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Intravenous Orexin Reduces LH Secretion in Castrated Camelus Dromedaries Fed a Sub-maintenance Diet

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ABSTRACT : It has been shown that orexin has an inhibitory effect on gonadotropin secretions in non-ruminant animals. The goal of this study was to determine whether orexin affects LH, and FSH secretions in the camel, as a pseudo-ruminant animal, under different dietary energy content. Sixteen castrated camels were randomly divided into 4 groups. Animals in groups 1 and 2 were fed 100% and animals in groups 3 and 4 were fed 50% energy content in their diet for 20 days. After 20 days, animals in groups 1 and 3 received infusions of 1 μg orexin and groups 2 and 4 received infusions of 2 μg orexin into their jugular vein. Blood samples were collected from the jugular vein every 20 minutes from 4 h before the first infusion of orexin until 4 h after the last orexin infusion. Lower dietary energy intake and infusions of 2 μg but not 1 μg orexin significantly ($p<0.01$) decreased the mean plasma concentrations and pulse amplitudes of LH of the animals. Infusion of 1 and 2 μg orexin did not change the secretions of LH of the animals fed NE. Different energy dietary intake and infusion of 1 and 2 μg orexin did not change the mean plasma concentrations of FSH of the animals in all groups. Infusions of 1 and 2 μg orexin significantly ($p<0.01$) decreased the glucose levels of animals fed LE but not in NE fed animals. Additionally, plasma glucose levels of the LE-fed animals in groups 3 and 4 were significantly ($p<0.01$) lower than those of the animals in groups 1 and 2 fed NE diet. The results of this experiment indicated that orexin may negatively affect LH and FSH in camels with negative energy balance, but not in those with positive energy balance. (**Key Words :** Orexin, LH, FSH, Camel)

INTRODUCTION

The desert camel (*Camelus dromedarius*), is a pseudo-ruminant, with certain physiological, biochemical and pharmacological peculiarities which make it distinct from other related ruminants. For example, higher plasma glucose level, less insulin responsiveness and fatty acid metabolism are some of the physiological peculiarities that make it different from a ruminant (Elmahdi et al., 1997; Kaske et al., 2001), and also they are well known for their exceptional ability to withstand long periods of food deprivation (MacFarlane et al., 1963; Perk, 1963), However, the neural mechanisms enabling camels to withstand food deprivation are poorly understood. It is well established that orexigenic peptide secretions increase during energy deprivation in ruminants and non-ruminants (Chaillou and Tillet, 2005). Among the orexigenic hormones, orexin is a 33-amino-acid neuropeptide that is mostly found in the hypothalamus (Arihara et al., 2000; Backberg et al., 2000; Antunes et al., 2001). Based on its neuronal distribution in the hypothalamus, orexin coexists with many other neurons. For example, orexin neurons are found in high concentrations in hypothalamic areas considered to be important in the regulation of many physiological effects such as food intake, adrenal secretions, and reproduction (Balasko et al., 1999; Antunes et al., 2001; Kohsaka et al., 2001). The effect of orexin on reproduction is not very clear. For example, it was shown that orexin decreases or increases GnRH and LH secretions. In humans, it was shown that plasma LH concentration is reduced in hypocretin-deficient narcoleptic men, whereas gonadal steroid hormone levels are normal (Kok et al., 2004). The mechanism of this inhibitory effect of decreased orexin is through the decrease in GnRH secretion. This data suggests that orexin increases LH secretions through the GnRH secretions. Moreover, other studies show that intra-cerebroventicular or rostal POA administrations of orexin induces GnRH and LH concentrations in rats (Pu et al., 1998; Kohsaka et al., 2001), whereas administration of orexin in medial POA and ARC/ME decreases GnRH and LH concentrations in rats (Pu et al., 1998; Russell et al., 2001). Furthermore, other studies showed that orexins suppressed the pulsatile secretion of LH in ovariectomized

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female rats (Tamura et al., 1999; Furuta et al., 2002). All of the above studies with the contradictory results were conducted in humans and rats as non-ruminants. It is assumed that the control of feeding behavior along with orexin secretion in ruminants is different from that of non-ruminants. There are no reports about the orexigenic effect of orexin on LH and FSH secretions in ruminants fed different dietary energy content. Therefore, the goal of this experiment was to determine whether orexin affects LH and FSH secretion in camels fed different energy content in the diet.

