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Effects of Thyroid Hormones on the Release of Calcitonin Gene-Related Peptide (CGRP) by Rat Prostate Glands *In Vitro*

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Abstract

It has been well known that calcitonin (CT) and calcitonin gene-related peptide (CGRP) are derived from the CT/CGRP gene which is localized in chromosome 11. CGRP is a 37-amino acid neuropeptide expressed predominantly in the nervous system and is one of the most potent endogenous vasodilatory peptides that have been found. Only few reports described the distribution of CGRP in reproductive organs. Moreover, the hormonal regulation of CGRP secretion is still not clear. The present study was designed to examine the presence of CGRP in rat prostates and the direct effect of thyroxine (T_4) on the release of CGRP by rat prostate glands in vitro. Male rats were thyroidectomized (Tx) or sham Tx for two weeks before decapitation. The ventral prostate glands were either extracted by phosphate buffer saline or bisected and preincubated with Locke's solution containing 10 mM glucose, 0.03% bacitracin, and 0.05% Hepes at 37°C for 90 min. The hemi-prostate tissues were then incubated with Locke's medium containing T_4 ($0\sim10^{-7}$M) for 1 hr. After incubation, the medium was collected, and the prostate tissues were weighed. The concentration of CGRP in both medium and prostate tissue extracts were measured by a specific radioimmunoassay (RIA) developed in our laboratory. Incubation of T_4 at 10^{-9} M was effective to increase the release of CGRP in rat prostate glands. Incubation of rat prostate glands with T_4 at 10^{-7} M resulted in a maximal release of CGRP (270% of the basal). These results suggest that thyroid hormones increase CGRP release by acting directly on rat prostate glands.

Key Words: CGRP, thyroxine, prostate

Introduction

Calcitonin gene-related peptide (CGRP) is a 37-amino acid peptide with a 2-7 disulphide bridge and a phenylalanine amide at the carboxyl terminal (2, 3, 33). CGRP is present mainly in the central nervous system (3, 33, 36) and is reported to be high in the spinal cord, amygdala and ventral striatum of the rat brain (11, 33). CGRP is also found with high concentrations in perivascular nerves throughout the body, including the coronary (26) and cerebral (18) vessels. It has been shown that CGRP is an extremely potent vasodilator in rabbit, hamster and man (7, 9). It has been demonstrated that CGRP has an important physiological role in the control of blood flow and vascular tone (17, 18).

The prostate is an accessory reproductive gland found in all orders of male mammals, which is localized around the urethra (1). The prostate surrounds the bladder and urethral in a ring-like manner, and tends to obstruct the urinary stream by growing inward and compressing the center of the ring (13). The prostate consists of two components, the stroma (connective and smooth muscle tissues) and epithelial components. The stroma is responsible for the androgenic induction necessary for tissue development. The epithelial component consists of secretory and excretory portions (1). It has been reported that CGRP was found in the sensory nerve and neuroendocrine cells of protate (39, 19). CGRP

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relaxes phenylephrine-induced contraction of smooth muscle, suggesting a modulating role in prostate contractile response (39). CGRP also stimulates adenosine 3':5' cyclic monophosphate (cAMP) accumulation in several cultured human prostate cancer cell lines (20), which indicates a possible function for CGRP in epithelial cells.

The decreased secretion of testosterone has been found in hypothyroid rats as compared with euthyroid animals (4, 6, 8, 34). Replacement of thyroxine (T_4) in hypothyroid rats restores the production of testosterone to euthyroid levels (6, 8). Since the growth of prostate tissue was androgen-dependent, T_4 may affect the prostate growth and function indirectly by altering testosterone secretion. It has been shown that T_4 affects the endocrine characteristics of prostate. For example, T_4 treatment increases prolactin receptor mRNA (37) and decreases the levels of thyrotropin-releasing hormone (TRH)-like peptides (5) in rat prostate. The hypothyroidism inhibits prostatic glycosidase and triiodothyronine (T_3) has a direct stimulatory effect on these enzymes (28). However, the direct effects of thyroid hormones on the CGRP release by the prostate is still not clear.

In the present study, the production of CGRP by rat prostate was characterized and the direct effect of T_4 on the release of CGRP by rat prostate *in vitro* was examined. We found that administration of T_4 at 10^{-9}~10^{-7} M was effective to increase the release of CGRP in rat prostate.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 300-350 g were housed in a temperature-controlled room (22±1 °C) with 14 hr of artificial illumination daily (0600-2000 hr) and were given food and water *ad libitum*. The rats were either thyroidectomized (Tx) or sham Tx for two weeks before use.

