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Effect of Galanin Infusion into the Third Ventricle on Plasma Concentrations of Metabolic Parameters in Goats Fed Diets of Different Energy Content

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ABSTRACT : The goal of this study was to determine whether galanin affects the mean plasma concentrations of metabolic parameters such as thyroxine (T4), triiodothyronine (T3), growth hormone (GH), insulin, glucagon, glucose, fatty acid and urea in goats fed diets differing in energy content. Sixteen goats 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 the diet for 20 days. After 20 days, animals in groups 1 and 3 received a daily infusion of 1 μg galanin and groups 2 and 4 received a daily infusion of 2 μg galanin into their third ventricle for 5 days. Blood samples were collected daily from the jugular vein before infusion on day 4 until 4 days after the last infusion of galanin. Samples were assayed for plasma T3, T4, GH, insulin and glucagon concentrations by double-antibody RIA. Glucose, fatty acid and urea concentrations were also measured. Lower dietary energy intake and infusions of 1 and 2 μg galanin significantly ($p<0.01$) decreased the mean plasma concentrations of T3, T4, insulin and glucose and significantly ($p<0.01$) increased the mean plasma concentrations of GH, glucagon, fatty acid and urea of the animals in groups 3 and 4. Different dosages of the galanin infusions did not change the plasma concentrations of the metabolic parameters in the animals fed a normal dietary energy content. The results of this experiment indicated that galanin may negatively affect T3, T4, insulin and glucose and increase GH, glucagon, fatty acid and urea in goats with negative energy balance, but not in those with positive energy balance. (**Key Words :** Galanin, Metabolic Hormones, Goat)

INTRODUCTION

Galanin is a 29-amino-acid neuropeptide that is mostly found in ventral structures (Skofitsch and Jacobowitz, 1985; Melander et al., 1986). Based on its neuron distribution in ventral structures, galanin coexists with many other neurons. For example, in the hypothalamic area, galanin coexists with neurons secreting different neurotransmitters such as GHRH, GABA, noradrenaline, 5-hydroxytryptamine (5-HT) and NPY (Hökfelt et al., 1987; Holets et al., 1988). Therefore, galanin controls different physiological actions on many different tracts (Goldstein and Deutch, 1989; Tsuda et al., 1990; Kyrkouli, 1992). One of the physiological actions is its effect on metabolism and feeding behavior that make galanin an orexigenic hormone (Crawley et al., 1990; Corwin et al., 1993). The orexigenic effect of galanin decreases plasma levels of insulin, glucagon, somatostatin and gastrin and increases the release of growth hormone (Kaplan et al., 1986; Hermansen, 1988; Merchentaler et al., 1990; Legakis, 2005; Stavroula et al., 2006). Most of the above studies were conducted in nonruminants, such as human and rats, fed at normal energy levels.Ruminants have a different metabolism from that of nonruminants (Harrison and Leat, 1975; Cho et al., 2006). It is assumed that the control of feeding behavior is different from that of nonruminants. There are very few reports about the orexigenic effect of galanin on metabolic hormones in ruminants fed diets of different energy content. Therefore, the first goal of this experiment was to determine whether galanin affects the mean concentrations of metabolic parameters in goats fed diets differing in energy content. Among many studies done on the effect of galanin on metabolic hormones, there are no reports about its effect on thyroid hormones. The importance of thyroid hormones in metabolism is well known. For example, thyroid hormones play an important role in the regulation of energy homeostasis via oxygen consumption and heat generation (de Jesus et al., 2001; Lanni et al., 2001). Changes in basal metabolic rate caused by different dietary energy content are accompanied by changes in secretion of thyroid hormones. Therefore, the second goal of this study was to

