Prolactin-releasing peptide, food intake, and hydromineral balance in goldfish

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Kelly, Scott P., and Richard E. Peter. Prolactin-releasing peptide, food intake, and hydromineral balance in goldfish. Am J Physiol Regul Integr Comp Physiol 291: R1474–R1481, 2006.—A potential role for prolactin-releasing peptide (PrRP) in appetite regulation and hydromineral balance in goldfish was examined. PrRP was found to be expressed in discrete regions of the goldfish brain, in particular, the hypothalamus. Intraperitoneal (IP) or intracerebroventricular administration of PrRP had dose-dependent effects to suppress food intake in goldfish. Hypothalamic PrRP mRNA expression significantly increased after feeding, as well as after 7 days of food deprivation. Refeeding fish after 7 days of food deprivation did not result in a postprandial increase in PrRP mRNA expression. These data suggest an anorexigenic role for PrRP in the short term around a scheduled meal time, but not over the longer term. IP injection of PrRP significantly increased pituitary prolactin (PRL) mRNA levels, suggesting involvement in the regulation of lactotrophic activity. Acclimating goldfish to an ion-poor environment decreased serum osmolality and increased PrRP and PRL mRNA levels, providing evidence for PrRP involvement in hydromineral balance through its actions on lactotrophs. Acclimation to ion-poor water diminished the anorexigenic properties of PrRP in goldfish, indicating that a role for PrRP in goldfish satiation is counterbalanced by alternate systemic needs (i.e., osmoregulatory). This was further supported by an ability to reinstate the anorexigenic actions of PrRP in fish acclimated to ion-poor water by feeding a salt-rich diet. These studies provide evidence that PrRP is involved in regulating appetite and hydromineral balance in fish, and that the degree of involvement in either process varies according to overall systemic needs in response to environmental conditions.

Prolactin-releasing peptide (PrRP) was first described as an endocrine factor of hypothalamic origin by Hinuma et al. (9) who unified the endogenous ligand with an orphan G-protein-coupled receptor (hGR3/GPR10). These initial studies and subsequent reports have suggested PrRP to be a prolactin-releasing factor (PRF) in at least two groups within the chorionate phyllum, namely mammals (9, 17) and bony fishes (18, 23, 27). However, in mammals, the exact nature and efficacy of PrRP as a classic hypophysiotropic PRF remains unclear (for a review, see Ref. 32). However, the widespread distribution of PrRP in the central nervous system (CNS) suggests involvement in a number of functions (for a review, see Ref. 29). In bony fishes, PrRP was initially described as a novel bioactive peptide with an RFamide C-terminal (8). As is the case with mammalian PrRP, fish PrRP is widely distributed in brain tissue of species studied so far (18, 25, 36), and this also suggests involvement in multiple functions.

In mammals, PrRP mRNA, peptide, and receptor have been localized to areas of the CNS known to be involved in the regulation of energy balance and appetite control (11, 22). Such observations suggested a physiological role for PrRP in the regulation of energy homeostasis. Lawrence et al. (13) first reported alterations in PrRP mRNA expression that were consistent with an anorexigenic role for PrRP. In the same communication, intracerebroventricular injection of PrRP was reported to reduce food intake in both free-fed rats, as well as fasted, re-feeding animals (13). A number of additional reports support appetite regulation as one of several potential physiological roles for PrRP in mammals (6, 14, 15, 26). Much less is known about the potential appetite-regulating effects of PrRP in nonmammalian vertebrates. Recent studies have suggested that PrRP may be involved in both the regulation of feeding and energy metabolism in aves (30, 31), and anecdotal reports have introduced a scenario whereby PrRP “induction” in bony fish may result in poor growth (24). However, to the best of our knowledge, there are no published studies that address a potential role for PrRP in the regulation of food intake in fish.

In bony fishes, prolactin (PRL) has been reported to have a broad range of systemic effects (10). Of these actions, PRL is principally considered to affect epithelial barriers that control hydromineral balance in hypersomoregulating fish (for a review, see Ref. 16). With evidence suggesting PrRP to be an important PRL secretagogue in fish (18, 23, 27), observations that brain PrRP mRNA and pituitary PRL mRNA expression elevate in fish acclimated to freshwater (FW), relative to those acclimated to seawater, are consistent with the generally accepted actions of PRL as a “FW osmoregulatory hormone” and PrRP as a PRF under such conditions (24).

