Third ventricle neuropeptide-Y infusion effect on metabolic parameters under different energy levels in diets

L. Hosseini¹ and H. Khazali²*

¹Humanities Department, Science and Research Branch of Islamic Azad University, Tehran, Iran. ²College of Biological Sciences, Shahid Beheshti University, GC, Tehran, Iran.

Accepted 1 March, 2013

The goal of this study was to determine whether neuropeptide-Y 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 the goats fed different energy content in diets. 16 goats were randomly divided into four groups. Animals in groups 1 and 2 were fed 100% energy content in diet and animals in groups 3 and 4 were fed 50% energy content in diet for 20 days. After 20 days, animals in groups 1 and 3 received daily infusion of 1 µg neuropeptide-Y and groups 2 and 4 received daily infusion of 2 µg galanin into their third ventricle for 5 days. Blood samples were collected daily from the jugular veins before infusions on day 4 until 4 days after the last infusions of neuropeptide-Y. Samples were assayed for plasma T3, T4, GH, insulin and glucagon concentrations by double-antibody radioimmunoassay (RIA). Glucose, fatty acid and urea concentrations were also measured. Lower dietary energy intake and infusions of 1 and 2 µg neuropeptide-Y significantly (P<0.01) decreased the mean plasma concentrations of T3, T4, insulin and glucagon 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 neuropeptide-Y infusions did not change the plasma concentrations of the metabolic parameters in the animals fed normal energy content in diets. The results of this experiment indicate that neuropeptide-Y may negatively affected T3, T4, insulin and glucagon and increased GH, glucagon, fatty acid and urea in the goats with negative energy balance, but not in those with the positive energy balance.

Key words: Neuropeptide-Y, metabolic hormones, goat, energy balance.

INTRODUCTION

Neuropeptide-Y is mostly found in hypothalamus (Takaya et al., 2000; Kamegai et al., 2001; Holst et al., 2004). Based on its neuron distributions in hypothalamus, neuropeptide-Y coexists with many other neurons. For example, in hypothalamic area, neuropeptide-Y coexists with neurons secreting different neurotransmitters such as GHRH, GABA, noradrenaline, 5-hydroxytryptamine (5-HT) and NPY (Kojima and Kangawa, 2005). Therefore, neuropeptide-Y controls different physiological actions in different glands (Ghigo et al., 2001; Gi et al., 2003; Barreiro et al., 2004; Fernandez-fernandez et al., 2005). One of the physiological actions is its effect on metabolism and feeding behaviors that make neuropeptide-Y a hormone that increases food intake (Gi et al., 2003). This orexigenic effect of neuropeptide-Y decrease or increase plasma levels of insulin, glucagon, somatostatin, gastrin and increase the release of growth hormone (Adeghate and Ponery, 2001; Arosia et al., 2001; Arvat et al., 2000, 2001; Broglio et al., 2003; Egido et al., 2002; Hataya et al., 2001). Most of the above studies were conducted in human and rat as a nonruminant. Ruminants have different metabolism from that of nonruminants (Harrison and Leat, 1975). For example, higher plasma glucose level,
Table 1. Experimental rations and prepared energy and nutrients.

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

less insulin responsivity and fatty acid metabolism are some of the physiological peculiarities that make them different from ruminant (Elmahdi et al., 1997; Kaske et al., 2001). It is assumed that the control of feeding behavior is different from that of nonruminants. There are few reports about the orexigenic effect of neuropeptide-Y on the metabolic hormones in ruminants fed different energy content in diet. Therefore, the first goal of this experiment was to determine whether neuropeptide-Y affects the mean concentrations of metabolic parameters in the goats fed different energy content in diets.

Among many studies done on the effect of neuropeptide-Y on metabolic hormone, there are no reports on the effect of the neuropeptide-Y on thyroid hormones under different energy intake. 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 (Data et al., 2003; Lanni et al., 2001). Changes in basal metabolic rate caused by different energy content in diet is accompanied by changes of thyroid hormones secretions. Therefore, the second goal of this study was to determine whether neuropeptide-Y alters the thyroid hormones secretion in the goats fed different energy content in diet.

