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The melanocortin agonist, melanotan II, enhances proceptive sexual behaviors in the female rat

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Abstract

Melanocortins have been reported to play a role in the control of both male and female sexual behavior. The present study examined the effects of melanotan-II (MT-II), a cyclic peptide analogue of alpha-melanocyte stimulating hormone on appetitive and consummatory aspects of female sexual behavior, including aspects of sexual proceptivity (solicitations, hops and darts, ear wiggling, pacing) and receptivity (lordosis). One group of ovariectomized Long-Evans rats ($n=7$) was primed subcutaneously with estradiol benzoate (EB) and progesterone (P) (10 μg and 500 μg respectively) and another group ($n=7$) with EB (10 μg) and oil (EB alone). Paced mating tests were performed with sexually experienced males in unilevel chambers, which were bisected by a Plexiglas divider containing three holes, through which only the female could pass. MT-II (1 and 3 mg/kg) or saline was injected intravenously 10 min before each 30-min paced mating test. Each female received the 3 treatments. In females primed with EB+P both doses of MT-II increased the number of hops and darts and ear wiggling significantly, but did not alter pacing or lordosis. With EB alone, no effect of MT-II was observed on any of the parameters measured. These results suggest that P can interact with MT-II to increase proceptive behaviors. Because hops and darts are essentially solicitations, made in close proximity to the male, that indicate a desire on the part of females to receive mounts and intromissions, these data suggest that activation of melanocortin receptors may represent a promising mode of action for the treatment of women with hypoactive sexual desire.

Keywords: Female sexual behavior; Proceptivity; Pharmacology

1. Introduction

Melanocortins (MCs) constitute a family of proteins derived from the common precursor proopiomelanocortin (POMC). Prohormone-converting enzymes cleave POMC into several bioactive peptides including α-, β-and γ-melanocyte-stimulating hormone (MSH), adrenocorticotropic hormone (ACTH), and the opioid β-endorphin. The first two of these peptides interact with specific MC receptors (MCRs). Although 5 G-protein-coupled MCRs, named MC1-5, have been identified (Mountjoy et al., 1994; MacNeil et al., 2002), only the MC3 and MC4 subtypes are expressed in the central nervous system (Lindblom et al., 1998; Kishi et al., 2003).

MCs are primarily known for their role in the regulation of adrenal steroid production and skin pigmentation, but the MC system is also involved in the modulation of a variety of other functions including fever, immunity and body weight homeostasis (Adan and Gispen, 1997; Luger et al., 2000; Wikberg et al., 2000). Recently, the role of MC receptors in the regulation of male sexual behavior, and more precisely of penile erection, has received increasing attention (Argiolas, 1999; Van der Ploeg et al., 2002; Giuliano, 2004). In the female rat, non-selective MC receptor agonists have been shown to increase lordosis, the dorsiflexion of the back denoting female sexual receptivity (Cragnolini et al., 2000; Scimonelli et al., 2000; Nocetto et al., 2004).

Sexual behavior in female rat has been divided into appetitive, precopulatory, and consummatory responses (Pfaus et al., 2003), which contain earlier denotations of sexual attractivity (e.g., scent-

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marking, ultrasonic vocalizations), proceptivity (e.g., solicitation, hoping and darting, ear wiggling, pacing), and receptivity (e.g., lordosis) (Beach, 1976; Madlafousek et al., 1976; McClintock and Adler, 1978; Erskine, 1989). These behaviors serve to attract males and allow females to control the initiation and rate of copulation. In addition to copulatory behaviors, other appetitive responses have been used as measures of sexual reward, including lever pressing to access a male, or crossing electrified grids to gain contact with a male (reviewed in Pfaus et al., 2001). Gonadal steroids exert a crucial influence on the central control of female sexual behavior (Pfaff and Schwartz-Giblin, 1994). Lordosis is dependent on the actions of estrogen (E) and is further facilitated to maximal levels by progesterone (P). On the other hand, proceptive behaviors are controlled primarily by the actions of P (Pfaff, 1980). At the molecular level, gonadal steroids control the synthesis and the activation of different proteins that serve as intermediaries in hormone function (Kow et al., 1994), or feed back to activate steroid receptors via second messenger systems (Mani et al., 1994).

There is evidence suggesting that the actions of ovarian steroids on lordosis, at the CNS level, are mediated in part by α-MSH. For example, intracerebroventricular infusions of α-MSH have been shown to facilitate, or inhibit, lordosis depending on the hormonal status and on whether the female rats were in a low or high state of sexual receptivity respectively (Raible and Gorzalka, 1986; Thody and Wilson, 1988; Gonzalez et al., 1996). In addition, it has been reported that estradiol benzoate (EB), either alone or with P, can significantly alter (increase or decrease depending hormonal regime) the concentration of α-MSH levels in hypothalamic nuclei involved in the control of female sexual behavior (Wilson et al., 1991; Medina et al., 1998), suggesting that α-MSH release may be one of the several intermediaries of E action.

