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Pineal-adrenal Relationship: Modulating Effects of Glucocorticoids on Pineal Function to Ameliorate Thermal-stress in Goats

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ABSTRACT : The purpose of the investigation was to establish how the pineal-adrenal axis plays an important role in thermoregulation in female goats under short-term heat stress. The study was conducted to observe the influence of glucocorticoids on pineal function in goats and its influence on stress alleviation capability. Melatonin and glucocorticoid secretions and several other endocrine and biochemical blood parameters reflecting the animals well being were determined over a one week period after goats had been exposed to 40°C and 60% relative humidity for 10 days. Six female goats were used in the study. These animals served as self controls prior to the start of the experiment. The study was conducted for a period of seventeen days in a psychrometric chamber at 40°C and 60% relative humidity. Chemical pinealectomy was achieved using propranolol followed by exogenous hydrocortisone treatment. Blood samples were drawn twice daily after each treatment to find the effect of hydrocortisone on plasma glucose, total protein, total cholesterol, cortisol, insulin, aldosterone, melatonin and corticosterone. Chemical pinealectomy significantly ($p \leq 0.05$) affected plasma levels of the parameters studied and these could be significantly ($p \leq 0.05$) counteracted by administration of hydrocortisone. Chemical pinealectomy aggravated thermal stress, although administration of hydrocortisone could ameliorate the condition. This indicated a role of the pineal in support of thermoregulation. The study establishes the modulating effect of glucocorticoids on pineal activity to relieve thermal stress in goats. (**Key Words :** Goats, Thermal-stress, Chemical Pinealectomy, Propranolol, Glucocorticoids, Hormones, Thermoregulation)

INTRODUCTION

The study of Pineal-Adrenal relationship probably began with Farrell's discovery of adrenoglomerulotropin in the pineal extracts of rats (Farrell, 1960). Since then, many investigators have attempted to establish this relationship (Demisch et al., 1988; Touitou et al., 1989; Konakchieva et al., 1998). Nevertheless, the contradictory nature of the results obtained so far make it difficult to draw any definitive conclusions about the pineal-adrenal axis (Heiman and Porter, 1980; Hasegawa and Mori, 1980; Demitrack, 1990; Hajak, 1997).

Heat stress associated with high humidity represents the most stressful constraint for animal production (Nardone et al., 2006). A variety of production system and/or geographical locations result in situations in which animals are exposed to environmental conditions outside of their thermoneutral range. Investigations of the physiological responses of animals to thermal-stress often includes quantification of the adrenal glucocorticoid response (Gould and Siegel, 1985; Hicks et al., 1998; Wolfensen et al., 2000).

Glucocorticoids are one among the variety of endogenous compounds that have been suggested to influence melatonin production in various vertebrate species, including human (Demisch et al., 1988; Bauer et al., 1989; Zawilska and Sadowska, 2002). However physiological and pathophysiological significance of this interplay still remains debatable. Furthermore the question of whether there is a direct link between glucocorticoids and melatonin production and secretion, still remains unsolved (Hajak et al., 1997). There is evidence that adrenalectomy in rats lead to significant increase in number of pinealocyte processes and increase in melatonin forming enzyme hydroxy indole-o-methyl transferase (HIOMT) which ultimately leads to an increase in pineal endocrine activity (Deuben-Schmitter et al., 1976).

Considering the views of above scientists that both pineal and adrenal gland influences each other's function

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through their endocrine secretions (Touitou et al., 1989; Konakchieva et al., 1998) and since adrenal cortex plays an important role in relieving thermal-stress through glucocorticoids (Gould and Siegel., 1985; Hicks et al., 1998; Wolfensen et al., 2000), the present study was conducted to establish the relationship between adrenal cortex and pineal gland under thermal-stress. The primary objective of the study was to observe the modulating role of glucocorticoids on pineal functions to relieve thermal-stress in goats.

