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Pineal-adrenal Relationship under Thermal Stress with Emphasis on Effect of Pineal Proteins on Endocrine Profiles in Chemically Adrenalectomized Does

V. Sejian and R.S. Srivastava

Neurophysiology Laboratory, Division of Physiology and Climatology,
Indian Veterinary Research Institute, Izatnagar, Bareilly- 243122, India

Abstract: Pineal-adrenal relationship was studied with the primary objective to determine the influence of total precipitated pineal protein on endocrine function in chemically adrenalectomized does to alleviate heat stress. Melatonin and glucocorticoids secretions and several other endocrine parameters reflecting the animals well being were determined over a period of a week after does had been exposed to 40°C and 60% RH for 10 days. The study was conducted for a period of seventeen days under psychrometric chamber. Animals were served as self controls prior to start of the experiment. Blood samples were drawn on day 0 and day 10 to establish control and thermal stress values, respectively. Chemical adrenalectomy was achieved using metyrapone followed by exogenous total precipitated pineal protein treatment. Chemical adrenalectomy significantly affected plasma levels of the different endocrine parameters studied. Total precipitated pineal proteins successfully and significantly relieved the animals from adverse affect of heat stress and metyrapone treatment. This is evident from the findings that most of the endocrine parameters studied were influenced by pineal protein indicating their antistress properties. In conclusion this result established that total precipitated pineal proteins have significant influence upon adrenal cortex secretions in relieving thermal stress in does.

Key words: Does · Hormones · Metyrapone · Cortisol · Thyroxine · Melatonin

INTRODUCTION

Pineal gland is considered as a neuroendocrine transducer of cyclic photic input, which is responsible for the seasonal changes in reproductive capability of various species [1]. Considerable evidence has now been accumulated to indicate its participation in a wide range of extra reproductive processes [2], among which Pineal-Adrenal, Pineal-Thyroid and Pineal-Immune system relationships are the thrust areas of research investigations.

The study of Pineal-Adrenal relationship probably began with Farrell's discovery of adrenoglomerulotropin in the pineal extracts of rats [3]. Since then, many investigators have attempted to establish this relationship [4 - 6]. Nevertheless, the contradictory nature of the results obtained so far make it difficult to draw any definitive conclusions about the pineal-adrenal axis [7, 8].

Different sources of evidence suggest that melatonin may act as an important regulator of adrenal function [6, 9]. *In vitro* and *In vivo* experiments revealed a suppressive action of melatonin on adrenal glucocorticoid production and release [10 - 12]. The relationship between the rhythms of plasma melatonin and cortisol as well as the presence of the melatonin receptors on adrenal cortex can be taken as evidence for the direct effect of melatonin on steroidogenesis [13].

Apart from melatonin, there are several other indoles and proteins produced by the pineal gland possessing properties similar to melatonin [14]. However, literatures were very scanty on the pineal protein influence on endocrine activity to relieve stress. Hence, the present study was undertaken to establish the relationship between pineal gland and adrenal cortex under thermal stress. The primary objective of the study was to ascertain the influence of pineal proteins on endocrine profile in chemically adrenalectomized goats to alleviate thermal stress.

MATERIALS AND METHODS

Animals: Six healthy female Marwari goats (8-12 months old/12-15 kg) were used in the present study. Does were served as self controls prior to start of experiment.

Corresponding Author: Dr. V. Sejian, Adaptation Physiology Laboratory, Division of Physiology and Biochemistry, Central Sheep and Wool Research Institute, Avikanagar, Via-Jaipur, Rajasthan- 304501, India, Tell: 091-9828624721

Prophylactic measures against goat diseases like goat pox, peste des petits ruminants, enterotoxaemia, endo and ectoparasitic infestations were carried out as prescribed by the health calendar of the institute to ensure that the animals were in healthy condition throughout the study.

Chemicals: Metyrapone was procured from Sigma Aldrich, USA and buffalo (*Bubalus bubalis*) pineal proteins were supplied by the Neurophysiology Laboratory, Division of Physiology and Climatology, IVRI, Izatnagar.

