Regular article

Sexual behavior attenuates the effects of chronic stress in body weight, testes, sexual accessory glands, and plasma testosterone in male rats

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ARTICLE INFO

ABSTRACT

The aim of this study was to evaluate whether continuous sexual behavior could attenuate the effects of chronic stress on spermatogenesis, sexual glands, plasma testosterone and corticosterone in sexually experienced male rats. Rats were exposed to stress by immersion in cold water (ICW) daily for 20 or 50 consecutive days. Plasma testosterone and corticosterone, masculine sexual behavior, as well as the number of offspring, the epithelial area of seminiferous, prostatic and seminal glands were assessed. In stressed males, body and testicular weights decreased, male sexual behavior was disrupted, and adrenal weights increased. In males stressed for 50 days, prostate and seminal glands had lower weights compared with controls. Prostate and seminal epithelial areas also decreased in these males. Seminiferous tubules in testes from rats stressed for 20 or 50 days showed several degenerative signs, such as vacuoles in the basal epithelium, with picnotic indicia; moderate to severe exfoliation of degenerative germinal cells in the tubule lumen was also observed. In males stressed for 50 days a significant decrease in seminiferous epithelial area was observed from stages I–VIII, regardless of copulation. The litters from females that copulated with males stressed for 50 days decreased significantly. Chronic stress caused increase in plasma levels of corticosterone, which were higher in males stressed for 20 days than in males stressed for 50 days. Testosterone decreased in stressed males and it was lower in males stressed for 50 days. In stressed males allowed to copulate, body and testicular weights were similar to controls. Adrenal, seminal glands, and prostate weights, as well as epithelial areas of males stressed for 50 days allowed to copulate were also similar to controls. Corticosterone was lower than in males stressed for 50 days, but still higher than in controls. Testosterone in males stressed for 50 days and allowed to copulate was higher than in stressed males not allowed to copulate and control males without copulation, but still lower than in control copulating males. These results show that chronic stress causes germ cell loss in testes and a decrease in prostate and seminal epithelium, possibly as a result of testosterone decrease, affecting fertility. Continuous copulation can attenuate the effects of stress on testosterone levels and on the epithelial area in male sexual glands, but not on the seminiferous epithelium after 50 days of stress.

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Introduction

The stress response to physical or psychological stressors can disrupt reproductive function. This disruption has been attributed to the activation of the hypothalamus–pituitary–adrenal (HPA) axis, which is associated with the inhibition of the hypothalamus–pituitary–gonadal (HPG) axis (Selye, 1946; Charmandari et al., 2005; Chrousos, 2007). Thus, the increase in corticosteroids during stress suppresses gonadotropin releasing hormone (GnRH) in the hypothalamus, as well as the release of gonadotropins from the pituitary gland (Gore et al., 2006), and

gonadal function, thus decreasing steroid hormone synthesis and inducing apoptosis (Whirledge and Cidlowski, 2010).

Stress is considered one of the factors that causes idiopathic infertility in male humans (for review, see Sheiner et al., 2003), reduces sperm count (Collodel et al., 2008; Lenzi et al., 2003), motility (Fukuda et al., 1996), and probably causes morphological defects (Auger et al., 2001), which can be causes of lower fecundity in men, mainly in those with low sperm concentration (Hjollund et al., 2004). It is known that reproduction in males can be disrupted by both acute (Fukuda et al., 1996) and chronic stressors (Sheiner et al., 2003; Wingfield and Sapolsky, 2003). The magnitude of the effects on reproduction also depends on the type and duration of the stressor (Retana-Márquez et al., 1996). Therefore, infertility has been a matter of research using animal models and different types of stressors to find what the effects of acute and chronic stress are in the testes and sperm quality. In rats, direct cooling

of the testes with Ringer solution at 0–1 °C for 60 min results in depletion of most germ cells in vivo 3–10 weeks after the treatment (Zhang et al., 2004). One exposure to immobilization in the supine position for 6 h is capable of causing reproductive dysfunction in male rats, decreasing sperm motility and count in the ejaculate several weeks after the exposure to the stressor (Potemina, 2008). Forced swimming stress (3 min at 32 °C) in rats for 15 days causes a decrease in spermatid production (Mingoti et al., 2003), although fertility did not decrease, possibly due to short exposure to the stressor. It has been shown that the effects of stress on fertility are apparent after a period of time necessary to complete a spermatogenic cycle, between 48 and 53 days in the rat (Clermont et al., 1959; Hess, 1990). In this species, immobilization stress during 6 h/day for 60 days causes a reduction in the amount of mature spermatids in the testis, as well as in spermatozoa concentration in the cauda epididymis (Almeida et al., 1998). Furthermore, chronic stress causes pre and post-implantation loss of embryos, probably due to a lower rate of fertilization and/or fertilization with damaged spermatozoa (Almeida et al., 2000). Forced swimming stress (3 min/day at 32 °C) for 50 days decreases sperm concentration, motility, and causes low fertilization rates (Saki et al., 2009, 2010). Immobilization during 4 h/day for two months causes a reduction in the number of spermatogonia, primary and secondary spermatocytes, as well as spermatids (Rai et al., 2003). Noise (300–350 Hz, 90–120 dB) during 1 h/day for 50 days causes a decrease in sperm concentration and motility (Jalali et al., 2012) and causes loss of germinal cells (Swami et al., 2007). Heat stress (water bath at 43 °C, 20 min) impairs spermatogenesis, with pachitene spermatocytes and early spermatids as the most susceptible to heat (for review, see Setchell, 2006), causing infertility (Rockett et al., 2001). Even when spermatogenic cells are not killed, some of them may complete their development, but appear as spermatozoa with damaged DNA (Banks et al., 2005). Immobilization (5 h/day) for 60 days or immobilization (1 h/day) followed by forced swimming (15 min/day) for 60 days causes a reduction in sperm count, viability and motility, as well as a decrease in the number of sperm (Hari Priya and Reddy, 2012) and the mean number of type A spermatogonia, mid-pachytene spermatocytes, stage 7 spermatids, and elongated spermatids in the seminiferous tubules. Most of these changes have been shown to be irreversible (Nirupama et al., 2012).

