In Vitro Studies on the Release of Intracelluar Prolactin from Lymphocytes Using Strees Related Amines and Hormones

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ABSTRACT : Circulating lymphocytes collected from control and heat-stressed buffaloes were subjected to *in vitro* culture with glucocorticoids, epinephrine or serotonin and their effect, if any, on the release of intracellular prolactin (PRL) was studied using ELISA and C-ELISA techniques. It was noted from the study that PRL level was higher in lymphocytes than in plasma of the control and heat-stressed animals, and that the PRL levels increased in the plasma of heat-stressed animals compared to that of non stressed animals with a significant decrease in lymphocytic PRL content by heat stress. Epinephrine and serotonin significantly increased the release of intracellular PRL from the lymphocytes of both in the control and the heat-stressed buffaloes but release of PRL from lymphocyte was not significantly changed by cortisol treatment in both control and heat-stressed buffaloes as compared to epinephrine and serotonin *in vitro*. When lympocytes were incubated with serotonin, it caused drastic lysis of the lymphocytes but epinephirine and cortisol did not show any lysis. It may be concluded from this study that hormones like epinephrine or serotonin known to increase during stress, release intracellular PRL from lymphocytes, the satellite PRL storage/synthesizing organ of blood, although the mechanism of the release is different. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1031-1034*)

Key Words : Buffalo, Serotonin, Epinephrine, Prolactin, Lymphocytes, *In Vitro*

INTRODUCTION

Stress is a complex phenomenon which alters physiological, psychological and behavioral responses of animals. It involves areas of brain and endocrine system thus causing threat to homeostasis. Occurance of diseases due to viral, bacterial or parasitic infection is common during this time. Different types of stress including heat stress have shown to increase plasma levels of prolactin (PRL), epinephrine (E) glucocorticoids, serotonin and β endorphin (Becker, 1987).

Besides pituitary, PRL has also been localized in the lymphocytes (Cevenger et al., 1991) and has been shown to bind and synthesize in these cells (Sharma et al., 1996). PRL helps in synthesis of IL (interleukin)-2 in B and T lymphocytes (Cevenger et al., 1991). IL-2 is both an autocrine/paracrine regulator of normal pituitary cell proliferation and a stimulator of the proliferation of a growth hormone (GH) producing pituitary adenoma cell line GH3 (Artsz, 1993). PRL antibody treatment to lymphocytes has shown to decrease IL-2 synthesis (Besedovsky et al., 1975).

So far no study has been conducted to examine effect of heat stress on the intracelluar prolactin content of lymphocytes and the interaction of the stressors (epinephrine, glucocorticoids, serotonin) on intracelluar prolactin content of lymphocyte. In the present study intracellular prolactin content of lymphocyte during prolonged heat stress and inter action between stressors viz. cortisol, epinephrine and serotonin on *in vitro* release of PRL from lymphocytes have been studied.

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MATERIALS AND METHODS

Ten healthy buffaloes were selected for this study and were divided into two groups of five each. One group was kept in the climatic chamber and exposed to hot environmental conditions, i.e. 35-40℃ 60% RH during the day; 30℃ and 60-70% RH during night for two weeks. Animals were fed with concentrate, hay and water ad libitum. Control group was kept at ambient temperature i.e. 20-30℃ (experiments were conducted during the month of December).

Preparation of bovine lymphocyte

For lymphocyte isolation, method of Boyum (1968) was followed. In brief blood was collected from all animals in tubes containing anticoagulant. This freshly collected blood was centrifuged at 1,800 rpm (600 g) for 30 minutes at room temperature to obtain a buffy coat. Buffy coat was collected and diluted 1:3 with RPMI-1640 medium (Sigma). It was centrifuged for 3 minutes at 1,500 rpm (400 g). Lymphocyte band was collected and pellet was suspended in Tris-NH_4Cl (0.1 ml packed cells/ml Tris-NH_4Cl) and were again centrifuged for 10 min. Cells were then washed two to three times with RPMI-1640 media, counted and put for culture as 1×10^6 cells/ml.

