Stimulation of the Secretion of Luteinizing Hormone by Ginsenoside-Rb1 in Male Rats

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Abstract

Ginsenoside-Rb1 is one of the pharmacologically active components of ginseng, the dry root of *Panax ginseng* C. A. Meyer (Araliaceae), a well-known traditional Chinese medicine. Ginseng enhanced mounting behaviour of male rats and increased sperm counts in rabbit testes. Some experimental results suggested no sex hormone-like function in ginseng but probably gonadotropin-like action. The present study was to explore the effect of ginsenoside-Rb1 on the secretion of luteinizing hormone (LH) both *in vivo* and *in vitro*. Male rats were orchidectomized (Orch) for 2 weeks or subjected to swim training for 1 week before catheterization via the right jugular vein. They were intravenously injected with ginsenoside-Rb1 (10 μg/kg) or saline at 15 min prior to a challenge of gonadotropin-releasing hormone (GnRH) or 10 min-swim. Blood samples were collected at several time intervals following intravenous injection of ginsenoside-Rb1. In the *in vitro* experiment, male rats were decapitated and their anterior pituitary gland (APs) were either bisceted or enzymatically dispersed. The hemi-APs were preincubated with Locke's medium at 37 °C for 90 min and then incubated with Locke's medium containing ginsenoside-Rb1 (10^{-7}~10^{-4} M) for 30 min. The dispersed AP cells (1×10^5 cells per well) were primed with dihydrotestosterone (DHT, 10^{-8} M) for 3 days, and then challenged with ginsenoside-Rb1 (10^{-6} and 10^{-5} M, n=8) for 3 h. The concentrations of LH or testosterone in samples were measured by radioimmunoassays. Administration of ginsenoside-Rb1 did not alter the levels of plasma LH in both intact and Orch rats but significantly increased plasma LH concentration at the termination of the 10 min swimming exercise. Administration of ginsenoside-Rb1 resulted in a lower testosterone response to GnRH challenge or swimming exercise as compared with saline-treated rats. Ginsenoside-Rb1 dose-dependently increased the release of LH from both hemi-AP tissues and the DHT-primed dispersed AP cells *in vitro*. These results suggest that ginsenoside-Rb1 increases LH secretion by acting directly on rat AP cells.

Key Words: ginsenoside-Rb1, LH, swimming

Introduction

The majority of ginseng effects reported could be explained by the pharmacological actions of ginsenosides (ginseng saponins). Ginsenoside-Rb1 is one of the pharmacologically active saponins of ginseng, the dry root of *Panax ginseng* C. A. Meyer (Araliaceae), a well-known traditional Chinese medicine. Ginseng enhanced mounting behaviour of male rats (2, 20) and increased sperm counts in rabbit testes (2). It has been reported that there is no sex hormone-like function but probably gonadotropin-like action in ginseng (2). Whether ginsenosides alter the secretion of gonadotropin is not clear.

The previous studies showed that no significant change in testosterone and luteinizing hormone (LH) was observed in the ginseng supplemented athletes (8) or male rats (20). However, Fahim et al. (7)

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showed a decreased blood testosterone level in 5% ginseng-received rats (7). Administration of American ginseng decreased the mount, intromission and ejaculation latencies in male rats (20). These results demonstrated that American ginseng significantly facilitates male copulatory behavior. Ginseng saponin abolished morphine induced release of plasma corticosterone and apoptosis of thymocytes (13). These studies provide theoretical background for ginseng as a therapeutic agent.

It is well known that serum steroid hormone levels are increased by the gonadotropin stimulation. Brief intense exercise elevates circulating steroid hormone levels (4, 10, 18). However, many studies have indicated that LH is not changed after exercise (6, 9, 19, 23). A previous study has reported that an increase in serum LH is synchronous with testosterone after cycling exercise in untrained males, which demonstrated a gonadotropin-independent increment without involvement of testosterone following exercise (3).

