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RESEARCH ARTICLE

Leptin and other Hormonal Responses to Different Stressors: Relationship with Stress-Induced Behavioral Deficits

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ABSTRACT

Leptin has a prime role in responses to stress and elicits antidepressant like effects. The present study investigates serum leptin, corticosterone and serotonin responses to noise, restraint and immobilization stress and their relationship with stress-induced behavioral deficits in rats. Animals exposed to 2h noise, restraint or immobilization stress were killed either immediately or 24h after the termination of stress to monitor serum leptin, corticosterone and serotonin. Stress-induced deficits in open field exploration as well as decreases of food intake were also monitored. Acute exposure to noise, restraint or immobilization stress resulted in an increase in serum corticosterone and leptin and a decrease in serum serotonin. The corticosterone response, but not leptin or serotonin response, was greater in rats exposed to immobilization than restraint than noise stress. Animals killed 24 h after the cessation of stress exhibited a decrease in serum leptin but corticosterone and serotonin levels were not altered. Stress-induced deficits of food intake and open field exploration were greater in rats exposed to immobilization than restraint than noise stress. The present study shows that together with increases in circulating levels of corticosteroids and leptin; decreases in serum serotonin are peripheral marker of acute stress response. A decrease in serum leptin associated with deficits of behavior in animals decapitated 24 h post stress termination suggest that an insufficiency of endogenous leptin contributes to stress-related illnesses.

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INTRODUCTION

In line with the association of depression with stressful life events, as per clinical evidence (Haleem, 2011a), several animal models exhibiting stressor controllability and learned helplessness have been developed (Chourbaji *et al.,* 2005; Maier and Watkin, 2005). Animal model of 'learned helplessness' is equated with a sense of 'giving up' as observed in humans suffering from depression (Richter *et al.,* 2013).

In similar studies, we have reported anorexia and decline in growth rates after 2-h immobilization stress (Haleem and Parveen, 1994; Haleem, 1999). Exposure to 2 h immobilization also decreased novel area (open field) exploration and anxiodenic/depressive profile in light dark transition test (Haleem, 2011a,b). The anorexigenic as well anxiety/ depression like effects of immobilization were not observed in animals injected with leptin (Haque *et al.,* 2013). It was suggested that the peptide hormone reduces stress perception to elicit antidepressant like effects.

Leptin is secreted by adipocytes and is a 16-kDa protein (Zhang *et al.,* 1994), which upon binding with hypothalamic receptors, controls homeostasis of energy by acting as adiposity feedback signal (Elmquist *et al.,* 1998). Evidence has indicated that systemic administration of leptin reduced stress perception by inhibiting the activity of hypothalamic pituitary adrenal (HPA) axis (Haque *et al.,* 2013) and elicited anti-depressant like effects (Lu *et al.,* 2006; Liu *et al.,* 2010; Haque *et al.,* 2013). Moreover, patients with major depression exhibited low levels of leptin in serum and cerebrospinal fluid (Jow *et al*., 2006; Westling *et al.,* 2004).

A decrease in central serotonin functions is also described in human depression (Quesseveur *et al.,* 2012). The inaccessibility of human brain and the ethical

limitations in human experiments have turned researchers to look for peripheral biomarkers to measure central serotonin activity in neuropsychiatric illnesses. These studies show a marked reduction in platelet serotonin level in major depression and post-traumatic stress disorder (Grah *et al.,* 2010; Silić *et al.,* 2012).

The purpose of the present study was to investigate a potential role of leptin in stress-induced behavioral deficits in rats. Effects of noise, restraint and immobilization stress were therefore determined on serum level of leptin. Associated changes in food intake and open field behavior were evaluated to understand a role of leptin in stress induced behavioral deficits. In view of an important role of HPA axis in response to stress (de Kloet and Sarabdjitsingh, 2008), serum levels of corticosterone were also determined. Moreover, attempt was made to identify serum serotonin as one of the peripheral marker of stress response.

MATERIALS AND METHODS

Animals: Locally bred male albino Wistar rats, weighing 180-200g purchased from Dow University of Health Sciences, Karachi, Pakistan were used in the present study. Housing conditions were same as described elsewhere (Ikram and Haleem, 2011). All animal experiments were conducted in accordance with NIH guidelines and approved by the institutional Ethics and Animal Care Committee.

Experiment 1: Twenty four animals were randomly divided into four equal groups: (i) no stress; (ii) noise stress; (iii) restraint stress; (iv) immobilization stress groups and were accordingly exposed to 2h noise, restraint or immobilization stress. Animals were killed immediately after the termination of stress. Serum samples were collected and stored at -70ºC.

