Original Article

Effects of Ghrelin on Sexual Behavior and Luteinizing Hormone Beta-subunit Gene Expression in Male Rats

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Abstract

Background: The hormones of hypothalamo-pituitary-gonadal (HPG) axis have facilitative effects on reproductive behavior in mammals. Ghrelin as a starvation hormone has an inhibitory effect on HPG axis' function. Hence, it is postulated that ghrelin may reduce the sexual behavior through inhibiting of HPG axis. The aim of this study was to examine the effects of ghrelin and its antagonist, [D-Lys^3]-GHRP-6, on sexual behavior and LH beta-subunit gene expression in male rats.

Methods: In this experimental study, 128 male Wistar rats were divided into two groups. Each group was further subdivided into eight subgroups (n=8 rats/subgroup) including the animals that received saline, ghrelin (2, 4 or 8 *nmol*), [D-Lys^3]-GHRP-6 (5 or 10 *nmol*) or co-administration of ghrelin (4 *nmol*) and [D-Lys^3]-GHRP-6 (5 or 10 *nmol*) through the stereotaxically implanted cannula into the third cerebral ventricle. The sexual behavior of male rats encountering with females and the hypophyseal LH beta-subunit gene expression were evaluated at two different groups. Data were analyzed by ANOVA and $p<0.05$ was considered statistically significant.

Results: Ghrelin injection (4 and 8 *nmol*) significantly ($p<0.01$) increased the latencies to the first mount, intromission and ejaculation as well as the post-ejaculatory interval. Also, 4 and 8 *nmol* ghrelin significantly ($p<0.05$) increased the number of mount and decreased the number of ejaculation. In co-administrated groups, [D-Lys^3]-GHRP-6 antagonized the effects of ghrelin. Ghrelin injection (4 and 8 *nmol*) reduced the LH beta-subunit gene expression while pretreatment with [D-Lys^3]-GHRP-6 improved the gene expression.

Conclusion: Ghrelin decreased the sexual behavior and LH beta-subunit gene expression in male rats, whereas [D-Lys^3]-GHRP-6 antagonizes these effects.

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Introduction

Ghrelin is a gut/brain peptide comprising 28 amino acids and is originally identified as the endogenous ligand of the growth hormone secretagogue receptor (GHSR) (1, 2). Ghrelin as a starvation hormone is mainly secreted by the stomach. This peptide is also expressed in the brain, primarily in the hypothalamic arcuate nucleus, an important region for controlling energy balance and reproduction (1, 3). In addition, the presence of ghrelin and its receptors has been reported in GnRH neurons (4) and gonadotrophs of pituitary (5-7). Previous studies have indicated that ghrelin has a direct inhibitory action on GnRH release. Also, ghrelin inhibits luteinizing hormone (LH) release from the pituitary *in vivo* and *in vitro* (8-11). The GnRH neurons are the central core of the hypothalamic-pituitary-gonadal (HPG) axis in all vertebrate species; therefore, according to the localization patterns of ghrelin, this peptide has a role in the regulation of HPG axis function (8). Moreover, this peptide has also a suppressive role in the release of testosterone in

male mammals (10, 12). On the other hand, ghrelin has been shown to have effects on all three tissues of the HPG axis (10, 12).

Several studies have demonstrated the facilitative effects of hormones secreted from HPG axis on reproductive behavior in male and female animals (13-17). It has been previously shown that GnRH-immunoreactive fibers are present in regions of the brain known to regulate sexual behavior including the bed nucleus of the stria terminalis (BnST), the medial amygdala (mAg) and most notably, the medial preoptic area (mPOA) (18). According to previous studies, administration of gonadotropins and androgens facilitated the sexual behavior in male mammals (13-15). In addition, there are immunohistochemical evidences for the endocrine/paracrine role for ghrelin in the reproductive tissues of mammals (8). These evidences led us to postulate that ghrelin may also reduce sexual activity in addition to inhibiting the HPG axis. To test this hypothesis, the effects of centrally administered ghrelin on sexual behavior in adult male rats were examined. This is the first study for analyzing the effects of ghrelin on all aspects of male sexual behavior in any mammalian species. Moreover, to test whether the locomotion prospects have a role in changes of sexual behavior, another experiment was developed to analyze rats' locomotor activity evaluated by an open field. Alongside the behavioral study, the luteinizing hormone beta-subunit gene expression was assessed following the ghrelin injection. The aim of this assessment was to evaluate the ghrelin's effect on synthesis of LH as one of the indices of HPG axis' activity.

