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Full Length Research Paper

Effect of intravenous injection of galanin on plasma concentrations of growth hormone, thyroid hormones and milk production in the Saanen goat

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The goal of this study was to determine whether intravenously galanin injection effect on plasma concentrations of growth hormone (GH), thyroxine (T4), triiodothyronine (T3) and milk production in the Saanen goats. Fifteen Saanen goats were randomly divided into 5 groups (n = 3 in each group). Each group received daily injection of 0, 0.2, 0.4, 0.8 and 1.6 g galanin/Kg for 10 days (period of injection). Blood and milk samples were collected daily on d-2 to d-1 (before treatment), d0 to d10 (during treatment), and d11 to d12 (after treatment) each morning. Injection of galanin significantly (P < 0.05) increased plasma concentrations of GH. Also, galanin decreased plasma concentrations of T3 and T4 throughout the experiment period, while it had no significant effect on milk production. The result of this study indicated that galanin may increase the plasma concentration of GH, and decrease the plasma concentration of T3 and T4, but fail to alter milk production in the Saanen goats.

Key word: Galanin, growth hormone, thyroxine, triiodothyronine, Saanen goats.

INTRODUCTION

Galanin, a 29 amino acid peptide originally isolated from porcine small intestine (Tatemoto et al., 1983), has also been found in the central and peripheral nervous system, gut and pancreas. Galanin-like immunoreactivity is distributed in the anterior pituitary and in neurons of peripheral and central nervous system. High concentrations of galanin are found in the hypothalamus, in the median eminence and in the hypophyseal portal blood (Lopez et al., 1990); the hypothalamus is particularly rich in cell bodies and fibers containing galanin-like immunoreactivity and high levels of galanin occur in the median eminence (ME). Galanin interferes with neuroendocrine, neural and metabolic systems (Vrontakis et al., 1991). The physiological role of galanin appears to be strictly influenced by species specificity and sex (Giustina et al., 1993). It is well established that metabolic hormones regulate milk production and secretion (Machlin, 1973). Many studies have demonstrated that long term administration of metabolic hormones such as GH and T4, either by injection or via feed as iodinated protein increases amount and constituents of milk by 10 - 40% (Chalupa et al., 1987; Davis et al., 1988; Gallo et al., 1991; Thomas et al., 1991; Binelli et al., 1995; Khal et al., 1995; Khazali et al., 2000). Hypothalamic growth hormone releasing hormone (GHRH) and thyrotropin releasing hormone (TRH) induce pituitaric GH and TSH followed by T3 and T4 secretions. GHRH and TRH secretions are under different neurotransmitters. It has been shown that neurons secreting galanin influence neurons secreting GHRH and TRH (Tatemoto et al., 1983). This indicates that galanin as a neurotransmitter may control GHRH and TRH secretion to increase GH, T3 and T4 secretion. The following experiment was designed to determine whether

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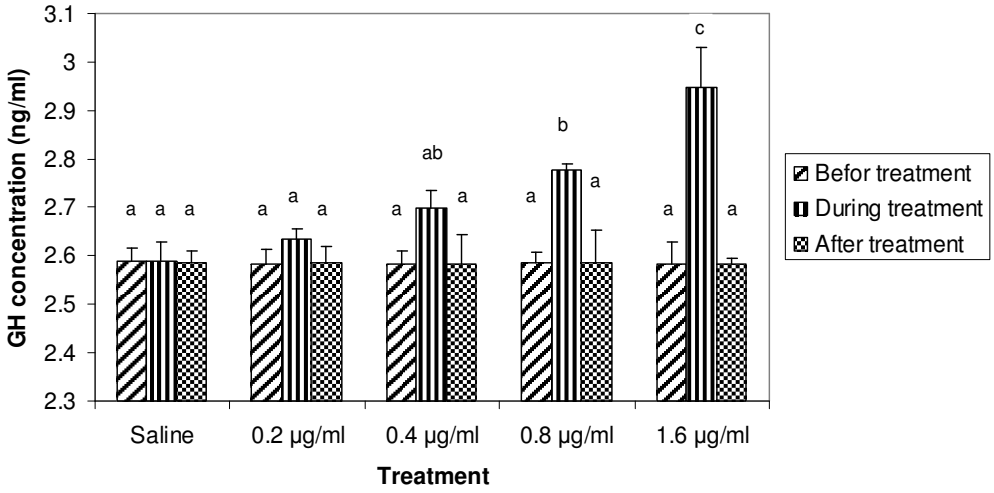

Figure 1. Mean (±SEM) plasma concentration of GH in goats that were received different doses of galanin and different times (Before, during and after treatment). Columns without a common letter differ significantly ($p < 0.05$).

galanin increase the plasma concentration of T3, T4 and GH and consequently milk amount in the Saanen goats.