Experiment 2: Twenty four animals were randomly divided into four equal groups: (i) no stress; (ii) noise stress; (iii) restraint stress; (iv) immobilization stress groups and were accordingly exposed to 2h noise, restraint or immobilization stress between 9:00 -10:00 h to 11:00-12:00h. Animals of the no stress group were left in their home cages during this time. After the termination of stress, animals of the stressed groups were also kept back in their home cages. Food Intake and open field exploration was determined 22 hr post termination of stress (Haleem *et al.,* 2013). Animals were killed immediately after monitoring the activity.

Stress procedures: The animals were exposed to immobilization, restraint or noise stress in separate rooms. The procedure of exposing animals to noise stress was essentially same as described earlier (Naqvi *et al.,* 2012). Restraint stress was produced by placing rats in adjustable (8" long and 2" diameter) Plexiglas tubes with air holes in the front, top and back (Haleem *et al.,* 2013).

Behavioral assessment

Food intakes and growth rates: 22 h cumulative food intakes (g) were determined by taking the difference of food weight (Ikram *et al.,* 2007) given immediately after the termination of stress on day 1 and food left before exposing animals to open field on day 2.

Activity in open field: Novel area (76×76 cm with walls 42 cm high) was used. Twenty five squares of equal size were marked on floor to divide its area equally. To monitor activity an animal was placed in the central square of the open field. Latency time (sec) to move from the center square and numbers of squares crossed with all four paws were counted for 5 min, as described previously (Ikram *et al.,* 2011).

Serum hormone analysis: The animals were decapitated to collect trunk blood. After clotting, the blood was centrifuged to obtain serum. Samples were stored at -70ºC until the assay of corticosterone, leptin and serotonin using respective ELISA kits (Cayman Chemical Company, Ann Arbor, MI, USA). Manufacturer's instructions were followed in the determination.

Statistical analysis: Effects of noise, restraint and immobilization on serum levels of corticosterone, leptin and serotonin, immediately and 24 h after the termination of stress were analyzed by two ways ANOVA. Behavioral data were analyzed by one way ANOVA. Post hoc comparisons were done by Tukey's test and p values less than 0.05 were taken as significant.

RESULTS

Table 1 shows the effects of three stresses on serum levels of corticosterone, immediately (A) and 24 h (B) after the termination of stress. Data analyzed by two way ANOVA showed significant effect of stress ($F=18.5$; $df=3,40$; $P<0.01$) and time ($F=173.4$ $df=1,40$ $P<0.01$) and significant interaction between stress and time ($F=43.9$; $df=3,40$; $P<0.01$). Post hoc comparison by Tukey's test showed that exposure to 2h noise, restraint or immobilization stress increased serum levels of corticosterone in animals killed immediately after the termination of stress. Immobilization-induced increases were greater than restraint or noise-induced increases of corticosterone. Animals killed 24 h after the termination of stress did not show any significant change in serum corticosterone level.

Table 1 shows the effects of different stresses on serum levels of leptin, immediately (A) and 24 h (B) after the termination of stress. Data analyzed by two way ANOVA showed significant effect of time ($F=190.3$; $df=1,40$; $P<0.01$) and significant interaction between stress and time ($F=25.2$; $df=3,40$; $P<0.01$). Effects of stress were non significant. Post hoc comparison by Tukey's test showed that exposure to 2h noise, restraint or immobilization stress increased serum levels of leptin in animals killed immediately after the termination of stress. The increases were comparable in the three groups. Animals killed 24 h after the termination of stress exhibited decrease in serum levels of leptin and the decreases were also comparable in the three groups.

Table 1 shows the effects of stresses on serum levels of serotonin, immediately (A) and 24 h (B) after the termination of stress. Data analyzed by two way ANOVA showed significant effect of stress ($F=8.8$; $df=3,40$; $P<0.01$) and time ($F=4.3$; $df=1,40$; $P<0.01$). Interaction between stress and time was also significant ($F=4.3$; $df=3,40$; $P<0.01$). Post hoc comparison by Tukey's test showed that

