Braz. J. vet. Res. anim. Sci.,
São Paulo, v. 30, n. 1, p. 17-9, 1993.

EFFECTS OF AMITRAZ ON ISOLATION-INDUCED AGGRESSION IN MICE

EFEITOS DO PRAGUICIDA AMITRAZ NO COMPORTAMENTO AGRESSIVO INDUZIDO PELO ISOLAMENTO SOCIAL EM CAMUNDONGOS

Michiko SAKATE[1]; Jorge Camilo FLÓRIO[2]; João PALERMO NETO[3]

SUMMARY

The effects of amitraz, a formamidine derivative on isolation-induced aggression were studied in mice. Results show that amitraz increased the latency to the first attack and increased not only the fight duration but also the frequencies of attacks and tail flickings. These results suggested that pesticide effects on aggressive behavior could be a consequence of it's inhibitory effects on monoamine oxidase activity within the Central Nervous System, most probably through the increments it produces on serotonin levels.

UNITERMS: Amitraz; Aggressive behavior; Social isolation; Serotonin; Catecholamines; Mice

Contrarily to other pesticides, amitraz, a formamidine derivative, widely used in Veterinary clinical practice for the treatment of demodicosis[12] does not stimulate Central Nervous System (CNS). Althought acute intoxications with this pesticide are not common, signs and symptons of CNS depression such as sedation, loss of the righting reflex, motor incoordination and coma were already described after it's use on large doses [7,9]. The dose of 195 mg/kg was that reported to be the oral and acute amitraz (Triatox®) LD_{50} in male rats[14].

Amitraz induced depression, however, is not unespecific. Pesticide affects were already related to an MAOI-like action within the CNS; indeed, it was shown that it inhibited monoamine oxidase (MAO) activity both in liver and brain of rats[1] and also that it crosses the blood brain barrier[6]. Recently, it was found that perinatal exposure to amitraz induced developmental changes and alterations in the motor function of rat pups*. Acute amitraz administration increased also pentobarbital sleeping time in mice and the convulsive threshold doses of rats to several convulsants[5]. Since amitraz administration induced many behavioral alterations in rats, increasing at the same time the levels of noradrenaline, dopamine and serotonin in different brain regions of these treated animals**, we thought it would be interesting to evaluate the effects of acute amitraz intoxication on isolation-induced aggression in mice. Indeed, the expression of this kind of aggressive behavior was already related to the activation of several brain amine pathways within the CNS[11]. Thus, the results to be observed could bring some new data to the understanding of the underlying mechanisms involved on amitraz effects.

Fifty six male Swiss Webster mice weighing initially 25-30 g at the beginning of the experiments and of the same strain were used. From 28 days before the beginning of the experiment, the mice were individually housed in opaque plastic cages (26 x 14 x 19 cm) in a controlled room temperature (22°C ± 1) with a 12 h light cycle (lights on from 7 a.m.); food and water were provided *ad libitum*, except during observation periods when they were withdrawn. Animals were used once.

The mice were equally and at random divided into four groups: one control and three experimental groups; the animals of the experimental groups were intraperitonially treated with 0.5; 20.0 or 50.0 mg/kg of Amitraz (Triatox® Coopers do Brasil S/A) 90 min. before aggressiveness evaluation. Animals of the control group were similarly treated but with distilled water (0.1 ml/10g), being also observed for aggressive behavior 90 min. after treatment. These pesticide doses were choosen since they were able to change animal's open-field behavior as observed on previous work[5].

Aggressive behavior was induced by isolation (28 days) being recorded for 10 min. according to the method described by VALZELLI et al.[15]. Hand-operated counters and stopwatches were employed to score the following parameters: latency to the 1st attack (time in s to the 1st attack), fight duration (total s of fight), attack frequency (number of attacks) and tail flicking frequency (number of tail flickings). To minimise possible circadian changes in mice's behavior, control and experimental observations were alterned, the mice being observed at the same time of the day (between 8:00 and 13:00 h). Since the obtained results were homocedastic an analysis of variance (ANOVA) followed by Duncan's test was used to analise all data. Results were considered significant when $p < 0.05$.

