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THYROTROPIN-RELEASING HORMONE AND A HOMOLOGOUS PEPTIDE IN THE REPRODUCTIVE SYSTEM OF THE FEMALE RAT AND PIG

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SUMMARY

TRH and a TRH homologous peptide have been shown to occur throughout the female rat and pig reproductive systems by TRH radioimmunoassay, SP-Sephadex C-25 cation exchange chromatography, and parallel line analysis of the assays. The total amount of TRH and TRH homologous peptide immunoreactivity was highest in the oviducts followed by the ovary and then uterus. The concentration of TRH immunoreactivity in all reproductive organs of the rat fell gradually from one month of age. TRH and the TRH homologous peptide were not parallel on serial dilution and measurement in the same TRH radioimmunoassay. The rapid degradation of TRH by pig follicular fluid may explain the higher measured concentration of TRH homologous peptide compared to TRH not only in pig follicular fluid but also in the pig ovary as a whole.

INTRODUCTION

Thyrotropin-releasing hormone and a homologous peptide are distributed throughout the male rat reproductive system (1). This finding coupled with the observation of TRH immunoreactivity in amniotic fluid (2) and TRH biosynthesis in human placental tissue (3,4) suggested that TRH or related peptides might occur in the reproductive organs of the nonpregnant female rat. Since TRH has recently been shown to have direct effects on smooth muscle in vitro (5), it was of particular interest to see if TRH also occurred in the oviducts, which transport ova by a combination of muscular contractions and ciliary movements (6). The TRH and TRH-like peptide content of pig follicular fluid, oviduct, ovary and uterus were also studied to permit some initial comparisons of the distrubion of these peptides in the female reproductive systems of two different mammalian species.

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Cholecystokinin immunoreactivity was also measured as part of a survey of brain and gut hormones within the male and female reproductive systems (1,7).

MATERIALS AND METHODS

Tissue extraction for TRH radioimmunoassay

Reproductive organs from immature and sexually mature female Sprague-Dawley rats were studied. After decapitation, ovaries, oviducts and uterus were rapidly excised together, the adhering fat trimmed away and the uterus detached at the utero-tubular junction. The oviducts were carefully dissected from the ovaries. In one experiment, abdominal fat was also removed. The four types of tissue were weighed separately and then placed into glass test tubes containing 0.5 M acetic acid at 97° C to inactivate the TRH-degrading enzymes. After at least 15 min all tubes were allowed to cool and their contents dispersed with a Polytron homogenizer. Homogenates were dried completely with a heater block and filtered air flowing into each tube. The dried residues were homogenized and extracted twice with methanol. The combined methanol supernatants were dried completely, and the residue was dissolved in 0.15 M NaCl-0.05 M phosphate buffer, pH 7.5 (PBS).

Fresh pig ovaries, uteri and oviducts, obtained at the Farmer John Packing Co., Vernon, California, were frozen on dry ice prior to transport to the laboratory. Pig follicular fluid was aspirated via a syringe and needle from other fresh ovaries. Part of this follicular fluid was injected immediately into a container in dry ice. An equal volume of 0.5 M acetic acid was added later to the frozen follicular fluid and then heated in boiling water for at least 20 min. These tissues and fluid were extracted for TRH as described above.

Half-time of disappearance ($t_{\frac{1}{2}}$) of TRH

The $t_{\frac{1}{2}}$ for pure TRH added to fresh, unfrozen pig follicular fluid at ambient temperature (20° C) and at 37° C was measured as follows. One ml of 4,000 pg TRH/ml deionized distilled water was added to 9 ml of fresh pig follicular fluid previously equilibrated with the experimental temperature. One ml aliquots were removed at 0, 1, 2, 3, 5, 7, 9, 12 and 15 min. The 1.0 ml samples were injected immediately into corresponding glass test tubes containing 3.0 ml of 0.5 M acetic acid heated to 97° C. After heating for at least 15 min, the TRH immunoreactivity within each tube was extracted for TRH radioimmunoassay as described above.

TRH radioimmunoassay

The TRH radioimmunoassay of 100 µl aliquots of a serial dilution of each extract was carried out with a modification (8) of the Bassiri and Utiger method (9). TRH antiserum was a generous gift from Dr. Robert Utiger, University of North Carolina. TRH concentrations were calculated with the aid of a parallel line and relative potency computer program modified for use on the Hewlett-Packard Model 9830 computer (10).