On the other hand, data from another study have shown that whole blood lactate increases in parallel with serum testosterone increments in humans after a heavy resistance protocol (14). Our previously study has reported that not only endogenous lactate in exercise but also lactate infusion increase the plasma testosterone, and the effect is LH-independent (17). Whether the negative feedback control of testosterone on LH release is involved in the regulation of ginsenoside-Rb1 on the secretion of LH is unknown.

This investigation was designed to examine the effects of ginsenoside-Rb1 on the secretion of LH both *in vivo* and *in vitro*. We found that ginsenoside-Rb1 may increase the basal, but not GnRH-induced, release of LH, by acting directly at rat anterior pituitary glands (APs).

Materials and Methods

Animals

Male rats of Sprague-Dawley strain weighing 300-350 g were housed in a temperature controlled room (22±1 °C) with 14 h of artificial illumination daily (0600-2000) and given food and water *ad libitum*.

Plasma LH Response to Ginsenoside-Rb1

Male rats were catheterized with polyethylene tubing under light ether anesthesia via the right jugular vein (12, 25, 26). Twenty hours later, they were intravenous injected with ginsenoside-Rb_1 (Rb_1, 10 μg/kg) or saline 15 min before challenge with gonadotropin releasing hormone (GnRH, 2 μg/kg). Blood samples (0.4 ml each) were collected at 0, 30, 60, 120, and 240 min (for measurement of LH) or at 0, 5, 10, 15, 30, 60, and 120 min (for measurement of testosterone) following hormone injection. An equal volume of donor rat blood (21) was replaced immediately after bleeding. Plasma was separated by centrifugation at 10000 × g for 3 min. The concentration of LH or testosterone in each plasma sample was measured by the radioimmunoassay (RIA) (12, 24).

Effect of Ginsenoside-Rb1 on Plasma LH in Response to Swimming

The normal and orchidectomized (Orch, 1 week) rats were subjected to swim training (10 min/time; 2 times/day) for further 1 week. Twenty-four hours before swimming (water temperature = 25 °C; flow = 18 l/min; time = 10 min), rats were catheterized via the right jugular vein. They were injected intravenously with ginsenoside-Rb_1 (10 μg/kg) or saline prior to a 10 min-swim. Blood samples (0.6 ml each) were collected via the catheter at 0, 5, 10, 30, and 60 min following injection of ginsenoside-Rb1. Plasma was separated by centrifugation at 10000 × g for 3 min. The concentration of LH or testosterone in each plasma sample was measured by RIA. The levels of plasma lactate and glucose were determined by a lactate plus glucose analyzer (Statplus-2300, Yellow Springs Instruments, OH, USA) (17).

LH Release in Pituitary Tissue

Male rats were decapitated. The anterior pituitary glands (APs) were excised, bisected, preincubated and then incubated with Locke's medium containing 10 mM glucose, 0.003% bacitracin, and 0.05% HEPES at 37 °C for 30 min (26). Each hemi-AP was assigned to a flask containing 1 ml medium. The medium was aerated with 95% O_2 and 5% CO_2. APs were then incubated with Locke's medium containing ginsenoside-Rb1 for 30 min. At the end of incubation, the tissues were weighed. The media were collected and stored at -20 °C until analyzed for LH by RIA.

LH Release in AP Cells

Each batch of pituitary cell cultures was prepared from a pool of five to seven anterior pituitaries excised from male rats, as previously described (16). The sliced tissue fragments were dissociated by collagenase and hyaluronidase after brief exposure to trypsin. Routinely, the dispersed cells (1×10^5 cells) in 1-ml aliquots were plated in 2-cm^2 wells (Falcon 24-well multiwell tissue culture plates, Taiwan Ivy Corp., Taipei, Taiwan, ROC) and cultured overnight at 37 °C under moist 5% CO_2 and 95% air. The culture medium contained 2.5% fetal bovine serum

