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Ivermectin impairs sexual behavior in sexually naïve, but not sexually experienced male rats

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ABSTRACT

Ivermectin (IVM) is an antiparasitic drug, widely used in domestic animals. In mammals, IVM act as a GABA agonist. This neurotransmitter has an important role in the regulation of sexual behavior. Thus, this study sought to investigate the effects of various medically relevant doses IVM on the sexual behavior of male rats. In particular, we also wished to examine if previous sexual experience modulated responses to IVM. In the first experiment, the sexual behavior of inexperienced male rats was analyzed after they received 0.2, 0.6, 1.0 or 2.0 mg/kg IVM, 15 min prior to behavioral testing. In the second experiment, the effects of four previous sexual experiences on IVM treated rats (1.0 or 2.0 mg/kg, 15 min prior to the 5th session) were assessed. The standard therapeutic dose (0.2 mg/kg) did not impair the sexual behavior of inexperienced male rats. At a more concentrated dose (0.6 mg/kg), which is still within the therapeutic range, the appetitive phase of sexual behavior of inexperienced male rats was impaired. Likewise, 1.0 mg/kg impaired the appetitive phase. Previous sexual experience blocked almost entirely this sexual impairment, suggesting that previous sexual experience exerts a positive effect in attenuating the sexual impairment produced by IVM treatment. Therefore, the standard therapeutic dose of IVM can be used without producing side effects on sexual behavior. Use of more concentrated therapeutic doses is not recommended during reproductive periods, unless the animals have had previous sexual experience.

1. Introduction

Avermectins, particularly Ivermectin (IVM), are broad-spectrum antiparasitic agents that are used widely in agricultural and domestic animals (Lifschitz et al., 2007). In endoparasites, IVM exerts an antiparasitic activity by selectively binding to receptors in the peripheral motor synapses, blocking chemical transmission of gamma-aminobutyric acid (GABA)-gated chloride channels localized in the central nervous system. This stimulates the discharge of GABA at nerve endings, increasing the affinity for GABA at its synaptic receptor and causes interruption of the nerve impulses, producing paralysis and death in parasites (Hicks and Elston, 2009).

In vertebrates, avermectin B_1 stimulates a GABA-mediated chloride conductance, liberates GABA from nerve endings and enhances the binding of GABA to its receptor (Spinosa et al., 2002). Previous studies performed in our laboratory have demonstrated that doramectin, another avermectin antiparasitic drug (Spinosa et al., 2000) and IVM (Spinosa et al., 2002) interfere with GABAergic-related behaviors, leading to anxiety and seizures, as a GABAergic agonist.

Moreover, several studies have suggested that GABAergic neurotransmission is involved in inhibitory processes underlying male sexual behavior (Agmo et al., 1987; Amikishieva and Semendyaeva, 2007; Fernandez-Guasti et al., 1986; Frye and Walf, 2008; Oropeza-Hernandez et al., 2002; Rodrigues-Alves et al., 2008). Both $GABA_A$ and $GABA_B$ receptor subtypes control sexual behavior (Bitran and Hull, 1987; Frye and Paris, 2009; Paredes and Agmo, 1989, 1995).

Furthermore, there are several complaints from farmers that IVM treatment has lead to impairment in the performance of sexual behavior in farm animals (Bernardi, personal communication). Despite these reports, IVM is used without prior consideration of the reproductive periods of the treated animals. Also, as far as we are aware, there are no studies investigating the effects of IVM on sexual behavior. Therefore, the purpose of this study was to investigate the effects of various medically relevant doses of IVM on the sexual behavior of male rats. The effects of previous sexual experience were also examined for practical reasons.

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2. Materials and methods

2.1. Animals

Male and female Wistar rats from our colony were used. The rats were housed by gender, at a density of five animals per cage. Cages were polypropylene with metallic cover (40 × 50 × 20 cm). Animals were housed under the following conditions: controlled room temperature (22 ± 2 °C), humidity (65–70%) and artificial lighting (12 h light/12 h dark cycle, lights on at 10:00 p.m.). Animals received free access to Nuvilab® rodent chow (Nuvital Company, São Paulo, Brazil) and filtered water. Sterilized and residue-free wood shavings were used as bedding. All the behavioral observations were conducted between 2:00 and 6:00 p.m., during the dark phase. Animals were divided randomly into control and experimental groups. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil. These guidelines are similar to those of the National Research Council, USA.

2.2. Drugs

IVM (Ivomec, Merck, Sharp & Dohme Farmacêutica e Veterinária do Brazil, Campinas, Brazil) was suspended in a 0.9% saline solution plus a drop of Tween-80 and was administered intraperitoneally (1 ml/kg). Saline solution plus a drop of Tween-80 was used as control solution.

