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THYROID FEEDING FROM CONCEPTION AND HYPOTHALAMO-PITUITARY-ADRENAL AXIS RESPONSE IN 30-DAY-OLD RATS[1]

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ABSTRACT. Elevation of thyroid status in pre- and postnatal rats has been previously shown to accelerate the development of the neuroendocrine axis controlling adrenal response to stress. The present study determined the influence of feeding desiccated thyroid powder (0.03%) in the maternal diet from conception until young were 30 d old. Circulating corticosterone levels were compared in control and thyroid-fed young before stimulation, after a general stress (one min exposure to ether fumes), direct adrenal stimulation (subcutaneous adrenocorticotropin (ACTH) injection) or control injection (physiological saline subcutaneously). Corticosterone levels were measured by both fluorometric assay and radioimmunoassay (RIA). These comparisons revealed that thyroid feeding did not significantly influence basal corticosterone levels, or response to general stress or saline injection. However, injected ACTH stimulated a significantly greater adrenal response in controls than thyroid-fed rats. Since responses relying on endogenous ACTH were not altered by thyroid feeding, it is suggested that absorption of subcutaneously injected ACTH was subnormal in the experimental animals. Results were similar for both assay techniques, indicating that the advantages of precision and specificity given by RIA over fluorometric assay were not critical in this study.

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INTRODUCTION

The hypothalamo-pituitary-adrenal (HPA) axis is the controlling mechanism for adrenocortical output of glucocorticoid hormone in response to stress. Between postnatal d three and 14 the HPA axis of the rat displays a relative stress non-responsive (SNR) period, during which a general stress results in a subnormal release of corticosterone, the primary glucocorticoid in the rat. When first described by Schapiro et al. (1962), the SNR period was felt to be absolute, with no corticosterone being released in response to stress during this time. However, technological advancements in assay techniques have revealed this period to be relative, with the stress response being present, but attenuated (Schoenfeld et al. 1980, Milkovic et al. 1982). From 15 d of age onward, the adrenal gland produces and releases a greater amount of corticosterone in response to a standard stress or an injection of adrenocorticotropic hormone (ACTH) until the adult response level is reached (Levine et al. 1967). The relative SNR period may be prolonged by depression of the thyroid status (Meserve and Leathem 1973, 1981), and shortened by elevation of thyroid status (Meserve and Leathem 1974, Poland et al. 1979). Indeed, Poland et al. (1979) have found chronic postnatal injection of thyroxine to increase corticosterone secretion in response to stress between four and 18 d of age, and to elevate basal corticosterone levels between 12 and 18 d of age.

The present study was conducted to determine the influence of thyroid hormone administration from conception to 30 d of age on the functional integrity of the HPA axis by measuring basal serum corticosterone levels and increases in corti-

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costerone after either general stress, ACTH injection, or physiological saline injection. Previous investigators have reported that determination of corticosterone concentration by radioimmunoassay (RIA) provides greater specificity and sensitivity than other assay methods (Gwosdow-Cohen et al. 1982). Because of these reports, corticosterone concentrations were measured by both RIA (Endocrine Sciences 1981) and by a less expensive and less time-consuming fluorometric method (Glick et al. 1964) to determine whether either method was superior in providing information about HPA axis response.

METHODS AND MATERIALS

Female rats derived from Sprague-Dawley stock in the Bowling Green State University animal facility were maintained under controlled conditions of temperature (21 ± 2°C) and light (12L:12D, L-0700 to 1900), and fed Wayne Lab Blox (Continental Grain Co., Chicago, IL) and tap water ad libitum. Upon reaching a body weight of 150-200 g, rats were mated to males of the same strain and isolated on the first day of pregnancy as determined by presence of sperm in a vaginal lavage. Pregnant rats which provided control pups were continued on Wayne Lab Blox. Animals which provided experimental young were given Wayne Lab Blox Mash, into which desiccated thyroid powder (Sigma, St. Louis, MO) was thoroughly mixed at a concentration of 0.03% (w/w), from the first day of pregnancy through gestation and lactation until the young were tested for HPA axis response at 30 d of age. All litters were reduced to eight pups at five d of age by randomly removing the excess young, regardless of sex since sex has been found to be a relatively unimportant factor in previous HPA studies (Meserve and Leathem 1973, 1981, Poland et al. 1979, Schoenfeld et al. 1980). It was necessary to introduce a variable with regard to presence or absence of the mother toward the end of the experiment. Young of mothers fed control diet were weaned at 21 d because prolongation of the together time resulted in the mother killing one or more members of the litter before 30 d of age. Conversely, removal of mothers fed thyroid powder from their litters at 21 d of age resulted in death of pups before 30 d of age, and mothers left with these litters continued to exhibit maternal behavior and care (these young had milk in their stomachs, as well as feed) through 30 d. Consequently, to keep litter size constant at eight young in both conditions control animals were weaned and thyroid-fed pups were not. Ultimately, five litters of eight pups each in each dietary condition were used in the experiment for a total of 40 control animals and 40 thyroid-fed animals.

