# **EFFECT OF SUBSTANCE-P ON GALACTOPOIETICS HORMONES SECRETIONS AND MILK PRODUCTION AND COMPOSITIONS**\*

# H. KHAZALI

Dept. of Biology, Shahid Beheshti University, Tehran, I. R. of Iran Email: h\_khazali@sbu.ac.ir

Abstract – It has been well established that the secretions of thyroid hormones and growth hormone (GH) is under the regulation of different neurotransmitters. The goal of this study was to determine whether substance-P, as a neurotransmitter, regulates the mean plasma concentrations of thyroxin (T4), triiodothyronine (T3), GH, milk amount and constituents in Sanan goats. Nine Sanan goats were randomly divided into 3 groups. Each group received a daily infusion of either 1, 2 or 4 Ug substance-P for 7 days into the third ventricle. Blood samples were collected daily for 11 days, and assayed for plasma T3, T4 and GH concentrations by double-antibody RIA. Milk samples were collected daily for each of the 11 days. Milk samples were assayed for protein, fat and lactose constituents. The daily amount of milk was determined throughout the experiment. Infusions of 1 and 2 Ug substance-P did not change the plasma concentrations of the T3, T4 and GH throughout the experiment. Infusions of 4 Ug substance-P significantly (P<0.01) increased the plasma concentrations of the T3, T4 and GH among all the animals, however, different dosages of substance-P did not change the amount, the protein, fat and lactose constituents of milk among all the animals in the different groups. The result of this experiment indicated that substance-P may increase the mean plasma concentrations of GH, T3, and T4 in Sanan goats.

Keywords – Substance-P, T3, T4, GH, Sanan goats

## 1. INTRODUCTION

Galactopoietics hormones induce the amount and constituents of milk. Many studies have demonstrated that the long term administrations of galactopoietics hormones such as growth hormone (GH) and thyroxine (T4), either by injection or via feed as iodinated protein increases the amount and constituents of milk by 10- 40% [1-11]. Both hypothalamic growth hormone releasing hormone (GHRH) and thyrotoph releasing hormone (TRH) induce pituitaric somatotroph GH and thyrotroph thyroid stimulating hormone (TSH), followed by T3 and T4 secretions respectively. GHRH and TRH secretions are under different neurotransmitters. It has been shown that neurons secreting substance-P are co-localized with neurons secreting GHRH and TRH [12-16]. This indicates that substance-P as a neurotransmitter may control GH and TSH secretion to increase GH, T3 and T4 secretions.

The following experiment was designed to determine whether substance-P increases the plasma concentrations of T3, T4 and GH, and consequently, the amount of milk and constituents in Sanan goats.

## 2. MATERIALS AND METHODS

#### a) Animals

Nine Sanan goats (weighing between 40 and 50 kg) were housed in controlled chambers at a constant 25 c and 70% humidity. The animals were anesthetized throughout the surgery for third ventricle cannulation

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under stereotaxic methods and jugular vein cannulations. The goats were cannulated into their third ventricle by the stereotaxic technique two weeks before day 1 of the experiment. They were fed ad libitum.

### b) Experimental Design

The goats were randomly divided into 3 groups. Each group received daily infusions of either 1, 2 or 4 ug substance-P into the third ventricle for 7 days.

#### c) Blood and Milk Collection

Blood samples were collected daily from the jugular vein on day 1 before the infusion until 3 days after the last 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 the plasma from clotting during storage. Plasma was stored at -20 C until assayed for T3, T4 and GH. Milk samples were collected daily from day 1 till day 11 of the experiment. Milk samples were kept at 4 C until the assay for protein, fat and lactose was completed. The daily amount of milk was determined throughout the experiment.

### d) Hormone Assays

Plasma T3, T4 and GH were measured by a hoterologous double-antibody radioimmunoassay (RIA). For the GH assay, bovine GH (USDA-oGH-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) were used for iodination. A seven-point standard curve ranging from 0.04 to 10 ng GH were used. An average assay binding of 40% was achieved using an initial 1:20,000 dilution of GH antiserum for GH assays. For the T3 assay, T2 were purchased from the Sigma Chemical Company and T3 antisera from the Chemicon Co. (Temmecula, Ca). T2 were used for iodination. A six-point standard curve ranging from 0.32 to 5.2 ng T3/ml were used. An average assay binding of 70% was achieved using an initial 1:5000 dilution of T3 antisera from the Chemicon Co. (Temmecula, Ca). T3 were used for iodination. A six-point standard curve ranging from 0.32 to 5.2 ng T3/ml were used. An average assay binding of 70% was achieved using an initial 1:5000 dilution of T3 antisera from the Chemicon Co. (Temmecula, Ca). T3 were used for iodination. A six-point standard curve ranging from 2.2 to 25 ng T4/ml were used. An average assay binding of 60%, was achieved using an initial 1:5000 dilution of T4 antiserum for T4 assays.

