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Identification of the Human Seminal TRH-Like Peptide pGlu-Phe-Pro-NH_2 in Normal Human Prostate

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GKONOS, P. J., C. K. KWOK, N. L. BLOCK AND B. A. ROOS. *Identification of the human seminal TRH-like peptide pGlu-Phe-Pro-NH_2 in normal human prostate.* PEPTIDES **15**(7) 1281–1283, 1994.—Human and rat prostate contain thyrotropin-releasing hormone immunoreactivity (iTRH) including TRH and an uncharged TRH-like peptide. Recently the uncharged TRH-like peptide pGlu-Phe-Pro-NH_2 was purified from human semen. To determine whether this peptide was of prostatic origin, human and rat prostate extracts were analyzed by ion-exchange chromatography and reversed-phase HPLC. The predominant uncharged iTRH comigrated exactly with synthetic pGlu-Phe-Pro-NH_2 on HPLC and had identical affinity to pGlu-Phe-Pro-NH_2 in a TRH radioimmunoassay. We conclude that prostate is a source of this peptide in humans and rats. This amidated TRH-like peptide may play a role in human reproductive physiology.

Neuroendocrine cells Thyrotropin-releasing hormone Prostate Amidated peptides

THE prostate is one of several tissues outside the central nervous system that contains thyrotropin-releasing hormone immunoreactivity (iTRH). Although a portion of iTRH in the prostate is authentic TRH (pGlu-His-Pro-NH_2) the majority of prostatic iTRH consists of one or more TRH-like peptides with the structure pGlu-X-Pro-NH_2 where X could potentially be any amino acid (10). pGlu-Glu-Pro-NH_2 was identified as the major iTRH in rabbit prostate (1) and was also found in rat ventral prostate (13) and human seminal fluid (2). However, the predominant iTRH in both rat and human prostate appears to be a novel peptide whose central amino acid has an uncharged side chain (5,10). Recently, the uncharged iTRH in human semen was purified and found to have the structure pGlu-Phe-Pro-NH_2 (7). We therefore investigated normal human prostate tissue to determine if this peptide is present in, and therefore likely to originate from, this tissue.

METHOD

Synthetic Peptides

Synthetic TRH, pGlu-Phe-Pro-NH_2, pGlu-NVal-Pro-NH_2, and pGlu-Glu-Pro-NH_2 were obtained from Peninsula Laboratories (Belmont, CA). pGlu-Trp-Pro-NH_2, pGlu-Leu-Pro-NH_2, pGlu-Tyr-Pro-NH_2, and pGlu-Met-Pro-NH_2 were synthesized by the peptide synthesis facility at the University of Miami. [^{3}H]-TRH was obtained from DuPont/New England Nuclear (Wilmington, DE).

Tissues

Ventral prostates were obtained from 3-month-old Sprague-Dawley male rats killed by CO_2 asphyxiation. A single 10-g normal human prostate was obtained at the time of organ donation from a previously healthy adolescent male who suffered brain death due to head trauma. This normal human prostate was obtained in accordance with federal and state regulations. All tissues were frozen immediately on dry ice and stored at −80°C until the time of extraction.

Extraction of Tissues

Tissues were extracted as previously described (5). In brief, tissues were boiled for 15 min in 10 volumes of 1 *M* acetic acid and cooled on ice. The chilled tissue was homogenized for 1 min with a Polytron homogenizer and then centrifuged at 30,000 × *g* at 4°C for 1 h. The supernatant was dried under vacuum and reextracted in 100% methanol (3 ml/g tissue), and the methanol extract was centrifuged at 16,000 × *g* for 10 min. The supernatant was dried under vacuum and stored at −20°C until analyzed.

