Acta Endocrinologica 1986, 112: 7–11

Postnatal development of brain TRH, serum TSH and thyroid hormones in the male and female rat

L. Gayo, B. Bonet, A. S. Herranz[1], R. Iglesias[2], M. J. Toro and E. Montoya

Departamento de Biología y Bioquímica, Facultad de Ciencias, Universidad de Cádiz, Spain, Servicio de Bioquímica[1], Departamento de Investigación, Centro Ramón y Cajal, Madrid, and Cátedra de Fisiología General[2], Facultad de Biología, Universidad de Barcelona, Spain

Abstract. The postnatal development of immunoreactive TRH in the central nervous system (CNS), serum TSH and thyroid hormones was studied in both male and female normal rats. While in most structures of the CNS, TRH increased until day 20–30, serum TSH values peaked at day 15 as did T_4. Significant differences were also obtained between both sexes in these parameters. These data further support the fact that pituitary-thyroid axis maturation is independent of brain TRH.

Two main functions have been established for the peptide thyrotropin-releasing hormone (TRH): its well known role as regulator of the pituitary-thyroid axis, and its role as a neurotransmitter or neuromodulator. In this particular aspect, it has been shown that TRH is widely distributed in the CNS of mammals (Winokur & Utiger 1975; Wilber et al. 1976), being also present in the nervous structures of some invertebrates (Grimm-Jorgensen et al. 1975), and that it has neurotransmitter and behavioural effects (Wilber et al. 1976).

The actions of TRH stimulating TSH synthesis and secretion are well documented, both from the pharmacological and physiological point of view (Montoya et al. 1975; Vale et al. 1977; Szabo & Froman 1977). Sex differences in serum TSH and thyroid hormones have been reported in adult animals (Fukuda et al. 1975; Kieffer et al. 1976; Simpkins et al. 1976; Fukuda & Greer 1978; Chen 1984). Although the prenatal and postnatal development of different parameters of the TRH-pituitary-thyroid axis has been previously reported (Dussault & Labrie 1975; Cons et al. 1975; Fisher et al. 1977; Fukuda & Greer 1978; Oliver et al. 1980; Lamberton et al. 1984) no data are available to relate the neonatal change of different sources of TRH (CNS) with the controlled parameters (TSH and thyroid hormones) in both sexes.

The present report represents an attempt to elucidate whether these parameters are interrelated during postnatal development and if there is sex dependence.

Requests for reprints: Eladio Montoya, Departamento de Biología y Bioquímica, Facultad de Ciencias, Apartado de Correos 40, Puerto Real, Cádiz, Spain.

Materials and Methods

Animals

Female Sprague-Dawley rats, fed standard laboratory rat chow, were housed under an automatically controlled temperature (20–23°C) and light-dark cycle (08.00–20.00 h); one adult (pregnant or with litter of 10 pups), or 6 pups (after weaning at 21 days) per cage. The animals were decapitated at 5, 10, 15, 20, 30 and 45 days after birth. Decapitation was carried out between 10.00 and 12.00 a.m.

Samples

Immediately after decapitation, the brain was removed and the olfactory lobes, hemispheres, cerebellum, diencephalon, hypothalamus, brain stem and spinal cord were dissected, placed in liquid nitrogen and kept at −70°C till processed. Thereafter, they were weighed and homogenized in 10 volumes (w/v) of chilled 90% methanol by sonication. After centrifugation at 2500 g for 30 min at 4°C, the supernatants were collected and brought to dryness under nitrogen flow at 37°C. Dry extracts were suspended in 0.15 M NaCl, 0.01 M phosphate (PBS), pH 7.4 and kept at −20°C until TRH assay was performed.

Trunk blood was allowed to clot. After centrifugation aliquots of sera were kept at −20°C until assayed for thyroid hormones and TSH. In animals aged less than 10 days, pools of sera (2−3 animals) were made.

Assays

TRH was measured by RIA as previously reported (Montoya et al. 1975) using synthetic TRH (Beckman) for radioiodination and as reference material. Minimal detectable dose was 20 pg/tube.

Serum TSH was measured by RIA using the immunoreactants kindly provided by the NIADDK, and goat anti-rabbit gamma globulin obtained in our laboratory.

Serum T_3 and T_4 were measured by RIA, as described by Obregón et al. (1978, 1979) using antibodies from Henning. L-T_2 and L-T_3 were used for radioiodination and L-T_3 and L-T_4 as reference. Iodothyronines were from Sigma. Antibody bound and free hormone were separated using polyethyleneglycol (Carbowax 6000) and bovine gamma globulin.

