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Influence of exercise and emotional stresses on secretion of prolactin and growth hormone in Thoroughbred horses

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The secretion of prolactin and growth hormone in response to exercise and emotional stresses was investigated in Thoroughbreds. Two experiments were performed: one with loading of only exercise stress and one with simultaneous loading of exercise and emotional stresses. Exercise stress was loaded in 4 steps using a treadmill for horses: pre-exercise period (5 min), walking period (6.5 min), galloping period (3 min), and cooling down period (10 min). Emotional stress was loaded by showing a loud video of an audience at a racetrack during the walking period. The results clearly demonstrated that exercise stress rapidly increased the secretion of prolactin and growth hormone and that secretion of them persisted for a specific period after the exercise. In addition, emotional stress promoted prolactin secretion.

Key words: *emotional and exercise stress, growth hormone, prolactin, Thoroughbred*

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Horses are nervous and easily excited animals, and the Thoroughbred is an especially nervous breed of horses. Racehorses are placed under various stresses other than those accompanying training and races, such as transport [25], and a high incidence of gastric ulcers has been reported [8, 21, 22]. To prevent these conditions, it is important to analyze the characteristics of the responses of racehorses to exercise and emotional stresses, identify the stress intensity and grade of biological reactions, and perform training and rearing management tailored to the individual horses. Improvement of their management and reduction of diseases and accidents are also important from the perspective of animal welfare.

In mammals, when the body feels stress, the hypothalamus-hypophyseal-adrenal system is activated. Stress reactions are observed regardless of the stress type (physical or mental) and onset pattern (acute or chronic) [26, 28, 29], and the system is activated by exercise stress [1, 12, 17, 18, 23]. In addition to the hypothalamus-hypophyseal-adrenal system, prolactin and growth hormone secreted from the anterior pituitary gland are essential hormones in the stress reaction as anti-stress hormones [29]. The secretion of prolactin and growth hormone by exercise in horses has been reported previously [30], but the study only compared the blood levels between before and after exercise.

In the present study, to elucidate the biological reactions of racehorses to various stresses accompanying races on the racetrack, exercise and emotional stresses were loaded on Thoroughbreds, and changes in the secretion of prolactin

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and growth hormone were investigated to endocrinologically analyze the biological reactions of the racehorses in races.

Exercise and emotional stress: Four male Thoroughbreds (4.1 ± 0.2 years of age, 466 ± 6 kg) were used. Two experiments were performed: one with loading of only exercise stress (ES group) and one with simultaneous loading of exercise and emotional stresses (EES group). The emotional stress experiment was performed one week after the exercise stress experiment with the same horses.

Exercise stress: As exercise stress, horses ran on a treadmill (Mustang 2000, Kagra, Fahrwangen, Switzerland) to simulate a horse race. Before the experiment, the horses were trained for 4 weeks to get them used to running on the treadmill. The exercise consisted of the following 4-step running program: (1) a pre-exercise period for warming up with a 5 min trot and gallop, (2) a 6.5 min walking period, (3) a 3 min galloping period in which the running speed was rapidly increased to 110% VHRmax (a speed corresponding to 110% of the maximum heart rate calculated from the correlation between the heart rate and running speed) until the horse was exhausted, and (4) a cooling down period with a 10 min walk.

Emotional stress: Novel stimuli causing anxiety in horses were loaded as the emotional stress. Recordings of a fanfare and cheering at horse races were played at a high volume (85 db) as acoustic stimuli, and a video of an audience was projected onto a screen as a visual stimulus. They were continuously loaded during the 6.5 min walking period. These stimuli were chosen as the emotional stress based on results concerning the behavior, heart rate, and endocrine responses of Thoroughbreds in a previous paper [12].

Blood collection: Blood was collected at rest, on entering the room containing the treadmill, before and after the novel stimulation, during exercise (at 30, 60, 90, 120, 150, and 180 sec), and after exercise (at 1, 5, 10, 20, 30, and 40 min). Blood was collected through a catheter inserted into the jugular vein (14-G, Angiocath, Becton Dickinson, Sandy, UT, U.S.A.). The catheter was placed more than 2 hr before the experiment. All protocols of the present experiments were approved by the Animal Welfare and Ethics Committee of the Equine Research Institute, Japan Racing Association (JRA).

Hormone assays: Plasma concentrations of prolactin were measured by radioimmunoassay using rat anti-sera against equine prolactin (AFP-261987) and purified equine prolactin (AFP-8794B) for radioiodination and the reference standard, as described previously [5]. The intra- and inter-assay coefficients of variance were 7.1% and 9.8%, respectively. Plasma concentrations of growth hormone were measured by radioimmunoassay using an anti-porcine growth hormone (GH-AFP10318545) and purified equine growth hormone (GH-AFP-5022) for radioiodination and the reference standard. The intra- and inter-assay coefficients of variance were 5.0% and 12.0%, respectively. These radioimmunoassay materials were provided by Dr. A.F. Parlow (National Hormone and Pituitary Program, NIDDK, NIH, Torrance, CA, U.S.A.).