The objectives of the present studies were several-fold. First, we hypothesized that PrRP may possess appetite-regulating effects in fish. We addressed this by examining the effects of central and peripheral administration of PrRP on food intake in goldfish. Secondly, we hypothesized that PrRP would participate in regulatory mechanisms that control hydromineral balance in a stenohaline FW fish. To examine this, we acclimated goldfish to a FW environment with lower ion content than “typical” FW. In the presence of an increased ionic/osmotic gradient between external (water) and internal (body fluids) environments (i.e., an increased demand on hypersomoregulatory mechanisms), it was anticipated that the role of PRL as a “FW-adapting hormone,” and, therefore the role of PrRP as a PRF, would be emphasized. Lastly, we sought to address how the role of PrRP as a potential PRF involved in osmoregulatory homeostasis and the role of PrRP as a potential appetite-regulating endocrine factor interacted in a physiologically relevant manner that facilitated both processes.

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Experimental animals. All experiments were conducted on mixed sex common/comet variety goldfish (Carassius auratus). Fish (~30–35 g) were purchased from Mt. Parnell Fisheries (Mercersburg, PA) and held in 600-liter flow-through freshwater (FW) aquaria at 17°C under simulated natural photoperiod (Edmonton, AB, Canada; 53.34°N/113.25°W). Edmonton dechlorinated FW was of the following composition (in μM): 330 [Na⁺], 120 [Cl⁻], 1100 [Ca²⁺], and 20 [K⁺]. During this period, fish were fed ad libitum once daily with commercial fish feed pellets (Corey Aquafeeds, Fredericton, NB, Canada). At least 2 wk before all experiments, fish were transferred to 65-liter flow-through glass aquaria at 20°C and fed a 2% wet body mass ration once daily at a scheduled feeding time (1000). All experiments were carried out according to the guidelines set out by the Canadian Council for Animal Care under the approval of the Biosciences Animal Service Unit, University of Alberta, Edmonton, Canada.

Cloning and sequence analysis of cDNA for prepro-goldfish PrRP. Goldfish were anesthetized in 0.05% tricaine methane sulfonate (MS 222; Syndel Laboratories, Vancouver, BC, Canada) and killed by spinal transection. Forebrain regions (telencephalon and hypothalamus) were removed and immersed in Trizol reagent (Invitrogen, Carlsbad, CA). Total RNA was extracted immediately from this tissue and Superscript II RNase H⁻ reverse transcriptase (Invitrogen) used for cDNA synthesis. To isolate goldfish prolactin-releasing peptide cDNA, primers PrRP1 (5'-CTT GTT ACT GAA GAG CAT AGC-3') and PrRP2 (5'-ATT ATA ACA GCT AAC ACA CC-3') were designed based on previously published sequence information for Japanese crucian carp RF-amide (PrRP) (25) and used for PCR amplification. The conditions for PCR were 5 min denaturation at 95°C followed by 35 cycles of denaturation at 95°C (1 min), annealing at 53°C (1 min), extension at 73°C (1 min), respectively, and a final, single, extension cycle at 73°C for 10 min. The PCR product was separated by agarose gel electrophoresis, excised, then isolated using a GeneClean II kit (Bio 101, La Jolla, CA). The purified cDNA fragment was then subcloned using a pGEM-T Vector System I (Promega, Madison, WI). Plasmid DNA containing cDNA inserts was isolated and purified by alkaline lysis (1, 2) and sequenced (Applied Biosystems Automated Sequencer, Perkin Elmer, Norwalk, CT).