Table 1: Behavioral and hormonal responses to different stressors

Parameter	Time	Treatment			
		No Stress	Noise Stress	Restraint Stress	Immobilization Stress
Serum hormones					
Corticosterone (μg/100ml)	A	13.66±3.14	27.83±4.87[a]	32.50±5.35[a]	43.33±4.84[ac]
	B	17.16±2.31	16.33±3.93[b]	13.33±3.07[b]	11.00±2.52[b]
Leptin (ng/ml)	A	3.01±0.52	4.03±0.29[a]	4.35±0.41[a]	4.60±0.57[a]
	B	3.10±0.53	2.15±0.26[a b]	2.25±0.29[ab]	1.95±0.22[ab]
Serotonin (ng/ml)	A	2.88±0.54	1.86±0.28[a]	1.71±0.21[a]	1.48±0.42[a]
	B	2.36±0.41	2.68±0.66[b]	2.10±0.71	1.93±0.21
Behavioral parameters					
Cumulative food intake (g)	B	12.56±1.75	9.23±1.10[a]	6.3500±0.81[ab]	4.3833±0.92[abc]
Open field activity (Squares crossed/ 5min)	B	89.00±9.40	56.33±9.66[a]	38.66±7.68[ab]	34.83±7.08[ab]

Values are means ± SD. Significant differences: [a]P<0.01 from unstressed controls, [b]P<0.01 from animals exposed to noise stress, [c]P<0.01 from animals exposed to restraint stress following one-way ANOVA. (Time: A=immediately after stress; B=22hrs after stress).

exposure to 2h noise, restraint or immobilization stress decreased serum levels of serotonin in animals killed immediately after the termination of stress. These decreases did not occur in animals killed 24 h after the termination of stress. Serum levels of serotonin in animals killed 24 h after the termination of noise stress were not different from the respective unstressed controls but these were significantly (P<0.01) greater than the levels observed in animals killed immediately after the termination of noise stress.

Table 1 shows the effects of three stresses on 22 h cumulative food intake. Data analyzed by one way ANOVA showed significant effect of stress (F=52.4; df=1,20; P<0.01). Post hoc comparison by Tukey's test showed that exposure to 2h noise, restraint or immobilization stress decreased food intake. Animals exposed to immobilization stress exhibited greater decreases than animals exposed to restraint or noise stress. The decreases were also greater in animals exposed to restraint than noise stress.

Table 1 shows the effects of noise, restraint and immobilization on exploratory activity in open field monitored next day. Data analyzed by one way ANOVA showed significant effect of stress (F=50.3; df=1,20; P<0.01). Post hoc comparison by Tukey's test showed that exposure to 2h noise, restraint or immobilization stress decreased open field exploration. The decreases were greater in animals exposed to immobilization or restraint stress than in animals exposed to noise stress. Animals exposed to restraint or immobilization exhibited comparable decreases of open field exploration.

DISCUSSION

Changes in circulating levels of corticosterone and leptin following exposure to an acute stressor have been a topic of great interest in many studies, since such studies help to understand role of these hormones in stress-induced behavioral deficits and related psychiatric illnesses. The present findings on the effects of stress on circulating hormones differ from earlier studies in many aspects. Firstly, whereas other studies reported an increase in circulating levels of corticosterone and leptin following acute exposure to a stressor (Patterson-Buckendahl *et al.,* 2007), the present study also investigated changes in the levels of these hormones 24 h after the termination of stress (Table 1). Moreover, we also determined acute and delayed effects of these stressors on serum serotonin.

We report that acute exposure to an uncontrollable stressor increases circulating levels of corticosterone and leptin but serum serotonin is decreased. Compared with previous studies measuring acute effects of a stressor on corticosterone and leptin, we compared acute and delayed effects of three different stressors on serum corticosterone, leptin and serotonin in relation to the behavioral deficits produced by these stressors.

The hormone, leptin, plays an important role in HPA-axis functioning (Roubos *et al.,* 2012). Thus, HPA axis is hyperactive in leptin-deficient (ob/ob) mice, and chronic administration of leptin to leptin-deficient mice decreases plasma corticosterone levels (Arvaniti *et al.,* 2001). Stress-induced increases in the activity of HPA axis are also prevented by exogenous leptin (Heiman *et al.,* 1997; Haque *et al.,* 2013). From these studies it may be speculated that an increase in the activity of HPA axis during exposure to a stressor will be associated with a corresponding decrease in leptin release. The present study however shows that exposure to noise, restraint and immobilization results in an increase in circulating levels of leptin as well as plasma corticosterone. Both of these hormones having wide ranging effects on metabolism and energy balance may facilitate and re-establish homeostasis following exposure to an acute stressor (Roubos *et al.,* 2012; Haque *et al.,* 2013).