### e) Chemical Analyses

Concentrations of fat, protein and lactose in milk were determined using a semiautomatic infrared analyzer (Milkoscan 104; A/s N. Foss Electric, Hillerod, Denmark).

### f) Statistical Analysis

All analyses were conducted using General Linear Model procedures [12]. Data were analyzed using an analysis of variance for a repeated measures design. Mean comparisons were evaluated by the least significant difference with a single degree of freedom. Data were analyzed by SAS [17].

## **3. RESULTS**

Infusions of 1 and 2 ug substance-P did not change the plasma concentrations of the T3, T4 and GH throughout the infusion period (Figs. 1-3). Infusions of 4 ug substance-P significantly (P<0.01) increased the plasma concentrations of the T3, T4 and GH during the experiment (Figs. 1-3). The increase of the T3

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and T4 plasma level occurred at day 2 and the increase of the GH plasma level occurred on day 1 of the infusion of 4 Ug substance-P. Infusions of 1, 2 and 4 ug substance-P did not change the amount or the protein, fat and lactose constituents of milk among all the animals in the different groups (Fig. 4).

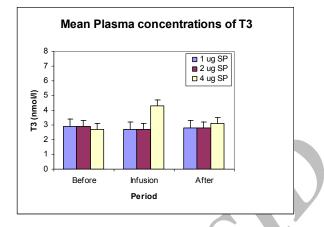


Fig. 1. Mean plasma concentrations of T3 among animals of three different groups before, during and after infusions of substance-P

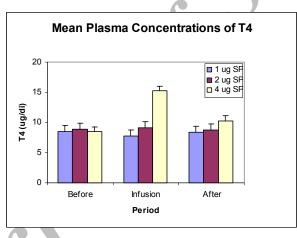


Fig. 2. Mean plasma concentrations of T4 among animals of three different groups before, during and after infusions of substance-P

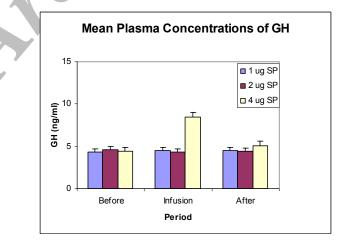


Fig. 3. Mean plasma concentrations of GH among animals of three different groups before, during and after infusions of substance-P

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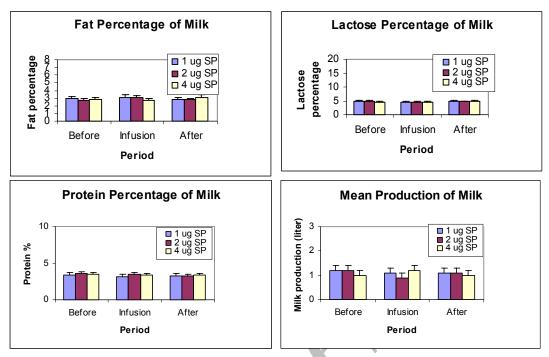


Fig. 4. Mean milk fat percentage, lactose percentage, protein percentage and milk amount of animals of different groups

# 4. DISCUSSION

One of the interesting findings of this experiment was the increase in plasma concentrations of the T3, T4 and GH in those animals that received 4 ug substance-P. This is the first report of the effect of substance-P on the plasma concentrations of the T3, T4 and GH in goats. This may be due to the co-localizations of substance-P neurons with neurons secreting GHRH and TRH, an indication of substance-P stimulation on T3, T4 and GH secretions [13-16]. Infusions of 1, 2 and 4 ug substance-P did not change the amount, the protein, fat and lactose percentage of milk in all animals in the different groups. This result is not similar to the previous finding [10], which reported that the GH treatment increased milk production. This may be due to the need for a higher increase in GH secretions to get an increase in the amount of milk.

In summary, Substance-P may induce the T3, T4 and GH secretions in Sanan goats.