MATERIALS AND METHODS

Experimental design

Sixteen goats (weighing 40 to 50 kg) were randomly divided into four groups. Animals in groups 1 and 2 were fed 100% energy (NE) and animals in groups 3 and 4 were fed 50% energy (LE) content in diet for 20 days. Gross energy and chemical compositions of feedstuffs consisted of dry mater, crude protein, crude fiber, ether extract, total ash, NDF, ADF, calcium and phosphorous which 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 was weighed based on body weight and individually given to each goat every morning. The goats had free access to fresh water. Diets 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 cannulations. 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 4 days recovery period. During recovery period, cannules were washed by PBS solution to prevent clotting. After surgery, on day 5, goats in groups 1 and 3 received 1 ug neuropeptide-Y and goats in groups 2 and 4 received 2 ug neuropeptide-Y into their third ventricles at 09.00 h for 5 days. Body weight of animals was measured on days 1 and 20 of the experiment.

Blood collection

Blood samples were collected from cannules that were put into the jugular veins, everyday from 4 days before first infusion of neuropeptide-Y until 4 days after the last neuropeptide-Y infusion. Blood samples were kept at 4°C until centrifugation. A saturated sodium citrate solution (40 ul 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 T3, T4, insulin, GH, glucagon, glucose, fatty acid and urea.

Hormone assays

Plasma T3, T4, insulin, GH, and glucagon were measured by a homologous double-antibody radioimmunoassay (RIA). For GH assay,
Mean plasma concentrations of T3 of the animals in the different groups of 1 (NE and 1 µg neuropeptide-Y), 2 (NE and 2 µg neuropeptide-Y), 3 (LE and 1 µg neuropeptide-Y) and 4 (LE and 1 µg neuropeptide-Y) before, during and after infusions of neuropeptide-Y (NE = normal energy; LE = low energy). a, b, c, Treatments with different letters are different at p<0.01.

Statistical analysis

All analyses were conducted using General Linear Model procedures SAS, 1996. Data were analyzed using analysis of variance for a repeated measure design. Mean comparisons were evaluated by least significant difference with single degree of freedom.

RESULTS

T3 and T4

Infusions of 1 and 2 µg neuropeptide-Y into third ventricle did not change the mean plasma concentrations of T3 and T4 of the animals in groups 1 and 2 that were fed NE. Mean plasma T3 levels of the animals in groups 1 and 2 were about 2.0, 2.1, 2.2 and 2.1, 1.9, 2.1 ng/ml before, during and after infusion of neuropeptide-Y, respectively (Figure 1). Also, mean plasma concentrations of T4 of the NE animals in groups 1 and 2 were about 41, 40, 40 and 39, 42, 41 ng/ml before, during and after infusion of neuropeptide-Y, respectively (Figure 2). Plasma T3 and T4 levels...
Mean plasma concentrations of the GH of group 1 were about 0.7 ng/ml, those of group 2 were about 2 ng/ml, those of group 4 significantly (P<0.01) were higher than the plasma levels of those animals fed NE. Mean plasma concentrations of the GH of group 2 were about 0.6, 0.6 and 0.6 ng/ml before, during and after infusion of neuropeptide-Y, respectively (Figure 3).

**Insulin**

Infusions of 1 and 2 µg neuropeptide-Y did not change the mean plasma concentrations of the insulin in the animals of groups 1 and 2 that were fed NE. Mean plasma concentrations of the insulin in the animals of groups 1 and 2 were about 45, 45, 44 and 42, 46, 44 ng/ml before, during and after infusion of neuropeptide-Y, respectively (Figure 4). Mean plasma concentrations of insulin in the animals of groups 3 (30 ng/ml) and 4 (29 ng/ml) fed LE were significantly (P<0.01) lower than the plasma insulin levels of those animals in groups 1 (45 ng/ml) and 2 (42 ng/ml) fed NE (Figure 4). Infusions of 1 µg neuropeptide-Y significantly (P<0.01) decreased the mean plasma levels of insulin in the animals of group 3 from 30 to 15, followed by rising of plasma levels of insulin from 15 to 26 after infusion of neuropeptide-Y. Also, mean of the plasma levels of insulin in the animals in group 4 significantly (P<0.01) decreased from 29 to 13 by infusion of 2 µg neuropeptide-Y (Figure 4).