Experimental Design: The study was conducted for a period of 17 days. The animals were kept on 12 h light and 12 h darkness for the entire study period. Prior to start of experiment blood samples were drawn from all six animals on day 0 to establish the control values for the different parameters studied. All does were exposed to thermal stress in the psychrometric chamber at a temperature of 40°C and relative humidity of 60% for four hours a day from 9:00 h to 13:00 h during the whole experimental period. Does were exposed to high ambient temperature and humidity for first 10 days without any experiments. This ensures that the effect of heat stress is established in these animals. Blood samples were collected after thermal exposure on 10^{th} day to establish the effect of heat stress on various studied parameters. On the 11^{th} day Metyrapone (2-Methyl-1, 2-di-3-pyridyl-1-propanone) was administered intravenously in these animals at the dose rate of 100-mg/kg body weight to induce chemical adrenalectomy. Metyrapone blocks 11α hydroxylase, which catalyzes the conversion of 11- deoxycortisol to cortisol, the final step in cortisol biosynthesis. Metyrapone injection was given at 10:00 h. Metyrapone induces temporal adrenalectomy as the half-life of metyrapone is two hours. But, the peak action of metyrapone was obtained between 15 minutes to 30 minutes after its administration. Generally, the action of metyrapone remains in the system for four hours although the action of the drug is reduced drastically after one hour of its administration. Hence, after one hour of metyrapone administration, while the action of the drug remains in the system, total precipitated pineal protein was injected to relieve its effects. This experimental procedure of injecting metyrapone and total precipitated pineal protein were followed everyday from 11^{th} to 17^{th} day of the study. Metyrapone injection was given at 10:00 h. After one hour of metyrapone treatment, five ml blood was drawn from jugular vein to ascertain chemical adrenalectomy. Total precipitated pineal protein was administered intravenously at the dose rate of 0.1 mg/kg body weight to counteract the metyrapone effect. Time of total precipitated pineal protein injection was at 11:00 h. After an hour of pineal protein administration, five ml blood was drawn from jugular vein to observe the metyrapone effect relieving capability of pineal proteins. The study was conducted after due approval from the Institute Ethical Committee.

Plasma Separation: Plasma was separated from blood by centrifugation at 4,500 rpm at room temperature for 20 minutes. The plasma was then divided into aliquots in micro centrifuge tubes and kept frozen at -20°C till further analysis.

Parameters Studied: The parameters analyzed in the study were plasma cortisol, aldosterone, insulin, tri-iodo-thyronine, thyroxine, melatonin and corticosterone. All these endocrine parameters are included in the study to know whether pineal proteins modulate the adrenal cortex activity to influence the levels of these parameters to ensure thermoregulation.

Analysis of Hormonal Parameters: Hormonal parameters such as cortisol, aldosterone, insulin, tri-iodo-thyronine, thyroxine and melatonin, were estimated by RIA method (*Immunotech*, France) using the Packard Cobra II gamma counter. Plasma corticosterone was estimated by ELISA method (*Neogen Corporation*, USA). As a representative sample, only day 17 plasma samples were subjected for estimation of both melatonin and corticosterone.

Statistical Analysis: Obtained data were statistically analyzed by paired t-test as per method described by Snedecor and Cochran [15]. The treatment values were compared both with control and thermal exposure values. Significant differences were determined at the levels of ($P< 0.05$).

RESULTS

Plasma Cortisol: The level of plasma cortisol increased ($P< 0.05$) after thermal exposure (Table 1). Plasma cortisol showed changes ($P<0.05$) for the treatment of both metyrapone and total precipitated pineal protein (Table 1). Metyrapone treatment) reduced ($P< 0.05$) the heat stress induced increase in cortisol level on day 11. Total precipitated pineal protein further reduced the cortisol level induced by metyrapone treatment on all experimental days when compared to heat stress value (Table 1). This effect of pineal proteins was significant ($P< 0.05$) only on days 11 and 17 of the experiment. The effect of both metyrapone and pineal protein are highly significant when compared to control value.

Table 1: Plasma level of cortisol, aldosterone and insulin in control, heat exposed, chemical adrenalectomized and pineal protein administered goats

EXPT DAYS	Treatment	Cortisol(nmol/L)	Aldosterone(Pg/mL)	Insulin (micro IU/ml)
Day 0		26.80±3.77	2.08±0.75	8.32±0.16
Day 10		80.20±5.75^{A}	2.70±1.09	6.32±1.16
Day 11	MET[1]	43.45±7.78^{Aa}	1.27±0.31	28.93±7.88^{Aa}
	PP[2]	36.42±5.89^{a}	0.38±0.13	67.36±2.64^{Aa}
Day 12	MET	76.72±2.06^{A}	0.54±0.24	24.73±9.65
	PP	69.75±4.88^{A}	0.14±0.04	34.71±7.25^{Aa}
Day 13	MET	64.96±9.83^{A}	0.09±0.03^{A}	33.66±3.90
	PP	59.73±7.29^{A}	0.04±0.02^{A}	36.92±2.61
Day 14	MET	71.64±4.14^{A}	0.10±0.02^{A}	13.06±1.07^{Aa}
	PP	65.14±11.63^{A}	0.25±0.12	17.28±1.42^{Aa}
Day 15	MET	73.04±2.68^{A}	0.46±0.22	12.26±2.72
	PP	68.58±5.87^{A}	0.08±0.03^{A}	14.41±1.52^{Aa}
Day 16	MET	80.68±5.36^{A}	1.17±0.04	13.93±5.05
	PP	75.35±7.61^{A}	0.09±0.02^{A}	28.29±4.82^{Aa}
Day 17	MET	63.96±5.58^{A}	3.92±0.89	17.95±1.85^{Aa}
	PP	48.00±9.30^{a}	1.87±0.48	15.43±0.32^{Aa}

Values are the mean and SEM of 6 animals each

[1] MET = Metyrapone; [2] PP = Total precipitated pineal proteins

^{A} Indicates statistical significance at P< 0.05 when compared with day 0 value.

