

The Cryptic Peptides, Prepro-Thyrotropin Releasing Hormone 186-199 and 194-199 Suppress Anterior Pituitary Prolactin Secretion *in vivo* and *in vitro*.

Emily Shortridge¹, Chad D. Foradori¹, Alicia M. Quihuis¹, Robert F. McGivern², T. John Wu³ and Robert J. Handa¹
¹University of Arizona College of Medicine-Phoenix; ²San Diego State University; ³Uniformed Services University of the Health Sciences

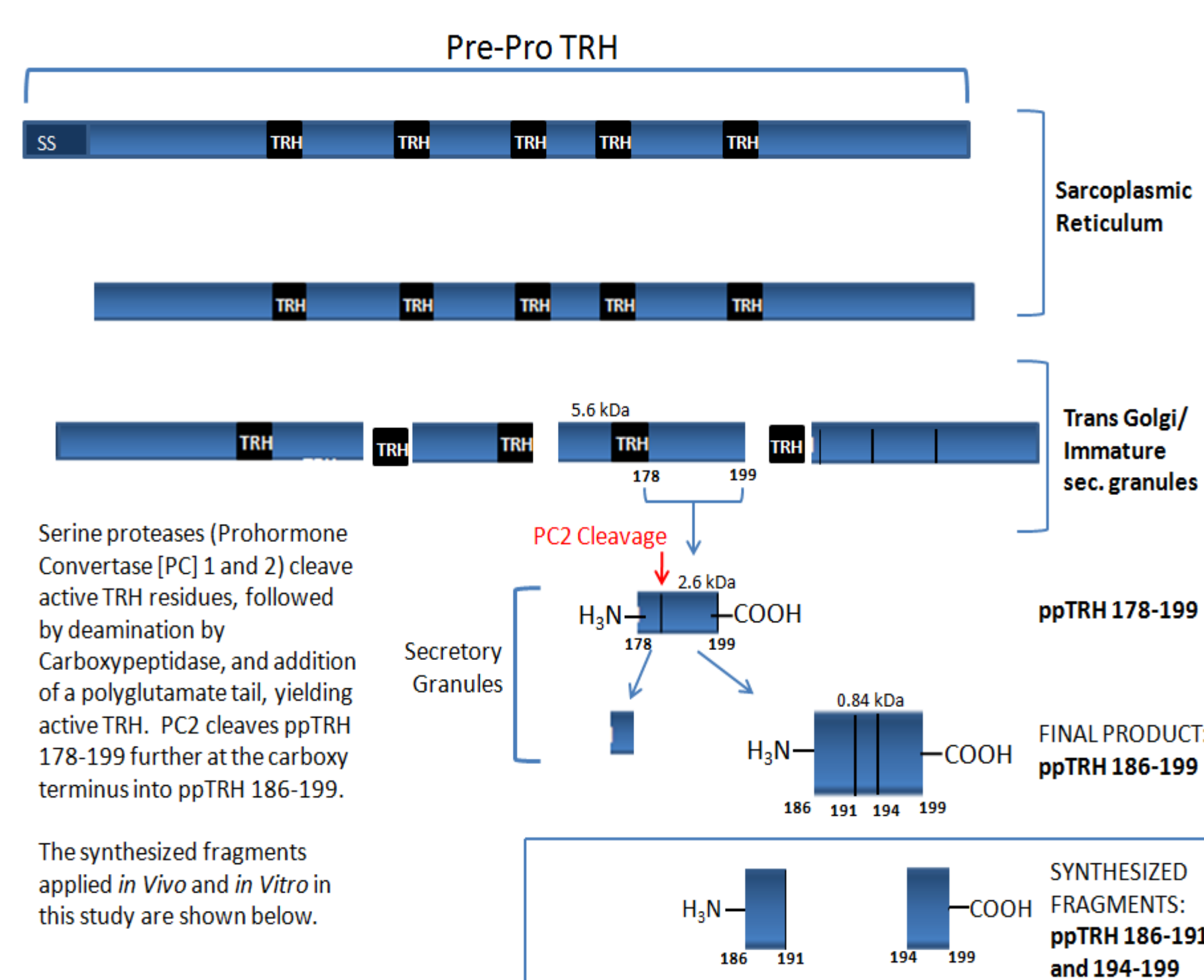


Objective

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)



2.3

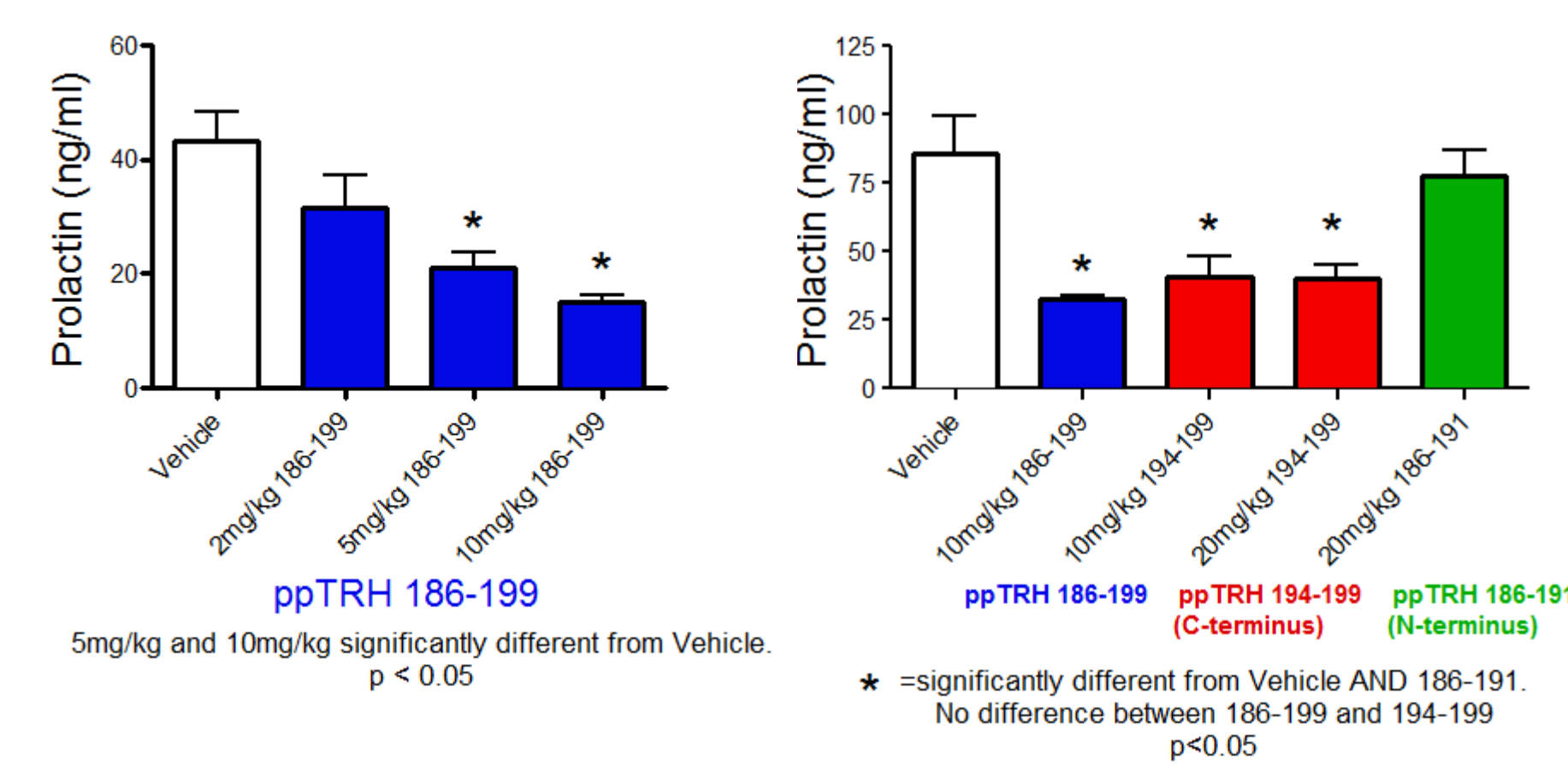
- Prepro-thyrotropin releasing hormone (ppTRH) 178-199 is one of several cryptic peptides cleaved during TRH synthesis
- TRH is well known to induce PRL secretion.
- ppTRH 178-199 has been shown to acutely reduce the stress-induced rise in PRL, ACTH, and CORT secretion¹.
 - ppTRH 178-199 has also been shown to reduce anxiety-related behaviors in rats⁴.
- 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.