Chromatography

Tissue extracts were subjected to SP-Sephadex C-25 cation exchange chromatography using a 0.9 x 58 cm column which was equilibrated and eluted with 0.2 M ammonium acetate, pH 6.2 (8). Fractions of 1.1 ml were collected. TRH radioimmunoassay measurements were carried out on these fractions without further processing.

Table 1. Distribution of TRH-like immunoreactivity in the reproductive organs of (A) 80-100 g (29-31 day-old), (B) 300 g postgravid and (C) 400 g female Sprague-Dawley rats. Results, uncorrected for recovery losses, are given as means ± SD. The observations per group were 6 for Section A and B and 8 for Section C.

	Ovaries		Oviducts		Uterus	
	Wet weight(mg)	TRH (ng/g)	Wet weight(mg)	TRH(ng/g)	Wet weight (mg)	TRH (ng/g)
(A)	12.4± 2.0	1.8 ±0.6	7.5 ±1.5	3.6 ±3.5	46. ± 10.	0.53± 0.44
(B)	112.5± 16.0	1.1 ±0.4	33.8 ±7.9	2.0 ±0.8	411.5± 51.5	0.45± 0.08
(C)	94 ±41	0.77± 0.58	50 ±16	0.68± 0.30	930 ±250	0.19± 0.06

Measurement of primary amines

The relative concentration of primary amine-containing compounds in the SP-Sephadex C-25 chromatography fractions were measured by diluting 50 µl of each fraction in 1.0 ml of 0.2 M borate buffer, pH 8.0, and then adding 0.33 ml of 28 mg fluorescamine (Sigma)/100 ml acetone with vortexing. The fluorescence was monitored with an Aminco-Bowman spectrophotofluorometer using a 390 nm excitation and a 487.5 nm emission wavelength (11).

Cholecystokinin (CCK) extraction and radioimmunoassay

Sexually mature male and female Sprague-Dawley rats were used for the RIA measurement of CCK in reproductive tissues. Following decapitation, the testes, cauda epididymis, prostate and seminal vesicles of males and uterus, ovaries and oviducts of females were weighed and dropped into boiling water (10 ml/g tissue) and boiled for 15 minutes. The tissues while in boiling water were homogenized and centrifuged. The supernatant was saved. The pellets were re-extracted with 5% acetic acid as above. The corresponding acetic acid and water extract supernatants were pooled, lyophilized and stored at -20° C. Prior to RIA, the lyophilized residues were dissolved in a small volume of distilled water.

The CCK-like immunoreactivity in the tissue extracts was measured by a previously described RIA method (12). Antisera 5135 used in this assay cross-reacts with all known molecular forms of CCK and gastrin. The minimum assay sensitivity was 0.025 pmoles/tube.

RESULTS

TRH radioimmunoassay

Recoveries of TRH added to boiling tissue from 250 g rats were: oviduct, 100%; ovary, 71.5%; uterus, 80%. Among the three female reproductive organs studied, the oviduct of both rat and pig, as seen in Tables 1 and 2, contained the highest tissue concentration of TRH immunoreactivity, on the average, followed by the ovary and then the uterus. The TRH levels within all female rat reproductive organs, Table 1, decline continuously from one month of age. The TRH concentration of abdominal fat from 200 g female rats was 1.1 ± 0.5 ng/g

Table 2. Distribution of TRH-like immunoreactivity in the reproductive organs of female pigs (n = 6). Results, uncorrected for recovery losses, are given as mean ±S.D.

Ovaries (ng/g)	Oviducts (ng/g)	Uterus (ng/g)
0.23 ± 0.18	0.32 ± 0.10	0.18 ± 0.09

wet weight (mean ± SD). The pooled pig follicular fluid frozen immediately after removal from the ovary contained 150 pg TRH/ml. Follicular fluid frozen 1 hr after aspiration contained no detectable TRH immunoreactivity. The serial dilution of extracts of rat ovarian, oviductal and uterine tissues, Fig. 1A, were parallel to the TRH standard curve while the extracts from pig ovary, oviduct, uterus and follicular fluid were parallel to the TRH homologous peptide obtained from pig ovary, Fig. 1B (See below). TRH and TRH homologous peptide were not parallel with each other, however, when measured in the same radioimmunoassay. Recoveries of TRH added to pig tissues boiling in 0.5 M acetic acid were: ovary, 84.5%, oviduct, 80.2% and uterus, 75.9%.