Detection of prepro-PrRP in goldfish tissues. Using the same primers and PCR conditions as described for the cloning of full-length cDNA encoding for prepro-PrRP, expression in discrete regions of the brain (olfactory bulbs, telencephalon, hypothalamus, midbrain, and hindbrain), and pituitary was examined using RT-PCR and Southern blot analysis. The reactions were electrophoresed, as previously described, and transferred to a Hybond-N membrane (Amersham Biosciences, Baie d’Urfe, QB, Canada) by capillary transfer. A [α-32P]dCTP-labeled probe covering the full-length PrRP cDNA was used for hybridization of membranes containing capillary transferred PCR product. Probe labeling was conducted using a redIPRIME II random prime labeling system (Amersham), and the labeled probe was purified to reduce nonspecific background signal using QIAquick spin columns (Qiagen, Santa Clarita, CA). Labeled membranes were exposed on a phosphorimaging screen (Molecular Dynamics, Sunnyvale, CA) for 1 h, after which the phosphorimage was scanned using a Phosphorimager 445 SI (Molecular Dynamics). As an internal control, PCR for a partial fragment of goldfish β-actin (GenBank accession number AF079831) was performed using the same cDNA generated from the tissues described above. These reactions were electrophoresed as previously described, and a gel image captured. Tissue distribution of prepro-PrRP was further examined using Northern blot analysis conducted on discrete regions of the goldfish brain (i.e., regions as described above). A total of 10 μg RNA from each brain region was electrophoresed in a denaturing agarose gel (1.5%) with formaldehyde after which, RNA was transferred to a Hybond-N membrane (Amersham Biosciences) by capillary transfer. Membranes were hybridized with full-length putative PrRP [α-32P]dCTP-labeled probe, exposed on a phosphorimaging screen, and analyzed with a Phosphorimager using a protocol identical to that described for Southern blot analysis except that exposure of the [α-32P]dCTP-labeled membrane on the phosphorscreen was allowed to proceed for a period of 5 days. Northern blot membranes were then stripped and hybridized a second time using a partial fragment of [α-32P]dCTP-labeled goldfish β-actin.

Slot blot analysis of prepro-goldfish PrRP and pituitary PRL mRNA. Total RNA was extracted from hypothalamic and pituitary samples, as described previously. RNA samples were denatured (denaturing solution; 42% formamide, 1.6% 3-(N-morpholino)propanesulfonic acid, 2 M formaldehyde; 65°C for 15 min) and immobilized by vacuum suction onto Hybond-N membrane using a Bio-Dot SF blotting apparatus. Membranes were fixed by UV exposure. The amount of RNA used for quantifying mRNA expression of prepro-PrRP and pituitary PRL was qualified by constructing standard curves to establish signal density as a function of total RNA immobilized within each slot (data not shown). Total RNA used for each tissue was selected so as to fall in the midrange of the curve under normal (control) conditions, giving optimal resolution for experimentally induced modulation of transcript abundance (for details, see Ref. 5). For pituitary PRL mRNA analysis, a partial fragment (~700 bp) of [α-32P]dCTP-labeled goldfish PRL cDNA (GenBank accession no. S82197) was generated using the techniques described above. The primer sequences used to amplify goldfish PRL cDNA were PRL1 (5'-GGT CTG ATG TGT GGG TTG G-3') and PRL2 (5'-TCT CTA ACA CAT CTC AGC TC-3'). The conditions for goldfish PRL cDNA PCR amplification were 5-min denaturation at 95°C followed by 30 cycles of denaturation at 95°C (1 min), annealing at 53°C (1 min), extension at 73°C (1 min), respectively, and a final, single, extension cycle at 73°C for 3 min.