As it can be seen in Fig. 1, amitraz administration was able to decrease isolation-induced aggression in mice. Thus, pesti-

1- PhD - Botucatu of Veterinary Medicine UNESP
2- PhD - School of Veterinary Medicine, University of São Paulo
3- Professor - School of Veterinary Medicine, University of São Paulo

* SAKATE, M.; FLÓRIO, J.C.; PALERMO NETO, J. Developmental and behavioral effects of prenatal amitraz administration in rats. Arch. Toxicol. (No prelo)

** FLÓRIO, J.C.; SAKATE, M.; PALERMO NETO, J. Effects of amitraz on motor function. Pharmacol. Toxicol. (No prelo)

cide administration increased in a dose-dependent way the latency to the 1st attack ($F=12.44$; $df=3/24$; $p<0.05$) and decreased also in a dose-dependent way, not only the attack frequency ($F=10.5$; $df=3/24$; $p<0.05$), but also the fight duration ($F=3.57$; $df=3/24$; $p<0.05$) and the tail flicking frequency ($F=4.71$; $df=3/24$; $p<0.05$). Further analysis of mice behavior after amitraz administration showed that animals presented not only sedation but also motor incoordinations; these signs were detected 10 min. after the 50.0 mg/kg dose, 20 min. after the 20 mg/kg dose and 80 min. after the smaller pesticide dose (0.5 mg/kg), lasting for at least 24 h.

According to FLÓRIO et al.** amitraz administration in rats, through it's MAOI-like activity, increased the whole brain levels of noradrenaline, the striatal levels of dopamine and the brain stem levels of serotonin, decreasing at the same time the striatal levels of homovanilic acid and the brain stem levels of 5-hydroxyindoleacetic acid. Since changes in aggressive behavior of laboratory animals are often associated with modifications in brain monoamine turnover or levels[11] the present results could be a consequence of pesticide effects on catecholaminergic and/or on serotoninergic pathways.

DOPA *per se* or combined with MAO inhibitors induces irritability and fighting in rats and mice[4]. Under certain conditions aggressiveness is positively related to brain noradrenaline levels[13]. Thus, amitraz effects might not be attributed to modifications in catecholaminergic pathways; indeed, pesticide administration reduced isolation-induced aggression and, as observed previously, induced sedation and motor incoordination[2,3,9].

Serotonin depletion causes excitability and aggressiveness[10] which suggests an inhibitory influence for this amine on behavior. It was already demonstrated that the firings of the axosomatic synapses containing 5-HT of the amygdaloid neurons, decreased sharply after 5-hydroxytryptophan (5-HTP) administration[3]. The administration of 5-HTP supresses not only the mouse-killing behavior of rats, but also the aggressive behavior induced in rats by PCPA administration and the isolation-induced aggression in mice[11]. Consequently, a possible hypothesis to explain the present observed decrease in mice aggressiveness would be the increment induced by amitraz on brain serotonin levels. Thus the possibility in raised that amitraz effects on isolation-induced aggression in mice were also a consequence of it's MAOI-like effects. Accordingly, MAOI agents such as pargyline decreased inter-male aggression in rats[11]. It should not be forgotten, however, that amitraz is also an agonist at alpha$_2$ adrenoceptors* since these receptors were already related to aggressive behavior[2].

RESUMO

Estudaram-se os efeitos do amitraz, um derivado formamidínico, no comportamento agressivo induzido pelo isolamento social em camundongos. Os resultados mostraram que o amitraz aumentou a latência para o primeiro ataque e diminuiu não somente a duração de briga como as freqüências de ataques entre esses animais. Estes resultados sugeriram que os efeitos do praguicida sobre o comportamento agressivo dos camundongos foram conseqüência de um efeito inibitório do mesmo sobre a atividade da enzima monoamina oxidase no Sistema Nervoso Central, e, conseqüentemente, de um aumento dos níveis cerebrais de serotonina.

UNITERMOS: Amitraz; Comportamento agressivo; Isolamento social; Serotoninas; Catecolaminas; Camundongos

AKNOWLEDGEMENTS

This work, part of the PhD thesis presented by M. Sakate to the School of Veterinary Medicine of the University of São Paulo, was supported by a fellowship from PADCT/CAPES.

REFERENCES

01-BONSALL, J.L.; TURBULL, G.J. Extrapolation from safety data to management of poisoning with reference to amitraz (a formadine pesticide) and xylene. **Human. Toxicol.**, v.2, p.578-92, 1983.

02-BUUS-LARSSEN, J. Piperoxan reduce the effects of clonidine on aggression in mice and on noradrenaline dependent hypermotility in rats. **Europ. J. Pharmacol.**, v. 47, p.47-9, 1978.

03-EIDELBERG, E.; GOLDSTEIN, G.P.; DEZAN, L. Evidence for serotonin as a possible inhibitory transmitter in some limbic structures. **Exp. Brain Res.**, v. 4, p. 73-80, 1976.

04-EVERETT, G.M.; BORCHERDING, J.W. l-DOPA effect on concentrations of dopamine, norepinephrine and serotonin in brains of mice. **Science**, v. 168, p.549-50, 1970.