KEY:
TRH – Thyrotropin-Releasing Hormone, PRL – Prolactin
CORT – corticosterone, ACTH – Adrenocorticotrophic Releasing Hormone

Results

In Vivo

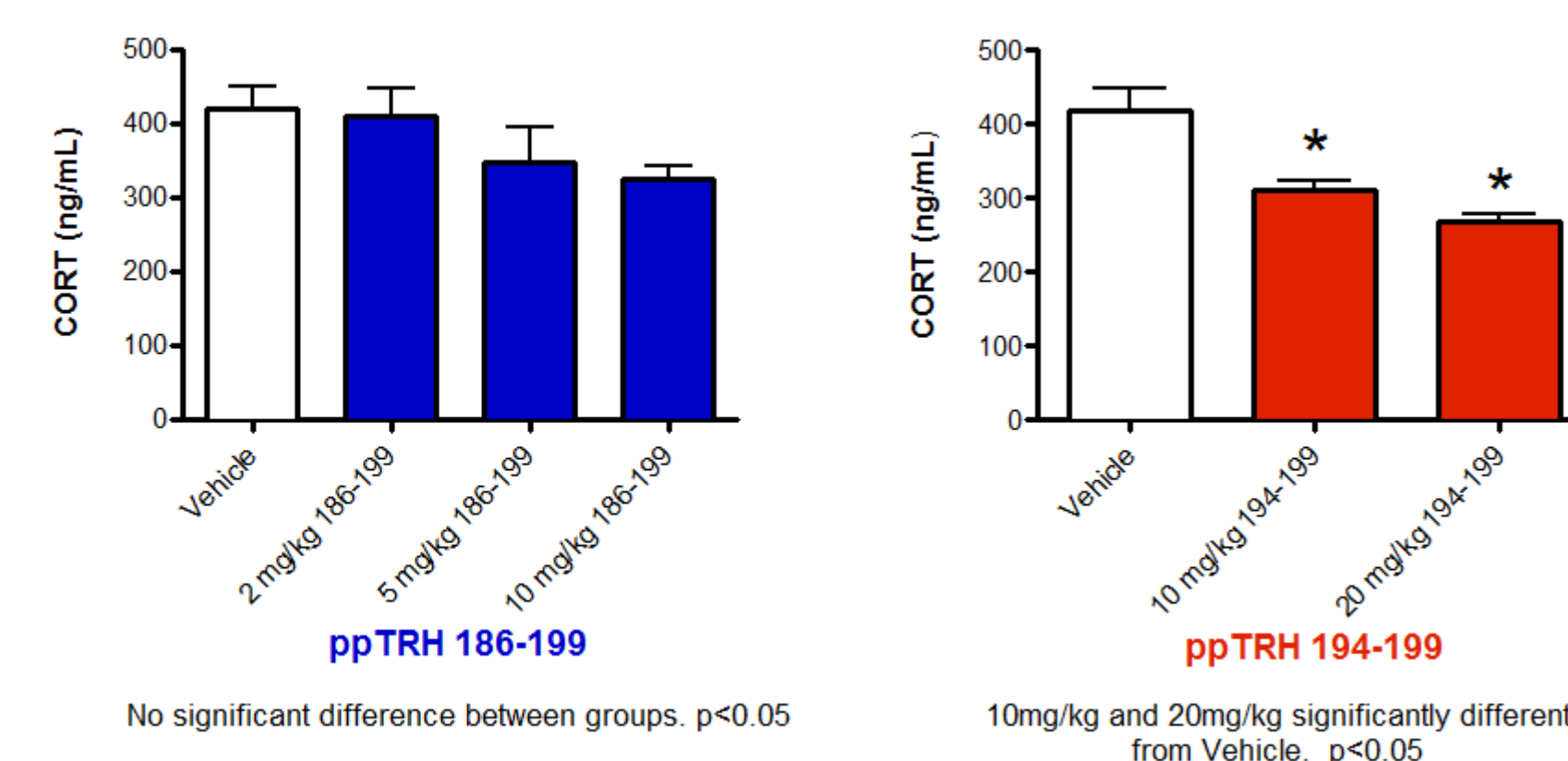
ppTRH 186-199 and 194-199 Reduce Stress-Induced Prolactin Rise



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. ($p < 0.05$)

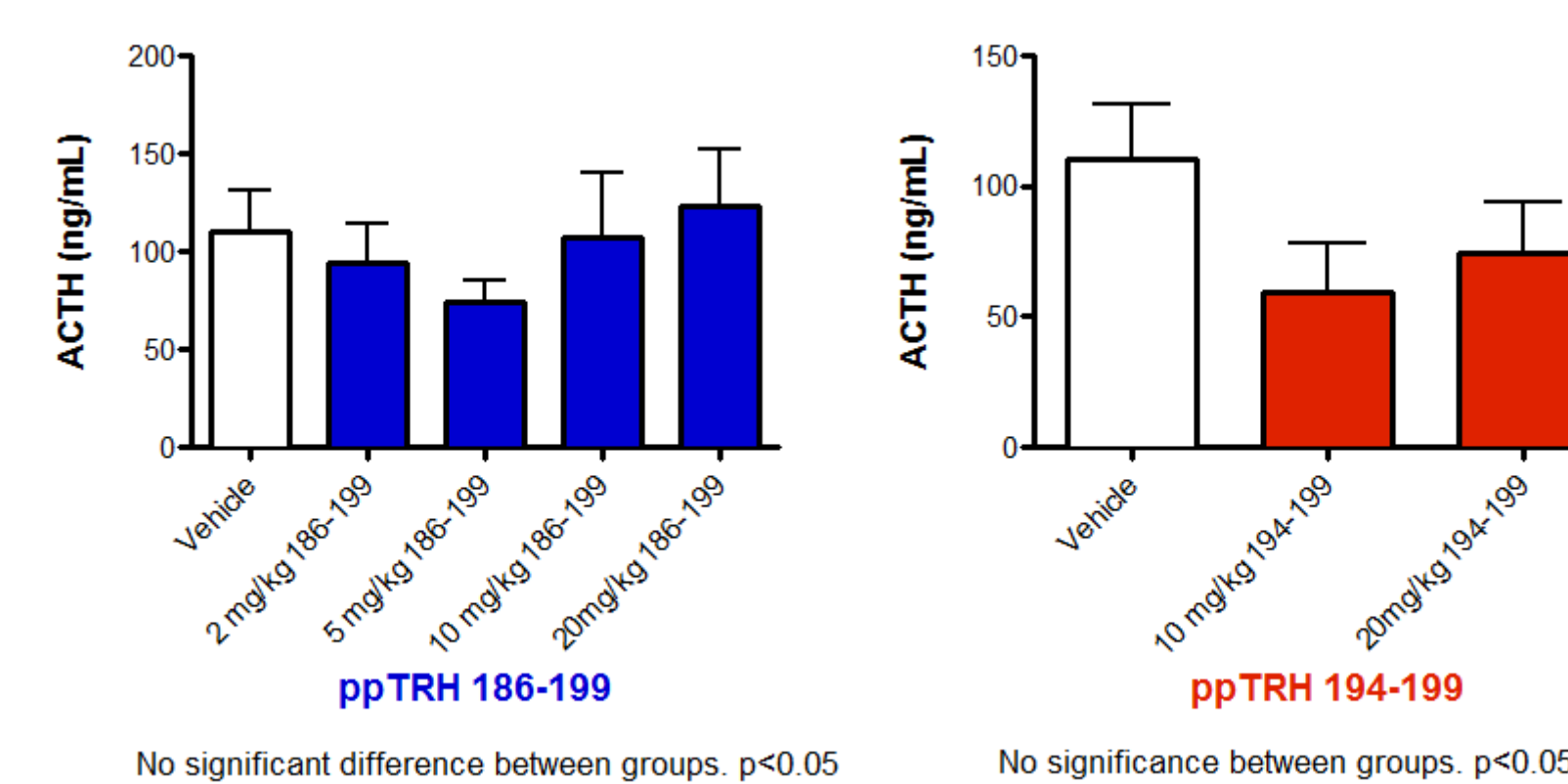
ppTRH 186-199 and 194-199 Reduce Stress-Induced Corticosterone Rise



ppTRH 186-199 caused a trend to reduction in plasma CORT.

ppTRH 194-199 caused significant reductions in plasma CORT. ($p < 0.05$)

