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EFFECT OF NALOXONE ON HORMONAL CHANGES DURING EXERCISE

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Synopsis It is well established that prolactin release during exercise is one of the important factors in exercise-induced menstrual dysfunction. The purpose of this study is to clarify the mechanisms of prolactin release during exercise.

Ten female athletes measured their BBT every morning. They performed incremental exercise on a cycle ergometer, with or without naloxone, on the 5th to 8th days of the follicular phase. Three minutes before the exercise, 0.4mg of naloxone was injected intravenously and a further 1.6mg/hr of naloxone was continuously infused during exercise. Blood samples were collected after 60 minutes bed rest (Rest), at the time when the heart rates reached 150 bpm (Submax), the point of exhaustion (Max) during exercise and after 60 minutes bed rest following exercise (After 1hr). The levels of prolactin in serum, dopamine, β-endorphin, VIP and ACTH in the plasma were measured.

Whereas prolactin increased significantly at Submax ($p<0.05$) and Max ($p<0.001$), the increase in prolactin was suppressed by the administration of naloxone ($p<0.05$). Dopamine showed no remarkable change during exercise, with or without naloxone. There were significant increases in β-endorphin at Max ($p<0.001$), VIP at Submax and Max ($p<0.001$), but these increases were suppressed by the administration of naloxone ($p<0.001$). ACTH which had markedly increased at Submax ($p<0.025$) and Max ($p<0.001$) showed a slight tendency to decrease following the administration of naloxone, but there were no significant differences in both groups.

These data suggested that the mechanism of prolactin release during exercise may result from the increase in opiate peptide (PRF) during exercise.

Key words: Exercise • Prolactin • Dopamine • β-endorphin • Naloxone

Introduction

During the past decade, the number of women who have begun to participate in strenous endurance sports has increased rapidly. Recent evidence indicating a causal relationship between athletic activity and a higher incidence of delayed menarche and menstrual dysfunction has aroused interest[21)22)]. However, the mechanisms of exercise-induced menstrual dysfunction have not yet been clarified.

There are three hypotheses for the factors involved in exercise-induced menstrual dysfunction: 1) physical and/or psychological stress due to exercise, 2) loss of weight (body fat) due to a diet to control body profile and/or exercising condition, 3) acute and/or chronic hormonal changes due to exercise. As we thought that the third hypothesis is the most important factor, we investigated hormonal changes during exercise[23)24)30)] and hypothalamo-pituitary function[25)] in female athletes. We have already reported that prolactin is the only hormone which increases significantly during exercise in both the follicular and luteal phases.

The relationship between prolactin secretion and the occurrence of menstrual dysfunction is well established. That is, it is believed that a significant, transient release of prolactin during exercise is an important factor in exercise-induced menstrual dysfunction. The purpose of this study is to clarify the mechanisms of prolactin release during exercise.

Subjects and Methods

Ten top-ranking Japanese female university basketball players, aged 18 to 21 years old, were subjected to an investigation of endocrinological responses during exercise. They measured their

basal body temperature (BBT) every morning and then exercised incrementally using a cycle ergometer with naloxone, an opiate antagonist, (Naloxone (+) group). or with normal saline control (Naloxone (−) group), on the 5th to 8th days of the follicular phase. During the incremental exercise, electrocardiogram (ECG) and heart rates were monitored.

The schedule of naloxone (Naloxone Hydrochloride, Sanky Co. LTD., Tokyo, Japan) administration was as follows. Three minutes before the incremental exercise, 0.4mg of naloxone was administered intravenously and a further 1.6mg per hour of naloxone was continuously infused during exercise using an infusion pump (ATOM AIP-S235) through the venous catheter (18G) inserted in the left forearm vein (median cephalic vein or ulnar vein).

Another venous catheter (18G) was inserted in the right forearm vein for the collection of blood samples. Blood samples were collected after 60 minutes bed rest before exercise (Rest), at the time when the heart rates reached 150 beats/minute (Submax), the point of exhaustion (Max) during exercise and after 60 minutes bed rest following incremental exercise (After 1hr) (Fig. 1).

