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Doramectin reduces sexual behavior and penile erection in male rats

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ABSTRACT

Doramectin (DOR) is an antiparasitic drug that is widely used in domestic animals. In mammals, DOR acts as a γ-aminobutyric acid receptor agonist. This neurotransmitter plays an important role in the regulation of sexual behavior. The present study investigated the effects of two medically relevant doses of DOR on sexual behavior in male rats. We also examined whether previous sexual experience modulates responses to DOR. General activity was first observed in an open field 24, 48, and 72 h after administration of 0.1 and 0.3 mg/kg DOR to determine the dose and time effects of the drug. Apomorphine-induced penile erection and sexual behavior in inexperienced male rats were then analyzed. The effects of previous sexual experience on subsequent sexual behavior in DOR-treated rats (0.3 mg/kg, 24 h prior to the test) were also assessed. The standard therapeutic dose (0.2 mg/kg) did not modify general activity or penile erection. A slightly concentrated dose of 0.3 mg/kg, which is still within the therapeutic range, decreased apomorphine-induced penile erection, whereas 0.2 mg/kg did not modify this behavior. Compared with controls, sexual behavior in inexperienced male rats was impaired after 0.3 mg/kg DOR. Previous sexual experience had little impact on the effects of 0.3 mg/kg DOR. In conclusion, the 0.2 mg/kg dose of DOR did not affect motor behavior or apomorphine-induced penile erection. At a more slightly higher dose level, the appetitive and consummatory phases of sexual behavior in inexperienced male rats were impaired. Previous sexual experience was unable to reverse this sexual impairment, suggesting that previous sexual experience does not exert a positive effect in attenuating sexual impairment produced by DOR treatment.

1. Introduction

Avermectins are produced through fermentation by soil-dwelling actinomyces from the genus *Streptomyces*. Doramectin (DOR; 25-cyclohexyl-5-*O*-demethyl-25-de[1-methylpropyl]avermectin A_{1a+}) was selected as a new avermectin prepared by mutational biosynthesis. It is a potent endectocide that can kill both endo- and ectoparasites (Mueller et al., 2011). Doramectin(25-cyclohexyl-5-O-demethyl-25-de(1-methylpropyl) avermectin A_{1A-} $C_{50}H_{74}0_{14}$] is a white-to-tan powder with 989.14 molecular weight (Conder and Baker, 2002) and in therapeutic doses the C_{max} was about 5.31 ± 0.35 days (Conder and Baker, 2002).

P-glycoprotein (P-gp), a plasma membrane protein belonging to the ATP-binding cassette superfamily, not only promotes the efflux of potentially toxic compounds from the blood into the gut lumen or from the brain into the blood (Bodo et al., 2003) but also limits the availability of a large number of drugs in many organisms (Kiki-Mvouaka et al., 2010; Schinkel and Jonker, 2003). P-gp has been clearly identified as the main factor that controls the concentration of avermectins by affecting their in vivo absorption, distribution, and elimination in the host (Kwei et al., 1999; Schinkel et al., 1996). Avermectins are poorly metabolized (Alvinerie et al., 2001; Chiu et al., 1987) and are good substrates and potent inhibitors of P-gp (Kiki-Mvouaka et al., 2010). P-gp protects the brain from avermectins by limiting their penetration across the blood–brain barrier and thus their subsequent neurotoxicity (Lankas et al., 1997; Roulet et al., 2003; Schinkel et al., 1994), and it contributes to the elimination of ivermectin via intestinal excretion (Ballent et al., 2006; Laffont et al., 2002).