At 30 d of age, young were randomly assigned to one of four treatment groups (10 rats/group of control or thyroid-fed animals): (1) Control — animals decapitated within two min of entering the animal room to prevent handling stress; (2) Ether Stress — animals exposed to ether fumes for 60 sec and decapitated 15 min later; (3) ACTH Injection — animals administered a subcutaneous injection of 4.0 IU ACTH (ACTHar Gel, Armour Pharmaceutical Co., Phoenix, AZ) and decapitated 15 min later; (4) Saline Control — animals administered a subcutaneous injection of a volume of physiological saline equal to that of the ACTH injection and decapitated 15 min later. Two pups from each control litter and two pups from each experimental litter were assigned to each condition.

On decapitation, blood was collected from severed neck vessels, allowed to clot, and centrifuged to separate serum which was stored at −20°C for future estimation of corticosterone content. Immediately after collection of the blood sample, body weight was determined to the nearest 0.1 g with a triple beam balance, and wet weights of pituitary, thyroid and adrenal glands were measured to the nearest 0.1 mg with an analytical balance.

Serum corticosterone concentrations were measured by both fluorometric assay (Glick et al. 1964) and by radioimmunoassay (Endocrine Sciences 1981). Briefly, the fluorometric assay involved extraction of serum samples with chloroform (recovery >95%), mixing the extract with sulfuric acid-ethanol fluorescing reagent, and comparison of the fluorescence of serum extracts with that of corticosterone standards using a Turner Model 111 fluorometer (G.K. Turner Associates, Palo Alto, CA) with an excitation wavelength of 470 nm and an emission wavelength of 530 nm. Serum samples for RIA were incubated with borate buffer; then the buffer-serum mixture was evaporated to dryness in a vacuum oven. Anti-corticosterone antibody (Endocrine Sciences, Tarzana, CA, <4% cross-reactivity with any other natural steroid) and [1,2−^{3}H]-corticosterone (New England Nuclear, Boston, MA) were concurrently added to the evaporated sample and incubated for 45 min at 37°C and 2 h at room temperature. Incubation was terminated by ammonium sulfate precipitation, and bound and free steroid were separated by centrifugation at 1110 xg for 10 min. Supernatant fluid was removed and radioactivity was determined by scintillation counting. Percent free labeled corticosterone was calculated and plotted by hand against a standard curve for known amounts of unlabeled corticosterone to determine serum content.

Body and organ weight data were statistically analyzed by Student's t-test. Corticosterone concentrations were analyzed by two-way analysis of variance with multiple comparisons. Significance was ascribed to $P < 0.05$.

RESULTS

Since statistical analysis revealed no influence of treatment group on body and organ weights, these data were collapsed and compared only relative to thyroid feeding. Furthermore, there was no significant effect of sex or litter on corticosterone concentration so individual pups were used as the unit of analysis.

Subjective observation of thyroid-fed pups gave the impression of very feeble and poorly developed animals. Locomotor ability appeared subnormal, and fur was juvenile in appearance. The altered developmental status of the thyroid-fed animals was substantiated by the inability to successfully wean them at 21 d as was done with control pups, and their significantly depressed body weight at 30 d of age (table 1). Mean wet weights of pituitary and adrenal glands appeared to be influenced minimally by thyroid feeding, and the small differences observed were eliminated by normalizing organ weight to body weight. On the other hand thyroid gland weight was depressed on both an actual weight and a relative weight basis, being 45% and 54% of control, respectively (table 1).