On the other hand, it has been reported that sexual contact in socially stressed males can attenuate the reproductive disruptions caused by stress, such as low sperm count, testes weight, and testosterone (Taylor et al., 1987). Furthermore, sexual behavior and mating have beneficial effects on the neuronal and endocrine responsiveness to stress (Waldherr et al., 2010) and has an anxiolytic-like effect (Justel et al., 2009; Waldherr and Newman, 2007). Therefore, the aim of this study was to evaluate whether sexual behavior could attenuate the effects of chronic stress on spermatogenesis, accessory glands, plasma testosterone and corticosterone in male rats. Fertility was also evaluated in sexually experienced stressed male rats.

Material and methods

Animals

Adult male Wistar rats, weighing 300–350 g were housed, five per cage (50 × 30 × 20 cm), under standard vivarium conditions. The colony room was maintained on a 12:12 reversed light cycle (lights off: 0900 h) and controlled temperature (24 ± 1 °C). Food and water were available ad libitum throughout the experiments.

Adequate measures were taken to minimize pain or discomfort to animals. Handling of the rats throughout the experiments, as well as the method of euthanasia was in accordance to NIH guidelines and to Mexican Official Rules (NOM-062-ZOO-1999).

Intact sexually experienced males (n = 40) were randomly assigned to one of the following groups (n = 5, each): 1) control 20 days without copulation; 2) control 20 days with copulation; 3) stress for 20 days without copulation; 4) stress for 20 days with copulation; 5) control 50 days without copulation; 6) control 50 days with copulation; 7) stress for 50 days without copulation; and 8) stress for 50 days with copulation.

Sexual behavior assessment

Males were tested for masculine sexual behavior three times in order to select those displaying ejaculation at least twice. Behavioral testing was performed under dim red lights, 3 h after the onset of the dark phase of the light/dark cycle. Male sexual behavior was assessed by placing the male in a Plexiglas arena (45 cm diameter) 5 min before a stimulus receptive female was presented. Female rats were brought into sexual receptivity by administering estradiol benzoate (Sigma Chemical, Co. St. Louis MO., 10 μg/100 μl oil, SC) 44 h before sexual tests. Progesterone (Sigma Chemical, Co. St. Louis MO., 1 mg/200 μl oil, SC) was administered 4 h prior to testing. After presentation of the female, tests lasted for 30 min. Upon presentation of the female, the following parameters were recorded: latency to the first mount, latency to the first intromission, and latency to the first ejaculation; number of mounts (mounts with pelvic thrusting) and intromissions (mounts with pelvic thrusting and penile insertion) of the first copulatory series. In addition, ejaculation frequency (number of ejaculations during 30 min of recording), and post-ejaculatory interval (time between ejaculation and subsequent intromission) were recorded. The full description of masculine sexual behavior parameters has been detailed elsewhere (Hull et al., 2006).

Stress and sexual assessment

The cages of stressed rats were transferred to another room. The order of the cages being taken to the stressor area was random and balanced from day to day. The stressors were applied after the onset of the dark phase of the cycle.

Stress by immersion in cold water (ICW): Rats were placed individually in a covered tank of cold water (depth = 15.5 cm; temperature = 15 °C), where they either swam or remained in an upright position, standing on their hind limbs, and keeping their head above water level. This situation lasted for 15 min. After this time, rats were picked up from water and towel dried.

Male rats from control groups remained undisturbed in their home cages throughout the experiment. Sexual behavior was tested at the same time as the stress groups. Masculine sexual behavior was assessed 30 min after ICW.

Rats were exposed to the stressor daily for 20 or 50 consecutive days. During this time, one group of stressed males and one group of control males were allowed to copulate with sexually receptive females. Sexual tests lasted 30 min, regardless of male sexual performance, and the aforementioned sexual parameters were evaluated. Sexual behavior was assessed every 10 days, which is a time span sufficient to maintain sexual activity in experienced males. Only data from days 20 and 50 of stress are presented. These days were chosen because we have previously reported the effects on sexual behavior of stress for 20 days (Retana-Márquez et al., 2003a,b). Fifty days were chosen in order to evaluate the effects of stress on the seminiferous epithelium, as the duration of spermatogenesis in rats is 48–53 days (Clermont, 1972). For sexual tests on days 20 and 50, female rats in natural estrous were used, in order to evaluate the number of offspring. Control and stressed rats were weighed at the end of the experiment.

Processing of biological material

Subjects were euthanized by an intraperitoneal overdose of sodium pentobarbital (100 mg/kg body weight, Pfizer, SA de CV, Toluca, Mexico) on days 20 or 50 of stress, immediately after the last sexual test, which occurred between 3 and 4 h after the onset of the dark period of the cycle.