EFFECTS OF LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH) ANALOGUE ON THE FUNCTION OF THE ISOLATED RABBIT CORPUS CAVERNOSUM

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An adverse effect of the administration of luteinizing hormone-releasing hormone (LH-RH) analogue is impotence. The effects of LH-RH analogue injection on the function of the rabbit corpus cavernosum were investigated. Eighteen male rabbits were divided into three groups, i.e., LH-RH analogue injection, castration, and sham-operated control groups. After measurement of serum testosterone, the LH-RH analogue (1.5 mg/kg leuprolide acetate) was injected once in the LH-RH group, castration in the castrated group, and sham surgery in the control group. Four weeks later, the rabbits were maintained in the same circumstance and serum testosterone was measured once a week. Four weeks after preparation, all rabbits were used for *in vitro* experiments.

At one week the serum testosterone level of the LH-RH and castrated groups decreased significantly from that in the sham-operated control group, which was sustained for the next 3 weeks. Although contractile strength of the corporal tissue taken from the castrated group was weakened in response to phenylephrine and KCl, corporal contractile strength of the LH-RH group was not. Under precontraction with 200 μM phenylephrine relaxation of the corpus cavernosum in response to field stimulation and sodium nitroprusside significantly decreased in both the LH-RH and castrated groups. However, there were no differences in the maximal relaxations induced by ATP and bethanechol between the three groups.

In conclusion, the LH-RH analogue impaired the relaxation of the corpus cavernosum induced by field stimulation and sodium nitroprusside as much as castration did. Although the corporal contraction to phenylephrine and KCl was decreased by castration, it was not altered by the LH-RH analogue.

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Key words: LH-RH analogue, Testosterone, Castration, Rabbit corpus cavernosum, Impotence

INTRODUCTION

Administration of luteinizing hormone-releasing hormone (LH-RH) analogue once a month has become a new standard treatment modality for advanced prostatic cancer instead of castration[1]. Although erectile dysfunction is one of the adverse effects of LH-RH analogue treatment, we could not find any study on the effects of LH-RH analogue on the local penile function.

The LH-RH analogue suppresses the serum testosterone level as much as castration does[1–5]. Although depletion of testosterone suppresses libido or sexual activities probably through the central nervous system, there are controversies in the relationship between the serum testosterone level and local function of the corpus cavernosum[6–12].

Herein, we investigated the effects of LH-RH analogue injection on the function of the rabbit corpus cavernosum *in vitro*.

MATERIALS AND METHODS

Animal preparation

Male Japanese white rabbits each weighing approximately 3 kg were purchased from Chubu Kagaku Co. (Aichi, Japan). Eighteen rabbits were separated into three groups (7 for LH-RH, 6 for castration, and 5 for sham). After measurement of serum testosterone in all rabbits, bilateral castration was done in the castrated group, and rabbits in the other two groups underwent sham surgery under anesthesia with ketamine/xylazine (ketamine: 30 mg/kg and xylazine: 3 mg/kg). Luteinizing hormone-releasing hormone (LH-RH) analogue (leuprolide acetate [D-Leu^6, des-Gly-$NH_2{}^{10}$, Pro-ethylamide^9]-LH-RH, 1.5 mg/kg, Takeda Chemical Industries, Ltd.) was injected once in the LH-RH group. In the other two groups 2 ml of vehicle including mannitol 100 mg, sodium carboxy methylcellulose 10 mg, and polysorbate 80 2 mg were injected. Following preparation all rabbits were

maintained for 4 weeks in the same breeding circumstance. During this time the serum testosterone level was measured by means of radioimmunoassay once a week (SRL Co, Tokyo, Japan).

In vitro muscle strip study

After four weeks all rabbits were sedated with an intramuscular injection of ketamine/xylazine (30 mg ketamine, 3 mg xylazine/kg), and anesthesia was maintained by intravenous injection of sodium pentobarbital (25 mg/kg). The penis was removed at the level of the attachment of the corporal bodies to the ischium. The grossly dissected organ preparation was then placed in Krebs' solution at room temperature. At this time, most of the overlying striated muscle was removed with care not to damage the underlying tunica albugniea. Once fully exposed, a slit was made in the proximal end of the tunica and extended distally. The corpus cavernosal tissue was sharply dissected free from the tunica bilaterally. Two tissue strips were obtained from a rabbit.

