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ORIGINAL PAPER

Prolactin-releasing peptide (PrRP) increases prolactin responses to TRH in vitro and in vivo

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Abstract The Prolactin-releasing Peptide (PrRP) is a 31-aminoacid peptide produced and secreted from the hypothalamus, and postulated to promote the prolactin release from the pituitary. However, the action of PrRP remain controversial, since it was described to have potency comparable enough to TRH, although there are many evidences that PrRP is less potent than TRH. Here we have studied the effects of PrRP alone or in combination with TRH in the prolactin levels of rat pituitary primary cell cultures in vitro and also in vivo prolactin responses in randomly cycling and estrogens-treated female rats. PrRP itself increased prolactin levels in vitro and in vivo, although in a magnitude several times lower than TRH. In vivo PrRP promotes an atypical non-peaking progressive and maintained prolactin increase. On the other hand, PrRP markedly increased the prolactin responses to TRH in vitro (10–30 fold increase) and in vivo (up to three-fold increase). In addition, FGF-2 and EGF, two important growth factors present in the pituitary, reduced the PrRP-induced prolactin increase in vitro. Taken together our results suggest that PrRP released from the hypothalamus may be relevant to modulate the circulating prolactin levels in the rat.

Keywords PrRP · Prolactin · Pituitary · EGF · FGF-2 · Estrogens

Abbreviations

PrRP	Prolactin releasing peptide
TRH	Thyrotropin releasing hormone
i.c.v.	Intracerebroventricular
FGF-2	Fibroblast growth factor-2
EGF	Epidermal growth factor
AUC	Area under curve

Introduction

Prolactin-Releasing Peptide (PrRP) was recently isolated from hypothalamus [1] and identified as an endogenous ligand of the orphan G-protein-coupled receptor hGR3 (GPR10) [2, 3] that is expressed mainly in the hypothalamus and the anterior pituitary [1, 4]. The peptide presents two molecular isoforms, the 31 amino acid peptide (PrRP-31) and the C-terminal 20 residue peptide (PrRP-20). Their sequences are highly conserved among several species, including bovine, rat and human [1], suggesting a physiological role in mammals.

The PrRP was originally reported to stimulate prolactin release in vitro [1, 5] and in vivo [6], being a selective modulator of prolactin release [6, 7]. Several studies revealed that PrRP effects on prolactin may be controversial, as it has been found to be equipotent to TRH [1], in another studies was less potent than TRH [6–10] or even

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completely ineffective to elicited prolactin release in vivo [4]. Recent studies have reported that PrRP might also play a significant role in ACTH secretion [11], and GH release from several somatotroph adenomas [12], although the intracerebroventricular (i.c.v.) administration of PrRP may reduce GH circulating levels in the rat [13]. In addition PrRP appears to be involved in gonadotrophin regulation, increasing LH and FSH levels in plasma after central administration of PrRP to male rats [14]. Finally, PrRP accounts for other effects at the hypothalamic level as being involved in the regulation of food intake and energy balance in rats [15], stress responses [16–18], cardiovascular function [9], sleep [19], and pain [20].

In previous reports we have shown that Fibroblast Growth Factor-2 (FGF-2) and Epidermal Growth Factor (EGF) modulated the prolactin responses to TRH and dopamine in pituitary primary cultures cells [21], likely through paracrine mechanisms. Other authors have suggested that PrRP may be considered as an autocrine/paracrine modulator within the pituitary, also using the classical endocrine pathway.

In the present study, firstly, we addressed the effects of PrRP on prolactin responses to TRH in vivo and in vitro. Secondly, we described previously the importance of FGF-2 and EGF as paracrine factors in the pituitary. Here, we want to check whether the PrRP was able to modulate the activity of both growth factors.

Results

Effect of PrRP on prolactin release, the in vitro study

The PrRP (10^{-9} M) alone, in a time-dependent manner, increased prolactin levels in the culture medium of pituitary primary cultures. The effects of PrRP on prolactin secretion appeared to be significant at 30 min of incubation (73.89 ± 3.70 ng/ml vs. 108.03 ± 12.90 ng/ml, $P < 0.05$), although the best responses were obtained at 3 h (167.44 ± 10.32 ng/ml vs. 231.90 ± 8.81 ng/ml, $P < 0.05$) (Fig. 1A). Afterward, we carried out the in vitro experiments at 3 h incubations.