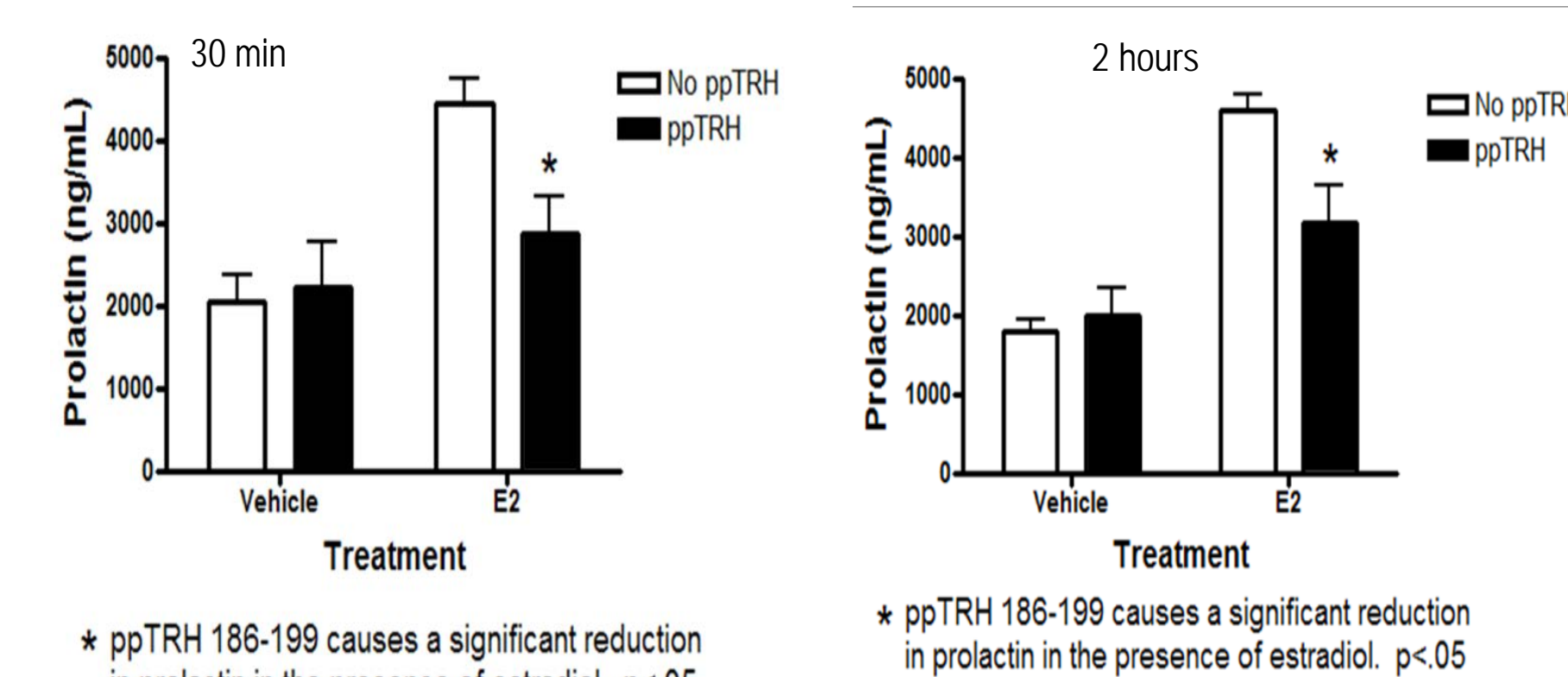
ppTRH 186-199 and 194-199 did not reduce ACTH



ppTRH 186-199 and 194-199 caused a trending reduction in plasma ACTH, without significance.

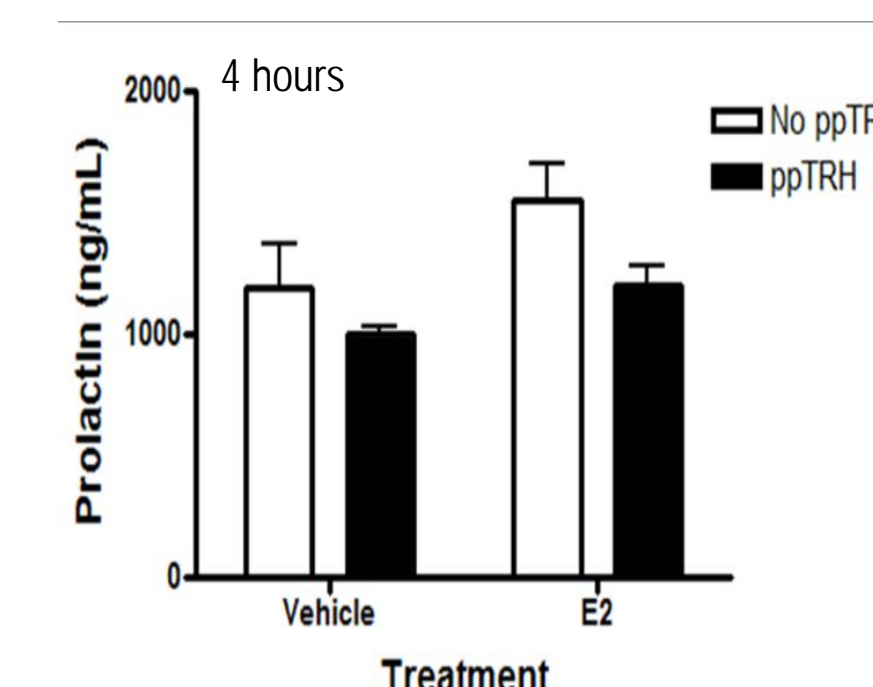
In Vitro

In MMQ cells, ppTRH 186-199 reduces the E2-induced rise in Prolactin



* ppTRH 186-199 causes a significant reduction in prolactin in the presence of estradiol. $p < 0.05$

* ppTRH 186-199 causes a significant reduction in prolactin in the presence of estradiol. $p < 0.05$



No significant difference in Prolactin made by ppTRH, with or without the presence of Estradiol.

E2 administration induced an increase in PRL secretion for up to 48 hrs, which was reduced by ppTRH 186-199 at 30 min and 2 hrs after treatment with ppTRH 186-199.

This effect became insignificant by 4 hours and was absent by 24 hrs.

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 (NH3 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.



In Vitro

PRL-secreting MMQ rat anterior pituitary tumor cells were treated with the same three ppTRH fragments. MMQ cell line obtained from ATCC® (Catalog No. CRL-10609) and plated 20-50% confluent. Media was first charcoal-stripped to remove steroids contained in FBS. Cells were treated with and without 10nM estradiol (E2), beginning 48 hours prior to administration of 100 nM ppTRH. Cell media was taken at 30 min, 2 hr, 4 hr, 24 h, and 48h after ppTRH exposure and PRL measured by EIA (Cayman Chemical®) by manufacturer instructions. Cells were harvested at 48h and PRL rPCR was performed. Standard curves ranged from 0.39 to 0.50 pg/mL. Intra-assay coefficients of variation: 4.5. Inter-assay coefficient of variation: 3.8. Analyses were performed with Multi-way ANOVA and Tukey post-hoc.

Discussion

SUMMARY

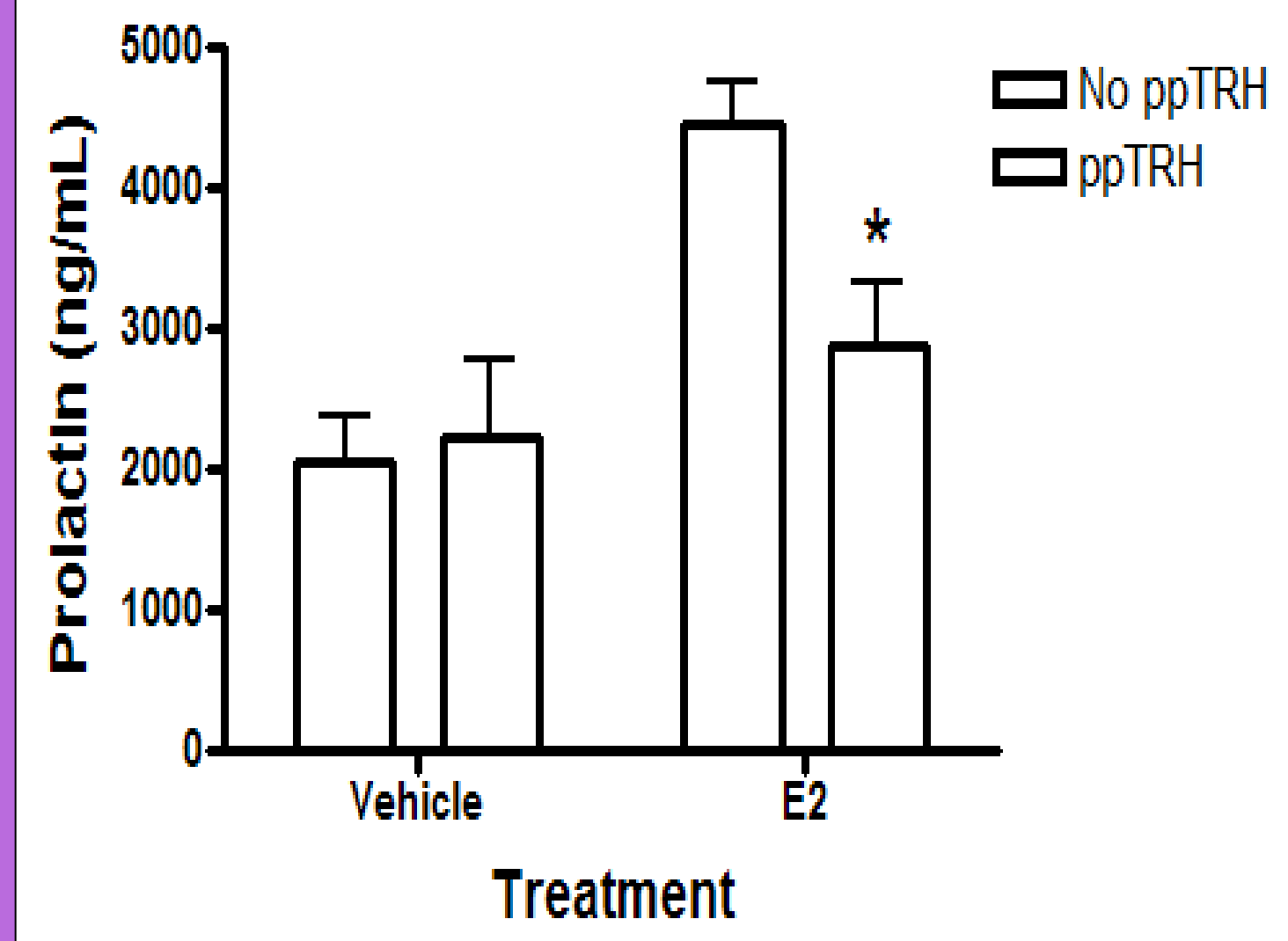
- PreproTRH 186-199 suppresses stress-induced rises in plasma PRL.
- PreproTRH 186-199 attenuates the stimulatory effect of estradiol on PRL secretion by rat MMQ anterior pituitary tumor cells.
- Prepro TRH 194-199 reduces the stress-induced rises in plasma PRL
- PreproTRH 186-191 fragment had no activity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