Recently, PT-141, a cyclic peptide analogue of α-MSH, was reported to induce a dramatic increase in solicitations and hops and darts, but not pacing or lordosis, in ovariectomized (OVX) rats primed with either EB+P or EB alone (Pfaus et al., 2004). The increase in solicitations occurred in both unilevel and bilevel pacing chambers, but was not secondary to an increase in locomotion. This suggests a specific effect of the peptide on systems in the hypothalamus, or elsewhere, that control appetitive sexual behavior. PT-141 is the deaminated metabolite of melanotan-II (MT-II) (Diamond et al., 2004). Like PT-141, MT-II has a high affinity for MC1, MC3, MC4 and MC5 receptors. Mock et al. (2002) reported that following intravenous administration of MT-II (1 mg/kg) the elimination was rapid with a half-life (average of four animals) of 0.5 h and that plasma levels of MT-II were detectable up to 4 h post-dosage. The present study examined the effect of MT-II on both appetitive and consummatory sexual behaviors in ovariectomized (OVX) rats primed with EB+P, or EB alone, in unilevel pacing chambers.

2. Methods

2.1. Animals

Adult female 225–250 g and male 350–400 g Long-Evans rats were purchased from Janvier (France). Rats were housed in same-sex groups of 3 or 4 in Plexiglas cages containing sawdust bedding with food and water available *ad libitum*. Rats were kept in our animal facilities for at least 3 weeks before the start of experiments in order for them to adapt to the laboratory's environmental conditions (lights off from 10.00 a.m. to 10.00 p.m. "dark phase", temperature 21±1 °C and 55% humidity). Seven days after their arrival in our animal facilities, all female rats were bilaterally OVX under isoflurane anesthesia (2.5–3% during 20 min) (Centravet, Plancoet France). All animal experiments were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC) on the use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

Beta-estradiol-3-benzoate (EB) and P (Sigma, France) were dissolved in paraffin oil and injected subcutaneously (s.c.). When administered alone (i.e. without P), EB was dissolved in sesame oil. MT-II, (Bachem, Germany) was dissolved in a sterile saline solution to 1 and 3 mg/ml. A volume of 1 ml/kg body weight of MT-II or of its vehicle was administered i.v., via the tail vein.

2.3. Behavioral tests

To become sexually experienced, males received 4 training test sessions (twice a week for 2 weeks) with non-experimental receptive females, i.e. the females used for the training of the males were not used for the rest of the study. These trainings began 1 week after arrival of the males in our animal facilities. Only males displaying at least 2 ejaculations during the 4 training test sessions were included in the final experiment. Immediately prior to the mating test with the experimental females, males were given brief access to fully receptive OVX, non-experimental females (each given 50 μg EB 48 h and 500 μg P 4 h prior to the mating test to ensure full receptivity) (Coolen et al., 1997). Only males displaying active mounting behavior were used with the experimental females.

After 2 weeks of recovery from the OVX surgery, experimental females received 4 preliminary sexual behavior tests with sexually experienced males in order to habituate them to the handling and testing procedure and to allow them to acquire the complete pattern of sexual behavior in the unilevel pacing chambers. All tests were conducted at 4-day intervals beginning 2 h into the dark phase. Before each test, females were primed (Pfaus et al., 2000) either with s.c. injections (in a volume of 0.1 ml/rat) of 10 μg EB and 500 μg P before each test (EB+P) or with 10 μg EB and 0.1 ml sesame oil (EB alone). In both cases, the injections were made 48 and 4 h before each test, respectively.

All behavioral observations were scored by a trained observer blinded for the treatment. Appetitive and consummatory behaviors were evaluated in unilevel pacing chambers (60 L×40 W×40 H cm). These were bisected by a transparent Plexiglas wall containing 3 small holes permitting the female to enter or exit the half of the cage in which the male was confined. The holes were too small for the male to go through, thus allowing the female to control or "pace" the sexual interaction. The two

groups of females (EB + P and EB alone) were injected with either saline or with one of the two MT-II dosings (1 or 3 mg/kg) and placed for 5 min in a cage. Following that period, each female, alone, was allowed to adjust to the unilevel chambers for 5 min. After this habituation period, a sexually active male was placed with the female for a 30-min paced mating test. Thus, the test of female sexual behavior started 10 min after treatment. Each female received each of the 3 treatments in a counterbalanced fashion separated by at least 3–4 days.