MATERIALS AND METHODS

Animals

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

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 the animals 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 whole experimental period. The animals 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 parameters studied. On the 11^{th} day propranolol (*Sigma*, USA) was administered intravenously in these animals at the dose rate of 2 mg/kg body weight to induce chemical pinealectomy. Propranolol a beta-adrenergic receptor blocker was used to selectively decrease melatonin production by acting on the pineal. Propranolol induces pinealectomy temporarily as the half-life of propranolol is two to three hours. Generally the action of propranolol remains in the system for five hours although the action of the drug is reduced drastically after three hours of its administration. But the peak action of propranolol is obtained after 30 minutes to 90 minutes of its administration. Hence after one hour of propranolol administration while the action of the drug remains in peak, hydrocortisone is injected to relieve its effects. This experimental procedure of injecting propranolol and hydrocortisone were followed everyday from 11^{th} to 17^{th} day of the study. Propranolol injection was given at 10:00 h. After one hour of propranolol treatment, five ml blood was drawn from jugular vein to ascertain chemical pinealectomy. Hydrocortisone (*SRL*, India) was administered intravenously at the dose rate of 2 mg/kg body weight to counteract the propranolol effect. Time of hydrocortisone injection was at 11:00 h. After an hour of hydrocortisone administration, five ml blood was drawn from jugular vein to observe the stress relieving capability of hydrocortisone. The doses of propranolol and hydrocortisone were established by repeated trials. Samples were drawn after one hour of each drug administration to ensure complete action of these drugs to induce changes in parameters studied.

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 microcentrifuge tubes, and kept frozen at -20°C till further analysis.

Parameters studied

The parameters analyzed in the study were plasma glucose, total protein, total cholesterol, melatonin, cortisol, corticosterone, aldosterone, and insulin.

Analysis of biochemical parameters

Plasma glucose (Tietz, 1976), total plasma protein (Tietz, 1995), and total plasma cholesterol (Allain, 1974) were estimated using *Span* diagnostic kits, India as per standard method using the Semi-auto analyzer (ERBA *CHEM*-5 Plus).

Analysis of hormonal parameters

Hormonal parameters such as melatonin (analytical sensitivity was 2 pg/ml; the intra-assay and inter-assay coefficient of variations were 12.1% and 12.3% respectively), cortisol (analytical sensitivity was 10 nM; the intra-assay and inter-assay coefficient of variations were 5.8% and 9.2% respectively), aldosterone (analytical sensitivity was 6 pg/ml; the intra-assay and inter-assay coefficient of variations were 9.5% and 9.9% respectively), and insulin (analytical sensitivity was 0.5 μIU/ml; the intra-assay and inter-assay coefficient of variations were 4.3% and 3.4% respectively), were estimated by RIA (Radio immuno assay) using the Packard Cobra II gamma counter employing RIA kits supplied by *Immunotech*, France. Plasma corticosterone was estimated by ELISA (Enzyme linked immuno sorbant assay) employing the corticosterone ELISA kit supplied by *Neogen Corporation*, USA. As a representative sample, only day 17 plasma samples were subjected for estimation of both melatonin and corticosterone. The two hormones melatonin and corticosterone were also estimated for other related work and hence the total number of samples to estimate the same

was more. As Melatonin RIA kit and corticosterone ELISA kit are costly and since the allocated budget for the project crossed the upper limit, its not been possible to estimate all seven day samples of melatonin and corticosterone. Hence only day 17 samples were subjected for the estimation of the same.

Glucocorticoids have direct control over glucose and protein metabolism and hence these parameters were included in the study. Insulin has direct control over glucose level in the body and hence it was obvious to study its level. Glucocorticoids are steroid hormones and since cholesterol is the precursor material for steroid hormone production, it was included in the study. Melatonin, Cortisol, corticosterone and aldosterone are obviously necessary for the present study. These parameters are included in the study to know the influence of pineal on these parameters and further to know whether pineal secretions modulate the adrenal cortex activity to influence the levels of these parameters to ensure thermoregulation.

Statistical analysis

The data obtained was analyzed statistically by paired t-test as per method described by Snedecor and Cochran (1989). Significant differences were determined at the levels of $p \leq 0.05$.

RESULTS

Chemical pinealectomy was induced using propranolol and sufficient time was given to observe its effects. Then synthetic corticosteroid, hydrocortisone was administered to relieve the propranolol effects in these animals. The results obtained are being discussed elaborately for each parameter as follows.

Plasma glucose

Plasma glucose showed increase in level after thermal exposure (Table 1). However this increase was not significant. Propranolol treatment reduced the mean plasma glucose. On the other hand hydrocortisone treatment was able to increase the mean plasma glucose, however this increase is not statistically significant. By 12th day hydrocortisone injection could successfully revert back the glucose level towards control (Table 1). After this period both propranolol and hydrocortisone injections increased the level of glucose significantly ($p \leq 0.05$).

Total plasma protein

The mean total plasma protein decreased significantly ($p \leq 0.05$) after thermal exposure (Table 1). Propranolol treatment further reduced the plasma protein level in a significant manner ($p \leq 0.05$). However hydrocortisone treatment was able to significantly ($p \leq 0.05$) increase the total plasma protein by 11th day when compared to both control and thermal stress value (Table 1). This trend continued until the end of experimental period when the total plasma protein returned to near control value.