CONCLUSIONS

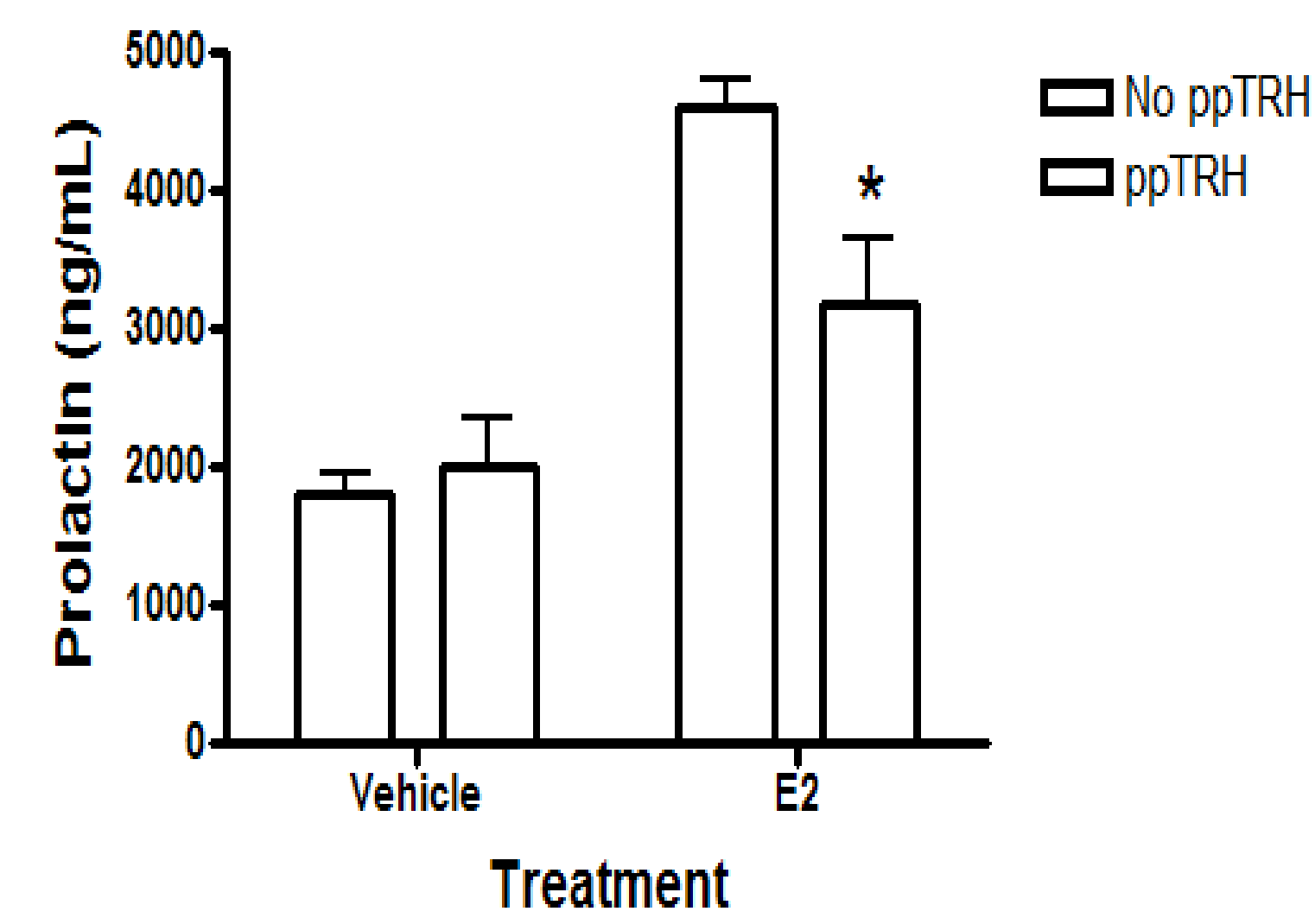
These findings suggest that the carboxy terminus of ppTRH 178-199 interacts with its receptor at the anterior pituitary gland since ppTRH 194-199 contains all the activity of this ppTRH cryptic peptide for the regulation of PRL and CORT secretion.

