Periprandial changes in growth hormone release in goldfish: role of somatostatin, ghrelin, and gastrin-releasing peptide

Luis Fabián Canosa, Suraj Unniappan, and Richard Ector Peter
Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

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Canosa, Luis Fabián, Suraj Unniappan, and Richard Ector Peter. Periprandial changes in growth hormone release in goldfish: role of somatostatin, ghrelin, and gastrin-releasing peptide. Am J Physiol Regul Integr Comp Physiol 289: R125–R133, 2005. First published March 3, 2005; doi:10.1152/ajpregu.00759.2004.—In goldfish, growth hormone (GH) transiently rises 30 min after meals, returning to baseline at 1 h postmeal. Somatostatin (SRIF) is the major inhibitor of GH release. Three cDNAs encoding pre-pro-SRIF (PSS) have been previously cloned from goldfish brain: PSS-I, which encodes SRIF-14; PSS-II, which is potentially processed into gSRIF-28 that has [Glu1,Tyr7,Gly10]SRIF-14 at the COOH terminus; and PSS-III, which encodes [Pro2]SRIF-14 at its COOH terminus. In goldfish, bombesin (BBS), mimicking the endogenous gastrin-releasing peptide (GRP), acutely suppresses food intake and also stimulates GH release. Ghrelin was recently characterized in goldfish as a GH secretagogue and an orexigen. In this paper, we studied the changes in SRIF mRNA levels during feeding and analyzed the influences of BBS and ghrelin peptides on forebrain PSS expression. The results showed a 60% reduction in PSS-II mRNA after meals, but no changes in the expression of PSS-I and PSS-III were found. Intraperitoneal injections of 100 ng/g body wt of BBS increased GH secretion and decreased PSS-I, while decreasing PSS-II gene expression. Intraperitoneal injection of goldfish ghrelin (100 ng/g body wt) blocked the effects of BBS (100 ng/g body wt) on PSS-I, but not on PSS-II expression. Coadministration of BBS and ghrelin decreased only the PSS-II gene expression. We conclude that the interactions between BBS/GRP and ghrelin can account for the postprandial variations in serum GH levels and the forebrain expression of PSS-II. Furthermore, we demonstrate that intraperitoneal administration of BBS reduces the ghrelin expression levels in the gut. Thus the inhibition of production of ghrelin in the gut may contribute to the satiety effects of BBS/GRP peptides.

THE INTERPLAY BETWEEN FACTORS involved in the control of growth and energy balance is currently evident (4, 16, 21, 68). The recent discovery of ghrelin, which functions as a hunger signal, meal initiator (3, 15), and growth hormone (GH) secretagogue in mammals (6, 38, 68), reinforces the notion of coordination of growth processes with feeding. Numerous aspects of the regulation of feeding behavior and food intake in fish and mammals are similar (for review, see Ref. 45), indicating the high conservation of these regulatory mechanisms during evolution.

In mammals, it is well established that regulation of GH secretion from the anterior pituitary gland is under the reciprocal control of two hypothalamic hormones: a stimulatory growth hormone-releasing hormone (GHRH) found in the arcuate nucleus and an inhibitory somatostatin (SRIF) synthesized in the periventricular nucleus. Several lines of evidence suggest that, in addition to the intricate patterns of GHRH and SRIF release regulating GH directly at the pituitary level, SRIF modulates GH secretion indirectly through central regulation of GHRH-containing neurons (70). In recent years, ghrelin, a Ser9-octanoylated, 28-amino acid peptide that was recognized as the endogenous ligand of GH secretagogue receptor, has been included in the regulatory model of GH secretion (68, 77). Ghrelin stimulates GHRH outflow at the arcuate nucleus level and, through direct pituitary actions, serves as a functional antagonist of SRIF at the pituitary level (69). Ghrelin is mainly synthesized in the stomach and acts as an orexigen, thereby linking growth and energy balance (16, 38).