Total plasma cholesterol

Thermal exposure increased significantly ($p \leq 0.05$) the total plasma cholesterol level (Table 1). Propranolol treatment significantly ($p \leq 0.05$) reduced total plasma

Table 1. Mean and standard error of biochemical parameters in blood plasma of control, heat exposed, chemically pinealectomized and hydrocortisone administered goats

Experiment days	Treatment	Glucose (mg/dl)	Total protein (g/dl)	Total cholesterol (mg/dl)
Control (day 0)	-	49.36±2.74	7.61±0.11	150.27±3.88
Heat stress (day 10)	-	56.66±2.11	6.70±0.29^{A}	163.00±4.44^{A}
Day 11	PRO[1]	46.53±3.92	6.26±0.32^{Aa}	131.14±2.62^{Aa}
	HYD[2]	53.05±3.74	7.91±0.30^{a}	159.28±3.37
Day 12	PRO	43.28±3.21^{a}	6.33±0.24^{A}	138.33±3.07^{Aa}
	HYD	50.95±3.88	7.02±0.28^{A}	165.87±2.73
Day 13	PRO	47.43±4.10	6.38±0.26^{A}	137.44±4.22^{a}
	HYD	48.66±2.46^{a}	7.07±0.19^{A}	173.34±3.04^{A}
Day 14	PRO	79.41±11.12^{A}	6.93±0.17^{A}	137.37±2.67^{a}
	HYD	68.21±7.46^{A}	7.38±0.28^{A}	175.05±4.27^{A}
Day 15	PRO	103.18±9.65^{Aa}	6.91±0.25^{A}	139.78±3.84^{a}
	HYD	69.13±6.71	7.20±0.23	173.38±4.50^{Aa}
Day 16	PRO	73.50±3.43^{Aa}	7.28±0.33^{a}	138.31±4.66^{a}
	HYD	73.38±5.61^{Aa}	7.51±0.34^{a}	174.97±7.60^{A}
Day 17	PRO	74.80±6.04^{Aa}	7.47±0.32^{a}	138.79±4.87^{a}
	HYD	77.90±7.08^{Aa}	7.73±0.32^{a}	170.91±6.95

Mean and standard error are based on 6 animals. [1] PRO = Propranolol; [2] HYD = Hydrocortisone.

[A] Indicates statistical significance at $p \leq 0.05$ when compared with day 0 value.

[a] Indicates statistical significance at $p \leq 0.05$ when compared with day 10 value.

Table 2. Mean and standard error of melatonin and corticosterone in blood plasma of control, heat exposed, chemically pinealectomized and hydrocortisone administered goats

Experiment days	Treatment	Melatonin (Pg/ml)	Corticosterone (ng/ml)
Control (day 0)	-	84.56±12.63	0.46±0.12
Heat stress (day 10)	-	118.56±8.81 [A]	1.55±0.33[A]
Day 17	PRO[1]	100.24±10.12	1.20±0.36
	HYD[2]	149.57±11.57[Aa]	3.60±1.13[A]

Mean and standard error are based on 6 animals. [1] PRO = Propranolol; [2] HYD = Hydrocortisone.
[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 3. Mean and standard error of cortisol, aldosterone and insulin in blood plasma of control, heat exposed, chemically pinealectomized and hydrocortisone administered goats

Experiment days	Treatment	Cortisol (nmol/L)	Aldosterone (Pg/ml)	Insulin (micro IU/ml)
Control (day 0)	-	5.73±0.84	2.87±0.58	10.67±0.69
Heat stress (day 10)	-	76.74±2.43[A]	1.50±0.87[A]	9.88±0.50[A]
Day 11	PRO[1]	70.14±2.96[A]	1.81±0.58	8.29±1.88
	HYD[2]	238.22±35.85[Aa]	2.89±0.60[A]	12.95±1.33
Day 12	PRO	73.46±9.37[A]	0.51±0.44[A]	10.56±1.43
	HYD	341.70±76.53[Aa]	1.36±1.25[A]	12.05±1.05
Day 13	PRO	229.04±36.23[Aa]	0.67±0.27[A]	13.82±1.21
	HYD	641.45±58.90[Aa]	0.89±0.36[A]	17.07±1.91[Aa]
Day 14	PRO	279.96±26.26[Aa]	0.33±0.11[A]	15.70±2.64
	HYD	586.23±97.50[Aa]	0.16±0.05[A]	17.53±3.61
Day 15	PRO	293.77±44.13[Aa]	0.36±0.16[A]	10.89±1.54
	HYD	633.65±87.09[Aa]	0.62±0.17[A]	13.28±1.03[a]
Day 16	PRO	312.39±38.67[Aa]	0.15±0.06[A]	9.99±0.88
	HYD	612.00±33.61[Aa]	0.11±0.04[A]	12.47±0.99
Day 17	PRO	177.92±29.01[Aa]	0.23±0.08[A]	15.39±4.10
	HYD	667.50±88.06[Aa]	0.19±0.06[A]	21.11±5.81