## REFERENCES

- Soderholm, C. G., Otterby, D. E., Linn, J. G., Ehle, F. R., Wheaton, J. E., Hansen, W. P. & Annexstad, R. J. (1988). Effect of recombinant bovine somatotropin on milk production body composition and physiological parameters. *J. Dairy Sci.*, 71, 355-361.
- Thomas, J. W., Erdman, R. A., Galton, D. M., Lamb, R. C., Arambel, M. J., Olson, J. D. & Madsen, K. S. (1991). Samuels, W. A., Response by lactating cows in commercial dairy herd to rBST. *J. Dairy Sci.*, 74, 945-952.
- Jenney, B. F., Grimes, L. W., Pardue, F. E., Rock, D. & Patterson, D. L. (1992). Lactational response of Jersey cows to bovine somatotropin administered daily or in a sustained release formulation. *J. Dairy Sci.*, 75, 3402-3408.
- 4. Gallo, L., Cassandro, M., Camier, P., Mantovani, R., Ramanzin, M., Tealdo, E. & Casson, P. (1991). Modeling response to slow-releasing somatotropin administered at 3- or 4- week intervals. *J. Dairy Sci.*, 77, 759-765.
- Binelli, M., Vanderkool, W. K., Chapinm L. T., VanaHaar, M. J., Tumer, J. D., Tucker, H. & Moseley, W. M. (1995). Comparison or growth hormone-releasing factor and somatotropin body growth and lactation of primiparous cows. J. Dairy Sci., 78, 2129-2135.

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- Chalupa, W., Baird, L., Soderholm, C., Palmaquist, D. L., Otterby, D., Annextad, D., Veichiarelli, B., Harmon, R. & Sinha, R. (1987). Responses of dairy cows to somatotropin. *J. Dairy Sci.*, 70, 1760-1765.
- Khal, S., Capuco, S., Binelli, M., Vanderkool, W. K., Tucker, W. & Moseley, W. (1995). Comparison of growth hormone-releasing factor and somatotropin: thyroid status of lactating primiparous cows. *J. dairy Sci.*, 78, 2150-2156.
- Davis, S. R., Coiller, R. J., MacNamara, J. P., Head, H. & Sussman, W. (1988). Effect of thyroxine and growth hormone treatment of dairy cows on milk yield, cardiac output and mammary blood flow. J. Anim. Sci., 66, 70-76.
- Davis, S. R., Coiller, R., MacNamara, J., Head, H. & Sussman, W. (1988). Effect of thyroxine and growth hormones treatment of dairy cows on mammary uptake of glucose, oxygen, and other milk fat precursors. *J. Anim. Sci., 66*, 80-87.
- Khazali, H., Khajavi, M. & Rokni, H. (2000). Effect of thyroxine and triiodothyronine on the amount and constituent of milk in the dairy sarabi cows treated with growth hormone. *Anim. Sci. J.*, 71, 481-485.
- Schmidt, G., Warner, R., Tyrrell, H. & Hansel, W. (1971). Effect of thyroprotein feeding on dairy cows. J. Dairy Sci., 54, 481-486.
- Balsa, J., Sanchez-Franco, F., Pazos, F., Lara, J., Lorenzo, M., Maldonado, G. & Cacicedo L. (1998). Direct action of serotonin on prolactin, growth hormone, corticotropin and luteinizing hormone release in cocultures of anterior and posterior pituitary lobes: autocrine and/or paracrine action of vasoactive intestinal peptide. *Neuroendocrinology*, 68, 326-333.
- 13. Bujatti, M. & Riederer, P. (1976). Serotonin, noradrenaline, dopamine metabolites in transcendental Meditation technique. *J. of Neural Transmission*, *39*, 257-267.
- Sánchez-Franco, F. & Razos, F. (2003). Direct Action of Serotonin on Prolactin, Growth Hormone, Corticotropin and Luteinizing Hormone Release in Cocultures of Anterior and Posterior Pituitary Lobes: Autocrine and/or Paracrine Action of Vasoactive Intestinal Peptide Regulation of Pituitary Cells, 68, 115-121.
- Savard, P. & Merand, Y. (1983). Effects of thyroid on substance P contents in discrete brain nuclei of adult rats. *Neurosci.* 10, 1399-404.
- Savard, P. & Blanchard, L. (1986). Substance-P content of discrete brain nuclei in rats made hypo-or hyperthyroid in the neonatal period: effect of growth hormone treatment. *Horm Metab Res*, 18(4), 234-237.
- 17. SAS user's guide. (1985). Statistics, SAS Institute Inc., Cary, NC.