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

Diet	100% energy	50% energy
Ingredients/nutrition		
Wheat straw g/d)	10	260
Alfalfa(hay) (g/d)	50	50
Corn(grain) (g/d)	10	220
Corn gluten meal (g/d)	210	85
Bone meal (g/d)	1.34	0.47
Salt (g/d)	1.66	1.22
Magnesium oxide (g/d)	0.69	-
Vitamin and mineral supplement	3.50	3.50
Metabolizable energy (MJ/kg)	13.03	9.73
Crude protein (%)	42.00	13.72
Calcium (%)	0.52	0.24
Phosphorous (%)	0.52	0.24
Sodium (%)	0.45	0.21
Magnesium (%)	0.24	0.11
Dry matter intake (g/d)	287	620
Metabolizable energy intake (MJ/kg)	3.74	6.03
Metabolizable protein intake (g/d)	56.00	55.37

determine whether galanin alters the secretion of thyroid hormones in goats fed diets of different energy content.

MATERIALS AND METHODS

Experimental design

Sixteen goats (weighing 40 to 50 kg) were randomly divided into 4 groups. Animals in group 1 and 2 were fed 100% energy (NE) and animals in group 3 and 4 were fed 50% energy (LE) content in the diet for 20 days. Gross energy and chemical compositions of feedstuffs, namely dry matter, crude protein, crude fiber, ether extract, total ash, NDF, ADF, calcium and phosphorous ,were analyzed in the Animal Science Research Institute of Karaj. Diets were formulated based on AFRC (1995) (Table 1). During the course of the experiment, daily feed offered was based on body weight and individually given to each goat every morning. The goats had free access to fresh water. Diet 1 and 2 comprised 100% and 50% of maintenance energy requirements, respectively. Other requirements were balanced at maintenance level. After 20 days, all animals were prepared for surgery. Goats were anesthetized throughout the surgery for third ventricle cannulation under stereotaxic methods and jugular vein cannulation. Surgical procedures were done under general anesthesia induced by sodium pentobarbital and maintained by halothane in a closed circuit system. Each goat was kept in a single cage for a 4-day recovery period. During recovery, cannulae were flushed with PBS solution to prevent clotting. After surgery, on day 5 goats in group 1 and 3 received 1 μg galanin and goats in group 2 and 4 received 2 μg galanin into their third ventricle for 5 days. Body weight of animals was measured on day 1 and 20 of the experiment.

Blood collection

Blood samples were collected from jugular vein cannulae daily from 4 days before first infusion of galanin until 4 days after the last galanin infusion. 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. Plasma was stored at -20°C until assayed for T3, T4, insulin, GH, glucagon, glucose, fatty acid and urea.

Hormone assays

Plasma T3, T4, insulin, GH, and glucagon were measured by an homologous double-antibody radioimmunoassay (RIA). For GH assay, ovine GH (TYN-OG) and antisera against GH were provided by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran). Ovine GH (TYN-OG) was used for iodination. A seven-point standard curve ranging from 0.04 to 10 ng GH was used. An average GH assay binding of 40% was achieved using an initial 1:20,000 dilution of GH antiserum. The inter- and intra-assay variations were 6% and 9%, respectively. For insulin assay, ovine insulin (TYN-OI) and antibody against insulin were provided by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran). Ovine insulin (TYN-OI) was used for iodination. A seven-point standard curve ranging from 0.02 to 10 ng insulin was used. An average insulin assay binding of 30% was achieved using an initial 1:5,000 dilution of insulin. The inter- and intra-assay variations were 8% and 5%, respectively. For glucagon assay, human glucagon (TYN-HC) and antibody against glucagon were provided by Tabeshyarnoor Co. (Industerial City of Bu-Ali, Hamadan, Iran). Human glucagon (TYN-HC) was used for iodination. A seven-point standard curve ranging from 0.02 to 10 ng insulin was used. An average glucagon assay binding of 35% was achieved using an initial 1:10,000 dilution of glucagon antiserum. The inter- and intra-assay variations were 7% and 6%, respectively. For T3 assay, T2 was purchased from Sigma Chemical Company and T3 antisera were purchased from Chemicon Co. (Temmecula, Ca). T2 was used for iodination. A six-point standard curve ranging from 0.32 to 5.2 ng T3/ml was used. An average T3 assay binding of 70% was achieved using an initial 1:5,000 dilution of T3 antiserum. The inter- and intra-assay variations were 7% and 7%, respectively. For T4 assay, T3 was purchased from Sigma Chemical Company and T4 antisera was purchased from Chemicon Co. (Temmecula, Ca). T3 was used for iodination. A six-point standard curve ranging from 2.2 to 25 ng T4/ml was used. An average T4 assay binding of 60% was achieved using an initial 1:5,000 dilution of T4 antiserum. The inter- and intra-assay variations were 7% and 5%, respectively. For glucose assay, ELISA kits were purchased from Sigma Chemical Company. A six-point standard curve