MATERIALS AND METHODS

These experiments were approved by the Committee of Research Ethics of Shahid Beheshti University.

Experimental design

Sixteen 5-year-old castrated camels (weighing between 340-350 kg) were randomly divided into four groups. Animals were fed either 100% (Groups 1 and 2) or 50% (Groups 3 and 4) of their maintenance energy requirements for 30 days. All animals were fed every day at 17.00 h, and took 1 to 3 h to finish eating all the diet. Gross energy and chemical composition of feedstuffs were analyzed in the Animal Science Research Institute of Yazd. Diets were formulated based on AFRC (1995) (Table 1). During the course of the experiment, the daily ration was weighed out based on body weight and individually given to each camel every morning. The camels had free access to fresh water. Other requirements were balanced at maintenance level. Body weight of animals was measured on day 1 and 30 of the experiment.

Table 1. Experimental ration ingredients and content of energy and nutrients

Ingredient	Diet of different energy content	
	100 (DM %)	50 (DM %)
Wheat straw	0	9,000
Alfalfa	1,000	0
Corn grain	3,000	0
Barley	3,000	0
Canola meal	1,000	300
Wheat bran	1,670	0
Urea	130	500
Lime	40	40
Salt	80	80
Trimix	80	80
Food intake	10,000	10,000

Surgery for cannulation of the jugular vein

After 30 days, animals of all groups were anesthetized throughout the surgery for jugular vein cannulation. Surgical procedures were done under general anesthesia induced by sodium pentobarbital and maintained by halothane in a closed circuit system (Dziuk et al., 1964). Catheters (70 cm) were made for the jugular vein with polysiloxane tubing with an inside diameter of 0.16 cm and a length of 70 cm. A piece of silk was attached to the catheter to secure it to the tissue near the jugular vein. Camels were anesthetized with 30-40 ml of sodium pentobarbital (65 mg/ml concentration) and scrubbed with iodized soap and sprayed with a tincture of prepodyne. The jugular vein was exposed as described by Pond and Houpt (1978). After cleaning the jugular vein, the catheter was inserted 15 cm into the vein. The silk was tied to tissue near the point of insertion. The catheter was passed subcutaneously to the dorsal aspect of the neck using a trocar. The incision was then closed with one stitch in the cut edge of the muscle, followed by suturing of the skin. A removable plastic plug was inserted into the external end of the catheter. No antibiotics were administered to the animals. Each camel was kept in a single cage for a 4-day recovery period. During recovery, catheters were flushed with PBS solution and heparin solution to prevent clotting.

Orexin (Tabeshyarnoor Co. Ltd.) with 99 percent purity was dissolved in ethanol and stored at -20°C as a stock solution (10 μg/500 μl) for no longer than two days. During the experiments, camels were kept in comfortable cages where they could lie down and have unrestrained access to hay. Camels in Groups 1 and 3 received 1 μg orexin/10 ml and camels in Groups 2 and 4 received 2 μg orexin/10 ml into their jugular vein every hour for 4 h at 08.00.

Blood collection

Blood samples were collected from the jugular vein cannula every 20 minutes from 4 h before first infusion of orexin until 4 h after the last orexin infusion following injection with GnRH. Blood samples were kept at 4°C until centrifugation. A saturated sodium citrate solution (40 μl sodium citrate solution/ml blood) was added to the samples before centrifugation to prevent clotting of plasma during storage. Plasma was stored at -20°C until assayed for LH and FSH.