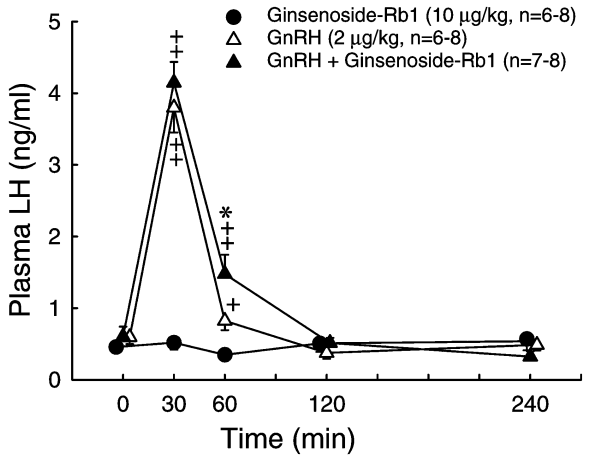

Fig. 1. Effect of ginsenoside-Rb1 on the basal and gonadotropin-releasing hormone (GnRH)-stimulated concentration of plasma LH in male rats. The experimental rats were catheterized via right jugular vein. They were intravenously injected with ginsenoside-Rb1 (10 µg/kg), GnRH (2 µg/kg), or GnRH plus ginsenoside-Rb1. Blood samples were collected at 0, 30, 60, 120, and 240 min following injection of ginsenoside-Rb1. The concentration of LH in rat plasma was measured by RIA. Each value represents mean±SEM. +, ++, $P<0.05$ and $P<0.01$ as compared with the value at 0 min, respectively. *, $P<0.05$ as compared with GnRH-injected rats.

(Hyclone Laboratories, Logan, UT, USA) and 10% defined supplemented bovine calf serum (Hyclone) in medium 199 without phenol red (Sigma, St. Louis, MO, USA) supplemented with Minimum Essential Medium (MEM) amino acids and vitamins (Gibco, Grand Island, NY, USA), 2.2 g/liter sodium bicarbonate, 1 mM sodium pyruvate, 4 mM L-glutamine, 25 mM HEPES, 50 µg/ml streptomycin, and 160 U/ml penicillin G. Phenol red was excluded from culture medium due to its weak estrogenic activity (11). All sera were pretreated with dextran-charcoal (15) to remove endogenous steroids.

After overnight culture for 16 h, 10 µl dihydrotestosterone (DHT, Sigma) in 0.1% ethanol-saline (diluent) were added to medium at a final concentration of 10 nM. Culture was continued for 72 h. Then cells were washed twice with 1.5 ml of PBS, respectively. Immediately after cell wash, 1-ml aliquots of BSA medium containing vehicle or various test drugs in the presence of DHT (10 nM) were added. Cells were incubated at 37 °C for 3 h under moist 5% CO_2 and 95% air. At the end of incubation period, medium was removed from cells and centrifuged. The supernatant was stored at -20 °C until assayed for LH.

RIA of LH and Testosterone

The concentration of LH in plasma and media samples were determined by RIA as described previously (26). The rat LH-I-6 used for iodination and the rat LH-RP-3 which served as standard preparation, were provided by the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the U.S. Department of Agriculture, USA. The sensitivity was 0.1 ng. The intra- and interassay coefficients of variation were 8.0%, and 4.5%, respectively.

The concentration of plasma and medium testosterone was determined by RIA as described previously (24). With anti-testosterone serum No. W8, the sensitivity of testosterone was 2 pg per assay tube. The intra- and interassay coefficients of variation were 4.1% (n=6) and 4.7% (n=10), respectively.

Statistical Analysis

All values are given as the mean ± standard error of the mean (SEM). The treatment means were tested for homogeneity by a two-way analysis of variance, and the difference between specific means was tested for significance by Duncan's multiple-range test (22). A difference between two means was considered statistically significant when $P<0.05$.

Results

Effect of Ginsenoside-Rb1 on Plasma LH and Testosterone in Male Rats

Administration of ginsenoside-Rb1 did not alter the basal and GnRH-stimulated peak levels of plasma LH, but enhanced the post-peak level of LH release ($P<0.05$) at 60 min following GnRH challenge in male rats (Fig. 1).