Analyses of serum corticosterone concentration by fluorometric assay and RIA provided results which were similar regardless of technique, although RIA estimates were generally slightly lower than fluorometric ones (fig. 1). Thyroid feeding depressed control corticosterone levels slightly, but not significantly ($P > 0.05$), and had no significant positive or negative effect on corticosterone response to ether stress. However, exogenously administered ACTH had significantly less stimulatory effect on corticosterone release from adrenal glands of thyroid-fed pups (76%) compared to controls. Saline injection proved to be a general stress comparable with exposure to ether fumes in control

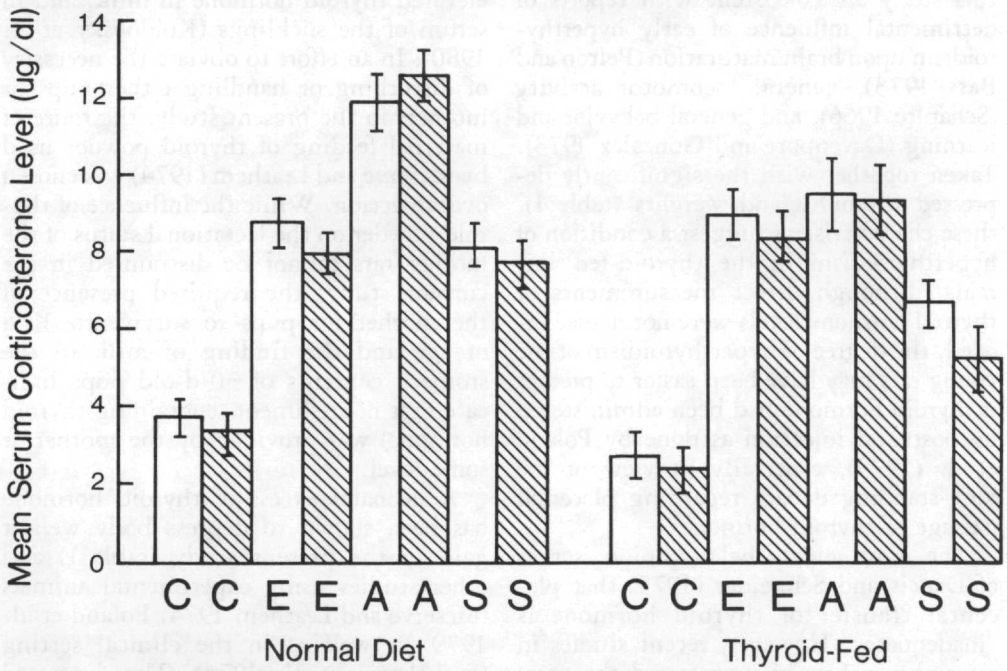

FIGURE 1. Circulating corticosterone levels in 30–d–old control and thyroid–fed rats. Each bar represents mean ±SEM for 10 rats. Open bars — fluorometric assay. Hatched bars — radioimmunoassay. C–basal levels. E — ether stress. A — ACTH injection. S — saline injection.

TABLE 1

Comparison of body weight and pituitary, thyroid, and adrenal gland weights of 30-d-old control and thyroid-fed rats.

	Control*	Thyroid Fed*
Body weight (g)	72.0 ±1.6	56.7 ±2.1**
Pituitary weight (mg)	2.5 ±0.1	2.1 ±0.1
(mg/100g b.w.)	3.5 ±0.2	3.7 ±0.2
Thyroid weight (mg)	15.2 ±0.8	6.9 ±0.5**
(mg/100g b.w.)	21.7 ±1.7	11.8 ±0.9**
Adrenal weight (mg)	22.6 ±0.7	19.2 ±0.9
(mg/100g b.w.)	34.5 ±1.3	34.2 ±1.1

*Values represent mean ±SEM for 40 rats.
**Significantly different from control ($P < 0.05$).

animals. The saline response was slightly depressed in thyroid-fed animals but only significantly so when measured by RIA.

DISCUSSION

The retarded developmental characteristics observed in the thyroid-fed young of this study are consistent with reports of detrimental influence of early hyperthyroidism upon brain maturation (Pelton and Bass 1973), general locomotor activity (Schapiro 1966), and general behavior and learning (Davenport and Gonzalez 1973). Taken together with the significantly depressed thyroid gland weights (table 1), these characteristics suggest a condition of hyperthyroidism in the thyroid-fed animals, although direct measurements of thyroid hormone levels were not made. Indeed, the degree of hyperthyroidism of the young rats may have been easier to predict if thyroid hormone had been administered by postnatal injection as done by Poland et al. (1979), especially in view of the long-standing debate regarding placental passage of thyroid hormones.

