Effects of Acute Restraint Stress on Sperm Motility and Secretion of Pituitary, Adrenocortical and Gonadal Hormones in Adult Male Rats

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ABSTRACT. The effects of acute restraint stress on sperm motility and reproductive endocrinology were investigated in adult rats. Sperm motility was determined by computer-assisted sperm analysis. Acute restraint stress reduced sperm motility starting after 30 min, and the sperm motility parameters, percentage of motile spermatozoa (%), straight-line velocity, curvilinear velocity, deviation of the sperm head from the mean trajectory and the maximum amplitude of lateral head displacement decreased. It also induced a significant elevation in plasma adrenocorticotropic hormone, prolactin, corticosterone and progesterone and decreased follicle-stimulating hormone, luteinizing hormone, testosterone and immunoreactive (ir-) inhibin. These results clearly demonstrated that the acute restraint stress rapidly suppressed sperm motility and increased the activity of the hypothalamus-pituitary-adrenal axis, whereas it disturbed hypothalamus-gonadal axis activity.

KEY WORDS: ACTH, corticosterone, LH, restraint stress, sperm motility.

Sperm motility is an important prerequisite determining the quality and fertilizing ability of semen. Several studies have demonstrated that there is a relationship between psychological stress and sperm concentration, motility and morphology [9, 14, 28]. In humans, psychological stress patients had decreased sperm count and motility [4]. Stress due to examination tests disturbed luteinizing hormone (LH) and testosterone concentrations [17].

Restraint stress has been used as a procedure to study the effects of stress on reproduction outcome in rodents. Several studies have reported reduced testis weight and viability or percentage of progressive epididymal spermatozoa and increased adrenal weight due to stress in rats [24, 34]. In rats, the activation of the hypothalamus-pituitary-adrenal axis by stress depends mainly on the characteristics of the stressor, and the response of this axis to stress also depends on the time of day in which the stressor is applied [29]; immobilization stress resulted in decreases in testicular weights along with viability and progressive epididymal spermatozoa [24].

To extend these observations and knowledge, adult male rats were used as an animal model to investigate whether and how acute restraint stress affects sperm motility and reproductive endocrinology in this study. Adult Wistar-Imamichi male rats, weighing between 340 and 430 g and aged 3 months, from Imamichi Institute for Animal Reproduction, Ibaraki, Japan, were used. The rats were housed in a room with controlled illumination (14 L:10 D) and temperature (22°C–25°C) and were given free access to commercial pellets (CE-2, Japan Clea Co., Tokyo, Japan) and tap water ad libitum. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals prepared by Tokyo University of Agriculture and Technology.

Adult male rats were stressed by immobilization in a small plastic bag (DecapiCone, Braintree Scientific Inc., Braintree, MA, U.S.A.) at 1,000 hr [16]. Groups of rats containing five animals each were decapitated at 0, 30, 60, 120 and 180 min, and blood samples were collected using heparinized centrifuge tubes. Then, blood samples were centrifuged at 4°C at 1700 × g for 15 min and separated for plasma. Plasma samples were stored at –20°C for adrenocorticotropic hormone (ACTH), prolactin (PRL), follicle-stimulating hormone (FSH), luteinizing hormone (LH), immunoreactive ir-inhibin, testosterone, corticosterone and progesterone assays.

