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Ontogenetic patterns of thyrotropin-releasing hormone-like material in rat hypothalamus, pancreas, and retina: Selective effect of light deprivation

(islets of Langerhans/radioimmunoassay/neurotransmitter/photoreception)

ENIO MARTINO*, HISAO SEO, ÅKE LERNMARK†, AND SAMUEL REFETOFF

Thyroid Study Unit, Department of Medicine and Biochemistry, University of Chicago, Chicago, Illinois 60637

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ABSTRACT **Recent observations have shown the presence of thyrotropin-releasing hormone-like material (TRH-LM) in rat pancreatic islets and in retina. Its immunological and biological properties are identical to those of synthetic thyrotropin-releasing hormone (thyroliberin). This communication deals with the ontogenesis of TRH-LM in rat pancreas and retina as compared to that of rat hypothalamus. Effects of sex and exposure to constant dark were also studied. Results show that asynchronous changes in the concentration of TRH-LM occur during the postnatal maturation of these tissues, presumably mediated by organ-specific control mechanisms—e.g., light affects only the accumulation of TRH-LM in the retina. TRH-LM may act as neurotransmitter in the regulation of pancreatic islet cell function and in the development of photoreception in the retina. Increases in hypothalamic TRH-LM seem to parallel the development of the pituitary–thyroid secretory activity, but the function of extrahypothalamic TRH-LM remains speculative.**

Thyrotropin-releasing hormone (TRH, thyroliberin) is a tripeptide that was isolated from mammalian hypothalami and characterized as pyroglutamylhistidylproline amide (1, 2). As evidenced by its name, TRH was initially considered to play a specific role in the regulation of pituitary thyrotropin secretion. Subsequently, it has been shown that TRH also stimulates pituitary prolactin and growth hormone secretion (3–5). Effects unrelated to the control of adenohypophyseal function have also been reported. These include: potentiation of behavioral excitation (6), mood elevation (7–9), stimulation of muscular activity of the intestine (10), induction of hypothermia (11), enhancement of cerebral noradrenaline turnover (12), inhibition of the electrical activity of some brain neurones (13), and potentiation of the excitatory action of acetylcholine (14). Thus, it is not surprising that significant amounts of TRH-like material (TRH-LM) have been found in areas of the central nervous system other than the hypothalamus (15–18). The presence of immunoreactive (IR) and bioreactive TRH-LM has also been reported outside the central nervous system in human placenta (19, 20), rat retina (21–23), frog skin (22), and rat gastrointestinal tract, including the pancreas (24–26). In the latter, the main source of TRH appears to be the islets of Langerhans (26).

Although it has been speculated that TRH may act as neurotransmitter or modulator of synaptic function, its function in extrahypothalamic tissues remains unknown, as is the precise cell of origin. Because there appears to be a good correlation between the postnatal changes in hypothalamic IR-TRH content and in pituitary and serum thyrotropin concentrations (27) and because exposure to dark has been shown to reduce the retinal IR-TRH content (21), we undertook a study of the ontogenesis of rat pancreatic and retinal TRH-LM under conditions of normal light–dark cycling or constant darkness. Results are compared to corresponding changes in hypothalamic TRH-LM content. The developmental changes of TRH-LM in these tissues do not appear to be synchronized, and light selectively affects the accumulation of TRH-LM in the retina.

METHODS

All rats were of the CD strain obtained from Charles River Breeding Laboratories. They were housed in individual cages in a temperature-controlled (19–21°C) and artifically illuminated (light from 0700 to 1900) room and allowed free access to Purina Rat Chow and water. Each female rat was impregnated by exposure to a single male for 24 hr. Prior to the expected day of delivery the animals were visited at frequent intervals, spaced at any time by not more than 6 hr. Thus, error in the determination of the pup's age was not greater than 6 hr. After delivery, litters were adjusted to 10 pups per mother. Some mothers with their pups were transferred on the fourth day after delivery to an adjacent room and maintained under the same conditions except for constant darkness.

Animals were killed by decapitation, at times between 1000 and 1100, at different intervals after birth. Tissues were immediately removed in the order of eyes, hypothalamus, and pancreas. Tissues were frozen on dry ice, weighed, and then pooled for extraction of TRH-LM. Animals used for the study of the effect of exposure to constant dark were killed and their eyes were enucleated and frozen in the dark. Three or four pools of 8–12 eyes each were obtained from pups up to 10 days of age. For older pups, each of the three pools for each age contained 4–6 eyes. Four hypothalami and pancreases were pooled from rats up to 8 and 15 days, respectively. Tissues from older animals were handled individually.

Islets of Langerhans were isolated from a pool of 10–15 pancreases from 3- and 23-day-old rats and from adult animals by methods previously described (26). Tissues from animals 8 days or older were divided according to sex. Only male rats were used for the isolation of islets of Langerhans and for the analysis of tissues from animals exposed to constant darkness. A total of 300 pups and 50 adult animals was used in the study.

