

*Full Length Research Paper*

# **Cortisol and epinephrine effects on galanin secretions in severe fasting**

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The goal of this study was to determine whether cortisol and epinephrine affects galanin secretions in the sheeps lower levels of their daily requirement. The sheeps were randomly divided into 6 groups. Animals in all groups were fed 100, 50 or 25% of their daily food requirements for 10 days followed by 48 hours of fasting. Consequently, the sheep's in all groups received infusions of either 1 or 3 ug cortisol/Kg BW, 1 or 3 ug epinephrine /Kg BW and 3 ug cortisol/Kg BW plus 3 ug epinephrine /Kg BW. Blood samples were collected from jugular veins at -30, +30, +90 and +180 minutes of infusions. Fifty and 25 % of daily food intake followed by 48 hours fasting decreased the body weights in the sheeps. Also, twenty five percent of daily food intake followed by 48 hours fasting significantly ( $P<0.01$ ) increased the mean plasma concentrations of glucose whereas these increases of glucose were not observed in the sheeps fed 100 or 50 % of daily food intake followed by 48 hours fasting. Injection of different dosages of cortisol, epinephrine and combination of cortisol and epinephrine significantly ( $P<0.01$ ) increased the mean plasma concentrations of glucose in the sheeps of all groups. Injection of different dosages of cortisol did not change the mean plasma concentrations of galanin in the animals of fed all groups. Injection of different dosages of epinephrine and epinephrine plus cortisol significantly ( $P>0.01$ ) decreased the elevated mean plasma concentration of galanin in the sheep fed 25 and 50% of their daily food intake for 10 days. The results of this experiment indicated that epinephrine may decrease mean plasma concentration of galanin in animal fed lower than their daily food requirement. Injections of cortisol did not change the mean plasma concentrations of galanin in sheep fed restricted.

**Key words:** Galanin, cortisol, epinephrine and food restricted.

## **INTRODUCTION**

There are three different physiological states during starvation. Simple starvation is characterized with the decreased of glucose levels, severe starvation is known with the increased of cortisol (Douyon and Schteingart, 2002) and very severe starvation is along with increased of epinephrine (Leiter *et al.*, 1984; Hilderman *et al.*, 1996; and Michalsen *et al.*, 2003) which lead to increase of ketone secretions. Different amount of daily food intake leads to different degree of physiological status of starvation. Galanin is a 33-amino-acid neuropeptide that is mostly found in hypothalamus (Arihara *et al.*, 2000;

Backberg *et al.*, 2000; Antunes *et al.*, 2001). Many studies have shown that starvation is along with the increased of galanin secretions in most mammals. The goal of these experiments is to determine the effects of different degree of starvations and injections of cortisol and epinephrine on the mean plasma concentrations of galanin in the sheeps.

## **MATERIALS AND METHODS**

### ***Experimental Design***

Forty five sheeps (weighing between 40 to 45 Kg) were randomly divided into 6 groups. Animals in all groups were fed 100, 50 or 25% of their daily food requirements for 10 days followed by 48

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**Table 1.** Mean plasma Concentrations of glucose after fasting and injections of cortisol and epinephrine in the sheeps fed different amount of their daily requirement.

	Plasma Concentrations of Glucose (mg/dl)		
	100% F	50% F	25% F
<b>Fasting for 10 days</b>	148	160	215
<b>Cortisol</b>	197	198	225
<b>Epinephrine</b>	211	230	269
<b>Cortisol and epinephrine</b>	221	233	284

hours of fasting. Body weights of animals were measured on day 1 and 10 of the experiments. All animals were cannulated into their jugular vein. Consequently, the sheeps in all groups received infusions of either 1 or 3 ug cortisol/Kg BW, 1 or 3 ug epinephrine /Kg BW and 3 ug cortisol/Kg BW plus 3 ug epinephrine /Kg BW.

#### **Blood Collection**

Blood samples were collected from cannules that were put into the jugular veins at -30, +30, 90 and 180 minutes of infusions. 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 galanin.

#### **Glucose Assays**

Plasma glucose was measured by a kit that was provided by Sigma Co.

#### **Hormone Assays**

Plasma galanin were measured by a homologous double-antibody radioimmunoassay (RIA). For galanin assay, galanin were provided by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran). Galanin was used for iodination. A seven-point standard curve ranging from 0.04 to 10 ng galanin was used. An average assay binding of 40% was achieved using an initial 1:20000 dilution of galanin antiserum for galanin assays. The inter- and intra-assay variations were 6% and 9%, 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 single degree of freedom.