TRH significantly increased prolactin levels in the culture medium of primary cultured cells from anterior pituitary in a dose-dependent manner at doses of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M, at 3 h incubations (grey bars, Fig. 1B). Doses of 10^{-9} M of PrRP greatly increased the prolactin levels in the culture medium in response to TRH (black bars, Fig. 1B). The increment is >200% (TRH 10^{-9} M) and >3,000% (TRH 10^{-5} M) in the responses to each TRH doses, showing a marked effect. Lower doses of PrRP

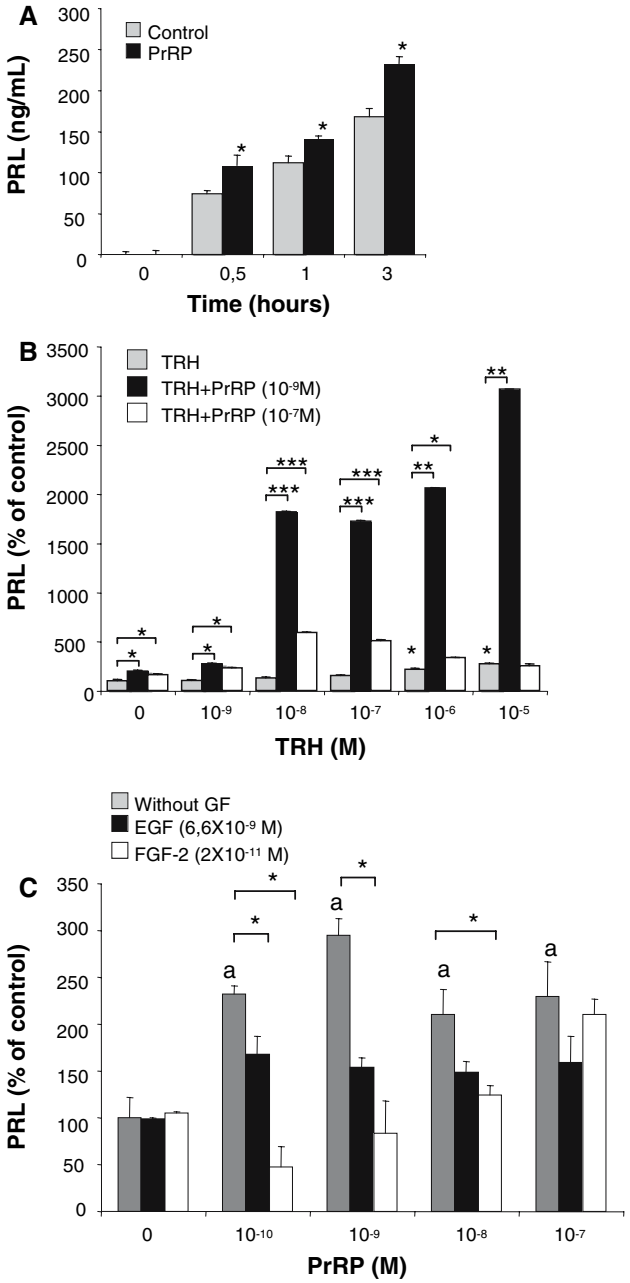

Fig. 1 Prolactin responses to PrRP in vitro in pituitary primary cultures. (**A**) Time-response curve to PrRP 10^{-9} M. Mann-Whitney test. *$P < 0.05$, Control group vs. PrRP group. Mean ± SD of three different experiments, run in quadruplicate (six pituitaries/experiment). (**B**) PrRP modulation of prolactin responses to TRH at 3 hours in primary culture cells. Duncan test: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, TRH treatment vs. PrRP treatment; Mean ± SD of three different experiments, run in quadruplicate (six pituitaries/experiment). (**C**) Dose-response of prolactin to PrRP in vitro: FGF–2 (2×10^{-11} M) and EGF (6.6×10^{-9} M) reduced prolactin responses to PrRP in primary culture cells. Mann-Whitney test. *$P < 0.05$, PrRP treatment vs. PrRP+FGF-2 or PrRP+EGF treatment. Duncan test: [a]$P < 0.05$, PrRP treatment vs. Control. Mean ± SD of three different experiments, run in quadruplicate (six pituitaries/experiment)

