Age-related differences in the secretion of calcitonin in female rats

CHIEN-CHEN LU, SHIOW-CHWEN TSAI, SHYI-WU WANG, WILLIAM J. S. HUANG, AND PAULUS S. WANG.

The mechanism that causes hypercalcitonemia in female rats and is associated with aging was investigated. Young (3 mo), adult (8 mo), middle-aged (12 mo), and old (21 mo) rats were infused with CaCl$_2$ and were bled from a jugular catheter after a CaCl$_2$ challenge. To mimic some of the hormonal changes caused by aging, the anterior pituitary (AP)-grafted rats with hyperprolactinemic syndrome were used to mimic the physiological status of aging. The increased release of thyroid CT in vitro in thyroid glands gradually increased with age. Compared with cortex (CX)-grafted rats, the AP-grafted rats possessed higher levels of plasma prolactin and CaCl$_2$-induced levels of plasma CT, and the release of thyroid CT in thyroid glands. After stimulation with oPRL, the in vitro release of thyroid CT increased in both CX- and AP-grafted rats. These results suggest that the hypersecretion of CT in old rats is due at least in part to hyperprolactinemia.

MATERIALS AND METHODS

Animals. The rats were purchased from the animal center of National Yang-Ming University. Old (21 mo), middle (mid)-aged (12 mo), adult (8 mo), and young (3 mo) female rats of Sprague-Dawley strain were housed in a temperature-
controlled room (22 ± 1°C) with 14 h of artificial illumination daily (0600–2000) and were provided food and water ad libitum. Vaginal smears were taken before the experiment. Young and adult rats at diestrus stage on the experimental day were used. Old rats in the persistent diestrus stage were employed. Both the constant diestrous (60%) and the constant estrous (40%) mid-aged rats were employed in this study.

A hyperprolactinemic rat model was used to mimic certain aspects of aging and to eliminate the possible confounding effects of differences in circulating gonadal steroids. All operations were performed under ether anesthesia. The 3-mo-old rats were implanted with anterior pituitary (AP) or brain cortex (CX, for control) under the capsule of the kidney (2). Castrations were performed 2 wks before the experiment.

Six weeks after implantation, rats were decapitated and the blood samples were collected. It has been shown that the levels of plasma PRL increased in AP-grafted rats but not in CX-grafted animals (2).

In vivo experiments. Rats were catheterized via the right jugular vein and left femoral vein under ether anesthesia (14, 25). Twenty hours later, CaCl2 (30 mg/kg body wt) was infused via the femoral catheter connected to a peristaltic pump (14, 25). Blood samples (0.6 ml each) were collected from the right jugular vein at 0, 30, 60, and 120 min post-CaCl2 challenge (14, 25).

Plasma was separated by centrifugation at 10,000 g for 1 min and stored at −20°C for radioimmunoassay (RIA) of CT. Plasma calcium concentration was determined by an automatic calcium analyzer (Calcette; Precision Systems, Natick, MA).

In vitro experiments. After decapitation, rat blood samples were collected, and the concentrations of CT, PRL, estradiol, and progesterone in the plasma were measured by the RIA. Rat thyroparathyroid glands 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. Thyroparathyroid glands were then incubated with vehicle (i.e., Locke's medium) or ovine PRL (oPRL; 40 or 80 ng/ml, Sigma) for 30 min. At the end of incubation the tissue was weighed, and the medium was collected and measured for CT by the RIA.

RIAs. The CT concentrations in each plasma and thyroid medium samples were measured by a human CT RIA kit purchased from Nichols Institute Diagnostics as described elsewhere (14, 25). The sensitivity was 4 pg/ml. The intra- and interassay coefficients of variability were 6.7 and 8.3%, respectively.

The concentration of PRL in the plasma samples was determined by RIA as described elsewhere (5). The rat PRL kit was provided by the National Institute of Diabetes and Digestive and Kidney Diseases. Rat PRL-RP-1 was used for radioiodination, whereas rat PRL-RP-3 was the standard. The sensitivity of rat PRL RIA was 3 pg per assay tube. The intra- and interassay coefficients of variability were 3.8 and 3.2% for PRL RIA, respectively.

The concentration of plasma progesterone was determined by RIA as described elsewhere (14, 16). With anti-progesterone serum no. W5, the sensitivity of progesterone RIA was 5 pg per assay tube. The intra- and interassay coefficients of variability were 4.8 (n = 5) and 9.5% (n = 4), respectively.

The concentration of plasma estradiol was determined by RIA as described previously (14). With anti-estradiol serum no. W1, the sensitivity of estradiol RIA was 1 pg per assay tube. The intra- and interassay coefficients of variability were 6.0% (n = 5) and 5.9% (n = 5), respectively.

Statistical analysis. The treatment means were tested for homogeneity with ANOVA, and the difference between specific means was tested for significance with Duncan's multiple range test (23). A difference between two means was considered significant when P < 0.05.

