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Anabolic androgens restore mating after sexual satiety in male rats

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Abstract

Androgen receptors and estrogen receptors importantly participate in the neuroendocrine control of masculine mating behavior. Sexual satiety is the long term inhibition of masculine mating behavior after repeated ejaculations and is associated to changes in both androgen receptor and estrogen receptor-α expression. Androgen receptor expression is up-regulated by systemic chronic administration of anabolic androgens, 5α-dihydrotestosterone or estradiol benzoate. This study was carried out to investigate the effect of these treatments on sexual satiety development and recovery; additionally flutamide or tamoxifen treatments – alone or together with anabolic androgens – were also included. Chronic 15-day treatment with 5α-dihydrotestosterone (5 mg/kg) or tamoxifen (15 mg/kg) inhibited, whereas estradiol benzoate treatment (5 mg/kg) facilitated, mating behavior during sexual satiety development. The proportion of animals that ejaculated 48 h after sexual satiety was increased after 17-day treatment with a mixture of anabolic androgens containing 2 mg/kg testosterone propionate, 2 mg/kg nandrolone decanoate and 1 mg/kg boldenone undecylenate. This effect was only blocked by the combined administration of flutamide plus tamoxifen. The data suggest that anabolic androgens metabolites synergize to restore mating behavior after sexual satiety.

Keywords: Anabolic androgens; Estradiol; 5α-Dihydrotestosterone; Flutamide; Tamoxifen; Androgen receptor; Estrogen receptor; Sexual satiety

1. Introduction

In rats, as in several species, masculine sexual behavior gradually vanishes after castration. Treating castrated males with aromatizable androgens fully restores their copulatory behavior, while treatment with non-aromatizable androgens or estrogens only partially re-establishes mating behavior (McGinnis and Dreifuss, 1989; Sachs and Meisel, 1994). On the other hand, treating intact males with antagonists of the androgen receptor or the estrogen receptor only partially inhibits their copulatory behavior (Sachs and Meisel, 1994).

Sexual satiety is the long term inhibition of masculine reproductive behavior which appears after repeated ejaculations (Beach and Jordan, 1956). Sexually experienced rats allowed ad libitum copulation ejaculate several times, but they eventually cease to mate (Rodriguez-Manzo and Fernandez-Guasti, 1994). The development of sexual satiety refers to the series of copulatory bouts preceding the inhibition of sexual behavior. In male rats, sexual satiety after ad libitum mating is manifested in two ways in the following 24 or 48 h: two-thirds of the animals do not exhibit any mating behavior, while the remaining one-third display one ejaculation without resumption of reproductive behavior afterwards (Rodriguez-Manzo and Fernandez-Guasti, 1994; Romano-Torres et al., 2007). Pharmacological treatments, that affect either the monoaminergic or opioidergic systems, restore mating after sexual satiety by augmenting the proportion of animals showing one or more ejaculations 24 or 48 h after sexual satiety (Fernandez-Guasti and Rodriguez-Manzo, 2003). The proportion of males that ejaculate increases dramatically 72 h after sexual satiety; thus, the recovery from sexual satiety starts at this time (Romano-Torres et al., 2007). Male rats regain their total mating capacity only after 15 days of sexual inactivity (Beach and Jordan, 1956).

Recently we reported a reduction in androgen receptor density accompanied by an increase in estrogen receptor-α density 24 h after sexual satiety in some of the forebrain areas that regulate male reproductive behavior (Fernandez-Guasti et al., 2003; Phillips-Farfan et al., 2007). In fact, androgen receptor density in the medial preoptic nucleus was still decreased

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48 h after sexual satiety (Romano-Torres et al., 2007). These variations in steroid receptors were not accompanied by changes in the systemic levels of testosterone or estradiol and coincided with an inhibition of mating activity, as evidenced by 70% of the males that did not copulate. Furthermore, 72 h after sexual satiety androgen receptor density increased in several brain areas (bed nucleus of the stria terminalis, lateral septum, medial nucleus of the amygdala and ventromedial hypothalamic nucleus) concurring with an important recovery of copulatory behavior, since 70% of the males ejaculate (Romano-Torres et al., 2007). This over-expression of androgen receptor was not associated with changes in the levels of serum testosterone. The data suggest that androgen receptor up-regulation in several brain areas may partly underlie the recovery of mating behavior after sexual satiety.