(10^{-7} M) (white bars, Fig. 1B) also increased the prolactin responses to TRH doses but to a lesser magnitude (range of increment: 100–500%). In both cases (10^{-9} and 10^{-7} M) PrRP significantly increased the prolactin levels in the culture medium of control wells without TRH (Control: 100 ± 15.79; vs. PrRP10^{-9} M: 200.24 ± 5.72, $P < 0.05$; Control: 100 ± 15.79 vs. PrRP10^{-7} M: 164.32 ± 8.05; $P < 0.05$) (Fig. 1B).

The dose-effect curve of PrRP (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} M, during 3 h incubation) on prolactin in vitro levels is shown in Fig. 1C (grey bars). The best responses were obtained with lower doses 10^{-9} M (Fig. 1C). Higher PrRP concentrations did not induce any additional increment in the prolactin response.

In the same set of experiments, we studied the effects of the growth factors FGF-2 and EGF upon prolactin release induced by PrRP. Recently, we reported that FGF-2 and EGF were important modulators of lactotroph cells [20, 21, 22]. We have shown that in primary culture cells from anterior pituitary, the optimal concentration for FGF-2 was 2×10^{-11} M and EGF 6.6×10^{-9} M [21, 22], and the best response of PrRP was during 3 h incubation (Fig. 1A). The co-incubation of PrRP and FGF-2 (2×10^{-11} M) produced a marked reduction of prolactin levels in response to PrRP at doses of 10^{-10} M (47.50 ± 21.32 with FGF-2 vs. 231.83 ± 9.30 without FGF-2, $P < 0.05$), 10^{-9} M (83.56 ± 34.31 with FGF-2 vs. 295.04 ± 17.24 without FGF-2, $P < 0.05$) and 10^{-8} M (124.05 ± 10.85 with FGF-2 vs. 209.96 ± 27.62 without FGF-2, $P < 0.05$). Moreover, with the lower doses of PrRP (10^{-10} M), FGF-2 induced a significant reduction of the prolactin levels even respect to control, indicating an effective blockage of prolactin release from the cells.

The co-incubation with PrRP plus EGF (6.6×10^{-9} M) yields similar results that FGF-2. The EGF blocked partially the elevation of prolactin levels induced by PrRP, but significantly at lower doses of PrRP (10^{-10} M: 168.01 ± 9.30 with EGF vs. 231.83 ± 9.30 without EGF, $P < 0.05$; 10^{-9} M: 154.09 ± 10.51 with EGF vs. 295.04 ± 17.24 without EGF, $P < 0.05$; Fig. 1C).

Effects of PrRP on prolactin levels, the in vivo study

We also studied whether i.c.v. administration of PrRP exogenous was able to module prolactin secretion in vivo, in female rats under pentobarbital anaesthesia. The PrRP administration induced a slow increase on prolactin levels in randomly cycling female rats. The response to PrRP do not resemble those of classical secretagogues which induced an abrupt peak-shape response, instead PrRP promotes a progressive prolactin increase with low-slope value, showing significant differences from 15 min onward to compare and to control saline-treated rats (45.33 ± 10.33 vs. 29.59 ± 2.03, $P < 0.05$), and maximal effects at 45 min 2-fold increase over basal, (60.52 ± 14.44 ng/ml vs. 31.55 ± 3.51 ng/ml, $P < 0.05$) (Fig. 2A). This particular response appeared clearly represented by the increase in the area under the curve (AUC) of the whole profile (Fig. 2A). Further, we measured the concentration of GH in the same samples, but PrRP did not influence the GH levels (data not shown), indicating that PrRP presents rather specific effects on prolactin control.

