Bull. Inst. Zool., Academia Sinica **15**(2): 77-79 (1976)

SCIENTIFIC NOTES

A Preliminary Report on the Effect of Thyroidectomy on Rat Hypothalamic Gonadotropin-Releasing Hormone (GnRH) Contents

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Received for publication, Dec. 30, 1976

Previous investigations on adenohypophyseal gonadotropins in thyroidectomized (Tx) female rats indicates lowered in LH content but varied in FSH[1,7~9]. It is understood that gonadotropins is under the control of hypothalamic gonadotropin-releasing hormone (GnRH). Thus, it is essential to study the variations of hypothalamic GnRH after the removal of thyroid.

MATERIALS AND METHODS

Forty Sprague-Dawley female rats reared in this laboratory, in a controled condition (room temperature: 23±1°C; light on 6:00 AM to 6:00 PM), were randomly divided into two groups, the intact control and Tx groups. The rats were on chicken feed (Taiwan Sugar Corp., Taipei) and tap water *ad lib* before and after Tx. One month after the operation, the Tx rats were subjected to vaginal inspection for proestrus. Rats of Tx and control groups that demonstrated proestrus were sacrificed by exsanguiation through dorsal aorta under light ether anesthesia at the time between 10:00 AM to 12:00 noon. The hypothalamic tissue were removed within 3 min after anesthesia. The hypothalami were dehydrated in precooled acetone (−20°C), which was refreshed twice within two days. The hypothalamic tissue was dissected with the approximations of the demarcations of optic chiasma, anteriorly; optic tracts, laterally; mammillary bodies, posteriorly and in a thickness of 5 mm. Control rats were sacrificed at a similar age. Hypothalamic extracts (HE) was prepared by a modification of the method introduced by Derry[2] and Steiner *et al.*[6]. After partial evaporation of acetone, groups of 20 hypothalamic tissue from the control or Tx were homogenized in cold acetone (−20°C) in a all-glass homogenizer. The resulting fine powder was then extracted with 0.1 N HCl at 4°C. The homogenate was then transfered to a pointed centrifuge tube and centrifuged at 1500 g for 60 min. The supernatant was neutralized with 1 N NaOH and the resulting cloudy precipitate removed by centrifugation at 1000 g for 30 min at 4°C. Remaining supernatant was lyophilized. The HE preparations were store in vaccum vials and reconstituted by double distilled water at the time for radioimmunoassay (RIA). The assay method of Koch *et al.*[4] was adapted for the present investigation.

Synthetic GnRH (LH-RH, Calbiochem Co., San Diago, USA) were utilized for both standard and radioiodination. Radioiodine (^{125}I Radiochemical Center, Amersham, England) was reacted with GnRH catalyzed by Chloramine-T and separated in Sephadex G-25 column. Anti-GnRH (Miles Laboratories, Ind., USA) in 1:50 dilution were served for 1st antibody and anti-rabbit

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globulin from sheep (Gibco Co., New York, USA) were used as 2nd antibody in present RIA. The sample GnRH level were determined by a logit transformed linear correlation equation of a standard curve.

RESULTS AND DISCUSSIONS

Synthetic unlabled GnRH of 5 pg to 10,000 pg were used to construct standard curves (Fig. 1). The minimum reliable measurements are in the range of 25 to 30 pg, while the actual quantity of GnRH of HE samples in assay is 400 pg in minimum. The inhibition curve of HE is showed in Fig. 1, the parallelism is evident.

In the present study, HE were from the pool

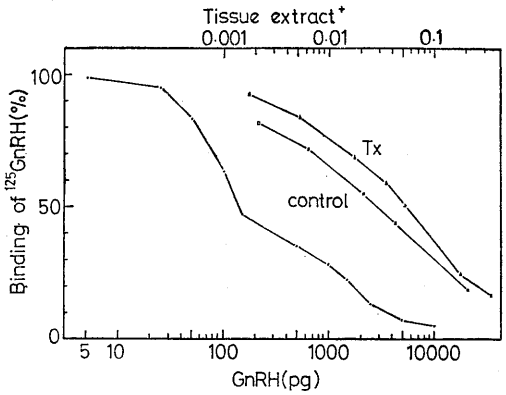

Fig. 1. Inhibition of binding of ^{125}GnRH to Gn RH antiserum by (1) unlabeled GnRH, (left) (2)Tx rat hypothalamic extract (right) and (3)intact rat hypothalamic extract (center). Anti GnRH-BSA serum used at 1:50 dilution. (+) Units indicate number of hypothalami from which the extract assayed was derived.

of 20 hypothalami. With 25 replicates, the averge of intact rats GnRH is 7.61 ± 1.33 ng/ hypothalamus and that of Tx rats, 4.72 ± 0.86 ng. By student t-test, the significant level is $p<0.01$. The randomized selection for rats of the present study may, at least, eliminate some of the individual variations that may contribute to the difference. Treatments designated to investigate on individual variations are in progress. The buffer dilution of the HE in assay may also influence the present estimated difference. An analysis of variance indicates that the difference of GnRH level between control and Tx is not due to the dilution procedure but the difference of treatment ($F=12.41$; $df=1, 32$; $p<0.01$). At this point, it is rather safe to believe that the estimated difference is reliable.

The accumulated data indicated repeatedly a lowered LH in adenohypophyses but varied in FSH level after thyroidectomy[1,7,8,9]. The present estimation on GnRH lowering considered to be responsible for the lowering of adenohypophyseal LH as estimated at the time of 10:00 AM to 12:00 noon of proestrus day, when no obvious pituitary hormonal fluctuation is predicted. This indicates the level of availibility of GnRH in hypothalamus. One component of the availibility must depend heavily on activity of synthesis. It is suggested that the production of GnRH is influenced by biogenic amine[5], and the thyroxine is proposed to have a regulatory effect on LH at the level of hypothalamus[3]. Thus, it is possible, a deficiency of thyroid hormone may influence the GnRH producing process generated by biogenic amine and in turn lowering LH content in pituitaries.

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甲狀腺切除雛鼠下視丘性釋激素之影響

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甲狀腺切除後一個月，將雌鼠之下視丘部份在動情前期日早上 10:00 至 12:00 間取出，用放射免疫測定法測量其萃取液中性釋激素 (GnRH) 之含量。經 25 次重覆測定，測得切除甲狀腺組每個下視丘中 GnRH 平均含量爲 4.72±0.86 ng 而對照組爲 7.61±1.33 ng。其可能之解釋爲，當甲狀腺素缺乏時，會影響下視丘中生源胺 (biogenic amine) 對於 GnRH 之促生過程；而此 GnRH 下降之結果，也可能是切除甲狀腺後腦下腺中 LH 下降之原因。