Pharmacological studies have reported that chronic high-dose treatment with aromatizable anabolic androgens (testosterone, nandrolone and boldenone), 5α-dihydrotestosterone (a non-aromatizable androgen) or estradiol benzoate up-regulates androgen receptor expression in various brain areas (Lynch and Story, 2000; Menard and Harlan, 1993). Based upon these studies, the same treatments were administered to male rats to investigate their effect upon the development of sexual satiety and the recovery from it. We observed that the treatment with the anabolic androgens restored male mating behavior after sexual satiety. Since the anabolic androgens that were used are metabolized to other androgens or to estrogens (Clark and Henderson, 2003); the androgen receptor-antagonist, flutamide (Lu et al., 1999; Sodersten et al., 1975) or the estrogen receptor-modulator tamoxifen (Lund et al., 2005; Shughrue et al., 1997), was administered alone or combined with the anabolic androgens to study the mechanism underlying their action.

2. Materials and methods

2.1. Animals

Adult male ($n=78$, 200–240 g) and female ($n=90$, 180–220 g) albino Wistar rats were kept separately four to eight per cage, with ad libitum access to rat chow and tap water, under an inverted light–dark cycle (12 h each, lights off: 10 AM) at 22 °C. Rat management was done according to the general principles of NIH publication 85-23, 1985. All the experiments were approved by the local committee of ethics on animal experimentation.

2.2. Compounds

Testosterone propionate, 5α-dihydrotestosterone, estradiol benzoate, flutamide, tamoxifen and progesterone were bought from Sigma-Aldrich Co. Nandrolone decanoate (Deca-Durabolin®) and boldenone undecylenate (Equi-gan®) were purchased, respectively, from Organon Mexicana S.A. de C.V. and Laboratorios Tornel S.A. All compounds were diluted in corn oil and injected s.c. chronically for 17 days in a volume of 1 ml/kg. Treatments, doses and schedules were based on previous studies (Lund et al., 2005; Lynch and Story, 2000; Menard and Harlan, 1993; Sodersten et al., 1975).

2.3. Training tests

After adapting at least for 1 week to the inverted light–dark cycle, all males were given eight mating training tests twice weekly. All the observations were done 1 h after the onset of darkness and under dim red light. All males were transferred, one each, to cylindrical observation cages. After a 5 min adaptation period, one receptive female was placed with each animal. Female receptivity was induced by sequential s.c. injections of estradiol benzoate (8 μg/rat) followed by progesterone (2 mg/rat) 24 and 4 h before the tests, respectively. The copulatory behavior parameters recorded were: the intromission latency, the number of mounts and intromissions and the ejaculation latency. The female was removed after the male displayed a single ejaculation or after a 30 min period if the animal did not show an intromission. Only the males that showed ejaculation latencies shorter than 15 min in at least half of the tests were used for further experiments.

2.4. Experimental treatments and observations

Chronic administration of compounds was started at least 4 days after the last training test and 39 days after acclimatizing the males to the inverted dark–light cycle condition. Nine independent groups were utilized, each received a different chronic treatment, as follows: corn oil ($n=8$), 5 mg/kg 5α-dihydrotestosterone ($n=5$), a mixture of anabolic androgens: 2 mg/kg testosterone propionate plus 2 mg/kg nandrolone decanoate plus 1 mg/kg boldenone undecylenate ($n=15$), 5 mg/kg estradiol benzoate ($n=6$), 50 mg/kg flutamide ($n=10$), 15 mg/kg tamoxifen ($n=7$), the anabolic androgens and flutamide ($n=10$), the anabolic androgens and tamoxifen ($n=8$) or the anabolic androgens plus flutamide and tamoxifen ($n=9$).