Growth in fish is regulated by the brain neuroendocrine–GH-insulin-like growth factor axis (58). The control of pituitary GH secretion in fish is multifactorial, with a balance of stimulatory and inhibitory neurohormones acting directly on the pituitary somatotrophs on a seasonal basis (57). SRIF is the major GH inhibitor in fish. Three cDNAs encoding for pre-pro-SRIF (PSS) have been cloned from goldfish brain (43): PSS-I, which encodes SRIF-14; PSS-II, which is potentially processed into gSRIF-28 that has [Glu1,Tyr7,Gly10]SRIF-14 at the COOH terminus; and PSS-III, which encodes [Pro2]SRIF-14 at its COOH terminus. All of these peptides were able to inhibit in vitro secretion of GH from goldfish pituitary cells (81). Moreover, in situ hybridization studies in goldfish also suggest that the three SRIF peptides could be involved in the control of pituitary function (8). On the other hand, GH release is stimulated by dopamine and a number of neuropeptides (57), including ghrelin (75). Notably, several of these neuropeptides such as CCK and bombesin (BBS)/gastrin-releasing peptide (GRP) and ghrelin have also been shown to regulate food intake in fish as well as in mammals (25, 59, 73, 74). In goldfish, a short-term relationship between circulating serum GH levels and feeding has been demonstrated (25). When fed a 2% wet body wt ration on a regular schedule, goldfish exhibit an acute elevation in serum GH at 30 min after feeding (25). It was speculated that CCK and/or BBS/GRP might integrate the regulation of satiation and the postprandial increase in circulating GH levels (45).

In goldfish, the effects of CCK and BBS on mRNA expression of SRIF isoforms have been recently studied (11). Both intraperitoneal and intracerebroventricular administration of CCK inhibits the expression of PSS-I but not PSS-II or PSS-III. On the other hand, BBS inhibits the expression of both...
PSS-I and PSS-II. These results suggest that, in addition to a possible direct effect at the pituitary level, both CCK and BBS can also act via SRIF peptides to control GH secretion (11). However, neither the involvement of SRIF peptides in the postprandial control of GH nor the effects of ghrelin on mRNA expression of SRIF peptides have been studied in fish. Furthermore, the possible interactions of ghrelin with BBS/GRP are also unknown in vertebrates.

In this report, we analyze the expression of mRNA for SRIF variants in the forebrain and ghrelin in the gut. The expression is studied in relation to feeding, postprandial GH secretion, and intraperitoneal injections of BBS and/or ghrelin peptides.

MATERIALS AND METHODS

Animals

Goldfish (Carassius auratus) of the common or comet varieties, with body weights ranging from 25 to 40 g, were purchased from Mount Parnell Fisheries (Mercersburg, PA) and maintained in 300-liter flow-through aquaria at 17°C under the simulated natural photoperiod of Edmonton, Alberta, Canada, for at least 2 wk before experiments were started. The fish were fed with commercially prepared Unifeed NU-Way trout ration (United Feeds, Calgary, AB, Canada). Sexually regressed or early recrudescent fish were used for the experiments. Goldfish were anesthetized with 0.02% tricaine methanesulfonate (MS-222, Syndel Laboratories, Vancouver, BC, Canada) for 2 min before treatments and tissue collection. The experimental procedures, approved by the University of Alberta, were performed according to standards of the Canada Council on Animal Care.

Peptides

BBS was obtained from American Peptide (Sunnyvale, CA). Goldfish ghrelin(1–19) with an octanoyl modification in the Ser3 residue was synthesized in the laboratory of Dr. Jean E. Rivier (Clayton Foundation Laboratories for Peptide Biology, The Salk Institute of Biological Sciences, San Diego, CA). Stock solutions were made in fish physiological saline (0.65% NaCl) or Milli-Q water, aliquoted, and stored at −20°C. Aliquots were subsequently thawed and diluted in fish physiological saline before use.

Intraperitoneal Injections

Fish were anesthetized, and 1–2 μl/g body wt of either fish saline (0.65% NaCl) or peptide dissolved in fish saline were administered into the peritoneal cavity just caudal to the pelvic fins, using a 25-gauge, 5/8-in. needle attached to a 100-μl Hamilton syringe. After injection, fish were returned to their respective aquaria and allowed to recover.

