Chinese Journal of Physiology 41(4): 211-216, 1998

Increase of Thyrotropin Response to Thyrotropin-releasing Hormone (TRH) and TRH Release in Rats during Pregnancy

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Abstract

Regulation of thyrotropin (TSH) release by thyrotropin releasing hormone (TRH) in the anterior pituitary gland (AP) of pregnant rats was studied. The pregnant (day 7, 14, and 21) and diestrous rats were decapitated. AP was divided into 2 halves, and then incubated with Locke's solution at 37 °C for 30 min following a preincubation. After replacing with media, APs were incubated with Locke's solution containing 0, or 10 nM TRH for 30 min. Both basal and TRH-stimulated media were collected at the end of incubation. Medial basal hypothalamus (MBH) was incubated with Locke's medium at 37 °C for 30 min. Concentrations of TSH in medium and plasma samples as well as the cyclic 3':5' adenosine monophosphate (cAMP) content in APs and the levels of TRH in MBH medium were measured by radioimmunoassay. The levels of plasma TSH were higher in pregnant rats of day 21 than in diestrous rats. The spontaneous release of TSH *in vitro* was unaltered by pregnancy. TRH increased the release of TSH by AP, which was higher in pregnant than in diestrous rats. Maternal serum concentration of total T_3 was decreased during the pregnancy. The basal release of hypothalamic TRH *in vitro* was greater in late pregnant rats than in diestrous rats. After TRH stimulation, the increase of the content of pituitary cAMP was greater in late pregnant rats than in diestrus animals. These results suggest that the greater secretion of TSH in pregnant rats is in part due to an increase of spontaneous release of TRH by MBH and a decrease of plasma thyroid hormones. Moreover, the higher level of plasma TSH in rats during late pregnancy is associated with the greater response of pituitary cAMP and TSH to TRH.

Key Words: T_3, TSH, TRH, pregnant rats

Introduction

The patterns and effectiveness of hormones secretion are altered during pregnancy in mammals. In rats, pituitary prolactin (PRL) surges abruptly terminate and plasma PRL concentrations remain low at the day 14 of gestation (27). Enhanced PRL serum concentrations occur before parturition (3, 12, 28) and these may be due to an increase in estrogen secretion (13). Maternal serum concentrations of estrogen increase between days 2 and 4, and on day 6 they decrease to the levels of day 0. The maternal serum estradiol remains low until day 12 of pregnancy (25). Since plasma T_3 and T_4 levels are decrease, which might induce an increase of plasma TSH. Meanwhile the secretion of T_3/T_4 and TSH is regulated by MBH TRH in mammals. However, the regulation of T_3/T_4 and TSH secretion by TRH in pregnant rats is not very clear. The fact that plasma levels of T_4 and T_3 decrease markedly during gestation in rats (23), has been demonstrated by many reports (4, 8, 9, 15, 18). The increased plasma TSH was reported by Kojima *et al.* (15) and Fukuda *et al.* (8). But, the changes of TSH response to TRH and TRH release in hypothyroid rats are still unknown during gestation in rats.

The aim of this investigation was to study the role of TRH in regulating the release of TSH. Both

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pituitary TSH release in response to TRH and TRH release by MBH were examined. We found that the increase of TSH response to TRH during pregnancy is associated with an increase of cAMP production.

Materials and Methods

Animals

Female Sprague-Dawley rats weighing 220-270 g were housed in a temperature controlled (22 ± 1 °C) room with 14 h of artificial illumination daily (0600-2000) and given food and water *ad libitum*.

Vaginal smears were taken every morning after overnight pairing of proestrus rats with males. The day of a positive sperm smear was designated as day 1 of pregnancy. Diestrous rats were used as control animals. Pregnancy was later confirmed by the presence of uterine implantation sites after sacrificing.

Experiments

Diestrous and pregnant rats were decapitated. The anterior pituitary glands (APs) and mediobasal hypothalami (MBHs) were excised, bisected, and preincubated with Locke's solution containing 10 mM glucose, 0.003% bacitracin and 0.05% HEPES at 37 °C for 90 min (33). One hemi-AP or one MBH was placed in a flask. After preincubation, basal incubations were performed for 30 min. The APs were then further incubated with or without 10 nM TRH. At the end of the incubation, AP and MBH tissues were weighed and extracted with 1 ml phosphate buffer saline (PBS, pH 7.0) and 0.5 ml 0.1 N HCl, respectively. The media of MBH incubation were removed , mixed with 20 µl 5 N HCl, and centrifuged at 10000 rpm for 1 min. The supernatant was collected , neutralized with 20 µl 5 N NaOH, and then stored at -20 °C until measurement of TRH by radioimmunoassay (RIA). The media following incubation of APs and rat plasma were collected and measured for TSH and/or total and free T_3 by RIAs.