The most widely held opinion is that of Dancis and Schneider (1975) that placental transfer of thyroid hormone is "inadequate." However, recent studies in rat embryos have demonstrated the presence of thyroid hormones four d after uterine implantation, well before onset of fetal thyroid function, suggesting important maternal transfer of the hormone (Obregon et al. 1984). Whether thyroid hormone passes from mother to fetus or not, the maternal milk is a known source of thyroid hormone in the suckling rat (Strbak et al. 1983) which can be increased by elevating thyroid status of the mother as shown by elevated thyroid hormone in milk, and in serum of the sucklings (Koldovsky et al. 1980). In an effort to obviate the necessity of disturbing or handling either pups or mothers in the present study, the route of maternal feeding of thyroid powder used by Meserve and Leathem (1974) was chosen over injection. While the influence of thyroid powder on the lactational status of the mother rats cannot be discounted in the current study, the required presence of the mother for pups to survive to 30 d of age and the finding of milk in the stomach contents of 30-d-old pups indicate that nourishment (containing thyroid hormone) was provided by the mother at some level.

A neonatal excess of thyroid hormone has been shown to depress body weight gain in the present study (table 1) and other studies using experimental animals (Meserve and Leathem 1974, Poland et al. 1979) as well as in the clinical setting (Finkelstein et al. 1974). The depressed growth has been correlated with deficient growth hormone secretion (Finkelstein

et al. 1974, Poland et al. 1979). In this regard, the pattern of endocrine status is similar to that of rats with the opposite thyroid alteration, that is, hypothyroidism (Froelich and Meserve 1982).

Previous studies have shown thyroid feeding to shorten the relative SNR period, with 12-d-old thyroid-fed pups responding to general stress with a greater adrenal corticosterone output than controls (Meserve and Leathem 1974). Furthermore, postnatal thyroxine injection has been found to elevate corticosterone above control levels, both basally and in response to stress, through d 18 (Poland et al. 1979). In the present study, thyroid feeding did not influence either basal corticosterone levels or HPA stress response in 30-d-old animals (fig. 1). It would appear that the effect of thyroid hormone administered via the maternal diet, seen at 12 d (Meserve and Leathem 1974) is a transient one which does not persist until 30 d of age. The more pronounced influence of thyroxine injection on HPA axis status is undoubtedly the result of direct delivery of larger doses of thyroid hormone to the HPA axis of developing rats.

In view of the response of thyroid-fed animals to endogenous ACTH, as seen by ether stress and saline injection (fig. 1), it was somewhat surprising that exogenously administered ACTH was less effective in stimulating corticosterone from the adrenal glands of thyroid-fed rats than from controls, even though the relatively large dosage of ACTH administered by Meserve and Leathem (1981) was duplicated in the present study. While this depressed response to injected ACTH may be the result of a direct interaction between thyroid hormones and HPA axis hormones, it seems unlikely that this interaction would appear only with ACTH injection. Since the thyroid-fed animals appeared debilitated as compared to controls, it is postulated that entry of subcutaneously injected hormone into their general circulation was less efficacious than in control animals. It would be worthwhile to measure circulatory uptake of other subcutaneously injected substances in 30-d-old thyroid-fed rats.

In the present study, the reported advantages of RIA over fluorometric assay with regard to specificity and sensitivity to corticosterone do not appear to outweigh the disadvantages of additional time and expense. While the antibody used by Gwosdow-Chen et al. (1982) was different from that provided by Endocrine Sciences (1981) and used in the present study, the sensitivity and specificity reported for the former do not differ drastically from those of the latter. The only difference between RIA and fluorometric results in the present study occurred in analyses of saline injected animals where RIA revealed a significantly depressed response in thyroid-fed rats which was not detected fluorometrically. The grossness of the difference between basal and stimulated levels of corticosterone (137%-305% increase) is the likely reason that extreme sensitivity is not an important factor in determination of the assay system of choice. Since RIA values were generally slightly lower than fluorometric measurements (fig. 1), especially in the case of saline injection, increased specificity of RIA might be important if more subtle differences were being determined.

In conclusion, thyroid feeding of rats from conception to 30 d of age resulted in depressed body and thyroid gland weights. As compared to controls, basal corticosterone concentrations and those as a result of stress response were not influenced by thyroid feeding. This finding suggests that the enhancement of HPA axis response occurring in 12-d-old thyroid-fed rats has disappeared by 30 d. Indeed, subcutaneously injected ACTH had less influence on adrenal glands of thyroid-fed than control rats. It would be of interest to investigate HPA functional integrity in thyroid-fed rats between 12 and 30 d of age and in thyroxine-injected animals after 18 d of age to determine whether the early thyroid-induced enhancement of HPA function declines gradually or abruptly.

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