The levels of prolactin in serum, β-endorphin, vasoactive intestinal peptide (VIP) and adrenocorticotrophic hormone (ACTH) in plasma were measured by radioimmunoassay (RIA). RIA kits were used for the measurement of serum prolactin (Daiichi Radioisotope Laboratory, Japan), plasma VIP (Immuno Nuclear, USA) and plasma ACTH (Euro-Diagnostics BV, Holland). Plasma β-endorphin was measured by the method of Furui et al.[11]. Then plasma dopamine was determined by the method of Ueda et al.[34] using high performance liquid chromatography (HPLC). The sensitivity for each assay were 3.0ng/ml for prolactin, 10pg/ml for ACTH, 5pg/ml for VIP, 3pg/ml for β-endorphin, 0.1ng/ml for dopamine, respectively. The inter- and intra-assay coefficients of variation were as follows: 5.2 and 2.0% for prolactin, 3.02 and 3.52% for ACTH, 8.7 and 7.6% for VIP, 3.68 and 7.25% for β-endorphin and 2.64 and 2.28% for dopamine, respectively.

The Student's t-test was conducted for statistical analysis and differences were considered to be significant at the level of $p<0.05$.

Results

A) Basal Levels of Hormones

Table 1 shows the basal levels of hormones at Rest, after 60 minutes bed rest before incremental exercise, for both the Naloxone (−) group and Naloxone (+) group.

In both groups, there were no significant differences in hormones and none of the subjects

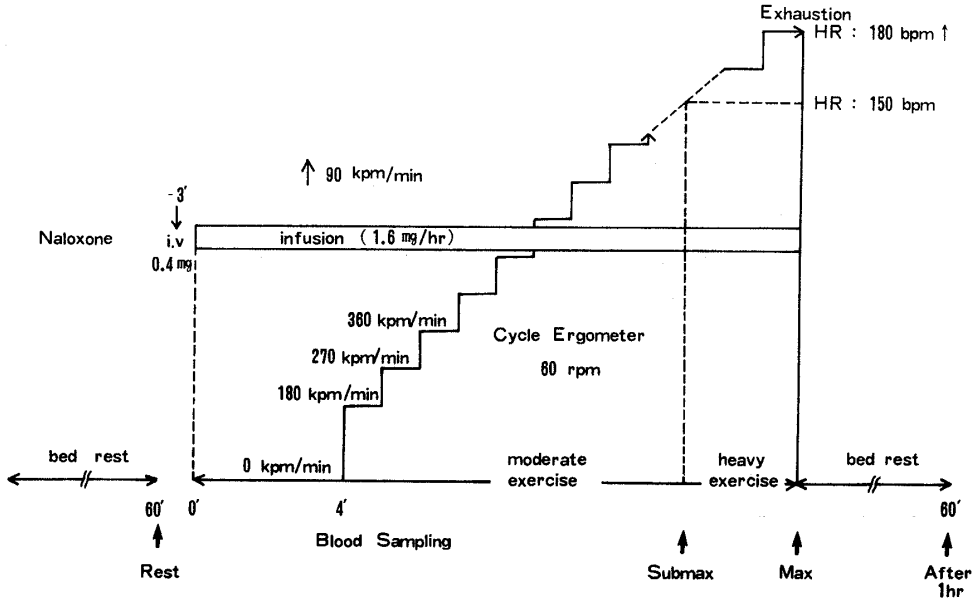

Fig. 1. Schedule of incremental exercise using cycle ergometer with or without the administration of naloxone.

Table 1. Basal levels of hormones at rest before incremental exercise using cycle ergometer in follicular phase.

Hormone		Naloxone (−)	Naloxone (+)	Normal Range
Prolactin	(ng/ml)	9.0±0.9	10.4±1.2	30↓
Dopamine	(ng/ml)	4.9±0.2	4.3±0.5	0.8~4.6
β-endorphin	(pg/ml)	19.9±4.3	13.1±1.0	7~27
VIP	(pg/ml)	31.6±4.5	24.6±2.8	100↓
ACTH	(pg/ml)	14.9±3.4	11.3±1.0	50↓

VIP : vasoactive intestinal peptide (Mean±SE)
ACTH : adrenocorticotropic hormone

showed signs of hyperprolactinemia. However, the levels of dopamine in both groups were slightly higher than the assay normal range.