For studying the effect of TRH on the accumulation of cyclic 3':5' adenosine monophosphate (cAMP) *in vitro*, pituitary tissues were incubated with the medium containing 1 mM 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor. At the end of incubation, tissues were mixed with 1 ml 65% ice cold ethanol, homogenized with polytron and centrifuged at 2000 X g for 15 min. The supernatants were lyophilized by a vacuum concentration (SpeedVac, Savant, Holbrook, USA) and reconstituted with assay buffer (0.05 M acetate buffer with 0.01% azide, pH 5.8) before measuring the concentration of cAMP by the RIA. The protein concentration in tissue extracts was determined by Lowry's assay (25).

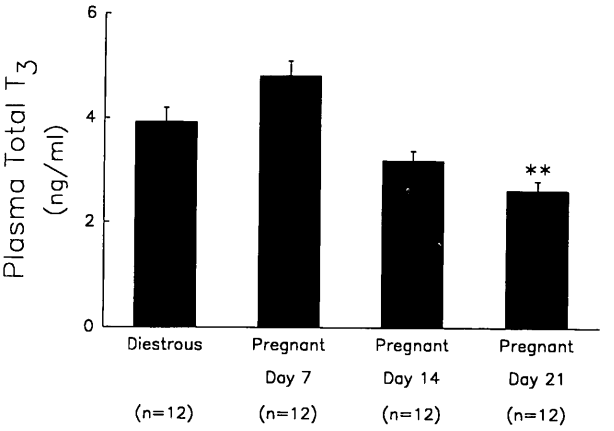

Fig. 1. Concentrations of plasma total T_3 in diestrous and pregnant rats at day 7, 14, and 21 of gestation. **, $p<0.01$ as compared with diestrous rats.

RIA of TSH, TRH, T_3 and cAMP

The concentrations of TSH, and TRH in media, tissue extracts, and/or plasma samples were determined by RIA using anti-TSH (S-5), and anti-TRH (S32) antisera, respectively (2, 21, 32). The rat TSH I-8, and synthesized TRH were used for iodination and the rat TSH RP-2, and synthesized TRH were served as the standard, respectively. The concentrations of total T_3 in plasma were determined by RIA kits. Concentration of cAMP in pituitary tissues extracted by ethanol was measured by RIA kits (30, 31). Both the T_3 and cAMP RIA kits were provided by Amersham Inc, Bucks, UK.

Statistical Analysis

All values are given as the mean±SEM. The treatment means were tested for homogeneity using a two-way analysis of variance, and the difference between specific means were tested for significance using Duncan's multiple-range test (29). In some cases, the Student's *t*-test was employed. Difference between two means were considered to be statistically significant when P is less than 0.05.

Results

Concentrations of Plasma Total T_3, and TSH in Rats during Pregnancy

The level of plasma total T_3 was gradually reduced in rats during pregnancy (Fig. 1). There was a significant difference ($p<0.01$) in plasma T_3 between

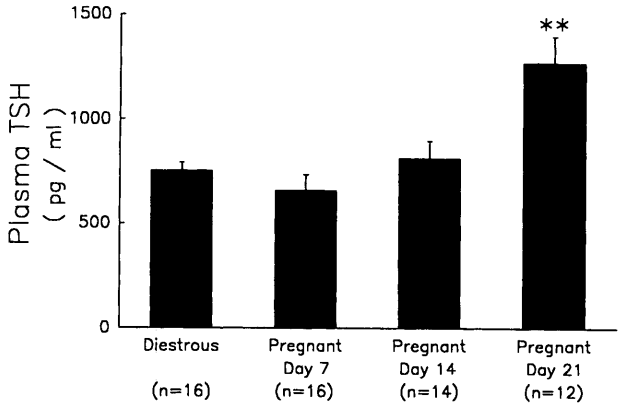

Fig. 2. Concentrations of plasma TSH in diestrous and pregnant rats at day 7, 14, and 21 of gestation. **, p<0.01 as compared with diestrous rats .

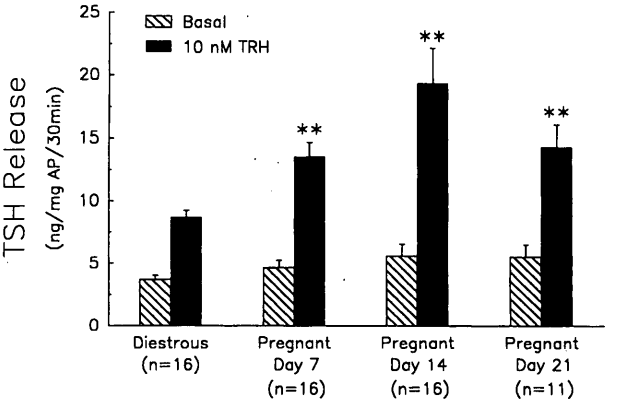

Fig. 3. Effect of pregnancy on the spontaneous and TRH-stimulated release of rat TSH *in vitro*. **, p<0.01 as compared with corresponding diestrous rats.

