



Rat Ghrelin Stimulates Growth Hormone and Prolactin Release in the Tilapia, *Oreochromis mossambicus*

Authors: Riley, Larry G., Hirano, Tetsuya, and Grau, E. Gordon

Source: Zoological Science, 19(7) : 797-800

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.19.797>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

[SHORT COMMUNICATION]

Rat Ghrelin Stimulates Growth Hormone and Prolactin Release in the Tilapia, *Oreochromis mossambicus*

Larry G. Riley, Tetsuya Hirano and E. Gordon Grau*

Hawaii Institute of Marine Biology, University of Hawaii, P.O. Box 1346,
Kaneohe, HI 96744, USA

ABSTRACT—Recently, ghrelin (Ghr), a new peptide which specifically stimulates growth hormone (GH) release from the pituitary, was identified in the rat and human stomach. Ghrelin has been shown to stimulate GH release by acting through a growth hormone secretagogue (GHS) receptor in the rat. The present study describes the *in vitro* effect of rat Ghr on the release of GH and two forms of prolactin (PRL₁₇₇ and PRL₁₈₈) in the tilapia, *Oreochromis mossambicus*. Rat Ghr stimulated the release of GH in a dose-related manner after 8 and 24 hr of incubation. Rat Ghr also significantly stimulated the release of PRL₁₇₇ and PRL₁₈₈ in a dose-related manner after 24 hr. Rat Ghr had no effect on the pituitary content of GH or PRL₁₈₈, but significantly increased PRL₁₇₇ content. These results show for the first time that rat Ghr significantly stimulates GH and PRL release in teleosts, and suggest that Ghr and a GHS receptor are present in fish.

Key Words: tilapia, *Oreochromis mossambicus*, ghrelin, growth hormone, prolactin

INTRODUCTION

In teleosts, growth hormone (GH) is involved in a variety of physiological processes such as growth, osmoregulation, metabolism, reproduction and development (McLean and Donaldson, 1993; Blazquez *et al.*, 1998). These processes influence one another within the animal, making it difficult to clarify the mechanisms that regulate the release of GH from the pituitary. It is well accepted that somatostatin (SRIF) is an important inhibitor of GH release in all vertebrates, including teleosts (Peng and Peter, 1997). By contrast, several factors have been implicated in the stimulation of GH release in fish. These include growth hormone-releasing factor (GRF), gonadotropin-releasing hormone (GnRH), pituitary adenylate cyclase-activating polypeptide (PACAP), thyrotropin-releasing hormone and dopamine (Nishioka *et al.*, 1988; Peng and Peter, 1997; Sherwood *et al.*, 2000).

In 1977, Bowers and colleagues developed a series of small peptides that exhibit weak GH stimulatory activity *in vitro* (Bowers *et al.*, 1977). Since then several synthetic peptides, collectively known as growth hormone secretagogues (GHS), have been developed. Studies in mammals have shown that GHS bind to a novel GH receptor distinct from

those of GRF and PACAP (Smith *et al.*, 1999; Kojima *et al.*, 2001). It has also been demonstrated that GHS stimulates GH release *via* different signal transduction pathways from those utilized by GRF (Smith *et al.*, 1999; Chen, 2000). Recently, Kojima *et al.* (1999) isolated an endogenous peptide, termed ghrelin (Ghr), from the rat stomach, which stimulates GH secretion by binding to the GHS receptor. In rats, Ghr exhibits a potent and specific stimulus of GH release both *in vitro* and *in vivo* (Peino *et al.*, 2000; Seoane *et al.*, 2000). In the bullfrog and human, ghrelin has also been shown to stimulate PRL release (Takaya *et al.*, 2000; Kaiya *et al.*, 2001). The present study was undertaken to examine *in vitro* effects of rat Ghr on GH and PRL release from the tilapia pituitary.

MATERIALS AND METHODS

Fish

Mozambique tilapia, *Oreochromis mossambicus*, weighing 20–70 g, were reared at the Hawaii Institute of Marine Biology, University of Hawaii, and maintained in fresh water (25±2°C). They were fed twice daily with ProForm (Agro Pacific, Chilliwack, BC, Canada), approximately 2% of body weight per day.