In Vitro Experiments

After decapitation, the mediobasal hypothalami (MBHs) and the ventral lobes of the prostates in normal rats were homogenized with phosphate buffer saline (PBS) by a polytron (PT-3000, Kinematica Ag., Luzern, Switzerland). The tissue homogenates were centrifuged at 1000×g for 30 min. The supernatants were collected. In another experiment, the ventral lobes of rat prostates were bisected and preincubated with Locke's solution containing 10 mM glucose, 0.03% bacitracin, and 0.05% Hepes for 90 min before incubation with or without T_4 at 37°C for 60 min under a controled atmosphere

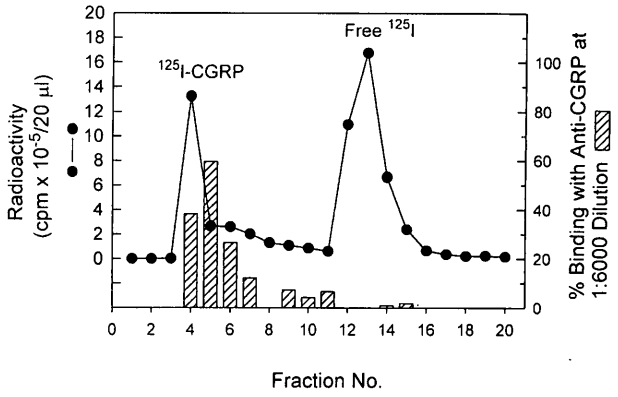

Fig. 1. Elution pattern of the purification of ^{125}I-rat-CGRP on Sephadex G-15 using 0.05 M phosphate buffer saline (PBS) as eluent.

(95% O_2 and 5% CO_2). After incubation, the media were collected , and the prostate tissues were weighed .

The concentrations of CGRP in both media and prostate tissue extracts were measured by a specific radioimmunoassay (RIA) developed in our laboratory.

RIA of CGRP

The synthetic rat CGRP was labeled with ^{125}I using the chloramine-T method (21). The labeled hormone and free iodide were separated on a 0.9×20 cm column of Sephadex G-15 using 0.05 M phosphate buffer as an eluent. Two radioactive peaks were observed in the system (Fig. 1). The first peak showed high immunoreactive activity of radioiodinated CGRP and was used in the following RIA of CGRP.

The antisera against rat CGRP (No. JYY 107) was generated by immunizing the rabbits with the conjugates of hormone with bovine serum albumin (BSA) by bisdiazotized-benzidine mixed with complete Freund's adjuvant (1:1, v/v) as the method previously described for the production of antiserum against thyrotropin releasing hormone (32).

In CGRP RIA systems, a known amount of unlabeled hormones, or of a heterologous peptide, or an aliquot of a medium or tissue extract adjusted to a total volume of 0.3 ml by a buffer solution (1 % BSA, 0.1% triton-PBS, pH 7.5) was incubated with 0.2 ml specific antiserum optimum diluted with normal rabbit serum (1:400) buffer and 0.1 ml ^{125}I-labeled hormone (approximately 10,000 cpm) at 4 °C for 24 hr. Triplicate standard curves with 7-11 points was assayed. An adequate amount of goat anti-rabbit gamma globulin (ARGG) was then added with a further incubation for 48 hr at 4°C. At the end of the incubation the assay tubes was centrifuged at 5000×g

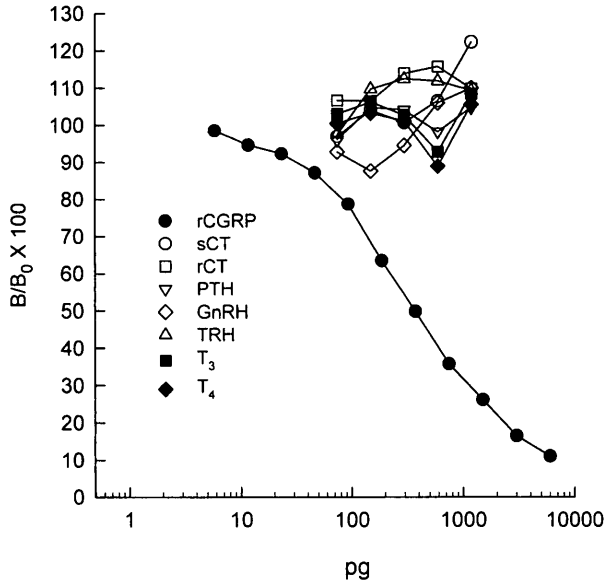

Fig. 2. Dose-response curve for rat CGRP standard and cross reactivities of anti-rCGRP with several hormones.