Statistics

Data are expressed as means (6−10 animals) ± SE. Comparisons between groups were done using the non-paired Student's *t*-test. Statistically significant differences were considered at $P < 0.05$.

Results

Immunoreactive TRH

In general, the concentrations of TRH found were highest in the hypothalamus, followed by the diencephalon, spinal cord, olfactory bulb, brain stem, cerebellum and hemispheres, in this order.

TRH concentration in the hypothalamus showed a progressive increase from day 5 to 30 (Fig. 1A) both in males and females. Values on day 45 were similar to those on day 30. No statistically significant differences were observed in this structure between sexes.

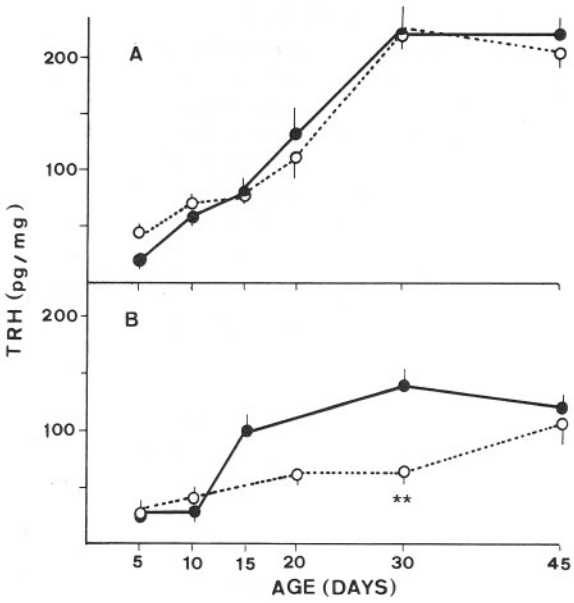

Fig. 1.
TRH concentration during postnatal development in the hypothalamus (A) and diencephalon (B) of male (●——●) and female (o− − −o) rats. Data are expressed as means of 6−8 samples ± SE. Asterisks represent statistically significant differences between males and females ($P < 0.01$).

In the diencephalon (Fig. 1B), a sharp increase in TRH concentration was observed at 15 days in males, with peak values at 30 days, whereas in females a plateau was observed between days 20 and 30, being significant the statistical comparison with males at this age.

In spinal cord (Fig. 2A) in males, maximal values were obtained at 20 days, decreasing thereafter. In females, values increased progressively up to day 20, and then maintained a plateau. This different behaviour led to significant differences between sexes at day 20.

TRH in the olfactory bulb showed similar behaviour to that described for the spinal cord, except that in males there was a further increase at 45 days (Fig. 2B). Statistically significant differences between both sexes were observed at days 20 and 45.

Brain stem TRH values were similar at all times in males and females. Peak values were attained on day 20 (Fig. 2C).

In the hemispheres, there was no detectable TRH at 5 days. The low values detected at day 10

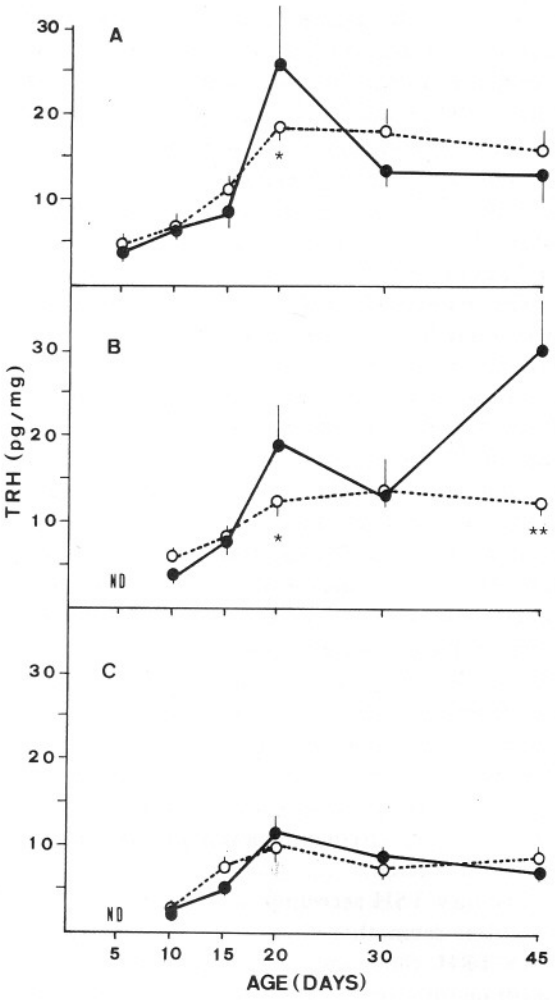

Fig. 2.