References

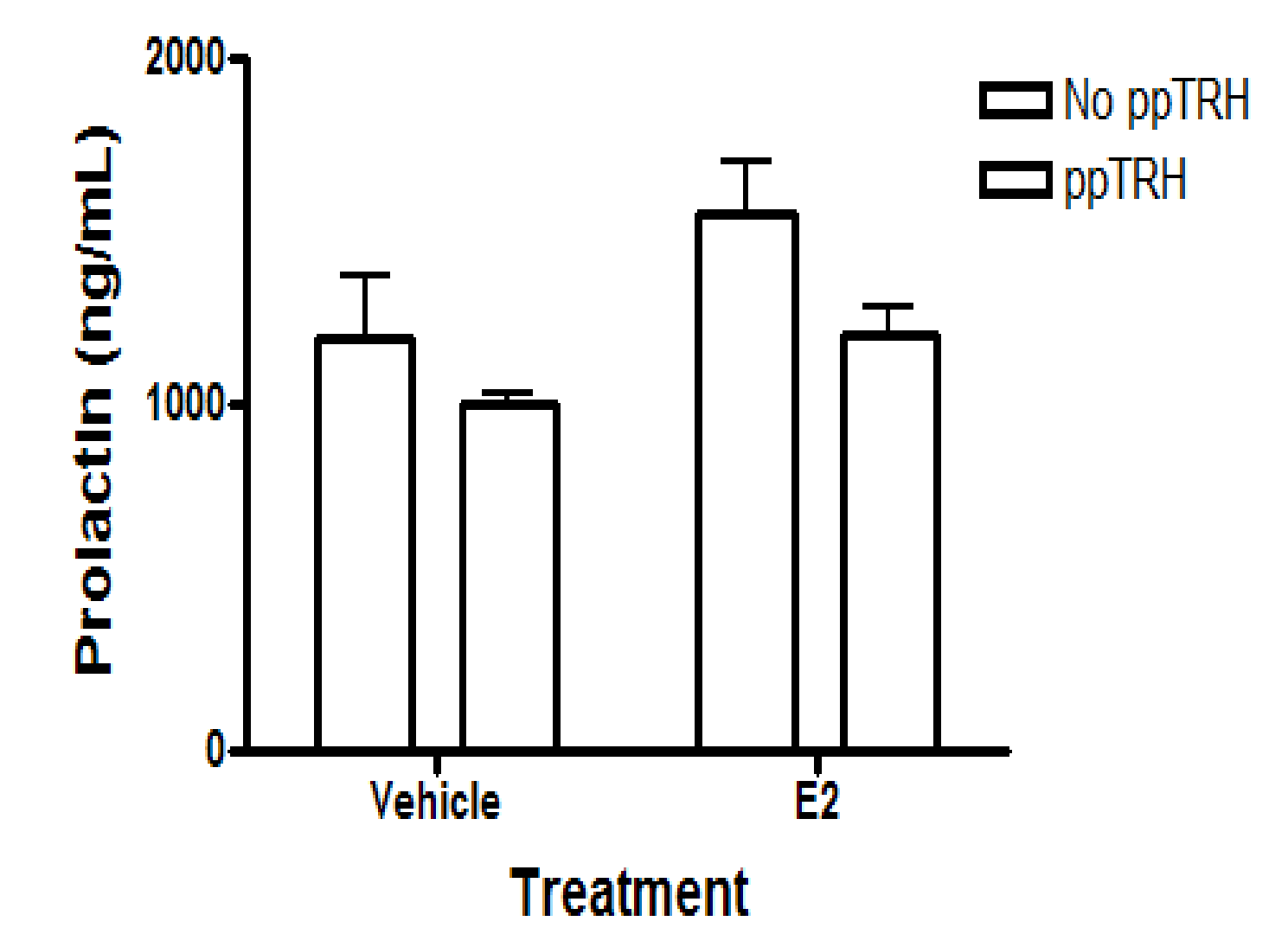
1. Robert F. McGivern, Peter Rittenhouse, Fraser Aird, et al. Inhibition of Stress-Induced Neuroendocrine and Behavioral Responses in the Rat by Prepro-Thyrotropin-Releasing Hormone 178-199. *Journal of Neuroscience* 1997 17(12):4886-4894
2. Eduardo A. Nillni, Kevin A Sevarino. The Biology of Pre-Pro Thyrotropin Releasing Hormone Peptides. *Endocrine reviews* 20(5):559-648.
3. Omparo Romero, Isin Kahir, Charles A. Vaslet. Role of Pro-Sequence in the Secretory Pathway of Prothyrotropin Releasing Hormone. *J of Biol Chem* Vol 273 No 46 pp. 31438-31448.
4. Giraldo, Pecori F, S. Pesce, P. Maroni, et al. Inhibitory Effect of Prepro-Thyrotropin-Releasing Hormone (178-199) on Adrenocorticotrophic Hormone Secretion by Human Corticotroph Tumours. *Journal of Neuroendocrinology* 22, 294-300.
5. Shotaro Suzuki, Leah C. Solberg, Eva E. Redei, et al. Prepro-thyrotropin releasing hormone 178-199 immunoreactivity is altered in the hypothalamus of the Wistar-Kyoto strain of rat. *Brain Research* 913 (2001) 224-233.
6. E. Redei, H. Hilderbrand, F. Aird, et al. Corticotropin release inhibiting factor is encoded within prepro-TRH. *Endocrinology* 136 (1995) 1813-1816.
7. E. Redei, H. Hilderbrand, F. Aird, et al. Corticotropin release-inhibiting factor is preprothyrotropin-releasing hormone-(178-199). *Endocrinology* 136 (1995) 3557-3563.



* ppTRH 186-199 causes a significant reduction in prolactin in the presence of estradiol. $p < .05$

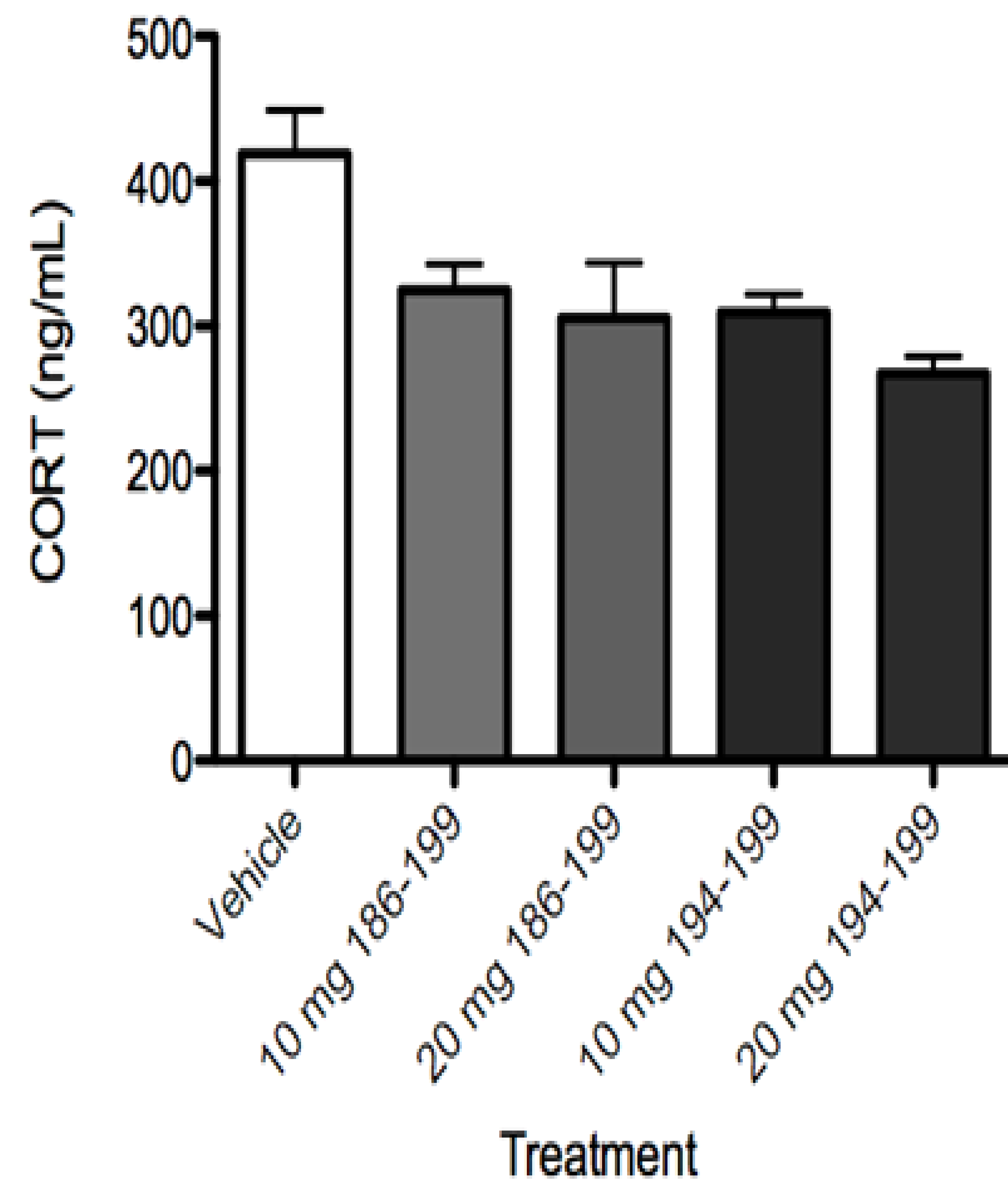


* ppTRH 186-199 causes a significant reduction in prolactin in the presence of estradiol. $p < .05$