RESULTS

Effects of aging on plasma PRL, estradiol, and progesterone in female rats. The thyroid weights were 17.11 ± 0.79 mg/thyroid in 3-mo-old rats, 23.87 ± 0.93 mg/thyroid in 8-mo-old rats, 26.23 ± 2.17 mg/thyroid in 12-mo-old rats, and 35.92 ± 3.17 mg/thyroid in 21-mo-old rats (sample size: 7–8). The correlation coefficient for aged rats and weights of the thyroid in rats was 0.80 (P < 0.01).

The levels of plasma PRL showed a gradual and an age-dependent increase in the different age groups (correlation coefficient = 0.91, P < 0.01, Fig. 1, top). Compared with young animals, the basal level of plasma PRL increased 2.5-fold in old rats (Fig. 1, top). There was a significant difference (P < 0.05) in plasma PRL between young and adult, mid-aged, or old rats or between adult and old animals (Fig. 1, top). The levels of plasma estradiol (Fig. 1, center) and progesterone (Fig. 1, bottom) showed a gradual and age-dependent decrease in the different age groups (correlation coeffi-
lients: estradiol, −0.41, P < 0.01; and progesterone, −0.47, P < 0.05). There was a significant difference (P < 0.05) in plasma estradiol and progesterone between young and old rats and between adult and old animals (Fig. 1, center and bottom).

Response of CT to CaCl₂ challenge in female rats with different ages. The changes of basal level of rat plasma calcium (Fig. 2, top) were not associated with age (correlation coefficient = −0.35, P = 0.053). Compared with young rats, the basal levels of plasma calcium decreased 4% in old rats (Fig. 2, top). The basal levels of plasma CT increased with age (correlation coefficient = 0.83, P < 0.01), and the lowest plasma CT was in young rats (Fig. 2, bottom).

After 30 min of CaCl₂ infusion, both calcium and CT increased in plasma (Fig. 2). Changes in plasma calcium after the challenge tended to differ according to age. At 30 min after the challenge, plasma calcium was higher in mid-aged rats than in young rats; however, this increase was not sustained in the old-age group. The plasma calcium had decreased more slowly in mid-aged and old rats than in the others at the end of calcium infusion. After this infusion, the maximal response of plasma CT showed an age-related increase. Meanwhile, the levels of plasma CT at 60 and 120 min after CaCl₂ infusion were higher in mid-aged and old rats than in young rats. In young rats, the plasma CT levels were always significantly lower (P < 0.01) than in others. A maximal increase in plasma CT occurred in old rats.

Effect of aging on CT release in vitro. The release of CT from thyroid glands gradually increased with age (Fig. 3, correlation coefficient = 0.49, P < 0.01), and the maximal release of CT was observed in old rats (Fig. 3).

Compared with young rats, the release of CT from thyroid glands increased 26, 71, and 94% in adult, mid-aged, and old rats, respectively.

Response of CT to CaCl₂ challenges in hyperprolactinemic rats. The levels of plasma PRL were higher in AP-grafted (85.70 ± 7.67 ng/ml) than in CX-grafted (64.07 ± 4.33 ng/ml) rats. Compared with CX-grafted rats, the basal levels of plasma calcium were not different from AP-grafted rats (Fig. 4, top). The basal levels of plasma CT were higher (P < 0.05) in AP-grafted (35.80 ± 4.25 pg/ml) than in CX-grafted (23.08 ± 2.75 pg/ml) rats.

After CaCl₂ infusion for 30 min, plasma calcium levels increased in all animals. The maximal increase of plasma calcium in response to CaCl₂ infusion from 0
to 30 min was greater (33.5%, $P < 0.01$) in AP-grafted than in CX-grafted Ovx rats (Fig. 4, top). Thereafter, calcium levels returned to basal level at 60 min or were below the basal level at 120 min (Fig. 4, top).

Infusion of CaCl$_2$ for 30 min increased plasma concentration of CT in all rats (Fig. 4, bottom). The maximal increase of plasma CT in response to CaCl$_2$ infusion from 0 to 30 min was greater (40.8%, $P < 0.05$) in AP-grafted than in CX-grafted Ovx rats (Fig. 4, bottom). Both CaCl$_2$-induced and post-CaCl$_2$ levels (120 min) of plasma CT in Ovx rats were altered by hyperprolactinemia.

Effects of hyperprolactinemia on the release of CT in vitro. The basal release of CT from thyroid glands was higher in AP-grafted than in CX-grafted rats (Fig. 5). Administration of oPRL (80 ng/ml) increased the in vitro release of thyroid CT in CX- and AP-grafted rats. After oPRL (40 ng/ml) treatment, the release of CT from thyroid glands was also higher in AP-grafted than in CX-grafted rats. The correlation coefficients between oPRL and CT were 0.52 ($P < 0.05$) and 0.50 ($P < 0.05$) in AP- and CX-grafted rats, respectively.