B) Hormonal Changes during Incremental Exercise

1. Changes in Serum Prolactin Levels

In the control Naloxone (−) group, serum prolactin levels increased significantly at Submax ($p<0.05$) and Max ($p<0.001$) during incremental exercise. However, the serum prolactin level decreased immediately after the end of the exercise, and the level at After 1hr was not significantly different from the level at Rest.

On the other hand, in the Naloxone (+) group, there were significant differences in serum prolactin levels compared with the Naloxone (−) group. That is, the increases in the serum prolactin level at Max was significantly suppressed ($p<0.05$) by the administration of naloxone (Fig. 2).

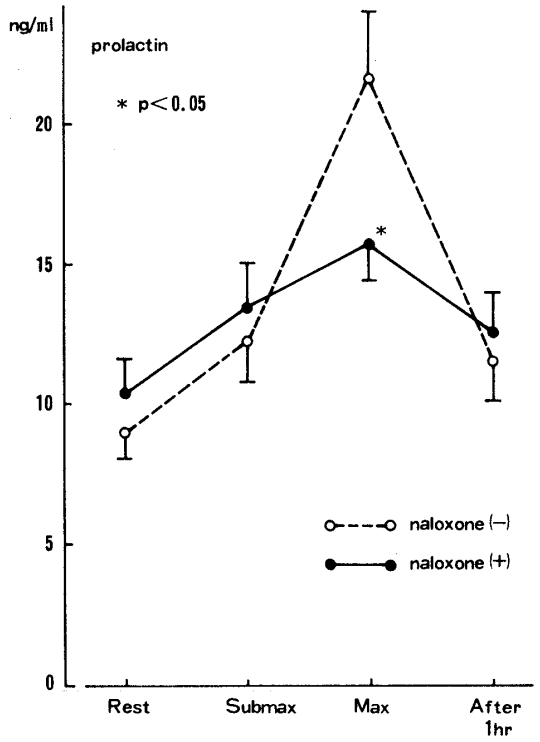

Fig. 2. Effect of naloxone on serum prolactin levels during incremental exercise using cycle ergometer in follicular phase. (Mean±SE)

2. Changes in Plasma Dopamine Levels

Plasma levels of dopamine in both groups tended to decrease during incremental exercise. However, there were no remarkable changes. Furthermore, there were no significant differences in both groups at any of the points examined during exercise (Fig. 3).

3. Changes in Plasma VIP Levels

In the Naloxone (−) group, plasma VIP levels increased significantly at Submax ($p<0.001$) and kept increasing until reaching a plateau at Max ($p<0.001$). Furthermore, there was an apparent decrease in plasma VIP level after the end of incremental exercise. However, the value was higher ($p<0.05$) at After 1hr than at Rest.

On the other hand, in the Naloxone (+) group, plasma VIP levels were suppressed significantly by the administration of naloxone at every point examined (Submax ; $p<0.02$, Max ; $p<0.001$, After 1hr ; $p<0.001$), and there were no significant variations during incremental exercise (Fig. 4).

4. Changes in Plasma ACTH Levels

Plasma ACTH levels increased significantly at Submax ($p<0.025$) and Max ($p<0.001$) during incremental exercise in the Naloxone (−) group but these responses were suppressed by the administration of naloxone. However, there were no significant differences in the Naloxone (−) group and Naloxone (+) group at all points examined