Experimental Design

Periprandial changes in serum GH levels and the forebrain expression of PSS genes. Goldfish were acclimatized to a scheduled feeding time for 2 mo. The fish were fed once daily with 2% body wt ration. On the day of the experiment, one group of fish was sampled at different times with respect to the scheduled feeding time. As a control, a group of fish did not receive food at the scheduled feeding time (unfed group). Under deep anesthesia, blood and forebrain tissue was collected, and forebrain samples were processed as described above.

Time course of the effects of ghrelin on serum GH levels and the forebrain expression of PSS genes. Ghrelin (100 ng/g body wt) was administered intraperitoneally; at different times postinjection, the animals were anesthetized, and blood and forebrain samples were collected and stored as described previously.

Effects of increasing dosages of ghrelin on serum GH levels and the forebrain expression of PSS genes. Ghrelin (0–100 ng/g body wt) was administered intraperitoneally. After 45 min, the fish were anesthetized, and forebrain samples were collected and processed as described above.

Interactions between ghrelin and BBS. Ghrelin (50 ng/g body wt) and/or BBS (100 ng/g body wt) were administered intraperitoneally. After 45 min, the animals were anesthetized, and forebrain samples were collected and processed as described above.

Effects of BBS on serum GH levels and the forebrain expression of PSS genes, before and after the feeding time, in fed and unfed conditions. Goldfish were randomly selected for placement into three groups and fed once daily with a 2% body wt ration. The fish were acclimatized to a scheduled feeding time for 2 mo. On the day of the experiment, 3 h before the scheduled feeding time, one group of fish received saline or BBS (100 ng/g body wt ip) injections and were sampled 35 min after injection. Of the remaining groups, only one group received food at the scheduled feeding time (fed group). Three hours after the scheduled feeding time, fish received saline or BBS (100 ng/g body wt ip) injections. Fish were sampled 45 min after injection under deep anesthesia. Forebrain tissue was stored at −70°C until RNA extraction. Blood was allowed to clot overnight at 4°C, centrifuged and serum collected, and stored at −20°C until GH and glucose determination. From the preprandial- and postprandial-fed groups, the most rostral portion of the gut, the “J” region (62), was also collected, cleaned in fish saline, and kept at −70°C until RNA extraction to determine ghrelin expression levels as described earlier (73).

GH Radioimmunoassay

Serum GH concentrations were determined using a double antibody radioimmunoassay, as described previously (47).

Preparation of Total RNA and Slot-Blot Quantification

We extracted total RNA from goldfish forebrain using Trizol reagent (Life Technologies, Gaithersburg, MD), based on the guanidinium thiocyanate-phenol-chloroform extraction (fed group). The integrity of the RNA was verified in a denaturing agarose gel, which was stained with ethidium bromide. Slot-blot analysis was used to quantitate mRNA levels for the three PSS genes. Total RNA was blotted onto a Hybond-N nylon membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK) using a Bio-Dot SF blotting apparatus (Bio-Rad Laboratories, Hercules, CA). Specific cDNA probes for PSS-I, PSS-II, PSS-III, ghrelin, and goldfish β-actin were amplified by PCR according to Canosa et al. (10) and Unniappan et al. (73) and labeled with [α-32P]dCTP using the Rediprime II Kit (Amersham Pharmacia Biotech) based on the random primer method (18). The labeled probes were purified through a silica cartridge using the QIAquick nucleotide removal kit (Qiagen, Santa Clarita, CA). The specificity of the probes and the quantitative relationship between amount of total RNA and the measured signal were previously demonstrated (10, 73). Hybridization was performed as previously described (10). Briefly, the membranes were prehybridized in hybridization solution (0.5 M Na2HPO4, 7% SDS, 1 mM EDTA, 1% BSA) for 3 h. The hybridization solution was then replaced with fresh hybridization solution, and 106 counts/min · ml−1 of labeled probe were added. After overnight hybridization at 65°C, the membranes were washed three times for 30 min with wash solution (40 mM Na2HPO4, 1 mM EDTA, 1% SDS) and exposed to a PhosphorImager screen for 72 h for PSSs or 10 days for ghrelin probes. As an internal control, the membranes were stripped and reprobed with [α-32P]dCTP-labeled cDNA probe for goldfish β-actin. The radioactive signal was scanned using a PhosphorImager 445 SI (Molecular Dynamics, Sunnyvale, CA) and quantified by Image Quant Software (Molecular Dynamics).
Data Analyses

The mRNA levels for each gene were expressed as a ratio of specific gene mRNA to β-actin and then normalized as a percentage of the control (saline or time 0) group. The normalized data were subjected to ANOVA followed by Tukey’s (assuming equal variance) or Dunnett’s (equal variance not assumed) multiple comparisons tests. Differences were considered significant when $P < 0.05$.