Additionally, an attempt was made to examine whether the possible effects of ghrelin on sexual behavior and LH beta-subunit gene expression depends on an interaction with GHSR-1a (5-7). GHSR-1a is the fully functional type of the ghrelin specific receptors. The broad range of biological processes exerted by ghrelin seem to be mediated by the interaction with specific receptors (5-7); most of these processes have been abolished by pretreatment with [D-Lys^3]-GHRP-6 (DLS), a selective GHS-R1a antagonist (19-22). Hence, DLS was used to determine the role of the GHS-R1a, and its antagonistic actions on ghrelin-induced effects.

The aim of present study was to investigate the effects of intracerebroventricular injection of ghrelin, DLS or co-administration of these peptides on sexual behavior and luteinizing hormone beta-subunit gene expression in male rats to understand the role of ghrelin as the starvation hormone on attenuation of the sexual behavior indices and LH synthesis in HPG axis.

Methods

Animals: One hundred and twenty-eight adult male Wistar rats weighing 200±20 *g* were used in this study. The animals were group-housed in polycarbonate cages. Rats were maintained in a temperature controlled room (22±2 ℃) under a 12 *hr*/12 *hr* light/dark cycle (lights on 08:00). Food and water were available at all times. All procedures for the maintenance and use of experimental animals were pre-approved by the Local Institutional Committee for the Ethical Use of Animals.

Surgery and Injections: All rats were anesthetized by intraperitoneal injection of a mixture of ketamine (80 *mg/kg* BW) and xylazine (20 *mg/kg* BW). For central injections, a guide cannula was stereotaxically implanted in the third cerebral ventricle according to the stereotaxic coordinates (AP=-2.3, ML=0.0, DV=6.5) published in the atlas of Paxinos and Watson (1989). The guide cannula consisted of a 22-gauge stainless steel needle secured to the skull with three stainless steel screws and dental cement. One 28-guage stainless steel removable obturator was inserted into the guide cannula to ensure that the cannula remained patent (23). Following the surgery, animals were housed individually. After the eight-day recovery period, these animals were divided into two groups. Each group (n=64) was further subdivided into eight subgroups (n=8 rats/subgroup). Sixty four rats in 8 groups intracerebroventricularly received saline (3 μl), ghrelin (2, 4 or 8 *nmol*/3 μl), DLS (5 or 10 *nmol*/3 μl), ghrelin (4 *nmol*/1.5 μl)+DLS (5 or 10 *nmol*/1.5 μl) in order to study the sexual behavior, and the other sixty four rats in 8 groups intracere-broventricularly received the same treatments for studying the locomotor activity and gene expression. Doses were chosen based on previous studies, which had established their stimulatory or inhibitory effects on HPG axis or feeding behavior. Ghrelin and DLS (Phoenix Pharmaceutical Inc., CA, USA) were dissolved in saline. Solutions were freshly prepared just before use and were injected by a Hamilton microsyringe (Hamilton Inc., USA). In coadministered group, DLS was injected 5 *min* before ghrelin injection.

Sexual behavior test: This experiment was conducted in a testing arena (56×32×32 *cm*) with a

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frontal glass. Each naïve male rat was individually placed in the arena 10 *min* after drug administration and allowed to habituate to testing chamber for 5 *min*; then a sexually active female rat was introduced and the sexual behavior test was performed in 40 *min* period. The female rats were tested for receptivity before being placed with the males; females that presented lordosis after the mount of a male rat were selected for studying the sexual behavior of naïve administrated males. All tests were performed during the late photo phase (15:00-19:00) and the following parameters for each male were recorded: the latencies of first mount (male places its forequarters on hindquarters of female from behind) (ML), first intromission (mount with vaginal insertion) (IL), and first ejaculation (EL); total number of mounts (NM), intromissions (NI) and ejaculations (NE) in 40 *min*; number of mounts (nM) and intromissions (nI) until the first ejaculation, and post ejaculatory interval (PEI). Additionally, other derived measures were calculated using mentioned parameters including: sum of the NM, NI and NE; copulatory efficiency (CE), and sexual activity index (SAI) based on Agmo et al. (24):

$$CE=(NI+NE/NM+NI+NE)\times 100$$

$$SAI=\log (1/ML\times t)+\log (1/IL\times t)+\log (1/EL\times t)\sqrt{(nM+nI)}+Y$$

The time of the observation was indicated with "t", and "Y" means 4 when an animal ejaculated and 0 when it did not. All latencies were calculated in seconds. The testing arena was cleaned with diluted ethanol after each trial.