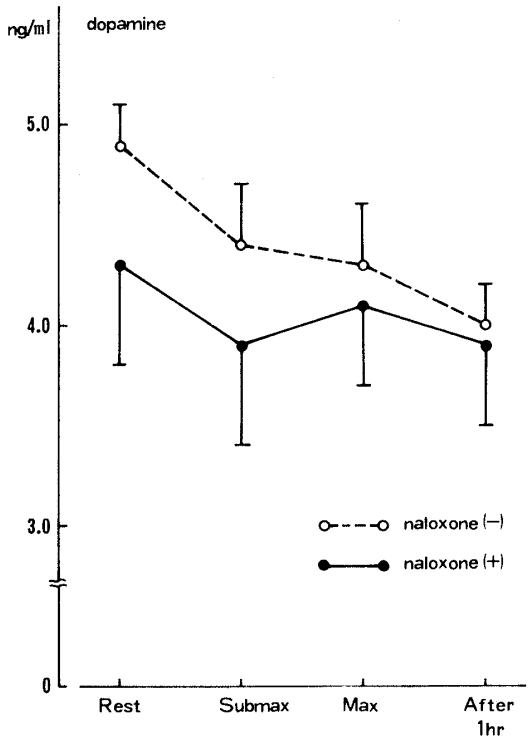

Fig. 3. Effect of naloxone on plasma dopamine levels during incremental exercise using cycle ergometer in follicular phase. (Mean±SE)

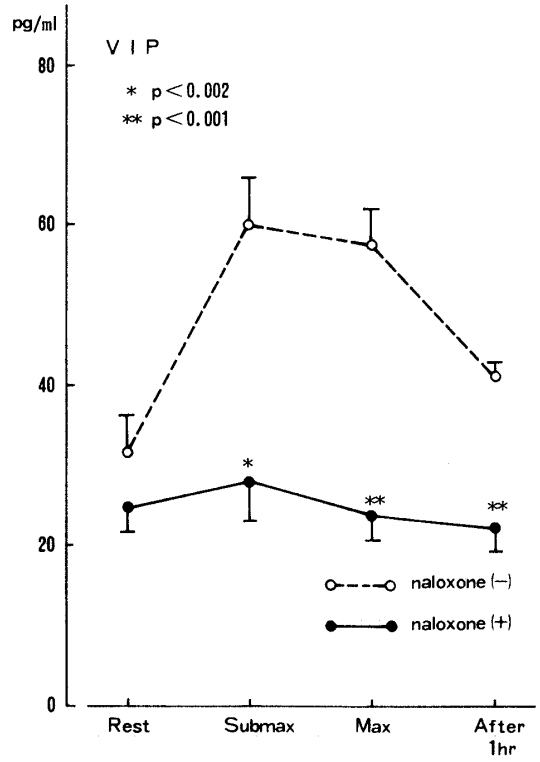

Fig. 4. Effect of naloxone on plasma vasoactive intestinal peptide (VIP) levels during incremental exercise using cycle ergometer in follicular phase. (Mean±SE)

(Fig. 5).

5. Changes in Plasma β-endorphin Levels

Plasma β-endorphin levels showed a significant increase at Max ($p<0.001$) during incremental exercise in the Naloxone (−) group.

On the other hand, in the Naloxone (+) group, plasma β-endorphin level increased significantly at Max ($p<0.01$). However, all values were significantly lower than those for the Naloxone (−) group (Submax ; $p<0.0025$, Max ; $p<0.01$, After 1hr ; $p<0.01$) (Fig. 6).

Discussion

The present study demonstrates that naloxone is effective in lowering serum prolactin levels during incremental exercise in the follicular phase.

The relationship between hyperprolactinemia and secondary amenorrhea/anovulatory cycle has been well documented in patients with functional hyperprolactinemia or bearing a prolactin-secreting adenoma. Recently, it is believed that transient hyperprolactinemia and occulted hyperprolactinemia at night are important to infertility[1] and anovulatory cycle[26]. Mori et al. have studied the circadian secretion of prolactin, and reported that a nocturnal hyperprolactinemic state occurred for several hours in the cases of women who needed bromocriptine for ovulation. These cases showed an increased prolactin secreting capacity after the administration of thyrotropin-releasing hormone (TRH)[26].

It is well established that exercise induces prolactin release[3)5)23)24)30)]. Furthermore, we reported that prolactin secreting capacities after the administration of TRH were significantly increased in athletes with menstrual disorders[25]. These data suggested that prolactin release during exercise is an important factor in exercise-induced menstrual dysfunction in female athletes.