05-FLÓRIO, J.C.; SAKATE, M.; PALERMO NETO, J. Some behavioral effects of the pesticide amitraz. **Braz. J. Med. Biol. Res.**, v. 22, p.1291-3, 1989.

06-FAO. Plant production and production paper. **Pestic. Residues Food Evaluation**, v. 26, p. 1-59, 1980. Suplemento.

07-FOLZ, S.D.; KAKUK, T.J.; HENKE, C.L.; RECTOR, D.L.; TESAR, F.B. Clinical evaluation of amitraz as a treatment for canine demodicosis. **Vet. Parasit.**, v. 16, p. 335-41, 1984.

08-GILBERT, M.E.; DYER, R.S. Increased hippocampal excitability produced by amitraz. **Neurotoxicol. Theratol.**, v. 10, p. 229-35, 1988.

09-HSU, W.H.; SCHAFFER, D.D. Effects of topical application of amitraz on plasma glucose and insulin concentrations in dogs. **Amer. J. vet. Res.**, v. 49, p. 130-1, 1988.

10-KOE, B.K.; WEISSMAN, A. The pharmacology of parachlorophenylalanine, a selective depletor of serotonin stores. **Advanc. Pharmacol.**, v. 6B, p. 29-47, 1968.

11-MASON, S.T. **Catecholamines and behavior.** Cambridge, Cambridge University Press, 1984. p. 96-165: Catecholamines and pathological behavior.

12-MULLER, G.H. Amitraz treatment of demodicosis. **J. Amer. Anim. Hosp. Ass.**, v. 19, p. 435-41, 1983.

13-RANDRUP, A.; MUNKVARD, I. Relation of brain cat-

* SAKATE, M.; FLÓRIO, J.C.; PALERMO NETO, J. Developmental and behavioral effects of prenatal amitraz administration in rats. **Arch. Toxicol.** (No prelo)
** FLÓRIO, J.C.; SAKATE, M.; PALERMO NETO, J. Effects of amitraz on motor function. **Pharmacol. Toxicol.** (No prelo)

excitation. In: GARATTINI, S.; SIGG, E.B., eds. **Agressive behavior.** New York, Willey, 1969. p. 228-35.

14-SAKATE, M. **Efeitos comportamentais do Amitraz (Triatox®).** São Paulo, 1991. Tese (Doutorado) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo.

15-VALZELLI, L. Drugs and agressiveness. In: GARATTINI, E.; SHORE, P. A., eds. **Advances in pharmacology.** New York, Academic Press, 1967. v. 5, p. 79-108.

Recebido para publicação em 10/08/92
Aprovado para publicação em 12/11/92

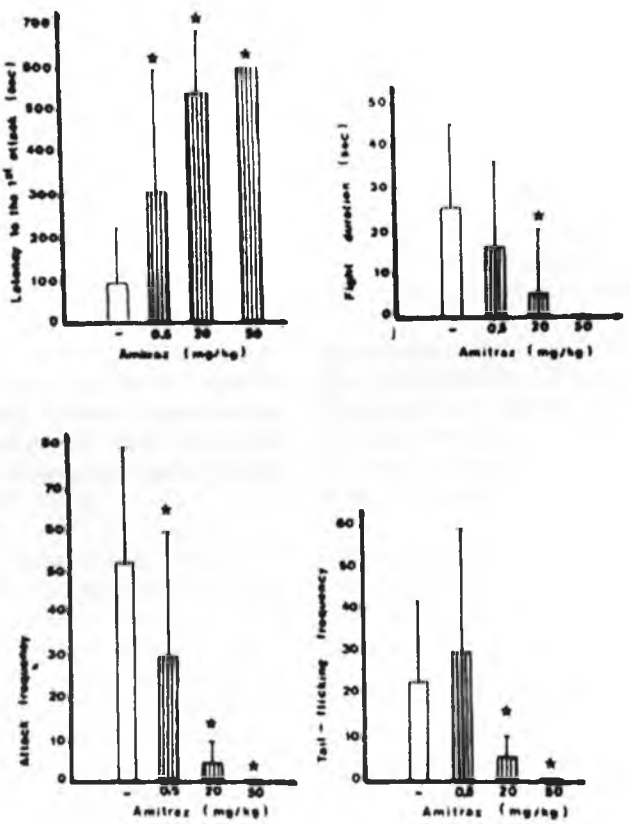

FIGURE 1
Effects of amitraz administration (stripped columns) on isolation induced aggression in mice. Amitraz was administered intraperitonially 90 min. before behavioral evaluations. * $p<0.05$ in relation to control data (open columns - analysis of variance, Duncan's test).