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Presence of thyrotropin-releasing hormone in porcine and bovine retina

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Summary. The thyrotropin-releasing hormone (TRH) has been found in porcine and in bovine retina, and it is indistinguishable from synthetic TRH in its immunological and biological properties. The role of retinal TRH is unknown, it probably acts as a neurotransmitter.

Thyrotropin-releasing hormone (TRH) has been isolated from the hypothalamus of various mammalian species[1,2] and it regulates the secretion of pituitary thyrotropin (TSH), prolactin and in certain circumstances growth hormone[3–5]. TRH is also distributed in extrahypothalamic regions of the brain in animals[6–9] and humans[10–13], and in regions outside the central nervous system, including the gastro-intestinal tract[14], islets of Langerhans of rat pancreas[15], frog skin[16], human placenta[17], and rat and frog retina[16,18].

In the present investigation we report the presence of TRH in the porcine and in bovine retina.

Materials and methods. The porcine and bovine eyes were obtained immediately after sacrifice at a slaughter-house. The retinas were isolated under direct microscopic control, immediately frozen in dry ice and stored at −25 °C until used. TRH was extracted from frozen tissues with 90% methanol as previously described[15]. The efficiency of the extraction procedure was above 90%, as assayed by the recovery of ^{125}I-labeled TRH added to the frozen tissues; the results were thus, not corrected for the extraction losses. TRH was measured by a double antibody radioimmunoassay[19] using ^{125}I-TRH and a specific antibody obtained in rabbits by immunization with synthetic TRH coupled with bovine serum albumin according to the method of Bassiri and Utiger[20]. The minimum detectable amount of TRH was 2 pg/tube. The results were expressed as pg/mg of wet tissue weight. The proteolytic degradation of TRH was evaluated by measuring the immunoreactivity of synthetic or retinal extracted TRH before and after incubation with fresh human serum at 37 °C for 2 h[19]. The biological activity of immunoreactive TRH extracted from porcine retinas was compared to that of synthetic TRH using an in vitro bioassay based on the ability of TRH to release rat pituitary thyrotropin[19].

Results. Immunoreactive TRH was found in the retinas of both animals at a concentration of 6.5 ± 0.3 pg/mg of wet tissue wt (mean ± SE) and 1.5 ± 0.5 pg/mg in porcine and bovine tissues respectively (table). The immunoreactivity of material extracted from porcine and bovine retinas was compared to that of synthetic TRH by analyzing the displacement of ^{125}I-TRH bound to specific antibody. A parallelism between the curves was observed. The fresh human serum incubation showed that it completely inactivated the immunoreactivity of 300–500 pg of synthetic TRH as well as that of equal amounts of immunoreactive TRH extracted from retinas. Finally, the quantity of TSH released into the medium from rat pituitary tissues incubated in vitro with equal amounts of synthetic or retinal-extracted TRH was similar, showing the same biological activity.

Concentration of thyrotropin-releasing hormone in porcine and bovine retinas

Retina (number)	TRH pg/mg wet wt (mean ± SE)
Porcine (16)	6.5 ± 0.3
Bovine (4)	1.5 ± 0.5

Discussion. The present data show that TRH is present in porcine and in bovine retinas. The identity of retinal-extracted and synthetic TRH has been established; they both showed the same immunoreactivity with a specific antibody, complete inactivation after incubation with human fresh serum, and finally similar biological activity as tested by the ability to release TSH from rat pituitaries in vitro.

These findings, obtained in pigs and oxen, extend the observations of Jackson and Reichlin[16] and Schaeffer et al.[18], who found significant amounts of immunoreactive TRH-like material in the retina in the frog and rat, respectively. Recently we observed that the TRH is present in the human retina[20] at a concentration similar to that observed in the human cerebral cortex.

The exact role of retinal TRH is unknown, in animals as well as in humans. The effect of light deprivation on TRH concentration in the retina has been recently reported in adult[18] and neonatal[22] rats; the results show that dark exposure is followed by a marked decrease in retinal TRH content. On the basis of these observations it is suggested that the TRH plays a significant role in retinal function, possibly by acting as a neurotransmitter.

