PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 239–242 (1987)

RAPID COMMUNICATION

EFFECT OF ILLNESS ON HORMONAL RESPONSE TO FOOTSHOCK STRESS

D.B. YELVINGTON, M.J. ROSENTHAL, AND A. RATNER

Departments of Physiology and Medicine
University of New Mexico School of Medicine
Albuquerque, NM 87131

Abstract: The corticosterone (CORT) and prolactin (PRL) responses to 1.0 mA of footshock were measured in healthy rats and rats with pneumonia. No differences in basal PRL levels were seen, but basal CORT levels were significantly increased in the sick animals. Healthy rats showed a significant increase in both PRL and CORT after receiving footshock whereas the sick rats showed no changes. The adapative value of the current findings are unclear at this time.

Introduction

Secretion of corticosterone (CORT) and prolactin (PRL) has been shown to increase in response to numerous stressors (1, 2). Recently, we noticed that a few animals which did not appear healthy showed a markedly blunted PRL stress response. These animals were later diagnosed as having pneumonia. Severe illness has been shown to decrease basal levels of several hormones (3-5). However, the effect of an additional stress upon hormonal responses of sick animals has not been investigated. In these studies, we examined the PRL and CORT response to moderate footshock stimulation in healthy rats and rats which exhibited signs of having pneumonia.

Materials and Methods

Animals: Male Sprague-Dawley rats (400-500 g) from Simonsen Labs (Gilroy, CA) were housed in a climate-controlled area (24 + 2 C) with a fixed light:dark cycle, lights on at 0500 and off at 1900 h. The rats were given food and water ad libitum. The animals were handled twice daily for one week before the experiment. Each time the rats were removed from their home cage and placed in a box equipped with an electric shock grid floor. This procedure allowed the rat to acclimate to both handling and exposure to the experimental chamber. We have recently shown that after repeated exposure to a novel environment, the PRL response becomes habituated (6). Similar findings have also been shown for the habituation of the adrenocorticosteroid response to handling or novelty (7,8).

Two groups of rats were exposed to electric footshock. One group was in good health as judged by external examination. The second group showed signs of moderate-to-severe illness including lethargy, respiratory distress and/or red discoloration around the eyes or nose. Both sick and healthy rats were examined postmortem. The healthy rats showed no signs of illness while the sick animals showed fluid in the lungs and exhibited typical pneumonic lesions.

Experimental Design: Right atrial cannulae (9) were inserted under ether anesthesia at least 3 days before the experiments were carried out and flushed daily with heparinized saline (15 U/ml). During the experiments, blood samples (0.4 ml) were collected in heparinized syringes connected to a polyethylene extension of the indwelling silastic cannula. This allowed the rats to

0037-9727/87 $1.50

move freely and permitted the experimenter to collect blood without disturbing the animals. To ensure that animals were kept close to an isovolemic state, an equal volume of fluid was replaced after each blood collection with Plasmanate TM (Cutter Labs, Berkeley, CA), a 5% human plasma protein fraction which is volume for volume osmotically equivalent to plasma.. On the day of the experiment, rats were moved from their home cages to a box equipped with an electric shock grid floor. After 30 min, a basal blood sample was taken. Ten min later, the rat was subjected to a 1.0-mA footshock. The footshock was delivered once per sec for 30 sec. Blood samples were collected 5, 10, 15, 25, and 45 min after footshock. All experiments were performed between 1300 and 1730 h.

Assay procedures: Blood samples were centrifuged at 3500 rpm for 20 min and the plasma was separated and stored at -20 C until assayed. PRL levels were determined in duplicate by RIA using a double antibody technique. Rat PRL antibody (rabbit) and rat PRL reference preparations were provided by the Hormone Distribution Program of the NIAMDD. Iodine-labeled PRL was obtained from New England Nuclear Corp. (Boston, MA). The limit of sensitivity of the assay was 3 ng/ml; the inter- and intra-assay variabilities were 9% and 4%, respectively. CORT was also assayed in duplicate by RIA using charcoal separation (10). The antibody was obtained from Radioassay Systems Laboratory (Carson, CA) and tritium-labeled CORT from New England Nuclear Corp. The limit of sensitivity of the assay was 0.5 ug/dl; the inter- and intra-assay variabilities were 5% and 2%, respectively. The data were analyzed using the Student-Neuman-Kuels procedure with analysis of variance. A p value of less than 0.05 was considered significant.

