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CAN PROLACTIN BE A MEASURABLE MARKER OF STRESS IN DROMEDARIES?

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Summary: Prolactin, a hormone produced by the anterior pituitary gland, has a well documented role in milk production and several studies have suggested its role in general adaptation syndrome. As dromedaries *(Camelus dromedarius)* are important animals of arid region, an investigation was carried out in adult dromedaries to assess the role of serum prolactin as a measurable marker of stress. Serum levels of prolactin and cortisol were determined by radioimmunoassay in the healthy and affected dromedaries (those with nasal peg wounds, saddle gall, sand in the third compartment and drought affected). The mean values of serum prolactin (pmol/L) and cortisol (nmol/L) in healthy group were 748.20± 17.82 and 25.93±0.82, respectively. Affected group showed higher levels of serum prolactin and cortisol as compared to healthy group. The mean level of prolactin was 4.94 times higher and cortisol was 4.75 times higher in affected camels as compared to healthy ones. The mean values of different subgroups of affected animals differed significantly and in comparison to healthy male mean value for both serum prolactin ($p\leq0.01$) and cortisol ($p\leq0.03$). The camels with sand in their third compartments had highest serum levels of prolactin and cortisol. Increase in serum cortisol suggested that affected camels were stressed and simultaneously many fold rise in serum prolactin clearly suggested that it can be a measurable marker of stress in different affections in dromedaries.

Key words: cortisol; dromedary camel; drought; nasal peg; prolactin; saddle gall; stress

Introduction

Prolactin (PRL) is a single chain polypeptide hormone produced by lactotrophes of anterior pituitary gland and is considered as most versatile pituitary hormone in function that acts directly on different tissues. The physiological actions of prolactin are mediated through specific membrane receptors in the cells of the mammary gland, liver, ovary, testis and prostate (1). Prolactin has been shown to stimulate intestinal calcium absorption, increase bone turnover, and reduce renal calcium excretion (2). Prolactin has multiple metabolic and behavioural effects that may contribute to the general adaptation syndrome as earlier studies (3) have shown the stress induced rise in prolactin secretion in animals and humans. Prolactin induces increased cortisol secretion (4), which is a glucocorticoid secreted by adrenal cortex and is associated with the stress, immune system and thermal regulation (5) besides its important role in many physiological functions including metabolism, mammogenesis, lactogenesis and galactopoiesis (6).

Although every animal has the inherent ability to withstand the stress, the problems arise when the degree of stress exceeds the limit what the body can handle. Generally the response to stress is in the form of neuroendocrine changes involving hormonal and metabolic variations. They can be evoked by anxiety, blood loss, tissue damage, visceral handling, and by the anaesthetic drugs and procedures (7). These reactions can be studied as general adaptation syndrome which enables an animal to adapt itself when suddenly confronted with a critical situation.

The stress syndromes vary in intensity according to the severity of the aggressive stimulus and also

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present with different hormone and metabolic profiles, depending on the kind of stressor. Though the camel resists extremes of desert environs, the production potential may became reduced with time (8). Not much attention has been given to either stress factors or health of the camel though stress may alter the physiological status of the individuals (9).For the better management of dromedaries in the stress free environment, different tests which can be carried out to assess the degree of the stress should be established. Acute prolactin responses are related to psychological stress in human being and much of the studies carried out to find the role of prolactin as a stress marker are performed on rats or humans (10, 11). As large animals also suffer from stress, the aim of the present investigation was to understand if prolactin could be an important tool in exploring the physiopathological consequences of certain disease/ affection patterns and a useful neuroendrocine correlate of the individual response to what we define as stress and to assess the role of prolactin in dromedaries as a measurable marker of stress.

Material and methods

The blood samples were collected from 32 adult dromedaries of arid region managed in similar conditions of feeding and watering by the private farmers kept for the purpose of farming and light load carrying. The camels were divided into two groups of 16 each i.e. healthy group and affected group. In the healthy group, the blood was drawn from healthy adult dromedaries of either sex (8 each). The affected group comprised of four sub-groups having males only. The first sub-group constituted of six adult dromedaries having nasal peg wounds, second subgroup of three adults having saddle gall, third subgroup of four drought affected adults and the fourth sub-group of three adults under observations having the history of pica, anorexia and depression, which upon post-mortem revealed the presence of sand in their third compartments.