RESULTS

PSS Gene Expression During Feeding

Similar to previous findings in our laboratory (28), serum GH levels were significantly increased at 20 min after feeding (Fig. 1A). Serum GH levels returned to baseline 60 min after feeding. On the other hand, in the unfed group, the serum GH levels remained unchanged (Fig. 1A).

In the same experiment, the PSS-II gene expression decreased significantly 60 min before the scheduled feeding time, reaching the lowest levels at 20 min after and remaining significantly lower for 2 h (Fig. 2B). On the other hand, neither PSS-I nor PSS-III gene expression changed during or after feeding (data not shown). These results suggest that PSS-II may be involved in the postprandial control of GH.

Ghrelin Effects on PSS Gene Expression

To identify the peripheral signal that triggers the changes in forebrain expression of PSS genes, we evaluated the effects of intraperitoneal administration of ghrelin on the expression levels of PSS in the goldfish forebrain. In this experiment, a dosage of 100 ng/g body wt of ghrelin(1–19) induced a transient rise of serum GH at 15 min after intraperitoneal injection (Fig. 2A), returning to the baseline at 45 min postinjection. The administration of ghrelin(1–19) had different effects on PSS gene expression (Fig. 2B). Ghrelin(1–19) treatment significantly increased the PSS-I expression levels, whereas it significantly decreased PSS-II gene expression at 45 min postinjection. No significant effects on PSS-III were found (Fig. 2B).

Interaction Between Intraperitoneally Administered Ghrelin and BBS

As previously shown, CCK and BBS act on SRIF peptide gene expression (11). It is believed that these peptides act postprandially, inducing satiety, and are also involved in GH regulation (28, 45). Table 1 summarizes the effects of CCK, BBS, and ghrelin on the forebrain expression of PSS-I and PSS-II. Although CCK had no effects, both BBS and ghrelin reduced PSS-II gene expression. Furthermore, the effects of ghrelin and BBS differed from one another in the regulation of PSS-I gene expression. Although BBS significantly decreased

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**Fig. 1.** Changes in serum growth hormone (GH) (A) and forebrain pre-prosomatostatin (PSS)-II gene expression (B) levels during feeding. Symbols represent means ± SE of 10 fish/group. Different letters represent significant differences ($P < 0.05$), with capital letters referring to the unfed series.

**Fig. 2.** Time course of changes in serum GH (A) and forebrain PSS gene expression (B) levels after intraperitoneal injection of 100 ng/g body wt of ghrelin(1–19). Symbols represent means ± SE of 12 fish/group. Different letters represent significant differences ($P < 0.05$), with capital letters referring to the PSS-II series. No significant differences were found in PSS-III gene expression.
PSS-I expression, ghrelin showed a nonsignificant tendency to increase it (Table 1); a higher dosage of ghrelin(1–19) (100 ng/g body wt) increased PSS-I expression (Figs. 2B and 3).

Thus we hypothesize that BBS and ghrelin could interact in the postprandial control of GH. In an attempt to verify this hypothesis, the effects of coadministration of these peptides on the expression levels of SRIF precursors were investigated. The results, shown in Fig. 4, confirm that intraperitoneal administration of 50 ng/g body wt of ghrelin(1–19) has no significant effect on PSS-I expression levels (Fig. 4A), whereas it inhibits the expression of PSS-II (Fig. 4B). On the other hand, 100 ng/g body wt of BBS reduced both PSS-I and PSS-II expression levels (Fig. 4, A and B). Neither ghrelin nor BBS had effects on PSS-III gene expression (Fig. 4C). Coadministration of ghrelin blocked the effects of BBS on the expression of PSS-I (Fig. 4A) but not on PSS-II (Fig. 4B).