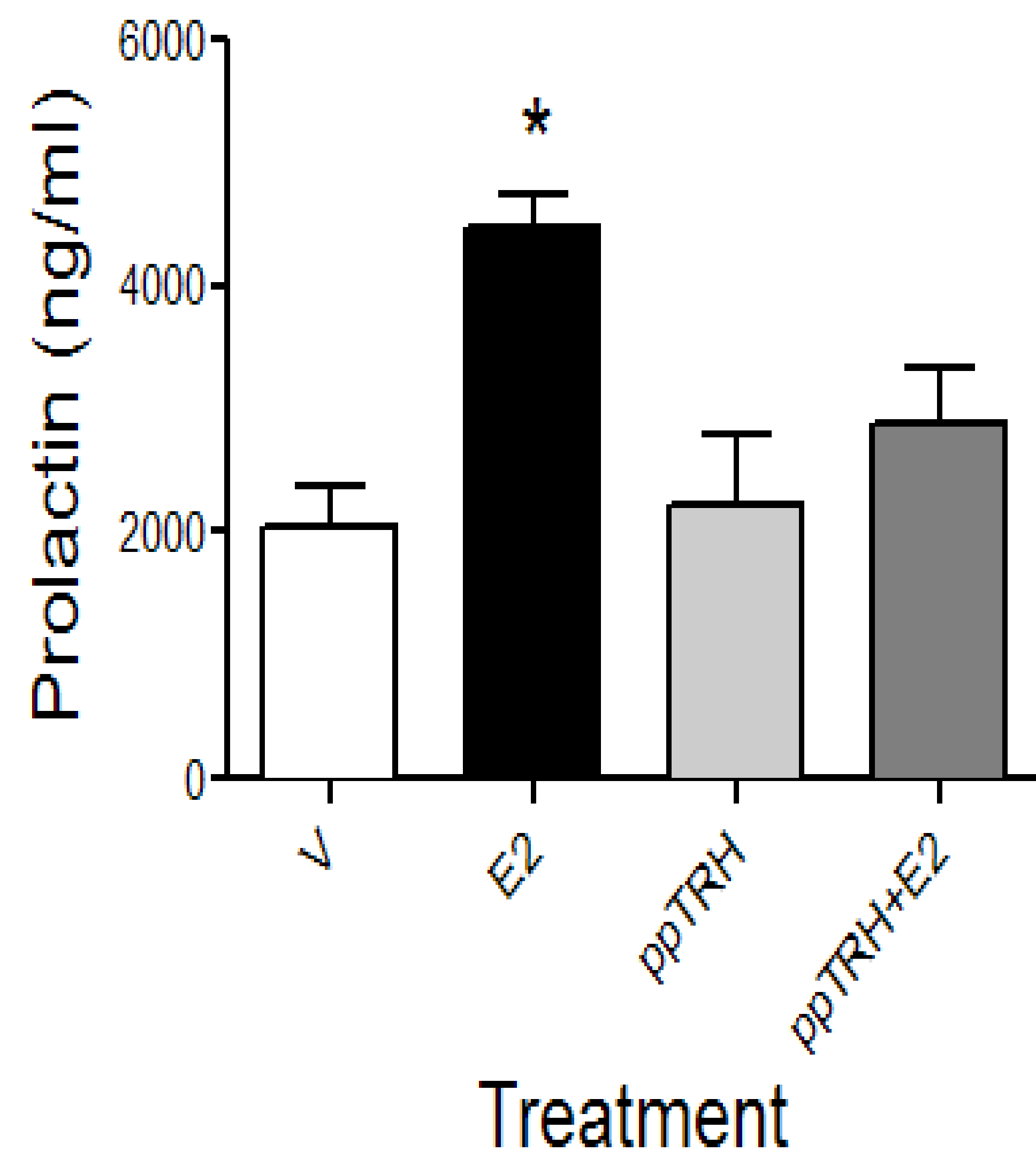


No significant difference in Prolactin made by ppTRH, with or without the presence of Estradiol.

ppTRH 186-199 inhibits CORT in the Stressed Rat



ppTRH 186-199 Inhibits E2-Induced PRL Release at 30min

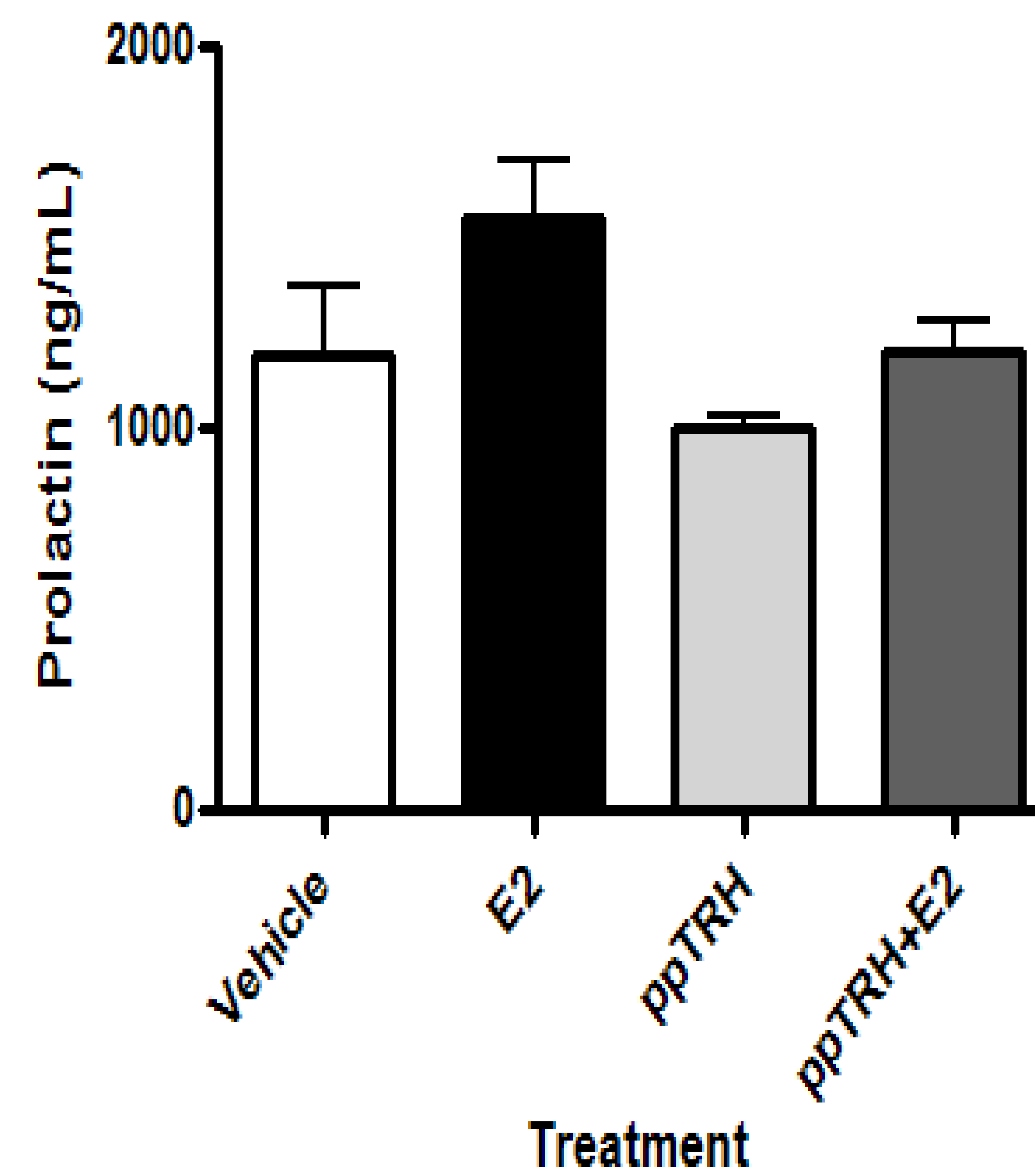


* = significantly different from vehicle; $p < .05$

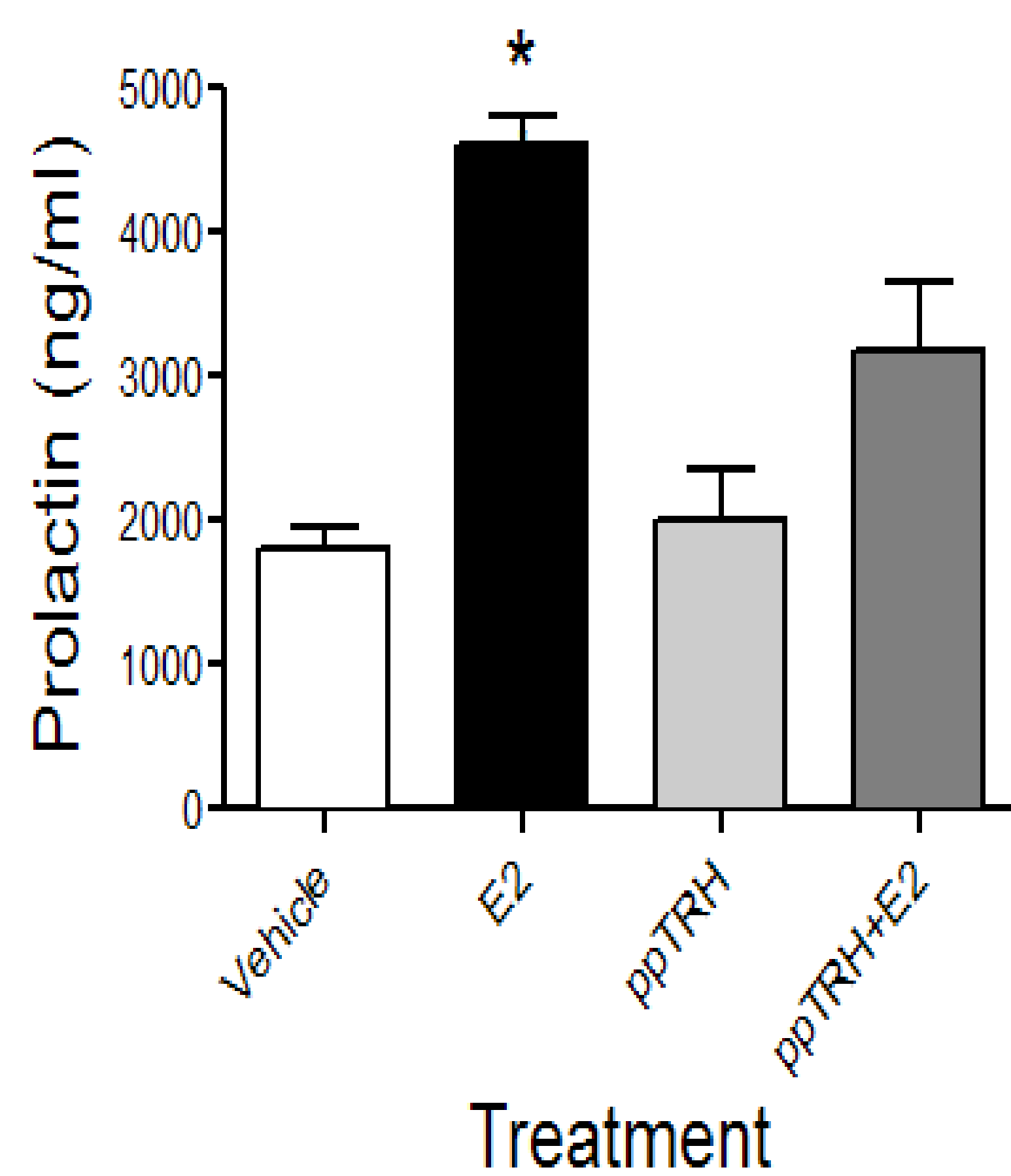
E2 was significantly different from V and ppTRH but not ppTRH+E2, however pp+E was not significant different from V.