MATERIALS AND METHODS

Animals and experimental design

Fifteen Saanen goats (weighing between 40 to 50 kg) were housed in controlled chambers at a constant 25°C and 70% humidity and fed *ad libitum* according to AFRC (1995). The goats were randomly divided into 5 groups. Each group received daily injection of 0 (control = 4 ml saline), 0.2, 0.4, 0.8 and 1.6 g galanin/KgBW via jugular vein for 10 days.

Blood and milk collection

Blood samples were collected from jugular vein by venepuncture. Samples were obtained daily on d-2 to d-1 (before treatment), d0 to d10 (during treatment), and d11 to d12 (after treatment) each morning. 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 GH, T3 and T4. Milk production was daily collected from day -2 till day 12 of the experiment in every morning.

Hormone assays

Plasma T3, T4 and GH were measured by a homologous double antibody radioimmunoassay (RIA). For GH assay, bovine GH (USDAoGH-I-1) and antisera against GH were provided by Dr. A. F. Parlow (Director of Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, 1000 West Carson Street, Torrance, CA). Ovine GH (USDA-oGH-I-1) was used for iodination. A seven-point standard curve ranging from 0.04 to 10 ng GH was used. An average assay binding of 40% was achieved using an initial 1:20,000 dilution of GH antiserum for GH assays. For T3 assay, T2 were purchased from Sigma Chemical Company and T3 antisera were purchased from Chemicon Co. (Temmecula, Ca). T2 were used for iodination. A six point standard curve ranging from 0.32 to 5.2 ng T3/ml was used. An average assay binding of 70% was achieved using an initial 1:5000 dilution of T3 antiserum for T3 assays. For T4 assay, T3 were purchased from Sigma Chemical Company and T4 antisera were purchased from Chemicon Co. (Temmecula, Ca). T3 were used for iodination. A six-point standard curve ranging from 2.2 to 25 ng T4/ml was used. An average assay binding of 60% was achieved using an initial 1:5000 dilution of T4 antiserum for T4 assays (Khazali et al., 2006).

Statistical analyses

Data were analyzed for repeated measures data using PROC MIXED of SAS (SAS, 1996 Inst., Inc., Cary, NC). The least squares means procedure (PDIFF option) was used to compare means when significant p-value ($P < 0.05$) was obtained.

RESULTS AND DISCUSSION

The results of this study showed that 0.8 and 1.6 g/Kg BW intravenous galanin injection increased mean GH plasma concentration (Figure 1). In sheep, galanin has been reported to increase plasma GH levels when given intracerebroventricularly (Spencer et al., 1994) at doses ranging from 0.2 ng to 20 mg per animal. Many evidences suggest that galanin acts at the hypothalamic level and that this peptide is very effective when injected into the third ventricle, not only in sheep but also in rat (Murakami et al., 1989). Specific binding sites for galanin have also been demonstrated in the rat medio-basal hypothalamus (Skofitsch et al., 1986). Although a signicant effect of galanin on GH release has been reported in both rat and human (Davis et al., 1987; Ottlecz et al., 1988; Giustina et al., 1992), the mechanism underlying this action is far to be understood. Furthermore, conflicting evidences exist in vitro about the direct effect of this pep-