2.4. Observations of sexual behavior

For each mount, the presence or absence of hops and darts and ear wiggling displayed by the female was noted. Solicitations were scored as a headwise orientation to the male followed by an abrupt runaway, regardless of whether the female remained in the male's side of the chamber (McClintock and Adler, 1978; Erskine, 1989; Pfaus et al., 2004). Pacing behavior was scored in two ways. 1) The percentage of exits after mount (number of times the female left the male's side of the chamber, defined as having all four paws on the other side within 30 s of a mount × 100 / total number of mounts received during the 30-min mating test), and the percentage of exits after intromissions and ejaculations. This last parameter was calculated as just described for mounts, except that the time after ejaculation was extended to "within 60 s". 2) The return latency after mounts was defined as the time between the female's exit from the male's compartment (within the 30 s following a mount) and her return. Return latencies after intromissions and ejaculations were calculated the same way as for mounts, except that the time after ejaculation was extended to

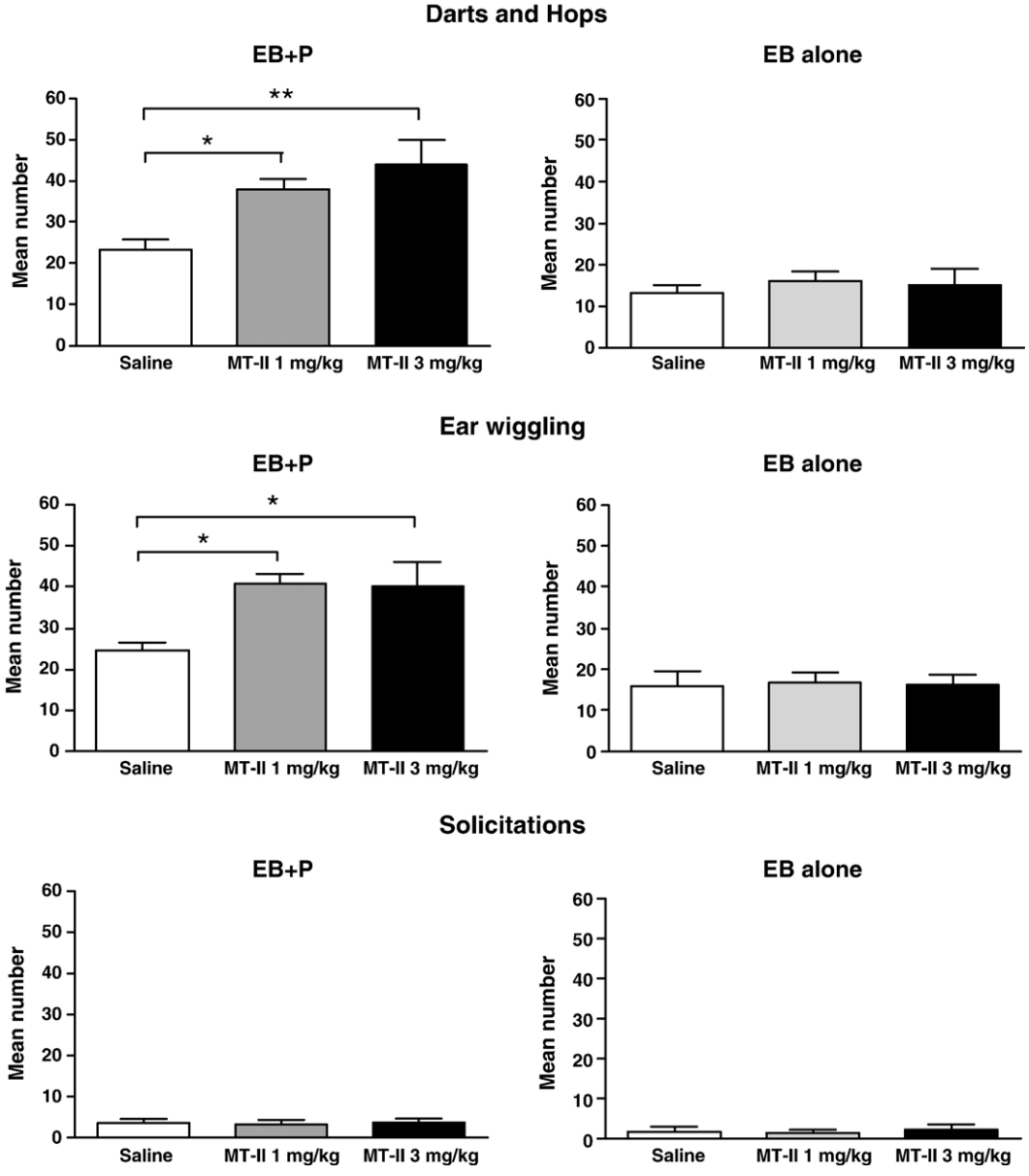