The concentration of plasma testosterone increased gradually from 15 min following IV injection of GnRH (Fig. 2). The maximal ($P<0.01$) response of plasma testosterone was observed at 60 min following GnRH injection. At 30 and 120 min following GnRH challenge, the administration of ginsenosides resulted in a lower testosterone response to GnRH challenge ($P<0.05$) as compared with vehicle-treated rats.

Effect of Ginsenoside-Rb1 on Plasma LH, and Testosterone in Response to Swimming

Plasma glucose levels were significantly higher ($P<0.01$) at 30 min after the beginning of the test swim compared with the pre-swim level (Fig. 3, top). The concentrations of plasma lactate were significantly increased ($P<0.01$) after 5 and 10 min of the swimming exercise (Fig. 3, middle). Plasma LH concentrations were not changed by swimming (Fig. 3, bottom).

Injection of ginsenoside-Rb1 (10 µg/kg)

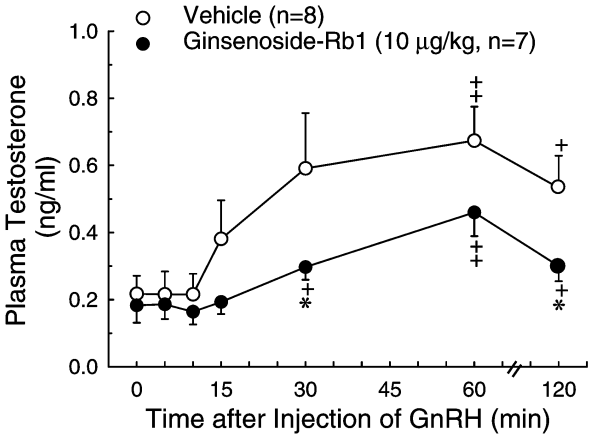

Fig. 2. Effect of ginsenoside-Rb1 on the levels of plasma testosterone in response to GnRH. Male rats were intravenously injected with ginsenoside-Rb1 (10 μg/kg) at 15 min prior to a single injection of GnRH (2 μg/kg) through a jugular catheter. Blood samples were collected at 0, 5, 10, 15, 30, 60, and 120 min following GnRH injection. The concentration of LH in rat plasma was measured by RIA. +, ++, P<0.05 and P<0.01 as compared with the corresponding levels at 0 min, respectively. *, **, P<0.05 and P<0.01 as compared with saline-treated animals. Each value represents mean±SEM.

significantly (P<0.05) increased plasma LH concentrations at the termination of the 10-min swimming exercise (Fig. 3, bottom). The concentration of plasma glucose and lactate were not significantly different between ginsenoside-Rb1- and saline-injected groups.

The levels of plasma glucose, lactate and testosterone increased in male rats after swimming for 10 min (Fig. 4). The levels of lactate and testosterone returned to basal levels at 120 min following beginning of swim. Administration of ginsenoside-Rb1 prior to swim did not alter the levels of plasma glucose and lactate but decreased the concentration of plasma testosterone in response to swim (Fig. 4).

LH Release in Pituitary Tissues

Incubation of rat pituitary tissues with GnRH (10 nM) for 30 min significantly increased (P<0.01) LH release (Fig. 5).

Incubation of rat pituitary tissues with ginsenoside-Rb1 at dose ranging from 10^{-6} to 10^{-4} M significantly increased (P<0.01) LH release in a dose-dependent manner (Fig. 5).

LH Release in AP Cells

Administration of GnRH for 3 hours, significantly increased (P<0.01) LH release of DHT-primed AP cells (Fig. 6).

