Prevention of adrenocortical hyperactivity by dietary casein in rats exposed to forced swimming stress

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Adrenal weight, adrenal hydroxysteroid dehydrogenase activity and serum corticosterone level were significantly higher in rats fed with 5% casein diet after 7 days of swimming stress (45 min/day) as compared to their controls. All the parameters were similar to their control levels in rats receiving 20% casein diet and exposed to swimming stress. The results suggest that casein can play an important role in preventing adrenocortical hyperactivity in swimming stressed rats.

Stress from different sources stimulates the hypothalamo-pituitary-adrenal (HPA) axis with increased secretion of glucocorticoids. Forced swimming stress is widely used to study physical stress in animals. Nagaraja and Jaganathan have shown that swimming stress in rats increases the weight of the adrenal glands significantly and that the animals develop leucopenia, eosinopenia and neutropenia. These changes indicate an increased release of corticosteroids during stress conditions. Though consumption of casein-enriched diet has been recently reported to prevent adrenal hyperactivity in estrogen-treated rats, the effect of casein diet in the prevention of stress-induced adrenal hyperactivity has not been studied.

Therefore, adrenal steroid dehydrogenase activity and serum corticosterone levels have been studied in rats fed on high casein diet and exposed to forced swimming stress.

Adult (120-140 days of age) male Wistar strain rats (24), obtained from University Animal House, were maintained at 30°C with 10 hr illumination daily. All the rats were fed ad libitum on two series of diets described previously. The total protein content of the diets was about 15%. 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 contained 20 g casein, 39 g wheat meal, 31 g chick-pea in addition to the 5 g corn oil, 1 g vitamin mixture and 4 g salt mixture in 100 g of each diet.

The animals were divided equally into 4 groups. Two groups of rats received diet containing 5% casein and other two groups 20% casein. Rats of one group each from 5% and 20% fed casein diet were forced to swim in cylindrical plastic vessels (height: 60 cm and diameter: 40 cm) filled with water to a height of 40 cm maintained at room temperature (30°C). The animals were forced to swim for 45 min every day for 7 days. The other two groups served as their respective controls. All the animals were sacrificed by decapitation 1 hr after the last swimming exposure, following protocols and ethical procedure.

Blood obtained from each of the animals was centrifuged for serum separation. The adrenal glands were excised and weighed in torsion balance. For assay of adrenal Δ\(^{5}\)-3β-hydroxysteroid dehydrogenase (Δ\(^{5}\)-3β-HSD) activity, each of the adrenal glands was immersed in ice-cold homogenizing medium consisting of equal parts of 0.9% NaCl and 0.1M sodium phosphate buffer, pH 7.4, to give a tissue concentration of 5 mg/ml. The enzyme was assayed by spectrophotometric measurement of the production of Δ\(^{4}\)-androstenedione from dehydroepiandrosterone.

Serum corticosterone was determined by spectrofluorometry according to the method of Glick et al. and modified by Silber. The fluorescence was measured at 463 nm (excitation) and 518 nm (emission) by setting the instrument at a spectrofluorometric reading 80 with a standard corticosterone (Sigma Chemical Company, St. Louis, MO, USA) solution having the concentration 1.6 μg/ml. A minimum 1.6 μg of corticosterone/100 ml serum can be measured by this method. All the chemicals used in the experiment were of analytical quality. The test for different between control and treated groups, 2-tailed Student
Fig. 1—Effect of casein diet and swimming stress on (a) adrenal weight, (b) adrenal $\Delta\delta-3\beta$-HSD activity and (c) levels of serum corticosterone in rats fed (i) 5% casein, (ii) 5% casein + exposed to swimming stress, (iii) 20% casein, and (iv) 20% casein + exposed to swimming stress. Values are mean ± SE from 6 rats in each group.*P<0.05 as compared with 5% casein group.

The results demonstrated that the forced swimming stress in rats fed with 5% casein diet produced adrenal hypertrophy and stimulated $\Delta\delta-3\beta$-HSD activity which in turn possibly increased the serum level of corticosterone. A similar possibility of an increased release of corticosteroids has been suggested on the basis of adrenal hypertrophy along with leucopenia, eosinopenia and neutropenia observed in swimming stressed rats. Exposure to stress increases adrenal secretion of glucocorticoids via hypothalamic-pituitary-adrenal axis. The precise mechanism by which swimming stress increases corticosterone release remains to be determined.

Stress induces an adrenomedullary response in humans. Giguere et al. have shown that stimulation of $\alpha$-receptor of rat anterior pituitary by epinephrine leads to a direct release of ACTH into culture medium. Therefore, the increased adrenal $\Delta\delta-3\beta$-HSD activity and serum corticosterone in swimming stressed rats fed on the 5% casein diet may possibly have been due to release of ACTH induced by epinephrine.

Little is known about the mechanism as to how a high casein diet may affect adrenocortical hyperactivity in swimming stressed rats. Bioactive peptide fragments originating from casein are considered as potential modulators of various regulatory processes in the body. Some bioactive peptides have been shown to behave like opioid receptor ligands and milk protein may elicit opioid effects.

Pharmacological studies have shown that opioids inhibit ACTH secretion while opiate antagonist naloxone stimulates ACTH release in humans. Wilson et al. have shown that opioid receptors play a role in the reduction of corticosterone level. Since chronic administration of the opiate alkaloid inhibits adrenocortical responses to stress, the bioactive peptides of casein possibly prevent hyperactivity-of the adrenal cortex in swimming stressed rats behaving like opioid receptors agonists. The prevention of hyperactivity of the adrenal cortex in swimming stressed rats by casein-enriched diet may suggest a strategy for protec-
tion of adrenocortical function by a high milk protein diet in human in a similar condition.

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