PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 147 510-512 (1974)

Neonatal Hyperthyroidism and Maturation of the Rat Hypothalamo–Hypophyseal–Adrenal Axis[1] (38376)

LEE A. MESERVE[2] AND JAMES H. LEATHEM[3]

Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey 08903

The neonatal rat exhibits a refractory period of the hypothalamo–hypophyseal–adrenal axis in response to stress which encompasses days 3–12 of life, an interval called the stress nonresponsive period (SNR) (1). Then, at 15 days of age, the rat responds equally to ether stress and exogenous ACTH by an increased synthesis and release of corticosterone (2). However, hypothyroidism induced by thiouracil feeding during pregnancy and lactation prevented the hypothalamo–hypophyseal–adrenal axis response to ether stress possibly by extending the SNR period (3). What effect the feeding of desiccated thyroid would have on the maturation of this axis has not been reported. Thus the purpose of the present study was to examine the effect of thyroid feeding on the response of 12-day-old rats to ether stress and to exogenous ACTH by measuring adrenal corticosterone (B) (an index of hormone synthesis) and serum corticosterone (an index of hormone release).

Materials and Methods. Female Sprague–Dawley rats weighing 150–175 g (Camm Research Institute, Wayne, NJ) were housed in a temperature (78° F) regulated room with a light–dark cycle of 12:12 hr. The rats were fed Purina Lab Chow *ad lib.* until a body weight of 180–200 g was attained. The animals were then mated with proven males of the same strain. On the first day of pregnancy, as determined by vaginal sperm, the females were isolated and fed a semipurified diet containing 20% casein (4) with or without 0.05% dessicated thyroid powder (Wilson). This dietary regime was continued through gestation and lactation until the offspring were tested at 12 days of age. At 5 days of age the litters were reduced to eight pups by removing the excess young but without disturbing those remaining. No litters with fewer than five pups were used. At 12 days of age, young of either sex were randomly placed in one of the following groups (10 rats/group): (1) control (removed from the cage and decapitated 15 min later); (2) ether stressed (exposed to ether fumes for 1 min and decapitated 15 min later); (3) adrenocorticotrophic hormone (ACTH) injected (given 20 IU/100 g body wt, Cortrophin-Gel, Organon, subcutaneously and decapitated 15 min later); or (4) saline injected subcutaneously and decapitated 15 min later. Blood from the severed neck vessels was collected and allowed to clot, centrifuged, and the serum frozen for future estimation of corticosterone content. Adrenals were excised, decapsulated, weighed and then homogenized in 2 ml of 33% ethanol to which 3 ml of distilled deionized water was added. Adrenal and serum corticosterone were determined by the sulfuric acid fluorometic method of Hilf *et al.* (5). Significant differences were estimated by Student's *t* test at the 0.01 level.

Results. Feeding dessicated thyroid (0.05%) throughout gestation and lactation did not influence body weight of the pups at 12-days of age (21.5 vs 21.0 g) but did result in smaller adrenals than in euthyroid controls (2.6 vs 3.2 mg). However the basal concentrations of adrenal (3.20 vs 2.88 μg/100 mg) and serum (11.7 vs 11.8 μg/100 ml) corticosterone were not affected by thyroid feeding.

[1] This study was supported in part by the Rutgers University Research Council and by the Charles and Johanna Busch Memorial Award to the Bureau of Biological Research.

[2] Current address: Department of Biology, Bowling Green State University, Bowling Green, OH 43403.

[3] Request for reprints: J. H. Leathem, Bureau of Biological Research, Rutgers University, New Brunswick, NJ 08903.

When the 12-day-old euthyroid rats were subjected to ether stress or exogenous ACTH adrenal corticosterone concentration and total adrenal corticosterone increased significantly (Table I). Similar responses were noted in the 12-day-old pups from mothers fed thyroid. Furthermore, the increase in adrenal corticosterone concentration was significantly greater in the "hyperthyroid" pups than the euthyroid pups. Saline injection was without effect in euthyroid pups but did increase adrenal B content in the thyroid feeding tests.

Despite the response of the 12-day-old euthyroid rat adrenal to stress, corticosterone was not released into the serum. On the other hand, the pups from mothers fed thyroid showed a significant increase in serum B concentration and in total serum corticosterone after ether stress and exogenous ACTH administration. Saline injection, however, did not incite release of corticosterone (Table I).

Discussion. Sensitivity of the hypothalamo–hypophyseal–adrenal axis of euthyroid rats to ether stress and exogenous ACTH increased gradually from birth until it reaches adult levels (6–8). The neonatal portion of this developmental pattern is a relative stress nonresponsive period between 3 and 12 days of age (1). Hypothyroidism, which alters maturation of metabolic mechanisms in the brain (9–13), also prolongs the stress nonresponsive period (13).

Neonatal hyperthyroidism accelerates maturation of metabolic activity in the rat. This metabolic maturation includes the brain, accelerating lipid synthesis (14–17), protein synthesis (10, 18), and carbohydrate metabolism (19) in brain tissue. Furthermore, hyperthyroidism accelerates appearance of such behavioral phenomena as audiogenic seizure (20), maze learning (21), general locomotor activity (22), startle response (23, 24), and swimming (25). The present study indicates that neonatal hyperthyroidism accelerates maturation of the hypothalamo–hypophyseal–adrenal axis as well. The hyperthyroid offspring respond to ether stress and exogenous ACTH with both corticosterone synthesis and release at an age (12 days) when euthyroid pups respond only by corticosterone synthesis (2). Thus, while hypothyroidism lengthens the stress nonresponsive period (3), hyperthyroidism appears to shorten it.

Although the initial effect of neonatal hyper-

TABLE I. Effect of Hyperthyroidism on Adrenal and Serum Corticosterone in 12-Day Old Rats.