Locomotor activity trial: The locomotor activity of administrated rats was evaluated by the open-field test. Ten minutes after injection, all rats were individually placed in a square arena (45×45×35 *cm*) divided in to nine equal sectors on the floor. The total number of sectors crossing with all four paws was recorded for 5 *min* (25).

Gene expression assay: All administrated male rats were decapitated 2 *hr* after injections; pituitaries were dissected and frozen in liquid nitrogen and stored at -80°*C* until processed for reverse transcriptase polymerase chain reaction (RT-PCR).

At first, total RNA was extracted using RNX-Plus solution (CinnaGen, Iran). Briefly, pituitaries were separately transferred to 1 ml ice cold RNX-Plus solution and homogenized. Chloroform (200 *µl*) was added to each sample and after vigorous mixing, centrifuged at 12000 *rpm* at 4°*C* for 15 *min*; the supernatant was gently mixed with an equal volume of cold isopropanol for 10 *min*; then the mixture was centrifuged at 12000 *rpm* at 4°*C* for 10 *min*. The aqueous phase was discarded and the RNA pellet was washed with 1 *ml* of 75% ethanol. The RNA pellet was quickly dried and reconstituted in 30 *µl* diethylpyrocarbonate (DEPC) water. The purified total RNA was quantified by the Nano-Drop spectrophotometer (Nano-Drop Technologies, USA).

Then, the synthesis of complementary DNA and the amplification of cDNA templates were carried out using the 2-step RT-PCR Kit (Vivantis Technologies, Malaysia), according to Kit's recommended protocol. For each gene of interest, cDNA template amplification was carried out in a final volume of 25 *µl*; the mixture consisted of 2.5 *µl* of synthesized cDNA solution, 1 *U* of Taq DNA polymerase, 2.5 *µl* of 10X PCR buffer (10X Vi Buffer A), 0.75 *µl* of 50 *mM* $MgCl_2$, 0.5 *µl* of 10 *mM* dNTPs mix, and 0.75 *µl* of forward and reverse primer mixture. The primers used were as follows: LHβ (accession no. NM_012858) forward: AGA GAA TGA GTT CTG CCC AGT CTG, reverse: AGG TCA TTG GTT GAG TCC TGG G (amplicon length 274 *bp*); and GAPDH [1] (accession no. BC_013915) forward: AAG AAG GTG GTG AAG CAG GCA TC, reverse: CGA AGG TGG AAG AGT GGG AGT TG (amplicon length 112 *bp*). The expression levels of LHβ were normalized by dividing by GAPDH mRNA expression levels. PCR cycling conditions were as follows: initial denaturation and enzyme activation at 94°*C* for 2 *min*, followed by 35 cycles of denaturation at 94°*C* for 30 *s*, annealing at 59°*C* for 30 *s* (LHβ), or 58°*C* for 30 *s* (GAPDH), and extension at 72°*C* for 30 *s*. As a final step, samples were kept at 72°*C* for 7 *min*. After the thermal cycling steps, PCR-amplified products were analyzed by electrophoresis on a 1.5% safe red solution mixed with agarose gel. The molecular size marker, a 100 *bp* plus, (CinnaGen, Iran) was run concurrently. Finally, gels were visualized under UV illumination and photographed. The intensity of each band was quantified using ImageJ software (version 1.41, USA) and the ratio of LHβ to GAPDH was determined.

Statistical analysis: Data are presented as mean± SEM. The Kolmogorov-Smirnov test was performed to evaluate the normality of the variables. Multiple comparisons were performed using one-way

1. Glyceraldehyde-3-phosphate dehydrogenase

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analysis of variance (ANOVA) followed by Tukey-HSD test by SPSS software (version 19.0, SPSS Inc., USA). Level of $p<0.05$ was considered statistically significant.