The TRH i.c.v. administration promoted a strong increase in prolactin circulating levels of randomly cycling female rats. The combined i.c.v. administration of TRH (1 μg/Kg) plus PrRP (10 μg/Kg), induced a marked increase in the peak response of prolactin respect to TRH alone, showing significant differences at times 5, 10 and 15 min (Fig. 2B,left) and also in the whole response considered as the AUC (TRH+PrRP 2439 ± 1029 vs. TRH 1166 ± 104, $P < 0.01$) (Fig. 2B.AUC, right).

Similar results were found in the combined administration of TRH (1 μg/kg) plus PrRP (10 μg/Kg) to previously in vivo estrogenized rats (see material and methods). In this case, the prolactin response to TRH was greatly exaggerated, reaching very high-peak levels (around 4,000 ng/ml at 5 mins), showing significant differences at times 5 and 10 min, after administration and also in the AUC (Fig. 2C).

Discussion

The present study clearly shows that PrRP is capable to stimulate prolactin secretion in vivo and in vitro, increasing prolactin circulating levels in plasma, and in the medium of primary culture cells from rat pituitary. Furthermore, PrRP markedly increases the prolactin responses to TRH in all experimental conditions studied, also in vivo and in vitro. Although PrRP do not work like a classical secretagogue, the magnitude effect on prolactin responses to TRH may play a significant role in the regulation of prolactin secretion in the pituitary.

In earlier studies PrRP was described to specifically stimulate the prolactin secretion from RC-4B/C (rat pituitary adenoma derived cell line) and to dispersed anterior pituitary cells obtained from lactating female rats, with potency comparable to TRH [1]. Later, PrRP was suggested to be a true and selective hypothalamic prolactin releasing factor because it does not affect the release of other pituitary hormones [1, 6]. However, this notion is controversial. Although it was reported that PrRP was equipotent to TRH in eliciting prolactin release, other later physiological studies found that PrRP is less potent than TRH [8, 23]. Even, Jarry et al. showed that PrRP did not stimulate prolactin release in vivo [4]. Moreover, some