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

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



ppTRH 186-199 Inhibits E2 Induced PRL Release at 2 hours

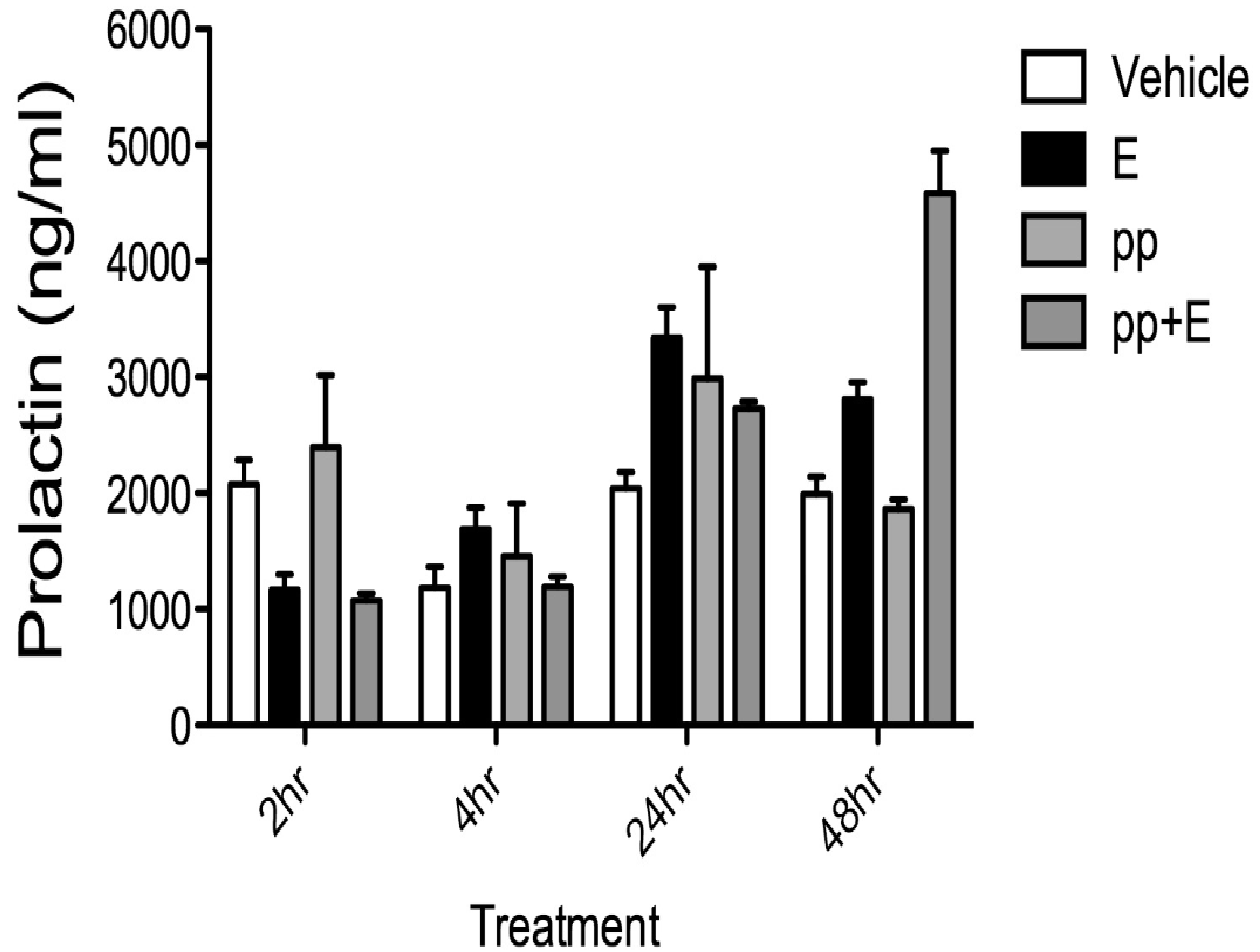


* = significantly different from all groups; $p < .05$

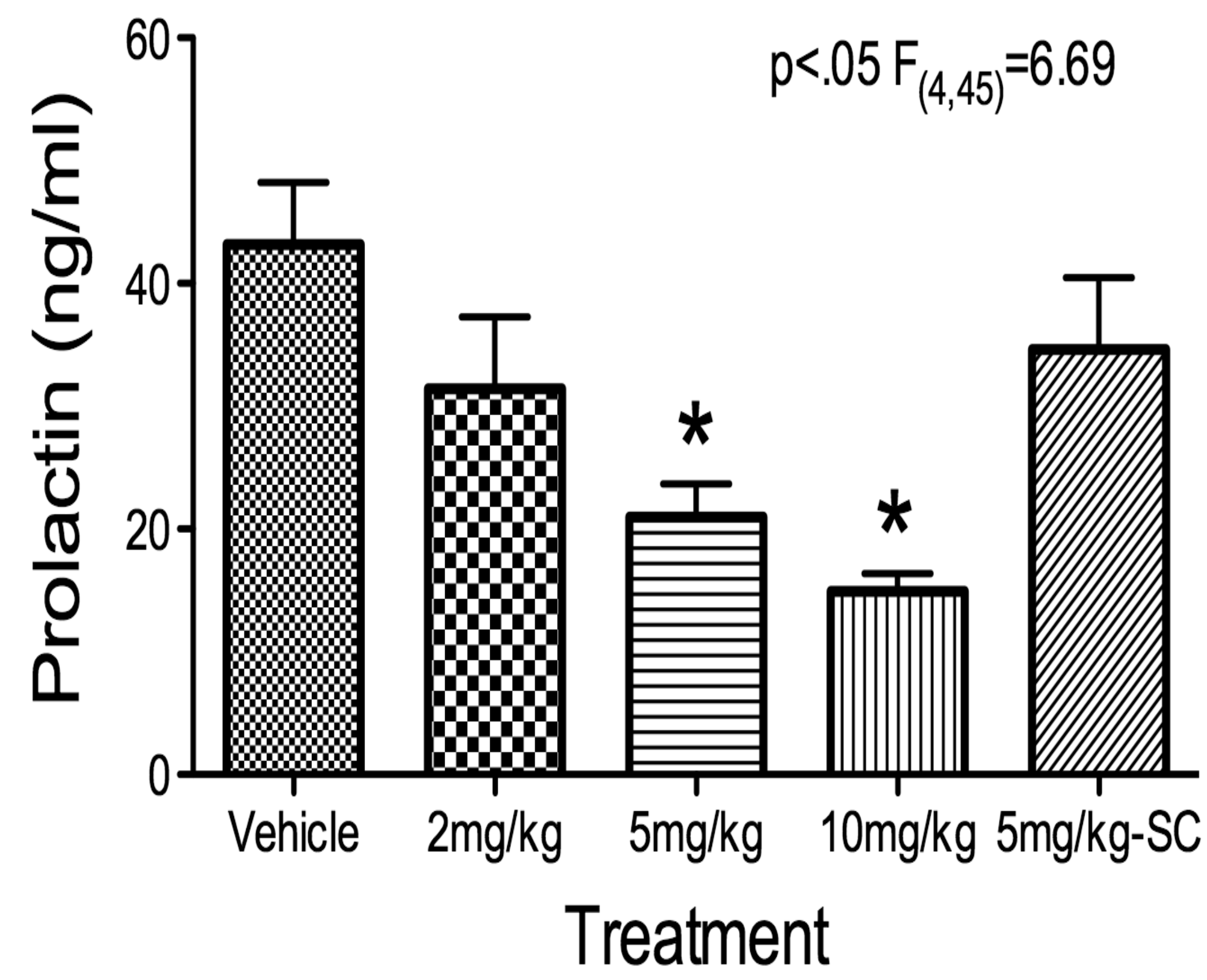
E2 was different from all other groups.