Results

Sexual behavior test: Figure 1 shows the sexual behavior latencies of ghrelin- or DLS-treated male rats. In relation to saline group, 4 or 8 *nmol* ghrelin significantly increased the mount, intromission and ejaculation latencies. No significant difference was observed between the effects of 5 or 10 *nmol* DLS and saline group on ML, IL and EL; whereas, a significant difference was observed between the 5 or 10 *nmol* DLS and 4 *nmol* ghrelin group. Co-injection of ghrelin (4 *nmol*) and DLS (5 or 10 *nmol*) significantly decreased the ML, IL and EL compared to ghrelin (4 *nmol*) group, but there was no significant difference between the effects of ghrelin (4 *nmol*)+DLS (5 or 10 *nmol*) groups and saline group on ML, IL and EL (Figures 1A, 1B, 1C). Different p-values are shown on the figure.

According to figures 2A and 2C, the total number of mount significantly increased and the total number of ejaculation significantly decreased in 40 *min* following the ghrelin (4 or 8 *nmol*) injection. No significant difference was observed between the effects of 5 or 10 *nmol* DLS and saline

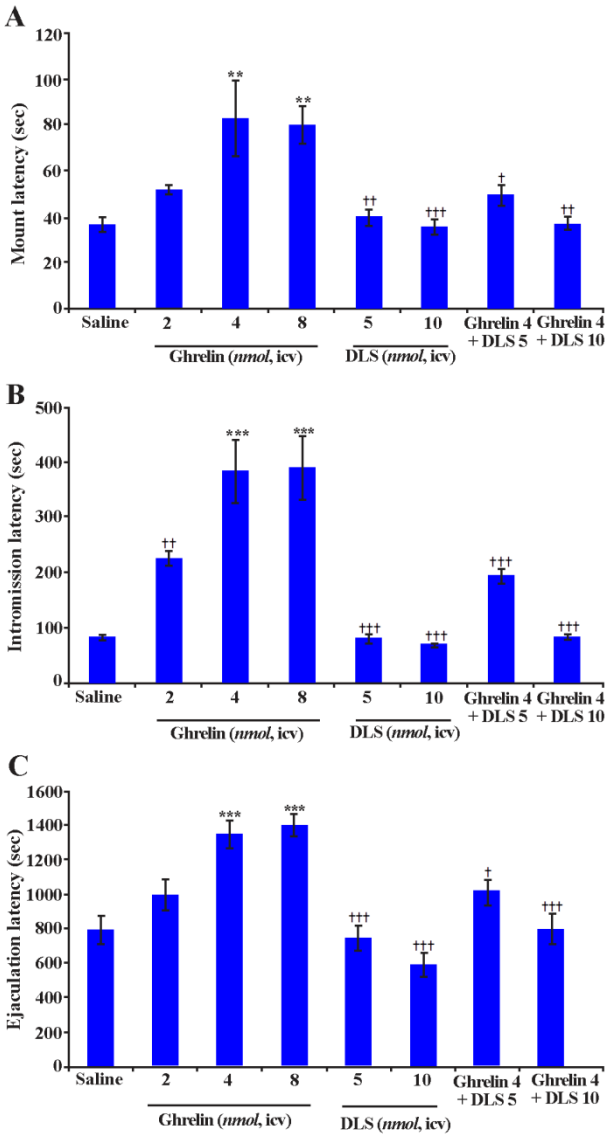

Figure 1. Effects of ghrelin, [D-Lys3]-GHRP-6 (DLS) or co-administration of ghrelin and DLS on mount; A: intromission; B: and ejaculation; C: latencies in male Wistar rats. Data are represented as mean±SEM; *p<0.05; **p<0.01; ***p<0.001 *vs*. saline group; †p<0.05, ††p<0.01; †††p<0.001 *vs*. ghrelin (4 *nmol*) group