TRH-LM was extracted from frozen pools of tissue by homogenization in 90% methanol/10% water (vol/vol), using a

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Abbreviations: TRH, thyrotropin-releasing hormone (thyroliberin); TRH-LM, TRH-like material; IR-TRH, immunoreactive TRH; somatostatin-LM, somatostatin-like material.

* Present Address: Cattedra di Patologia Speciale Medica e Metodologia Clinica 2, via Bonanno 48, 56100 Pisa, Italy.
† Present Address: The Steno Memorial Hospital Research Laboratory, DK-2820, Gentofte, Denmark.

glass–glass homogenizer. The residue was pelleted by centrifugation and the supernatant was dried in a water bath at 37°C under a stream of air. The dried extract was diluted in phosphate-buffered saline (0.01 M sodium phosphate/0.15 M NaCl, pH 7.6) and used as such or delipidated with an equal volume of chloroform prior to assay. The efficiency of the extraction was 90% and the recovery of total IR-TRH was not affected by the delipidation procedure.

TRH-LM was measured by a specific and sensitive radioimmunoassay (28). All samples were analyzed in duplicate and at two different dilutions in a single assay. The results are expressed as pg of synthetic TRH per mg of wet tissue weight for hypothalamus, pancreas, and islets of Langerhans and as pg synthetic TRH per single eye for retinal TRH-LM.

We previously established that TRH-LM from islets of Langerhans, whole pancreas, retina, and hypothalamic tissue possesses immunologic and biologic properties quantitatively identical to those of synthetic TRH (23, 26). More specifically, when TRH-LM extracted from each of these tissues was compared to synthetic TRH, it exhibited a parallel dilution displacement curve in the radioimmunoassay, identical inactivation by fresh serum, and a quantitatively similar biologic potency in an assay measuring release of thyrotropin from rat pituitaries in vitro. However, as shown in Fig. 1, the tissue extract affected the elution pattern of TRH on gel chromatography.

RESULTS AND DISCUSSION

Fig. 2 depicts the changes in concentration of TRH-LM in the rat hypothalamus, whole pancreas, islets of Langerhans, and retina during postnatal life. TRH-LM was detectable in the hypothalamus of the 1-day-old rat and increased from a mean level of 43 pg/mg to a peak level of 338 pg/mg at age 23 days. The adult concentration of 212 pg/mg was reached between ages 30 and 45 days. These results, in terms of both absolute values (17, 18, 29) and ontogenetic pattern (27), are in agreement with data obtained by other investigators. We failed to show sex differences or changes related to rearing under conditions of constant darkness. Thus, if the nyctohemeral variation in the concentration of hypothalamic TRH-LM described for the adult rats (29) was present in the neonatal life, it could not be related to the light cycle.

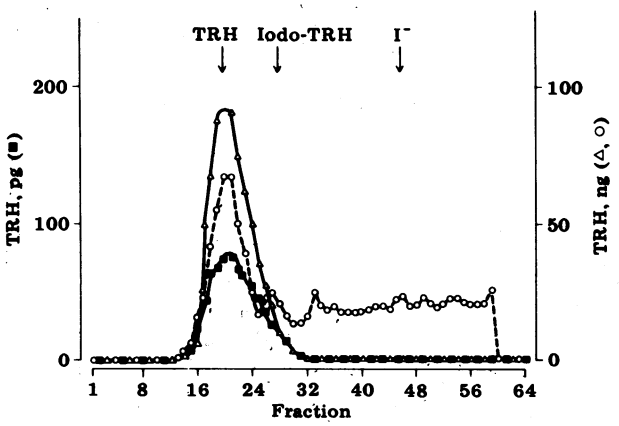

FIG. 1. Sephadex G-10 elution patterns of TRH-LM extracted from rat retina, and synthetic TRH added to retinal extracts. Delipidated and nondelipidated retinal extracts were chromatographed before and after enrichment with 2.5 μg of synthetic TRH (1500 times the concentration of TRH-LM measured in the extract). IR-TRH was measured in each fraction after appropriate dilution. ■, Delipidated extract; △, delipidated extract + TRH; ○, extract + TRH. As shown, the nondelipidated retinal extract altered the elution pattern of synthetic TRH but not its immunoreactivity. The TRH-LM in nondelipidated retinal extracts (not shown) was also polydisperse. The conditions of gel filtration chromatography have been published elsewhere (28). Arrows indicate the elution positions of synthetic TRH, monoiodinated TRH, and iodide in the absence of tissue extract.