TRH concentration during postnatal development in the spinal cord (A), olfactory bulb (B) and brain stem (C) of male (●——●) and female (o– – –o) rats. Data are expressed as means of 6–8 samples ± SE. Asterisks represent statistically significant differences between males and females (* = $P < 0.05$; ** = $P < 0.01$). ND = non detectable.

were maintained with minor changes throughout the different times studied (Fig. 3A). No differences were observed between both sexes.

At 5 and 10 days, TRH was not detectable in cerebellum. Maximal values were seen on day 20, and then decreased. A further increase was observed at 45 days in females (Fig. 3B).

Serum TSH

On day 5, TSH values in males were lower than those in females (Fig. 4). However, values in both sexes increased up to day 15, decreasing later on day 20. This decrease was maintained in females, whereas males showed a further increase at day 30. Significant differences were therefore observed between both sexes at days 30 and 45.

Serum thyroid hormones

Serum T_3 values (Fig. 4) increased both in males and in females from day 5 to 20 (females) or day 30 (males). A slight decrease was seen after this time. No significant differences were observed between both sexes except at 15 days.

A similar behaviour was seen in serum T_4 (Fig. 4), except the decrease at 30 and 45 days was more pronounced in females than in males ($P < 0.05$ at day 45).

Discussion

The developmental pattern of the hypothalamic-pituitary-thyroid axis, as well as the ontogenesis of CNS TRH has been studied previouly (Dussault &

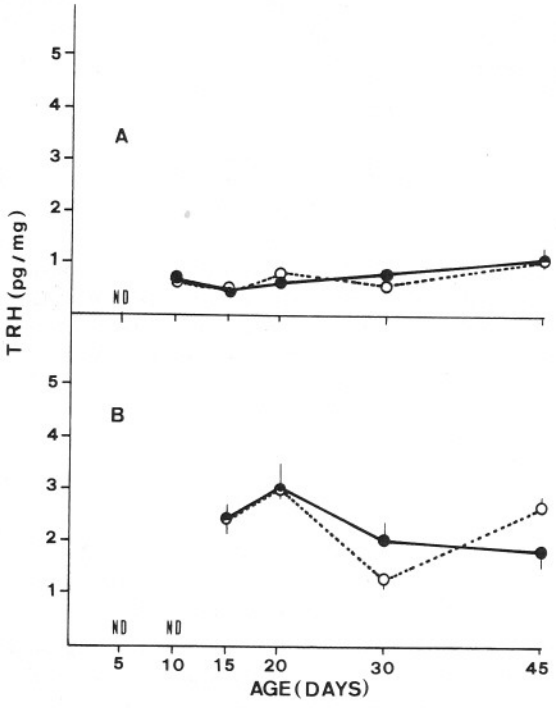

Fig. 3.

TRH concentration during postnatal development in the hemispheres (A) and cerebellum (B) of male (●——●) and female (o– – –o) rats. Data are expressed as means of 6–8 samples ± SE. ND = non detectable.