Sperm from a single cauda epididymis were collected into a 1.5-ml tube containing 1 ml of modified Tyrode's medium [27] (3 µl sperm sample from the cauda epididymis diluted with 1 ml modified Tyrode’s medium). The sperm motility was measured by computer-assisted sperm analysis (CASA) by using a C-Imaging C-Men system (C-Imaging systems, Compix Inc., Tualatin, OR, U.S.A.). Briefly, diluted sperm suspensions were placed in prewarmed slide chambers at a depth of 20 µm. The slides were viewed using an Olympus microscope (Olympus BX50F, Olympus Optical Co., Ltd., Tokyo, Japan) equipped with ×4 dark field optics and a video camera (CCD XC77, Sony Corporation,
Tokyo, Japan) connected to personal computer. The temperature of the microscope stage was maintained at 37°C throughout observation by a stage warmer (MP-10DM, Kitazato Supply Co., Ltd., Tokyo, Japan). CASA was performed using the C-Imaging C-Men system operating with the C-Imaging software. The CASA system was based upon the analysis of 15 consecutive, digitalized photographic images obtained from a single field. These 15 consecutive photographs were taken with a time lapse of 0.5 sec. Two to 3 separate fields were taken for each sample. Percentage of motile spermatozoa, straight-line velocity (VSL, $\mu$m/s), curvilinear velocity (VCL, $\mu$m/s), linearity index (ratio of the straight line distance to the actual tracked distance) [40], deviation of sperm head from the mean trajectory (ALH, mean $\mu$m/s), maximum amplitude of lateral head displacement (ALH, max $\mu$m/s) and beat frequency of centroids crossing the average trajectory (BCF, Hz) were determined.

Plasma concentrations of FSH, LH and PRL were determined by NIDDK kits for each hormone. Iodinated preparations were rat FSH-I-7, rat LH-I-7 and rat PRL I-5. The antisera used were anti-rat FSH-S-11, anti-rat LH-S-10 and anti-rat PRL-S-9. Results were expressed as means ± SEM. One-way analysis of variance (ANOVA) was used to compare means among groups. Post-hoc analyses were performed with the Least Significant Difference (LSD) test when the F ratio for the ANOVA was significant ($P<0.05$).

Data for sperm motility parameters are shown in Fig. 1. The restraint stress induced significant decreases in VSL (Fig. 1D) and the mean and max of ALH (Fig. 1B, C) throughout the stress period and significant decreases in VCL (Fig. 1G) and the percent ages of sperm motility (Fig. 1A) at 30 and 60 min, respectively, when compared with the control. There were no significant differences in linearity index (Fig. 1E) and BCF (Fig. 1F) as compared with the control group. However, BCF showed a decrease trend at 30 and 60 min compared with the control group during the stress period (Fig. 1F).

The plasma concentrations of ACTH, PRL, corticosterone and progesterone are shown in Fig. 2. The plasma concentrations of ACTH (Fig. 2A) of the stress rats were higher than those in the control and showed significant increases at 30, 60 and 120 min. The plasma concentration of PRL (Fig. 2B) also showed significant increases at 60 and 120 min in the stress period. The plasma concentrations of corticosterone (Fig. 2C) and progesterone (Fig. 2D) of the stress rats were higher as compared with the control group, and there were significant increases at 30, 60, 120 and 180 min for corticosterone; and at 30, 60 and 120 min for progesterone.

The plasma concentrations of LH, FSH, testosterone and ir-inhibin are shown in Fig. 3. Compared with the control, the plasma concentrations of LH (Fig. 3A) decreased significantly starting at 60 min after the restraint stress. The plasma concentrations of FSH (Fig. 3B) and testosterone (Fig. 3C) both showed significant decreases at 120 min and ir-inhibin (Fig. 3D) showed significant decreases at 120 and 180 min.

The present study provided new evidence that the acute restraint stress induced a rapid decrease in sperm motility in the rats. It also demonstrated that restraint stress increased the circulating levels of ACTH, PRL, corticosterone and progesterone. On the other hand, decreased plasma concentrations of FSH, LH, testosterone and ir-inhibin were observed in the treatment rats.

In the present study, we first demonstrated that some of the sperm motility parameters, such as VSL, mean and max of ALH and VCL decreased within 30 min after restraint stress. This suggests that acute restraint stress exerts a harmful influence on male reproductive activity by suppressing sperm motility. Up to now, the mechanism of this influence has not been clarified. However, previous studies have proposed a variety of mechanisms to explain the activation of mammalian sperm motility; these have been based on such factors as ion movements, changes in the viscosity of the surrounding medium, cytoplasmic alkalization, the presence of HCO$_3^-$ and increases in intracellular cAMP [18].