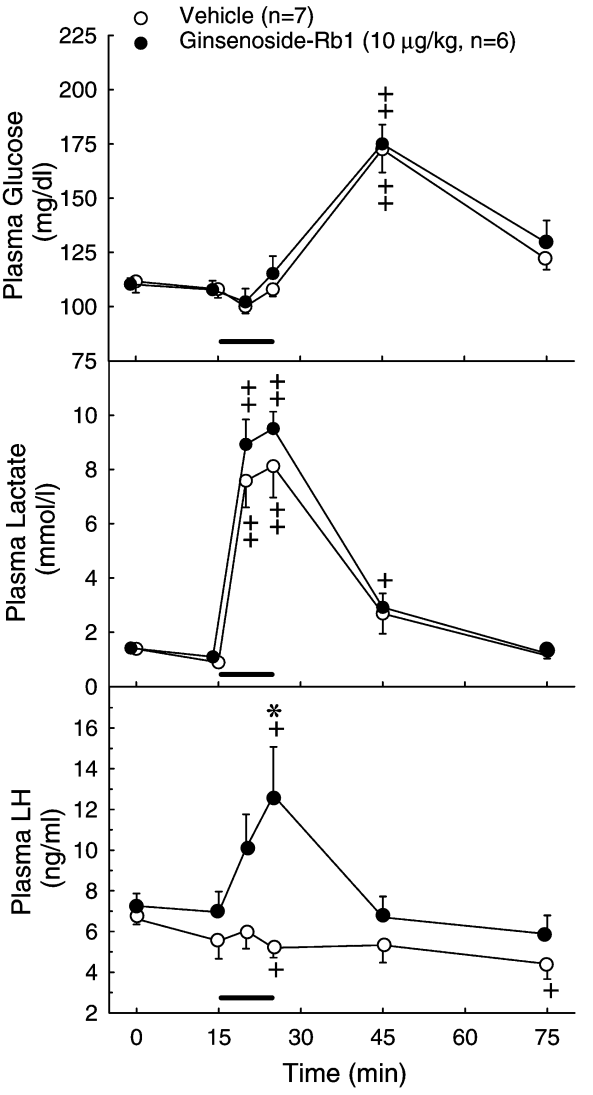

Fig. 3. Effect of ginsenoside-Rb1 on the concentration of plasma glucose (top), lactate (middle), and LH (bottom) in response to swimming exercise in orchidectomized (Orch) male rats. Male rats were Orch for 2 weeks. The experimental rats were catheterized via right jugular vein. They were intravenously injected with ginsenoside-Rb1 (10 μg/kg) or saline at 15 min prior to a 10-min swimming as shown by a horizontal bar. Blood samples were collected at 0, 15, 20, 25, 45, and 75 min following injection of ginsenoside-Rb1. The concentration of LH in rat plasma was measured by RIA. Each value represents mean±SEM. +, ++, P <0.05 and P<0.01 as compared with the value at 0 min. *, P< 0.05 as compared to the corresponding saline group, respectively.

Incubation of DHT-primed rat AP cells with DHT plus ginsenoside-Rb1 at the doses of 10^{-6} and 10^{-5} M significantly increased (P<0.05) LH release in a dose-dependent manner (Fig. 6).

Discussion

The present results demonstrated that ginsenoside-Rb1 increased the spontaneous release