Effects of BBS on PSS Gene Expression at Different Times of the Feeding Schedule

It is already known that serum ghrelin levels are higher before meals and in fasted animals, whereas serum ghrelin levels are significantly decreased after a meal (73). To verify the interaction between ghrelin and BBS in a more physiological approach, the serum ghrelin levels were manipulated by the feeding status. Intraperitoneal administration of BBS 3 h before feeding increased serum GH levels (Fig. 5A, left). Under these conditions, BBS had no effects on PSS-I expression levels, whereas it decreased PSS-II gene expression levels (Fig. 5A, right). In unfed animals, 3 h after the scheduled feeding time, BBS administration had no effects on serum GH levels or PSS-I gene expression levels but still significantly reduced the expression of PSS-II (Fig. 5B). On the other hand, when the fish were fed and the effect of BBS administration was assayed 3 h after feeding, BBS significantly increased serum GH levels, whereas it reduced both PSS-I and PSS-II gene expression (Fig. 5C).

BBS Regulation of Ghrelin Gene Expression in the Gut

BBS mimics the actions of endogenous GRP, which controls gastric function shortly after meals, reducing the gastric acid secretion and increasing the gastrin release (49). In this experiment, we tested the hypothesis that ghrelin gene expression in the gut could be under the control of GRP. We determined ghrelin mRNA expression levels in the goldfish gut 3 h before (preprandial) or 3 h after (postprandial) the scheduled feeding time in fish injected with saline or BBS (100 ng/g body wt). As shown in Fig. 6, administration of BBS during the preprandial period significantly decreased ghrelin expression. Confirming previous results, we found significantly lower ghrelin expression in the postprandial period than in the preprandial period. BBS treatment did not cause a further decrease in ghrelin expression in the postprandial condition (Fig. 6).

Table 1. Effects of intraperitoneal administration of CCK, BBS, and ghrelin on the forebrain PSS-I and PSS-II gene expression

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PSS-I</th>
<th>PSS-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100±6.42</td>
<td>100±9.27</td>
</tr>
<tr>
<td>CCK (100 ng/g body wt)</td>
<td>60.02±6.07*</td>
<td>95.13±9.24*</td>
</tr>
<tr>
<td>BBS (100 ng/g body wt)</td>
<td>61.62±4.61*</td>
<td>56.36±4.99*</td>
</tr>
<tr>
<td>Ghrelin (50 ng/g body wt)</td>
<td>118.86±3.79</td>
<td>80.94±4.99*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Pre-pro-somatostatin (PSS) gene expressions presented here are from tissue collected 45 min after intraperitoneal administration of peptides. BBS, bombesin. *Significant differences (P < 0.05) with respect to control (saline) group.
DISCUSSION

Our results show, for the first time, a correlation between the postprandial rise in serum GH levels and the expression of PSS-II, suggesting that a reduction in gSRIF-28 precursor mRNA occurs at the same time as postprandial GH secretion. At present, the level of protein for the three forms of SRIF cannot be determined because there are no specific antibodies for goldfish SRIF peptides. Moreover, some SRIF peptides are structurally so similar that immunodetection will likely not discriminate the different variants. Thus the strategy chosen in the present work was to detect changes in gene expression levels. Because our in situ hybridization studies showed that the three types of SRIF transcripts have specific brain distributions (8), we have measured the mRNA levels as an indication of the activation or inactivation of a particular neuronal system. We recognize that mRNA levels do not measure physiological effects produced by proteins but may give a clue as to which SRIF system in the brain is activated. From our results, we interpret that a decrease in activity of PSS-II-expressing neurons occurs at the same time as postprandial GH secretion rise.

The goldfish pituitary expresses several SRIF receptor subtypes, with types 2 and 5 (sst2 and sst5) being the most highly expressed (40–42, 44). Goldfish sst5 shows a four- to fivefold higher affinity to mammalian SRIF-28 and gSRIF-28 (42) and has similar pharmacological characteristics to mouse sst5 (55), which also binds preferentially to mammalian SRIF-28. It was recently shown that estradiol treatment reduces the in vitro GH responsiveness to SRIF-14 and simultaneously reduces the expression levels of sst2 in goldfish pituitary (12). However, estradiol treatment increases the expression levels of sst5 (9). Thus it seems that sst5 may be specialized in the transduction of gSRIF-28 actions in the pituitary. Taking all of these results together, we consider it plausible that a reduction in gSRIF-28 inhibitory tone occurs during meals as a postprandial control mechanism of GH secretion. However, this hypothesis needs further experimental demonstration.

