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Effect of melatonin administration on thyroid hormones, cortisol and expression profile of heat shock proteins in goats (*Capra hircus*) exposed to heat stress

S. Sharma, K. Ramesh, I. Hyder, S. Uniyal, V.P. Yadav, R.P. Panda, V.P. Maurya, G. Singh, P. Kumar, A. Mitra, M. Sarkar*

Physiology & Climatology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh 243122, India

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ABSTRACT

Heat stress had been a constant challenge to goat farming under tropical climate as more chance exist for exposure to solar radiation for most of the day while grazing with exaggeration by nutritional constraints. Menacing global rise in surface temperature compelled more focus of research over mitigation of heat stress. With 6 female goats of uniform age and weight experimental group ($n=6$) and were acclimatized for initial 5 days in psychrometric chamber and kept at 25 °C. From 6th day, the animals were exposed daily 6 h (07:00 h to 13:00 h) for 6 days each at 35 °C and 40 °C in psychrometric chamber. Melatonin was administered I/V at the dose rate of 0.1 mg/kg at 12:00 h throughout the experimental period from 5th day to 17th day. Cardinal physiological response assessment was done at 30 min before and after melatonin administration respectively. Blood samples were collected after 1 h of melatonin administration on alternate experimental days starting from 5th day. Level of hormones T3, T4 and cortisol was assessed in serum samples and relative expression of genes like HSP 60, HSP 70, HSP 90 and ubiquitin was studied in peripheral blood mononuclear cells. In control group as well as treatment group rectal temperature and pulse rate increased significantly ($P<0.05$) as the exposure temperature increased. No significant change was observed in T3, however T4 significantly ($P<0.05$) decline at 40 °C in melatonin treated group. The cortisol level rose significantly ($P<0.05$) with increase in exposure temperature in control animals. Significantly ($P<0.05$) low level of cortisol was observed in melatonin treated group at all exposure temperature. HSP 60, HSP 70, HSP 90 and ubiquitin showed significant ($P<0.05$) up regulation as a protective effects under heat stress in both the groups. The relative expression of HSP 60 was increased manifold in melatonin treated group at 40 °C exposure temperature. In conclusion, the present study clearly supported heat alleviating and cell protective role of melatonin with suppression in serum cortisol level and upregulation of genes like HSP-60 in PBMCs.

1. Introduction

Globally goat plays an important role in the economy of thousands poor livestock owners who earn their livelihood by rearing them in different terrain and climatic condition. Goats are primarily raised on grazing resources under extensive range management in different parts of the country. India, being a tropical country has wide range of climatic condition, and the animals such as goat, which browse for many hours a day, is regularly subjected to extreme weather conditions. Beyond the upper limit of thermoneutral zone, the animal experiences heat stress.

* Corresponding author.
E-mail address: msarkar24@gmail.com (M. Sarkar).

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Physiological mechanisms including endocrinal responses and cellular heat stress responses are triggered to maintain homeostasis. Thermal stress redistributes the body resources including protein and energy at the cost of decreased growth, reproduction, production and health.

At cellular level the ability to survive and adapt to thermal stress involves biochemical responses and gene expression (Fujita, 1999; Lindquist, 1986). Activation of heat shock transcription factor 1 (HSF1) and subsequent increased expression of heat shock proteins (HSP) is integral to cellular response to heat stress (Collier et al., 2008).

At endocrinal level, thyroid and adrenal hormones secretions having impact on the productivity of animal show change in plasma levels under heat stress. Secretion of thyroid hormones is decreased under heat stress thereby hampering the productivity of the animal for which optimum levels of thyroid hormones are required (Todini, 2007). Similarly, pituitary–adrenal axis is stimulated under heat stress which increases levels of glucocorticoids. This further takes toll on animal's productivity by bringing substantial change in body metabolism.

Melatonin (N-acetyl-5-methoxytryptamine), best known for its role in seasonality of reproduction, plays prominent role in relieving heat stress by influencing cardiovascular system and evaporative heat loss (Harlow, 1987). Melatonin also interacts with other hormones to alleviate heat stress possibly with thyroxine and successfully modify adrenal function to relieve thermal stress (Sejian and Srivastava, 2009). It has also been reported to modulate gene expression of antioxidant enzymes in body, HSP 60 in heat stressed pancreatic cells (Bonior et al., 2005), HSP 27 in HL-60 cells (Cabrera et al., 2003) HSP 70, HSP 90 and HSP 40 in rat liver cells under oxidative stress (Catala et al., 2007).