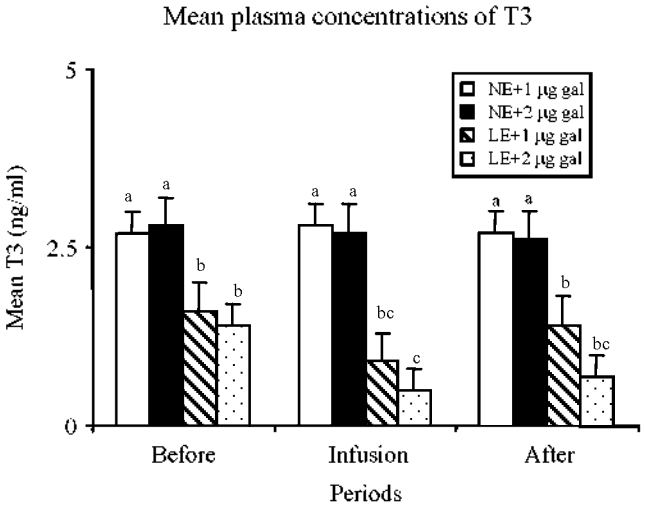

Figure 1. Mean plasma concentrations of T3 of animals in groups 1 (NE and 1 µg galanin), 2 (NE and 2 µg galanin), 3 (LE and 1 µg galanin) and 4 (LE and 1 µg galanin) before, during and after infusions of galanin (NE = Normal energy; LE = Low energy). [a, b, c] Treatments with different letters are different at $p<0.01$.

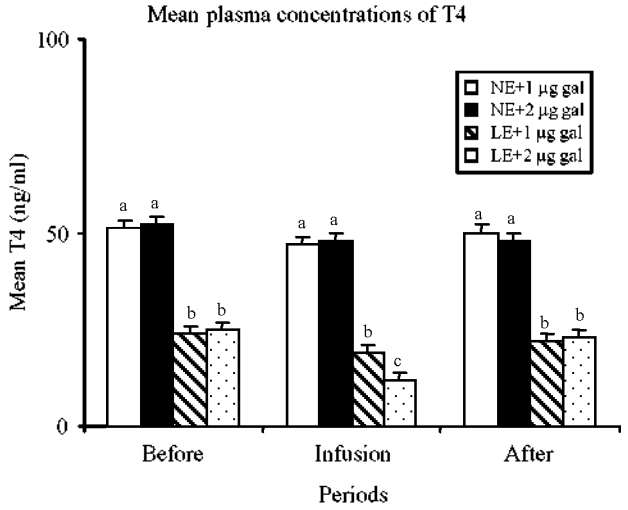

Figure 2. Mean plasma concentrations of T4 of animals in groups 1 (NE and 1 µg galanin), 2 (NE and 2 µg galanin), 3 (LE and 1 µg galanin) and 4 (LE and 1 µg galanin) before, during and after infusions of galanin (NE = Normal energy; LE = Low energy). [a, b, c] Treatments with different letters are different at $p<0.01$.

ranging from 20 to 250 mg glucose/dl was used. An average assay binding of 35% was achieved. The inter- and intra-assay variations were 4% and 6%, respectively. For fatty acid assay, ELISA kits were purchased from Sigma Chemical Company. A six-point standard curve ranging from 10 to 150 mg fatty acid/dl was used. An average assay binding of 45% was achieved. The inter- and intra-assay variations were 5% and 8%, respectively. For urea assay, ELISA kits were purchased from Sigma Chemical Company. A six-point standard curve ranging from 10 to 150 mg urea/dl was used. An average assay binding of 32% was achieved. The inter- and intra-assay variations were 4% and 6%, respectively.