As in the male reproductive system (1) two major peaks of TRH immunoreactivity are apparent in the SP-Sephadex C-25 cation exchange profile for the TRH extract of combined rat ovary and oviduct, Fig. 2A, and rat uterus, Fig. 2B. These peak fractions, designated I and II, appear at tube number 33 and 62, respectively. Peak II, corresponding to pure TRH, clearly pre-

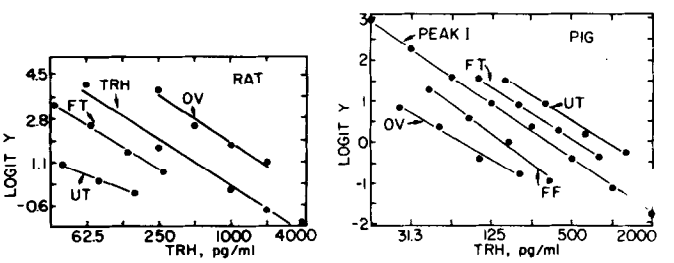

Fig. 1. Parallel line analysis of serial dilutions of tissue extracts from the reproductive system of rats and pigs: OV, ovary; FT, fallopian tubes; UT, uterus; FF, follicular fluid. Synthetic TRH and peak I (TRH homologous peptide) from pig ovaries were used as the reference materials for rat and pig tissues, respectively.

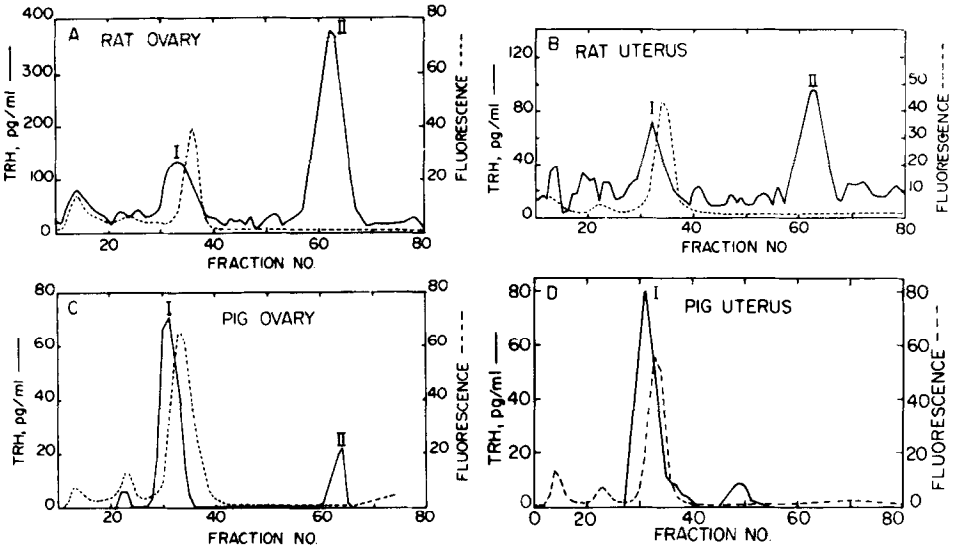

Fig. 2. SP-Sephadex C-25 cation exchange chromatography of (A) extract of ovary and oviduct from 250 g rats, (B) uterine extract from 250 g rats, (C) extract of pig ovaries and (D) extract of pig uteri. Pure TRH elutes at peak II. See Materials and Methods section for details of chromatographic procedures.

dominates. Peak I is termed the TRH homologous peptide (1). A qualitatively similar pattern is seen in the corresponding profiles for the pig ovary, Fig. 2C, and pig uterus, Fig. 2D. The only major difference was that the area of Peak I in these latter chromatograms was much larger relative to Peak II. In all reproductive tissues examined so far, it is characteristic for the bulk of the contaminant peptides and other primary amine-containing compounds to coelute in the same region as peak I, as seen in the dashed fluorescence profiles of Figs. 2 and 3.

The $t_{\frac{1}{2}}$ for TRH added to fresh pig follicular fluid is highly temperature dependent, as seen in Fig. 3A. The rapidity of TRH degradation may explain the absence of TRH in the cation exchange profile of pig follicular fluid, Fig. 3B, which showed no TRH immunoreactivity at tube number 62.

