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Testosterone prevents but not reverses anhedonia in middle-aged males and lacks an effect on stress vulnerability in young adults

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ABSTRACT

Middle-aged male rats are more vulnerable than young adult ones to develop anhedonia when exposed to chronic mild stress (CMS). Clinical studies support the idea that in aged subjects the low testosterone (T) levels are related with their higher stress vulnerability and that this hormone possesses antidepressant-like actions. In this study we evaluated the role of gonadal hormones — mainly T — on the depressive-like behavior of middle-aged and young adult male rats submitted to CMS. In middle-aged rats we analyzed the effect of T restitution (at the levels of young adult animals) given 3 weeks before (experiment 1) or 3 weeks after (experiment 2) anhedonia development (indicated by a reduction in sucrose solution intake). T restitution before CMS effectively prevented anhedonia but failed to reverse it once installed. In young adult rats we studied if orchidectomy increased stress vulnerability and found that it failed to modify sucrose intake. These results indicate a stress-dependent differential effect of T in middle-aged rats an age differential role of gonadal hormones on the vulnerability to develop anhedonia. The results suggest that T is a resilience factor in middle-aged but not in young adult males.

Introduction

Depression is the most common psychiatric disease in the elderly with a prevalence ranging from 22 to 46% in patients over 65 years old (Lebowitz et al., 1997). This disorder is characterized by anhedonia (incapacity to experience pleasure), feelings of sadness, depressed mood and guilt (American Psychiatric Association, 2000). It is estimated that by the year 2020, depression will be the second cause of disability and mortality worldwide (Murray and Lopez, 1997). In the USA, the highest rate of suicides associated to depression is found in men older than 65 years and this rate doubles at around 85 years of age (Reynolds and Kupfer, 1999). These features make depression in late life a major public concern that acquires more importance as world life expectancy increases (United Nations, 2010).

Aging is a complex process (Lamberts et al., 1997; Smith et al., 2005) that involves changes in various neuroendocrine systems that have been associated with depression. There are numerous reports describing that aged men, in contrast with young, can develop depression when exposed to everyday life stress (Bogdan and Pizzagalli, 2006; Millom and Davis, 1999; Plotsky et al., 1998) and that as aging advances there is a steady reduction in testosterone (T) levels as a result of an alteration of the hypothalamus–pituitary–gonadal (HPG) axis (Lamberts et al., 1997). Interestingly, such reduction in T levels (<2.5 ng/ml) in old men predicts a higher incidence of depressive illness (Shores et al., 2005). The relevance of T on vulnerability to depression is supported by several clinical studies in aged or young adult hypogonadal men; in this line, it has been shown that in men aged 50–89 the severity of depression (indicated by high scores in the Beck Depression Inventory) increases with age and is inversely related to bioavailable T (Barret-Connor et al., 1999). There are also evidences showing that, in hypogonadal men (22–62 years old), low serum T levels are related with negative mood symptoms and these correlations disappeared after T replacement therapy (Wang et al., 1996). Accordingly, Shores et al. (2004) found that T treated hypogonadal patients (mean age 65 years) were 2 to 3 times less susceptible to develop depression than untreated patients. Furthermore, a study done to examine the effectiveness of T replacement as an antidepressant treatment shows that aged (more than 50 years old) eugonadal men with late-life depression (first episode after 45 years of age) responded better to T treatment than those with early-life first-episode depression (Perry et al., 2002). These data suggest that the levels of T in males are inversely related to depression vulnerability, and that T restitution could reverse this pathology; however, there is not a direct study demonstrating this relationship.

Chronic mild stress (CMS) is an animal model used to study the neurobiological bases of depression (Willner, 1997). This model simulates anhedonia, a core symptom of depression, which is reflected as a decrease in sucrose solution consumption produced by the chronic

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exposure to mild stressors (Willner, 1997, 2005; Willner et al., 1987). It has been consistently observed that the stress-dependent sucrose-intake reduction is specifically reversed by antidepressants and steroid hormones with antidepressant properties (Montgomery et al., 2001; Romano-Torres and Fernández-Guasti, 2010; Willner, 1997).

In a previous study we found that, after CMS, a higher percentage — 73% — of middle-aged rats (13–15 months) developed anhedonia as compared with only a 35% of young adult ones (3–5 months). In addition, middle-aged male rats presented around a fourth of the basal levels of T and estradiol than young adult animals (Herrera-Pérez et al., 2008). These data, as clinical studies, suggest that the increased susceptibility to develop anhedonia is inversely related to the levels of male gonadal hormones.

This study was aimed to evaluate the role of gonadal hormones — mainly T — on the depressive-like behavior of middle-aged and young adult male rats submitted to the CMS. In middle-aged rats we analyzed the effect of T restitution (at the levels found in young adult animals) given before (experiment 1) and after (experiment 2) anhedonia development. Results showed that T replacement prevented, but not reversed, the anhedonic action of CMS. On these bases, in a third experiment we studied in young adult rats if the withdrawal of gonadal hormones — by orchidectomy — increased stress vulnerability and found that this surgery failed to reduce sucrose intake.

