior (on the 6th day of lactation) and the histopathology of the ovaries and the thyroid gland of the dams. The fipronil caused a disturbance of the maternal aggressive behavior; the aggression against a male intruder decreased at the lowest dose, but increased at the highest dose, without interfering with the general activity of the dams in the open field test at either dose. The histopathological analysis revealed no abnormalities. The differential effects of fipronil behavior appeared to be a consequence of actions on central nervous system areas that control these behaviors. We suggest that fipronil acts on maternal aggressive behavior through $GABA_A$ receptors.

1. Introduction

Fipronil is a second-generation phenilpirazol insecticide that is used in agriculture and veterinary medicine for protection against fleas, ticks, ants, cockroaches and other pests (Ikeda et al. 2004; Mohamed et al. 2004). The mode of action of the pesticide is to inhibit the chloride channels that are coupled to the gamma-amino butyric acid (GABA) receptors by acting as a noncompetitive blocker (Caboni et al. 2003; Zhao et al. 2004). The antagonist action of the fipronil is to prevent the flux of chloride ions, which leads to excessive neural activation, causing the death of the insect by hyperarousal (Ohi et al. 2004).

Fipronil has a higher affinity for the GABA receptors of invertebrates than for the receptors of mammals, which makes the pesticide more toxic to insects than to mammals. There are three different reasons for this affinity to insect receptors: 1) the nervous system of insects is less complex compared with the nervous system of mammals; 2) the GABAergic receptors in insects and mammals are structurally different (Narahashi et al. 2007); and 3) the insects also have a glutamatergic system that is coupled to the chloride channels, a critical target for the fipronil (Zhao et al. 2005; Narahashi et al. 2007).

The GABAergic system participates in the expression of maternal behaviors (Lee and Gammie 2010). Consisting of multiple activities performed by the females, maternal behaviors ensure the survival of the young (Lonstein and De Vries 2000). In addition to the maternal behaviors, lactating rats also exhibit aggressive behavior to the presence of an intruder (Lee and Gammie 2010). Therefore, the assessment of maternal care includes not only the model maternal behavior but also the maternal aggressive behavior.

According to Udo et al. (2014), female rats that received 0.1 mg/kg of fipronil showed a maternal behavior that was disturbed, and for female rats that received 10.0 mg/kg of this pesticide, the disturbed behavior increased; however, the study did not evaluate specifically the maternal aggressive behavior. Thus, one of the aims of this study was to evaluate the maternal aggressive behavior. Furthermore, changes in animal behavior can also be caused by changes in hormones (Ferreira et al. 2002). Because the action of fipronil is similar to that of an endocrine disruptor, modifying the thyroid gland function (Jackson et al. 2009) and the estrus cycle of rats (Ohi et al. 2004), selected endocrine tissues (ovaries and thyroid gland) were sampled to verify histopathological changes associated with the exposure of the dams.

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2. Methods

2.1. Animals

In this study, forty female Wistar rats (90 days old) and twenty male Wistar rats (100 days old) were used from the Department of Pathology (School of Veterinary Medicine and Animal Science, University of São Paulo). All of the rats were housed in 40 × 50 × 16 cm polycarbonate cages that were maintained in the specific conditions of a constant 12 h light/dark cycle (light: 06:00–18:00), a controlled temperature (21 ± 2°C) and free access to food and water. All animal manipulations followed the Ethical Principles in Animal Research that were adopted by the Ethics Committee on the Use of Animals by the School of Veterinary Medicine and Animal Science of the University of São Paulo (protocol no. 3153/2013).

2.2. Chemical

The fipronil was obtained as the commercial product Regent® 800 WG (BASF–Agro Brazil, São Paulo, SP, Brazil) in powdered form (80% fipronil) and was dissolved in tap water. The pregnant rats received 0.1, 1.0 and 10.0 mg/kg of fipronil or tap water (control group) by gavage in volumes that did not exceed 1.0 ml/kg body weight. The administration of fipronil or water occurred daily in the afternoon, from the 6th to the 20th day of gestation.

2.3. Experimental design

A total of forty pregnant rats received different doses of fipronil (0.1, 1.0 or 10.0 mg/kg) or water by gavage daily (n = 10 pregnant rats/group) from the 6th until the 20th day of gestation. The general motor activity of the female rats was evaluated on the 6th day of lactation in the open field test, which was followed by the analysis of maternal aggressive behavior. On the 8th day of lactation, some of the female rats were euthanized to collect the ovaries and the thyroid gland for histopathological analyses.