The mechanisms underlying the control of SRIF gene expression during meals has not yet been established. However, in a recent report (11), our group analyzed the effects of intraperitoneal administration of CCK and BBS, two good candidates that link food intake and GH secretion in goldfish. CCK is widely distributed in both peripheral nerves and endocrine cells in the gastrointestinal tract in mammals, as well as in nonmammalian vertebrates and is involved in many gastrointestinal functions, including gallbladder contraction, pancre-
BBS is a tetradecapeptide that was first isolated from the skin extracts of the fire-bellied toad, *Bombina bombina* (2). The structurally related GRP, a 27-amino acid peptide, has been isolated from several mammalian species, and closely related forms of the peptide are also found in other vertebrates (30, 51, 78). This group of peptides is characterized by a highly conserved COOH terminus, which is important for biological actions. Although BBS and antibodies against BBS have been used in most physiological and immunohistochemical studies, GRP is the peptide expressed in the gut and brain of vertebrates. Exogenous BBS most likely mimics the function of endogenous GRP (49). BBS/GRP peptides are widely distributed in the gastrointestinal tract and central nervous system (50) and have been shown to be potent in suppressing food intake when administered either intraperitoneally or intracerebroventricularly (25, 26). Furthermore, intraperitoneal administration of BBS reduces PSS-I and PSS-II but not PSS-III gene expression in goldfish forebrain (11). These actions on SRIF neurons likely represent the route of action of BBS/GRP on anterior pituitary hormone release since only low densities of BBS/GRP binding sites are present in the anterior pituitary (29). On the other hand, high densities of binding sites are present in the neurointermediate lobe and the hypothalamus. Even if BBS/GRP actions on the anterior pituitary are mediated by forebrain SRIF neurons, its actions on forebrain SRIF gene expression do not allow a full explanation for the postprandial changes in expression of SRIF precursors shown in the present paper. This is because BBS affected not only PSS-II but also PSS-I.

Ghrelin is synthesized mainly in the stomach and was first identified as the endogenous ligand to the GH secretagogue receptor in mammals (38, 39). It is also a potent stimulus to feeding (79, 80), and both the mRNA expression and the circulating levels are upregulated in fasted conditions (3, 79). Periprandial changes in plasma ghrelin levels in humans suggest ghrelin has a role as a meal initiator (15). Ghrelin has also been identified in several nonmammalian species (33–37, 56), including goldfish (74). As in mammals, goldfish ghrelin acts as GH secretagogue, both in vivo and in vitro, and stimulates food intake (73, 75). Ghrelin induces an increase in serum GH levels 15–30 min after intraperitoneal administration in goldfish (Ref. 75 and present results). In mammals, ghrelin stimulates GHRH-producing neurons and acts as a functional antagonist of SRIF-14 (69, 77). In goldfish, perfusion of dispersed cells with SRIF-14 abolishes the stimulatory effects of a ghrelin pulse (75). Ghrelin in fish, as in mammals, represents a link between food intake and GH secretion. In goldfish, serum ghrelin levels are high before meals and are significantly lower 1 h after food intake (73). Our present results show that intraperitoneal administration of ghrelin had a dual effect on PSS-I and PSS-II gene expression. Thus, although changes in ghrelin release cannot completely explain the postprandial changes in forebrain expression of PSS genes, it may be part of the regulatory mechanism for postprandial GH secretion. In support of this view, we found that ghrelin is able to block the inhibitory effects of BBS on PSS-I gene expression but not on PSS-II. Therefore, the interaction between ghrelin, which increases postprandially, and GRP, which is believed to act shortly after food intake, could account for the postprandial variations of forebrain expression levels of SRIF precursors.