**DISCUSSION**

In this study, we found that 1) the time it took for CaCl$_2$-stimulated levels of plasma CT to return to pre-CaCl$_2$ levels was longer in old and mid-aged than in young and adult rats; 2) the release of thyroid CT in thyroid glands was the greatest in old rats; 3) compared with CX-grafted rats, the AP-grafted rats possessed higher levels of plasma PRL and CT; 4) maximal increase of plasma CT in response to CaCl$_2$ infusion was greater in AP-grafted than in CX-grafted rats; and 5) the release of thyroid CT in thyroid glands was greater in AP-grafted than in CX-grafted rats.

Age-associated morphological and physiological changes of the thyroid have been known for some time. In elderly humans, thyroid weight has been shown to decrease, remain substantially unchanged, or increase; the last is unrelated to physiological aging but is rather the consequence of an increased prevalence of nodular goiter in the elderly (4, 17). An age-dependent increase in thyroid weight also occurs in rats (20, 21). This finding is similar to our results. Admittedly, a number of C cells have been shown to increase in rats from birth to 120 days (18). In humans, however, the number of C cells appears to decrease rather than increase with age (28, 29). This may be one of the reasons why the plasma levels of CT are decreased in elderly humans but increased in aged rats.

Age-associated or age-specific physiological changes in CT secretion have been extensively investigated in both humans and experimental animals. Deftos et al. (7) found that, irrespective of age, the increase in CT in response to calcium infusion was greater in men than in women but that basal levels of CT and the response to calcium waned with age in both sexes. Queener et al. (20) found that hypercalcitonemia occurs in aged Buffalo rats, as does hyperparathyroidism, and further suggested that the concentrations of CT in blood are modulated by $\beta$-adrenergic agonists. In this study, we found that the pre- and post-CaCl$_2$ plasma CT and the in vitro release of CT reach the highest levels in old rats compared with young rats. After CaCl$_2$ challenge, the plasma level of calcium at 60 min was higher in mid-aged and old rats than in young rats. Thus mid-aged and old rats had a low recovery ability on plasma calcium from CaCl$_2$ stimulation. The increased in vitro release of CT in aged rats has also been observed by Wongsurawat and Armbricht (30). Tsai et al. (25) have reported that the basal CT secretion and the maximal CT level in female rats during calcium infusion are increased with age. The link between estrogen and the hypocalcemic effect of CT observed in rats is actually an age-related phenomenon instead of a physiological regulation of all ages (25). Thus hypercalcitonemia occurs in aged rats (20, 25, 30), and the increased secretion of CT is probably due to $\beta$-adrenergic agonists (20), an aging-related decline in estrogen secretion (25), or regulation of secretion by calcium (30).

Previous studies have demonstrated that the circulating PRL concentration in rats (10) and humans (26) increases during aging. In humans, it has been shown that gonadal steroid deficiency may affect malfunction of CT secretion (9). Meanwhile, the basal plasma CT levels are slightly reduced in hyperprolactinemic women (24). These phenomena may explain why aged humans possessing low ovarian steroid hormones and high PRL have a low level of basal plasma CT.

In this study, we have confirmed that the level of plasma PRL is gradually and age-dependently increased and that the levels of plasma estradiol and progesterone are gradually and age-dependently decreased in rats. Because lower plasma levels of estrogen and/or progesterone result in a decrease of the levels of plasma CT in Ovx rats (6), the hypercalcitonemia in female rats during aging is not due to the decline of ovarian steroid hormones.

Previous studies have demonstrated that hyperprolactinemia is associated with decreased bone mineral
density (24) and that plasma CT levels are slightly reduced in hyperprolactinemic women (24), which is similar to the CT levels in older humans. In this study, the hyperprolactinemic rats induced by AP graft were used as an aged-animal model. We found that the basal, CaCl$_2$-induced, and oPRL-stimulated levels of CT release from thyroid glands were markedly higher in AP-grafted hyperprolactinemic rats than in CX-grafted animals. Furthermore, the in vitro release of CT was increased by oPRL in AP- or CX-grafted rats. The maximal increase of plasma calcium in response to CaCl$_2$ infusion from 0 to 30 min was greater in AP-grafted than in CX-grafted rats and greater in the old than in the young rats. Recently, Ohkubo et al. (19) reported that the gene encoding the PRL receptor is expressed in the thyroid gland of the domestic chicken. These data indicate that hyperprolactinemia was involved in the mechanism regulating hypersecretion of CT in rats during aging.

In summary, the present results demonstrate that the hypersecretion of CT both in vivo and in vitro in female rats during aging is associated with an increase in the plasma PRL arising from hyperprolactinemia.

The authors greatly appreciate A. L. Vendouris for English editorial assistance.

This study was supported by Grant CI-85–03 from the Yen Tjing Ling Medical Foundation and Grant NRICM-84104 from the National Research Institute of Chinese Medicine, Republic of China. This work was also supported by awards from the Medical Research and Development Foundation in memory of Dr. Chi-Shuen Tsou (ROC) to P. S. Wang.


Received 20 March 1998; accepted in final form 15 July 1998.

REFERENCES