Experimental design

Plasma collected by centrifugation was analysed by ELISA to estimate PRL levels. After preparation of lymphocytes, some cells were kept separately without

any treatment for C-ELISA to check the cellular PRL. Rest of prepared lymphocytes from all animals of two groups were suspended it DPBS without serum so that each mililiter contains 1×10^6 lymphocytes and used for the treatment with epinepherine, serotonin and cortisol dissolved in DPBS as follows :

Treatment	Dose	Time of incubation with lymphocytes
Epinephrine	10 μg/ml	2 h
Serotonin	1 μg/ml	2 h
Cortisol	0.16 nmol/ml	2 h
Control	-	-

After two hours of incubation at 37℃ with 5% CO_2 in air, culture fluid was collected after centrifugation and the pellet was washed with PBS (0.01 M, pH 7.4). Cells were used for measuring PRL concentration by C-ELISA. Epinephrine, serotonin and cortisol were used as stressors in this study.

The cells (1×10^6) and culture fluids (100 μl) were then coated in ELISA plate. PRL level was then determined according to Sharma et al. (1996) with an antibody of prolactin raised in the laboratory, antirabbit HRP conjuate purchased from Sigma, USA. Colour was developed by OPD. The reaction was blocked after 30 min. by 5N H_2SO_4. The OD was measured by an ELISA reader at 450 nm and data were calculated from the standard curve. The specific binding efficiency of prolactin antibody was found to be 90%. Statistical analysis was done using students' test according to Snedecor and Cochran (1967).

RESULTS

Prolactin level of lymphocytes collected from the buffaloes which was maintained at ambient temperature (control) was found to be higher compared to the heat-stressed buffalo (53.60±23.13 vs. 31.80±9.34 μg). When freshly isolated lymphocytes from heat-stressed and non-stressed buffaloes were cultured in serum free DPBS for 2 h in presence and absence of epinephrine, serotonin or crotisol, all the three stressor released PRL from the lymphocytes as shown in table 1. PRL in supernatent was significantly higher in all the treatment groups compared to the control group. In the non-stressed animals 48.20±8.73, 51.80±26.0 and 34.20±2.85 μg of PRL were measured in the supernatant of culture medium for epinephrine, serotonin and cortisol respectively as against 7.40± 1.24 μg PRL in the medium of cortisol group. Amongst the treatment groups cortisol did release intracellular PRL which is significantly higher than the control group but apparently it was lower than the PRL levels of epinephrine and serotonin group. In the heat-stressed animals intracelluar PRL release by epinephrine, serotonin and cortisol was significantly higher than the non-stressed contrsol group. Within the three treatment groups intracellular PRL release was significantly ($p<0.05$) higher by the epinephrine (41.50 ±10.3 μg) treatment than serotonin (29.00±1.70 μg) or cortisol (31.80±0.91 μg) treatment. *In vitro* culture of the lymphocytes with all the three stressors showed a decline in the intracellular PRL levels compared to the levels determined before treatment (table 1) and also after the incubation of lymphocytes

Table 1. *In vitro* effect of epinephrine, serotonin and glucocorticoides on intracellular release of PRL from lymphocytes (μg)

Groups	No treatment		Epinephrine		Serotonin		Glucocorticoids	
	Cells	Supernaptant	Cells	Supernaptant	Cells	Supernaptant	Cells	Supernaptant
Control	27 μg	48 μg	08 μg	20 μg	38 μg	03 μg	93 μg	24 μg
	34 μg	10 μg	10 μg	63 μg	28 μg	30 μg	30 μg	36 μg
	82 μg	09 μg	18 μg	68 μg	10 μg	72 μg	22 μg	41 μg
	61 μg	04 μg	18 μg	20 μg	15 μg	28 μg	31 μg	39 μg
	72 μg	05 μg	23 μg	52 μg	34 μg	34 μg	23 μg	28 μg
Mean ± SE	61.20^a	7.41^b	17.80^b	48.20^a	18.00^b	51.80^a	26.00^b	34.20^a
	±7.26	±1.24	±8.73	±8.73	±11.43	±26.06	±1.87	±2.85
Heat stressed	42 μg	4 μg	21 μg	59 μg	20 μg	27 μg	30 μg	32 μg
	28 μg	3 μg	22 μg	38 μg	13 μg	25 μg	22 μg	30 μg
	28 μg	5 μg	20 μg	30 μg	34 μg	28 μg	19 μg	32 μg
	32 μg	7 μg	18 μg	44 μg	20 μg	35 μg	21 μg	35 μg
	27 μg	6 μg	21 μg	36 μg	31 μg	30 μg	26 μg	30 μg
Mean ± SE	31.40^a	5.00^a	20.40^b	41.50^c	23.60^b	29.00^b	23.60^b	31.80^b
	±2.78	±0.70	±5.57	±10.30	±3.88	±1.70	±1.96	±0.91

Values with different superscript are significantly different ($p<0.05$) from each other.

for the similar time as was given for the treatment groups.