Animals were weighted every day of chronic treatment to adjust the injection volume according to the animal's weight. The anabolic androgens were mixed prior to injection, but all other compounds were injected separately. On day 15 of chronic treatment, the development of sexual satiety was observed. Animals were allowed to ejaculate ad libitum until they displayed a 90 min period without an ejaculation (sexual satiety criterion). Sexual satiety recovery was observed on day 17 of chronic treatment (i.e. 48 h after the males copulated to satiety); those animals that did not copulate were observed for 45 min, while those that did mate were observed until they showed a 60 min interval without an ejaculation. The mating behavior parameters recorded on days 15 and 17 were: the intromission latency, the number of ejaculations, the satiation latency (the time from the first intromission of the first mating series to the last ejaculation or last intromission) and the number of mounts and intromissions, the ejaculation latencies, the post-ejaculatory intervals and the proportion of animals ejaculating in each mating series.

2.5. Statistical tests

To test whether the weight changes were significantly different from either the basal condition (day 1) and from the oil-treated group, a two-way ANOVA followed by a Holm–Sidak test was

performed, where one factor was treatment and the other was day (group mean-weight on days 3, 6, 9, 11, 13, 15 and 17 normalized to group mean-weight on day 1).

For every group, the proportion of males that ejaculated in each mating series displayed during both the development of and recovery from sexual satiety was compared pair-wise against all other groups' proportions utilizing the Fisher *F* exact probability test. Chronic estradiol benzoate, 5α-dihydrotestosterone or tamoxifen administration affected the length of post-ejaculatory intervals and the number of ejaculations during sexual satiety development; thus, Mann–Whitney Rank Sum tests were used to compare the effects of these treatments versus the oil-treated control group.

3. Results

Chronic estradiol benzoate treatment significantly reduced the subjects' weight by 45.2±3.4 g (mean±SE) after 17 days. All other groups gained weight (28.5±2 g) and none gained significantly more weight than the controls. Thus, the two-way ANOVA revealed a significant difference for treatment ($F_{(8,56)}=11.89$, $p\leq 0.001$) and for day ($F_{(7,56)}=5.69$, $p\leq 0.001$). No significant interaction between these factors was found.

Table 1 shows the proportion of males that ejaculated in each mating series shown during the development of sexual satiety. As can be seen, chronic 5α-dihydrotestosterone or tamoxifen administration significantly reduced the proportion of males that ejaculated in the fifth (Fisher *F* test: 5α-dihydrotestosterone vs. oil $p=0.035$, tamoxifen vs. oil $p\leq 0.001$) and sixth (5α-dihydrotestosterone vs. oil $p\leq 0.001$, tamoxifen vs. oil $p\leq 0.001$) copulatory series. Indeed, the tamoxifen effect persisted even if animals were treated with anabolic androgens since the proportion of animals that ejaculated in the fifth (anabolic androgens+tamoxifen vs. oil $p=0.007$) and sixth (anabolic androgens+tamoxifen vs. oil $p=0.001$) mating series was significantly lower than that of the oil-treated control group. The proportion of males that ejaculated in the sixth copulatory series after treatment with anabolic androgens plus flutamide and tamoxifen was also significantly smaller than that shown by the control group (anabolic androgens+flutamide+tamoxifen vs. oil $p=0.002$). On the other hand, the proportion of estradiol benzoate-treated animals that ejaculated was significantly higher than that shown by the control group (estradiol benzoate vs. oil $p=0.015$) from the eighth to the eleventh mating series. The average number of ejaculations before sexual satiety was significantly reduced by the chronic treatments with 5α-dihydrotestosterone (Mann–Whitney *U* test: 4.6±0.4 vs. oil 6.4±0.2, $p=0.006$) or tamoxifen (3.6±0.2 vs. oil 6.4±0.2, $p\leq 0.001$), and was increased by chronic administration of estradiol benzoate (9.8±1.7 vs. oil 6.4±0.2, $p=0.059$).