Results

Figure 1 shows the PRL and CORT response of healthy and sick rats to footshock. Basal PRL levels were not significantly different between the groups, but basal CORT levels were significantly higher in the sick rats. In the healthy rats, both PRL and CORT were significantly increased at every time point after receiving

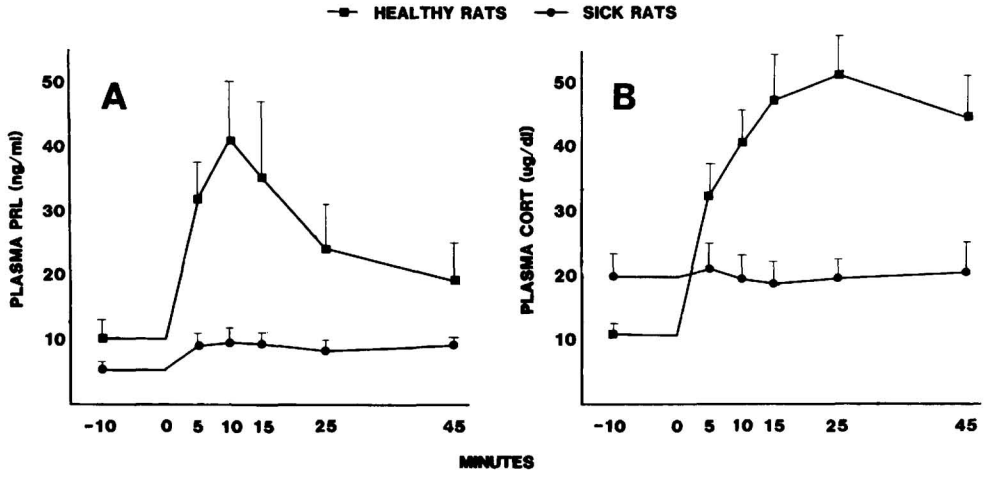

Figure 1. Plasma prolactin (PRL) (A) and corticostrone (CORT) (B) concentrations before and after application of footshock stress in healthy and sick rats. A blood sample was collected 10 min prior to footshock stimulation. At time zero, the rats received 1.0 mA footshocks. The footshocks were given at the rate of 1 shock/sec for 30 sec. Blood samples were collected 5, 10, 25, and 45 min after footshock. Each data point represents the means ± SEM of at least six animals.

footshock. PRL values were increased 5 min after stimulation and reached a peak level 10 min after footshock. CORT levels were also increased at 5 min but continued to increase up to 25 min after footshock; peak values were some four times higher than basal levels. In contrast, the sick rats showed no significant change in either PRL or CORT at any time after footshock.

Discussion

According to Selye's classic description of the General Adaptation Syndrome, increased corticoid levels occur during either the alarm reaction or the state of exhaustion (11, 12). Selye presented limited histological evidence for the stage of exhaustion and never directly confirmed whether such animals could mount a glucocorticoid response to stress. The sick animals in our study showed increased resting CORT levels and were unable to increase either CORT or PRL levels in response to the footshock stress. These animals had spontaneously acquired clinical pneumonia.

How can the absent PRL and CORT responses be explained? Glucocorticoids can blunt the ACTH response to stress (13) and we have recently shown that CORT can also suppress stress-promoted PRL release (14). In this study we found that the basal CORT levels were increased in the sick rats. These high basal CORT levels could possibly have acted to suppress the PRL and CORT response to footshock stress via negative feedback mechanisms at a pituitary or hypothalamic site. It is also possible that severe illness *per se* could directly affect the hypothalamic-pituitary response to stress.

The concept that systemic illness decreases basal hormone levels has been most widely studied in connection with thyroid hormones. Severe illness and/or starvation decreases triiodothyronine and thyroxine levels by affecting extrathyroidal hormone conversion, binding, and metabolism (15, 16). In addition, the normal feedback control of the pituitary-thyroid axis is blunted (5, 17, 18). Gonadotropins are also decreased by severe illness (3). Transient hypogonadotropic hypogonadism has also been shown to develop in patients with severe burns (4) and other critical illnesses (19).