Experimental Protocol

Whole pituitaries were removed and pre-incubated in a 96-well plate for 24 hr in 100 µl bicarbonate-Ringer solution (330 mOsm) with essential additives as described by Wigham *et al.* (1977) and

* Corresponding author: Tel. +1-808-956-7031;
FAX. +1-808-956-3014.
E-mail: grau@hawaii.edu

supplemented with 0.025 µg/ml gentamycin. The pre-incubation medium was removed and replaced with fresh medium (100 µl) containing synthetic rat Ghr. Rat Ghr, obtained from Peptide Institute (Minoh, Osaka, Japan), was dissolved in distilled water at a concentration of 10 µM and stored at -20°C. Final concentrations of Ghr (0.01, 0.1, 1 and 10 nM) were dissolved in the culture medium.

Medium samples were removed at 4 and 8 hr and replaced with media containing appropriate doses. After 24 hr of incubation, media and pituitaries were collected. Data are presented as cumulative release at each time point. The pituitaries were sonicated in 200 µl of radioimmunoassay buffer (0.01 M sodium phosphate, 0.1% Triton-X, 1% BSA). All samples were stored at -20°C until they were analyzed for GH, PRL₁₇₇ and PRL₁₈₈ by homologous radioimmunoassays according to Ayson *et al.* (1993) as modified by Yada *et al.* (1994).

Statistical Analysis

Group comparisons were performed using a two-way ANOVA followed by the least significant difference test. Calculations were performed using a computer program, Statistica (Statsoft, Tulsa, OK). Data are expressed as means±S.E.M.

RESULTS AND DISCUSSION

As shown in Fig.1A, rat Ghr stimulated the release of GH from the tilapia pituitary in a dose-related manner after 8 hr ($r^2 = 0.44$, $P < 0.0001$) and 24 hr ($r^2 = 0.55$, $P < 0.0001$) of incubation. Significant increase in GH release was observed at 0.01, 0.1 and 1 nM after 4 h and at all doses assayed after 8 and 24 hr. No significant dose-related effect of Ghr on GH release was seen after 4 hr. The effect was primarily on the release of GH since no change was observed in the pituitary content (Table 1) or gene expression after 24 hr (data not shown).

In the rat, a single injection of Ghr induced a rapid increase in circulating GH. Maximum stimulation was observed at 15–20 min: GH returned to the baseline level after 30–60 min (Kojima *et al.*, 1999; Date *et al.*, 2000). Kojima *et al.* (1999) also demonstrated that a 15-min exposure of the rat pituitary to Ghr *in vitro* stimulated a rapid release of GH. In this study, rat Ghr stimulated the release of GH after 4 hr of incubation. The use of whole pituitaries versus dispersed pituitary cells may have delayed the effect of Ghr. We have shown, however, that SRIF inhibits GH release from whole pituitaries within 1 hr (unpublished results). Thus, the delayed response observed in the tilapia may be due to the fact that we used a heterologous Ghr. Further studies using homologous Ghr in teleosts are required to ascertain if Ghr exhibits rapid action on GH release as in mammals. According to Kaiya *et al.* (2001), bullfrog Ghr stimulated the release of GH in dispersed bullfrog pituitary cells, with a potency that was 2–3 orders of magnitude greater than that of rat Ghr. By contrast, bullfrog Ghr was only minimally effective in elevating plasma GH levels following intravenous injection into rats. In mammals, Ghr has been shown to stimulate GH release by acting at the pituitary and hypothalamus through an orphan receptor, GHS receptor, which is different from the GRF and PACAP

