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Author(s)
ITOH, Yuichi; ANDO, Masashi; OHMURA, Masaharu; KONDO, Atsuo; MIYAKE, Koji; SAITO, Masahiko

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

Yuichi Itoh and Masashi Ando
From the Department of Urology, Kasugai Municipal Hospital

Masaharu Ohmura, Atsuo Kondo, Koji Miyake and Masahiko Saito
From the Department of Urology, Nagoya University School of Medicine

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.

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. 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. 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.

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-Leu6, des-Gly-NH210, Pro-ethylamide]-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 albuginea. 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, NaHCO3 25 mM, MgSO4 1.2 mM, KH2PO4 1.2 mM, CaCl2 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 I).

Contraction 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 significantly 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(3). 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
stimulates or inhibits gonadal function depending on the dose and duration of treatment in men and laboratory animals. 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. 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.

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%, 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 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 testo-
sterone 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
corporeal 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.\(^4\,5\)

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.\(^9\,5\) 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.\(^22\,23\)
Field stimulation brings relaxation via the intramural
erves of the cholinergic, adrenergic, and non-
adrenergic non-cholinergic. ATP induces corporal
relaxation through purinergic receptors,\(^24\) 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.\(^27\)
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
findings.\(^27\,28\) 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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インピテンスは luteinizing hormone-releasing hormone (LH-RH) analogue の副作用の一つである。LH-RH analogue 治療の陰茎硬体機能におよぼす影響を家児を使用して実験した。18匹の雄家児を 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。実験終結内における陰茎硬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陰茎硬体の弛緩実験では、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 betahanechol 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 投与により陰茎硬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。