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[SHORT COMMUNICATION]

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

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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 (PRL_{177} and PRL_{188}) 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 PRL_{177} and PRL_{188} in a dose-related manner after 24 hr. Rat Ghr had no effect on the pituitary content of GH or PRL_{188}, but significantly increased PRL_{177} 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.

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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, Chilliwalck, 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

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 cummulative 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, PRL_{177} and PRL_{188} 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

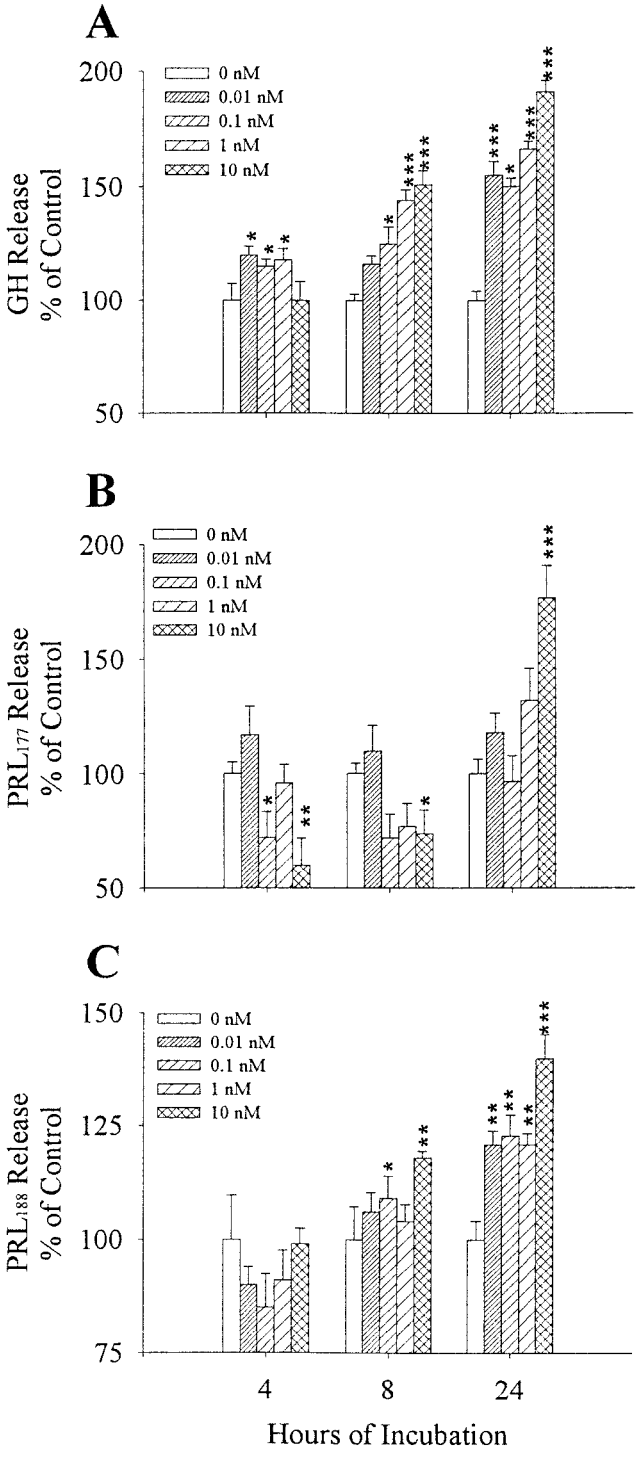

Fig. 1. Effect of rat Ghr on GH (A), PRL_{177} (B), PRL_{188} (C) release *in vitro*. Pituitaries were pre-incubated for 24 hr in isotonic medium (330 mOsm), and then exposed to 0.01–10 nM rat Ghr for an additional 24 hr . Incubation medium was changed at 4 and 8 hr. Hormone release is expressed as percent 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, PRL_{177}, and PRL_{188} 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 intracerebroventricluar 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 PRL_{177} after 24 hr and PRL_{188} after 8 and 24 hr (Fig. 1B, C). Interestingly, PRL_{177} release was inhibited after 4 and 8 hr of incubation with rat Ghr. The effect on PRL_{177} and PRL_{188} 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 PRL_{177} but not PRL_{188} (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.

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