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PRO EXPERIMENTIS

Trichromatic fluorescent vital labeling of bone in the fetal macaque[1]

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Summary. Mineralizing tissue was labeled in the macaque fetus by administering sequential vital bone labels to the pregnant female. The 3 labels used (DCAF, xylenol orange and minocycline) fluoresce different colors, thereby facilitating identification of discrete lines in the rapidly growing bone and a quantitative analysis of bone deposition in utero.

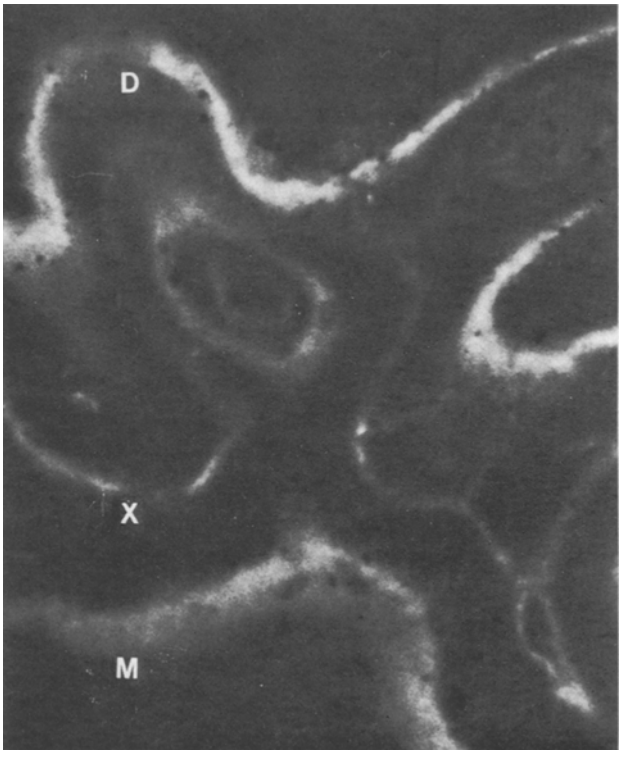
Various fluorescent compounds, particularly antibiotics of the tetracycline group, have been used to label mineralizing bone and dentin in diverse species. Although transplacental transmission of various tetracyclines has been demonstrated in both the rat and human fetus[2,3], there has been no systematic attempt to label mineralizing fetal tissues for the purpose of analysis. We report here a method for labeling in utero bone in the fetal macaque *(Macaca nemestrina)* using 3 fluorescent substances administered sequentially. The substances were DCAF (2,4Bis) N,N'Di (carboxymethyl) aminomethyl fluorescein[4] obtained from ICN Pharmaceuticals, Inc., xylenol orange[5] from Mallenckrodt, Inc., and minocycline hydrochloride from Lederle Laboratories Division, chosen on the basis of reported minimal interference with bone growth compared with that of other vital labels[6,7].

Four pregnant macaques *(Macaca nemestrina),* 1 of known conception date and 3 of estimated conception dates, were started on the marking schedule when the fetus was estimated to be about 145 gestational days. The female was anesthetized and 100 cm^3 of saline solution containing the label compound was administered i.v. by slow drip; fetal heart beat was monitored throughout. After birth the left humerus was fixed, dehydrated in alcohol and dioxane, and embedded in Bioplastic® from Wards Biological Supply. Blocks 250-µm-thick were cut at midshaft with a diamond saw and ground to 40 µm. The sections were mounted and viewed with a bright field condenser microscope with a high pressure mercury lamp as the UV light source. A

In utero trichromatic vital bone labeling of the macaque fetus

Label	Dosage (mg/kg)	Birth	Interval	Age
D, X, M	50, 50, 35	dead	3	170
D, X, M	50, 50, 35	viable	9	170 E
D, X, M	35, 35, 35	dead	1	145 E
D, X, M	20, 20, 20	viable	21	160 E

Labels are given in order of administration at 9-day intervals; D = DCAF, X = xylenol orange, M = minocycline. Interval refers to the number of days between the last prenatal label and parturition. Age given is gestational days at parturition; E = estimated gestational age as obtained by age-predictive regression equations[9].



Contrasting trichromatic fluorescent labels in the humerus from the fetus of estimated 160 gestational days at birth (D, DCAF; X, xylenol orange; M, minocycline). Midshaft transverse section, original magnification: × 52.