Statistical analysis

All analyses were conducted using General Linear Model procedures (SAS, 1996). Data were 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.

RESULTS AND DISCUSSIONS

T3 and T4

Infusions of 1 and 2 µg galanin into the third ventricle did not change the mean plasma concentrations of T3 and T4 in groups 1 and 2 that were fed NE. Mean plasma T3 levels in groups 1 and 2 were about 2.7, 2.8, 2.7 and 2.8, 2.7, 2.5 ng/ml before, during and after infusion of galanin, respectively (Figure 1). Also, mean plasma concentrations of T4 of the NE animals in groups 1 and 2 were about 51, 47, 50 and 52, 48, 48 ng/ml before, during and after infusion of galanin, respectively (Figure 2). Plasma T3 and T4 levels of LE fed animals in groups 3 and 4 were significantly ($p<0.01$) lower than for the NE fed animals (Figures 1 and 2). Galanin infusions significantly ($p<0.01$) decreased plasma T3 and T4 levels in the LE fed animals (Figures 1 and 2).

Our study is the first to report the effect of galanin infused into the third ventricle on thyroid hormones in ruminants. The effects of galanin on mean plasma T3 and T4 levels of goats fed LE were similar to previous findings (Le´gra´di et al., 1997) which reported that peripheral injection of galanin increased the plasma level of thyroid stimulating hormones (TSH) in non-ruminants, such as rat and human, but there were no data on plasma levels of T3 and T4 in that study. It is well established that increased plasma TSH level is accompanied by decreased plasma T3 and T4 in NE-fed humans (Felig and Frohman, 2001; Reasner and Ralbert, 2002). Our results indicate that the NE-fed ruminant goat is not as sensitive to galanin as nonruminants. Only when ruminant animals are in a long-term fasting period, are they sensitive to the effect of galanin on plasma T3 and T4 levels. The hypothalamus pituitary thyroid (HPT) axis plays an important role in the regulation of energy homeostasis (de Jesus et al., 2001; Lanni et al., 2001) via the effects of thyroid hormone which increase oxygen consumption and heat generation (de Jesus et al., 2001; Lanni et al., 2001). Thus, inhibition of the HPT axis during fasting would appear to be an important adaptive mechanism to conserve energy stores (Rondeel et al., 1992; van Haasteren et al., 1995; Le´gra´di et al., 1997). The state of central hypothyroidism induced by fasting is orchestrated by changes in circulating levels of galanin,

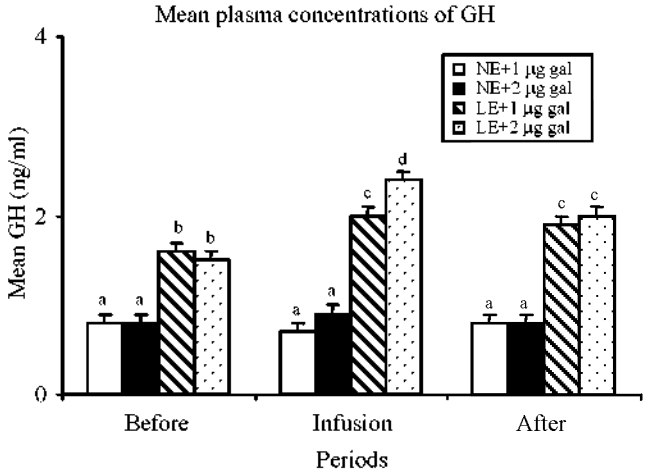

Figure 3. Mean plasma concentrations of GH of animals in groups 1 (NE and 1 µg galanin), 2 (NE and 2 µg galanin), 3 (LE and 1 µg galanin) and 4 (LE and 1 µg galanin) before, during and after infusions of galanin (NE = Normal energy; LE = Low energy). [a, b, c, d] Treatments with different letters are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