## **RESULTS**

Fifty and 25 % of daily food intake for 10 days followed by 48 hours fasting decreased the body weights from 300 to

250 grams in the sheeps. Also, twenty five percent of daily food intake followed by 48 hours fasting significantly ( $P < 0.01$ ) increased the mean plasma concentrations of glucose whereas these increases of glucose were not observed in the sheeps fed 100 or 50 % of daily food intake followed by 48 hours fasting (Table 1). Injection of different dosages of cortisol, epinephrine and combination of cortisol and epinephrine significantly ( $P < 0.01$ ) increased the mean plasma concentrations of glucose in the sheeps of all groups (Table 1). Injection of different dosages of cortisol did not change the mean plasma concentrations of galanin in the animals of fed all groups (Figure 1). Injection of different dosages of epinephrine and epinephrine plus cortisol significantly ( $P > 0.01$ ) decreased the elevated mean plasma concentrations of galanin in the sheep fed 25 and 50% of their daily food intake for 10 days (Figures 2 and 3).

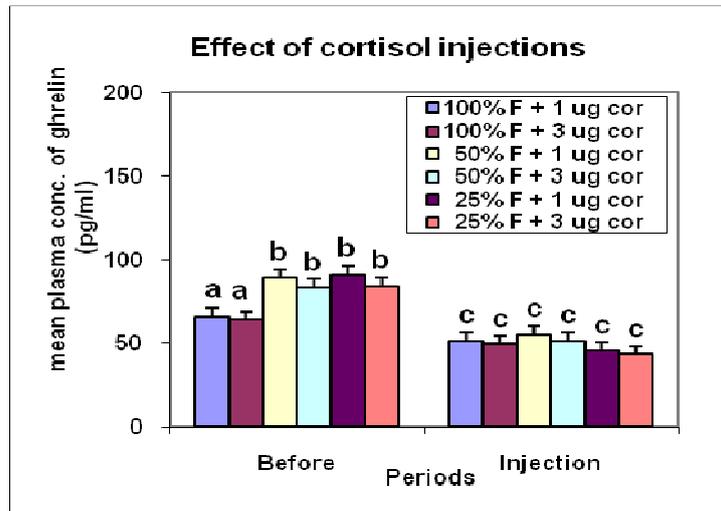
## **DISCUSSION**

### **Fasting, glucose and galanin**

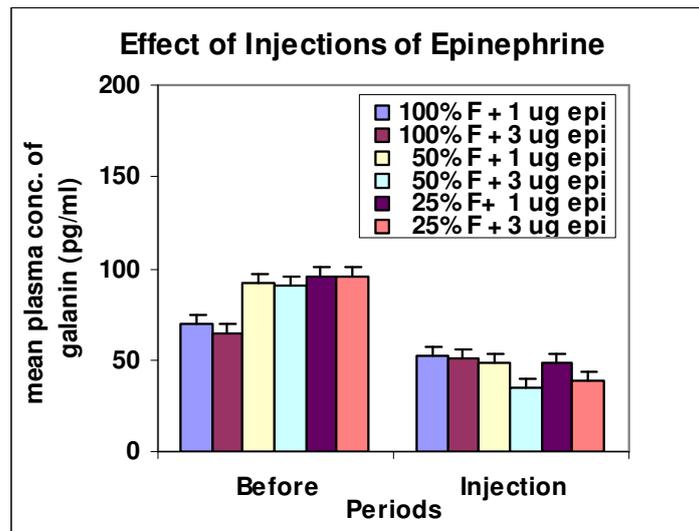
The results of these experiments showed that mean plasma concentrations of galanin is significantly ( $P > 0.01$ ) higher in the sheeps fed 25 or 50 percent of their daily food requirements than those animal fed ad-lib. This may be due to the decreased plasma level of glucose in the sheeps fed lower. Feeding lower food intake for long term cause weight loss that might be a good physiological model to investigate the relations between the degree of fasting and galanin. Our results are similar to the other findings (Komaki *et al.*, 2001; Kirchgessner, 2002, Xu *et al.*, 2002; Ehrstrom *et al.*, 2005; Lee *et al.*, 2006; Sun *et al.*, 2006) that indicated galanin concentrations increased during fasting in human.