Fig. 1. Effect of MT-II or saline on hops and darts leading to mounts (top), ear wiggling (middle) and solicitations (bottom) in OVX rats ($n = 7$) primed with EB + P (left) or EB alone (right). Results are expressed as mean ± S.E.M. *$P < 0.05$ and **$P < 0.01$, comparison between MT-II (1 or 3 mg/kg) and saline (Dunnett's post hoc comparison).

"within 60 s". Finally, the lordosis quotient was scored as the total number of lordoses/total number of mounts (L/M ratio). Whenever the female displayed kicking, boxing, or rolling over, she was considered to be resistive to the male's attempts to mount.

2.5. Statistical analysis

Data was subjected to one-way ANOVA with repeated measures to assess the overall effect of treatment on each measure. All significant ANOVAs were followed by Dunnett post hoc test of significance between means after MT-II treatments (1 and 3 mg/kg), as compared to saline. For all analyses, $P<0.05$ determined significance.

3. Results

3.1. Hops and darts, ear wiggling and solicitations

3.1.1. Females primed with EB+P

There was a significant main effect of treatment on hops and darts ($F(2,12)=6.76$, $P=0.01$). Post-hoc comparisons with Dunnett's test revealed that hops and darts were significantly increased by MT-II 1 mg/kg (38.00±2.56; $P<0.05$) and MT-II 3 mg/kg (43.86±6.13; $P<0.01$) when compared to saline (23.14±2.70) (Fig. 1, top left).

There was also a significant main effect of treatment on ear wiggling after MT-II 1 mg/kg (40.71±2.37) and 3 mg/kg