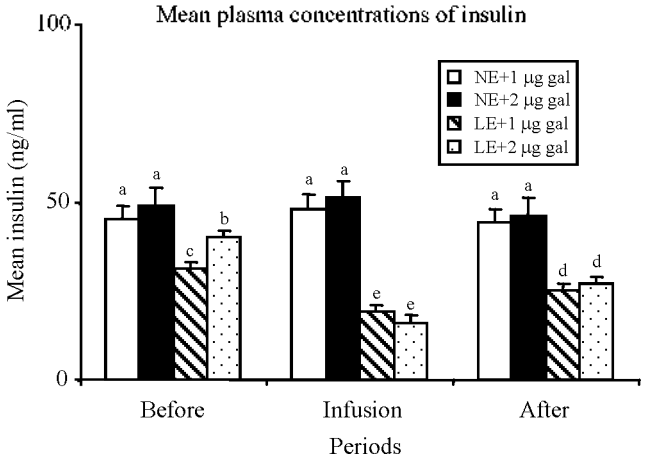

Figure 4. Mean plasma concentrations of insulin of animals in groups 1 (NE and 1 µg galanin), 2 (NE and 2 µg galanin), 3 (LE and 1 µg galanin) and 4 (LE and 1 µg galanin) before, during and after infusions of galanin (NE = Normal energy; LE = Low energy). [a, b, c, d, e] Treatments with different letters are different at $p<0.01$.

which rise with fasting and are restored to normal levels by re-feeding (Rondeel et al., 1992). Thus, if galanin is administered exogenously to fasting animals, greater decrease in circulating levels of thyroid hormones can be observed (Le´gra´di et al., 1998).

GH

Low energy content in the diet significantly ($p<0.01$) increased the plasma GH levels of group 3 (1.6 ng/ml) and 4 (1.5 ng/ml) in comparison with animals fed NE. Further to the effect of lower dietary energy intake, infusions of 1 µg galanin significantly $p<0.01$) increased mean plasma GH levels in group 3 (from 1.6 to 2), followed by declining GH level from 2 to 1.9 after infusion of galanin. Also, mean GH level of group 4 significantly ($p<0.01$) increased from 1.5 to 2.4 by infusion of 2 µg galanin (Figure 3). Infusions of 1 µg galanin did not change the mean plasma GH concentrations of group 1 that were fed 100% energy content in the diet for 20 days. Mean plasma GH concentrations of group 1 were about 0.8, 0.7, and 0.8 ng/ml before, during and after infusion of galanin, respectively (Figure 3). Infusion of 2 µg galanin did not change the mean plasma concentrations of GH in group 2 that were fed NE which were about 0.8, 0.9 and 0.8 ng/ml before, during and after infusion, respectively (Figure 3).

Our study is the first to report the effect of galanin into the third ventricle on GH in ruminants fed LE. Our results are similar to those of other studies which indicated that galanin is a hypophysiotropic hormone that elicits GH secretion (Bauer et al., 1986; Giustina et al., 1993) and enhances the GH response to GHRH in NE-fed nonruminants (Davis et al., 1987). Furthermore, conflicting evidence exists *in vitro* about the direct effect of galanin on GH, with an inhibitory influence on GH secretion observed in the rat (Meister and Hulting, 1987) and a stimulatory one observed in the rat (Lindstromm and Sävendhal, 1993) and bovine (Baratta et al., 1997). Our finding about the effect of galanin on GH in the NE goats fed normal energy content in the diet is different to the results of other studies that showed injections of galanin increase GH in the rat and human (Kaplan et al., 1986; Merchentaler et al., 1990). This may be due to normal plasma insulin level and the inhibitory effect of normal concentrations of plasma glucose (Holl et al., 1999) in the NE-fed goats on GH secretion.