All results are expressed as the mean ± standard error of the mean (SEM). Statistical comparisons between the two groups were performed by Student's *t*-test when uniformity of variance was confirmed by the *F*-test. When the variance was not uniform, the unpaired *t*-test with Welch's correction was used. $P<0.05$ was considered to be statistically significant.

Effects of emotional stress on hormone secretion: The emotional stress loaded during 6.5 min walking period significantly elevated the circulating prolactin level (Fig. 1a). No significant elevation was noted in growth hormone (Fig. 1b).

Effects of exercise and emotional stress on hormone secretion: In both the ES and EES groups, the circulating prolactin level rose immediately after the initiation of the pre-exercise period, and it continuously rose without a decrease during the walking period. The level peaked at 120 sec and 60 sec after the initiation of the galloping period in the ES and EES groups, respectively, and then slowly decreased until 40 min after the completion of exercise in both groups, but it was still higher than the resting level at 40 min. The circulating prolactin level in the galloping period tended to be higher in the EES group than in ES group (Fig. 2). The circulating growth hormone level rose throughout the pre-exercise, walking, and galloping periods in both the ES and EES groups, with no significant difference between them. The level peaked at 150 sec after the initiation of the galloping period and then slowly decreased until 40 min after the completion of exercise in both groups, but it was still higher than the resting level at 40 min. The resting and peak growth hormone levels were 1.4 ± 0.7 and 118.8 ± 22.1 ng/m*l* in the ES group, respectively, showing that the peak level was very high (85 times higher; Fig. 3).

To clarify the influences of exercise and emotional stresses on the endocrine function of racehorses, stresses were loaded on Thoroughbreds by simulating a horserace using a treadmill, and changes in prolactin and growth hormone were investigated.

In the present study, the circulating prolactin and growth hormone levels rose with the initiation of the pre-exercise period, did not decrease in the walking period, and further increased and peaked in the galloping period. After exercise completion, the levels slowly decreased and were still higher than the resting levels at 40 min after exercise completion. The present study clarified, for the first time, the changes in the secretion of prolactin and growth hormone during

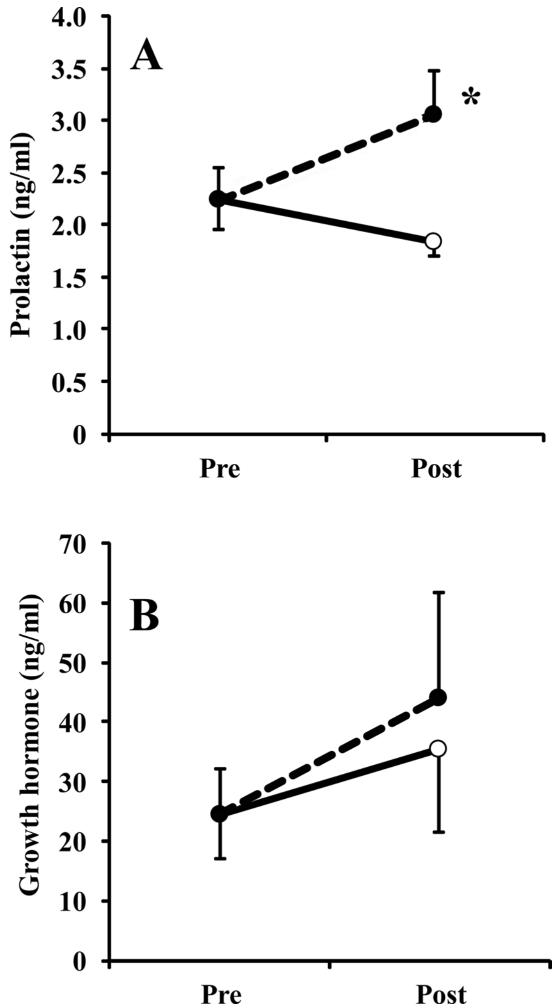

Fig. 1. Endocrine changes of prolactin (A) and growth hormone (B) with emotional stress (●) and without emotional stress (○) at before (pre, n=8) and after (post, n=4) stress. Values are expressed as the mean ± SEM. *P<0.05 versus the value for without emotional stress.

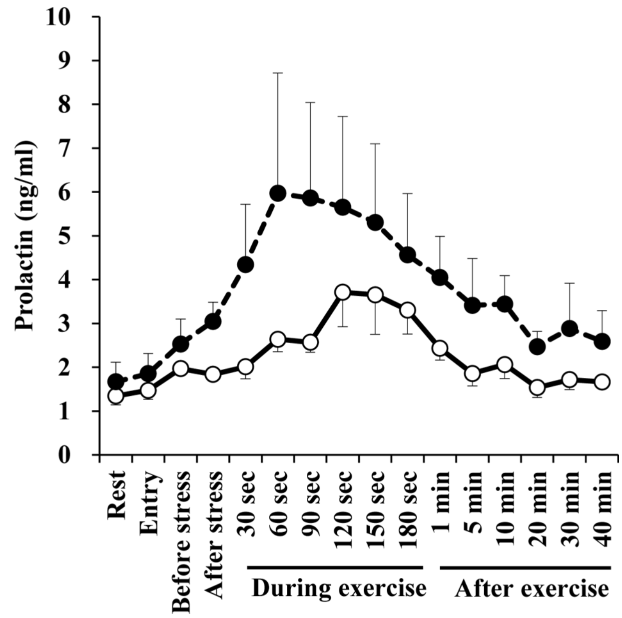

Fig. 2. Time course of plasma prolactin concentrations during the treadmill exercise with emotional stress (●) and without emotional stress (○). Values are expressed as the mean ± SEM (n=4).