Buckman et al.[4] have shown that prolactin levels vary in women, depending on the phase of their menstrual cycle. Levels appear to peak during the periovulatory phase, remain relatively elevated throughout the luteal phase, and fall to appreciably lower levels during the follicular phase. In addition, this rise in prolactin has been found to correlate directly with levels of circulating estradiol, and not with those of luteinizing hormone (LH), follicle-

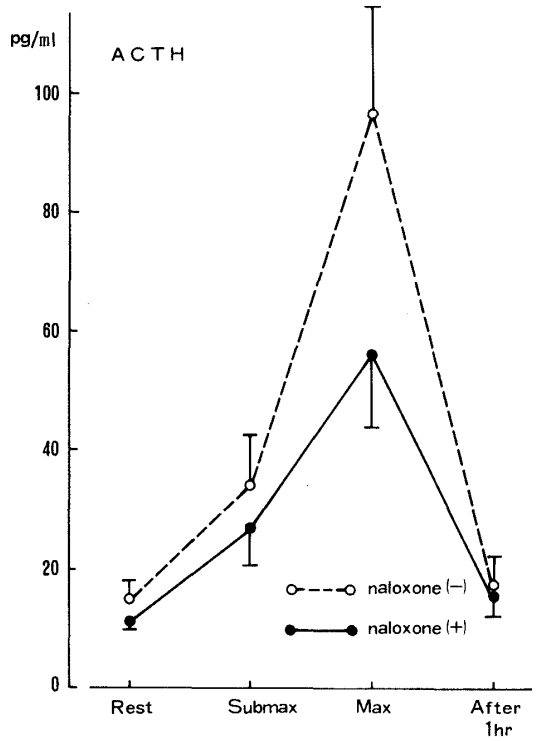

Fig. 5. Effect of naloxone on plasma adrenocorticotrophic hormone (ACTH) levels during incremental exercise using cycle ergometer in follicular phase. (Mean±SE)

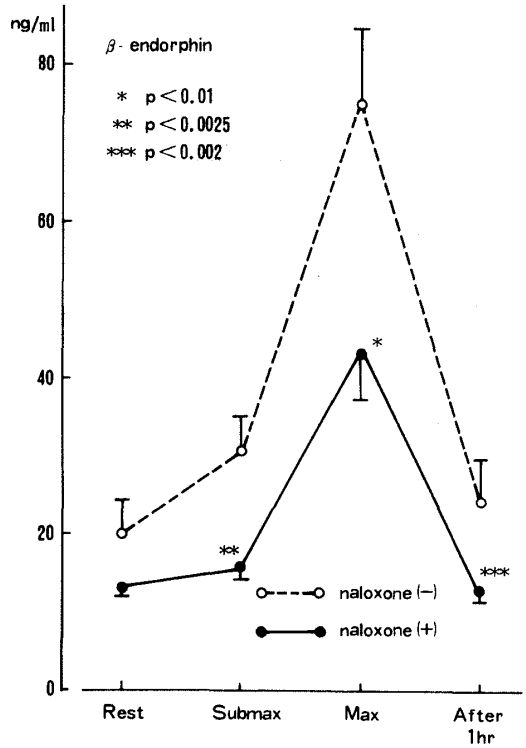

Fig. 6. Effect of naloxone on plasma β-endorphin levels during incremental exercise using cycle ergometer in follicular phase. (Mean±SE)

stimulating hormone (FSH), estrone, or progesterone. Accordingly, in this study, we investigated the changes in prolactin and other hormone levels during incremental exercise in the follicular phase.

It is well known that prolactin release from the pituitary anterior lobe is regulated by two mechanisms: the prolactin inhibitory factor (PIF) and the prolactin releasing factor (PRF). There is growing skepticism of the view that dopamine is the sole PIF mediating tonic hypothalamic inhibition. Furthermore, it is thought that dopamine tonically stimulates the secretion of an unidentified PIF[13]. However, Peters and co-workers[2)28)] reported that the extracts of pituitary posterior lobe contained significant prolactin-inhibiting activity that could be attributed to dopamine since inhibition was reversed by cotreatment with a dopamine antagonist. Another PIF for which evidence has rapidly accumulated is gamma aminobutyric acid (GABA). Schally et al.[31)] reported that, while dopamine was responsible for a large part of the prolactin inhibiting activity of porcine median eminence extracts, a significant part was associated with GABA. It was reported that GABA directly inhibits spontaneous prolactin release in vitro[8)], and GABA receptors are found on pituitary membranes[15)]. On the other hand, a large amount of GABA is required to inhibit prolactin release[29)]. Furthermore, hypophyseal stalk plasma from diestrous rats contains low levels of GABA not significantly different from circulating levels in peripheral plasma[27)]. These data indicate that dopamine may be the most important PIF.