Longitudinal sections of the rabbit corpus cavernosum with the unstretched length about 8 mm were placed in organ baths containing 10 ml Krebs' solution (NaCl 119 mM, KCl 4.7 mM, $NaHCO_3$ 25 mM, $MgSO_4$ 1.2 mM, NH_2PO_4 1.2 mM, $CaCl_2$ 2.5 mM, and glucose 11 mM) at 37°C. Each tissue was equilibrated with a mixture of 95% oxygen and 5% carbon dioxide. One end of each strip was connected to a force displacement transducer, and changes in muscle tension were measured and recorded on a Rectigraph 8 K (San-ei Co. Tokyo, Japan).

Field stimulation was delivered via platinum electrodes set on both sides of the muscle strip in each organ bath. Transmural nerve stimulation was performed with a DPS-160 field stimulator (Dia Medical System Co, Tokyo, Japan) delivering biphasic square wave pulses of 50 V, 0.5 ms duration. The interval of stimulations was two minutes.

After equilibration for 1h at 2 g tension, tension increases responding to phenylephrine in doses from 0.8 to 200 μM and KCl Krebs' solution (124 mM KCl) were determined. KCl Krebs' solution was prepared by replacing NaCl with an equimolar amount of KCl. Relaxation effects induced by field stimulation, ATP, bethanechol, and sodium nitroprusside were studied under precontraction with 200 μM phenylephrine. After the tissue contraction induced by 200 μM phenylephrine reached its plateau, various frequencies of field stimulation (2–60 Hz), and maximal dose of ATP (2 mM), bethanechol (600 μM), and sodium nitroprusside (100 μM) were applied and subsequent relaxation was recorded.

Drugs and statistical analysis

ATP, phenylephrine, bethanechol, and sodium nitroprusside were purchased from Sigma Co. The contractile response was expressed as absolute gram tension of the tissue, and relaxation as percent relaxation of the total tonic tension (basal tissue tension plus increased tension by 200 μM phenylephrine). Statistical comparisons were made by using analysis of variance with Fisher's protected least significant difference with a $p<0.05$ accepted as indicating statistical significance.

RESULTS

Serum testosterone levels in both the LH-RH group and the castrated group significantly decreased one week after preparation, which was sustained for the next 3 weeks. There were no differences in serum testosterone level between the LH-RH and castrated groups at any time (Table 1).

Contractions of the corpus cavernosum in response to phenylephrine (Fig. 1) and KCl (Fig. 2) were significantly decreased, compared to the sham-operated control, by castration, but not by the LH-RH analogue injection.

The relaxation induced by field stimulation under precontraction with 200 μM phenylephrine was sigficantly decreased by both castration and LH-RH analogue injection (Fig. 3). The same response to sodium nitroprusside was observed in the castrated and LH-RH groups (Fig. 4). However, the responses to ATP and bethanechol were similar in all 3 groups.

DISCUSSION

Luteinizing hormone-releasing hormone (LH-RH), which promotes the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland in men and experimental animals, has been clinically used for the treatment of sterility[13] The potent LH-RH analogue either

Table 1. Effects of LH-RH analogue injection and castration on serum testosterone (ng/ml).