	Control		Ether stress		ACTH		Saline	
	Euthyroid	Hyperthyroid	Euthyroid	Hyperthyroid	Euthyroid	Hyperthyroid	Euthyroid	Hyperthyroid
Adrenal Corticosterone								
μg/100 mg	2.88[a] ±0.19	3.20 ±0.18	4.33[b] ±0.23	5.48[b,c] ±0.39	4.47[b] ±0.20	5.97[b,c] ±0.29	3.34 ±0.26	4.57[b,c] ±0.34
Ng/pair adrenals	99.4 ±5.1	87.6 ±5.6	137.6[b] ±9.3	147.5[b] ±10.7	125.0[b] ±5.9	149.1[b,c] ±7.2	101.3 ±8.4	117.5[b] ±10.2
Serum Corticosterone								
μg/100 ml	11.84 ±0.27	11.73 ±0.24	11.88 ±0.59	19.87[b,c] ±0.67	12.85 ±0.84	20.31[b,c] ±0.84	11.72 ±0.33	11.48 ±0.28
Ng — total	153.1 ±5.0	148.5 ±8.9	155.4 ±9.2	262.4[b,c] ±10.2	148.2 ±9.2	259.4[b,c] ±9.4	150.2 ±8.0	140.9 ±7.2

[a] Mean ± SE for 10 rats.
[b] Significantly different from control, $P < 0.01$.
[c] Significantly different from euthyroid, $< P$ 0.01.

thyroidism is an acceleration of brain maturation and behavioral development, as adults these animals demonstrate retarded physiological (26), brain (27, 28), and behavioral (29) development. Indeed, the adult rat made hyperthyroid at birth resembles the animal made hypothyroid at birth in many aspects (27, 30). The status of the hypothalamo–hypophyseal–adrenal axis of the adult rat made hyperthyroid neonatally remains to be determined.

Summary. The 12-day-old pups of rats fed 0.05% thyroid during gestation and lactation revealed normal levels of adrenal and serum corticosterone. However, thyroid feeding accelerated the maturation of the hypothalamo–hypophyseal–adrenal axis as ether stress and ACTH resulted in both corticosterone synthesis and release in 12-day-old pups whereas steroid release was not invoked in euthyroid rats. Hyperthyroidism may shorten the stress nonresponsive period.

1. Schapiro, S., Geller, E., and Eiduson, W., Proc. Soc. Exp. Biol. Med. **109**, 937 (1962).

2. Levine, S., Glick, D., and Nakane, P. K., Endocrinology **80**, 910 (1967).

3. Meserve, L. A., and Leathem, J. H., Develop. Psychobiol. **6**, 123 (1973).

4. Wolf, R. C., and Leathem, J. H., Endocrinology **57**, 286 (1955).

5. Hilf, R., Burnett, F. F., and Borman, A., Cancer Res. **20**, 1389 (1960).

6. Butte, J. C., Kakihana, R., Farnham, M. L., and Noble, E. P., Endocrinology **92**, 1775 (1973).

7. Levine, S., *in* "Progress in Brain Research" (D. de Weid and J. J. A. W. M. Weijnen, eds.), Vol. 32, p. 79, Elsevier, Amsterdam (1970).

8. Levine, S., and Mullins, R. F., Jr., Science **152**, 1585 (1966).

9. Balazs, R., Brooksbank, B. W. L., Davison, A. N., Eayrs, J. T., and Wilson, D. A., Brain Res. **15**, 219 (1969).

10. Cocks, J. A., Balazs, R., Johnson, A. L., and Eayrs, J. T., J. Neurochem. **17**, 1275 (1970).

11. Dainat, J., Rebiere, A., and Legrand, J., J. Neurochem. **17**, 581 (1970).

12. Meisami, E., Valcana, T., and Timiras, P. S., Neuroendocrinology **6**, 160 (1970).

13. Schwark, W. S., Singhal, R. L., and Ling, G. M., J. Neurochem. **19**, 1171 (1972).

14. Hamburgh, M., and Bunge, R. P., Life Sci. **3**, 1423 (1964).

15. Myant, N. B., and Cole, L. A., J. Neurochem. **13**, 1299 (1966).

16. Hamburgh, M., Gen. Comp. Endocrinol. **10**, 198 (1968).

17. Walravens, P., and Chase, H. P., J. Neurochem. **16**, 1477 (1969).

18. Klee, C. B., and Sokoloff, L., J. Neurochem. **11**, 709 (1964).

19. Moore, T. J., Lione, A. P., and Regen, D. M., Amer. J. Physiol. **255**, 925 (1973).

20. Hamburgh, M., and Vicari, E., Anat. Rec. **127**, 302 (1957).

21. Eayrs, J. T., Anim. Behav. **12**, 195 (1964).

22. Schapiro, S., Endocrinology **78**, 527 (1966).

23. Schapiro, S., and Norman, R. I., Science **155**, 1279 (1967).

24. Schapiro, S., Gen. Comp. Endocrinol. **10**, 214 (1968).

25. Schapiro. S., Salas, M., and Vukovitch, K., Science **168**, 147 (1970).

26. Bakke, J. L., Lawrence, N., and Robinson, S., Neuroendocrinology **10**, 183 (1972).

27. Nicholson, J. L., and Altman, J., Brain Res. **44**, 25 (1972).

28. Pelton, E. W., and Bass, N. H., Arch. Neurol. **29**, 145 (1973).

29. Davenport, J. W., and Gonzalez, L. M., J. Comp. Physiol. Psychol. **85**, 397 (1973).

30. Bakke, J. L., and Lawrence, N., J. Lab. Clin. Med. **67**, 477 (1966).

Received June 18, 1974. P.S.E.B.M., 1974, Vol. 147.