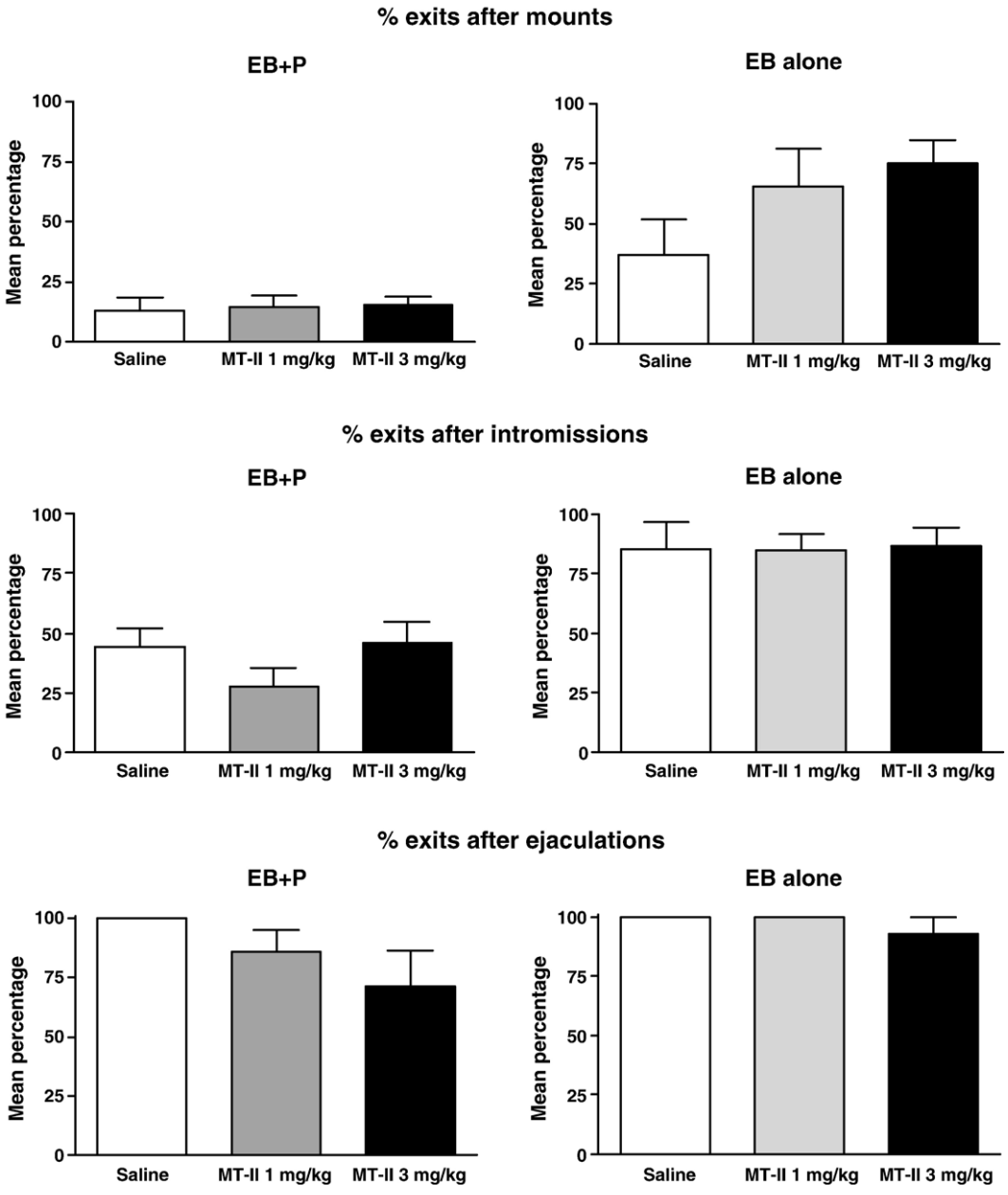

Fig. 2. Effect of MT-II or saline on the percentage of exits after mounts (top), intromissions (middle) and ejaculations (bottom) in OVX rats ($n=7$) primed with EB+P (left) or EB alone (right). Results are expressed as mean±S.E.M.

(40.29±5.94) when compared to saline (24.71±1.92;$P<0.05$), $F(2,12)=6.51$, $P=0.01$ (Fig. 1, middle left).

No significant effect of MT-II 1 mg/kg (3.29±1.06) and 3 mg/kg (3.71±0.94) was observed on solicitations ($F(2,12)=0.04$, $P=0.96$) when compared to saline (3.57±0.97) (Fig. 1, bottom left). Females did not display any rejection behaviors with any treatment during the paced mating test.

3.1.2. Females primed with EB alone

No significant effect of MT-II 1 mg/kg (16.29±2.25) and 3 mg/kg (15.29±3.64) was observed on darts and hops ($F(2,12)=0.45$, $P=0.65$) when compared to saline (13.29±1.90) (Fig. 1, top right). There was no significant effect of treatment on ear wiggling ($F(2,12)=0.036$, $P=0.96$) (Fig. 1 middle right).

No significant effect of MT-II 1 mg/kg (1.43±0.84) and 3 mg/kg (2.29±1.21) was observed on solicitations ($F(2,12)=0.25$, $P=0.78$) when compared to saline (1.57±1.41) (Fig. 1, bottom right). Females rarely displayed rejection behavior, whatever the treatment, during the paced mating test.

3.2. Pacing

3.2.1. Females primed with EB+P

Although the effect of MT-II on the proportion of exits after mounts, intromissions, or ejaculations did not reach statistical significance, there was a trend toward a dose-dependent decrease in the percentage of female exits after ejaculations following MT-II 1 and 3 mg/kg (85.71±9.22 and 71.43±14.87)