	Before preparation	Post preparation			
		1 wk	2 wks	3 wks	4 wks
LH-RH	2.70±1.80	0.80*±0.21	0.63*±0.29	0.34*±0.13	0.48*±0.17
Castrated	1.09±0.18	0.50*±0.23	0.28*±0.12	0.13*±0.07	0.27*±0.13
Sham	1.58±1.21	2.14 ±0.68	2.30 ±1.17	1.74 ±0.62	1.84 ±1.12

* Significant difference from the value of the sham-operated control: $p<0.05$

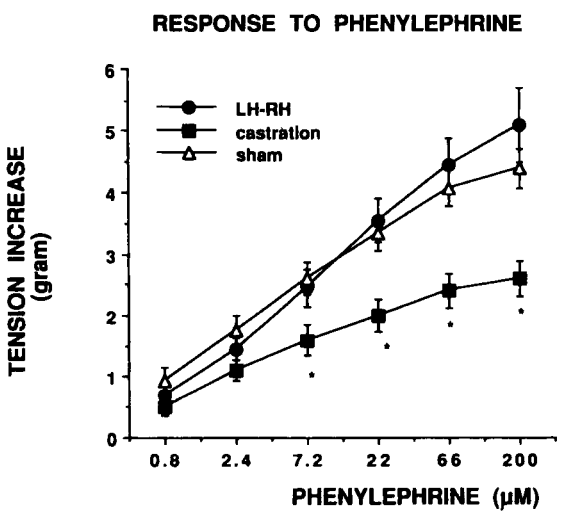

Fig. 1. Effects of LH-RH analogue injection and castration on the contraction of the rabbit corpus cavernosum in response to phenylephrine. Each point is mean±SEM of 5–7 duplicate observations. * Significant difference from the value of the sham-operated control ($p<0.05$).

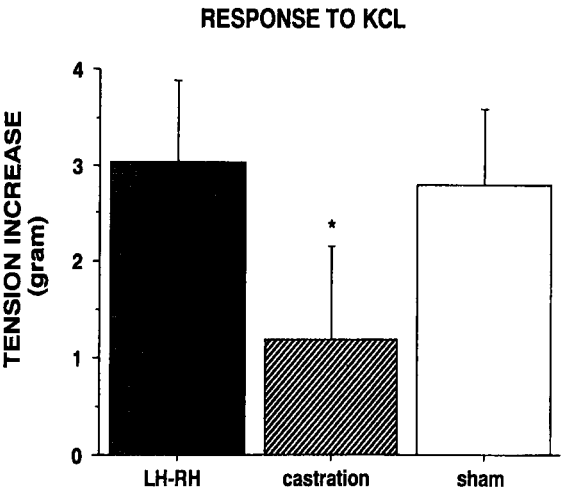

Fig. 2. Effects of LH-RH analogue injection and castration on the KCl-induced contraction of the rabbit corpus cavernosum. Each bar is mean±SEM of 5–7 duplicate observations. * Significant difference from the value of the sham-operated control ($p<0.05$).

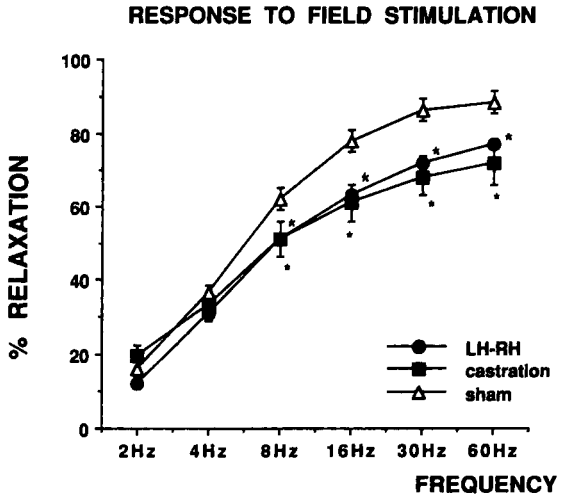

Fig. 3. Effects of LH-RH analogue injection and castration on the field-stimulated relaxation of the rabbit corpus cavernosum. Each point is mean±SEM of 5–7 duplicate observations. * Significant difference from the value of the sham-operated control ($p<0.05$).