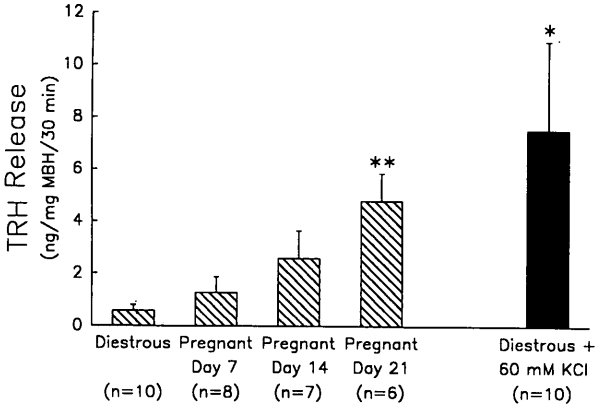

Fig. 4. Effect of pregnancy on the release of rat TRH *in vitro*. *; **, p<0.05 and p<0.01 as compared with diestrous rats, respectively.

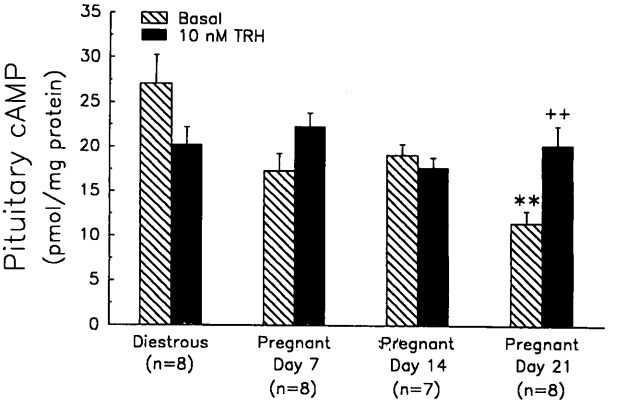

Fig. 5. Effect of pregnancy on the concentration of cAMP in rat anterior pituitary gland (AP) after incubation with TRH. **, p<0.01 as compared with diestrous rats. ++, p<0.01 as compared with basal.

rats at the day 21 of gestation and diestrous animals.

The changes of plasma TSH are illustrated in Fig. 2. TSH concentrations increased markedly in rats at day 21 of gestation as compared with diestrous animals.

Effects of TRH on TSH Release in Pregnant Rats in Vitro

The spontaneous release of TSH by rat APs *in vitro* was unaltered by pregnancy (Fig. 3). TRH increased the release of TSH from AP by 2- to 3-fold. The TRH-stimulated release of TSH was significantly higher (p<0.01) in pregnant than in diestrous rats. The concentration of rat pituitary TSH following incubation with or without TRH was not altered by pregnancy (data not shown).

Effects of Pregnancy on TRH Release in Vitro

The spontaneous release of TRH *in vitro* was greater in late pregnant than in diestrous rats (p<0.01, Fig. 4). The mean level of medium TRH in pregnant rats on day 21 of gestation was 3-fold of that in diestrous and early pregnant rats. Potassium of 60 mM increased the release of TRH from MBH in diestrous rats. The TRH concentration in MBH following incubation was not altered by pregnancy (data not shown).

Effects of Pregnancy on cAMP Accumulation in Vitro

The basal concentration of pituitary cAMP was significantly lower in pregnant rats at day 21 than in diestrous rats (Fig. 5). In the presence of TRH, the concentration of cAMP in AP was not altered by the status of pregnancy. A significant difference in the pituitary cAMP concentration between basal and TRH-treated groups was observed in the pregnant rats at day 21 (Fig. 5). TRH resulted in an increase of pituitary cAMP concentration by 68% on day 21 of pregnancy (Fig. 6).

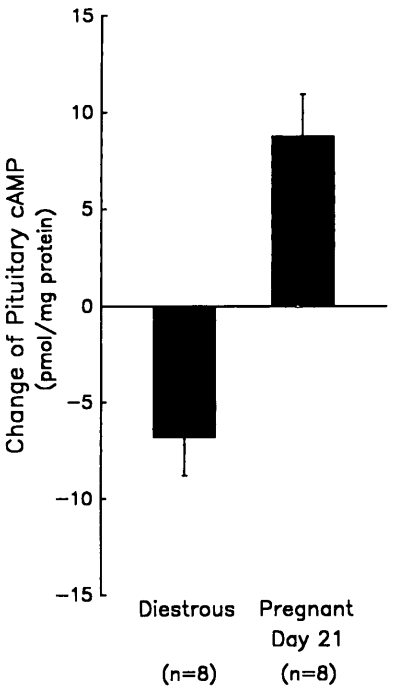

Fig. 6. Change of the concentration of AP cAMP in pregnancy and diestrous rats after incubation with TRH *in vitro*.