^{a} Indicates statistical significance at P< 0.05 when compared with day 10 value.

Table 2: Plasma level of T_3 and T_4 in control, heat exposed, chemical adrenalectomized and pineal protein administered goats

EXPT DAYS	Treatment	T_3(nmol/L)	T_4(nmol/L)
Day 0		1.27±0.20	86.49±10.82
Day 10		1.78±0.28^{A}	116.74±10.60^{A}
Day 11	MET[1]	1.17±0.16	116.09±12.70
	PP[2]	1.36±0.12	99.53±5.93
Day 12	MET	0.76±0.11^{a}	66.58±6.76^{a}
	PP	0.89±0.09^{a}	65.27±6.91^{Aa}
Day 13	MET	0.83±0.10^{a}	52.72±3.51^{Aa}
	PP	1.28±0.15	49.03±4.14^{Aa}
Day 14	MET	0.75±0.02^{a}	52.01±3.18^{Aa}
	PP	1.36±0.12	42.14±2.87^{Aa}
Day 15	MET	0.76±0.12^{Aa}	44.73±6.22^{Aa}
	PP	1.04±0.13^{a}	49.02±7.64^{Aa}
Day 16	MET	0.77±0.09^{Aa}	54.35±3.88^{Aa}
	PP	1.13±0.10	53.40±5.54^{Aa}
Day 17	MET	0.72±0.13^{Aa}	52.70±6.71^{Aa}
	PP	1.15±0.07^{a}	63.00±6.46^{Aa}

Values are the mean and SEM of 6 animals each

[1] MET = Metyrapone; [2] PP = Total precipitated pineal proteins

^{A} Indicates statistical significance at P< 0.05 when compared with day 0 value.

^{a} Indicates statistical significance at P< 0.05 when compared with day 10 value.

Plasma Aldosterone: The level of plasma aldosterone non-significantly increased after thermal exposure (Table 1). Metyrapone generally reduced this heat stress induced increase in aldosterone level. Total precipitated pineal protein further reduced non-significantly the aldosterone level induced by metyrapone treatment on all experimental days when compared to heat stress value (Table 1). However, the effect of both metyrapone and pineal protein were significant on day 13, 14 and 16 when compared to the control value.

Plasma Insulin: Thermal exposure reduced the insulin level when compared to control value (Table 1). Metyrapone treatment increased its level (P< 0.05) from that of control and heat exposure values (Table 1). Total precipitated pineal protein treatment further increased insulin level (P< 0.05) when compared to both control and thermal exposure value.

Plasma T_3: Thermal exposure reduced (P< 0.05) the plasma T_3 level when compared to control value (Table 2). Metyrapone treatment reduced (P< 0.05) this increase in

Table 3: Plasma level of melatonin and corticosterone in control, heat exposed, chemical adrenalectomized and pineal protein administered goats

EXPT DAYS	Treatment	Melatonin(Pg/mL)	Corticosterone(ng/mL)
Day 0		163.95±32.65	0.88±0.19
Day 10		195.20±31.99[A]	1.68±0.21[A]
Day 17	MET[1]	178.45±22.54	1.07±0.33
	PP[2]	208.89±22.86[A]	0.60±0.16[a]

Values are the mean and SEM of 6 animals each

[1] MET = Metyrapone; [2] PP = Total precipitated pineal proteins

[A] Indicates statistical significance at P< 0.05 when compared with day 0 value.

[a] Indicates statistical significance at P< 0.05 when compared with day 10 value.

T_3 level due to heat stress. But, total precipitated pineal protein treatment increased the T_3 level when compared to heat stress value (Table 2). This effect of pineal protein was significant only on days 12, 15 and 17. However, pineal proteins could bring back the T_3 level towards control value by the end of study period (Table 2).

Plasma T_4: Thermal exposure reduced (P< 0.05) the plasma T_4 level when compared to control value (Table 2). Metyrapone treatment reduced (P< 0.05) this increase in T_4 level due to heat stress. Pineal protein treatment further reduced (P< 0.05) T_4 level when compared to both control and thermal exposure value (Table 2).