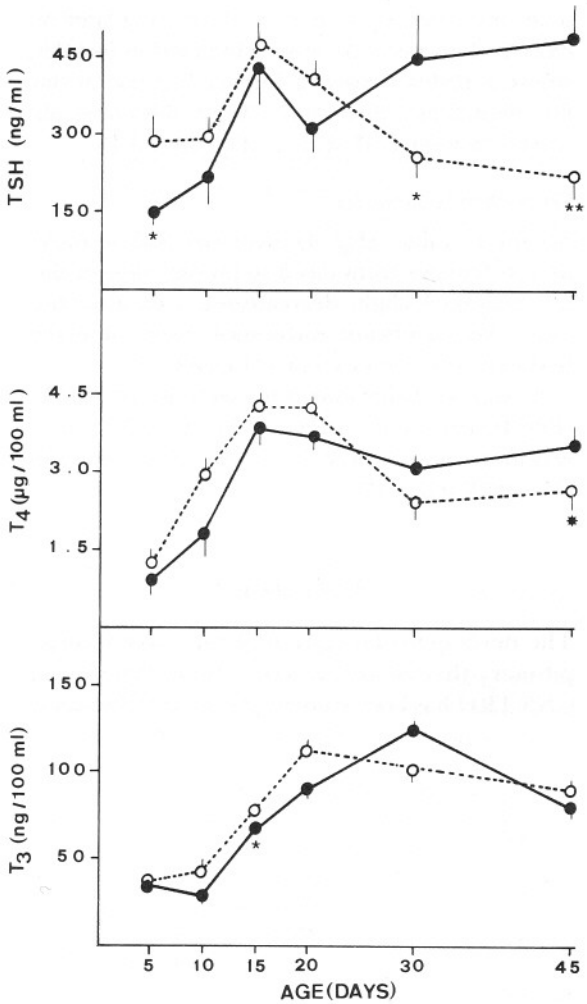

Fig. 4.

Serum levels of TSH (A), T_4 (B) and T_3 (C) during postnatal development in the rat. (●——●) male, (o– – –o) female. Data are expressed as means of 6–8 samples ± SE. Asterisks represent statistically significant differences between males and females (* = $P < 0.05$; ** = $P < 0.01$).

Labrie 1975; Cons et al. 1975; Fisher et al. 1977; Fukuda & Greer 1978; Sowers et al. 1980; Oliver et al. 1980; Lamberton et al. 1984). Most studies have been carried out in one sex or pooling data from both sexes. To our knowledge, the results reported here represent the first study in which the developmental pattern of CNS TRH, and serum TSH and thyroid hormones has been investigated in both sexes.

We found that although there is a progressive increase in the concentrations of CNS TRH, serum TSH and thyroid hormones, there is no parallelism in this increase: while in most structures TRH attained maximal values at day 20 or 30, TSH and T_4 peak values were obtained at day 15. This would be in agreement with results found by others (Theodoropoulus et al. 1979; Strbak & Greer 1979): that TSH secretion is independent of TRH in the perinatal rat.

The pattern of TSH and thyroid hormones presented here is very similar to that found by Fukuda & Greer (1978), who also showed the presence of sexual differences in this parameter from day 30 which marks the onset of puberty in the rat. The existence of sex difference in these parameters has also been reported by others (Fukuda et al. 1975; Kieffer et al. 1976; Simpkins et al. 1976; Chen 1984) and has been shown to be testosterone-mediated (Christianson et al. 1981). Since most of the T_3 present in the pituitary is of intrapituitary T_4 monodeiodination (Larsen et al. 1981), the increase in serum TSH seen until day 15 may reflect the progressive decrease in the pituitary T_4 5'-monodeiodination described by Cheron et al. (1980). As stated by these authors, this phenomenon would also explain the coexistence of low circulating levels of thyroid hormones and TSH.

Pituitary TSH secretion and thyroid hormone secretion seem therefore to be independent of CNS TRH. Since most of the parameters of rat brain maturation, including cell proliferation and protein synthesis, are not achieved until day 17 of postnatal development (Winick & Noble 1965, 1966), the TRH increase in CNS during this time may represent the progressive maturation of TRH synthetising neurons and mechanisms. Whether this maturation depends on thyroid hormone or not remains to be established. Besides thyroid hormone, other hormones so far unknown may also influence TRH synthesis, since sex differences in TRH concentration in different parts of the CNS were present at different times of postnatal development.

Acknowledgments

The authors wish to express their gratitude to the NIADDK, NIH for generously providing the TSH immunoreactants, and to Ms. P. Peers for editorial assistance.

References

Chen H J (1984): Age and sex difference in serum and pituitary thyrotropin concentration in the rat: influence by pituitary adenoma. Exp Gerontology 19: 1–6.

Cheron R G, Kaplan M M & Larsen P R (1980): Divergent changes of thyroxine-5'-monodeiodination in rat pituitary and liver during maturation. Endocrinology 106: 1405–1409.

Christianson D, Roti E, Vagenakis A G & Braverman L E (1981): The sex related difference in serum thyrotropin concentration is androgen mediated. Endocrinology 108: 529–535.

Cons J M, Umezu M & Timiras P S (1975): Developmental patterns of pituitary and plasma TSH in the normal and hypothyroid female rat. Endocrinology 97: 237–240.

Dussault J H & Labrie F (1975): Development of the hypothalamic-pituitary-thyroid axis in the neonatal rat. Endocrinology 97: 1321–1324.