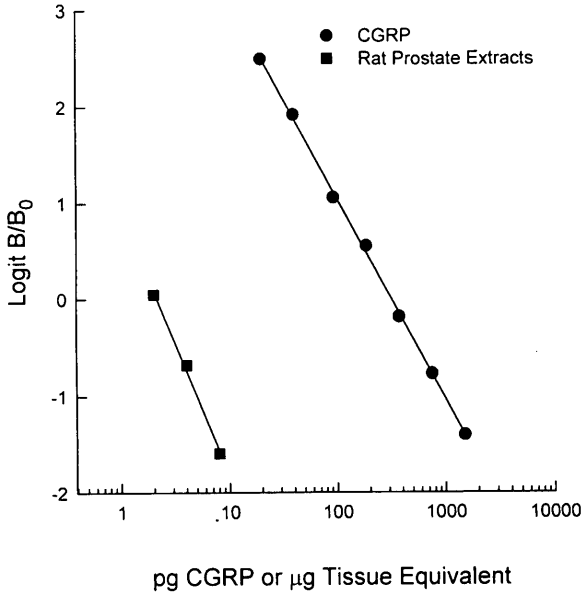

Fig. 3. Dose-response curves for rat CGRP standard and the extracts of rat prostate after a logit-log transformation.

for 30 min. The supernatant was discarded before counting the radioactivity of the precipitates in an automatic gamma counter (1277 Gamma Master, Pharmacia, Turku, Finland). The inhibition curve produced by the rat CGRP standard (6~6000 pg) is shown in Fig. 2. The cross-reactivity are less than 0. 1% with salmon calcitonin (sCT), rat CT (rCT), parathyroid hormone (PTH), gonadotropin-releasing hormone (GnRH), TRH, T_3 and T_4 (Fig. 2).

A parallelism between the inhibition curves generated by rat prostate extracts and CGRP was shown in Fig. 3.

Statistical Analysis

Data were expressed as mean±SEM. Treatment means were tested for homogeneity using a one-way analysis of variance (ANOVA) and the differences between the specific means were tested for the significance by Dunnett's test or by Student's *t*-test (35). The level of significance chosen was $p<0.05$.

Results

Characterizations of CGRP RIA System

Two radioactive peaks were observed in the radioiodination system (Fig. 1). The first peak showed high immunoreactive activity of radioiodinated CGRP and was used in the following RIA of CGRP. The inhibition curve produced by the rat CGRP standard (6~6000 pg) is shown in Fig. 2. The cross-reactivity are less than 0.1% with SCT, rCT, PTH, GnRH, TRH,

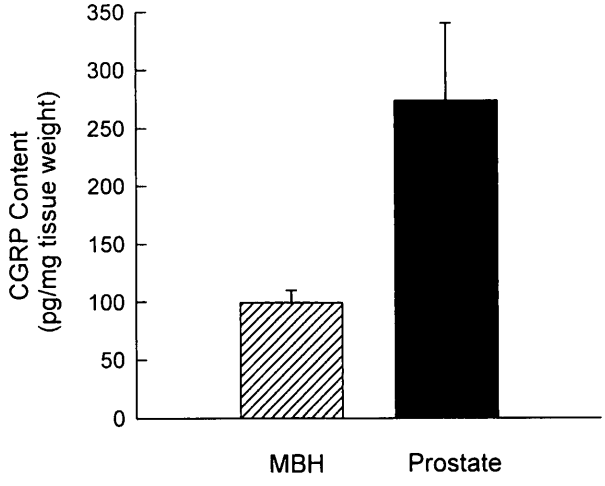

Fig. 4. Concentration of CGRP in rat mediobasal hypothalamus (MBH) and prostate.

T_3 and T_4 (Fig. 2). Fig. 3 represents the parallelism of rat prostate extracts and rat CGRP. The intra- and interassay coefficients of variability were 1.6 % and 8.5 %, respectively.

CGRP Concentration in Prostate Extract

Fig. 4 illustrates the CGRP concentration in rat prostate and MBH. Abundant CGRP was detected in rat prostate by using our CGRP RIA system.

Effect of T_4 on CGRP Release In Vitro

The basal level of CGRP release by normal rat

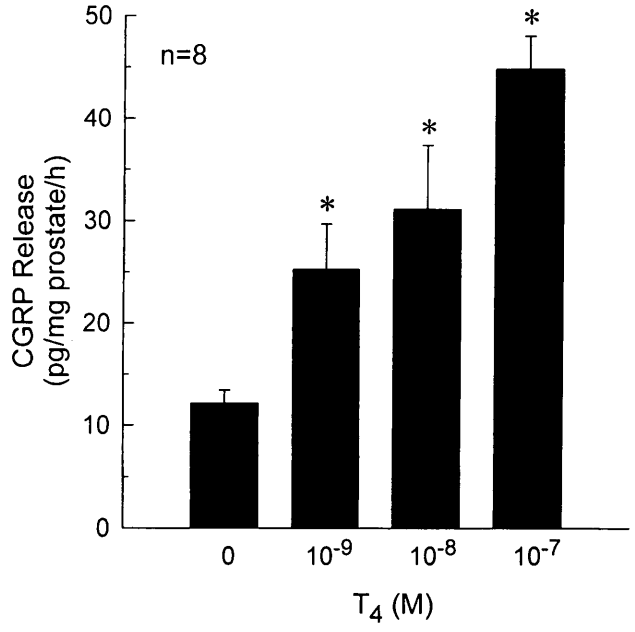

Fig. 5. Effect of T_4 at different doses on the release of CGRP by rat prostate *in vitro*.