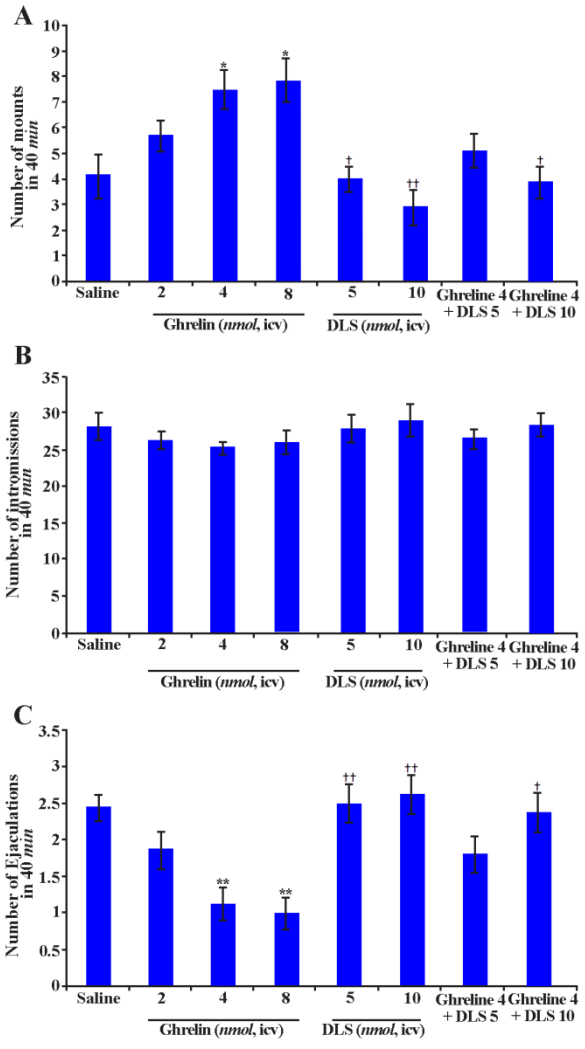

Figure 2. Effects of ghrelin, [D-Lys3]-GHRP-6 (DLS) or co-administration of ghrelin and DLS on total number of mounts; A: intromissions; B: and ejaculations; C: in 40 *min* in male Wistar rats. Data are represented as mean±SEM; *p< 0.05, **p<0.01 *vs*. saline group; †p<0.05; ††p<0.01 *vs*. ghrelin (4 *nmol*) group

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Table 1. Sexual behavior parameters of male Wistar rats following ghrelin or [D-Lys^3]-GHRP-6 (DLS) injection

	Saline	Ghrelin			DLS		DLS 5 *nmol*+ Ghrelin 4 *nmol*	DLS 10 *nmol*+ Ghrelin 4 *nmol*
		2 *nmol*	4 *nmol*	8 *nmol*	5 *nmol*	10 *nmol*		
nM	2.9±0.5	4.1±0.5	6.7±0.9***	7±0.7***	2.2±0.4†††	1.9±0.4†††	3.4±0.4††	2.9±0.6†††
nI	11.9±0.9	13.1±1.1††	20±2.2***	21.2±1.7***	10.4±0.9†††	8.8±0.7†††	14±1.2†	12±0.6††
PEI	423.8±12.2	476.7±10.7†	548±15.1***	562±10.3***	404.8±16.2†††	336.5±20.6**†††	452.1±12.5†††	422.1±13.7†††
NM+NI+NE	34.8±1.5	33.9±0.9	33.9±0.97	34.9±1.3	34.4±1.8	34.6±1.8	33.4±1.03	34.6±1.02
SAI	8.5±0.2	7.7±0.03	6.8±0.7**	6. 6±0.7**	7.3±0.1	7.4±0.1	6.8±0.1**	7.3±0.03
CE	87.4±2.5	82.9±2.1	77.9±2.3	77.1±3.1*	88.1±1.6††	91.4±2.3	84.4±2.2	88.5±2†

nM=Number of Mounts Until the First Ejaculation; nI=Number of Intromissions Until the First Ejaculation; PEI=Post Ejaculatory Interval; NM+NI+NE=Sum of the Total Number of Mount, Intromission and Ejaculation in 40 *min*; SAI=Sexual Activity Index; CE=Copulatory Efficiency.
Data are represented as mean±SEM; *p<0.05, **p<0.01, ***p<0.001 *vs*. saline group; †p<0.05, ††p<0.01, †††p<0.001 *vs*. ghrelin (4 *nmol*) group

group on NM and NE; whereas, a significant difference was observed between the 5 or 10 *nmol* DLS and 4 *nmol* ghrelin group. Co-administration of ghrelin (4 *nmol*) and DLS (10 *nmol*) significantly decreased the NM and significantly increased the NE compared to ghrelin (4 *nmol*) group, but a significant difference was not observed between the effects of ghrelin (4 *nmol*)+ DLS (5 *nmol*) and ghrelin (4 *nmol*) group on NM and NE (Figures 2A, 2C). According to figure 2B, there were no significant differences among the groups on number of intromission in 40 *min*. Different p-values were shown on the figure.

