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Gender-Biased Activity of the Novel Prolactin Releasing Peptides

Comparison with Thyrotropin Releasing Hormone Reveals Only Pharmacologic Effects

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The prolactin- (PRL) releasing activities of the newly described PRL-releasing peptides (PrRPs) were compared to that of thyrotropin-releasing hormone (TRH) in dispersed, rat anterior pituitary cell cultures. A dose-related stimulation of PRL release by TRH was observed in cells harvested from both intact male and random cycle female pituitary donors. The minimum effective dose of TRH ranged from 1 to 10 n*M*. Neither PrRP-20 nor PrRP-31 significantly altered PRL secretion in cells from male donors even at doses as high as 1 μ*M*. In cells harvested from females, only the highest doses of PrRP-20 and PrRP-31 tested (0.1 and 1.0 μ*M*) significantly stimulated PRL secretion. The PRL-releasing action of TRH was observed already at 15 min of incubation, whereas those of PrRP-20 and PrRP-31 appeared only after 1 and 2 h of incubation, and the magnitude of PRL release in the presence of 1 μ*M* PrRPs was significantly less than that of a similar dose of TRH. These data do not suggest a physiologically relevant role for the PrRPs in the neuroendocrine regulation of PRL secretion in intact male and nonlactating, random-cycle female rats.

Key Words: Pituitary; prolactin; releasing factors.

Introduction

Multiple evidence indicates the presence of yet-to-be identified prolactin- (PRL) releasing activities in the mammalian hypothalamus *(1)* and neurointermediate lobe of the pituitary gland *(2)*. Several peptides possessing PRL-releasing activity have been identified, but only one, thyrotropin-releasing hormone (TRH), has been demonstrated uniformly to be active and potent in both male and female animal models, in vivo and in vitro. Recently, two novel peptides, identified as ligands that bound the orphan receptor, hGR_3, in the pituitary gland, have been identified to possess prolactin-releasing factor (PRF) activity *(3)*. These peptides, termed prolactin-releasing peptides (PrRPs), are overlapping, posttranslational products of the same preprohormone, and have been designated PrRP20 and PrRP-31 on the basis of their bioactivity and their amino acid content. In the original description of these peptides, their PRF activity was demonstrated in a rat pituitary adenoma cell line and in anterior pituitary cells harvested from lactating female rats *(3)*. It has been our experience that some peptides possessing PRF activity in cells harvested from lactating female rats are also bioactive in cells harvested from random-cycle female rats, but not those from male pituitary donors *(4,5)*. This and their weaker biopotency (4–7) distinguish those peptides from TRH, which is active across a wide dose range in cells harvested from both female and male donors. Thus, we endeavored to determine if PrRP-20 and PrRP-31 were bioactive in both male and female cells, and compared their relative potency to that of TRH.

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Results

In two separate cell harvests, derived from random-cycle female pituitary donors, both PrRP-20 and PrRP-31 exerted PRF activity, although only at very high doses. In the first experiment, only the highest doses (10^{-7} *M*) of both PrRP-20 and PrRP-31 exerted significant ($p < 0.05$) PRF activity (113 and 123% of control, respectively). In the second experiment, the dose range was extended in log-molar doses from 10^{-11}–10^{-6} *M*. Again both peptides exerted significant PRF activity at the 10^{-7} *M* doses (Fig. 1), and, although a greater PRF activity was present at the higher dose of 10^{-6} *M*, there were no significant differences in the PRF activities of each peptide when the amounts of PRL released by these two doses (10^{-6} vs 10^{-7} *M*) were compared. TRH, on the other hand, stimulated PRL release at lower doses, and a significant dose relationship was present ($p < 0.001$). The magnitude of the PRF activity of TRH when compared to the PrRPs on