**GH**

Low energy content in diet increased the GH plasma levels of the animals in group 3 (0.7 ng/ml) and 4 (0.9 ng/ml) in comparison with plasma GH levels of those animals fed NE. Further to the effect of lower energy dietary intake, infusions of 1 µg neuropeptide-Y significantly (P<0.01) increased the mean plasma GH levels in the animals of group 3 (from 0.7 to 2), followed by declining GH level from 2 to 1.2 after infusion of neuropeptide-Y. Also, mean GH level of the animals of group 4 significantly (P<0.01) increased from 0.9 to 3 by infusion of 2 µg neuropeptide-Y (Figure 3). Infusions of 1 µg neuropeptide-Y did not change the mean plasma concentrations of the GH in the animals of group 1 fed 100% energy content in diets for 20 days. Mean plasma concentrations of the GH of group 1 were about 0.5, 0.6, and 0.6, ng/ml before, during and after infusion of neuropeptide-Y, respectively (Figure 3). Two micrograms neuropeptide-Y did not change the mean plasma concentrations of the GH in the animals of group 2 that were fed NE. Mean plasma concentrations of the GH of group 2 were about 0.6, 0.6 and 0.6 ng/ml before, during and after infusion of neuropeptide-Y, respectively (Figure 3).
Figure 3. Mean plasma concentrations of GH of the animals in the different groups of 1 (NE and 1 µg neuropeptide-Y), 2 (NE and 2 µg neuropeptide-Y), 3 (LE and 1 µg neuropeptide-Y) and 4 (LE and 1 µg neuropeptide-Y) before, during and after infusions of neuropeptide-Y. (NE = normal energy; LE = low energy). a, b, c, Treatments with different letters are different at p<0.01.

Figure 4. Mean plasma concentrations of insulin of the animals in the different groups of 1 (NE and 1 µg neuropeptide-Y), 2 (NE and 2 µg neuropeptide-Y), 3 (LE and 1 µg neuropeptide-Y) and 4 (LE and 1 µg neuropeptide-Y) before, during and after infusions of neuropeptide-Y. (NE = normal energy; LE = low energy). a, b, c, Treatments with different letters are different at p<0.01.
Glucagon

Neuropeptide-Y infusions did not change the mean plasma concentrations of the glucagon in the animals of groups 1 and 2 that were fed NE. Mean plasma levels of the glucagon of the animals in groups 1 and 2 were about 3, 3.2, 3 and 3.1, 3.1, 3 ng/ml before, during and after infusion of neuropeptide-Y, respectively (Figure 5). Mean plasma concentrations of glucagon of the animals in groups 3 (8.2 ng/ml) and 4 (8 ng/ml) were significantly $P<0.01$ higher than that of the animals fed NE (Figure 5). Infusions of 1 µg neuropeptide-Y significantly ($P<0.01$) increased the mean of plasma levels of glucagon in the animals of group 3 from 2.9 to 5, followed by decreasing glucagon plasma levels from 5 to 3.6 after infusion of neuropeptide-Y. Also, mean of plasma levels of glucagon of the animals in group 4 significantly ($P<0.01$) increased from 3 to 126 by infusion of 2 µg neuropeptide-Y (Figure 5).

Glucose

Neuropeptide-Y did not change the mean plasma concentrations of the glucose of the animals in groups 1 and 2 that were fed NE. Plasma concentrations of the glucose of groups 1 and 2 were about 50, 48, 50 and 52, 50, 50 mg/dl before, during and after infusion of neuropeptide-Y respectively (Figure 6). Plasma glucose levels of the LE fed animals in groups 3 (32 mg/dl) and 4 (28 mg/dl) were significantly $P<0.01$ lower than the mean plasma concentrations of glucose of those animals in the group 1 (50 mg/dl) and 2 (52 mg/dl) fed NE (Figure 4). Infusions of 2, but not 1 µg neuropeptide-Y significantly ($P<0.01$) decreased the glucose levels among the animals of group 3 fed LE (Figure 6).