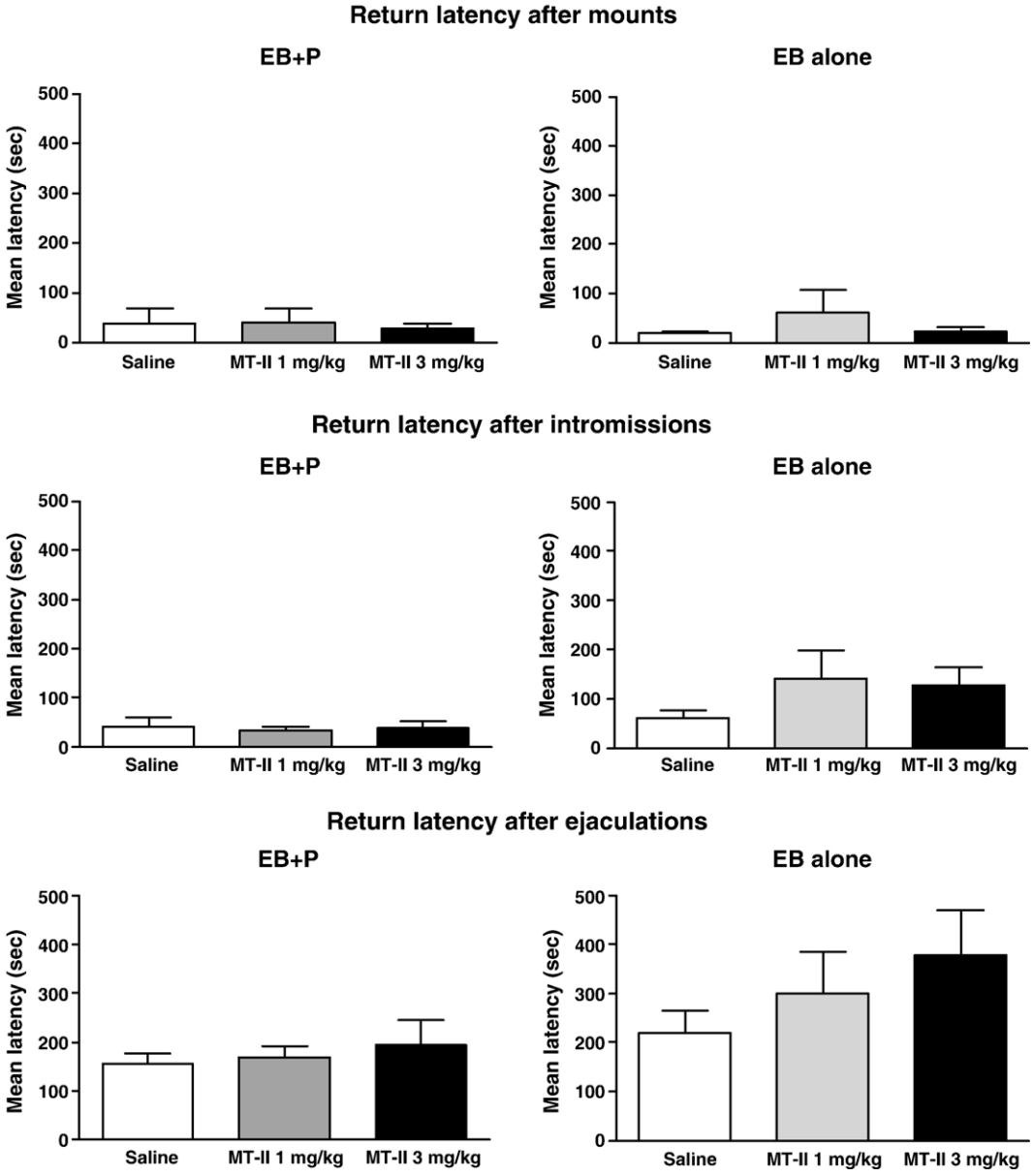

Fig. 3. Effect of MT-II or saline on the return latency after mounts (top), intromissions (middle), and ejaculations (bottom) in OVX rats ($n=7$) primed with EB+P (left) or EB alone (right). Results are expressed as mean±S.E.M.

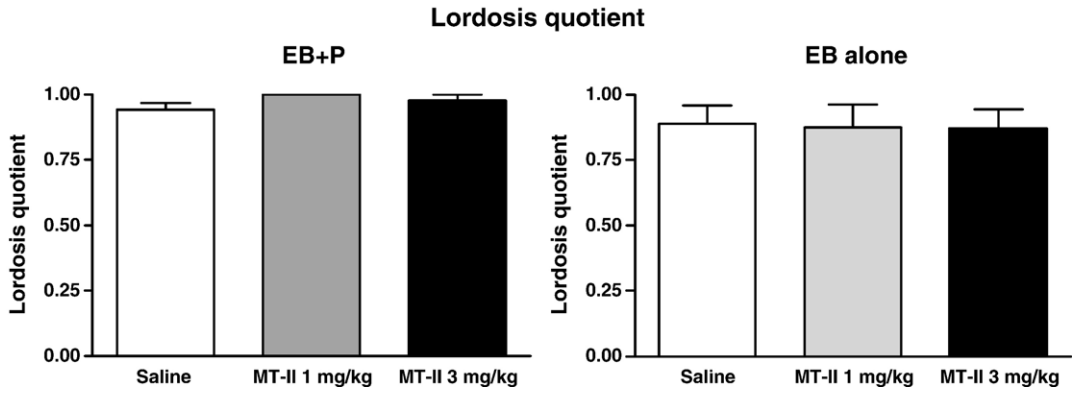

Fig. 4. Effect of MT-II or saline on the lordosis quotients of OVX rats ($n=7$) primed with EB+P (left) or EB alone (right). Results are expressed as mean±S.E.M.

when compared to saline (100±00) ($F(2,12)=2.4$, $P=0.13$) (Fig. 2, bottom left). There was no significant effect of MT-II on the female return latencies after mounts ($F(2,12)=0.09$, $P=0.92$), intromissions ($F(2,12)=0.26$, $P=0.77$), or ejaculations ($F(2,13)=0.38$, $P=0.69$) (Fig. 3 left).