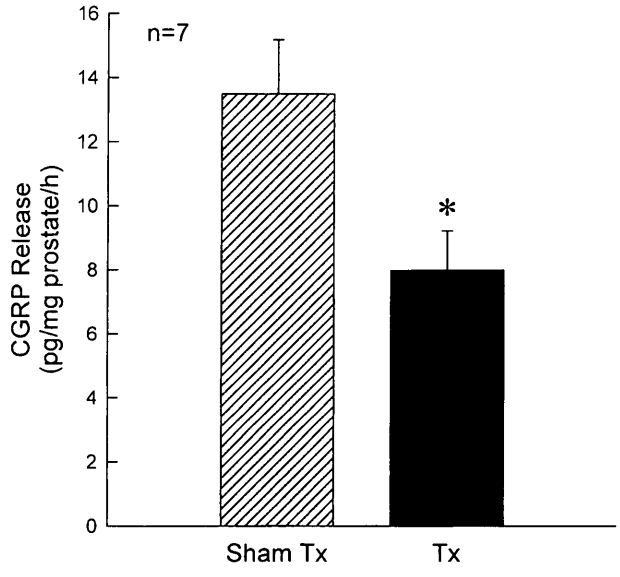

Fig. 6. Effect of thyroidectomy on the release of CGRP by rat prostate *in vitro*.

prostate *in vitro* was 12.1±1.2 pg/mg/hr (Fig. 5). T_4 at 10^{-9}~10^{-7} M dose-dependently increased CGRP release by 108~269% ($p<0.05$). The F value of one-way ANOVA was 10.4 ($p<0.01$).

Thyroidectomy resulted in a reduction of CGRP release by 41% ($p<0.05$) in rat prostate (Fig. 6).

Discussion

The prostate diseases including benign prostatic hyperplasia (BPH) and prostate cancer are serious problems in males, especially in elderly or old men (24, 25). Patients with BPH suffer from hesitancy, straining to void, nocturia and weak stream. Many of the BPH patients develop cancer of prostate, although there is still controversial about the relationship between BPH and prostate cancer. All prostatic carcinomas are androgen dependent initially but become independent of the hormone in the long term (1). Circulating androgen are required for normal growth and maintance of functions of the prostate. However, the prostate also contains neuroendocrine peptides, found either in nerve terminals or in prostatic neuroendocrine cells, including neuropeptide Y, CGRP, and substance P (9, 10, 25) which are likely to regulate prostate growth or function. In addition to the well established vasodilator role and myotropic effects of the peptide, CGRP exerts a subtle influence on the growth or secretion in the prostate under physiological and pathophysiological conditions (40). The neuroendocrine peptides in malignant prostate tissue have also been described (12). CGRP increases the invasive potential of PC3, the androgen-independent prostate cancer cells (30).

The physiological role of CGRP is only partially understood. Some investigators found that CGRP was existed in the peptide-containing nerves of rat or human prostate (22, 31). Some previous studies demonstrated that CGRP can be released upon activation of the peripheral branches of sensory nerves by capsaicin, nicotine, ouabain, and ischemia (14, 15, 16, 29). It has been demonstrated that endocrine cells of the prostate secrete TRH, and TRH-like peptide (23). Serum total and free T_4 and T_3 were significantly reduced in ventral prostatectomized rats. It has been suggested that the ventral prostatic secretions have a stimulatory role on the endocrine function of the thyroid gland (27). It has been well documented that T_4 treatment increases prolactin receptor mRNA (37) and decreases the levels of thyrotropin-releasing hormone (TRH)-like peptides (5) in rat prostate. The hypothyroidism inhibits prostatic glycosidase and T_3 has a direct stimulatory effect on these enzymes (28). Our data indicate that there is an abundant of CGRP stored in rat prostates compared with that in rat MBH. Furthermore, the *in vitro* secretion of rat prostate CGRP can be dose-dependently increased by a physiological dose of T_4. We also found that thyroidectomy significantly decreased the CGRP secretion by rat prostate. It is apparent that thyroid hormone is necessary for the maintenance of CGRP production in rat prostate.

In summary, the present results demonstrate that rat prostate is abundant in CGRP and T_4 stimulates CGRP release by acting directly on rat prostates.

Acknowledgements

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