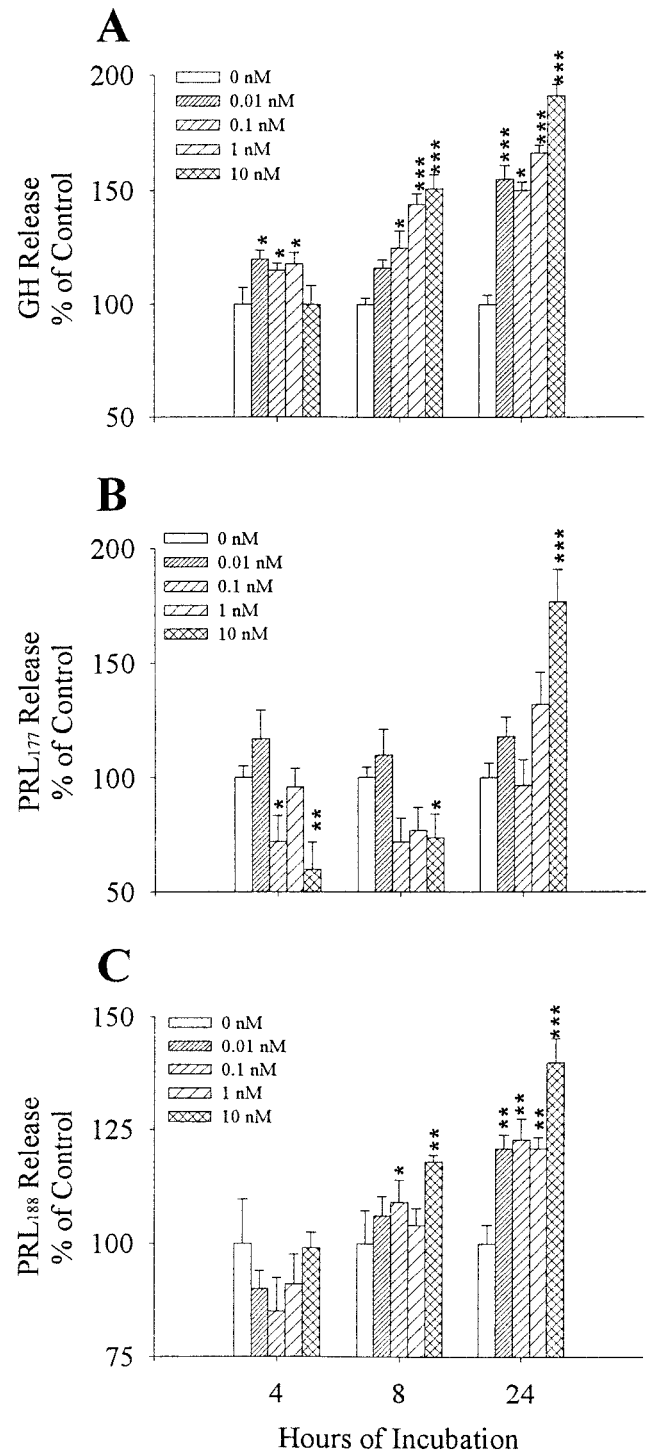


Fig. 1. Effect of rat Ghr on GH (A), PRL₁₇₇ (B), PRL₁₈₈ (C) release *in vitro*. Pituitaries were pre-incubated for 24 hr in isotonic medium (330 mOsm), and then exposed to 0.01–10 nM rat Ghr for an additional 24 hr. Incubation medium was changed at 4 and 8 hr. Hormone release is expressed as percent of control. Significantly different from the control (0 nM) at each time point at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, respectively ($n = 8–12$).

Table 1. Effect of rat Ghr on pituitary content of GH, PRL₁₇₇, and PRL₁₈₈ after 24 hr of incubation.

Treatment	GH	PRL ₁₇₇	PRL ₁₈₈
0 nM (control)	123.6 ± 18.5	59.3 ± 7.9	91.7 ± 15.1
0.01 nM	149.7 ± 13.3	86.6 ± 9.1	108.9 ± 13.8
0.1 nM	130 ± 15.0	94.3 ± 9.8*	83.7 ± 6.7
1 nM	121.9 ± 17.4	80.8 ± 6.0	87.9 ± 9.3
10 nM	130.5 ± 8.3	125.7 ± 21.4***	91.2 ± 15.4

Data are presented as ng/pit/100g. *, *** Significantly different at $P < 0.05$ and $P < 0.001$, respectively. (n=10–12)

receptors (Smith *et al.*, 1999; Chen, 2000). Growth hormone gene expression is dependent on the pituitary-specific transcription factor, Pit-1. Garcia *et al.*, (2001) have demonstrated that Ghr activates the pit-1 transcription factor through the GHS receptor. However, single and continuous intracerebroventricular administration of Ghr did not alter steady state GH mRNA levels in the rat pituitary (Date *et al.*, 2000). According to Shepherd *et al.* (2000), intraperitoneal injection of KP-102, one of the GHSs, at a concentration of 1 ng/g significantly increased plasma GH levels in the tilapia 6 h after the injection, suggesting that a specific GHS receptor is also present in teleosts.