ppTRH+E2 was different from all other groups except ppTRH alone

Time Course Summary

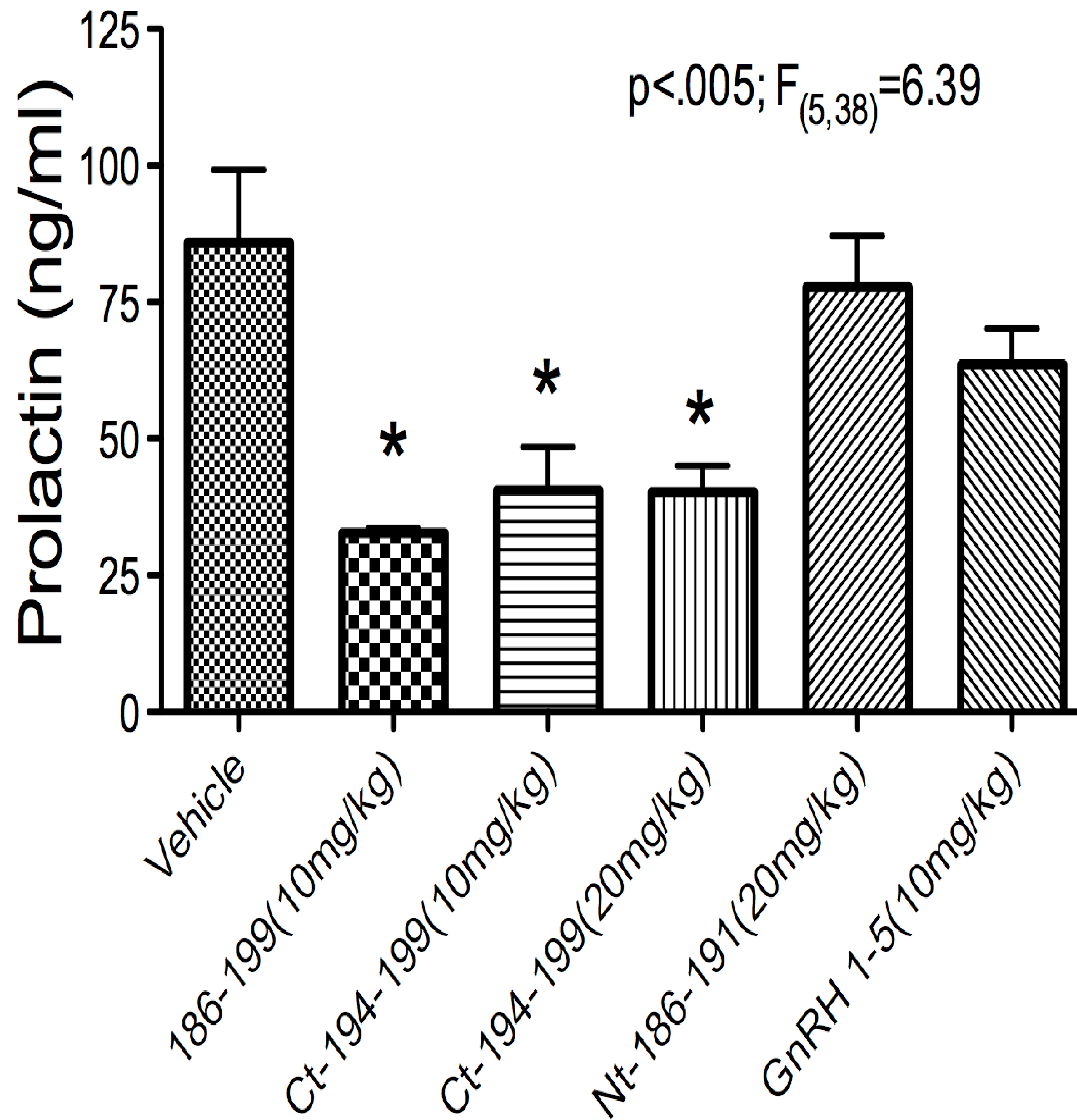


Intraperitoneal injection of ppTRH
reduces stress induced prolactin rise



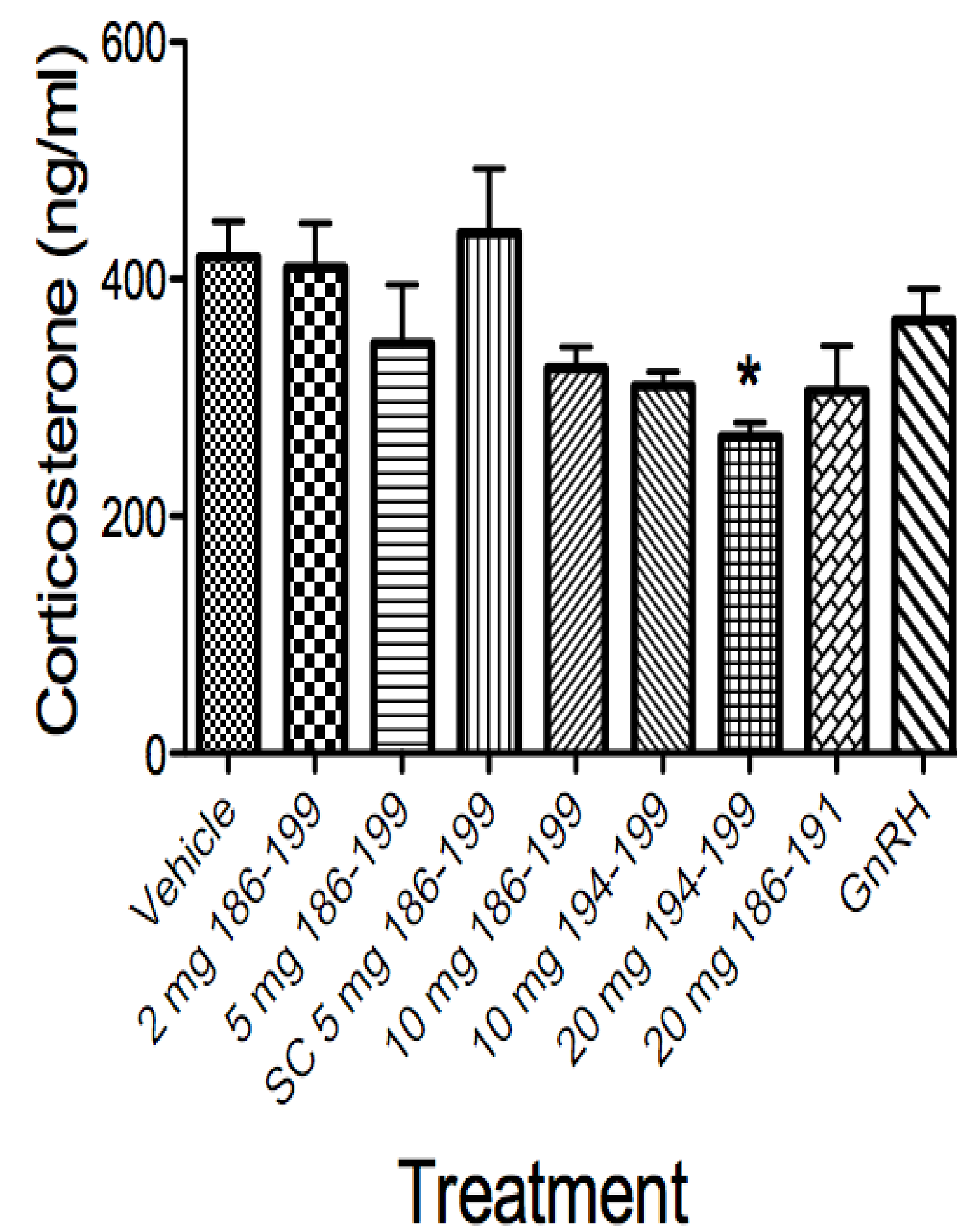
35 minutes after injection
20 minutes after onset of stress, 10 minutes
after end of stress.

Intraperitoneal injection of ppTRH
reduces stress induced prolactin rise



* =significantly different from Vehicle and Nt-186-191

CORT Levels



* =significantly different from Vehicle treated animals