In addition to an inhibitory mechanism of control for prolactin secretion, the concept of supporting stimulatory mechanism has enjoyed wide acceptance. Unlike the prolactin inhibitory mechanism, the clear requirement for a prolactin stimulating mechanism has been difficult to demonstrate unambiguously. The growing list for PRF includes TRH[33)], VIP[12)], β-endorphin[14)], methionin enkephalin, leucine enkephalin[18)], serotonin[36)], neurotensin[9)], gonadotropin-releasing hormone (GnRH)[6)], oxytocin[19)], vasopressin[32)], substance P[17)], epidermal growth factor[16)] and estradiol[37)]. Even though these substances have the capacity to effect the release of prolactin, this fact alone does not establish a physiological role for the substance as a prolactin-releasing hormone. It is thought that the major PRFs are endogenous opiate peptide β-

endorphin and VIP. In addition, the control of dopamine release or turnover in the central nervous system, therefore, may be mediated by β-endorphin[7)20)35)]. Furthermore, the interaction between β-endorphin and gonadotropin release is complex and appears to involve GnRH with a modulating effect on ovarian sex steroids[10)].

We measured the concentration of serum prolactin, plasma dopamine as PIF, and plasma β-endorphin, VIP and ACTH as PRFs during incremental exercise using a cycle ergometer in female athletes in the follicular phase. The data on hormonal changes, with or without naloxone (opiate antagonist), indicated as follows. During incremental exercise without naloxone (with saline as control study), serum prolactin and plasma PRFs (β-endorphin, VIP and ACTH) levels increased significantly. On the other hand, plasma dopamine (PIF) levels showed a decreasing tendency but it was not significant. The administration of naloxone resulted in significant changes in serum and plasma hormonal levels during incremental exercise. This indicated that serum prolactin levels and plasma PRFs levels were significantly suppressed by the administration of naloxone. Plasma dopamine levels, however showed no significant changes compared to plasma dopamine levels during incremental exercise without naloxone.

In conclusion, these data suggested that the mechanism of prolactin release during exercise may result from the increase in PRFs by exercise, under the condition in which there were no significant changes in dopamine as PIF.

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概要 女子運動選手の月経異常発現機転において重要な要因と考えられる，スポーツ活動時の prolactin 分泌亢進機構について検討した．

女子運動選手を対象とし，月経周期の 5 ～ 8 日に自転車 ergometer を用いる最大運動負荷試験を naloxone 投与の有無により 2 回行つた．naloxone は，運動開始 3 分前に0.4mg を静注，運動中は infusion pump を用い1.6mg/hr で持続投与した．前腕静脈に翼状針を留置し，安静臥床 1 時間後（Rest），運動負荷試験中は心拍数 150bpm 時（Submax）と最大運動負荷時（Max），運動終了 1 時間の安静臥床後（After 1hr）に採血した．血清中 prolactin，血漿中 dopamine, β-endorphin, vasoactive intestinal peptide（VIP），ACTH 濃度を測定した．

prolactin は，運動負荷試験時に Submax, Max と著明に増加するが，naloxone の投与により，その分泌亢進は明らかに抑制された．dopamine には naloxone 投与の有無による差は認められなかつた．し

かし，β-endorphin は Max 時に著明に増加するが，naloxone 投与によりその上昇は明らかに抑制された．また，VIP は Submax および Max と著明に上昇するが，naloxone 投与によりこの上昇は明らかに抑制された．ACTH の運動時の上昇は naloxone 投与により抑制傾向を示した．

以上の成績より，スポーツ活動時の prolactin 分泌亢進は，内因性 opioid peptides の上昇が重要な因子として関与していることが示唆された．