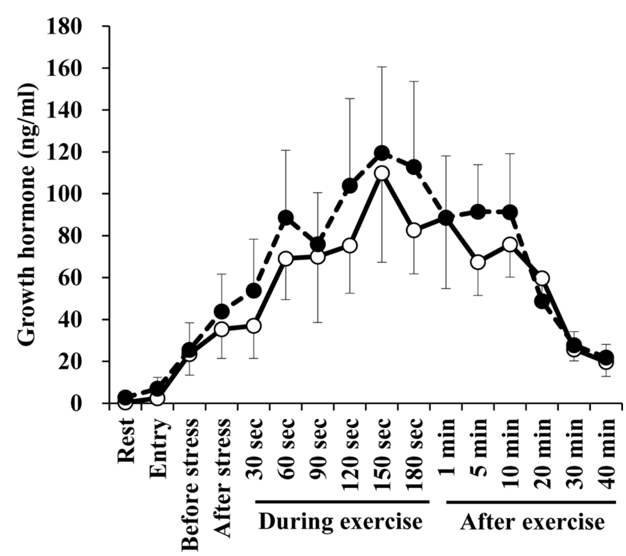

Fig. 3. Time course of plasma growth hormone concentrations during the treadmill exercise with emotional stress (●) and without emotional stress (○). Values are expressed as the mean ± SEM (n=4).

exercise in detail.

Regarding the physiological roles of prolactin and growth hormone secreted during exercise, it is thought that there is an association with immune function [16]. Previous studies have clarified that prolactin and growth hormone stimulate the immune system in humans and laboratory animals [10, 15, 24]. Prolactin acts on the mammary gland and promotes milk secretion; however, in addition to this, its immune reaction-activating actions, such as the activation of interferon production by helper T cells and of interferon- and T-cell-dependent cytotoxic activity in macrophages, have been clarified. Prolactin-induced activation of lymphocyte proliferation, antibody production, and NK cells and potentiation of phagocytosis by macrophages have been reported [4, 24, 32]. In humans, an exercise stress-induced increase in the number of prolactin receptors expressed on the lymphocyte cell membrane and a positive correlation between the circulating prolactin level and number of prolactin receptors expressed have been reported [6]. Furthermore, prolactin promotes glucocorticoid secretion from adrenocortical cells and prevents gastric ulcer, and it is attracting attention as an anti-stress hormone [2, 3, 9, 13, 14]. In addition, a previous study demonstrated that chronic elevation of prolactin levels within the brain results in reduced neuronal activation within the hypothalamus, specifically within the paraventricular hypothalamic nucleus, in response to an acute stressor. Thus, prolactin acting in various relevant brain regions exerts profound anxiolytic and anti-stress effects and is likely to contribute to the attenuated stress responsiveness found in the female rat in the peripartum period, when brain prolactin levels are physiologically upregulated [7, 31]. Although the exact mechanism of increase of prolactin in response to the emotional stress is not known, it could be due to suppression of the secretion of dopamine, a suppressor of prolactin secretion, by the high concentration of β-endorphin in the hypothalamus.

Growth hormone is secreted from the anterior pituitary and mainly promotes growth. Growth hormone induces the growth of all tissues of the body. It increases the sizes of cells and promotes increases in cell number via mitosis. It also acts on the liver to produce insulin-like growth factor I (IGF-I), and IGF-I acts on cells in each tissue and promotes growth [11]. In humans, growth hormone secretion is promoted by sleep, exercise, food ingestion, and stress [19, 20, 27]. Growth hormone promotes protein anabolism and is necessary to repair muscle tissue damaged by exercise. Growth hormone secreted during exercise is also assumed to promote immunological enhancement [10, 19]. Marked stress decreases the strength of the immune system [16]. The prolactin and growth hormone secreted in response to exercise stress are assumed to activate the immune system to protect the body against a stress-induced decrease in immune function. Prolactin has been reported to inhibit stress-induced ulceration of the stomach in rats [3, 9], suggesting that rapid increases in prolactin and growth hormone secretion during exercise are also important elements to maintain the health of racehorses, which have a high incidence of gastric ulcers.

In the present study, it was suggested based on the endocrine changes investigated in detail in the exercise and emotional stress experiments that racehorses approaching a race are aware of the atmosphere and noise of the audience at the racetrack. Emotional stress potentiated prolactin and growth hormone secretion. Prolactin and growth hormone enhance the immune function of racehorses, and prolactin may exert a profound anxiolytic effect in the brain during and after exercise. However, the grade of stress reactions markedly varies among individual horses. Further accumulation of data is necessary.

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