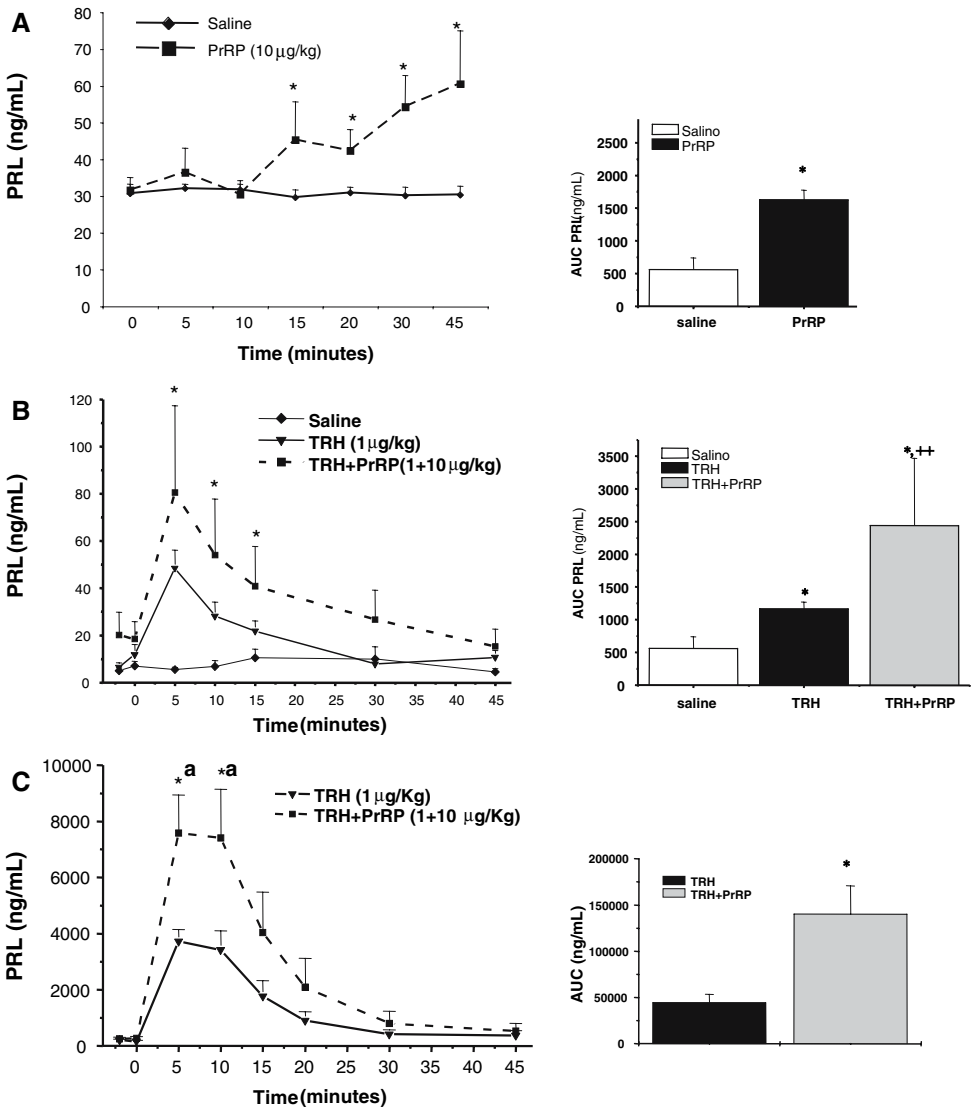

Fig. 2 Prolactin responses to PrRP in vivo in randomly cycling female rats. (**A**) Prolactin serum profile and AUC, ten rats per group. PrRP(10 µg/Kg) administered i.c.v. increased weakly serum prolactin levels in vivo. Mann-Whitney test: $*P < 0.05$, PrRP treatment (■) vs. saline treatment (●). (**B**) Prolactin serum profile and AUC, seven to nine rats per group. TRH (1 µg/Kg), and TRH (1 µg/Kg)+PrRP(10 µg/Kg) administered i.c.v. PrRP modulated prolactin levels induced by TRH in vivo. Mann-Whitney test: $*P < 0.05$, TRH (▼) treatment vs. TRH+PrRP treatment (■). (**C**) Prolactin serum profile and AUC to estrogens-treated rats, seven to nine rats per group. TRH (1 µg/Kg), and TRH (1 µg/Kg)+PrRP(10 µg/Kg) administered i.c.v. PrRP modulated strongly prolactin levels induced by TRH in vivo. Mann-Whitney test: $*P < 0.05$, TRH (▼) treatment vs. TRH+PrRP treatment (■)

data suggest some relationship between PrRP and lactotroph cells. In this study, we showed that PrRP is capable of stimulating prolactin secretion in vivo, although did not inducing a classical peak-shape response but a progressive and maintained prolactin increase. Also, PrRP increased in vitro prolactin levels in monolayer cultures from primary pituitary cells, however, the magnitude of the stimulatory prolactin response to PrRP is rather modest compared to TRH [7, 8, 10]. In support of this fact, it was described that PrRP-induced prolactin secretion was less potent than those induced by Vasoactive Intestinal Peptide (VIP) [10].

Many other factors are reported to promote prolactin secretion in vivo and in vitro [24]. Otherwise, PrRP seems to be distinct from all these factors in its ability to induce specific prolactin secretion, suggesting that PrRP may be an autocrine or paracrine modulator within the pituitary. We showed that PrRP markedly increased the prolactin secretion induced by TRH. Furthermore, it was recently published that PrRP was effective in counteracting the inhibitory action of dopamine in the pituitary cell aggregate system [10]. Taken together these data, it appears that PrRP may work as modulator of TRH activity in the anterior pituitary instead of being a secretagogue in the lactotroph cells.