Fatty acid

Infusions of 1 and 2 µg neuropeptide-Y did not change the mean plasma concentrations of the fatty acid of the animals in groups 1 and 2 that were fed LE. Mean plasma concentrations of fatty acid of the animals in the groups 3 (70 mg/dl) and 4 (68 mg/dl) fed LE were significantly $P<0.01$ higher than the mean plasma concentrations of fatty acid of the animals in groups 1 (40 mg/dl) and 2 (2 mg/dl) fed NE (Figure 7). Infusions of 1 and 2 µg neuropeptide-Y significantly ($P<0.01$) increased the fatty acid levels among the animals fed LE (Figure 7).

Urea

Neuropeptide-Y did not change the mean plasma concentrations of the urea of the animals in all groups. Mean plasma concentrations of urea of the animals in groups 3 and 4 fed LE were significantly $P<0.01$ higher than the
mean plasma concentrations of urea of the NE fed animals in groups 1 and 2 (Figure 8).

**Body weight**

Low energy dietary intake for 20 days significantly (P<0.01) decreased the mean body weight of the animals from 47 to 35 kg.

**DISCUSSION**

**T3 and T4**

Our study is the first to report the effect of infusion of neuropeptide-Y into the third ventricle, on thyroid hormones in the ruminants. The results of the effect of neuropeptide-Y on mean plasma T3 and T4 levels of the goats fed LE is similar to the previous finding of Dalkjar and Hansen (2003) which showed that the peripheral injection of neuropeptide-Y increased the plasma level of thyroid stimulating hormones (TSH) in nonruminants such as rat and human, but there was no data on the plasma level of T4 in that study. It is well established that increase of plasma TSH level is accompanied by decrease of plasma T3 and T4 in NE fed human (Felig and Frohman, 2001; Reasner and Ralbert, 2002). Our results indicate that the NE fed goats as a ruminant are not sensitive to neuropeptide-Y as compared to nonruminant. It is only when the ruminant animals are in long term fasting period, that they become sensitive to the effect of neuropeptide-Y on plasma T3 and T4 levels. The hypothalamus pituitary thyroid (HPT) axis plays important role in the regulation of energy homeostasis (Data et al., 2003; Lanni et al., 2001) via the effects of thyroid hormone to increase oxygen consumption and heat generation (Data et al., 2003; Lanni et al., 2001). Thus, inhibition of the HPT axis during fasting would appear to be an important adaptive mechanism to conserve energy stores (Reasner and Ralbert, 2002; van Haasteren et al., 1995; Le’gradi et al., 1997, 1998). The state of central hypothyroidism induced by fasting is orchestrated by changes of circulating levels of neuropeptide-Y, which increases with fasting and is restored to normal levels by refeeding (Reasner and Ralbert, 2002). Thus, if neuropeptide-Y is administered exogenously to fasting animals, the more decrease in circulating levels of thyroid hormones can be observed (Le’gradi et al., 1998).

**GH**

Our study is the first to report the effect of infusion of neuropeptide-Y into the third ventricle, on GH in the ruminants fed LE. Our result on the effect of neuropeptide-Y on GH in the goats fed LE is similar to other studies, indicating that neuropeptide-Y is a hypophysiotropic hormone that elicits GH secretion (Takaya et al., 2000;
Figure 7. Mean plasma concentrations of fatty acid of the animals in the different groups of 1 (NE and 1 µg neuropeptide-Y), 2 (NE and 2 µg neuropeptide-Y), 3 (LE and 1 µg neuropeptide-Y) and 4 (LE and 1 µg neuropeptide-Y) before, during and after infusions of neuropeptide-Y (NE = normal energy; LE = low energy).

Figure 8. Mean plasma concentrations of fatty acid of the animals in the different groups of 1 (NE and 1 µg neuropeptide-Y), 2 (NE and 2 µg neuropeptide-Y), 3 (LE and 1 µg neuropeptide-Y) and 4 (LE and 1 µg neuropeptide-Y) before, during and after infusions of neuropeptide-Y (NE = normal energy; LE = low energy).