Hormone assays

Plasma LH and FSH were measured by a homologous double-antibody radioimmunoassay (RIA) as described previously (Pelletier et al., 1982). For LH assay, bovine LH (TYN-bLH) and antisera against LH were provided by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran). Bovine LH (TYN-bLH) was used for iodination. A seven-point standard curve ranging from 0.04 to 10 ng LH was used. An average assay binding of 40% was achieved using an initial 1:20,000 dilution of LH antiserum for LH

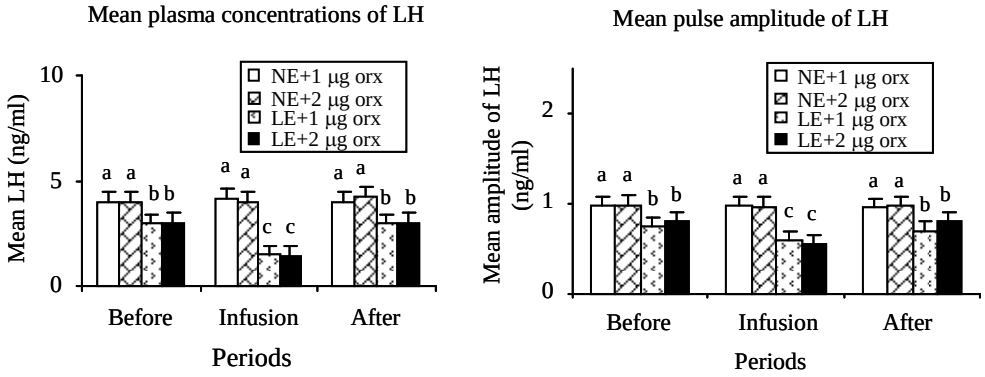

Figure 1. Mean plasma concentrations and pulse amplitude of LH of animals in Groups 1 (NE and 1 µg orexin), 2 (NE and 2 µg orexin), 3 (LE and 1 µg orexin) and 4 (LE and 1 µg orexin) and before, during and after infusions of orexin (NE = Normal energy; LE = Low energy). [a, b, c] Treatments with different letters are different at p<0.01.

assays. The inter- and intra-assay variations were 6% and 9%, respectively. For FSH assay, bovine FSH (TYN-bFSH) and antisera against FSH were provided by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran). Bovine FSH (TYN-bFSH) was used for iodination. A seven-point standard curve ranging from 0.05 to 20 ng FSH was used. An average assay binding of 70% was achieved using an initial 1:10,000 dilution of FSH antiserum for FSH assays. The inter- and intra-assay variations were 3% and 5%, respectively.

Statistical analysis

All analyses were conducted using General Linear Model procedures (SAS, 1996). Data was analyzed using an analysis of variance for a repeated measure design. Mean comparisons were evaluated by least significant difference with a single degree of freedom. The number of LH pulses was determined by the PC-PULSAR computer program according to the method of Merriam and Wachter (1982) with the following G parameters: G1 = 3.98; G2 = 2.40; G3 = 1.68; G4 = 1.24 and G5 = 0.93.

RESULTS

Body weight

Fifty percent energy content in the diet for thirty days significantly (p<0.01) decreased the mean body weight of animals in Group 2 from 345 kg to 273 kg. This was similar to our previous finding reporting that negative energy balance decreased body weight in camels and ewes (Dahlborn et al., 1992; Towhidi et al., 2007). In those studies, an 80% energy diet decreased body weight by about 10-12 kg.