Table 1
Effect of various treatments on the proportion of males that ejaculate (E) in each mating series (1–14) displayed during sexual satiety development

	Oil	Tam	Flu	EB	DHT	AA	AA+Tam	AA+Flu	AA+Flu+Tam
E1	8/8	7/7	10/10	6/6	5/5	15/15	8/8	10/10	9/9
E2	8/8	7/7	10/10	6/6	5/5	15/15	8/8	10/10	9/9
E3	8/8	7/7	10/10	6/6	5/5	14/15	8/8	10/10	9/9
E4	8/8	4/7	9/10	5/6	5/5	14/15	7/8	9/10	9/9
E5	8/8	0/7*	8/10	5/6	2/5*	13/15	2/8*	7/10	5/9
E6	8/8	0/7*	7/10	5/6	1/5*	10/15	1/8*	6/10	2/9*
E7	3/8	0/7	2/10	5/6	0/5	6/15	0/8	2/10	0/9
E8	0/8	0/7	0/10	4/6*	0/5	0/15	0/8	1/10	0/9
E9	0/8	0/7	0/10	4/6*	0/5	0/15	0/8	0/10	0/9
E10	0/8	0/7	0/10	4/6*	0/5	0/15	0/8	0/10	0/9
E11	0/8	0/7	0/10	4/6*	0/5	0/15	0/8	0/10	0/9
E12	0/8	0/7	0/10	3/6	0/5	0/15	0/8	0/10	0/9
E13	0/8	0/7	0/10	1/6	0/5	0/15	0/8	0/10	0/9
E14	0/8	0/7	0/10	1/6	0/5	0/15	0/8	0/10	0/9

Abbreviations: Tam, tamoxifen; Flu, flutamide; EB, estradiol benzoate; DHT, 5α-dihydrotestosterone; AA, a mixture of the anabolic androgens: testosterone propionate, nandrolone decanoate and boldenone undecylenate. Fisher's *F* test values vs. the control oil-treated group: *$p\leq 0.05$.

The length of each post-ejaculatory interval during sexual satiety development was dramatically affected by chronic administration of tamoxifen or estradiol benzoate (Fig. 1). Tamoxifen treatment significantly increased the duration of the post-ejaculatory interval on the first (Mann–Whitney *U* test vs. the oil-treated group, $p\leq 0.001$), second ($p\leq 0.001$) and third ($p=0.004$) copulatory series; while estradiol benzoate administration had the opposite effect on the fourth (Mann–Whitney *U* test vs. the oil-treated group, $p=0.003$), fifth ($p=0.011$) and sixth ($p=0.016$) copulatory series. In fact, the exponential increase in the length of each post-ejaculatory interval which normally occurs during the development of sexual satiety (Rodriguez-Manzo and Fernandez-Guasti, 1994) was drastically blunted by chronic estradiol benzoate treatment. All the other treatments did not affect any parameter of mating behavior during sexual satiety development (data not shown).

Fig. 2 shows the proportion of animals that ejaculated 2 days after sexual satiety. As previously shown (Romano-Torres et al., 2007), 38% of the control animals ejaculated once and did not recover sexual behavior afterwards; thus, none (0%) of the oil-treated animals ejaculated two times 48 h after sexual satiety. Chronic tamoxifen, flutamide, estradiol benzoate or 5α-dihydrotestosterone treatments did not modify the proportion of males that ejaculated 48 h after sexual satiety. Chronic administration of anabolic androgens dramatically increased the proportion of males that ejaculated 48 h after sexual satiety. In fact, 87% of the treated animals displayed one ejaculation (anabolic androgens vs. oil, Fisher *F* test: $p=0.026$), 80% showed a second ejaculation (anabolic androgens vs. oil, $p=0.021$) and 40% exhibited a third ejaculation (data not shown). The drastic action of anabolic androgens on the proportion of males that ejaculated was not mimicked by estradiol benzoate or 5α-dihydrotestosterone. Chronic treatment with either flutamide or tamoxifen did not prevent the anabolic androgen effect on the first copulatory series; since the proportion of animals that ejaculated (anabolic androgens+tamoxifen vs. oil, Fisher *F* test: $p=0.026$; anabolic androgens+flutamide vs. oil, $p=0.043$) was still significantly higher than that shown by the control oil-treated group. However, chronic tamoxifen administration antagonized the effect of anabolic androgens on the second copulatory series by reducing the proportion of animals that ejaculated (anabolic androgens vs. anabolic