The effect of rat Ghr on the tilapia pituitary does not appear to be limited to GH cells. In the present study, rat Ghr also stimulated the release of PRL₁₇₇ after 24 hr and PRL₁₈₈ after 8 and 24 hr (Fig. 1B, C). Interestingly, PRL₁₇₇ release was inhibited after 4 and 8 hr of incubation with rat Ghr. The effect on PRL₁₇₇ and PRL₁₈₈ was dose-related after 24 hr of incubation ($r^2 = 0.24$, $P < 0.02$) and ($r^2=0.28$, $P < 0.0005$), respectively. Interestingly, rat Ghr increased the pituitary content of PRL₁₇₇ but not PRL₁₈₈ (Table 1). Ghrelin was without effect on mRNA expression on the PRLs after 24 hr (data not shown).

Our observation that rat Ghr stimulates the release of GH and PRL from the tilapia pituitary is not surprising, since GH and PRLs are members of the same peptide family and both exhibit many overlapping and antagonist functions (Goffin *et al.*, 1996). However, according to Kojima *et al.* (1999), Ghr's action was limited to GH secretion in the rat; no effect was observed on the release of PRL, ACTH, TSH or GTHs. On the other hand, Wren *et al.* (2000) found that Ghr also elevated ACTH levels and decreased TSH levels in the rat. Likewise, Takaya *et al.* (2000) reported that intravenous injection of human Ghr increased not only plasma GH but also plasma levels of ACTH, cortisol and PRL in humans. In the tilapia, intravenous injection of KP-102 stimulated GH release, without affecting PRL release (Shepherd *et al.*, 2000). However, in the bullfrog, homologous Ghr was effective in stimulating secretion of both GH and PRL (Kaiya *et al.*, 2001). These results suggest that Ghr is involved in a greater array of pituitary functions than just regulating GH cell function.

In conclusion, the fact that rat Ghr stimulated GH and PRL release in the tilapia provides evidence that a novel mechanism is involved in the regulation of GH and possibly PRL cell function also in teleosts.

ACKNOWLEDGMENTS

We are grateful to Dr. N. Harold Richman III, Mr. Steven K. Shimoda, and Ms. Claire Ball, Hawaii Institute of Marine Biology, University of Hawaii, for their invaluable suggestions and encouragement during the course of this study. This study was funded in part by grants from University of Hawaii Sea Grant College Program, #NA86RG0041, a grant from State of Hawaii, DLNR 40402, and USDA grant # 9835206644.