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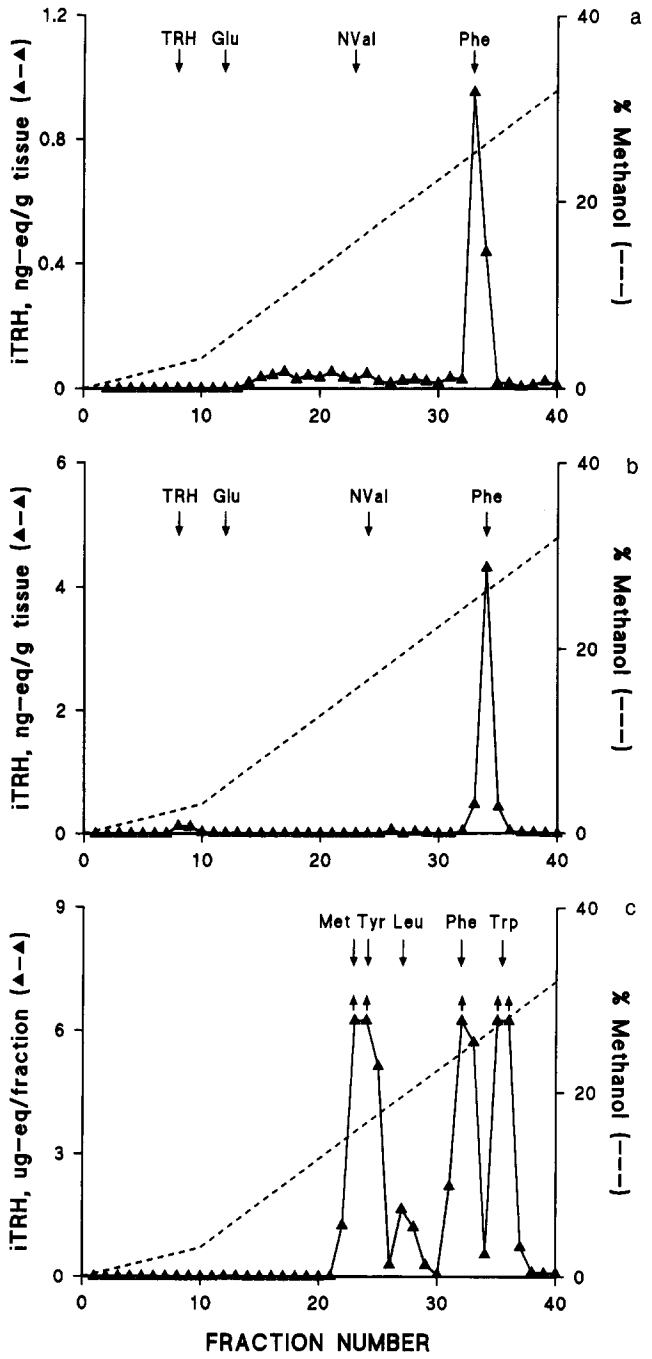

FIG. 1. Reversed-phase HPLC of iTRH from prostate. (a) Uncharged iTRH from normal human prostate. (b) Uncharged iTRH from rat ventral prostate. (c) Uncharged synthetic peptide standards (up arrows indicate values off-scale in the assay). Down arrows indicate the elution positions of synthetic peptide standards. The elution position of "TRH" is indicated. The other standards are of the structure pGlu-X-Pro-NH_2 where the identity of the middle amino acid in the tripeptide is indicated. The identities of the individual immunoreactive standards were determined by previously chromatographing each standard individually.

Chromatography

SP and QAE Sephadex minicolumn ion-exchange chromatography were performed as previously described (5). Recovery of iTRH from these columns is regularly greater than 80%. For one experiment an extract of 48 rat ventral prostates was applied to a 1 × 35 cm column of SP Sephadex equilibrated in 50% acetic acid to determine the size of the uncharged rat iTRH. The unretained iTRH was pooled, dried under vacuum, and applied to a 1 × 35 cm column of QAE Sephadex equilibrated in 0.01 *M* ammonium acetate, pH 8.0, and the unretained iTRH was pooled and dried. Gel filtration chromatography was performed using a 1 × 50 cm column of Sephadex G-25 with 25% acetic acid as the mobile phase. [^{3}H]-TRH, 10,000 cpm, was included as a size marker.

To prepare samples for HPLC analysis, the extract of 0.5–1 g of prostate tissue was applied to an SP Sephadex minicolumn in 50% acetic acid, and the unretained fraction was collected and dried under vacuum. This fraction was applied to a QAE Sephadex minicolumn in 10 m*M* ammonium acetate, pH 7.0, and the unretained fractions were collected and dried. Samples were dissolved in 50–100 μl of 0.1% trifluoroacetic acid prior to injection. Pooled synthetic peptide standards were chromatographed in a separate run immediately after each sample was analyzed. Reversed-phase HPLC was performed on a 4.6 × 250 mm C-18 silica column (Synchropak RP-P, SynChrom Inc., Lafayette, IN) using a Beckman System Gold gradient programmer, pumps, and UV detector. The mobile phase consisted of a methanol gradient in 0.1% trifluoroacetic acid pumped at a flow rate of 1 ml/min. One-milliliter fractions were collected.

Radioimmunoassay

The TRH radioimmunoassay and specificity of the TRH antibody have been previously described (5).

RESULTS

Analysis of normal human prostate extracts by minicolumn ion-exchange chromatography revealed that greater than 90% of iTRH was not retained by either the anion or cation exchange resins. These results indicate that the predominant iTRH in normal human prostate is uncharged. Uncharged iTRH was partially purified from extracts of normal human prostate and rat ventral prostate by sequential chromatography on anion and cation-exchange minicolumns. The iTRH not retained by either column was analyzed by reversed-phase HPLC (Fig. 1). In both tissues a single predominant species of iTRH was identified that eluted at the identical position to synthetic pGlu-Phe-Pro-NH_2 chromatographed immediately after the prostate sample. This HPLC method readily separates synthetic pGlu-Phe-Pro-NH_2 from other hydrophobic TRH-like peptides [Fig. 1(c)]. To confirm the size of the uncharged iTRH, the extract of 48 rat ventral prostates was partially purified by sequential chromatography on SP and QAE Sephadex. When this uncharged iTRH was chromatographed on Sephadex G-25, the iTRH coeluted with a ^{3}H-TRH marker (data not shown).