Mean and standard error are based on 6 animals. [1] PRO = Propranolol; [2] HYD = Hydrocortisone.
[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.

cholesterol while hydrocortisone treatment increased cholesterol values significantly (p≤0.05). This trend continued till the end of experimental period (Table 1).

Plasma melatonin

Thermal exposure increased significantly (p≤0.05) the plasma melatonin level (Table 2). Propranolol treatment on day 17 non-significantly decreased plasma melatonin when compared to thermal exposure level (Table 2). However the level was higher than the control value after propranolol treatment although this higher level was not significant. Hydrocortisone treatment on day 17 significantly (p≤0.05) increased the melatonin level when compared to both control and thermal exposure values (Table 2).

Plasma cortisol

Thermal exposure increased significantly (p≤0.05) the plasma cortisol level (Table 3). Propranolol appeared to have no effect on cortisol on days 11 and 12 as the values remained close to day 10 values. Beyond day 12 propranolol treatment significantly (p≤0.05) increased the plasma cortisol level as compared to control value. Further hydrocortisone treatment increased the plasma cortisol level significantly (p≤0.05) when compared to both control and thermal exposure values on all days of experimental period (Table 3).

Plasma corticosterone

Thermal exposure significantly (p≤0.05) increased the plasma corticosterone level (Table 2). Propranolol treatment on day 17 non-significantly reduced plasma corticosterone when compared to thermal exposure value while increased the level significantly (p≤0.05) when compared to control. Hydrocortisone treatment on day 17 significantly (p≤0.05) increased the plasma corticosterone when compared to both control and thermal exposure values (Table 2).

Plasma aldosterone

The mean plasma aldosterone showed significant (p≤0.05) reduction in its level after thermal exposure (Table 3). On day 11 propranolol increased significantly (p≤0.05) aldosterone level when compared to both control and

thermal exposure values. Hydrocortisone increased aldosterone level on day 11 and brought the value towards control. From day 12 onwards both propranolol and hydrocortisone treatment gradually decreased significantly ($p \leq 0.05$) the aldosterone level when compared to both control and thermal exposure values (Table 3).

Plasma insulin

The mean plasma insulin decreased after thermal exposure although this decrease is not statistically significant (Table 3). Propranolol decreased plasma insulin while hydrocortisone treatment increased it on day 11. From 13th day onward both propranolol and hydrocortisone treatment increased the plasma insulin (Table 3) from that of both control and heat stress value but this increase is not statistically significant.

DISCUSSION

The results obtained reveal the chemical pinealectomy relieving effects of the glucocorticoid. This shows that during thermal-stress glucocorticoids were able to successfully modify the level of melatonin so that melatonin could exhibit anti-stress effects. This action of glucocorticoids on the level of pineal melatonin shows that there should be some mechanism existing by which glucocorticoids are acting on the pineal to control melatonin level.

Biochemical parameters

Thermal exposure increased the plasma glucose level while propranolol treatment reduced this increase although these effects were non-significant. Similar effects were observed by Milcu et al. (1971) and Mahata et al. (1988), indicating that after pinealectomy there was a significant ($p \leq 0.05$) reduction in glucose level in rats. After 13th day propranolol treatment increased the glucose level gradually and this finding coincided with the findings of Diaz and Blazquez (1986); Rodriguez et al. (1989); Zeman et al. (1993); and Llma et al. (2001) who showed a significant increase in glucose level in rats after pinealectomy. The hydrocortisone treatment successfully kept the level of plasma glucose under check leading to maintenance of the level towards control. Among the biochemical parameters, total plasma protein showed highly significant ($p \leq 0.05$) changes for the effects of both propranolol and hydrocortisone treatment. The biological significance of thermal-stress reducing protein is to support hepatic gluconeogenesis by glucocorticoids to increase glucose level (Kamiya et al., 2006; Korde et al., 2007). After thermal exposure total plasma cholesterol increased significantly ($p \leq 0.05$). This increase in cholesterol by thermal-stress could be to increase glucocorticoid hormone production to combat stress, as cholesterol is the precursor material for glucocorticoid hormones. Chemical pinealectomy induced significant decrease in the level of total plasma cholesterol. This effect contradicted with the findings of Cunnane et al. (1979), who reported significant elevation of serum Cholesterol in pinealectomized rats. However hydrocortisone treatment successfully relieved the effects of chemical pinealectomy leading to significant elevation in total plasma cholesterol. Hydrocortisone treatment successfully maintained the level of cholesterol towards control level by the end of experimental period.