PrRP administration and food intake. A 20-amino acid mature goldfish PrRP was synthesized according to the amino acid sequence deduced from the above analysis [SPEIDFPWYVGPRIPGRF-NH₂]. PrRP was synthesized by solid-phase approach with Boc chemistry on a CS-Bio Peptide Synthesizer (Model CS536). The synthesis was performed on 4-methylbenzhydrylamine resin. The following amino acid side-chain protections were used: Arg(Tos), Asp(OcHex), Glu(OcHex), Ser (Bzl), and Thr(Bzl). The couplings were mediated with diisopropylcarbodiimide and N-hydroxysuccinimide. Boc removal was achieved in 20 min with trifluoroacetic acid (80% in CH₂Cl₂, 1–2% m-cresol). The completed peptide was cleaved from the resin by anhydrous HF/anisole (9:1, vol/vol). The crude peptide was determined by analytical HPLC and capillary zone electrophoresis analysis and showed a purity of >95% by both methods. The observed monosotopic (M + H)+ value of the peptide corresponded with the calculated (M + H)+ value (2347.2) determined by matrix-assisted laser desorption/ionization-mass spectrometry. For intraperitoneal injections, anesthetized fish were weighed, and an injection of goldfish PrRP administered through the ventrolateral side of the body wall using a Hamilton syringe. The volume of each injection varied according to fish body weight at 2.5 μg/fish. Varying doses of PrRP were administered intraperitoneally for determination of food intake and for determination of pituitary PRL mRNA expression. Fish that received intracebroventricular injections were used for analysis of food intake only. Procedures for intracebroventricular administration of goldfish PrRP were conducted according to methodology previously outlined (20, 33). Briefly, anesthetized fish were placed in a custom-designed stereotaxic apparatus, and varying doses of test solution were injected into the preoptic region of the brain third ventricle (coordinates +1.0, M, D 1.2), according to the stereotaxic atlas of the goldfish brain (20). The volume of injection varied relative to fish body weight at 0.034 μg/g fish mass. After intracebroventricular injection, the cranial cavity was filled with teleost physiological saline (4). The skull flap was put back in place and secured with surgical thread. Fish were then...
placed in fresh water where they generally regained equilibrium within 2–5 min. After regaining equilibrium, fish were given a 10- to 15-min postoperative recovery period before conducting experiments to determine food intake. No mortality was observed as a result of the surgical procedures. Goldfish will feed immediately after a 10–15 min postoperative recovery period. Therefore food intake was determined 10–15 min after recovery from surgical anesthetic using fish held in pairs in a single observational tank. Each fish pair was administered the same treatment (i.e., untreated, saline, or PrRP dose). Fish were provided with a preweighed excess of pellets (approx. 4–5% body weight) and allowed to feed undisturbed for 1 h. During the first set of experiments, fish were observed directly so that individual food intake rates could be recorded. After 1 h of feeding, all remaining pellets were recovered and dried to a constant weight for the determination of dry pellet weight. Dry pellet weights were corrected for pellet weight lost to the water during the 1-h feeding period, and food intake was calculated by weight differences. From the direct observations of food intake, it was evident that each fish within a paired treatment ate approximately the same amount of food relative to body weight. This was confirmed by regression analysis using untreated and sham-treated groups, where food intake of fish 1 = 0.8756(food 2) + 1.9468, r = 0.82, n = 24, P < 0.0001. From this point forward, fish were not observed. Instead, fish were allowed to feed undisturbed for 1 h, after which time, all remaining pellets were gathered, dried, and weighed. Using the weight differences (corrected for pellet weight lost to the water) the total amount of food eaten was calculated relative to the mass of the two fish combined.

**PrRP administration and pituitary PRL expression.** To examine whether PrRP had a stimulatory action on pituitary lactotrophs in goldfish, pituitary PRL mRNA expression was measured after IP administration of PrRP. A single injection of PrRP was administered to multiple groups of goldfish at doses effective in altering food intake (i.e., 25 and 250 ng/g body mass), and pituitaries were collected at times –1, 1, 2, 4, and 8 h preinjection and postinjection. As a control, saline was administered to a separate series of groups and pituitaries collected at identical time periods. PRL mRNA expression was analyzed in RNA samples extracted from individual pituitaries. PRL mRNA was examined using slot blot analysis, as previously outlined, and expressed relative to goldfish β-actin after stripping and reprobing the same membranes.

**Food deprivation and postprandial PrRP mRNA expression.** To examine the effects of food deprivation on hypothalamic PrRP mRNA expression, fish were food deprived for periods of 3 and 7 days. Total RNA extracted from hypothalamic tissues was examined for alterations in PrRP mRNA expression using slot blot analysis, as described above. To examine postprandial responses of goldfish PrRP, hypothalamic PrRP mRNA expression was measured over a period of 24 h after a single feeding event. In these studies, two experimental regimes were used. The first regime comprised six groups of fish that were fed once daily at a scheduled time (for 2 wk) until sampling. The second comprised six groups of fish that were food deprived for 7 days and then fed a single meal. Temporal sampling of groups took place around the single feeding event at –1, 0, 3, 6, 12, and 24 h before and after feeding. In all cases, the single feeding event comprised a 2% body mass ration presented at time 0. PrRP mRNA expression in the hypothalamus was measured by slot blot analysis, as previously described.