The data presented in this study show that stress-mediated hormonal responses are altered during severe acute illness. Decreases in thyroid hormone and gonadotropins may benefit the severely ill by shifting metabolism toward more essential functions, but the significance of the current results remains to be determined. Studies are underway to investigate the response of sick animals to other stresses and to probe the mechanisms behind the altered hormonal responses.

Acknowledgements

We are grateful for the generous gift of assay materials provided by Dr. A. F. Parlow and the Rat Pituitary Hormone Distribution Program of NIAMDD.

References

1. Kant GJ, Lenox RH, Bunnell BN, Mougheу EH, Pennington LL, Meyerhoff JL. Comparison of stress response in male and female rats: pituitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. Psychoneuroendocrinology 8: 421-428, 1983.
2. Brown GM, Martin JB. Corticosterone, prolactin, and growth hormone responses to handling and new environment in the rat. Psychosom Med 36: 241-246, 1974.
3. Quint AR, Kaiser FE. Gonadotropin determinations and thyrotropin-releasing hormone testing critically ill post-menopausal women with hypothyroxemia. J Clin Endocrinol Metab 60: 464-471, 1985.
4. Vogel AV, Peake GT, Rada RT. Pituitary-testicular axis dysfunction in burned men. J Clin Endocrinol Metab 60: 658-665, 1985.
5. Wehmann RE, Gregerman RI, Burns WH, Saral R, Santos GW. Suppression of thyrotropin in the low-thyroxine state of severe nonthyroidal illness. New Engl J Med 312: 546-552, 1985.
6. Yelvington DB, Weiss GK, Ratner A. Habituation of the prolactin

response in rats to psychological stress. Psychoneuroendocrinology 10: 95-102, 1985.
7. Bassett JR, Cairncross D, King MG. Parameters of novelty, shock, predictability and response contingency in corticosterone release in rats. Physiol Behav 10: 901-907, 1973.
8. Mason JW. A review of psychoendocrine research on the pituitary-adrenal cortical system. Psychosom Med 30: 576-607, 1968.
9. Harms PG, Ojeda SR. A rapid and simple procedure for chronic cannulation of the rat jugular vein. J Appl Physiol 36: 391-392, 1974.
10. Abraham GE. Handbook of RIA. Clin Biochem Anal 5: 591-656, 1977.
11. Selye H. General adaptation syndrome and the diseases of adaptation. J Clin Endocrinol 6: 117-135, 1946.
12. Seyle H, Stone H. On the Experimental Morphology of the Adrenal Cortex. Springfield, Ill: CC Thomas, 1950.
13. DeSouza H, Van Loon GR. Stress-induced inhibition of the plasma corticosterone response to subsequent stress in rats: a non-adrenocorticotropin-mediated mechanism. Endocrinology 10: 23-33, 1982.
14. Yelvington DB, Weiss GK, Ratner A. Effect of corticosterone on the prolactin response to psychological and physical stress in rats. Life Sci 35: 1705-1711, 1984.
15. Chopra IJ. Thyroid function in nonthyroidal illnesses. Ann Int Med 98: 946-957, 1983.
16. Tibaldi JM, Surks MI. Animal models of nonthyroidal disease. Endocrin Rev 6: 87-102, 1985.
17. Borst GC, Osburne RC, O'Brian JT, Georges LP, Burman KD. Fasting decreases thyrotropin responsiveness to thyrotropin-releasing hormone. J Clin Endocrinol 57: 380-383, 1983.
18. Gardener DF, Kaplan MM, Stanley CA, Utiger RD. Effect of triiodothyronine replacement on the metabolic and pituitary responses to starvation. N Engl J Med 300: 579-584, 1979.
19. Woolf PD, Hamill RW, McDonald JV, Louyse AL, Kelly M. Transient hypogonadotropic hypogonadism caused by critical illness. J Clin Endocrinol Metab 60: 444-450, 1985.

Received September 22, 1986
P.S.E.B.M. 1987, Vol. 184
Accepted December 8, 1986