Plasma Melatonin: Thermal exposure increased (P< 0.05) the plasma melatonin level when compared to control value (Table 3). Metyrapone treatment non-significantly reduced this increase in melatonin level due to heat stress. But, total precipitated pineal protein increased the level of plasma melatonin. However, these effects of pineal protein are significant (P< 0.05) only with control value (Table 3).

Plasma Corticosterone: Thermal exposure increased (P< 0.05) the plasma corticosterone level when compared to control value (Table 3). Metyrapone treatment reduced corticosterone level when compared to heat stress value. But total precipitated pineal protein induced a reduction (P< 0.05) in corticosterone level when compared to heat stress value (Table 3).

DISCUSSION

The significant increase in plasma cortisol and corticosterone after thermal exposure establishes clearly the effect of heat stress in the present study. Elevated glucocorticoid level indicates that the animals are in severe stress. Further significant reduction in cortisol level after metyrapone treatment indicates the effectiveness of chemical adrenalectomy in the study. This shows that metyrapone @ 100mg/kg BW was able to induce chemical adrenalectomy in the present study. Further total precipitated pineal protein significantly reduced this thermal stress induced increase in glucocorticoids. This shows that a relationship exists between pineal and adrenal gland to relieve thermal stress in does. Similar relationship was established in the same species with a different experiment in our laboratory [16]. The results obtained in the present study revealed that pineal proteins have significant influence upon adrenal cortex function in relieving thermal stress in goats. The reports on the influence of pineal proteins on adrenal cortex functions with respect to the parameters included in the study were very scanty. However, there are several reports on anti-stress properties of melatonin, the principal hormonal product from pineal gland [5, 13]. The obtained results from this study suggest that apart from melatonin, pineal proteins also possess similar properties that relieve stress in goats.

Plasma cortisol showed highly significant changes for the treatment of both metyrapone and pineal proteins with respect to relieving thermal stress. Lommer [17] showed significant reduction in the level of cortisol after pineal extract treatment. In our study, pineal proteins were given to counteract the metyrapone effect and this could be the reason for the increased level of cortisol when compared to control level. Plasma aldosterone level showed less significant changes when compared to other endocrine parameters for the treatment of metyrapone and pineal proteins. The reduction trend on aldosterone level associated with pineal protein treatment in our study was similar to the findings of Lommer [17] and Ng [18] who reported that after exogenous pineal extract treatment, there is significant reduction in aldosterone level in rats. Plasma insulin also showed statistical significance for the treatment of both metyrapone and total precipitated pineal protein. [19] identified melatonin receptors in the pancreas and further exogenous melatonin increased serum insulin level [20, 21]. Similar results in our study indicate that

there is a possibility for the presence of receptors mediated action of pineal proteins on pancreas to control insulin level.

Thermal exposure significantly increased T_3 and T_4 level in the experimental animals. Our result contradicted with the findings of Abecia *et al.* [22] who reported that during summer the level of thyroxine was lowest in the ewes. Metyrapone treatment reduced significantly the level of T_3 and T_4 as compared to thermal stress values. This shows that in absence of glucocorticoids, levels of thyroid hormones were reduced under thermal stress. The effect of total precipitated pineal protein to reduce the thyroid hormones under thermal stress could be to reduce the metabolic heat to minimize the thermal stress. There were no reports available on the effect of either pineal protein or pineal extract on thyroid hormone levels. However, there are available reports on the effect of melatonin, the major hormonal product from pineal gland on the circulating level of thyroid hormones [23, 24]. They reported that melatonin reduced plasma level of T_3 and T_4 in rats. They explained the probable reason for this could be that melatonin inhibits iodine uptake by thyroid gland leading to reduction in the thyroid hormone level. Sakamoto *et al.* [25] reported that melatonin administration in rats regulates TSH release from pars tuberalis showing that melatonin could control level of thyroid hormone by controlling level of TSH in pituitary. The results indicate a possibility for the pineal proteins to mediate similar action like that of melatonin to control thyroid hormone level. The significant increase in plasma melatonin after exposing the animals to thermal stress indicates some role for melatonin during thermal stress. Further exogenous pineal protein increased the melatonin level to counteract the metyrapone effect under thermal stress. Total precipitated pineal protein brought about significant reduction in the plasma corticosterone level. This was similar to previous findings [17,26]. The present study clearly indicated that pineal proteins were able to reverse the metyrapone effects on plasma corticosterone under thermal stress. This acts as the direct evidence for the relationship of pineal and adrenal gland to combat thermal stress in these animals.

In conclusion, total precipitated pineal protein successfully and significantly relieved the animals from adverse effect of heat stress and metyrapone treatment. This is evident from the findings that most of the parameters studied were influenced by pineal protein indicating their antistress properties. This shows that total precipitated pineal proteins have significant influence upon adrenal cortex function in relieving thermal stress in goats.

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