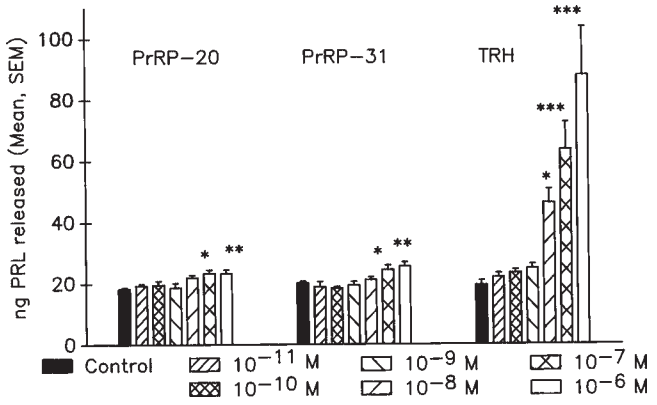

Fig. 1. Prolactin release from dispersed anterior pituitary cells harvested from random cycle female rat donors during 1 h of incubation. TRH and PrRPs (PrRP-20 and PrRP-31) were added in doses ranging from 10 p*M* to 1 μ*M*, $n = 6$, except controls (no peptide) where $n = 12$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs control.

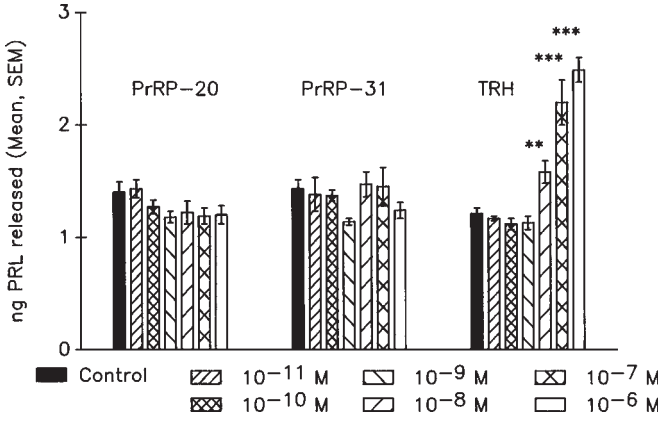

Fig. 2. PRL release from dispersed anterior pituitary cells harvested from intact male rat donors during 1 h of incubation. TRH and PrRPs (PrRP-20 and PrRP-31) were added in doses ranging from 10 p*M* to 1 μ*M*. $n = 6$, except controls (no peptide) where $n = 12$.**$p < 0.01$, ***$p < 0.001$ vs control.

the basis of the amount of hormone released in the presence of the minimal effective doses (0.1 μ*M* for the PrRPs vs 1.0 n*M* for TRH) was significantly greater in all cases ($p < 0.001$). None of the peptides tested, at any dose, significantly altered the release of growth hormone (data not shown).

In cells harvested from male rat donors, significant PRF activity was expressed by TRH already at the 10^{-8} *M* dose (Fig. 2), and a significant dose relationship was evidenced ($p < 0.001$). In the first experiment employing male cells, doses of PrRP-20 and PrRP-31 ranging from 10^{-12} to 10^{-7} *M* were without significant effect on PRL release (data not shown). In the second experiment with cells harvested from male rats (Fig. 2), neither PrRP-20 nor PrRP-31, even at a dose as high as 10^{-6} *M* significantly altered PRL release

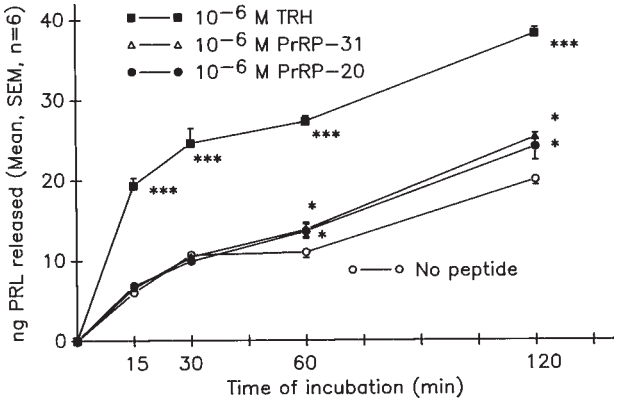

Fig. 3. PRL release from dispersed anterior pituitary cells harvested from random-cycle female rat donors as a function of time in the absence or presence of 1 μ*M* TRH or PrRPs (PrRP-20 and PrRP-31), $n = 6$. *$p < 0.05$, ***$p < 0.001$ vs control.

when compared to controls. Again, growth hormone release was not significantly altered by the three peptides at any dose (data not shown).