3.2.2. Females primed with EB alone

There was no effect of MT-II on the proportion of female exits after mounts ($F(2,11)=1.95$, $P=0.19$), intromissions ($F(2,18)=0.01$, $P=0.99$), or ejaculations ($F(2,17)=0.92$, $P=0.42$) (Fig. 2, bottom right). Furthermore there was no significant effect of MT-II on the return latencies after mounts, $F(2,11)=0.59$, $P=0.57$, intromissions, $F(2,12)=1.72$, $P=0.22$, or ejaculations, $F(2,15)=1.16$, $P=0.34$ (Fig. 3 right).

3.3. Lordosis

3.3.1. Females primed with EB+P

There was a trend toward a significant increase in the lordosis quotient by MT-II, 1 mg/kg (1.00±0) and 3 mg/kg (0.98±0.02) when compared to saline (0.94±0.03), ($F(2,12)=3.25$, $P=0.07$) (Fig. 4, left).

3.3.2. Females primed with EB alone

There was no effect on lordosis of MT-II, 1 mg/kg (0.88±0.09) and 3 mg/kg (0.87±0.07) when compared to saline (0.89±0.07), ($F(2,12)=0.01$, $P=0.99$) (Fig. 4, right).

4. Discussion

In the present experiments, MT-II (1 and 3 mg/kg) enhanced appetitive proceptive sexual behaviors selectively in OVX rats primed with EB+P, but not in those primed with EB alone. In females primed with EB+P, MT-II increased hops and darts and ear wiggling, precopulatory behaviors that females use in close proximity to males to arouse them (Pfaus et al., 1999). PT-141 has also been reported to selectively stimulate solicitations and hops and darts in OVX rats primed not only with EB+P, but also with EB alone, at the same priming dosings as in the present experiments (Pfaus et al., 2004). With PT-141 (0, 50, 100, or 200 μg/kg s.c. delivered) no effect on pacing or lordosis was found in OVX rats primed with EB+P, or EB alone.

In contrast to PT-141, MT-II did not increase female solicitations. This difference is not due to different hormonal priming of the OVX females, because they were the same when both compounds were tested (Pfaus et al., 2004). Such a difference between PT-141 and MT-II could be due to differences in testing procedure, and in particular to the amount and type of female sexual experience, which might have altered the expression of solicitations in the present experiments. Indeed, we performed only 4 preliminary mating tests in unilevel pacing chambers, whereas in the study with PT-141 (Pfaus et al., 2004) 10 preliminary mating tests were performed in bilevel pacing chambers. In bilevel pacing chambers, solicitations and pacing (level changes) are used almost exclusively to regulate sexual contact (Pfaus et al., 1999). We note that in the present experiment, in females primed with EB+P, hops and darts were observed at a relatively high rate in contrast to the low rate of solicitations overall, and that fewer than half of the mounts or intromissions were followed within 30 s by an exit, regardless of saline or drug treatment. (see Fig. 2).

The influence of melanocortins on consummatory aspects of female sexual behavior has been known for more than two decades (Raible and Gorzalka, 1986; Thody et al., 1979, 1981). Infusions of α-MSH to the ventromedial nucleus of the hypothalamus (VMN) stimulated lordosis in OVX and adrenalectomized rats primed with either or EB+P or EB alone (Gonzalez et al., 1993; Cragnolini et al., 2000). It has been proposed that these effects may be mediated through MC3 receptors (Cragnolini et al., 2000). Alpha-MSH delivered into the median eminence of OVX and adrenalectomized female rats primed with 10 μg of EB increased lordosis (Scimonelli et al., 2000). Furthermore bilateral infusions of α-or γ-MSH in the medial preoptic area (mPOA) also increased lordosis in OVX rats primed with EB (Nocetto et al., 2004). These authors again proposed that MC3 receptors in this hypothalamic area could mediate the effects of MSH peptides on lordosis behavior.

The ability of MT-II and its metabolite PT-141 to increase certain proceptive behaviors (hops and darts, solicitations) in females primed with EB+P suggests that central MC pathways are part of the neurochemical network that evokes appetitive sexual behavior in female rats. MT-II is a 10-100 fold more potent agonist of MC3 and MC4 receptors than α-MSH (Al-Obeidi et al., 1989). The precise brain structures targeted by both MT-II and PT-141 when increasing female appetitive sexual behaviors still remain so far

