REVISTA ESPAÑOLA DE FISIOLOGIA, 34, 301-308. 1978

Changes in Parameters of Growth Hormone and Thyrotrophic Hormone, and of Thyroid Function, During the Early Postnatal Period in the Rat

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(Received on November 25, 1977)

A. M. PASCUAL-LEONE, M. D. GARCIA and F. HERVAS. *Changes in Parameters of Growth Hormone and Thyrotrophic Hormone, and of Thyroid Function, During the Early Postnatal Period in the Rat.* Rev. esp. Fisiol., 34, 301-308. 1978.

The body length, and the weight of the body, liver, kidney and brain have been measured daily in rats during the first 10 days after birth. The plasma and pituitary growth hormone and thyrotrophic hormone levels were also determined, as well as the thyroidal ^{127}I content and the plasma PBI. This observation period comprises a critical stage during which administration of large doses of thyroid hormones result in a permanent derangement of the thyroid-pituitary interrelations, and impairment of body growth and pituitary GH economy.

The rate of growth of body, liver and kidney has been found to decrease significantly from day 7th to 9th of post-natal age, laer to increase again. The pattern of the changes observed in the plasma and pituitary GH levels during the same period might well account for the alterations in growth patterns. The rate of growth of the brain, however, is not decreased during this stage, and appears to be independent of the changes in GH economy. No clearcut pattern of changes was observed in plasma TSH levels; the pituitary TSH and thyroidal ^{125}I contents increased progressively during the entire observation period. Plasma from suckling rats often contained high concentrations of non identified iodinated compounds, which were not thyroid hormones. Results are discussed in terms of the possible relationships between thyroid hormone and GH economy during a critical developmental period.

The syndrome resulting from the administration of high doses of thyroxine (T_4) or tri-iodothyronine (T_3) during the early post-natal period (1, 9), the so-called «neo-T_4» syndrome, has recently been studied by us with respect to GH economy (16). It was found that rats treated with high T_4 doses at an early post-natal

age, had a decreased pituitary GH content, when studied at 22 days of age. This was not an unexpected finding, considering that EAYRS and HOLMES (9) reported a decreased body weight and pituitary ecidophil count in similarly treated rats, studied at 25 and 100 days of age.

It appeared interesting to us to obtain a more precise knowledge of thyroid hormone. THS and GH economies in *normal* neo-natal rats, during the period when they are specially sensitive to excessive thyroid hormone exposure. This period comprises the first 14 days of life (7). With the development of specific radioimmunoassays (RIA) for rat TSH and GH, several studies have appeared describing changes in the plasma and/or pituitary content of these hormones as a function of the age of the rat (3, 4, 6, 15, 19, 22, 24).

To our knowledge the changes in the pituitary and plasma concentrations of both TSH and GH, as determined almost daily for the first 14 days of life have not been reported. As will be seen, this period appears to comprise one of definite changes in the GH economy of the normally developing rat.

Materials and Methods

Animals: Wistar rats were used throughout. On the day of birth, litters were distributed in such a way that there were 8 female pups/mother. The day of birth was considered as 0 days of post-natal age. Pups were sacrificed always between 9-10 am by bleeding under ether anaesthesia from the iliac vessels, into heparinized glassware. For the study of changes of the length and the weights of the body, liver, kidney, and brain, and of thyroidal iodine content and plasma PBI, 8 pups were sacrificed on days 1, 2, 3, 4, 12, and 14 and 24 pups were sacrificed on days 6, 7, 8, 9 and 10 of post-natal age. Immediately after sacrificed, the liver, kidneys, brains and thyroids were dissected out and weighed, the pooled thyroids being kept at —20° C. The length was measured from nose to rump. The anterior pituitaries were dissected out, pooled in groups of 8 and homogenized in phosphosaline buffer, pH 7. The homogenate was stored at —20° C.

Because of the large variability of the TSH and GH data, additional litters were sacrificed, so that there were pools, each of 8 pituitaries and plasma, on days 1, 6 and 14, and at least 2-3 pools of 8 samples on the other days included in this study. The milk curdle was obtained from the stomach of a few pups when they were sacrificed, and a weighed aliquot was homogenized in water. An aliquot of the homogenate was used for total iodine determination.