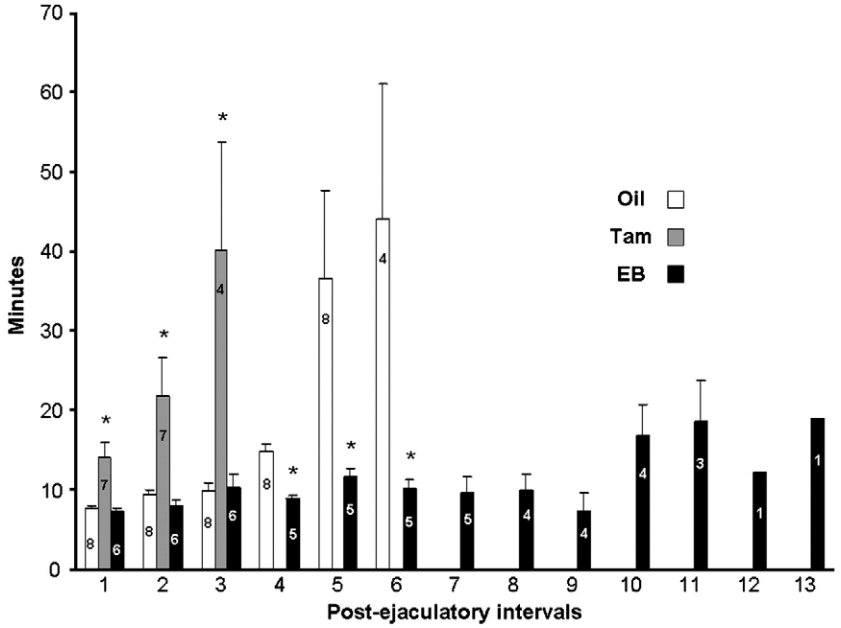

Fig. 1. Effect of chronic administration of oil, tamoxifen (Tam) or estradiol benzoate (EB) on the length of each post-ejaculatory interval in the successive ejaculations preceding sexual satiety. The bars represent the mean ± SE, the *n* is shown inside each bar. Mann–Whitney *U* test vs. the control oil-treated group $^{*}p \leq 0.05$.

androgens + tamoxifen, Fisher *F* test: $p = 0.023$). On the other hand, chronic flutamide plus tamoxifen treatment completely antagonized the anabolic androgen effect by reducing the proportion of rats that ejaculated in the first mating series (anabolic androgens + flutamide + tamoxifen vs. anabolic androgens, Fisher *F* test: $p = 0.021$, anabolic androgens + flutamide + tamoxifen vs. anabolic androgens + flutamide $p = 0.020$, anabolic androgens + flutamide + tamoxifen vs. anabolic androgens + tamoxifen $p = 0.009$) and in the second mating series (anabolic androgens + flutamide + tamoxifen vs. anabolic androgens, Fisher *F* test: $p \leq 0.001$, anabolic androgens + flutamide + tamoxifen vs. anabolic androgens + flutamide $p \leq 0.001$).