2.4. Open field test

The open field test was conducted in a round arena with a diameter of 96 cm that was painted white with cardboard walls that were 30 cm high. The floor of the arena was divided into 25 regions of approximately equal area and was bounded by two concentric circles of different radii with straight radial segments marked in black on the surface. The arena was situated on a counter inside the observation cubicle and was illuminated with white fluorescent lamps. Hand operated counters were used to score the locomotion frequency (i.e., the number of floor sections entered), the rearing frequency (i.e., the number of times the animal stood on its hind legs) and the self-grooming frequency (i.e., the number of times the animal touched its forepaws to the head and snout). A chronometer was used to measure the duration of immobility (i.e., the total time in seconds without spontaneous movement). For the open field observations, each individual rat was placed in the center of the arena, and the behavioral parameters were recorded for 5 min. The apparatus was washed with a 5% ethanol solution before each behavioral test. The control and the experimental rats were intermixed for the observations, and the observations were recorded on the 6th day of lactation between 14:00 and 17:00.

2.5. Maternal aggressive behavior

After the open field test, the maternal aggressive behavior test was performed on the same day. The offspring were removed from the home cage for 15 min. After this period, the pups were placed back in the cage in a corner, with their respective dam. Then, a male intruder was placed with the dam and her pups, and the behaviors were recorded for 10 min. The accepted difference between the male intruder and the female weights did not exceed 10 g.

The following parameters were evaluated in the maternal aggressive behavior test: 1) social interaction: the time that the mother interacted with the male intruder and vice-versa; 2) intruder sniffing: the time that the male intruder sniffed the pups; 3) boxing frequency: the attacks by the mother with the forelimbs and hind limbs; 4) latency period for the first fight: time spend for the mother to struggled with the male intruder rolling into the cage, for the first time; 5) frequency of fights and bites: frequency that the mother fights and bites the male intruder; 6) time for maternal care: the time that the mother spent with her pups licking, sniffing or being with them; and 7) mother self-grooming frequency.

2.6. Histopathological analyses

On the 8th lactation day the dams were euthanized (5 mg/kg xylazine and 50 mg/kg ketamine, by intraperitoneal route) and the thyroid and the ovaries were collected for histopathological analysis. The organs were fixed in 10% neutral-buffered formalin. After fixation, the tissues were embedded in paraffin and 5 μm sections were sliced and stained with hematoxylin and eosin (HE) to visualize pathologic abnormalities and potential treatment-related effects. The microscopic evaluation of the ovaries was based on a qualitative analysis to observe any abnormalities/lesions, such as ovarian atrophy, neoplasia, inflammation, infectious agents, and/or congenital anomalies. The evaluation of the thyroid gland included analyses of follicular colloid content and follicular epithelial cell height (Keane et al. 2015). These histopathological analyses were also performed in female control rats (n = 10) to determine the range of spontaneous physiological and background changes that may be normal for the specific strain and ages of the rats that were used in the study.

2.7. Statistical analyses

The data were analyzed with the software GraphPad Prism 5 for Windows (GraphPad Software, Inc., San Diego, CA, USA). An analysis of variance (ANOVA) was used, which was followed with the Dunnett posttests. The results were expressed as the means ± standard errors, and the differences among the means were statistically significant at $p < 0.05$.

3. Results

3.1. Open field test

For most of the parameters, no significant differences among the groups were observed in the open field test: locomotion ($F(3/36) = 1.740$, $p > 0.05$), self-grooming ($F(3/36) = 3.658$, $p > 0.05$) and immobility($F(3/35) = 0.805$, $p > 0.05$). However, for the rearing frequency, the one-way ANOVA found significant differences among the groups ($F(3/36) = 3.197$, $p < 0.05$), and the Dunnett posttests showed that the dams from the 10.0 mg/kg group had a higher frequency of rearing behavior than the control group($p < 0.05$—Fig. 1).

3.2. Maternal aggressive behavior

Only two of the parameters examined for maternal aggressive behavior (Fig. 2) were not significantly different among the groups, i.e., social interaction ($F(3/36) = 1.390$, $p > 0.05$) and boxing frequency ($F(3/36) = 1.164$, $p > 0.05$). Regarding the intruder sniffing of the pups, the dams in the 0.1 mg/kg group let the male sniff their pups much longer than in the control group ($F(3/35) = 8.835$, $p < 0.001$ — Dunnett's posttest showed significance at $p < 0.0001$). For the latency period before the first fight, the dams in the 0.1 mg/kg group took longer to start a fight with the male intruder than the dams in the control group