DOR, like others avermectins, exerts central nervous system effects in mammals by interfering with γ-aminobutyric acid (GABA)-sensitive neurons, which can lead to neurotoxicity, such as tremors, ataxia, and gait abnormalities. Turner and Schaeffer (Turner and Schaeffer, 1989) summarized the mode of action of avermectins, demonstrating GABA release, [^{3}H]GABA binding, benzodiazepine binding sites, and chloride uptake. (Dawson et al., 2000) correlated the toxicity and anticonvulsant activity of avermectins with $GABA_A$ receptor activity, which may contraindicate the potential use of avermectins as anticonvulsants. We previously suggested that DOR has a pharmacological profile that indicates that it is an anxiolytic/anticonvulsant drug with GABAergic properties. In fact, DOR reduced anxiety-like behavior in the elevated plus maze and susceptibility to seizures induced by picrotoxin.

Several studies suggested that GABAergic neurotransmission is involved in inhibitory processes that underlie male sexual behavior

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(Agmo et al., 1987; Amikishieva and Semendyaeva, 2007; Fernandez-Guasti et al., 1986; Frye and Paris, 2009; Frye and Walf, 2008; Oropeza-Hernandez et al., 2002; Rodrigues-Alves et al., 2008). In mammals, both $GABA_A$ and $GABA_B$ receptor subtypes control sexual behavior (Bitran and Hull, 1987; Frye and Paris, 2009; Paredes and Agmo, 1989, 1995). Doramectin showed efficacy as an antiparasitic drug and enhanced animal productivity (weight gain) (Borges et al., 2013; Höglund et al., in press), but some reports have shown that avermectin drugs interfered with sexual performance in cattle (Bernardi, personal communication). Studies on maternal/embryotoxic and teratogenic effects of Doramectin were performed in many animals including rats, mice and rabbits. Doramectin passes the placental barrier and was detected in rat and mouse fetuses as well as induced embryo mortality in rabbits (EMEA, 1997). Some studies on avermectins were also reported on female cattle (Muniz et al., 1995), goats (Dupuy et al., 2001; Imperiale et al., 2003), rats (de Souza Spinosa et al., 2000) and guinea pigs (Loyacano et al., 2002). Therapeutic suppression of nematode infections by ivermectin or doramectin resulted in significantly increased bodyweight, bodyweight gain and subjective body condition scores in replacement heifers, but not in significantly higher pregnancy rates (Loyacano et al., 2002). In addition, exposure to abamectin, another avermectin, induces testicular damage and affects sperm dynamics in rats (Celik-Ozenci et al., 2011) as well as decreased sperm maturity in Turkey farmworkers (Celik-Ozenci et al., 2012). Ivermectin increased postnatal pup mortality and decrease pup weights in the surviving offspring of mice compared with those of the control groups at doses as low as 0.4 mg/kg body weight/day. This toxic effect is consistent with the postnatal formation of the blood–brain barrier in this species (Lankas et al., 1989). Additionally, this group of drugs interferes with the GABAergic system. We previously found that ivermectin, another avermectin drug, impaired sexual behavior in sexually naive rats (Bernardi et al., 2011). Thus, we investigated the effects of DOR on sexual-related behavior in male rats.

The doses used in the present study was the therapeutic dose (0.2 or 0.3 mg/kg) administered subcutaneously to cattle, pigs and sheep (Franco and Hamann, 2004; Pachaly et al., 2009) and in a previous study on the effects of DOR in rats that used 0.1, 0.3, and 1 mg/kg (de Souza Spinosa et al., 2000). The choice of rats to investigate the effects of DOR on sexual-related behavior was based on very well-known methods for studying such behavioral changes in rats and practical and economic considerations.

2. Material and methods

2.1. Animals

Male and female Wistar rats (100–110 days of age) from the Pathology Department, FMVZ facilities, were used. The animals were housed in groups of five in polypropylene cages with a metal cover (40 × 50 × 20 cm) under controlled room temperature (22 ± 2 °C) and a 12 h/12 h light/dark cycle (lights on 8:00 AM). The open field behavior was performed between 2:00 PM and 6:00 PM during the light phase and the sexual behavior and penile erection experiments were conducted under reverse lighting conditions (lights on 11:00 PM). Male and female rats used in sexual behavior observations were maintained in the reverse lighting conditions for at least 15 days before the experiments and the sexual behavior observations were performed between 2:00 PM and 6:00 PM during the light dark phase. All testing of the control and the DOR-treated rats was intermixed to minimize the effects of the circadian rhythm. Food and water were freely provided. The rats were randomly distributed into control and experimental groups. The animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, USA (1996). All efforts were made to minimize suffering of animals.