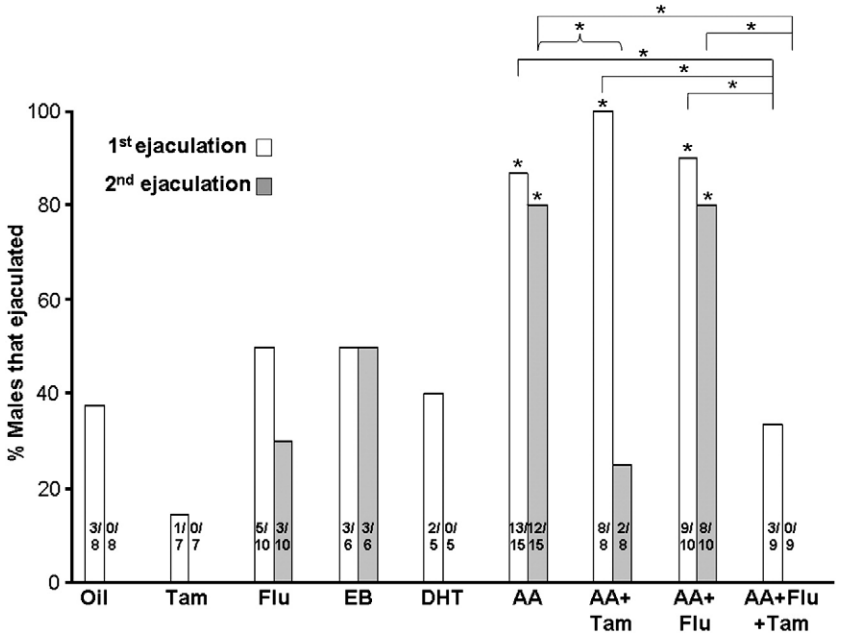

Fig. 2. Effect of different chronic treatments on the percentage of males that ejaculated one or two times 48 h after sexual satiety. The proportion is shown inside each bar. The asterisks above the bars represent the comparisons vs. the oil-treated control group. The square brackets represent the comparisons vs. anabolic androgens + flutamide + tamoxifen. The curly bracket represents the comparison between anabolic androgens + tamoxifen vs. anabolic androgens for the second ejaculation. Fisher's *F* test, $^{*}p \leq 0.05$ (exact probabilities are in the Results section). Abbreviations: Tam, tamoxifen; Flu, flutamide; EB, estradiol benzoate; DHT, 5α-dihydrotestosterone; AA, a mixture of anabolic androgens.

4. Discussion

The main findings of this study were that chronic estradiol benzoate administration increased the proportion of males that ejaculated and the number of ejaculations, whereas it reduced the length of each post-ejaculatory interval during sexual satiety development. Tamoxifen treatment produced the opposite effects: it diminished the proportion of animals that ejaculated and the number of ejaculations, while augmenting the duration of each post-ejaculatory interval before the onset of sexual satiety. 5α-dihydrotestosterone administration reduced the proportion of males that ejaculated and the number of ejaculations during the development of sexual satiety. On the other hand, chronic anabolic androgen treatment restored mating behavior 2 days after sexual satiety and this effect was only antagonized by administration of flutamide and tamoxifen combined.

The mechanism underlying the action of estradiol benzoate, 5α-dihydrotestosterone or tamoxifen on sexual satiety development is unknown. The effect of chronic estradiol benzoate on the length of each post-ejaculatory interval resembles the action of GABAergic antagonists injected directly into the medial preoptic area (Fernandez-Guasti et al., 1986). In fact, estradiol reduces GABAergic transmission in the substantia nigra (Nicoletti and Meek, 1985) and the hippocampus (Murphy et al., 1998). To the contrary, tamoxifen (Chesnoy-Marchais, 2003) and 5α-3α-androstanediol – a 3α-reduced metabolite of 5α-dihydrotestosterone – (Aikey et al., 2002; Rosellini et al., 2001) enhance GABAergic transmission; that, in turn, may inhibit male mating behavior (Fernandez-Guasti et al., 1986). The inhibitory effect of 5α-dihydrotestosterone upon the development of sexual satiety was surprising, since it partly facilitates sexual behavior in castrated males (Sachs and Meisel, 1994). These differences may be due to the dose used and the endocrine condition of the animals.

Regarding the recovery from sexual satiety, we observed that most (80%) of the animals chronically treated with the anabolic androgens ejaculated twice 48 h later, while only 38% of the males in the control group displayed a single ejaculation without recovering mating behavior afterwards. The dramatic effect of anabolic androgens may have been mediated by steroid-receptor-independent processes (Bitran et al., 1993; Clark and Henderson, 2003); however, combined treatment with flutamide and tamoxifen effectively cancelled the action of the anabolic androgens; suggesting its dependence upon steroid receptors. Among many other possibilities, two non-exclusive mechanisms may be proposed: one entails synergism between androgenic and estrogenic metabolites, and the other involves a specific androgen receptor over-expression pattern.