The synthesis of serotonin in the brain as well as at the periphery is dependent on the supply of its precursor tryptophan which is an essential amino acid. About 90% of the tryptophan is metabolized via kynureninenicotinamide pathway located in the liver (Marazziti *et al.,* 2013). Factors that increase plasma levels of corticosterone also increase utilization of tryptophan via kynureninenicotinamide pathway (Badawy *et al.,* 2009) resulting in a decrease in tryptophan concentration in circulation. The present results on stress-induced decreases of serum serotonin are explainable in terms of stress-induced increases of corticosterone (Table 1). On the other hand, stress-induced greater increases of corticosterone in rats exposed to 2 h immobilization do not result in greater decreases of serum serotonin, suggesting factors other than the availability of tryptophan are also important in the stress-induced decreases of serum serotonin.

Corticotropin-releasing factor (CRF), a component of HPA axis, is known to have an anorexigenic effect (Ohata *et al.,* 2011). Restraint stress-induced anorexia was antagonized by CRF antagonist suggesting that the releasing factor is involved in the stress-induced inhibitory mechanism of feeding behavior (Ohata *et al.,* 2011). Immobilization stress induced anorexia is also explained in terms of stress-induced increases of CRF, because immobilization-induced anorexia and activation of HPA axis were both inhibited by exogenous leptin (Haque *et al*.,

2013). Increases of serum corticosterone in rats exposed to noise and restraint stress observed in the present study suggest that noise and restraint stress-induced anorexia are also mediated via an increase in CRF release. Thus, stress-induced increases of serum corticosterone and decreases of food intake followed the order immobilization > restraint > noise stress (Table 1).

Injected leptin also decreases food intake and these effects of the peptide hormone are produced via its receptors within the ventromedial hypothalamus. An increase in malonyl-CoA level in the hypothalamic arcuate nucleus (Gao *et al.,* 2011) resulting in a decrease in the expression of the orexigenic neuropeptide Y is also involved in the anorexigenic effects of leptin. The present study suggests that stress-induced increases in leptin are also, at least in part, involved in anorexia observed following exposure to an uncontrollable stressor.

Exogenous leptin increases open field exploration in unstressed animals by inhibiting novelty-induced stress (Lu *et al.,* 2006; Liu *et al.,* 2010; Haque *et al.,* 2013). Immobilization-induced decreases of open field exploration are also reversed by the injected leptin. These studies suggest that the peptide hormone elicits an antidepressant like effect. It is therefore tempting to relate the decreases of serum leptin observed in rats 24 h after the termination of immobilization, restraint and noise stress with the deficits of behavior in open field exploration. It may be noted that serum corticosterone and serotonin are not different in stressed and unstressed animals and returned to normal 24 h after the termination of stress but serum leptin levels are smaller in stressed rats killed 24 h after the termination of stress.

Conclusion: The present study shows that acute exposure to an uncontrollable stressor not only stimulates HPA axis but an increase in leptin release and a decrease in serum serotonin also occurs. It suggests that together with circulating levels of corticosteroids, serum levels of serotonin and leptin are peripheral markers of acute stress exposure. The present state of knowledge supports a role of CRF and leptin in stress-induced anorexia. On the other hand, stress-induced decreases in open field exploration associated with decreases in serum leptin support the notion that an insufficiency of the peptide hormone contributes to stress-induced behavioral deficits.

REFERENCES

Arvaniti K, Q Huang and D Richard, 2001. Effects of leptin and corticosterone on the expression of corticotropin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. Neuroendocrinology, 73: 227-236.

Badawy AA-B, S Bano and A Steptoe, 2011. Tryptophan in alcoholism treatment I: Kynurenine metabolites inhibit the rat liver mitochondrial low km aldehyde dehydrogenase activity, elevate blood acetaldehyde concentration and induce aversion to alcohol. Alcohol, 46: 651-660.

Chourbaji S, C Zacher, C Sanchis-Segura, C Dormann, B Vollmayr and P Gass, 2005. Learned helplessness: validity and reliability of depressive-like states in mice. Brain Res Brain Res Protoc, 16: 70-78.

de Kloet C, E Vermetten, E Lentjes, E Geuze, J van Pelt, R Manuel, C Heijnen and H Westenberg, 2008. Differences in the response to the combined DEX-CRH test between PTSD patients with and without co-morbid depressive disorder. Psychoneuroendocrinology, 33: 313-320.

Elmquist JK, C Bjørbaek, RS Ahima, JS Flier and CB Saper, 1998. Distributions of leptin receptor mRNA isoforms in the rat brain. J Comp Neurol, 395: 535-547.

Grah M, M Mihanović, P Svrdlin, SV Pisk and B Restek-Petrović, 2010. Serotonin and cortisol as suicidogenic factors in patients with PTSD. Coll Antropol, 34: 1433-1439.