Hormones

On subjecting the animals to thermal-stress, the level of melatonin increased significantly ($p \leq 0.05$). This shows the protective role of melatonin during thermal-stress. Further melatonin plays an important role in thermoregulation (John et al., 1978). Perhaps higher concentration of melatonin is required to combat the stressful condition in order to maintain the homeothermy. Propranolol treatment reduced the level of melatonin in a non-significant manner. However the efforts made by these animals to withstand the stress are so vigorous that the high level of melatonin was maintained to relieve stress in these animals. Hence the propranolol treatment could not bring about significant changes in the melatonin level when compared to both control and heat stress level. Further the hydrocortisone treatment significantly increased melatonin as compared to both control and thermal-stress level. No reports were available for the level of melatonin during thermal-stress to compare our results. This condition indicates complex physiological intricacies operating under life threatening situations. Barriga et al in 2001 reported direct effect of corticosterone on pinealocytes in reducing melatonin level in rats. From this, it is evident that glucocorticoids have direct action on pinealocytes to alter melatonin level. Hence during adverse pinealectomy condition in these goats, glucocorticoids could have stimulated the pinealocytes to release melatonin in these animals in order to relieve thermal-stress. It remains to be established though that whether melatonin control thermal-stress by acting directly on adrenal cortex or by acting on hypothalamus or anterior pituitary. Thermal-stress increased cortisol level significantly. Generally the level of cortisol increased to a significant level after thermal exposure in sheep (Sheikheldin et al., 1988; Nazifi et al., 2003). Plasma cortisol showed significant ($p \leq 0.05$) changes for both the treatments. Chemical pinealectomy induced significant increase in plasma cortisol level. Vaughan and co-workers (1972) obtained similar finding in the experiment conducted on mice. But exogenous hydrocortisone increased plasma cortisol significantly ($p \leq 0.05$). This could be explained easily as exogenous hydrocortisone combines with high concentration of

cortisol during thermal exposure, leading to very high level of cortisol in these animals (Hirayama and Katoh, 2004). Plasma corticosterone showed non-significant changes to propranolol and significant ($p \leq 0.05$) changes to hydrocortisone treatment. Ogle and Kitay, in 1978 reported significant relationship between pineal and adrenal corticosterone levels. They tested the corticosterone 5α reductase activity in the adrenals of simultaneously overiectomized and pinealectomized female rats. The activity of this enzyme declines following pinealectomy indicating a change in the metabolism of corticosterone within the adrenal. However exogenous glucocorticoids were able to increase the plasma corticosterone level significantly ($p \leq 0.05$).

Plasma aldosterone also showed significant ($p \leq 0.05$) changes to both propranolol and hydrocortisone treatments. The interesting finding is that exogenous hydrocortisone was able to bring back the level to the control value on first day itself. From then on, these effects of hydrocortisone gradually reduced leading to the basal level of aldosterone by the end of study period. Of all the hormonal parameters, insulin showed lower significance for both treatments. After thermal exposure plasma insulin decreased but this decrease was not significant. Chemical pinealectomy did not seem to have much influence on the level of insulin in these animals in first few days but from 13th day onwards it increased its level though non-significantly. Diaz and Blazquez (1986); Rodriguez et al. (1989) and Llma et al. (2001) reported reduction in insulin level in pinealectomized rats while Gorray et al. (1979) reported increase in insulin level after pinealectomy in rats.

IMPLICATIONS

Pineal gland is known to have anti-stressogenic effect in mammals and birds. It is also known to have a tranquilizing effect on the animals. Melatonin is included in the feed of pigs raised in commercial piggeries to protect them against occurrence of peptic ulcers. Our finding from this study indicates the anti-stress properties of melatonin in goats. The significant effect of synthetic glucocorticoid on melatonin level during thermal-stress establishes the relationship between these two endocrine glands. Given the importance of thermal-stress in hampering animal productivity to a greater extent in tropical countries, our finding has greater significance in terms of improving economy of farm households as well as poor farmers are concerned. The data generated from this study help us to understand the functional relationship between these glands, and how they influence each other for the well being of the domestic and farm animals. Further detailed studies are required to fully understand the mechanisms of interactions involved between these glands.

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