Fish I & Winick M (1969): Effect of malnutrition on regional growth on the developing rat brain. Exp Neurobiol 25: 534–540.

Fisher D A, Dussault J H, Sack J & Chopra I J (1977): Ontogenesis of the hypothalamic-pituitary-thyroid function and metabolism in man, sheep and rat. Recent Prog Horm Res 23: 59–116.

Fukuda H, Greer M A, Roberts L, Allen C F, Critchlow V & Wilson M (1975): Nyctohemeral and sex-related variations in plasma thyrotropin, thyroxine and triiodothyronine. Endocrinology 97: 1424–1431.

Fukuda H & Greer M A (1978): Postnatal development of pituitary-thyroid function in male and female rats: comparison of plasma and thyroid T_3 and T_4 concentration. J Endocrinol Invest 1: 311–314.

Grimm-Jorgensen Y, McKelvy J F & Jackson I M D (1975): Immunoreactive thyrotrophin releasing factor in gastropod circumoesophageal ganglia. Nature 254: 620–623.

Kieffer J D, Mover H, Federico P & Maloof F (1976): Pituitary thyroid axis in neonatal and adult rats: comparison of sexes. Endocrinology 98: 295–304.

Lamberton P R, Lechan R M & Jackson I M D (1984): Ontogeny of the thyrotropin-releasing hormone and histidyl proline diketopiperazine in the rat central nervous system and the pancreas. Endocrinology 115: 2400–2405.

Larsen P R, Silva J E & Kaplan M M (1981): Relationships between circulating and intracellular thyroid hormones: physiological and clinical implications. Endocr Rev 2: 87–101.

Montoya E, Seibel M J & Wilber J F (1975): Thyrotropin-releasing hormone secretory physiology: studied by radioimmunoassay and affinity chromatography. Endocrinology 96: 1413–1418.

Obregón M J, Morreale de Escobar G & Escobar del Rey F (1978): Concentrations of triiodo-L-thyronine in the plasma and tissues of normal rats as determined by radioimmunoassay: comparison with results obtained by isotopic equilibrium technique. Endocrinology 103: 2145–2153.

Obregón M J, Pascual A, Morreale de Escobar G & Escobar del Rey F (1979): Pituitary and plasma thyrotropin, thyroxine and triiodothyronine after hyperthyroidism. Endocrinology 104: 1467–1473.

Oliver C, Eskay R L & Porter J C (1980): Developmental changes in brain TRH and in plasma and pituitary TSH and prolactin levels in the rat. Biol Neonate 37: 145–152.

Simpkins J W, Bruni J F, Mioduszewski R J & Meites J (1976): Serum and pituitary TSH and response to TRH in developing male and female rat. Endocrinology 98: 1365–1369.

Sowers J R, Resch G, Walker S & Sollars E (1980): Developmental patterns of the hypothalamic-pituitary axis in the euthyroid and hypothyroid male rat. Neuroendocrinology 30: 257–261.

Strbak V & Greer M A (1979): Acute effects of hypothalamic ablation on plasma thyrotropin and prolactin concentrations in the suckling rat: evidence that early postnatal pituitary-thyroid regulation is independent of hypothalamic control. Endocrinology 105: 488–492.

Szabo M & Froman L A (1977): Suppression of cold-stimulated thyrotropin secretion by antiserum to thyrotropin-releasing hormone. Endocrinology 101: 1023–1033.

Theodoropoulus T, Braverman L E & Vagenakis A G (1979): Thyrotropin-releasing hormone is not required for thyrotropin secretion in the perinatal rat. J Clin Invest 63: 588–594.

Vale W, Rivier C & Brown M (1977): Regulatory peptides of the hypothalamus. Annu Rev Physiol 39: 473–527.

Wilber J F, Montoya E, Plotnikoff N P, White W F, Gendrich R, Renaud L & Martin J B (1976): Gonadotropin-releasing hormone and thyrotropin-releasing hormone: distribution and effects in the central nervous system. Recent Prog Horm Res 32: 117–159.

Winick M & Noble A (1965): Quantitative changes in DNA, RNA and protein during prenatal an postnatal growth in the rat. Dev Biol 12: 451–466.

Winick M & Noble A (1966): Cellular response in rats during malnutrition at various ages. J Nutr 89: 300–306.

Winokur A & Utiger R D (1975): Thyrotropin-releasing hormone: regional distribution in the rat brain. Science 185: 265–266.

Received on June 27th, 1985.