Sera were separated and analysed for prolactin and cortisol. The serum prolactin was determined by immunoradiometric assay using RIA kit (IRMA CT, RADIM, Italy) following manufacturer protocol. The method uses of two anti-PRL monoclonal antibodies which recognised two different epitopes of the molecule. One antibody was adsorbed in solid phase in the coated tube (mouse monoclonal anti-PRL antibody) and the other as radioactive conjugate labelled with iodine-125 (^{125}I anti-PRL mouse monoclonal antibody in serum matrix). The serum samples and labelled antibodies were incubated simultaneously in the coated tubes. The amount of bound conjugate was directly proportional to the hormone concentration in the sample and standard. At the end of incubation the unbound material was removed by an aspiration and washing cycle (Tris-HCl and Tween 20). The radioactivity in the tubes was measured in a ^{125}I Gamma counter (ECIL,India).

The serum cortisol was determined by using the Gamma coat (^{125}I) cortisol radioimmunoassay kit procedure based on the competitive binding principles of radioimmunoassay (DiaSorin, USA). Serum samples and standards were incubated with cortisol tracer in antibody-coated tubes (Rabbit anti-cortisol serum coated) where the antibody was immobilised onto the lower inner wall of the Gamma Coat Tube. After incubation the contents of the tubes were decanted and the tube was counted in a ^{125}I Gamma counter (ECIL, India).

Statistical significance was assessed between male and female animals of healthy group by paired 't' test (12). As affected animals were comprised of male animals only, their mean values were compared with respective healthy male mean value only. This was carried out by analysis of variance. Further post-hoc (Bonferroni's) test was applied. Mixed model least square and maximum likelihood computer programme PC-I (Copyright, 1987, Walter R. Harvey) were used to determine analyses of variance. Adjustment to multiple comparison was made by Bonferroni's procedure (13,14).

Results

The mean ± SEM values of serum prolactin and cortisol in the dromedaries are presented in table 1.

Serum prolactin value was significantly ($p \leq 0.05$) higher in healthy female camels than in males whereas non significant ($p > 0.05$) change was observed for serum cortisol. Affected groups showed higher levels of serum prolactin and cortisol in comparison to healthy group. The mean rise in prolactin and cortisol levels in affected camels was calculated from that of respective healthy mean value in terms of times. It was 4.94 times higher for serum prolactin level and 4.75 times higher for cortisol. In order to assess the increase in serum prolactin and cortisol in affected animals statistically, analysis of variance was performed which revealed significant changes at 0.01 level of probability for both hormones. Further Bonferroni's adjustments were carried out and the adjusted probability level was 0.01 for prolactin and 0.03 for cortisol. On this basis it was observed that the mean values of different subgroups of affected camels i.e. nasal peg wounds, saddle gall, drought affected and having sand in the third compartment differed significantly from each other for serum prolactin ($p \leq 0.01$) and cortisol ($p \leq 0.03$). Each value of affected subgroup differed significantly from respective healthy male mean value of prolactin ($p \leq 0.01$) and cortisol ($p \leq 0.03$). The camels with sand in their third compartments were having highest serum levels for prolactin and cortisol.

Table 1: Serum levels of prolactin and cortisol in dromedaries. Figures in the parentheses indicate number of animals ('b' = Significant ($p \leq 0.05$) variation from healthy male mean value; 'a' = Non significant ($p \leq 0.05$) variation from healthy male mean value; 'c' = Significant ($p \leq 0.01$) variation from each other for prolactin; 'd' = Significant ($p \leq 0.03$) variation from each other for cortisol)

Groups	Sub-groups	Prolactin (pmol/L)	Cortisol (nmol/L)
I. Healthy (16)	Overall healthy mean value	748.20± 17.82	25.93± 0.82
	Male (8)	607.60 ± 21.73[c]	27.03± 1.13[d]
	Female (8)	888.80 ± 26.08[b]	24.83± 1.07[a]
II. Affected (16)	Overall affected mean value (Male)	3004.12±167.0[c]	128.5±7.5[d]
	Nasal peg wounds (6)	1856.8 ±158.40[c]	84.14 ± 8.74[d]
	Saddle gall (3)	2574.3 ±167.2[c]	124.43 ± 6.89[d]
	Drought affected (4)	3220.22± 130.5[c]	140.80± 6.0[d]
	Camels with sand in third compartment (3)	4365.19±217.39[c]	166.09 ± 7.17[d]

Discussion

There is little data in the literature about serum prolactin levels in dromedaries. Commercially available human radioimmunoassay (RIA) kits were used in few studies (15) for PRL determination in one-humped camel (*Camelus dromedarius*) who suggested that serum concentrations of prolactin reflected age and seasonal differences. In the present study the mean value of serum prolactin in healthy camels was similar to those reported by earlier workers in cows (5) whereas it was lower than those reported for ewes (16). Increase in prolactin levels in affected dromedaries indicated that animals were stressed as it was accompanied by a rise in serum cortisol, which is a well documented marker of stress in animals (9). In the present study only one time sampling was carried out as earlier studies suggested consistent secretion pattern (16).