CCK radioimmunoassay

The CCK levels in all male and female rat reproductive tissues examined were below the assay minimum detectable dose. The upper limit for the

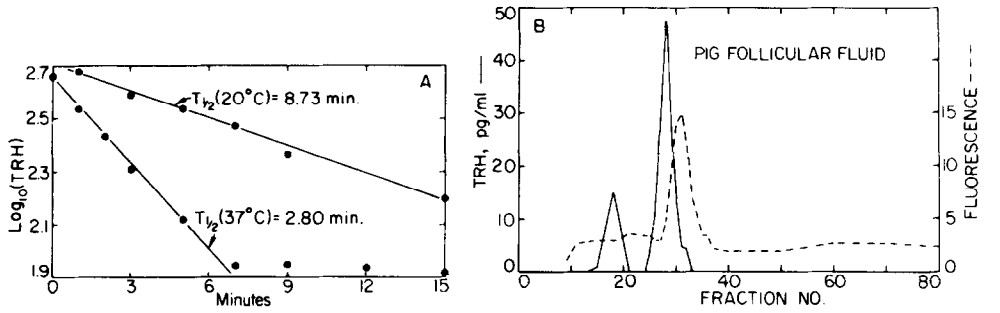

Fig. 3. (A) Half-time of disappearance, $t_{1/2}$, for synthetic TRH added to pig follicular fluid. (B) SP-Sephadex C-25 cation exchange chromatographic profile for extract of fresh pig follicular fluid frozen immediately in dry ice.

possible CCK concentrations, given as pmoles/g wet weight were: oviducts, 0.8; ovaries, 0.3; uterus, 0.05; seminal vesicles, 0.25; epididymus, 0.06; prostate, 0.07; testis, 0.016. This same assay readily measured brain CCK (13).

DISCUSSION

The average TRH immunoreactivity within the female rat reproductive system falls gradually from one month of age. This observation is consistent with the recent demonstration of high circulating TRH levels in the neonatal rat which are derived from the pancreas and other extrahypothalamic sources (14). Mature graffian follicles and corpora lutea are absent from the ovaries of the sexually immature 80-100 g female rat, Table 1. On the other hand, the number of primordial follicles is maximal at birth and declines continuously throughout the reproductive life of the female rat (15). The lower TRH immunoreactivity detected in pig follicular fluid compared to the ovary as a whole suggested the possibility of rapid degradation of TRH (and TRH homologous peptide) by TRH-degrading enzymes within the antrum of the preovulatory follicle. As seen in Fig. 3A, the $t_{1/2}$ for TRH added to fresh pig follicular fluid at 37°C is much shorter than the 15.4 min $t_{1/2}$ measured in fresh human serum at the corresponding temperature (16). Because the cellular composition of the oviducts and uterus is complex, immunohistochemical techniques may be required to establish the cellular origin of TRH in these

organs. Previous reports of TRH in human placenta (3,4) and amniotic fluid (2) suggest an endometrial origin for TRH in the nonpregnant uterus.

As seen in Fig. 3 there are at least two peptides extractable from the ovary, oviduct and uterus, labeled Peaks I and II, which crossreact in the TRH radioimmunoassay. As previously demonstrated for the male reproductive organs using high pressure reversed phase liquid chromatography (1) peak II corresponds to TRH and peak I to a structurally homologous peptide. Moreover, in the female rat reproductive tract, TRH appears to be the predominant peptide. This is also reflected in the consistent parallelism of the TRH standard curve with the displacement curves resulting from the serial dilution of the female rat reproductive tissue extracts, Fig. 1A. Conversely, in the female pig reproductive organs, the TRH homologous peptide predominates. Extracts of these tissues more consistently parallel the Peak I standard curve upon serial dilution.

The observation of several "hypothalamic" peptides within the mammalian reproductive system including LH-RH (17), β-endorphin (7), Met-enkephalin and Leu-enkephalin (18) and TRH (1) suggested that other peptides that have recently been identified in the brain, such as CCK octapeptide (19), might also be detected in the reproductive system. Unfortunately, this peptide as well as another cross-reacting peptide, gastrin, could not be detected. The other peptides which have been found, however, provide a unique opportunity for studies on their phylogenetic and embryologic distribution. In addition, these peptides may serve as markers permitting immunohistochemical identification of new classes of cells not previously distinguishable by traditional histological criteria.

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