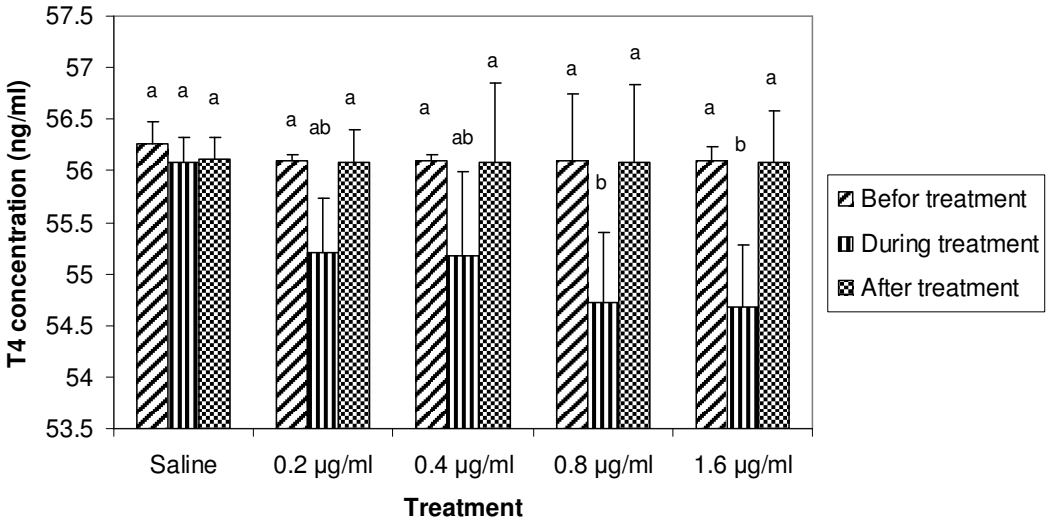

Figure 2. Mean (±SEM) plasma concentration of T_4 in goats that were received different doses of galanin and different times. Columns without a common letter differ significantly ($p < 0.05$).

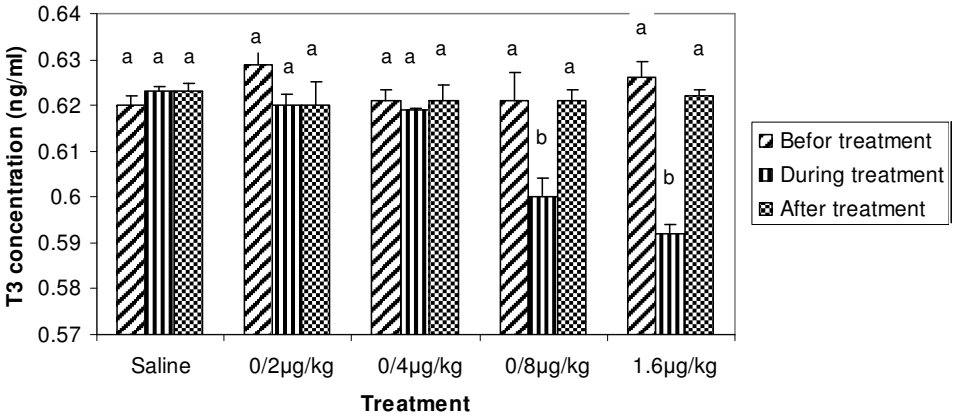

Figure 3. Mean (± SEM) T_3 plasma concentration of T_3 in goats that were received different doses of galanin and different times. Columns without a common letter differ significantly ($p < 0.05$).

tide on anterior pituitary, with an inhibitory influence on GH secretion observed in rat (Meister and Hulting, 1987) and a stimulatory one observed in rat (Lindstromm and SaÈvendhal, 1993) and bovine (Baratta et al., 1997).

The results of this study also showed that injection of 0.8 and 1.6 µg/Kg BW intravenous galanin injection decrease ($p < 0.05$) mean plasma concentration of T_4 and T_3 (Figures 2 and 3). These results indicated that galanin receptor probably is more sensitive to higher dose of galanin. Furthermore, studies showed that galanin can decrease T_4 and T_3 plasma concentration via decrease of TSH levels. For example, Ottlecz et al. (1988) documented that TSH concentration were decrease by galanin injection.

It was established that GH and thyroid hormones influence on milk production in ruminant, such as cow (Khazali et al., 2000). In the current study, galanin injections in all doses failed to alter milk production. This may be due to the transient changes of GH and thyroid hormones concentration in this study. The result of this study indicated that galanin may increase the plasma concentration of GH, and decrease the plasma concentration of T_3 and T_4, but fail to alter milk production in the Saanen goats. Further experimentation is warranted to verify this finding.

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