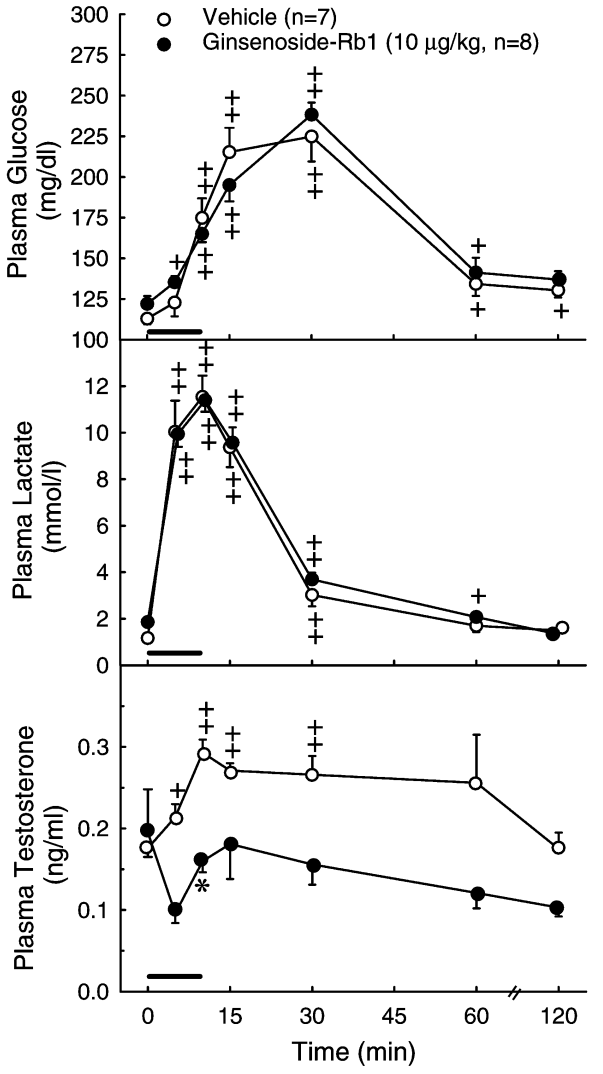

Fig. 4. Effects of ginsenoside-Rb1 (10 μg/kg) on the levels of plasma glucose, lactate and testosterone in response to swim. Male rats were intravenously injected with saline or ginsenoside-Rb1 (10 μg/kg) through the jugular catheters at 0 min prior to a 10 min swimming as shown by a horizontal bar. Blood samples were collected at 0, 5, 10, 30, 60, and 120 min following injection of ginsenoside-Rb1. +, ++, $P<0.05$ and $P<0.01$ as compared with the corresponding levels at 0 min, respectively. *, $P<0.05$ as compared with saline-treated rats. Each value represents mean±SEM.

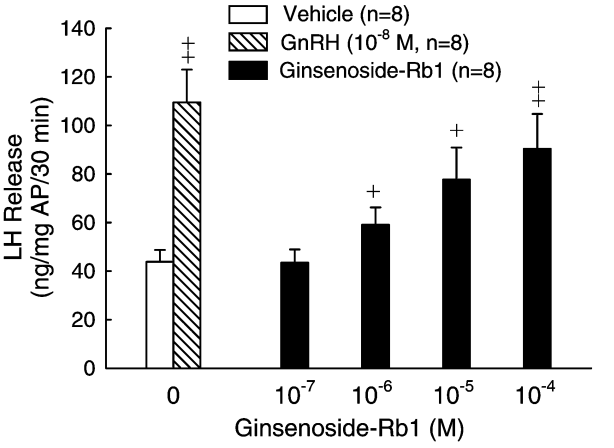

Fig. 5. Effect of ginsenoside-Rb1 on the release of LH from rat anterior pituitary gland (AP) *in vitro*. Rat APs were preincubated at 37 °C for 90 min and then incubated with Locke's medium containing ginsenoside-Rb1 (10^{-7} ~ 10^{-4} M) for 30 min. The concentration of LH in medium was measured by RIA. Each value represents mean±SEM. +, ++, $P<0.05$ and $P<0.01$ as compared to the control group, respectively.

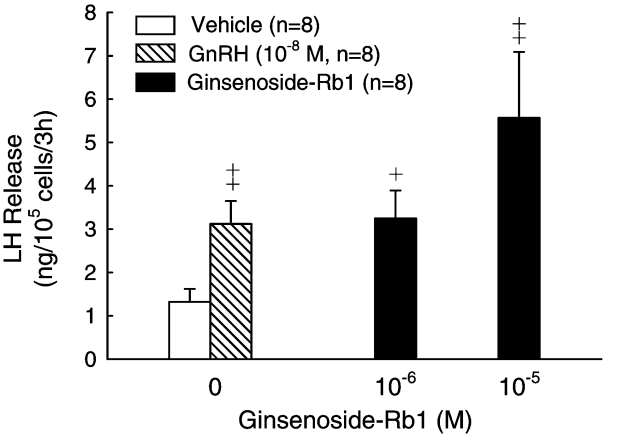

Fig. 6. Effect of ginsenoside-Rb1 on the release of LH in AP cells *in vitro*. The dispersed rat AP cells were primed with 10^{-8} M DHT for 72 h, and then challenged with ginsenoside-Rb1 (10^{-6} and 10^{-5} M) in the presence or absence of GnRH (10^{-8} M) for 3 h. The concentration of LH in medium was measured by RIA. Each value represents mean±SEM. +, ++, $P<0.05$ and $P<0.01$ as compared to control group, respectively.