LH

Mean plasma concentrations and pulse amplitudes of LH were significantly (p<0.05) lower in animals of Groups 3 and 4 than in Groups 1 and 2. Also, infusion of 2 µg, but not 1 µg, orexin significantly (p<0.01) decreased mean plasma concentrations and pulse amplitudes of LH of the animals in Groups 3 and 4 that were fed LE. Mean plasma LH levels of animals in Groups 3 and 4 were about 3, 1.5, 3 and 2.9, 1.4, 3 ng/ml before, during, and after infusion of orexin, respectively (Figure 1). Also, Mean pulse amplitudes of LH of the animals in Groups 3 and 4 were about 0.7, 0.6, 0.97 and 0.8, 0.5, 0.8 ng/ml before, during, and after infusion of orexin, respectively (Figure 1). Mean plasma LH levels of the animals in Groups 1 and 2 were about 4, 4.1, 4 and 3.9, 4.1, 4 ng/ml before, during, and after infusion of orexin, respectively (Figure 1). Also, mean pulse amplitudes of LH of the animals in Groups 3 and 4 were about 0.97, 0.95, 0.98 and 0.95. 0.95, 0.96 ng/ml before, during, and after infusion of orexin, respectively (Figure 1). Lower dietary energy intake also significantly (p<0.01) decreased mean plasma concentrations of LH in animals of Groups 3 and 4 (Figures 3 and 4).

FSH

Low dietary energy intake did not change mean plasma FSH level in animals of all groups, but infusion of orexin decreased (not significantly) the mean plasma FSH level of animals in Groups 3 and 4. Mean plasma FSH levels of the animals in Groups 1, 2 and 3, 4 were about 4, 4.1, 4 and 4, 3.5, 3.8 ng/ml before, during, and after infusion of orexin, respectively (Figure 2).

Glucose

Orexin did not change the mean plasma glucose concentrations of the camels in Groups 1 and 2 that were fed NE. Mean plasma glucose concentrations of the animals in Group 1 and 2 were 45, 50, 40 and 50, 47, 48 mg/dl before, during, and after infusion of orexin, respectively (Figure 3). Plasma glucose levels of the LE-fed animals in Groups 3 (25 mg/dl) and 4 (24 mg/dl) were significantly

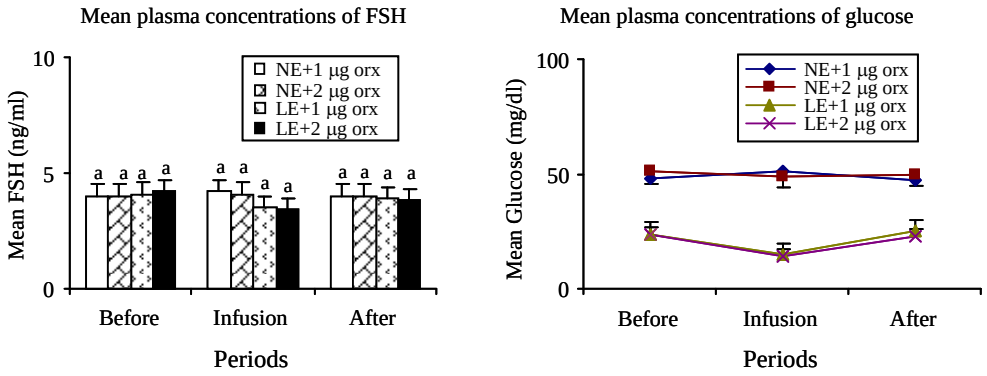

Figure 2. Mean plasma concentrations of FSH and glucose of animals in Groups 1 (NE and 1 µg orexin), 2 (NE and 2 µg orexin), 3 (LE and 1 µg orexin) and 4 (LE and 1 µg orexin) and before, during and after infusions of orexin (NE = Normal energy; LE = Low energy). [a] Treatments with different letters are different at $p<0.01$.

($p<0.01$) lower than in the NE-fed Group 1 (45 mg/dl) and 2 (50 mg/dl) (Figure 4). Infusions of 1 and 2 µg orexin significantly ($p<0.01$) decreased the glucose levels of LE-fed animals in Group 3 and 4 (Figure 3). Mean plasma glucose concentrations of the animals in Groups 3 and 4 were 25, 15, 22 and 24, 14, 23 mg/dl before, during and after infusion of orexin, respectively (Figure 2).

