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Objective

To define the active structure of prepro-thyrotropin releasing hormone fragments in suppressing/regulating basal and stress-induced prolactin secretion by the anterior pituitary gland.

Introduction

Synthesis of Thyrotropin-Releasing Hormone (TRH)

- Prepro-thyrotropin releasing hormone (ppTRH) 178-199 is one of several cryptic peptides cleaved during TRH synthesis.
- TRH is well known to induce PRL secretion.
- ppTRH 178-199 has been shown to acutely reduce the stress-induced rise in PRL, ACTH, and CORT secretion.1
- ppTRH 178-199 has also been shown to reduce anxiety-related behaviors in rats.2
- ppTRH 178-199 is cleaved at the amino terminus to yield ppTRH 186-199, which is thought to be the active fragment in the regulation of PRL, ACTH, and CORT secretion.

Methods

In Vivo

ppTRH fragments were administered IP to 15 week-old intact male Sprague-Dawley rats 15 min. prior to a ten min. restraint stress. Ten minutes following stress animals were sacrificed and blood collected. Fragments administered: (1) ppTRH 186-199 (2) ppTRH 194-199 (COOH terminus) (3) ppTRH 186-191 (NH2 terminus).

Plasma PRL was measured using enzyme immunoassay (EIA) (Cayman Chemical ®) by manufacturer instructions. Standard curves ranged from 0.39 to 0.50 pg/mL. Intra-assay coefficients of variation: 4.2, 5.2. Intra-assay coefficients of variation: 3.4. Plasma CORT and ACTH were measured using radioimmunoassay.

Results

In Vivo

- ppTRH 186-199 and 194-199 reduce stress-induced corticosterone rise.
- ppTRH 186-199 caused a trend to reduction in plasma CORT.
- ppTRH 194-199 caused significant reductions in plasma CORT. (p<0.05)

In Vitro

- ppTRH 186-199 significantly reduced the stress-induced rise in rat serum PRL in a dose-dependent fashion (2, 5, and 10 mg/kg). (p<0.05)
- ppTRH 194-199 also reduced the stress-induced rise in PRL (at 10 and 20 mg/kg) while ppTRH 186-191 did not. (p<0.05)

Discussion

SUMMARY

- PreproTRH 186-196 suppresses stress-induced rises in plasma PRL.
- PreproTRH 186-196 attenuates the stimulatory effect of estradiol on PRL secretion by rat MMQ anterior pituitary tumor cells.
- Prepro TRH 194-199 reduces the stress-induced rises in plasma PRL.
- PreproTRH 186-191 fragment had no activity.
- Prepro TRH 194-199 suppresses plasma CORT in the stressed rat.

CONCLUSIONS

These findings suggest that the carboxy terminus of ppTRH 178-199 interacts with its receptor at the anterior pituitary gland since ppTRH 194-199 contains all the activity of this ppTRH cryptic peptide for the regulation of PRL and CORT secretion.

References

2. Eduardo A. Nillni, Kevin A. Seravalli. The Biology of Pre-Pro Thyrotropin Releasing Hormone Peptides. Endocrine reviews 205(159-164).
ppTRH 166-199 causes a significant reduction in prolactin in the presence of estradiol, p < 0.05.
ppTRH 186-199 inhibits CORT in the Stressed Rat

![Graph showing the inhibition of CORT by ppTRH 186-199 with different treatments.]
**ppTRH 186-199 Inhibits E2-Induced PRL Release at 30min**

* = significantly different from vehicle; p < .05

E2 was significantly different from V and ppTRH but not ppTRH+E2, however pp+E was not significant different from V.

Analysis was done with 1-way anova and tukey post-hoc

**ppTRH 186-199 Has No Effect on PRL at 4 Hrs**

* = significantly different from all groups; p < .05

E2 was different from all other groups.

ppTRH+E2 was different from all other groups except ppTRH alone.
Intraperitoneal injection of ppTRH reduces stress induced prolactin rise

35 minutes after injection
20 minutes after onset of stress, 10 minutes after end of stress.

* = significantly different from Vehicle and Nt-186-191
preproTRH, 29 KDa

proTRH, 26 KDa

15 KDa

10 KDa

9.5 KDa

16.5 KDa

5.1 KDa

Lys-Arg-Gln-His-Pro-Gly-Lys-Arg

CPE or CPD

Gln-His-Pro-Gly (TRH-Gly)

PAM

pGln-His-Pro-NH₂ (TRH)