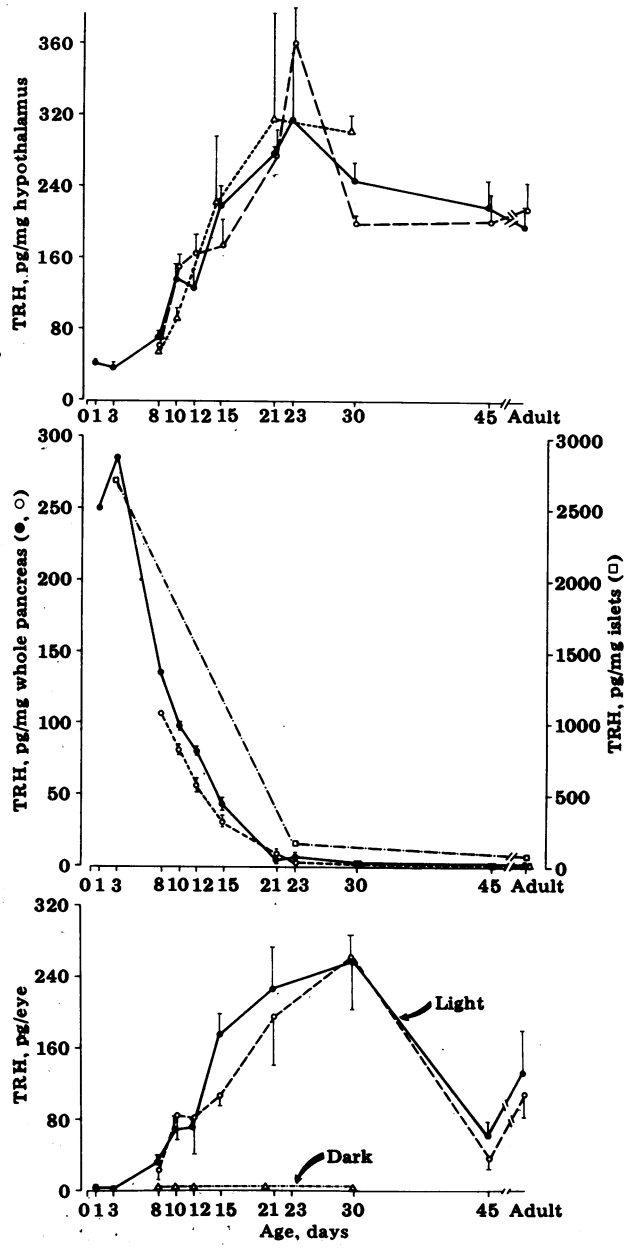

FIG. 2. Changes in the concentration of TRH-LM with age in rat hypothalamus, whole pancreas, isolated islets of Langerhans, and retina. TRH-LM was measured by radioimmunoassay and results are expressed in pg of synthetic TRH per mg of wet tissue weight. Error bars indicate SD. ● and ○, males and females, respectively, reared under normal light cycle (0700–1900); △, males reared in constant darkness; □, males, normal light cycle. Note the asynchrony among tissues in the ontogenesis of TRH-LM and the specific effect of light exposure on retinal TRH-LM. Pups opened their eyes on day 8.

In contrast to the ontogenesis of rat hypothalamic TRH-LM, concentration of pancreatic TRH-LM declined from a mean level of 249 pg/mg (wet weight) at day 1 to a mean adult level of 2.0 pg/mg reached at 45 days of age. TRH-LM concentration in the pancreas of the neonatal rat was approximately 6-fold higher than that in the corresponding hypothalamus, and was similar to the highest hypothalamic TRH concentration achieved at 23 days of age. Between 8 and 15 days of age the concentration of TRH in whole pancreas of female rats was

about 20% lower than in males. As in the case of hypothalamic TRH-LM, rearing of the pups in complete darkness had no effect on the ontogenesis of pancreatic TRH-LM (not shown in Fig. 2). Islet TRH-LM concentrations were 2600 pg/mg at 3 days, 131 pg/mg at 23 days, and 75 pg/mg in the adult. They corresponded to the following mean concentrations for TRH-LM in whole pancreas: 285, 9.5, and 2.0 pg/mg. These results support our previous findings in the adult rat suggesting that the islets of Langerhans are the main, if not the sole, source of pancreatic TRH-LM. Indeed, the concentration ratios of islet to whole pancreas TRH-LM in the 3-day-old and adult rat were 9.12 and 37.5, respectively. The 4.1-fold difference between these ratios is in keeping with the known 4.0-fold difference in the volume of islet tissue relative to total pancreatic weight in young rats as compared to adult animals (30).