In line with this hypothesis, we found that, 3 h before the scheduled feeding time (high serum ghrelin levels), intraperitoneal administration of BBS increased GH, while, conversely, it only reduced PSS-II gene expression. This experimental result resembles the natural variations during spontaneous feeding. When BBS was administered to unfed animals 3 h after the scheduled feeding time (high serum ghrelin levels), it was correlated with a reduction in PSS-II gene expression, although no changes in serum GH levels.
were found; the reason for this behavior is not clear at present. One explanation could be that in these experimental conditions (27-h food restriction) some other mechanisms controlling GH secretion are activated in a similar fashion as during starvation, a condition in which GH is elevated. Interestingly, when BBS was administered 3 h after feeding (low serum ghrelin levels), it was correlated with an increase in serum GH levels and a decrease of both PSS-I and PSS-II gene expression, supporting the interpretation that the preprandial levels of ghrelin blocks BBS effects on PSS-I gene expression.

The regulation of production and secretion of ghrelin from the stomach is not fully understood. Plasma ghrelin levels are upregulated under conditions of negative energy balance, such as starvation, cachexia, and anorexia nervosa, and downregulated during positive energy balance, such as following feeding and obesity (15, 53, 64, 71, 72). Similar to mammals, in goldfish, serum ghrelin levels decrease after meals (73). To date, there are no reports on the hormonal regulation of ghrelin in fish. In humans, it has been shown that insulin may reduce ghrelin secretion (19, 46). Also, SRIF-14 infusions into normal subjects reduce plasma ghrelin levels to 70–80% of normal control values (7, 54). Furthermore, perfusion with SRIF-14 or the SRIF agonist octreotide reduces ghrelin secretion in isolated rat stomach, suggesting that this is a direct effect of SRIF on the stomach in a paracrine fashion (46, 65). Our present results show, for the first time, that intraperitoneal administration of BBS reduces ghrelin mRNA expression in the gut. GRP has a role in gastric physiology (49) and also induces satiety and meal termination through vagal and nonvagal afferents (52). Therefore, a concomitant inhibition of the hunger signal ghrelin (15) should be expected. It is known that BBS/GRP induces SRIF-14 secretion from gastric fundus and antrum in mammals (17, 48, 63), and, as mentioned before, SRIF-14 reduces ghrelin secretion (65). Then, it may be that SRIF mediates BBS/GRP actions on ghrelin gene expression at the gut level.

In conclusion, the present results show that an interplay between BBS/GRP and ghrelin controls GH secretion after feeding and occurs at the same time as a reduction in the expression of PSS-II. This interplay is schematically represented in Fig. 7, which shows preprandial and postprandial situations. In preprandial conditions, it was previously shown that ghrelin levels are increased (73); as a consequence, 1 h before the scheduled feeding time, circulating levels of GH levels tend to increase in goldfish (Figs. 1A and 7A). This increase in serum GH levels may represent the actions of increasing levels of ghrelin on GH secretion (75), as well as on PSS-II expression (Figs. 1B and 7A). However, high doses of ghrelin can stimulate PSS-I expression, which if translated into peptide secretion would exert a counterbalance to a GH rise. Therefore, GH secretion would be kept close to normal basal levels. Soon after feeding, BBS/GRP exerts a satiety signal. It should be noted that meal termination or satiety occurs before the digestion and/or absorption of food nutrients, and BBS/GRP may represent one of the physiological mediators of satiety signals between gut and brain (52). Additionally, BBS/GRP inhibits PSS-II expression, and thus serum GH levels increase significantly while at the same time PSS-II expression decreases (Figs. 1 and 7B). The expression levels of PSS-I are not affected during BBS/GRP activity due to the presence of ghrelin (Figs. 4 and 7B). We also demonstrated in the present paper that BBS/GRP peptides inhibit ghrelin transcription in the gut (Fig. 6). The reduction of ghrelin expression by BBS is a likely explanation for the postprandial reduction of serum ghrelin levels, and it may contribute to the satiety effect of BBS/GRP peptides.

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Present address for S. Unniappan: Laboratory of Molecular and Cellular Medicine, Dept. of Cellular and Physiological Sciences, D. H. Copp Bldg., 2146 Health Sciences Mall, University of British Columbia, Vancouver, BC, Canada V6T 1Z3.

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