A time-course comparison for the PRF activities of PrRP-20, PrRP-31, and TRH, all at 10^{-6} *M*, was conducted in cells harvested from random-cycle female rats (Fig. 3). At the earliest time-point sampled (15 min of peptide exposure), a significant stimulation ($p < 0.001$) was observed in cells exposed to TRH. Significantly ($p < 0.001$) more PRL was released at all time-points from cells exposed to TRH when compared to cells incubated in culture medium alone. PRL release in response to PrRP-20 and PrRP-31 was not significantly different from cells incubated in control medium at 15 and 30 min. In agreement with results from the initial experiment displayed in Fig. 1, after 60 min of exposure to 10^{-6} *M* PrRP-20 and PrRP-31, significant stimulation of PRL release was observed ($p < 0.05$). However the magnitude of the PRF activity of those two peptides was significantly less than that of TRH ($p < 0.001$). Similar results were obtained following 120 min of incubation.

Discussion

Like several other putative PrRPs, most notably vasoactive intestinal peptide *(4)* and oxytocin *(5)*, the recently discovered PrRPs *(3)* are significantly less potent in cells derived from random-cycle rats than TRH in terms of both the magnitude of stimulation of PRL secretion and the latency to onset of hormone release. Importantly, neither PrRP-21 nor PrRP-30, even at micromolar doses, was an effective PRF in cells derived from male rats. This sexual dimorphism is reminiscent of the situation with other PRFs *(4,5)*, which although demonstrated to exert apparently physiologic effects in certain models *(8–12)*, are not potent hormone releasers in males in vivo or in male cells in vitro. Thus, the physiologic relevance of the newly described

PrRPs remains to be established, and their efficacy in the male rat remains unproven. Our data do not support a physiological role for PrRP-20 or PrRP-31 in the hypothalamic regulation of PRL secretion in intact male and random-cycle female rats.

It will be important to quantitate the amount of PrRP-20 and PrRP-31 present in the hypothalamus and pituitary gland, so that the physiologic relevance of a bioactivity expressed only at micromolar concentrations can be gauged. In addition, our studies do not rule out the possibility that the PRF activities of these two peptides are limited, in a physiologic sense, to the lactating female *(3)* or are possibly more relevant as potentiators of the actions of other, previously recognized PRFs. At least in our hands, in dispersed, cultured pituitary cells harvested from intact male and female rats, the PRF activity of both peptides is unremarkable.

Materials and Methods

Intact male and random-cycle female rats (250–300 g, Harlan Sprague Dawley, Indianapolis, IN) were sacrificed by decapitation as approved by the university animal care and use committee. Anterior pituitary glands were collected and mechanically dispersed in the presence of trypsin *(4)*. Cells were aliquoted into 24 well plates (approx 300,000 cells/well) and incubated for 72 h in Medium 199 (pH 7.3) containing 20 m*M* HEPES, 10% horse serum, and 1% antibiotic/antimycotic (all Gibco-BRL, Grand Island, NY) in room air at 37°C. On the day of experimentation, cells were washed with fresh medium and exposed for 1 h, with the exception of the time-course study, to test substances diluted in 1.0 mL test medium (Medium 199, 20 m*M* HEPES, 1% penicillin–streptomycin [all Gibco] and 0.1% BSA and 0.02 n*M* bacitracin [both Sigma, St. Louis, MO], pH 7.3, 37°C. Incubations were terminated by removal of medium.

Peptides were obtained from Phoenix Pharmaceuticals, Inc. (Mt. View, CA). PrRPs *(3)* were synthesized by solid phase, and protected resin was cleaved by HF. Crude peptides were purified by high-performance liquid chromatography (HPLC) and the final products verified by analytical HPLC and mass spectroscopy (rat PrRP-20: TPDINPAWYTGRGIRPVGRF-NH_2; human PrRP-31: SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH_2).

PRL content of the incubation medium was determined by radioimmunoassay (RIA) using the rat kit provided by the National Hormone and Pituitary Program (NHPP, NIDDK) with the rPRL-RP-3 standard, as previously described *(4,5)*. Growth hormone content was determined by RIA using the kit provided by the NHPP, NIDDK with the rGH-RP-2 standard. Significant within and between groups differences ($p < 0.05$) were determined by analysis of variance and the Student Newman Keuls multiple-comparison procedure.

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