In a further attempt to find a putative autocrine/paracrine function of PrRP, we studied, whether other paracrine factors like FGF-2 and EGF were able to modulate the prolactin secretion induced by PrRP. The source of FGF-2 within the pituitary is the folliculostellate cells [25], while EGF appeared to be released by some subpopulations of endocrine cells [26]. In our previous work we demonstrated that FGF-2 and EGF modulated the responses of the lactotroph cells to TRH and Dopamine [21]. Here, we show

that FGF-2 and EGF blocked the prolactin secretion induced by PrRP. Both growth factors, EGF and specially FGF-2 have been previously involved in the induction of proliferative changes of lactotroph cells and prolactinomas [27–30]. Moreover, FGF-2 mediates the proliferative response of lactotroph cells to in vivo estrogens treatment in rats, and inducing the expression of the *pttg* gene [31]. It might be possible that while FGF-2 promotes lactotroph cells to enter to proliferative state, interferes to make cells refractory to secretion. These results are quite intriguing, and show the complex network that regulates the prolactin secretion in the pituitary.

The relevance of the possible role of PrRP on prolactin secretion became clear in the in vivo experiments. Although it was originally reported that PrRP was equipotent with TRH [1], in our study, we found that the magnitude of the stimulatory prolactin response to PrRP is rather modest compared to TRH. However, PrRP markedly increased the prolactin secretion in response to effective doses of TRH both in normal and in previously in vivo estrogens-treated rats. Therefore, we argued that PrRP is not an important prolactin secretagogue. PrRP seems to be a paracrine modulator of prolactin secretion in the pituitary, working in the fine tuning role with TRH and dopamine.

In conclusion, our results suggest that PrRP is involved in the fine-tuning action of lactotroph cells in the anterior pituitary. Although PrRP has been shown to induce the secretion of prolactin in vivo and in vitro, it does not show the typical profile of other prolactin releasing factor. However, it is remarkable the efficiency of PrRP to greatly increase the prolactin secretion in response to TRH. Therefore we suggest that PrRP release from the hypothalamus may effectively modulate the circulating prolactin levels.

Materials and methods

Adult female Sprague-Dawley rats weighing 150–200 g were housed in a constant dark–light cycle (12:12 h). Standard pellet chow (A04, Panlab S.L., Barcelona, SP) and tap water were available *ad libitum*. Animal manipulations were carried out following the conventions and ethical rules included in Directive 86/609/CEE of the European Union.

In vivo experiments

The in vivo experiments were done with randomly cycling female rats. A silicone catheter was placed in the right jugular vein of the rats under anaesthesia with pentobarbital (50 mg/Kg, in 10% w/v CO_3Na_2). A bolus of heparin (1,000 IU/Kg) was immediately administered for anticoaugulation. A basal blood sample was taken at time 0 and stimuli were immediately administered in the following doses: PrRP31 (10 μg/Kg) (Sigma-Aldrich Quimica SL, Alcobendas, SP), TRH (1 μg/Kg) (Sigma-Aldrich Quimica SL, Alcobendas, SP) or TRH + PrRP (1 + 10 μg/Kg). Blood samples were collected at—2, 0, 5, 10, 15, 20, 30 and 45 min. The samples were centrifuged at 3500 rpm/5 min and serum was frozen at –20°C until measurements of prolactin and growth hormone by specific RIA (reagents gently supplied by Dr. A.F.Parlow NHPP-NIH, USA). Intrassay coefficients of variation for prolactin were lower than 8%.

A group of rats were in vivo estrogenized by the administration of 3-benzoate 17β-estradiol (Sigma-Aldrich Quimica SL, Alcobendas, SP), 5 mg/Kg every 3–4 days, sc, during 2 week. Those animals were challenged with TRH or TRH + PrRP, as described above, to study prolactin responses.

In vitro experiments

The anterior pituitaries were collected, by rat decapitation under pentobarbital anaesthesia (50 mg/Kg in 0.1 M CO_3Na_2), in EBSS (Sigma-Aldrich Quimica SL, Alcobendas, SP). Pituitaries were washed with fresh EBSS, minced and incubated in EBSS containing 0.1% trypsin (Sigma-Aldrich Quimica SL, Alcobendas, SP) at 37°C, for 30 min. The cell suspension was centrifuged for 5 min at 720 g, and then washed three times in EBSS. Pellets were resuspended in DMEM (Seromed) containing antibiotics, and cells were mechanically dispersed, yielding viability greater than 95%. The culture medium was DMEM supplemented with 10% FBS (Gibco BRL) and antibiotics: 20 mg/mlampicilin (Sigma-Aldrich Quimica SL, Alcobendas, SP) plus 0.6 mg/ml streptomycin (ICN). Cells were seeded in a 24-well plastic culture dishes (Corning) at a density $5–10 \times 10^4$ cells/well (2–3 pituitaries/dish) and incubated at 37°C in a humidity-saturated atmosphere containing 5% CO_2 for 4 days. On the fifth day the medium was replaced with fresh serum-free DMEM plus 1% antibiotics, and exposed to the test substances. Primary cultures of dispersed pituitary cells contained approximately 30% of lactotroph cells.