Analytical procedures. The pituitary and plasma GH contents were determined by RIA, using the immunoreactants kindly supplied by Dr. A. Parlow on behalf of the Rat Pituitary Agency of the National Institutes of Arthritis, Metabolic and Digestive Diseases (NIAMDD) at the National Institutes of Health (NIH, Bethesda, U.S.A.), modifications already described (11) being followed. The plasma and pituitary THS contents were determined by the RIA method described by GARCÍA *et al.* (10) data being given in terms of the NIAMDD rat TSH RP 1 reference preparation, also kindly supplied by Dr. A. Parlow. Plasma T_4 was determined in a few plasma pools by RIA, at the Nichols Institute for Endocrinology in Wilmington (California).

All stable iodine determinations were carried out using a modified semi-automated, Zak chloric acid procedure (2). A modification required for maximun sensitivity, permitting detection of 2 mg iodine (J. Benotti, personal communication) was used for the iodine estimations on paper chromatograms of plasma. These chromatograms were prepared i) by transferring 200 μl of whole plasma onto

2.5 cm wide Whatmann 1 paper strips, or ii) by adding a tracer amount of high specific activity ^{125}I-labeled-L-thyroxine to a 1.5 ml aliquot of pooled plasmas and extracting three times with 5 ml each of ethanol containing 10^{-4} M propylthiouracil. The ethanol extracts were evaporated to dryness in a Büchli Rotavapor at 30° C and the dried extracts were transferred with small aliquots of methanol-ammonia (99:1) onto 2.5 cm wide Whatmann 1 paper strips. The chromatograms were developed in n-butanol-ethanol-1 N ammonia (5:1:2). They were dried and cut into strips (2 cm for i, or 1 cm for ii), which were digested with chloric acid for the determination of their iodine content.

Calculations. Mean values, standard deviations, standard errors and the degree of significance of differences between means, were calculated as outlined by SNEDECOR and COCHRAN (23).

Results

There is a progressive increase in body length from day 1 to 7 (fig. 1): the differences between mean values corresponding to groups sacrificed every two days were statiscally significant within this period. However, body length stopped increasing between days 7-9, after which it started augmenting again. The weight of the body, the liver and kidneys increased with age, following a pattern similar to that described for body length. The weight of the brain of the same pups, however (fig. 2), did not stop increasing between days 7 and 9. During this period, the relative weight of the brain (referred to 100 g b.w.) was increasing steadily, the difference between mean values on consecutive days being statistically significant. On the contrary, the relative weights of the liver and kidneys remained constant during the same period (7-9 days).

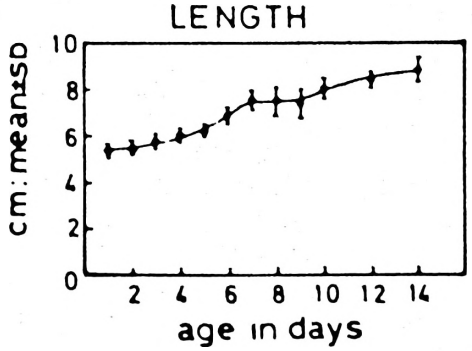

Fig. 1. *Body length is plotted as a function of post-natal age.*

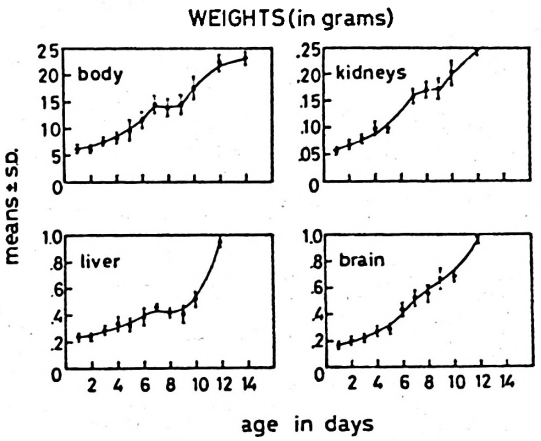

Fig. 2. *The weights of the body, liver, kidneys and brain are plotted as a function of post-natal age.*
The data correspond to the same pups as those of figure 1.