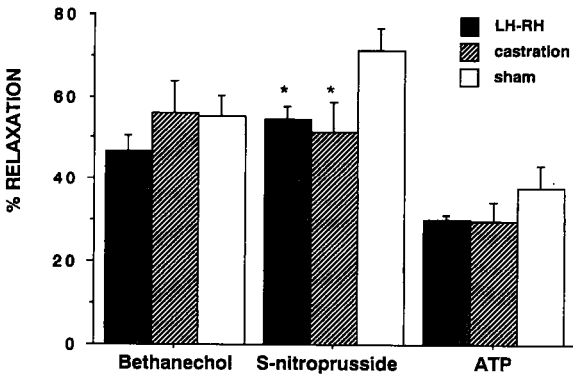

Fig. 4. Effects of LH-RH analogue injection and castration on the relaxation of the rabbit corpus cavernosum responding to ATP, bethanechol, and sodium nitroprusside (S-nitroprusside). Each point is mean±SEM of 5–7 duplicate observations. * Significant difference from the value of the sham-operated control ($p<0.05$).

stimulates or inhibits gonadal function depending on the dose and duration of treatment in men and laboratory animals[1,2,4] The inhibition of gonadal function caused by chronic treatment with the potent LH-RH analogue, i.e., paradoxical effects, has been clinically utilized to induce medical castration for treatment of a variety of hormonally responsive clinical disorders such as prostate cancer[2,3] Recently biodegradable microcapsules of copoly lactic/glycolic acid (PLGA) containing potent LH-RH analogue (leuprolide acetate) were developed to slowly and constantly release it for one month[4,14].

The LH-RH analogue decreases the serum testosterone as low as surgical castration through down-regulation on the pituitary gland resulting in a decrease in luteinizing hormone (LH). Serum testosterone, which works mainly through the central nervous system, is essential for the male erectile function. Following castration, which reduces serum testosterone levels by 90%[6], there is in general a decline in libido and erectile potency. However, local effects of depletion of testosterone on the corpus cavernosum are in controversy. In the dog and rat, surgical castration markedly reduced maximal intracavernous pressure during cavernous nerve stimulation[7,8] while other researchers confirmed no

significant effects of castration on the dog's erection induced by cavernous nerve stimulation[9,10]

The corpus cavernosum consists of the smooth muscle which is integrated by autonomic regulation. Several reports claimed that changes in hormonal inner-circumstance could alter the autonomic receptors and/or smooth muscle contractility[11,15,16] We observed that castration significantly weakened contractile strengths to phenylephrine, and confirmed a report of Baba[17] who found that castration reduced the responses to both alpha-1 and alpha-2 sympathomimetic agents, while injection of testosterone propionate increased them. Our observation of reduced contractile power to KCl following castration was in accord with that of Holmquist et al.[12] who suggested that surgical castration would deteriorate the smooth muscle/connective tissue ratio or would deprive the corporal tissue of responding to depolarization. Although both castration and LH-RH analogue similarly decreased the testosterone level, the response to phenylephrine was different. This implies that it is not the serum testosterone level alone which governs the responsiveness of the corporal tissue to an alpha adrenergic agonist. There are 2 possibilities : one is the testis itself and its intratesticular autoregulation mechanism which is obviously not under the influence of the pituitary gland, and the other is a transient increase in the testosterone level following a sudden increase in LH which is released from the anterior pituitary gland. The latter situation is clinically known as "a flare up" The period of a transiently high level of testosterone following LH-RH analogue injection is dependent on the species : 3 days for rats and 21 days for men[1,4,5] Since the testosterone level was decreased 7 days after administration, the latter speculation is unlikely in the present situation. The LH-RH analogue injection has been reported to reduce the weight of the male reproductive organ including testis as much as by half[4,5] Although testicular function is primarily regulated by the pituitary testicular axis, there is evidence that the intratesticular autoregulation works, for instance, as an interaction between the Leydig cell and the adjacent tubular cells[18] A possible candidate for this local Leydig cell regulator is estradiol[19] Moreover, in the testis there is a difference in the sensitivity to gonadotropine between enzymes which contribute to synthesis of steroid hormone[20,21] Although we confirmed that the LH-RH analogue injection reduced the testosterone level significantly, the other effects of the LH-RH analogue on the testis plays are not known. The testis and its autoregulation mechanism helped maintain the contractile strength of the corpus cavernosum to phenylephrine and KCl in the LH-RH group.