Insulin

Infusions of 1 and 2 µg galanin did not change the mean plasma concentrations of insulin in groups 1 and 2 that were fed NE, which were about 45, 48, 44 and 49, 51, 46 ng/ml before, during and after infusion of galanin, respectively (Figure 4). Mean plasma concentrations of insulin in groups 3 (34 ng/ml) and 4 (39 ng/ml) fed LE were significantly $p<0.01$) lower than the plasma insulin levels of groups 1 (45 ng/ml) and 2 (49 ng/ml) fed NE (Figure 4). Infusions of 1 µg galanin significantly $p<0.01$) decreased the mean plasma levels of insulin in group 3 from 34 to 19, followed by rising of plasma insulin levels from 19 to 24 after infusion of galanin. Also, mean plasma levels of insulin of group 4 were significantly ($p<0.01$) decreased from 39 to 15 by infusion of 2 µg galanin (Figure 4).

Our data are different from studies in nonruminants that indicated galanin may slightly decrease the plasma level of insulin (Stavroula et al., 2006). In those studies, there was no effect of galanin on the long-term fasting subject. Our

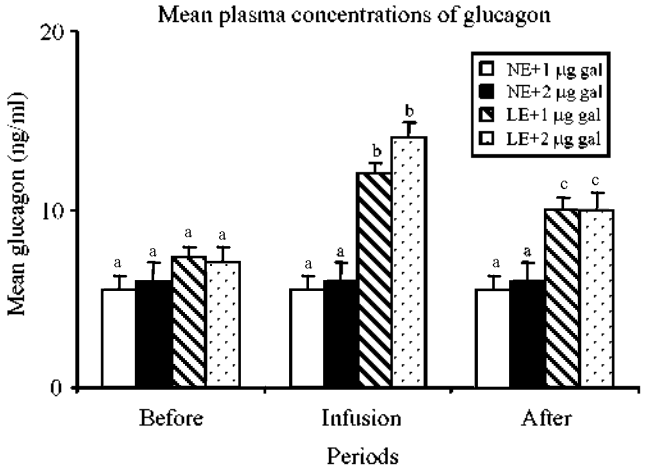

Figure 5. Mean plasma concentrations of glucagons of animals in groups 1 (NE and 1 µg galanin), 2 (NE and 2 µg galanin), 3 (LE and 1 µg galanin) and 4 (LE and 1 µg galanin) before, during and after infusions of galanin (NE = Normal energy; LE = Low energy). [a, b, c] Treatments with different letters are different at $p<0.01$.

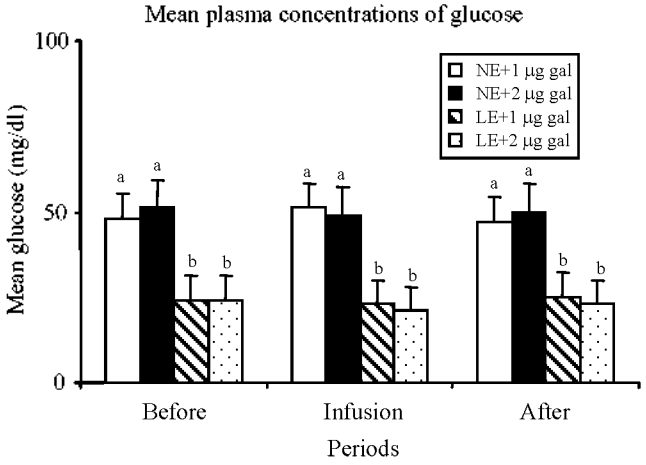

Figure 6. Mean plasma concentrations of glucose of animals in groups 1 (NE and 1 µg galanin), 2 (NE and 2 µg galanin), 3 (LE and 1 µg galanin) and 4 (LE and 1 µg galanin) before, during and after infusions of galanin (NE = Normal energy; LE = Low energy). [a, b] Treatments with different letters are different at $p<0.01$.

result is similar to previous findings that intravenous administration of galanin into fasted conscious dogs decreased plasma insulin levels (McDonald et al., 1985). The mechanism of inhibition of galanin on insulin release most likely occurs through the inhibition of adenylate cyclase, involving a pertussis-toxin-sensitive inhibitory GTP-binding regulatory protein and activity of protein kinase C and cyclic AMP (Amiranoff et al., 1988; Lindskog and Ahren, 1991).