The weights of the thyroids are not shown, as it was often difficult to dissect them properly from other tissues. However, it was clear that the increased during the observation period from a mean (± SD) of 1.5 ± 0.6 mg on day 4 to 5.4 ±1.7 mg on day 12 of age. The thyroidal iodine content (fig. 3) increased progressively during the period studied. The plasma PBI on day 1 and 3 was comparable to that found in our laboratory for adult rats (2-4 μg/100 ml). Between the 3rd and 4th day of age, a sudden increase in the plasma PBI is observed, to values which are very high, and variable from one plasma pool to another even for pups of the same age. Plasmas obtained

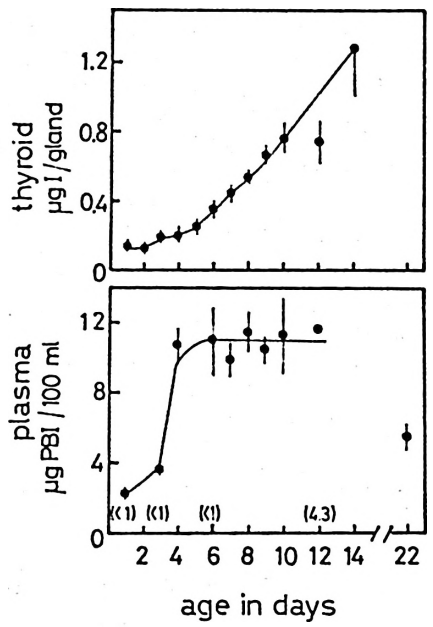

Fig. 3. *The thyroidal iodine content and the plasma PBI of newborn rats are plotted as a function of their post-natal age.*
The number in brackets in the lower panel corresponds to circulating T_4 values (in µg/100 ml) as determined by RIA. Data shown are means ± S.E.

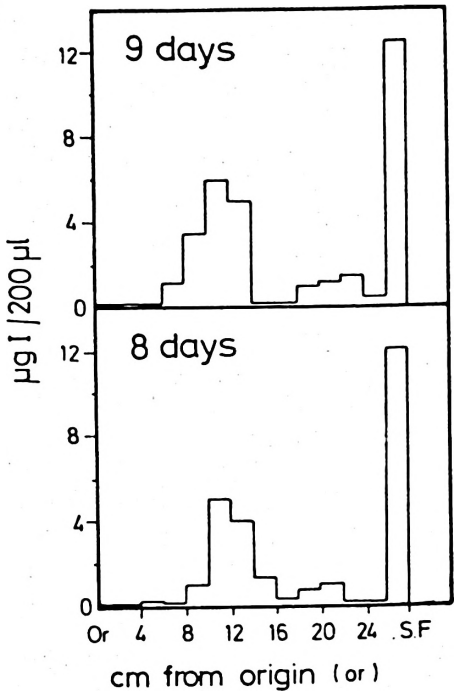

Fig. 4. *Aliquots (200 µl) of pooled plasma from rats of 8 and 9 days of age were submitted to paper chromatography and the stable iodine distribution was determined.*

from 22 days old rats a mean PBI which was again lower, and more similar to the values found for adult animals. The T_4 concentration of some of the plasma pools was determined (fig. 3) and found to be low. Aliquots (200 µl) of plasma pools obtained from 8 and 9 days old pups were submitted to paper chromatography (fig. 4). The total iodine recovered on the chromatograms agreed with the PBI value of the corresponding plasma pool. However, a large part of the iodine was found in regions of the chromatograms which did not correspond to the R_f of the iodothyronines in the chromatographic system used. This was verified by paper chromatography of an ethanol extract of a different plasma pool from 8 days old rats, to which a tracer amount of high specific activity ^{125}I labeled T_4 was added prior to extraction and chromatography. Though results are not shown here, it was found that the chemically determined ^{127}I did not accumulate at the solvent front, as shown in figure 4, but was somewhat retarded; the ^{127}I detected in the first half of the chromatogram did not coincide with the ^{125}I labeled T_4 tracer. The very hight plasma PBI of the 8-14 days old rats was not a constant finding, some plasma pools showing lower values. The distribution of the stable iodine on paper chromatograms was also not well defined and variable from one plasma pool to another, and the nature of the circulating iodinated compounds was not further characterized. This variability might be related to the fact that the total and protein bound ^{127}I content of milk curdles obtained from the stomach of pups of varying ages (1-14 days) was also highly variable. The ^{127}I content ranged from