Experiments were carried out in serum-free medium containing PrRP31 (Sigma-Aldrich Quimica SL, Alcobendas, SP) at 0, 10^{-10}, 10^{-9}, 10^{-8} and 10^{-7} doses for the dose-response curves during 3 h, and 10^{-9} M for the time-response curve (0, 0.5, 1 and 3 h of incubation). TRH (Sigma-Aldrich Quimica SL, Alcobendas, SP) at 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M doses, with/without PrRP31 at 10^{-9} and 10^{-7} M with incubation of 3 h. Finally, we carried out co-incubations of PrRP31 at 0, 10^{-10}, 10^{-9}, 10^{-8} and 10^{-7} with/without FGF-2 (Sigma-Aldrich Quimica SL,

Alcobendas, SP) at 2×10^{-11} M or with/without EGF (Sigma-Aldrich Quimica SL, Alcobendas, SP) at 6.6×10^{-9} M.

The medium was stored at –20°C until measurements of prolactin and growth hormone by specific RIA (reagents gently supplied by Dr. A.F.Parlow NHPP-NIH, USA). Intrassay coefficients of variation were lower than 8%, and assay sensitivity was 1.35 ng/ml, and assay sensitivity was 1.35 ng/ml, and for growth hormone was lower than 9.5%, and assay sensitivity was 0.25 ng/ml.

Total protein in culture medium was used as loading control for each well, measured by the Bradford method [32]. Hormone levels are expressed by micrograms of total protein in the culture medium. Thus, units of the graphs are percent over control.

Statistical analyses were carried out using the Mann-Whitney non-parametric test for comparison among groups, and the Duncan test for comparison among the doses effects respect to the control value. Significance was considered at $P < 0.05$.

