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Protection of adrenocortical activity by dietary casein in ether anaesthetized rats

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Adrenal Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) activity and serum corticosterone level were significantly higher in rats fed with 5% casein or 4% albumin diets after 1 hr of ether anaesthetic stress as compared to the controls, 5% casein and 20% casein (equivalent to 4% albumin) respectively. Ether anaesthesia to 20% casein fed rats caused no change in adrenal Δ^5-3β-HSD activity and serum corticosterone level when compared with controls fed 20% casein diet. The results suggest that high milk protein diet may prevent acute stress effects by protecting adrenocortical activity. The present investigation opens up a new area of management of stress.

Stress can be considered as the sum of the biological reactions to physical, mental or emotional stimuli that influence the onset and propagation of number of disorders leading to change the balance of hormones in the body[1]. Almost any type of physical or mental stress will cause within minutes an increase in ACTH secretion followed by cortisol secretion as much as 20-fold[2]. Stress effect of anaesthesia is a serious problem in clinical surgery. Ether anaesthesia stress causes a significant increase in the concentration of plasma glucocorticoids in rats[3]. In chronic stressful state excess cortisol secretion helps to restore and maintain homoeostasis[4]. But in certain forms of mild stress basal levels of glucocorticoids exert permissive effects that protect against stress by normalizing homoeostatic functions[5,6]. Similarly, basal levels of glucocorticoids can control response to moderate stress while stress-induced levels may be necessary to cope with severe stress[7].

So, in acute stress when man and animals are subjected to short or single exposure to stress, high levels of glucocorticoids may lead to profound catabolic effects on protein and other components of peripheral tissue and to a redistribution of fat[8]. Since high milk protein diet prevents adrenocortical hyperactivity in induced physical swimming stress in rats[9], the present investigation was undertaken to determine whether milk protein diet can protect adrenocortical activity in acute ether anaesthetic stress in rats. This report is perhaps the first evidence to show that milk or milk products containing casein has a protective role against moderate stress.

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Animals and ether exposure—The experiments were carried out on 40 male adult albino rats of a laboratory-bred Wistar strain, weighing 150-160 g. The animals were housed in a laboratory controlled environment (25° ± 1°C, 98% R.H.) and 12:12 hr L:D cycle. All the rats were fed *ad libitum* on three series of diets described previously[9,10]. First series of diets contained 5 g casein (P.C. Dutta and Bros., Calcutta, India), 38.5 g wheat meal and 46.5 g chick-pea; second series, 20 g casein, 39 g wheat meal, 31 g chick-pea; and third series 4 g albumin (B.S.A. Sigma Chemical Co., St. Louis, Missouri, USA); 70 g wheat meal and 16 g chick-pea in addition to the corn oil 5 g, vitamin mixture 1 g, salt mixture 4 g in 100 g of all diets. The total protein content of the diet was about 15%. The protein content of 20% casein was about 4% and was equivalent to 4% albumin. Serum albumin was used to compare its effect with casein. The animals were divided equally into five groups. The animals of the 1st and 2nd groups received diet containing 5% casein, 3rd and 4th group 20% casein and 5th group 4% albumin diets for 7 days. On the 8th day one group of 5% and 20% casein, and 4% albumin diet were anaesthetized by ether in an wooden box (with a glass window to watch the movement of the animal) for 1 hr. Ether soaked in cotton was applied inside the box. When the rats were fully anaesthetized the cotton was removed immediately and applied again just before awakening of the rats. Thus the stable level of anaesthesia for one hour was maintained. All the animals were sacrificed under light ether anaesthesia. Blood was drawn from the abdominal vein by heparinized syringe and centrifused to separate serum, which was kept at

−20°C until corticosterone was assayed. Adrenals were removed and dissected free of surrounding connective tissue.

Measurement of adrenal Δ^5-3β-HSD enzyme and serum corticosterone — Adrenal kept at −20°C was immediately processed for assay of Δ^5-3β-HSD activities following method of Rubin *et al.*[11] and subsequently modified by Sarkar *et al.*[12]. Serum corticosterone was determined by spectroflurometry according to the method of Glic *et al.*[13] and modified by Silber[14]. The fluorescence was measured at 463 mm (excitation), 518 mm (emission) by setting the instrument at a spectrofluorometric reading 80 with a standard corticosterone (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) solution having 1.6 μg/ml concentration. A minimum 1.6 μg of corticosterone/ 100 ml serum can be measured by this method. To test differences between control and treated groups, 2-tailed Student's *t* test was used, $P<0.05$ was considered to be significant. Each value represents means ± SE of 8 rats in each group.