The sperm motility showed a rapid decrease starting at 30 min, which may be controlled by the chemistry and neurochemistry [26]. In the nervous system, stress provokes cholinergic hyperactivation and subsequently increases acetylcholine (ACh) action. The levels of acetylcholinesterase (AChE), which plays a role in degradation of ACh signals and is localized in many tissues including the brain, muscle and sperm head, were elevated by forced swimming-induced stress [11, 21]. In addition, plasma membrane ‘neuronal’ receptor types in sperm appear to be involved in influencing motility [25]. Taken together, we assume that the rapid decrease in sperm motility parameters in the stress rats might be the result of either androgenic hormone or neurohormone or both. Though the mechanism is not clear at the present time, the molecular mechanisms and cellular substrates for this relationship need to be further investigated.

In addition, stress is believed to influence male reproduc-
Glucocorticoids are known to inhibit testicular function, both in vivo and in vitro [2, 7, 31]. Chronic stress decreased viability or the percentage of progressive epididymal spermatozoa in rats [24]. Sperm become motile during the maturation process in the epididymal tract and depend on the presence of testicular androgens [30]. Epididymal proteins at the cauda epididymis such as clusterin (sulfated glycoprotein-2, SGP-2), apoER2 (apolipoprotein E receptor-2) and 5-α reductase played a role in sperm motility and were regulated by androgens [5, 13]. Also, glucocorticoid receptors (GRs) are present in basal cells of the epididymis in rats [29, 30]. Previous studies indicated the presence of GRs in Leydig cells [8], Sertoli cells [23] and the epididymis sperm [22].

The present study demonstrated that the restraint stress increased the circulating levels of ACTH, PRL, corticoster-
one and progesterone. It is well known that stress can activate the hypothalamo-pituitary-adrenal (HPA) axis and increase the release of corticotropin releasing hormone (CRH) from the hypothalamic paraventricular nucleus [1]. It can cause secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. In rats, corticosterone and progesterone are the predominant adrenal steroid hormones, and their production is directly stimulated by adrenocorticotropic hormones secreted from corticotrophs in the anterior pituitary gland. And because progesterone is a precursor to corticosterone, the increase in corticosterone has been caused by acute restraint stress results in a high concentration of progesterone. In addition, it demonstrated that ether stress induces a rapid increase in plasma PRL in rats [6, 15, 16]. On the other hand, ACTH stimulates the secretion of glucocorticoids from the adrenal cortex [39]. High concentrations of corticosterone induced by restraint stress might directly suppress FSH and LH secretions or indirectly suppress them through GnRH suppression [10]. A previous study found decreases in GnRH mRNA levels and GnRH secretion in adult male rats treated with corticosterone [10].

The effects of glucocorticoids on gonadotropin synthesis and release may be mediated both through hypothalamic GnRH neurons and their neural inputs, as well as through pituitary gonadotropes [10]. Moreover, in the present study, the plasma concentrations of ACTH, PRL and progesterone showed decreases 180 min after restraint stress, which showed a trend to return to the levels before restraint stress. These results may be due to negative feedback by the high corticosterone level, which can suppress the secretions of CRH and ACTH [16]. Furthermore, the present study demonstrated that the plasma LH, FSH, ir-inhibin and testosterone concentrations decreased under restraint stress. Our previous studies had indicated that the decreased levels of testosterone may be caused by a stress-induced decrease in plasma LH concentrations and increased secretion of corticosterone in the restraint stressed rats [36]. Moreover, the low levels of the plasma inhibin in stressed rats were also probably due to the direct effect of corticosterone on the Sertoli cells [37]. Taken together, these results strongly supported the views that the suppressive effects of the HPA system on HPG function are manifested at least in part as an
inhibition of gonadotropin release through effects of glucocorticoid hormones on the hypothalamus and/or anterior pituitary gland [3, 19].

In summary, the present study first demonstrated that the sperm motility decreased within 30 min after restraint stress and then that the plasma concentrations of ACTH, PRL, corticosterone and progesterone increased. The mechanism of this is not clear at the present time. We presume that the decreased motility of sperm may be caused by androgenic hormone or neurohormone or both or that it may be caused by increased secretion of corticosterone, which directly affects the epididymis and epididymal sperm or neuron system control through the neuronal receptor of the sperm in the restraint stressed rats.

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