Table 2. Prolactin levels in the lymphocytes and plasma of heat-stressed and nonstressed buffaloes

Groups	Lymphocytes	Plasma
Control	30 μg	15 μg
	70 μg	12 μg
	90 μg	14 μg
	46 μg	12 μg
	32 μg	10 μg
Mean ± SE	$53.6^{a} \pm 23.13$	$12.60^{c} \pm 1.74$
Heat stressed	30 μg	29 μg
	24 μg	26 μg
	26 μg	22 μg
	29 μg	30 μg
	50 μg	20 μg
Mean ± SE	$31.80^{b} \pm 9.34$	$25.40^{b} \pm 3.87$

Values with different superscript are significantly different ($p<0.05$) from each other.

DISCUSSION

It is evident from the results that serum of heat-stressed buffaloes were having significantly higher PRL than that of buffaloes maintained in ambient temperature. Similar observation has been made by other workers in the animals during heat stress (Vanjonack and Johnson, 1974; Kaprowski and Tucker, 1973), cold stress (Reid and Mills, 1962) and also psychological stress (Friend and Polan, 1974; Nienabar et al., 1974; Raud et al., 1971). The intracelluar PRL level of lymphocytes, collected from heat-stressed buffaloes were significantly lower in comparison to non stressed buffaloes indicating that during heat stress either PRL liberated out of lymphocytes or that it did not bind to lymphocytes as PRL receptors have been localized on lymphocytes (Blalock, 1994). It is also possible that during heat stress the body temperature increases and PRL synthesis from lymphocytes decreases as lymphocytes also synthesise prolactin (Blalock, 1994). To understand the probable cause of increase in PRL level during stress we cultured lymphocytes collected from heat-stressed and non-stressed buffaloes in presence of cortisol, epinephrine and serotonin. It was noted from the results that all these three hormones enhanced PRL release from lymphocytes of both heat-stressed and non-stressed buffaloes showing that cortisol, epinephrine or serotonin that releases during stress (Becker, 1987) released PRL from lymphocytes. thus, if not total, surely partial increase in PRL level of plasma during stress is contributed due to PRL of lmphocytes. The release of PRL was higher in case of lymphocytes of non-stressed buffaloes than those of heat-stressed buffaloes may be due to the fact that heat-stressed buffaloes continously release PRL and have adapted to some extent with the changed environment while lymphocytes of non-stressed buffaloes experienced first time the effect of these stressors or that the heat-stressed buffalo lymphocytes contained less PRL than that of non-stressed buffaloes and thus release was comparatively less in lymphocytes heat-stressed buffalo than non-stressed ones.

There was more PRL release when incubated with epinephrine followed by serotonin and cortisol showing that epinephrine acts very quickly than that of cortisol. Epinephrine is also known to act very quickly in other body system and catabolised quickly (Guyton, 1995).

The-lower efect of crotisol in case of heat-stressed lymphocytes on PRL release may be due to less receptors of cortisol available in the lymphocytes as Tornello et al. (1982) observed down regulation of corticosterone receptors and no receptor affinity during crowding stress in mice. Koch et al. (1983) also reported decreased glucocorticoids binding in cytosol and nuclear receptor under stress. During the experiment, a large number of cell lysis was noted in the group of lymphocytes cultured with serotonin though no such cell lysis was observed in case of epinephrine or cortisol group. This may be due to the higher dose of serotonin used in this experiment. Serotonin is known to increase maximally in the afternoon as associated with photoperiod (Aleandri et al., 1996). It is not known whether they have any role in elemination of lymphocytes *in situ*.

It may be concluded from the study that cause of hyperprolactinemia during stress if not fully but partially is due to PRL released from the lymphocytes under the influence of epinephrine, glucocorticoids, serotonin, the stressors released during the stress.

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