Retinal TRH-LM shown in Fig. 2, is expressed in terms of IR-TRH content per eye, or approximately 1 mg of retinal tissue. We have established, in preliminary experiments, that the amount of TRH-LM measured in the extract from whole eyes corresponded to that found in the retina. TRH-LM was undetectable, or less than 1 pg, in eyes of 1- and 3-day-old pups. At day 8, after opening of the eyes, the mean concentration was 29 pg/eye; it rose to a peak level of 258 pg/eye at 30 days of age. The concentration of TRH-LM then declined to a nadir at 45 days. Adult levels ranged from 75 to 200 pg/eye (mean value 132 pg/eye) and are thus in agreement with values reported by Schaeffer *et al.* (21). There were no sex differences. Most striking was the failure to detect TRH-LM in eyes of rats reared under the condition of constant darkness for periods up to 30 days.

The ontogenetic patterns of appearance of TRH-LM in rat hypothalamus, pancreatic islets, and retina are asynchronous. Hypothalamic and retinal concentrations of TRH-LM increase from birth until 23 and 30 days of age, respectively, accompanied by a marked decline in pancreatic islet TRH-LM over the same period of time. Thus, the postnatal development of TRH-degrading activity in rat plasma (31, 32) is unlikely to play a role in the observed changes of TRH-LM in the islet. This contention is further supported by the observation that pups reared in the dark show a normal developmental pattern for hypothalamic and pancreatic TRH-LM in the absence of appearance of detectable TRH-LM in the retina. Our studies thus suggest organ independence in the regulatory control of TRH accumulation, and possibly synthesis.

The function of hypothalamic TRH in the control of pituitary thyrotropin secretion is well established. Hypothalamic lesions depleting TRH or administration of anti-TRH serum cause thyrotropin deficiency and hypothyroidism (33, 34). The ontogenesis of rat hypothalamic TRH-LM is in agreement with the pattern of development of pituitary and thyroid gland activity for the secretion of thyrotropin and thyroid hormone (27). Though TRH stimulates secretion of prolactin and growth hormone (4, 5), its role in the physiologic control of these hormones remains unclear.

The action of extrahypothalamic TRH-LM has not been fully defined. Because of its ubiquitous distribution, it has been speculated that it may function as a neurotransmitter. Because somatostatin is known to antagonize all known stimulatory effects of TRH on pituitary hormones (4, 35), it is possible that islet TRH-LM serves as an antagonist to somatostatin-like material (somatostatin-LM) present in the D cells of the islet (36, 37). If so, the physiologic contribution of TRH-LM in the control of the islet cell should diminish with age due to its marked decline in concentration, while the concentration of somatostatin-LM continues to rise during the postnatal period (38).

The presence of TRH-LM in the rat retina was shown by Schaeffer *et al.* (21). Properties identical to those of authentic TRH were demonstrated immunologically, by cochromatography, and by inactivation with pyroglutamate aminopeptidase. We confirmed the findings of immunologic identity and enzymatic inactivation and furthermore demonstrated that TRH-LM in retinal extracts measured by radioimmunoassay has a biologic potency quantitatively identical to that of synthetic TRH (23). On the basis of studies using thin-layer chromatography, Youngblood *et al.* (39, 40) questioned the biological significance of IR-TRH detected in extrahypothalamic brain tissues, in rat pancreas and eye, and in human placenta. However, in a more recent publication (41), the same group of investigators detected bioreactivity, specific for thyrotropin release, in human placental extracts. Although these authors argue that the bioreactivity failed to coelute with authentic TRH on gel chromatography, results from several investigators have been at variance. In fact, they showed that IR-TRH extracted from rat pancreas and from eyes coeluted with authentic TRH on gel filtration, on thin-layer chromatography in several solvents, and on high-pressure liquid chromatography (21, 24, 25). As shown in Fig. 1, different treatments of tissue extracts may influence the elution pattern of TRH on chromatography.

Our findings that retinal TRH-LM becomes detectable in the neonatal period only after opening of the eyes, and that it remains undectable when the pups are reared in constant darkness, are in agreement with the observations of Schaeffer *et al.* (21). These authors showed diurnal variation of retinal TRH-LM in the adult rat that was totally dependent upon environmental lighting rather than upon the time of the day. It is not known if pups reared in the dark recover their retinal TRH-LM after return to light, and if not, after what length of exposure to the dark. If, as suggested by Schaeffer *et al.*, retinal TRH-LM is involved in photoreception (21), failure to recover the TRH-LM after prolonged absence of light may be related to the development of disuse blindness (amblyopia ex anopsia). Of interest is also the recent demonstration of immunoreactive and bioreactive somatostatin-LM in rat retina (42). The observed increase in the relative content of somatostatin-LM in retinae of blind rats, after optic nerve transection or as a result of hereditary photoreceptor degeneration, again suggests that the reciprocal changes in islet-cell TRH- and somatostatin-LM during ontogenesis and in streptozotocin-induced diabetes mellitus (26, 38, 43) may be related to their possible antagonistic action in these as in other tissues (4, 5, 35).

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