Table 1 shows the other parameters of sexual behavior. The post-ejaculatory interval and the parameters related to motor activity (mount and intromission frequencies until the first ejaculation) increased in the ghrelin-received animals compared to control group; co-administration of ghrelin and DLS decreased the mentioned parameters to control range. However, the sum of the total mount, intromission and ejaculation frequencies did not alter under several treatments. The sexual activity index (SAI) and the copulatory efficiency (CE) decreased following the ghrelin injection; DLS pretreatment abolished the inhibitory effect of ghrelin on CE in both 5 and 10 *nmol* and on SAI at the high dose (10 *nmol*). Different p-values depended on different doses and are shown in table 1.

Locomotor activity trial: Figure 3 shows the locomotor activity on administrated rats in 5 *min*. Total number of sectors crossing significantly increased in ghrelin injected rats as compared to control group, while the injection of high dose of DLS (10 *nmol*) significantly decreased the locomotor activity as compared to saline received rats.

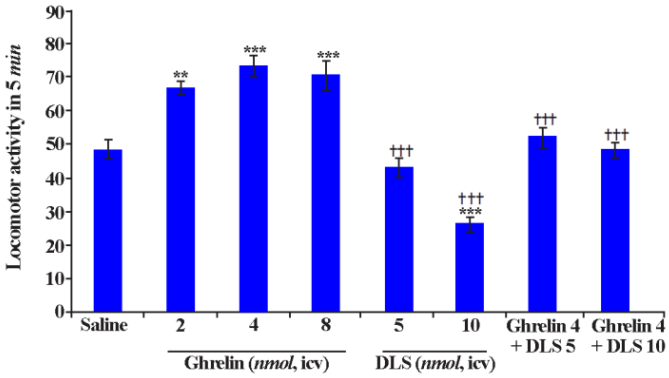

Figure 3. Effects of ghrelin, [D-Lys3]-GHRP-6 (DLS) or co-administration of ghrelin and DLS on locomotor activity in 5 *min* in male Wistar rats. Data are represented as mean±SEM; **p<0.01; ***p<0.001 *vs*. saline group; †††p<0.001 *vs*. ghrelin (4 *nmol*) group

Co-administration of ghrelin (4 *nmol*) and DLS (5 and 10 *nmol*) significantly decreased locomotion as compared to ghrelin received animals. Different p-values are shown on the figure.

Gene expression assay: Expression of LHβ and GAPDH mRNA in the pituitary of male rats is shown in figures 4A and 4B, respectively. The ratio of LHβ to GAPDH was determined after the quantification of each band using imageJ software and is shown in figure 4C. Relative LHβ mRNA expression significantly (p<0.001) decreased following the ghrelin (4 or 8 *nmol*) injection, while the administration of DLS (5 or 10 *nmol*) had no significant effect on gene expression levels. However, co-injection of the lowest effective dose of ghrelin (4 *nmol*) and the highest non-effective dose of DLS (10 *nmol*) significantly (p<0.01) increased the relative expression of LHβ mRNA compared to ghrelin (4 and 8 *nmol*) groups.

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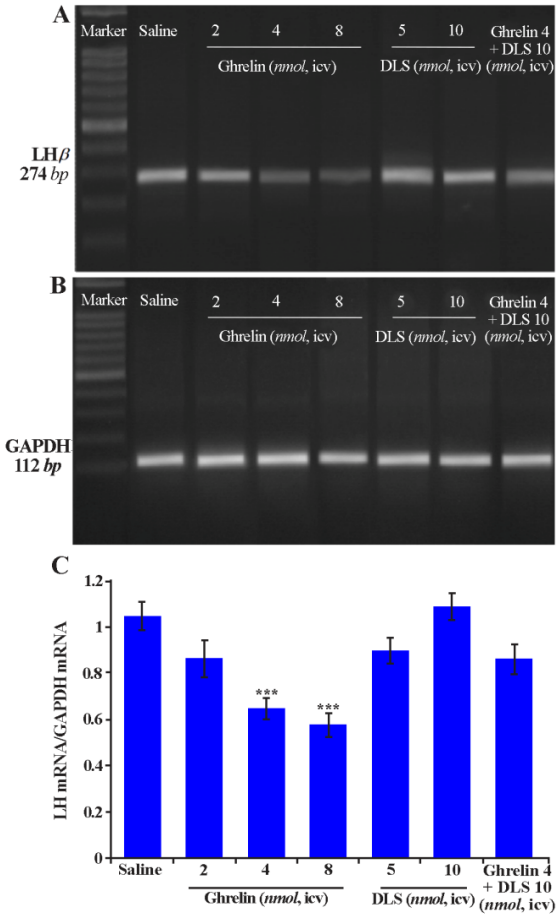