Regarding the former, previous reports showed that estrogens may act upon the androgen receptor (Roselli and Fasasi, 1992; Tyagi et al., 2000; Yeh et al., 1998) and also that androgens might affect estrogen receptor function (Brown et al., 1994; Panet-Raymond et al., 2000; Thakur and Sharma, 2007). For example, chronic estradiol benzoate treatment can up-regulate androgen receptor expression in many brain areas (Lynch and Story, 2000); whereas 5α-dihydrotestosterone and estradiol synergize to down-regulate estrogen receptor levels in specific brain regions (Brown et al., 1996). Together, all these data suggest that the androgenic and estrogenic metabolites of the anabolic androgens may have synergized on either the androgen receptor or the estrogen receptor (which ever was not blocked) to change its expression and/or function to promote the recovery of reproductive behavior after sexual satiety. These data may explain the observation that both steroid receptors antagonists are required to block the anabolic androgen action.

As to a specific androgen receptor over-expression pattern, it was shown that chronic anabolic androgen administration up-regulates androgen receptor expression in the medial preoptic area (MPOA), medial nucleus of the amygdala (MeA), ventromedial hypothalamic nucleus (VMN) and in other brain areas (Lynch and Story, 2000; Menard and Harlan, 1993). 5α-dihydrotestosterone (a non-aromatizable androgen) treatment up-regulated androgen receptor expression to a similar extent in the MPOA, but to a lesser degree in both the MeA and VMN compared to the action of the anabolic androgens (Lynch and Story, 2000). Estradiol benzoate treatment up-regulated androgen receptor expression in the MeA and VMN to an even lesser extent than 5α-dihydrotestosterone and completely lacked an effect on the androgen receptor in the MPOA (Lynch and Story, 2000). These results suggest that a particular androgen receptor over-expression pattern was produced by anabolic androgens, and not by 5α-dihydrotestosterone or estradiol benzoate, and that this pattern may partly underlie the restoration of mating after sexual satiety. In support, the physiologically-mediated androgen receptor up-regulation that occurs 72 h after sexual satiety – coinciding with a significant recovery of mating behavior – (Romano-Torres et al., 2007) is very similar to the androgen receptor over-expression induced by anabolic androgens.

Interestingly, chronic anabolic androgen treatment only affected the recovery from sexual satiety and not its development. This result suggests that anabolic androgens specifically affect sexual motivation and not sexual performance. In support, chronic treatment with testosterone (Lumia et al., 1994) or anabolic androgens (Bronson, 1996; Clark et al., 1997) minimally affected the display of mating behavior in intact adult males, while chronic treatment of pre-pubertal animals with testosterone (Feinberg et al., 1997; Wesson and McGinnis, 2006) significantly increased the percentage of rats that ejaculated (an evidence for an increased sexual motivation). These results suggest that pharmacological androgen receptor manipulation by anabolic androgen treatment primarily affects the motivational aspects of masculine mating behavior, which are importantly reduced during sexual satiety (Agmo et al., 2004; Rodriguez-Manzo, 1999). Chronic treatment with anabolic androgens should be tested in other models that might reflect decreased sexual motivation.

In summary, the present results show that 5α-dihydrotestosterone and tamoxifen had inhibitory effects and estradiol benzoate had stimulatory actions upon mating behavior before the onset of sexual satiety. Chronic administration of anabolic androgens (testosterone, nandrolone and boldenone) restored mating after sexual satiety by increasing dramatically the proportion of males that ejaculated. The latter effect probably

involves the synergistic action of androgenic and estrogenic metabolites upon either the androgen receptor or the estrogen receptor, since it was only antagonized after combining flutamide plus tamoxifen. The behavioral effects of the anabolic androgens were presumably underlied by a unique pattern of androgen receptor up-regulation in brain areas involved in the control of sexual satiety.

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