DISCUSSION

The results of this study demonstrated that 1 or 2 µg of orexin into the jugular vein significantly ($p<0.01$) decreased the mean plasma concentrations and pulse amplitudes of LH in the LE-fed camels for 30 days. Our results are different from previous studies that showed a stimulatory effect of orexin on the secretory activity of the GnRH/LH axis in humans and rats (Pu et al., 1998; Kohsaka et al., 2001; Kok et al., 2004). This may be due to the fact that the orexinergic effect of orexin causes an increase in food intake that results in the increased secretion of GnRH and LH in the subjects fed *ad libitum*. By contrast in our study, one group of animals fed LE resulted in a decreased secretion of LH. One theory for the mechanism of this effect is that, in the central nervous system, orexin producing neurons are colocalized with the neurons in the lateral hypothalamic area that are responsible for food intake (Date et al., 1999; Dun et al., 2000; Ehmke and Just, 2000). Therefore, orexin activates the lateral hypothalamic neurons that indirectly inactivate the GnRH secreting neurons (Hagan et al., 1999; Horvath et al., 1999; Al-Barazanji et al., 2001; Jones et al., 2001). The other mechanism is the inhibitory effect of orexin on the GnRH-secreting neurons at the median eminence (ME), as it is suggested that a subset of GnRH neurons responsible for episodic GnRH, and hence LH secretion, is located within the medial basal hypothalamus (MBH) (Boukhliq et al., 1999). Moreover, much of the GnRH axons have terminals in the ME, a structure located on the ventral part of the MBH (Silverman et al., 1994). Administration of orexin in the NE camels did not affect the LH secretions. This may be due to the normal energy diet that the animals of Groups 1 and 2 received throughout the experiment; thus, they would stay in positive energy balance as indicated by

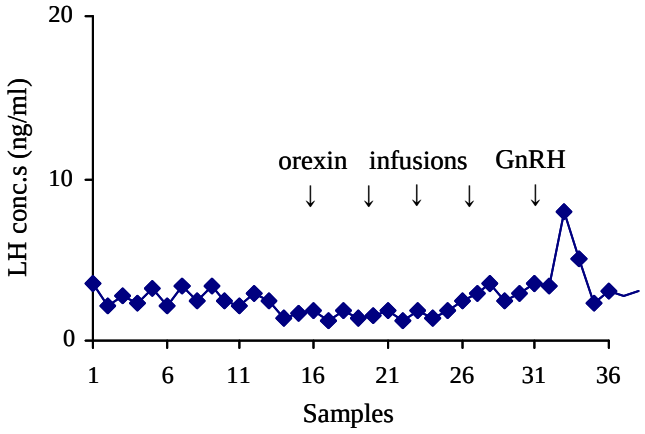

Figure 3. LH concentrations of animal fed low energy which received 1 µg orexin infusions.

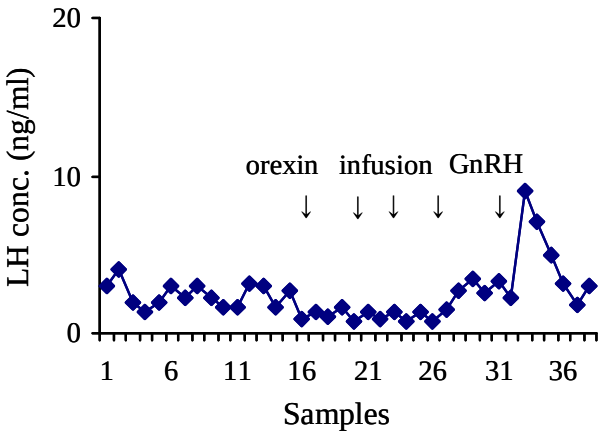

Figure 4. LH concentrations of animal fed low energy which received 2 µg orexin infusions.