Kamegai et al., 2001; Holst et al., 2004; Kojima and Kangawa, 2005; Ghigo et al., 2001; Gi et al., 2003; Barreiro et al., 2004; Fernandez-ferrandez et al., 2005; Adeghate and Ponery, 2002; Arosia et al., 2003; Arvat et al., 2000; 2001); and enhances the GH response to GHRH in NE fed nonruminant (Arvat et al., 2001). Furthermore, conflicting evidences exist in vitro for the direct effect of neuropeptide-Y on GH, with an inhibitory
Influence on GH secretion observed in rat (Kamegai and Tamura, 2004) and a stimulatory one observed in rat (Hataya et al., 2001). Our findings on the effect of neuropeptide-Y on GH in the NE goats fed normal energy content in diet is different from the results of other studies that showed that injections of neuropeptide-Y increase GH in rat and human (Arosia et al., 2003; Arvat et al., 2000; 2001). This may be due to normal plasma level of insulin and the inhibitory effect of normal concentrations of plasma glucose (Holl et al., 1999) in the goats fed NE on the GH secretions.

**Insulin**

Our data are different from that from the studies with nonruminants that indicated that neuropeptide-Y may slightly decrease the plasma level of insulin (Adeghate and Ponery, 2002; Broglio et al., 2003; Data et al., 2003). In those studies, the effect of neuropeptide-Y was not on the long term fasting subject. Our result is similar to the previous findings reported which showed that intravenous administration of neuropeptide-Y into fasted conscious dogs decreased plasma insulin levels (Egido et al., 2002). The mechanism of inhibitory effect of neuropeptide-Y on insulin release most likely occurs through the inhibition of cyclic AMP (Egido et al., 2002).

**Glucagon**

Our results are different from the previous studies that reported that neuropeptide-Y has no effect on glucagon level in the rats (Adeghate and Ponery, 2002). This may be due to the plasma glucose concentrations in the fasted dog (Cherrington et al., 1978). Also, some studies indicated that neuropeptide-Y inhibited glucagon secretion in rat. All the above studies were conducted to determine the effect of neuropeptide-Y on glucagon via in vitro or peripheral injections. In our study, decreased plasma level of glucose caused by lower energy intake (Marsoobian et al., 1995) and neuropeptide-Y infusions may be the reason for increase level of glucagon and decrease level of insulin.

**Glucose**

It is well established that low energy content in diet 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 neuropeptide-Y infusion and mean plasma level of glucose in the fasted ruminants whereas in other study, it is reported that there is a positive correlation between these two parameters in nonfasted nonruminant (Broglio et al., 2003).

**Fatty acid**

Infusions of 1 and 2 µg neuropeptide-Y significantly (P<0.01) increased the fatty acid levels among those animals fed LE (Figure 7). This may directly be due to the effect of negative energy balance which caused severe weight lost together with the lipolysis of adipose tissue (Harrison and Leat, 1975).

**Urea**

Our result on the effect of neuropeptide-Y on urea in the LE fed goats is similar to other studies done in the non ruminant that indicated that 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 endproduct of protein catabolism (Reese et al., 1982).

**Body weight**

Low energy dietary intake for 20 days significantly (P<0.01) decreased the mean body weight of the animals. This was similar to our previous findings which showed that negative energy balance decrease body weight in the ewes (Towhidi et al., 2007).

**Implication**

The results of our studies indicate that the third ventricle infusion of neuropeptide-Y may increase the plasma levels of GH, glucagon, fatty acid and urea, and decrease the plasma levels of T3, T4, insulin and glucose in the goats with severe body loss. The effect of neuropeptide-Y infusion into third ventricle on metabolic parameters is different from the effect of neuropeptide-Y injections in peripheral circulation. Also, different metabolic system of ruminant and nonruminant animals offers different changes of metabolic status under neuropeptide-Y effect.

**REFERENCES**


van Haasteren GA, Linkels E, Kootwijk W, van Toor H, Rondeel JM, Themmen AP, de Jong FH, Va