The findings of present investigation regarding an increase in levels of prolactin and cortisol in affected dromedaries were in agreement with the earlier reports where both prolactin and cortisol increased significantly in stressed cows (5) and stressed rats (17). Many other studies have reported an increase in prolactin levels in stressed rats (18). In one study 10-14 fold increase in prolactin secretion was observed after 5 minutes of restraint stress (19). Though cortisol is well established as an important marker of stress (9), in present study prolactin was also found as an important marker in physiological adjustments of stress. Trauma and other affections probably produced a complex set of hormonal and metabolic changes which were evoked by anxiety, blood loss, tissue damage or visceral handling (7).

Increase in prolactin and cortisol could be considered as measurable markers of coping strategies to stress (10). In the affected camels stress most likely developed due to trauma and nervousness which elevated blood prolactin levels and cortisol levels (4). Resutls of our study therefore suggest that prolactin could serve as a sensitive marker of both physical and psychological stress in camels (20). It is important to understand the physiological significance of stress induced prolactin release. Earlier studies (21) have attributed the prolactin surge to the general increase in the adrenergic activity of the hypothalamus which leads to the secretion of PRL-releasing factors (22) and inhibits the tubero infundibular dopaminergic neurons, which are tonic inhibitors of PRL secretion (23). Dopamine regulates cortisol and prolactin secretion in animals (5) and stress-induced PRL release is a rapid and strong response that can be evoked by a large number of medical and surgical procedures (24).

Rise in prolactin levels in affected animals was probably a mechanism to increse the pain threshold (25) and defensive behaviour (26). In camels with sand in their third compartments increased prolactin possible also acted as a protective factor against acute gastric ulceration (4), while raised serum prolactin in camels with nasal peg wounds and saddle gall might have enhanced inflammatory responses (27) as it has been shown before that prolactin has an immuno modulatory effects (28). In drought affected camels scarcity of feed and low quality feed coupled with environmental stress could have been the cause of increased levels of prolactin(29).

Increase in serum prolactin in affected camels suggested its role in stress adaptation to unfavourable conditions. Increase in serum cortisol confirmed that affected camels were stressed. Present study therefore suggest that increase in prolactin could be directly related to the stressful condition of the dromedaries, hence making its measurement a practical stress marker to determine stress in affected dromedaries.

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ALI JE LAHKO PROLAKTIN IZMERLJIV OZNAČEVALEC STRESA PRI ENOGRBIH KAMELAH?

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Povzetek: Prolaktin, hormon, ki ga proizvaja adenohipofiza, ima dokumentirano vlogo pri proizvodnji mleka, mnoge študije pa so pokazale še njegovo vlogo pri splošnem sindromu prilagajanja. Ker so enogrbe kamele *(Camelus dromedarius)* pomembne živali na izsušenih področjih, je raziskava potekala na odraslih enogrbih kamelah, z namenom ugotoviti vlogo serumskega proteina kot izmerljivega označevalca stresa. Raven prolaktina in kortizola v serumu so določili z radioimunskim testom pri zdravih in prizadetih enogrbih kamelah (z ranami zaradi nosnih čepov, s sedelnimi odrgninami, s peskom v tretjem predelku želodca ter pri živalih prizadetih zaradi suše). Serumske vrednosti prolaktina (pmol/L) in kortizola (nmol/L) so bile pri zdravi skupini 748.20± 17.82 ter 25.93±0.82. Pri prizadetih kamelah so bile opažene višje ravni serumskega prolaktina in kortizola v primerjavi z zdravo skupino. Srednja vrednost prolaktina je bila 4.94-krat višja ter kortizola 4.75-krat višja pri prizadetih kamelah, v primerjavi z zdravimi. Povprečna vrednost v različnih podskupinah prizadetih živali se je značilno razlikovala od srednje vrednosti pri zdravih živalih za serumski prolaktin ($p \leq 0.01$) in kortizol ($p \leq 0.03$). Kamele s peskom v tretjem predelku želodca so imele najvišje serumske ravni prolaktina in kortizola. Povišan nivo kortizola kaže na to, da so bile prizadete enograbe kamele pod stresom, hkratno povišam nivo serumskega prolaktina pa nakazuje, da je lahko izmerljiv označevalec stresa povzročenega z različnimi dejavniki pri enogrbih kamelah.

Ključne besede: kortizol; enogrba kamela; suša; nosni čep; prolaktin; sedelne odrgnine; stres