unknown. However, MT-II's selective binding to central MC3 and MC4 receptors makes it plausible to consider hypothalamic and/or limbic sites of action, given that these regions are involved in the control of appetitive aspects of female sexual behavior (Mountjoy et al., 1994; Lindblom et al., 1998; Kishi et al., 2003). With in vitro receptor autoradiography ^{125}I-MT-II binding in the absence or presence of 0.5 μM unlabeled MT-II was reported in the mPOA and VMN (Trivedi et al., 2003), two hypothalamic structures involved in the control of female sexual behavior (Pfaff, 1999). Nevertheless, in vivo brain autoradiography, following systemic administration of ^{125}I-MT-II, showed significant labeling only in a group of circumventricular organs (CVOs) such as the subfornical organ, choroids plexus, median eminence, organum vasculosum of lamina terminalis and area postrema (Trivedi et al., 2003). It has been recently reported that s.c. administration of PT-141 (100 or 200 μg) activates the immediate-early gene product Fos in several hypothalamic and limbic structures important for sexual behavior, including the nucleus accumbens, piriform cortex, mPOA, hypothalamic paraventricular nucleus (PVN), and ventral tegmental area (VTA) (Gelez et al., 2005).

The absence of effect of MT-II in females primed with EB alone leads to the assumption that P might interact with MT-II to increase proceptive behaviors. Membrane progesterone binding sites have been evidenced in the same rat brain areas as those containing MC3 and MC4 receptors; e.g. hypothalamus and circumventricular organs (Meffre et al., 2005). In OVX rats, a basal level of progestin receptors has been detected in a variety of preoptic, hypothalamic and limbic structures (Parsons et al., 1982). In OVX rats, 10 μg EB alone raised levels of α-MSH in VMN, POA and arcuate nucleus but did not alter α-MSH in extra-hypothalamic areas. In OVX rats, the addition of P to EB raised the α-MSH levels in the septum, amygdala, hippocampus and caudate putamen (Medina et al., 1998), indicating that the melanocortinergic system is differentially impacted depending on the hormonal regimen and the brain areas examined. In OVX rats, drugs can induce behavioral effects on sexual activity after EB + P, but not after EB alone. For example, depletion of hypothalamic 5-HT levels by *p*-chlorophenylalanine methyl ester (PCPA), was shown to inhibit sexual activity in females normally induced by EB + P, but not in females made receptive by EB alone (Wilson and Hunter, 1985). There are other drugs that enhance female sexual behaviors only in EB + P-primed animals, but not in animals primed with EB alone, which indicates that an interaction with P or even P receptors is a fairly common mechanism for facilitation. Ocytocin, for example, markedly increased lordosis behavior in OVX (Gorzalka and Lester, 1987) or OVX + adrenalectomized (Schumacher et al., 1989) rats treated with EB and P, but not in rats treated with EB alone. Effects on proceptive behaviors were not investigated in the two mentioned studies.

Although sexual behavior greatly differs between rat and human, the effects of various pharmacological agents on erection are similar, if not identical, in male rats and men; and a number of drugs that alter dopamine or opioid function have homologous, or analogous effects on appetitive and consummatory aspects of male sexual behavior (Pfaus et al., 2003). This suggests that there are aspects of the neurochemistry and neuroanatomy of sexual responding that have been conserved across evolution in different mammalian species, and thus allows sexual responses of male rats to be used as models for human male sexual response. This applies in particular to the PT-141 and MT-II proerectile effect reported in male rats (Molinoff et al., 2003; Wessells et al., 2003; Giuliano et al., 2005) and in humans (Molinoff et al., 2003; Diamond et al., 2004; Wessells et al., 1998, 2000). It may also very well apply in the case of females when comparing the data with PT-141 in rats (Pfaus et al., 2004) and in premenopausal women with sexual arousal disorder (Diamond et al., 2006).

According to epidemiological studies, the most common complaint in women seeking treatment for sexual dysfunction is hypoactive sexual desire disorder (Lewis et al., 2004). According to the DSM-IV-TR (American Psychiatric Association, 2000), hypoactive desire disorder is characterized by persistently or recurrently deficient (or absent) sexual fantasies and desire for sexual activity. This set of symptoms has been reported in approximately 30% of women in population-based studies, and is associated with a wide variety of medical and psychological causes (Rosen, 2000). Considering that desire in women is homologous to appetitive motivation in animals (Ågmo et al., 2004), or to appetitive aspects of sexual activity in female rats (Pfaus et al., 2003), the present results reinforce the concept that non-selective melacortin agonists may, by targeting central melanocortin receptors, represent a promising pharmacological target for the treatment of women with hypoactive sexual desire disorder. Recent data from an early clinical trial with PT-141 reinforce this strategy (Diamond et al., 2006). One important question that remains unanswered is which subtype of MC receptor, i.e. MC3 and/or MC4, mediates the appetitive prosexual effects of MT-II and PT-141.

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