Figure 4. Effects of ghrelin, [D-Lys3]-GHRP-6 (DLS) or co-administration of ghrelin and DLS on amplification products of PCR; LHβ subunit; A: GAPDH; B: Gene expression levels and the relative expression levels of LHβ to GAPDH; C: In male Wistar rats. Data are represented as mean±SEM; *** p< 0.001 *vs*. saline group; ††p<0.01 and †p<0.05 *vs*. ghrelin (4 *nmol*) group

Discussion

Identification of ghrelin and its receptors in hypothalamus, pituitary and gonads (3-8) and the subsequent demonstration of the facilitative effects of hormones secreted from these tissues on reproductive behavior (10, 13-16, 18) led us to hypothesize that in addition to affecting on HPG axis, ghrelin may also alter the sexual behavior. This is the first study to analyze the effects of ghrelin and DLS ([D-Lys^3]-GHRP-6) on male sexual behavior in any mammalian species. Present results show that ghrelin injection distorted some aspects of sexual behavior and reduced LHβ subunit gene expression while enhanced locomotor activity of male rats.

In this study, it was found that ghrelin has similar inhibitory effects on sexual behavior in male rats as previously reported for female mice in a dose-dependent manner (26). Ghrelin dose dependently increased mount, intromission and ejaculation latencies. These latencies are commonly used for evaluating male sexual motivation (27) characterized by a synchronization of sexual desire arising in the brain and its transmission to the periphery, resulting in penile erection (16, 17). It has been suggested that testosterone positively control both the initiation and the end of the penile erection and that it is a main synchronizer of sexual activity (16, 17). In the absence of testosterone, the sexual desire arising is delayed and uncoordinated with penile erection (16, 17). Previous studies have shown the suppressive role of ghrelin in the release of testosterone in male mammals (10, 12). Ghrelin presumably postponed the beginning of sexual activity through decreasing testosterone in HPG axis. However, this presumption should be investigated. Also, as was observed, pretreatment with DLS reduced the latencies of studied rats.

In this study, despite the decreased number of intromission and ejaculation in ghrelin-received animals, the number of mounts increased in these rats. Nevertheless, there was no difference in sum of the total number of mounts, intromissions and ejaculations among different groups. It suggests that the decrease in the number of intromission and ejaculation after ghrelin injection was compensated by an increase in the number of mounts. In this study, it was demonstrated that ghrelin increased the locomotor activity in the open field test. Presumably, increasing the number of mounts in ghrelin-received animals due to the effects of this peptide on locomotor activity may be a compensatory mechanism to prevent the decreasing of locomotor activity during the intercourse. Previously, a number of studies have been conducted into the effect of ghrelin on locomotion indicating the facilitative effects of this peptide on locomotor activity (28, 29). Nevertheless, there is controversy over the increasing effect of ghrelin on locomotor activity. These discrepancies among studies may be due to the differences in the animal species used, rout or dose of peptide administrated or the photoperiodic condition of experiments (30, 31). Also, few studies showed that DLS treatment or ghrelin receptor deficiency reduced the locomotor activity (29, 32, 33). These findings are compatible with our observations showing that DLS attenuated the enhanced ghrelin-induced locomotor activity and mount frequency. Altogether, it is concluded that the damage in sexual be-

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havior following the ghrelin injection might be the result of other variables rather than locomotion prospects. In other words, this possibility is rejected that reduced sexual behavior after ghrelin injection may result from decreased motor activity.

Considerable decrease in the number of ejaculation as the main factor of success in reproduction process caused a significant decrease in copulatory efficiency in ghrelin-received animals. It may be argued that ghrelin significantly reduced mating success rate. This peptide led to a decrease in the body's ability to perform the ejaculation presumably through the mechanisms regulating energy balance. Nowadays, it is proved that negative energy balance and many food intake-enhancing hormones such as ghrelin have inhibitory effects on the reproductive axis (10, 12, 34, 35). Accordingly, our findings indicating the inhibitory effect of ghrelin on sexual behavior is compatible with the fact that starvation conditions and negative energy balance prevent the loss of energy in the reproductive pathways. Moreover, ghrelin also increased the interval between the copulatory cycles (PEI), probably due to the delay in the recovery of energy for the next copulation. Calculation of sexual activity index (SAI) in the studied animals confirmed the suppressive effects of ghrelin on reproductive behavior. However, pretreatment with DLS increased the number of ejaculations and in turn improved the mating efficiencies.