Materials and methods

Animals

Adult young and middle-aged (3–5 and 12–15 months, respectively) male Wistar rats were obtained from the *Instituto Nacional de Psiquiatría "Ramón de la Fuente Muñiz"*. Animals were individually housed in small cages (35×25×15 cm) one week before starting the experiment and maintained on a 12:12 h dark–light inverted cycle (lights off at 10:00 h), with free access to water and food, under controlled temperature and humidity. These conditions varied according to requirements of the CMS procedure. Animal management was done following the general principles of laboratory animal care (NIH publication 85-23, 1985). All experimental procedures were performed in accordance with the Mexican official norm for animal care and handling (NOM-062-ZOO-1999) and approved by the Ethical Committee of the "CINVESTAV-IPN" and *Instituto Nacional de Psiquiatría "Ramón de la Fuente Muñiz"*. All efforts were made to minimize the number of animals used and their suffering.

Testosterone restitution of middle-aged rats

To restore the T levels of middle-aged animals to those found in young adults we used T-containing pellets, which were prepared with polydimethyl silicone tubes (Silastic Rx 50, Dow Corning, ID: 1.57 mm, OD: 3.18 mm). The tubes, of 1 cm long, were filled with T propionate (~9 mg, Sigma Chemicals) and their ends were sealed with pure silicone. These pellets were individually placed in the rats' cervical region under tribromoethanol (200 mg/kg, Sigma-Aldrich) anesthesia. The incision was sutured and cleaned with antiseptic solution and the animals returned to their home cages.

In order to determine the time-course of hormone delivery of these T pellets *in vivo*, groups (n = 4–5) of unstressed middle-aged animals were sacrificed by decapitation at different intervals (3, 8, 15, 21 and 29 days) after the T pellet placement; then their trunk blood was collected in cold tubes for determining T serum levels, as described below.

Once established the suitability of the T restitution method, another group of middle-aged males received T pellets and was left undisturbed for one week before starting the sucrose consumption training. Thereafter these animals were subjected to the CMS as described below. Proper controls were included (see experiment 1 in Fig. 1).

To evaluate the antidepressant-like effect of T, a group of anhedonic middle-aged rats (*i.e.* after 3 weeks of CMS exposure) received a T pellet. In parallel, an unstressed control group of rats received the pellet 3 weeks after baseline sucrose consumption determination (see experiment 2 in Fig. 1).

Orchidectomy in young adult rats

Young adult males were orchidectomized under anesthesia with tribromoethanol (200 mg/kg, Sigma-Aldrich). Briefly, a single midline incision was made in the low abdominal area to expose the testes; the vas deferens was bilaterally ligated and the testes were removed. The muscle and skin were sutured and the ventral area was cleaned with an antiseptic solution. Animals were returned to their home cages following surgery and left one week of recovery before sucrose consumption training (see experiment 3 in Fig. 1). In order to evaluate the T levels of orchidectomized young rats, a group was left undisturbed for three weeks after surgery and then sacrificed.

Chronic mild stress model

Sucrose consumption training

Rats were allowed to adapt to the taste of a palatable sucrose solution (1%) for two consecutive weeks. During this period, 1 h every day, a bottle containing sucrose solution was presented to the rats at the beginning of the dark phase (10:00 h). After these two weeks, the baseline sucrose consumption was determined. For this purpose, the rats were water and food deprived for 20 h and thereafter presented with two bottles for 1 h: one containing sucrose solution (1%) and the other tap water (to control for non specific liquid intake changes). Fluid (sucrose solution or tap water) consumption was calculated by weighing the bottles before and after the test. In the CMS paradigm the animals were exposed, for 3–7 weeks, to several stressors: white noise (~90 dB), overcrowding (2–3 animals per cage), continuous light, soiled cage (250 ml water spilled into bedding), stroboscopic light (300 flashes/min), 45° cage tilt along the vertical axis and water deprivation. The stressors' schedule followed in this study (Table 1) has been reported to induce anhedonia in young adult and middle-aged rats (Herrera-Pérez et al., 2008). The males of the unstressed groups were maintained for three weeks without stress, but under comparable handling and storage conditions than the stressed animals.

In all groups, the rats' sucrose solution and tap water consumption was determined weekly after a 20 h period of water and food deprivation (TEST, Table 1 for the stressed groups). This TEST consisted of an hour exposure to two bottles: one containing sucrose solution and the other containing tap water. The anhedonic state generated on the rats by the CMS was reflected as a reduction in their sucrose consumption of at least 2 g (Herrera-Pérez et al., 2008). Classification of the animals as anhedonic or no anhedonic was done after 3 weeks of CMS, on the basis of their weekly-evaluated sucrose solution intake. To avoid changes determined by differences in baseline sucrose-intake levels, individual sucrose consumption data were expressed as relative sucrose intake, which was calculated by dividing the sucrose intake at a given time between the baseline sucrose consumption.

Experimental design

Fig. 1 shows the time flow for the different experimental manipulations.

Experiment 1: preventive effect of testosterone restitution on stress vulnerability of middle-aged rats. Middle-aged male rats (intact or with T restitution) were divided into two groups, control condition or exposed to CMS, matched for similar baseline sucrose consumption. In