Glucagon

Galanin infusions did not change the mean plasma concentrations of glucagon in group 1 and 2 that were fed NE, which were about 5.5, 5.1, 5.4 and 6, 5.5, 5.6 ng/ml before, during and after infusion of galanin, respectively (Figure 5). Mean plasma concentrations of glucagon of groups 3 (8.2 ng/ml) and 4 (8 ng/ml) were significantly $p<0.01$) higher than in the NE-fed animals (Figure 4). Infusions of 1 µg galanin significantly ($p<0.01$) increased the mean plasma levels of glucagon in group 3 from 8.2 to 11, followed by decreasing of levels from 11 to 7.8 after infusion of galanin. Also, mean plasma glucagon levels in group 4 were significantly ($p<0.01$) increased from 8 to 12 by infusion of 2 µg galanin (Figure 5).

Our results are different from previous studies that reported galanin had no effect on glucagon level in isolated, perfused dog pancreas (Hermansen, 1988) and in the fasted dog (McDonald et al., 1985). This may be due to low plasma glucose concentrations in the fasted dog (Manabe et al., 2003). Also, some studies indicated that galanin inhibited glucagon secretion in the rat. All the above studies were conducted to determine the effect of galanin on glucagons via *in vitro* or peripheral injections. In our study, decreased plasma level of glucose caused by lower energy intake (Marsoobian et al., 1995; Achmadi et al., 2007) and galanin infusion may be the reason for increased level of glucagon and decreased level of insulin.

Glucose

Galanin did not change the mean plasma glucose concentrations of group 1 and 2 that were fed NE, which were about 45, 50, 40 and 50, 47, 48 mg/dl before, during and after infusion of galanin, respectively (Figure 6). Plasma glucose levels of the LE-fed animals in groups 3 (25 mg/dl) and 4 (24 mg/dl) were significantly ($p<0.01$) lower than in the NE-fed group 1 (45 mg/dl) and 2 (50 mg/dl) (Figure 4). Infusions of 2 µg, but not 1 µg, galanin significantly ($p<0.01$) decreased the glucose levels of LE-fed group 3 (Figure 6). It is well established that low dietary energy content decreases mean plasma concentrations of glucose in most mammals (Marsoobian et al., 1995), as we observed in the goats fed LE. Also, there is a negative correlation between galanin infusion and mean plasma level of glucose in fasted ruminants, whereas another study reported a positive correlation between these two parameters in non-fasted non-ruminants (Leibowitz et al., 1998).

Fatty acid

Infusions of 1 and 2 µg galanin did not change the mean plasma fatty acid concentrations of groups 1 and 2 that were fed NE. Mean plasma concentrations of fatty acid in group 3 (65 mg/dl) and 4 (66 mg/dl) fed LE were significantly $p<0.01$) higher than in groups 1 (45 mg/dl) and 2 (47 ng/dl) fed NE (Figure 7). Infusions of 1 and 2 µg galanin