2.3. Sexual behavior

Behavioral testing was conducted in a wooden box (56 × 32 × 32 cm) provided with a moveable cover and frontal glass. Pine shavings covered the floor. The test room was illuminated by two 25 W red lamps. To investigate sexual behavior, male rats were allowed to mount ovariectomized females sexually activated with exogenous estradiol (50 μg/kg subcutaneously (s.c.), 54 h before the tests) and progesterone (2.0 mg/kg s.c., 6 h before the tests). The female lure rats were tested once for receptivity before being placed with the males. Male rats were individually allowed to acclimate to the behavior box for 5 min; then a receptive female was introduced, and the sexual behavior were assayed in 40 min time periods. The following parameters were recorded: (a) first mount latency (mount without intromission); (b) first intromission latency (mount with vaginal insertion); (c) number of mounts before ejaculation; (d) number of intromissions before ejaculation; (e) ejaculation latency; (f) first post-ejaculatory mount latency and (g) first post-ejaculatory intromission latency. The sexual activity index (SAI), a derived measure, was calculated as proposed by Agmo et al. (1987):

$$\mathrm{SAI} = \log(1/\mathrm{ML} \times t) + \log(1/\mathrm{IL} \times t) + \log(1/\mathrm{EL}) \times t)\sqrt{(\mathrm{NM} + \mathrm{NI})} + \mathrm{Y}$$

where ML is first mount latency, IL is first intromission latency, EL is first ejaculation latency, NM is number of mounts, NI is number of intromissions, t is the time of observation and Y means four when an animal's ejaculation occurred and zero when it did not.

All latencies were calculated in minutes and only the animals that ejaculated were included in statistical analysis, except for SAI.

Two experiments were performed. In the first experiment, the sexual behavior of inexperienced rats was investigated, using 95 male rats divided in five equal groups (n = 19 for each group). The experimental groups received 0.2, 0.6, 1.0 or 2.0 mg/kg of IVM, 15 min prior to the behavioral test. The control group received 1 ml/kg 0.9% saline solution at the same time as experimental animals.

The second experiment investigated the effects of previous sexual experience on IVM treated rats. Animals of control and experimental groups were submitted to four experiences of sexual behavior, as described above. In the 5th sexual experience, which constituted the test phase, 42 animals were divided into three equal groups (n = 14 for each group): one control and two experimental groups which received either 1.0 or 2.0 mg/kg of IVM 15 min prior to the test.

2.4. Statistical analysis

Results were expressed as mean ± SEM. Homoscedasticity was verified through the Bartlett's test. Normality was verified through Kolmogorov–Smirnov test. One way ANOVA followed by the Dunnett test was used to compare all data.

All analyses were performed with GraphPad Instat software (San Diego, USA). In all cases, results were considered significant if $P < 0.05$.

3. Results

3.1. Effects of an increasing dose of IVM on the sexual behavior of inexperienced rats

Fig. 1 shows the effects of increasing IVM dose on first mount, first intromission, first post-ejaculatory mount and first post-ejaculatory intromission latencies of inexperienced rats. The ANOVA revealed significant differences in the first mount latency ($F(4/90) = 14.97$, $P = 0.0001$), first intromission latency ($F(4/90) = 14.76$, $P = 0.0001$), first post-ejaculatory mount latency ($F(4/90) = 11.25$, $P = 0.0001$) and first post-ejaculatory intromission latency ($F(4/90) = 5.19$, $P = 0.0008$). The Dunnett's post hoc test showed that in relation to the control group, 0.6 and 1.0 mg/kg IVM increased both first mount and first intromission latencies. The 2.0 mg/kg IVM dose decreased the first mount, first intromission, first post-ejaculatory mount and first post-ejaculatory intromission latencies. No differences were detected between groups in ejaculatory latency ($F(4/90) = 0.59$, $P = 0.66$, data not shown).

Fig. 2 shows the effects of increasing IVM doses on number of mounts and intromissions before ejaculation and SAI of inexperienced rats. The ANOVA revealed significant differences in the number of mounts before ejaculation ($F(4/90) = 14.41$, $P = 0.0001$), number of intromissions before ejaculation ($F(4/90) = 7.89$, $P < 0.0001$) and on SAI ($F(4/90) = 3.89$, $P = 0.005$). The Dunnett's post hoc test showed that only 2.0 mg/kg IVM was sufficient to increase these parameters.

3.2. Effects of an increasing dose of IVM on the sexual behavior of experienced rats

Table 1 shows the effects of an increasing IVM dose on experienced male rats. Relative to the control group, 1.0 and 2.0 mg/kg IVM administration was not able to modify the first mount ($F(2/39) = 0$, $P = 1.0$), first intromission ($F(2/39) = 0$, $P = 1.0$), ejaculation ($F(2/39) = 2.52$, $P = 0.09$), first post-ejaculatory mount ($F(2/39) = 0.01$, $P = 0.98$), or first post-ejaculatory intromission ($F(2/39) = 0.01$, $P = 0.98$) latencies. The number of intromissions before ejaculation ($F(2/39) = 0.92$, $P = 0.38$) and the SAI ($F(2/39) = 0.16$, $P = 0.87$) were also not statistically different. The number of mounts before ejaculation was significantly increased only after the 2.0 mg/kg dose ($F(2/39) = 4.25$, $P = 0.02$).