Haleem DJ and T Parveen, 1994. Brain regional serotonin synthesis following adaptation to repeated restraint NeuroReport, 5: 1785-1788.

Haleem DJ, 1999. Serotonergic mechanism of antidepressant action and adaptation to stress. J Col Phys Surg Pak, 3: 139-146.

Haleem DJ, 2011a. Behavioral deficits and exaggerated feedback control over raphe-hippocampal serotonin neurotransmission in restrained rats. Pharmacol Rep, 63: 887-897.

Haleem DJ, 2011b. Raphe-hippocampal serotonin neurotransmission in the sex related differences of adaptation to stress: focus on serotonin-1A receptor. Curr Neuropharmacol, 9: 512–521.

Haleem DJ, H Ikram, S Haider, T Parveen and MA Haleem, 2013. Enhancement and inhibition of apomorphine-induced sensitization in rats exposed to immobilization stress: Relationship with adaptation to stress. Pharmacol Biochem Behav, 112: 22-28.

Haque Z, N Akbar, F Yasmeen, MA Haleem, and DJ Haleem, 2013. Inhibition of Immobilization stress-induced anorexia, behavioral DEFICITS and plasma corticosterone secretion by injected leptin in rats. Stress, 16: 353-362.

Heiman ML, RS Ahima, LS Craft, B Schoner, TW Stephens and JS Flier, 1997. Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology, 138: 3859-3863.

Ikram H and DJ Haleem, 2010. Haloperidol-induced tardive dyskinesia: role of 5HT2C receptors. Pak J Sci Ind Res, 53: 136-145.

Ikram H and DJ Haleem, 2011. Attenuation of apomorphine-induced sensitization by buspirone. Pharmacol Biochem Behav, 99: 444-450.

Ikram H, S Ahmed and DJ Haleem, 2011. Effects of apomorphine on locomotor activity and monoamine metabolism: a dose related study. Pak J Pharm Sci, 24: 315-321.

Jow GM, TT Yang and CL Chen, 2006. Leptin and cholesterol levels are low in major depressive disorder, but high in schizophrenia. J Affect Disord, 90: 21-27.

Liu J, JC Garza, J Bronner, CS Kim, W Zhang and XY Lu, 2010. Acute administration of leptin produces anxiolytic-like effects: a comparison with fluoxetine. Psychopharmacology (Berl), 207: 535-545.

Lu X-Y, CS Kim, A Frazer and W Zhang, 2006. Leptin: A potential novel antidepressant. Proc Nat Acad Sci USA, 103: 1593-1598.

Maier SF and LR Watkins, 2005. Stressor controllability and learned helplessness: The roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci Biobehav Rev, 29: 829-841.

Marazziti D, S Baroni, M Picchetti, APiccinni, S Silvestri and L Dell'Osso, 2013. New developments on the serotonin hypothesis of depression: shunt of tryptophan. Riv Psichiatr, 48: 23-34.

Ohata H and T Shibasaki, 2011. Microinjection of different doses of corticotropin-releasing factor into the medial prefrontal cortex produces effects opposing anxiety-related behavior in rats. J Nippon Med Sch, 78: 286-292.

Patterson-Buckendahl P, LA Pohorecky and R Kvetnansky, 2007. Differing effects of acute and chronic stressors on plasma osteocalcin and leptin in rats. Stress, 10: 163-172.

Rainer Q, HT Nguyen, G Quesseveur, AM Gardier, DJ David and BP Guiard, 2012. Functional status of somatodendritic serotonin 1A autoreceptor after long-term treatment with fluoxetine in a mouse model of anxiety/depression based on repeated corticosterone administration. Mol Pharmacol, 81: 106-112.

Richter SH, B Zeuch, MA Riva, P Gass and B Vollmayr, 2013. Environmental enrichment ameliorates depressive-like symptoms in young rats bred for learned helplessness. Behav Brain Res, 252: 287-292.

Roubos EW, M Dahmen, T Kozicz and L Xu, 2012. Leptin and the hypothalamo-pituitary-adrenal stress axis. Gen Comp Endocrinol, 177: 28-36.

Silić A, D Karlović and A Serretti, 2012. Increased inflammation and lower platelet 5-HT in depression with metabolic syndrome. J Affect Disord, 141: 72-78.

Westling S, B Ahrén, L Träskman-Bendz and A Westrin, 2004. Low CSF leptin in female suicide attempters with major depression. J Affect Disord, 81: 41-48.

Zhang Y, R Proenca, M Maffei, M Barone, L Leopold and JM Friedman, 1994. Positional cloning of the mouse obese gene and its human homologue. Nature, 372: 425-432.