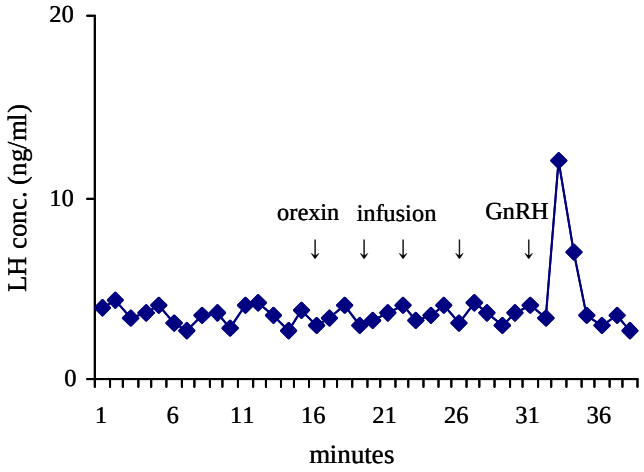

Figure 5. LH concentrations of animal fed normal energy which received 1 μg orexin infusions.

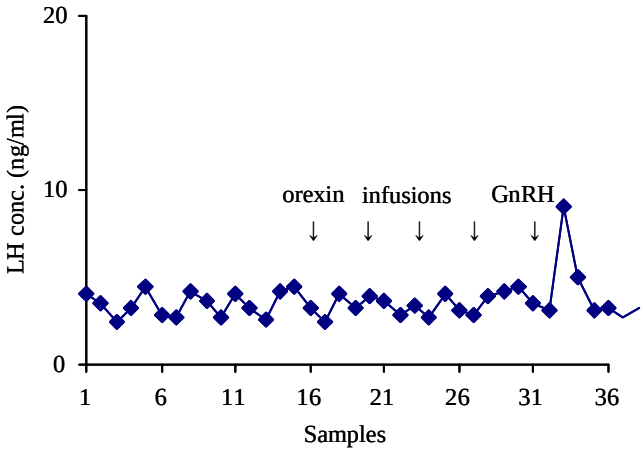

Figure 6. LH concentrations of animal fed normal energy which received 2 μg orexin infusions.

their normal glucose concentrations. Removing the main source of testosterone by castration destroys the inhibitory mechanism and leads to increased LH secretion, which was observed in the present study. Our results demonstrate that orexin decreased the amplitudes of LH pulses in camels deprived of the negative feedback of testosterone, along with mean plasma LH concentration. These observed changes in pulsatile LH secretion were transient and occurred only during the period of the infusion. Injection of GnRH increased the mean plasma concentrations of LH in all animals of different groups. This is similar to a previous finding of Khazali (1992) that indicated GnRH injection increased the plasma LH level. This may be due to gonadotrophin responsiveness to GnRH in animals either fed NE or LE diets.

The purpose of our study was first to show that orexin infusion decreases the plasma FSH level only in camels with a lower plasma glucose level, which is an indication of negative energy balance, but not in animals fed a NE diet with normal glucose concentrations. The result of our study is different from the previous finding of Kok et al. (2004) that showed plasma FSH level of hypocretin-deficient narcoleptic men is similar to that in normal men. This may be due to lower energy dietary intake that caused severe body weight loss.

Furthermore, it is well established that low dietary energy content decreases mean plasma concentrations of glucose in most mammals (Khazali, 1992; Marsoobian et al., 1995), as we observed in the camels fed LE. The result of our study regarding the effect of orexin on glucose concentration is similar to the previous findings of Quedraogo et al. (2003) and Willie et al. (2002) that indicated a negative correlation between orexin and mean plasma glucose level *in vitro*. This may be due to decreased level of glucagon (Meghan and Samson, 2003; Gonez et al., 2008) along with an increase in insulin concentrations (Meghan and Samson, 2003; Novak et al., 2005). The results of our study showed that fasted animals are more susceptible to the physiological effect of orexin.

CONCLUSION

Orexin reduced LH secretion in sub-maintenance fed camels and had no effect in animals fed at maintenance level.

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