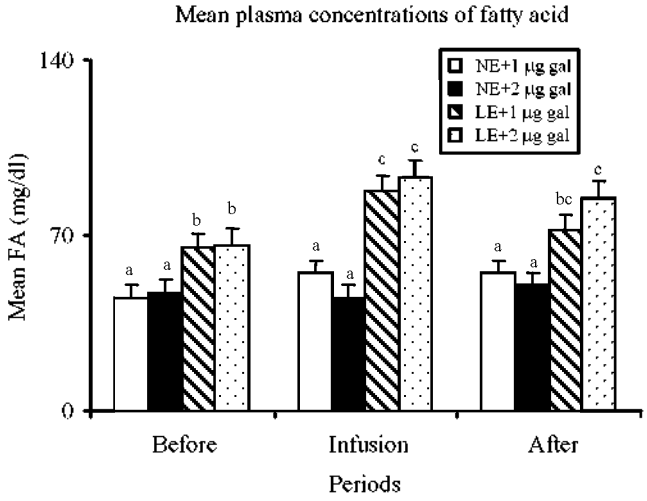

Figure 7. Mean plasma concentrations of fatty acid of animals in groups 1 (NE and 1 µg galanin), 2 (NE and 2 µg galanin), 3 (LE and 1 µg galanin) and 4 (LE and 1 µg galanin) before, during and after infusions of galanin (NE = Normal energy; LE = Low energy). [a, b, c] Treatments with different letters are different at $p<0.01$.

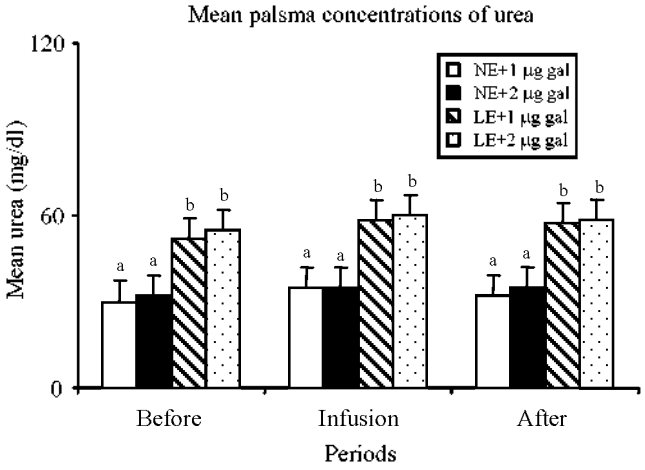

Figure 8. Mean plasma concentrations of urea of animals in groups 1 (NE and 1 µg galanin), 2 (NE and 2 µg galanin), 3 (LE and 1 µg galanin) and 4 (LE and 1 µg galanin) before, during and after infusions of galanin (NE = Normal energy; LE = Low energy). [a, b] Treatments with different letters are different at $p<0.01$.

significantly ($p<0.01$) increased the fatty acid levels of those animals fed LE (Figure 7). This may be directly due to the effect of negative energy balance which caused severe weight loss along with lipolysis of adipose tissue (Rees et al., 1982).

Urea

Galanin did not change the mean plasma urea concentrations of all groups. Mean plasma concentrations of urea in group 3 and 4 fed LE were significantly ($p<0.01$) higher than in NE-fed animals of groups 1 and 2 (Figure 8). This effect of galanin on urea in the LE-fed goats is similar to other studies in the nonruminant which indicated that a low energy diet increased plasma urea level (Khazali, 1992).

When energy intake is inadequate, proteins can serve as an energy source and plasma urea level is considered as an end-product of protein catabolism (Ruiz et al., 1971).

Body weight

Low energy dietary intake for 20 days significantly ($p<0.01$) decreased the mean body weight of the animals from 45 to 36 kg. This was similar to our previous finding which reported that negative energy balance decreased body weight in ewes (Towhidi et al., 2007; Gao et al., 2008).

IMPLICATION

The results of our studies indicated that infusion of galanin into the third ventricle may increase the plasma levels of GH, glucagons, fatty acid and urea, and decrease the plasma levels of T3, T4, insulin and glucose in goats undergoing severe body loss. The effect of galanin infusion into the third ventricle on metabolic parameters is different from the effect of galanin injections in the peripheral circulation. Also the different metabolic systems of ruminant and non-ruminant animals respond with different changes of metabolic status under the effect of galanin.

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