2.2. Drugs

The following drugs were used: DOR (Dectomax®, 1%, Agroline, Pfizer, São Paulo, Brazil), apomorphine chloridrate (Uprima®, 2 mg/kg, São Paulo, Abbott), almond oil (Industrial Leclerc, São Paulo, Brazil), and estradiol valerate (Primogyina®, 1 mg, Bayer Schering Pharma, Socorro, São Paulo, Brazil). Doramectin was dissolved in almond oil and administered subcutaneously (SC) at dose of 0.2 or 0.3 mg/kg. Almond oil was also administered in the same form as a control solution (1 ml/kg). Estradiol valerate (0.5 mg/kg) and apomorphine (0.08 mg/kg) were diluted in distilled water and administered SC. All of the solutions were prepared immediately before use and administered in a volume of 1 ml/kg body weight.

2.3. Behavioral procedures

2.3.1. Open field behavior

The apparatus was similar to the one described by (Broadhurst, 1957). It consisted of a round, 96 cm diameter arena surrounded by a 25 cm high enclosure painted white and subdivided into 25 parts that were painted black. Hand-operated counters were used to score locomotion frequency (i.e., the number of floor sections entered), and a chronometer was used to measure the duration of immobility (i.e., the total time in seconds without spontaneous movements). For open-field observations, each rat was individually placed in the center of the arena, and its behavioral parameters were recorded for 3 min. The apparatus was washed with a 5% ethanol solution before each behavioral test. Control and experimental rats were intermixed, and the observations were made between 2:00 PM and 6:00 PM.

Twenty-four naive male rats were distributed into three equal groups: two experimental groups received 0.2 and 0.3 mg/kg of DOR, respectively, and one control group received 1 ml/kg almond oil. Open field behavior was observed 24, 48, and 72 h after treatment.

2.3.2. Penile erection

Each rat was tested once for penile erection induced by 0.08 mg/kg apomorphine (SC). Moderate doses of 0.05–0.2 mg/kg apomorphine were previously shown to facilitate male sexual responses associated with the genitals, including penile erection (Melis et al., 1987). Immediately after this treatment, each animal was individually placed into a glass box (30 × 30 × 40 cm) with a mirror wall. This box was elevated 15 cm above a mirror (50 × 45 cm) by four metal feet. The rats were observed for 60 min after apomorphine administration, during which the latency and number of spontaneous penile erections (only when the rat displayed full erection and bent down to lick its penis) were recorded. The frequency of spontaneous penile erections was defined as the total number of genital reflexes divided by the number of rats ($n = 8$), and latency was defined as the time that elapsed between the injection to the first penile erection. The box was cleaned thoroughly and washed with a 5% ethanol solution following each animal.

Twenty-four naive male rats were distributed into three equal groups: two experimental groups received 0.2 and 0.3 mg/kg DOR, respectively, and one control group received 1 ml/kg almond oil 24 h before the penile erection observation.

2.3.3. Sexual behavior

This test was conducted in a glass box (60 × 40 × 40 cm) that had a removable cover and pine shavings on the floor. The test room was illuminated by one 25 W infrared lamp. To investigate sexual behavior, male rats were allowed to mount females that were sexually activated with estradiol valerate (0.5 mg/kg, SC) 24 h before the experiments. These female lure rats were tested for receptivity before being placed with the males. Females that presented lordosis after a male mount were selected for the study. Each male rat was individually allowed to acclimate to the behavior box for 5 min. A receptive female was then introduced, and sexual behavior was observed for 30 min time periods.