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References

1. S. Hinuma, Y. Habata, R. Fujii, Y. Kawamata, M. Hosoya, S. Fukusumi, C. Kitada, Y. Masuo, T. Asano, H. Matsumoto, M. Sekiguchi, T. Kurokawa, O. Nishimura, H. Onda, M. Fujino, Nature **393**, 272–276 (1998)
2. S.K. Welch, B.F. ÓHara, T.S. Kilduff, H.C. Heller, Biochem. Biophys. Res. Commun. **209**, 606–613 (1995)
3. A. Marchese, M. Heiber, T. Nguyen, H.H.Q. Heng, V.R. Saldivia, R. Cheng, P.M. Murphy, L.C. Tsui, X. Shi, P. Gregor, S.R. George, B.F. ÓDowd, J.M. Docherty, Genomics **29**, 335–344 (1995)
4. H. Jarry, H. Heuer, L. Schomburg, K. Bauer, Neuroendocrinology **71**, 262–267 (2000)
5. Y. Kawamata, R. Fujii, S. Fukusumi, Y. Habata, M. Hosoya, S. Hinuma, C. Kitada, H. Onda, O. Nishimura, M. Fujino, Endocrine **12**, 215–221 (2000)
6. H. Matsumoto, J. Noguchi, Y. Horikoshi, Y. Kawamata, C. Kitada, S. Hinuma, H. Onda, O. Nishimura, M. Fujino, Biochem. Biophys. Res. Común. **259**, 321–324 (1999)
7. R. Tokita, T. Nakata, H. Katsumata, S. Konishi, H. Onodera, J. Imaki, S. Minami, Neurosci. Lett. **276**, 103–106 (1999)
8. W.K. Samson, Z. Resch, T.C. Murphy, Endocrine **9**, 289–291 (1998)
9. W.K. Samson, Z. Resch, T.C. Murphy, Brain Res. **858**, 19–25 (2000)
10. E. Swinnen, M. Boussemaere, C. Denef, J. Neuroendocrinol. **17**, 379–386 (2005)
11. T. Abe, N. Koga, M. Tomita, T. Tonoike, M. Kushima, K. Takahashi, Y. Sano, M. Taniyama, Acta Neuropathol. **106**, 495–500 (2003)
12. T. Rubinek, M. Hadani, G. Barkai, S. Melmed, I. Shimon, J. Clin. Endocrinol. Metab. **86**, 2826–2830 (2001)
13. N. Iijima, Y. Matsumoto, T. Yano, M. Tanaka, T. Yamamoto, K. Kakihara, Y. Kataoka, Y. Tamada, H. Matsumoto, N. Suzuki, S. Hinuma, Y. Ibata, Endocrinology **142**, 3239–3243 (2001)
14. L.J. Seal, C.J. Small, M.S. Kim, S.A. Stanley, S. Taheri, M.A. Ghatei, S.R. Bloom, Endocrinology **141**, 1909–1912 (2000)
15. C.B. Lawrence, F. Celsi, J. Brennand, S.M. Luckman, Nat. Neurosci. **3**, 645–646 (2000)
16. H. Matsumoto, M. Maruyama, J. Noguchi, Y. Horokishi, K. Fujiwara, C. Kitada, S. Hinuma, H. Onda, O. Nishimura, K. Inoue, M. Fujino, Neurosci. Lett. **285**, 234–238 (2000)
17. L.J. Seal, C.J. Small, W.S. Dhillo, A.R. Kennedy, M.A. Ghatei, S.R. Bloom, Neuroendocrinology **76**, 70–78 (2002)
18. W.K. Samson, C. Keown, C.K. Samson, B. Lane, J.R. Baker, M.M. Taylor, Endocrine **20**, 59–66 (2003)
19. S.Q. Zhang, M. Kimura, S. Inoue, Clin. Neurosci. **54**, 262–264 (2000)
20. M.L. Kalliomaki, A. Pertovaara, A. Brandt, H. Wei, P. Pietila, J. Kalmari, M. Xu, E. Kalso, P. Panula, Neurophamarcolgy **46**, 412–424 (2004)
21. C. Spuch, Y. Diz-Chaves, D. Pérez-Tilve, F. Mallo, Endocrine **29**, 317–324 (2006)
22. C. Spuch, Y. Diz-Chaves, D. Pérez-Tilve, F. Mallo, Endocrine **24**, 131–136 (2004)
23. J.D. Curlewis, D.H.L. Kusters, J.L. Barclay, S.T. Anderson, J. Endocrinol. **174**, 45–53 (2002)
24. W.K. Samson, M.M. Taylor, J.R. Baker, Regul. Pept. **114**, 1–5 (2003)
25. N. Ferrara, L. Schweigerer, G. Neufeld, R. Mitchell, D. Gospodarowicz, Proc. Natl. Acad. Sci. USA. **84**, 5545–5549 (1987)
26. A. Mouihate, D. Verrier, J. Lestage, J. Endocrinol. **146**, 319–324 (1996)
27. S. Ezzat, Brain Pathol. **11**, 356–370 (2001)
28. H. Fukuro, C. Mogi, K. Yokoyama, K. Inoue, Endocr. Pathol. **14**, 145–149 (2003)
29. J.H. Mukdsi, A.L. De Paul, J.P. Petiti, S. Gutierrez, A. Aoki, A.I. Torres, Acta Neuropathol (Berl). **112**, 491–501 (2006)
30. N.G. de la Torre, H.E. Turner, J.A. Wass, J.H. Mukdsi, A.L. De Paul, J.P. Petiti, S. Gutierrez, A. Aoki, A.I. Torres, Pituitary **8**, 17–23 (2005)
31. A.P. Heaney, G.A. Horwitz, Z. Wang, R. Singson, S. Melmed, Nat. Med. **5**, 1317–1321 (1999)
32. M.M. Bradford, Anal. Biochem. **72**, 258–254 (1976)