A significant increase in adrenal Δ^5-3β-HSD activity and serum level of corticosterone were recorded in rats fed on 5% casein or 4% albumin diet and exposed to ether anaesthesia in comparison with controls of 5% casein and 20% casein (equivalent to 4% albumin) group (Fig.1). Anaesthetic stress in rats fed on 20% casein diet showed no change in adrenal Δ^5-3β-HSD activity or serum level of corticosterone when compared with control rats fed on 20% casein diet. Both the parameters were similar among the control group of rats receiving either 5% or 20% casein diet.

The results demonstrate that ether anaesthetic stress in rats fed with 5% casein or 4% albumin diet produced adrenal hypertrophy and stimulated Δ^5-3β-HSD activity, which in turn increased the serum level of corticosterone. A similar increase in corticosteroids has been observed in rats and man exposed to anaesthesia[3,15].

The present study shows that feeding on 20% casein diet and exposure to anaesthesia resulted no change in adrenal Δ^5-3β-HSD activity or serum levels of corticosterone when compared with control rats fed on 20% casein diet. The mechanism by which high casein diet protects adrenocortical activity in anaesthetized rats cannot be determined from the present experiment. It is now well established that in several subprimate species hypothalamic implantation of atropine, an anticholinergic agent inhibits plasma ACTH response to ether anaesthesia[16]. Biologically

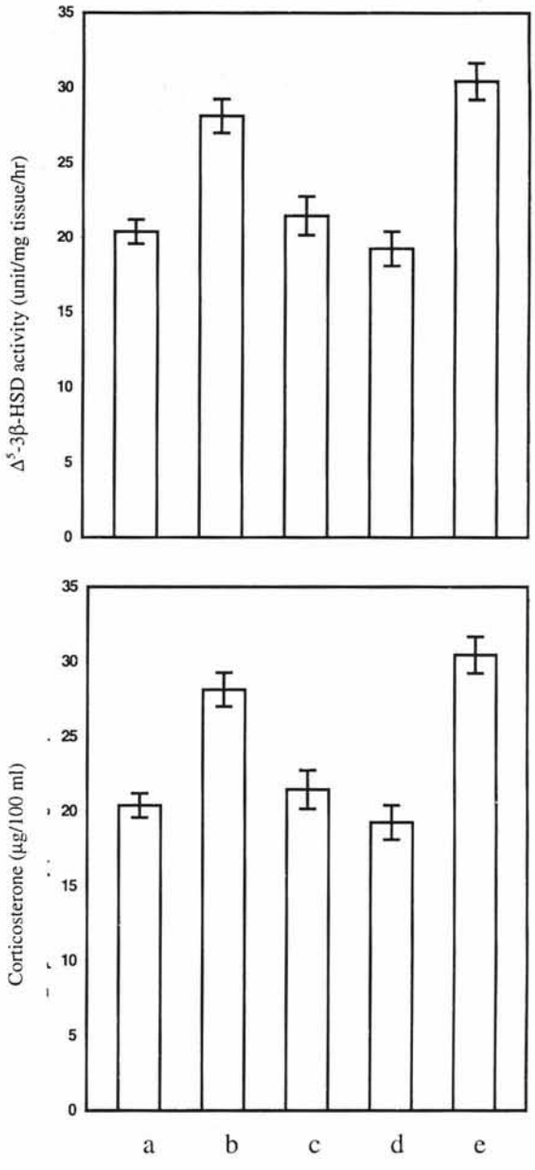

Fig. 1 — Effect on casein or albumin diet and ether anaesthesia on (A) adrenal Δ^5-3β-HSD activity and (B) levels of serum corticosterone in rats fed with (a) 5% casein, (b) 5% casein, (c) 20% casein, (d) 20% casein + ether, (e) 4% albumin + ether.
[Values are mean ± SE from 8 animals in each group.]
ANOVA (model 1 or fixed model[21]) followed by Student's *t* test.
*$P<0.05$ compared with control group.

active peptides derived from casoprotein and probably not from albumin during intestinal digestion are considered to be potential modulator of various regulatory process in the body[17]. Some bioactive peptides have been shown to behave like opioid receptor ligands and casein may elicit opioid effects[18]. Opiate peptides such as β-endorphin in physiological concen-

tration inhibits both basal and ACTH-stimulated corticosterone synthesis[19].

In the present experiment short exposure to anaesthesia increased corticosterone significantly above the normal level only with 5% casein and 4% albumin diet. Although stress-induced excess glucocorticoids contribute to maintain homoeo-stasis in severe stress but in moderate stress, basal level of glucocorticoid can protect stress response after exerting its permissive effects[5-7]. Therefore excess corticosteroids which are known to alter biochemical parameters of the system, result significant changes in stress responsiveness and behaviour in short single experience of stress[20].

Therefore, the protection of normal level of corticosterone in acute stress in rats by high levels of milk protein diet may suggest a strategy for prevention of catabolic effects of stress-induced corticosterone in human in a similar condition.

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