The potential implications of these findings are clear; ghrelin has a deleterious effect on male rat's sexual behavior. Up to now, there is no report about the involvement of ghrelin in regulation of the male sexual behavior. However, Bertoldi et al. demonstrated that ghrelin was able to inhibit the sexual behavior of female mice and that it was involved in receptivity reduction after food scarcity (26). Also, Shah and Nyby showed that ghrelin quickly suppressed the androgen-dependent behaviors including the ultrasonic mating calls to a female and the latency to attack a stimulus male through its direct effects on the brain in male house mice, Mus musculus (36). Additionally, in this study DLS, the GHSR-1a antagonist, abolished the prejudicial effects of ghrelin on sexual behavior. The sexual behavior aspects under DLS treatment alone had no significant differences with those in saline-treated rats, while DLS modified the behavioral effects of ghrelin in co-administrated animals. These data suggest that the inhibitory effect of ghrelin on sexual behavior is mediated by GHSR-1a. Previously, DLS has been investigated in food intake (19, 20), inflammatory pain (37), Ischemia-Reperfusion (21), hormone secretion (22) and some other studies in which the several effects of ghrelin were or were not attenuated in animals pretreated with DLS. However, this is the first study evaluating the effects of DLS on male sexual behavior.

It was also revealed that ghrelin and DLS had regulatory effects on LHβ subunit gene expression in male rats. In this study, central administration of ghrelin declined the LHβ subunit gene expression. Previous neuroanatomical analysis indicated that many ghrelin fibers have been projected to LH contained neurons (6-8) and ghrelin secretion decreased Fos induction in LH neurons and suppressed the LH secretion (8, 10, 11). Therefore, our findings showing the inhibitory effects of ghrelin on LH synthesis are in line with previous studies based on reducing effects of ghrelin on LH release. Notwithstanding the original reports, where ghrelin decreased LH release in several species (8, 10, 11), few studies to date had addressed the suppressive role of ghrelin in LHβ subunit gene expression. Moreover, to see whether the effect of ghrelin on LHβ expression is mediated through the GHS-R1a or not, DLS was used in this study. Notably, blocking the ghrelin receptor (GHS-R1a) with the antagonist DLS prevented the ghrelin-induced effects on LHβ expression. Several lines of evidence suggested the direct and indirect stimulatory effect of luteinizing hormone on regions of the brain which have a role in regulating the male sexual behavior (10, 18, 38). According to these evidences, inhibiting the LH synthesis or release may lead to copulatory deficiency. All in all, our data suggest that ghrelin reduces the LH synthesis and in turn decreases sexual behavior. It is worth mentioning that more studies are required to clarify the direct effects of ghrelin on regions of the brain known to regulate male sexual behavior.

Furthermore, the dose dependent effects of ghrelin were observed on both sexual behavior and locomotor activity in male rats. Therefore, ghrelin in the lowest dose had no significant effect on most of the aspects of sexual behavior while at the same dose, it significantly affected the locomotor activity. It is suggested that the sensitivity of ghrelin receptors in locomotor activity regulating system is more than the ones in the brain regions which control the sexual behavior. Presumably, ghrelin as a starvation hormone in low physiological doses has a negligible effect on mating behav-

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ior but continuity of ghrelin release has a remarkable effect on reproductive behavior. Therefore, the negative energy balance that causes the surge of ghrelin may affect reproduction and sexual activities.

Conclusion

In this study, copulatory actions of ghrelin and its specific antagonist, DLS, were examined. To do so, behavioral tests and LHβ subunit gene expression assessment were implemented in male rats after injection of different doses of ghrelin or its antagonist through the central rout of administration. Overall, our results suggest that ghrelin in a dose-dependent manner decreased the sexual desire and ability, and postponed the beginning of the intercourse. DLS antagonizes the putative inhibitory role of ghrelin in the regulation of sexual behavior, which involves indirect actions through inhibition of LH synthesis. Additional studies are required about the possible direct effects of ghrelin on brain regions which regulate the male sexual behavior and also about the efficacy of ghrelin following chronic administration.

Conflict of Interest

Authors declare no conflict of interest.

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