REFERENCES

- Ayson F G, Kaneko T, Tagawa M, Hasegawa S, Grau E G, Nishioka R S, King D S, Bern H A, Hirano T (1993) Effects of acclimation to hypertonic environment on plasma and pituitary levels of two prolactins and growth hormone in two species of tilapia *Oreochromis mossambicus* and *Oreochromis niloticus*. Gen Comp Endocrinol 89: 138–148
- Blazquez M, Bosma P T, Fraser E J, Van Look K J W, Trudeau V L (1998) Fish as models for the neuroendocrine regulation of reproduction and growth. Comp Biochem Physiol C 119: 345–364
- Bowers C Y, Chang J P, Momany F, Folkers K (1977) Effects of the enkephalins and enkephalin analogs on release of pituitary hormones *in vitro*. In "Proceedings of the Sixth International Conference on Endocrinology" Ed by I. MacIntyre, Elsevier/North-Holland, Amsterdam, p 287
- Chen C (2000) Growth hormone secretagogue actions on the pituitary gland: multiple receptors for multiple ligands? Clin Exp Pharmacol Physiol 27: 323–329
- Date Y, Murakami N, Kojima M, Kuroiwa T, Matsukura S, Kangawa K, Nakazato M (2000) Central effects of a novel acylated peptide, ghrelin, on growth hormone release in rats. Biochem Biophys Res Comm 275: 477–480
- Garcia A, Alvarez C V, Smith R G, Dieguez C (2001) Regulation of the Pit-1 expression by ghrelin and GHRP-6 through the GH secretagogue receptor. Mol Endocrinology 15: 1484–1495
- Goffin V, Shiverick K T, Kelly P A, Martial J A (1996) Sequence-function relationships within the expanding family of prolactin, growth hormone, placental lactogen, and related proteins in mammals. Endocrine Reviews 17: 385–409
- Kaiya H, Kojima M, Hosoda H, Koda A, Yamamoto K, Kitajima Y, Matsumoto M, Minamitake Y, Kikuyama S, Kangawa K (2001) Bullfrog ghrelin is modified by n-octanoic acid at its third threonine residue. J Biol Chem 276: 40441–40448
- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402: 656–659
- Kojima M, Hosoda H, Matsuo H, Kanagawa K (2001) Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 12: 118–122
- McLean E, Donaldson E M (1993) The Role of Growth Hormone in the Growth of Poikilotherms. In "The Endocrinology of Growth, Development, and Metabolism in Vertebrates," Eds by C G Scanes and P K T Pang, Academic Press New York, pp 43–71
- Nishioka R S, Kelley K M, Bern H A (1988) Control of prolactin and growth hormone secretion in teleost fishes. Zool Sci 5: 267–280
- Peino R, Baldelli R, Rodriguez-Garcia J, Rodriguez-Segade S, Kojima M, Kangawa K, Arvat E, Ghigo E, Dieguez C, Casan-

- ueva F F (2000) Ghrelin-induced growth hormone secretion in humans. *Euro J Endocrinol* 143: R11–R14
- Peng C, Peter R E (1997) Neuroendocrine regulation of growth hormone secretion and growth in fish. *Zool Studies* 36: 79–89
- Seoane L M, Tovar S, Baldelli R, Arvat E, Ghigo E, Casanueva F F, Dieguez C (2000) Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. *Euro J Endocrinol* 143: R7–R9
- Shepherd B S, Eckert S M, Parhar I S, Vijayan M M, Wakabayashi I, Hirano T, Grau E G, Chen T T (2000) The hexapeptide KP-102 (D-Ala-D-B-Nal-Ala-Trp-D-Phe-Lys-NH₂) stimulates growth hormone release in a cichlid fish (*Oreochromis mossambicus*). *J Endocrinol* 167: R7–R10
- Sherwood N M, Krueckl S L, McRory J E (2000) The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. *Endocrine Reviews* 21: 619–670
- Smith R G, Palyha O C, Feighner S D, Tan C P, McKee K K, Hreniuk D L, Yang L, Morriello G, Nargund R, Patchett A A, Howard A D (1999) Growth hormone releasing substances: Types and their receptors. *Horm Res* 51: 1–8
- Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K (2000) Ghrelin strongly stimulates growth hormone (GH) release in humans. *J Clin Endocrinol Metab* 85: 4908–4911
- Wigham T, Nishioka R S, Bern H A (1977) Factors affecting *in vitro* activity of prolactin cells in the euryhaline teleost *Sarotherodon mossambicus* (*Tilapia mossambica*). *Gen Comp Endocrinol* 32: 120–131
- Wren A M, Small C J, Ward H L, Murphy K G, Dakin C L, Taheri S, Kennedy A R, Roberts G H, Morgan D G A, Ghatel M A, and Bloom S R (2000) The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 141: 4325–4328
- Yada T, Hirano T, Grau E G (1994) Changes in plasma levels of the two prolactins and growth hormone during adaptation to different salinities in the euryhaline tilapia, *Oreochromis mossambicus*. *Gen Comp Endocrinol* 93: 214–223

(Received February 25, 2002 / Accepted April 15, 2002)