To overcome detrimental effects of the heat stress, the basic strategy is to alter the surrounding environment of the animal by using sheds, fans and evaporative cooling (Bucklin et al., 1991). Owing to the tropical climate of the India, semi-intensive nature of rearing goats and economic backwardness of farmers, it becomes pertinent to search for novel ways to counteract the adverse effect of the heat stress. To devise new strategy, it becomes important to fully understand the underlying physiological mechanism and the role that different bio-molecule may play under heat stress.

Therefore we have undertaken the present experiment to investigate the role of melatonin under heat stress. The present study was conducted with a purpose to analyze the effect of administrating exogenous melatonin on thyroid hormones and cortisol and expression profile of HSP 60, HSP 70, HSP 90 and ubiquitin in goats exposed to heat stress in the psychrometric chamber.

2. Materials and methods

2.1. Site of study

The present study was carried out at the Division of Physiology and Climatology, IVRI. Location of this institute is 28° north and 79° east having sub-tropical climate. The experiment was conducted in the month of November when mean environmental temperature and relative humidity was 19–25 °C and 60–82%, respectively.

Table 1
Group, age, weight and sex of experimental animals used in the present study.

Group	Age	Weight	Sex
Control ($n=6$)	3.17 ± 0.3	17.67 ± 1.41	Female
Treatment ($n=6$)	2.92 ± 0.26	16.5 ± 1.56	Female

2.2. Animals

Twelve healthy barbari goats of uniform age and weight were selected for the study (Table 1). They were randomly divided into two groups, of six animals ($n=6$) each as control and treatment. The animals were housed in well ventilated and hygienic shed with ad libitum access to feed and drinking water.

2.3. Experimental design

The experiment was conducted for the period of 17 days during which animals were housed in the shed adjoining to psychrometric chamber and in the chamber during exposed heat stress exposure. The size of the chamber was 7.5 × 7.5 m, equipped with individual feeders and waterers. Rectal temperature, heart rate and respiratory rate were measured daily from day 5 to day 17, 30 min before and after melatonin administration in both the groups. During first five days animals were kept in psychrometric chamber at thermo-neutral zone (25 °C) for 6 h (7:00 h to 13:00 h) each day to make them acclimated to climatic chamber. For next 6 days animal were to 35 °C temperature for 6 h (7:00 h to 13:00 h) each day, followed by exposure at 40 °C for 6 h (7:00 h to 13:00 h) each day for next 6 days. Dose rate of melatonin was as per previous experiments conducted in our lab (Sejian and Srivastava, 2010). Melatonin (MP Biomedicals, France) was dissolved in normal saline and administered I/V @ 0.1 mg/kg BW, to animals in treatment group from 5th day to 17th day at 5th hour (12:00 h). Sham injection of normal saline was administered I/V to animals in control group. After 1 h of melatonin administration blood samples (10 ml) were collected in serum vacutainer (5 ml) and heparinized vacutainers (5 ml) from both control and treatment group on alternate days starting from 5th day.

Serum was separated and stored in microcentrifugation tubes at −20 °C for further analysis. Heparinized blood was used for mRNA isolation.

2.4. Hormones assay procedure

Triodothyronine (T3) was estimated by using commercial kit (ERBA Thyrokit, Germany) with sensitivity of 0.1 ng/ml and coefficient of variation for within assay 3.01% and for between assay is 2.62%.

Thyroxine (T4) was estimated by using commercial kit (ERBA Thyrokit, Germany) with sensitivity of 10 nmol/l and coefficient of variation for within assay is 2.50% and between assay is 5.78%.

Cortisol was estimated by a highly sensitive EIA procedure on microtiterplates using the second antibody coating technique and cortisol-HRP as a label in unextracted goat plasma following the procedure described by Sarkar et al. (2007). Intra- and interassay coefficients of variation (CV) determined using pooled plasma was found 7.25% and 10.34%, respectively. The analytical sensitivity of the assay was 20 pg/ml.

2.5. Primers

Primers were designed by the Fast PCR (Version: 6.2.73) software. The sequences and expected PCR Product length are shown in Table 2.

2.6. PBMCs isolation

PBMCs were isolated by density gradient centrifugation method using Histopaque 1077 (Sigma, USA). Briefly, the blood was layered carefully onto the Histopaque to produce a clean interface between the two layers. Further it was centrifuged at 2000 rpm for 30 min at room temperature. The white opaque mononuclear fraction from the interface was collected. The cells were washed thrice in PBS (pH 7.4) and finally the cell pellet was obtained.