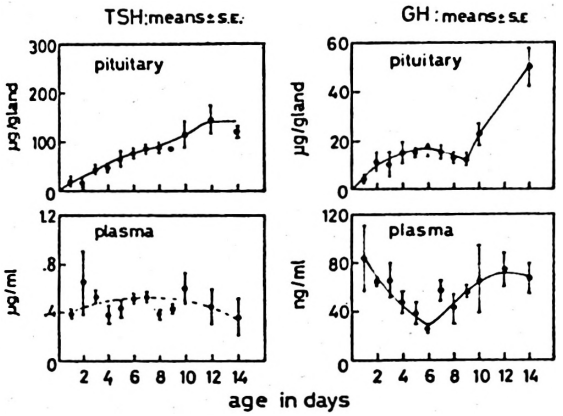

Fig. 5. *The pituitary content of TSH and GH, and the circulating levels of both hormones are plotted as a function of post-natal age.* The data shown are mean values ± S.E. of the values obtained for different pools. Each pool contained 8 pituitaries, or the plasma from 8 pups.

0.6-1.5 µg/g of curdle. Wide fluctuations of the iodine content were also found in several samples of milk taken directly from the lactating mothers.

In these animals a progressive increase of the pituitary TSH contents from day 1 to day 12 was found (fig. 5). The plasma TSH data were extremely variable, even for pups sacrificed at the same age, and a definite pattern of changes could not be discerned. The plasma TSH levels for adult rats of our colony are lower (0.13-0.29 µg/ml).

The pituitary GH content (fig. 5) increased from day 1 to 4, the difference between mean values being statistically significant ($p < 0.01$). It stopped increasing between day 4 and 9, starting to increase quite sharply thereafter. The difference between the mean values corresponding to days 9 and 10 was statistically significant ($p < 0.05$).

The plasma GH levels were higher on day 1 then usually found in adult rats (16.4 ± 2.6 ng/ml, for 110 adult male rats ranging from 100-260 g b.w.). The circulating GH level decreased during the first days of life, reaching the lowest value at 6 days. It then appeared to increase again, the difference between the mean values on day 6 and 9 being statistically significant ($p < 0.001$). By 14 days of age the plasma GH levels were again higher than those usually found in adults; on day 22 it was 17.5 ± 4.3 ng/ml, which is the same as that of the adult rats.

Discussion

It is known that one of the most important phases of cerebral growth occurs during the first 9 days of post-natal life (5, 13), and that during this period important maturational, morphological and biochemical changes are occurring. Present data confirm this, as regards the intense rate of increase in the weight of the brain even during a period (7-9 days) when the body, liver and kidneys stopped increasing in weight.

Circulating GH levels were found to be quite variable during the first few days after birth, and high as compared to those of adult rats. They decreased gradually, reaching a nadir at 6 days post-natal age, and later increased again. RIEUTORT (19) and STROSSER (24) have also described a rapid decrease in circulating GH during the first days after birth, from very high values to the levels observed in the adult rats. However, no data were given by these authors between 5 and 10 days of post-natal age, and it is not possible to know whether a nadir was reached at about 6 days of post-natal age. Further comparisons between these different studies are also not possible, because of the different manner in which the blood samples were obtained. The pituitary GH content increased gradually until day 4, and then remained relatively constant, until a rapid increase started between day 9 and 10 days of post-natal age. In a previous study BIRGE *et al.* (3) described that pituitary GH content increased between day one and 10 of post-natal age, though no values were given for the intervening

period. The GH content found at 10 days (22.0 + 2.4 μg/gland) agrees with the present data. So do the pituitary GH data reported for 10 day old pups by RIEUTORT (19).