The uncharged iTRH from human and rat prostate was further analyzed by comparing the displacement by these peptides of radiolabeled TRH in the TRH radioimmunoassay with that caused by synthetic TRH or pGlu-Phe-Pro-NH_2 (Fig. 2). pGlu-Phe-Pro-NH_2 is recognized with higher affinity by our antiserum than is TRH as evidenced by both a shift to the left and by a steeper slope of the curve produced by serial dilutions of pGlu-Phe-Pro-NH_2 when compared to that of TRH. Serial dilutions of uncharged rat and human prostatic iTRH yielded curves that were parallel to the pGlu-Phe-Pro-NH_2 curve but steeper than the TRH curve.

DISCUSSION

Our results indicate that the predominant iTRH in normal human prostate is an uncharged TRH-like tripeptide with iden-

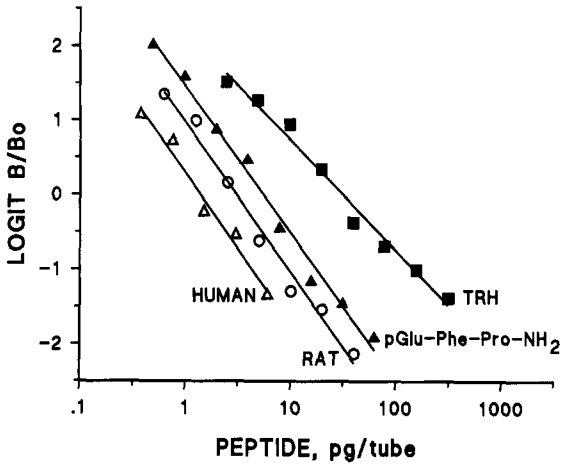

FIG. 2. Radioligand displacement curves of synthetic peptides and partially purified, uncharged iTRH in the TRH radioimmunoassay. Serial 1:1 dilutions of(■) synthetic TRH and (▲) pGlu-Phe-Pro-NH_2 are indicated by solid symbols. Serial 1:1 dilutions of (○) rat ventral prostate and (△) normal human prostate extracts are positioned arbitrarily against the X-axis. The rat extract was partially purified by ion-exchange chromatography and reversed-phase HPLC. The human extract was partially purified by ion-exchange chromatography.

tical HPLC mobility to pGlu-Phe-Pro-NH_2. Our HPLC method readily resolved synthetic pGlu-Phe-Pro-NH_2 from other hydrophobic TRH-like synthetic peptides including pGlu-Trp-Pro-NH_2, pGlu-Tyr-Pro-NH_2, pGlu-Leu-Pro-NH_2, pGlu-NVal-Pro-NH_2, and pGlu-Met-Pro-NH_2. Because pGlu-Phe-Pro-NH_2 has been unequivocally demonstrated in human semen by sequence analysis and mass spectroscopy (7), this finding suggests that the prostate is an important source of this seminal peptide. Furthermore, we found no evidence in normal human prostate for any additional uncharged TRH-like peptides. pGlu-Gln-Pro-NH_2 has been found in tryptic digests of human semen, but did not exist as a free peptide (7). Human prostate also produces the charged peptides TRH (pGlu-His-Pro-NH_2) (9) and pGlu-Glu-Pro-NH_2 (8).

Our results are also consistent with previous reports of a peptide in rat ventral prostate with identical HPLC mobility to pGlu-Phe-Pro-NH_2 (4,11). Our present results also confirm this finding in the rat. Although there has been no structural analysis of the uncharged rat TRH-like peptide, it is likely that pGlu-Phe-Pro-NH_2 is present in the prostates of both rats and humans. In contrast to these findings, rabbit prostate contains predominantly pGlu-Glu-Pro-NH_2 is also secreted into semen. Rabbit prostate also contains an uncharged iTRH whose identity is not yet determined (1). Therefore, rat ventral prostate is a closer model system of the human than is the rabbit for the study of prostatic TRH-like peptides.

pGlu-Phe-Pro-NH_2 is a member of the growing list of neuroendocrine peptides that are produced in the human prostate and secreted into the semen including TRH, pGlu-Glu-Pro-NH_2, calcitonin, β-endorphin, Met-enkephalin (3), somatostatin (12), and a bombesin-like peptide (6). Preliminary evidence has already been presented for effects of calcitonin (3), TRH, and pGlu-Glu-Pro-NH_2 (13) on sperm motility. Therefore, it will now be important to determine whether pGlu-Phe-Pro-NH_2 may also have effects on sperm function or may play some other role in fertility. Alternatively, it is possible that pGlu-Phe-Pro-NH_2 or other TRH-related peptides could have local effects in the prostate. Further investigation of the biology of these seminal peptides should help to further define the role of the prostate gland in male reproductive function.

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