of LH by rat AP cells. However, the enhanced levels of plasma testosterone in response to GnRH or swim were decreased by the administration of ginsenoside-Rb1.

The mechanism of ginsenoside-Rb1 to enhance the plasma LH level after swimming might be explained in two ways. First, ginsenoside-Rb1 might have direct effect on pituitary glands and increase LH secretion, or it could interfere the negative feedback effect of testosterone on the pituitary or hypothalamus. If ginsenoside-Rb1 interacts with testosterone, they might exert feedback on testosterone receptor binding or testosterone release. Our results showed that ginsenoside-Rb1 abolished GnRH or swim-enhanced testosterone release (Fig. 2 and 3). The reasons why ginsenoside-Rb1 decreased the testosterone response to GnRH *in vivo* were not known, but might be due to the hypersecretion of glucocorticoids induced by ACTH-like actions of ginsenosides after the administration of ginsenosides or ginseng root (2). Since both GnRH and ginsenoside-Rb1 increase LH

release, the possibility of increased LH secretion can not be involved in the reduction of testosterone secretion in response to the administration of ginsenoside-Rb1. More studies need to be conducted to figure out the mechanism of ginseng action on testosterone release.

Because the major source of circulating LH is secreted by the anterior pituitary glands (APs), it is reasonable to hypothesize that ginsenoside-Rb1 may stimulate LH release by acting directly on rat APs. After incubation of AP tissues and dispersed cells with ginsenoside-Rb1, our *in vitro* data indicate that despite the presence or absence of androgen, ginsenoside-Rb1 dose-dependently increased the spontaneous release of LH by acting directly on rat APs. This is the first report for describing the stimulatory effect of ginsenosides on LH release by rat anterior pituitary glands.

Administration of ginsenoside-Rb1 did not alter the levels of plasma LH in both intact and Orch rats. However, the basal level of plasma LH following I.V. injection of ginsenoside-Rb1 was enhanced by 10 min-swimming exercise in Orch rats. It is apparently that swim is a good model for examining the *in vivo* effect of ginsenoside-Rb1 on LH secretion. Ginsenoside-Rb1 increased LH release *in vitro* in the presence or absence of androgen suggested that the ginsenoside enhanced LH release is independent of testosterone feedback. Our previous report has demonstrated that swimming exercise increases the concentration of plasma lactate, and lactate stimulated GnRH release *in vitro* (17). We suggest that the output of hypothalamic GnRH might be further enhanced by ginsenoside-Rb1 during exercise.

Results from sport studies indicate that sex steroid response to exercise is determined by the mode, intensity, and duration of the exercise (5). Early studies have shown that circulating testosterone was elevated by brief intensive exercise (1, 23) and reduced by prolonged submaximal exercise (1, 19). In agreement with our previously study, rats were subjected to a swimming protocol that resulted in an elevation of plasma glucose, and lactate concentrations. Although the post-exercise plasma LH levels showed no change, which suggests that the swim-induced testosterone increase is independent of gonadotropin regulation, the change of testosterone production following administration of ginsenoside-Rb1 during exercise is not known at the present time.

In summary, the present data of *in vitro* experiments demonstrate that ginsenoside-Rb1 possesses a direct effect on LH release in rat AP. In consideration of the stimulatory effect of ginseng on copulatory behavior, ginseng has the latent capacity to develop as a therapeutic agent for hormonal related ejaculation deficiency patient.

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