Discussion

The present results indicate that 1) plasma total T_3 were lower in term; 2) TSH concentration of plasma was higher in pregnant rats of day 21 than in diestrous animals; 3) the basal release of TSH was similar and the responses to TRH were greater in pregnant than in diestrous rats; 4) the cAMP content in APs in response to TRH increased in pregnant rats of day 21; and 5) the spontaneous release of TRH was greater in late pregnant than in others. These results strongly suggest that large secretion of TRH stimulate APs to secrete TSH in pregnant rats of day 21.

The factors in regulating TSH, and T_3 secretion have been well reported. The plasma levels of T_3 and T_4 decreased markedly during gestation in rats (5, 8, 9, 15, 18). The plasma TSH was expected to be elevated (7), which is in agreement with our results, and has been reported by Kojima *et al.* (15) and Fukuda *et al.* (8). The plasma TSH response to TRH was greater in pregnant rats at 21 day gestation than in non-pregnant rats (5). Our results confirmed that TSH response to TRH *in vitro* was greater in pregnant than in diestrous rats. The mechanism by which TRH induces TSH release is associated with the mobilization of intracellular Ca^{2+} and influx of extracellular Ca^{2+} (10). It has been shown that cAMP is not a physiological mediator for TRH-induced TSH secretion. This is because TRH stimulated TSH release at concentrations that had no effect on intracellular cAMP levels, and the derivatives of cAMP and the cyclic nucleotide phosphodiesterase inhibitor, methylisobutyl-xanthine, caused an increase in TSH release which was additive to TRH effects (11). Brozmanova has shown that the activity of adenylate cyclase is increased after TRH challenge and the level of cAMP is increased after 20 nM TRH *in vitro* in rat AP (4). Davis and Sheppard suggest that cAMP acts to increase the availability of TSH for acute release by TRH by modulation of an intracellular releasable hormone pool, and indicate synergistic interactions between the adenylate cyclase system and the phospholipid-calcium stimulus-release coupling mechanism of TRH (6). However, we found that TRH treatment increased AP cAMP in late pregnant rats, although no change of AP cAMP in diestrous rats.

Thyroid hormone secretion is regulated by TSH from the AP. TSH is controlled by TRH of hypothalamic origin (20). Thyroid hormones feedback at the hypothalamic and pituitary levels to inhibit TSH secretion (26). Kurt *et al.* (17) indicated that removal of thyroid may change the secretion pattern of AP hormones. In rats during pregnancy, the decreased level of plasma T_3 may cause a feedback to increase both the TRH secretion in MBH and the TSH response to TRH in rat AP. This might be the reason why rat plasma TSH concentration increases in late pregnancy. Furthermore, the cAMP production might be involved in the stimulatory effect of TRH on TSH secretion by AP in rats during late pregnancy as shown in the present study.

Krulich *et al.* (16) suggested that there existed an inhibitory dopaminergic mechanism and a facilitatory noradrenergic mechanism in the regulation of TSH secretion in rats. Andersson *et al.* (1) found that catecholamine (CA) neuronal systems are involved in the regulation of TSH secretion. Therefore, the influence of CA on TSH secretion in rats during pregnancy might not be excluded.

It has been well known that estradiol increases both TSH and PRL in humans and rats. Modulation of pituitary TRH receptor levels are mainly by estrogens (14) but lesser by thyroid hormones. Estrogens increase but thyroid hormones decrease the level of pituitary TRH binding and TRH effect on TSH secretion (19). Application of T_3 *in vitro* prevents the release of TSH in response to TRH (22). The present data demonstrated that the responsiveness of pituitary TSH secretion to TRH was greater in pregnant than in diestrous rats and the level of plasma TSH was higher in day 21 of gestation than in diestrus animals. These results implied that the increase in TSH secretion in late pregnancy might be due to the increase in the pituitary responsiveness to TRH, TRH release, and/or the decreased secretion of thyroid hormones.

However, pituitary responsiveness to TRH was higher on the days 7 and 14 of pregnancy, but the plasma TSH was indifferent among days 7 and 14 of pregnancy and the diestrus stage. These conflict results might be due to unaltered of thyroid hormone and TRH in early and mid-term pregnancy.

In summary, the present results suggested that the increased release of TRH play an important role in the greater secretion of TSH in late pregnant rats.

Acknowledgments

This study was supported by the National Research Institute of Chinese Medicine, NRICM-81019, and awards from the Medical Research and Advancement Foundation in memory of Dr. Chi-Shuen Tsou (ROC) to P. S. W. The RIA kits of rat prolactin and thyrotropin were kindly supplied by NIAMDD, USA.

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