While contraction of the corpus cavernosum, related to detumescence of the penis, is sustained by alpha adrenergic stimulation, relaxation of the tissue provoking tumescence and penile erection is initiated and maintained by cholinergic, beta adrenergic and non-adrenergic non-cholinergic stimulation[22-25] Field stimulation brings relaxation via the intramural nerves of the cholinergic, adrenergic, and non-adrenergic non-cholinergic. ATP induces corporal relaxation through purinergic receptors[25], and bethanechol through local release of nitric oxide[26] Sodium nitroprusside relaxes the smooth muscle nonspecifically via suppression of the intracellular smooth muscle contractile mechanisms. The effects of castration on the corporal relaxation are controversial[11,12] Baba demonstrated a significant decrease in relaxation of the rabbit corporal tissue in response to acetylcholine and VIP (vasoactive intestinal polypeptide) after castration[17] We found that the tissue taken from the castrated and the LH-RH groups exhibited less relaxation in response to field stimulation and sodium nitroprusside. On the contrary, Holmquist et al.[12] reported that castration increased field stimulated-relaxation of the rabbit corpus cavernosum. Although the origin of this divergence is not clear, reports which had showed significant reduction of the intracavernous pressure increases in response to cavernous nerve stimulation and intracavernous drug injection support our findengs[27,28] Since the increase in intracavernous pressure is closely related to tumescence of the penis induced by relaxation of cavernous smooth muscle, relaxation of corpus cavernosum in response to field stimulation should be decreased by castration.

The degree of relaxation in response to sodium nitroprusside was significantly inhibited in the LH-RH and castrated groups while there were no differences in response to ATP and bethanechol in the 3 groups. This observation suggests that the serum testosterone level will affect the receptor independent relaxation mechanisms of the corporal smooth muscle, which in the clinical setting might impair sexual function for those who are treated by the LH-RH analogue.

In conclusion, both LH-RH analogue injection and surgical castration reduced the testosterone level and altered erectile function of the rabbit corpus cavernosum *in vitro*. Although contractile strength in response to phenylephrine and KCl was significantly decreased by the latter, this was not observed by the former. Relaxation of the tissue in response to field simulation and sodium nitroprusside was significantly impaired in the LH-RH analogue and castrated groups.

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和文抄録

Luteinizing hormone-releasing hormone (LH-RH) analogue の遊離家兎陰茎海綿体機能におよぼす影響

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インポテンスは luteinizing hormone-releasing hormone (LH-RH) analogue の副作用の一つである．LH-RH analogue 治療の陰茎海綿体機能におよぼす影響を家兎を使用して実験した．18匹の雄家兎を LH-RH 群，精巣摘除群，コントロール群に分け，血中テストステロン測定後それぞれ LH-RH analogue (leuprolide acetate 1.5 mg/kg) の皮下注射を LH-RH 群に，精巣摘除術を精巣摘除群に，sham 手術をコントロール群に施した．毎週血中テストステロンを測定し，4週後に実験に供した．

1週後血中テストステロン濃度は LH-RH 群および精巣摘除群でコントロール群に比較して有意に低下し，これはその後3週間持続した．実験浴槽内における遊離海綿体の各種刺激に対する反応性を調べた結果，phenylephrine，および KCl に対する収縮反応は精巣摘除群で有意に低下したが，LH-RH 群ではコントロールとの差は認めなかった．200 μM の phenylephrine による収縮状態における海綿体の弛緩実験では，電気刺激および sodium nitroprusside に対する弛緩反応が LH-RH 群と精巣摘除群で有意に低下した．しかし ATP と bethanechol による弛緩反応には3群間で有意差を認めなかった．以上の結果より LH-RH analogue 投与により陰茎海綿体の収縮機能は変化しないものの，電気刺激と sodium nitroprusside に対する弛緩反応が精巣摘除術同様に低下し，これが同薬剤による性機能低下の原因の一つと考えられた．

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