The pattern of changes in pituitary and plasma GH levels could account for the type of curves observed when body length and weight and liver and kidney weights are plotted as a function of post-natal age, though other hormones not measured in the present study might be involved.

The growth of the brain during this early developmental period, however appears unrelated to GH economy, a conclusion which agrees with the early observation (25) that hypophysectomy of the rat on the 6th day after birth did not impair cerebral weight increase.

The mean circulating TSH values found in the present study were usually higher than those of adult rats; no clearcut pattern of changes with age was observed. This would agree with the results of DUSSAULT (6). On the contrary, in the studies reported by CONS *et al.* (4) and by KIEFFER *et al.* (15) an increase in the circulating levels of TSH took place between days 4 and 20 of post-natal age. It is possible that discrepancies in results from different laboratories are due to variables such as the use and duration of anaesthesia.

Pituitary TSH content of the pups of the present study appears to be increasing gradually within the period of observation in agreement with the data reported by CONS *et al.* (4) for the same post-natal ages. It also showes that the major changes in pituitary TSH content occur after the period included in the present one.

The few low plasma T_4 values obtained in the present study appear to agree with the observation of DUSSAULT (6) and of KIEFFER *et al.* (15). In both studies circulating T_4 levels are very low during the firts few days after birth and were reaching values normal for the adult rat (4-6 μg/100 ml) from about 12 days of age onwards. Circulating T_3 levels were low during the first days of post-natal life (6). Thus, it seemed very unlikely that the very high plasma PBI values often found in the present study represent high levels of thyroid hormone. This was confirmed by the paper chromatographic distribution of the iodine containing compounds. Though the compounds were not identified, they did not correspond to T_4 and T_3. No iodinated material was found with a chromatographic Rf = 0, which would be the one corresponding to iodinated proteins. However, present results are compatible with the idea that the iodinated components found in the plasma of the pups and moving between 7-16 cm from the origin of the chromatograms (fig. 4), might be iodotprosines derived from digested milk: POTTER *et al.* (18) found that most of the iodine in the milk was in this form. High plasma PBI have also been reported in suckling kittens, the values being five fold higher than those of adult cats (14).

Maternal T_4 and T_3 may also be transferred to the suckling rats through the milk (18). Thus, at present, it is very difficult to assess the respective contributions of the thyroidal secretion of the pups and of their mother to the circulating hormone levels during the early post-natal phase, a point which has usually been overlooked in studies of the development of the thyroid-pituitary-hypothalamic complex. Transfer of hormones from the mother, however, does not appear to be enough to significantly substitute for the thyroidal secretion of the pups, considering that thyroidectomy of newborn rats results in hypothyroidism, before weaning (8, 21).

Acknowledgements

We wish to thank Dr. G. Morreale de Escobar for suggestions and fruitful discussions, and Mrs. Blanca Sánchez and Socorro Durán

for skilled technical help. We are grateful to Dr. A. Parlow and the Rat Pituitary Agency of the NIAMDD at NIH for the supply of immunorealtants for the determination of rat GH and TSH.

Resumen

Se determina el peso del cuerpo, hígado, riñón y cerebro durante los diez primeros días de vida de la rata, y también los niveles en plasma y pituitaria de la hormona somatotropa y tirotropa. Asimismo, se dosifica el contenido tiroideo de I^{127} y el PBI plasmático.

La proporción del crecimiento del cuerpo, hígado y riñón decrece significativamente desde el día 7 al 9 de edad y se incrementa más tarde de nuevo. Estos cambios en el crecimiento parecen corresponder a las variaciones en los niveles de GH hipofisario y plasmático. La evolución del crecimiento cerebral, sin embargo, no disminuye en el mismo período, y se presenta como independiente de las variaciones en la economía de GH.

No se observan cambios en los niveles de TSH plasmático. El TSH hipofisario y el contenido del I^{125} tiroideo crecen progresivamente durante este tiempo estudiado. El plasma de rata lactante contiene concentraciones altas de compuestos iodados, no identificados, los cuales no son hormonas tiroideas.

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