### **Cortisol, glucose and galanin**

Diferent dosage of cortisol injections did not change the elevated plasma level of galanin in the sheeps fed 25 or 50 percent of their daily food requirements. Our results are different from those findings (Mazzocchi *et al.*, 2001; Yang *et al.*, 2007; Goncz *et al.*, 2008; Ramanjaneya *et al.*, 2008, 2009; Wenzel *et al.*, 2009). That indicated



**Figure 1.** Mean plasma concentrations of ghrelin of the animals in the different groups after injections of cortisol in the sheep fed different amount of their daily requirement.



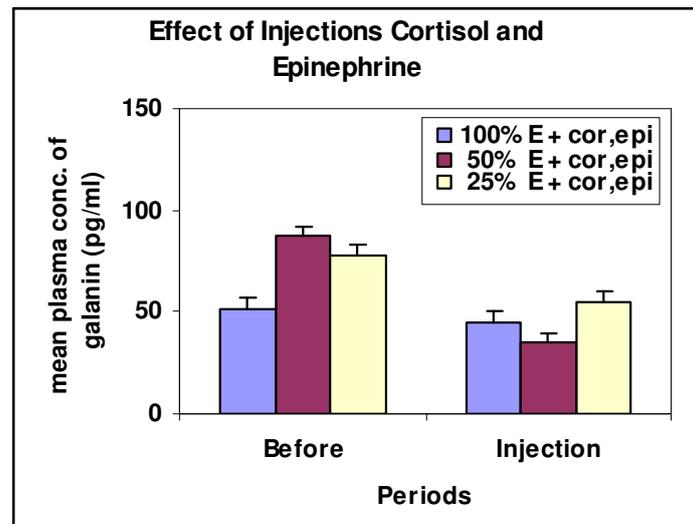
**Figure 2.** Mean plasma concentrations of galanin of the animals in the different groups after injections of epinephrine in the sheep fed different amount of their daily requirement.

injections of galanin increased the cortisol synthesis in adrenal cells. Our study was conducted in a different degree of fasting whereas theirs were in vitro. Injections of cortisol significantly ( $P > 0.01$ ) increased the plasma level of glucose in the sheep fed 25, 50 or 100 percent of their daily food requirements. The results of this experiment are similar to the findings of (Komaki *et al.*, 2001) that indicated the plasma level of glucose and cortisol are positively related. This may be due to the

effect of cortisol to cause glucogenolysis and gluconeogenesis reactions in liver.

**Epinephrine, glucose and galanin**

This is first to report that injections of different dosages of epinephrine significantly ( $P < 0.01$ ) decreased the elevated plasma level of galanin in the sheep fed 25



**Figure 3.** Mean plasma concentrations of galanin of the animals in the different groups after injections of cortisol in the sheeps fed different amount of their daily requirement.

and 50 percent of their daily food intake. Our results are different from those finding (Matsumura *et al.*, 2001; Spinazzi, *et al.*, 2006; Goncz *et al.*, 2005; Hou *et al.*, 2007; Huang *et al.*, 2010; Young *et al.*, 2005). That indicated injections of galanin increased the epinephrine secretions. Our study was conducted in a different degree of fasting whereas theirs were in vitro. One of the reason that epinephrine decreased the elevated plasma level of galanin is because of a direct effect of epinephrine on neurons or cells synthesized galanin, and this may not be related to plasma glucose level. Injections of different dosages of epinephrine significantly ( $P > 0.01$ ) increased the plasma level of glucose in the sheeps fed 25, 50 or 100 percent of their daily food requirements. The results of this experiment are similar to the findings of (Komaki *et al.*, 2001) that indicated the plasma level of glucose and epinephrine are positively related. Epinephrine is one of the neurotransmitters that have a potential effect on glycogenolysis and gluconeogenesis increase in liver during severe fasting. This is what we observed that injection

#### **Epinephrine, cortisol, glucose and galanin**

The result of these experiments showed that the injections of cortisol plus epinephrine decreased the elevated plasma galanin level in the sheeps fed 25 and 50 percent of their daily food requirements. This decrease was same as when just epinephrine is injected. This is first time to report that injections of cortisol did not boost the effect of epinephrine effect on decreasing the elevated plasma level of galanin. It seemed that epinephrine has a highly

inhibitory effect on galanin secretion. We could not find any results from other studies to compare with our results

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