**Acclimatization of goldfish to ion-poor water.** Goldfish were acclimated to ion-poor FW by gradually replacing flow-through regular dechlorinated Edmonton FW with flow-through reverse osmosis water until a ratio of ~30:70% (regular dechlorinated FW: reverse osmosis water) was obtained. Fish were allowed to acclimate to these conditions for at least 3 wk. Water ion levels in ion-poor water were measured by atomic absorption spectrometry and were, in μM: 100 [Na⁺], 50 [Cl⁻], 260 [Ca²⁺], 8 [K⁺]. Tissues for mRNA analysis were collected and analyzed, as described previously. As well as tissues for mRNA analysis, blood was collected from these fish for analysis of serum osmolality. Blood was allowed to clot at room temperature, and serum was obtained after centrifugation. Osmolality was measured using a vapor pressure osmometer (Wescor).

**PrRP and food intake in fish acclimatized to ion-poor water.** Feeding studies in fish acclimatized to ion-poor water were conducted as outlined above. Only IP injections (the least invasive of techniques) were used to administer PrRP in these studies. In fish held in ion-poor conditions and fed salt-rich diets, diets were prepared by grinding commercial pellets to a powder and in diets designated for salt addition, adding NaCl before reconstituting powdered diets with distilled water into a paste. The paste was run through a commercial pasta maker to make moist “noodles” which were then dried in an oven at 40–45°C. Control diets were treated in an identical manner without the addition of NaCl. Fish used for salt-rich diet-feeding studies were fed salt-rich diets for 2 wk before study. Control diets had a nominal Na⁺ content of 0.6 g/100 g, whereas salt-rich diets had a nominal Na⁺ content of 6 g/100 g. Measured dietary Na⁺ content was 0.45 ± 0.01 g/100 g (n = 3) and 5.99 ± 0.04 g/100 g (n = 3) for control and salt-rich diets, respectively.

**Statistical analysis.** All data are expressed as mean values ± SE. Data were either subjected to a t-test or an ANOVA followed by a Student Neuman-Keuls multiple comparison test (Sigmastat software, Jandel Scientific, Richmond, CA) as appropriate. A fiducial level of P ≤ 0.05 was used throughout.

**RESULTS**

**Goldfish prepro-PrRP cDNA.** Analysis of PCR product amplified using primers PrRP1 and PrRP2 (see MATERIALS AND METHODS) by agarose gel electrophoresis revealed a single major band of ~450 bp. Sequence analysis indicated that this product was 466 bp in length comprising a 5′-untranslated region (54 bp), an open reading frame (351 bp), a stop codon (TGA), and a 3′-untranslated region (58 bp). The deduced amino acid sequence demonstrated the putative goldfish PrRP to be part of a 117-aa precursor with several potential cleavage recognition sites as outlined previously (25). By ClustalW analysis, the goldfish 117 aa precursor sequence was found to be identical to that of the previously characterized Japanese crucian carp (25).

**PrRP expression profile.** Expression of goldfish PrRP was investigated in discrete brain regions using RT-PCR and Southern blot analysis. All regions within the brain contained a transcript with the exception of the olfactory bulbs (Fig. 1A). The pituitary contained a weak positive signal. Northern blot analysis revealed an ~0.8-Kb band, and analysis of discrete brains regions suggested that transcript abundance was greatest in the hypothalamus, followed by the telencephalon. Signal was not detected in other regions of the brain or the pituitary by Northern blot analysis within the parameters used in our analysis.

**PrRP administration and food intake.** Goldfish exhibited a dose-dependent reduction in food intake in response to both intraperitoneal and intracerebroventricular injections of PrRP. Systemic administration (i.e., ip injection) of PrRP only reduced food intake at tested doses of 25 ng/g body wt and above (Fig. 2A). In contrast, central administration (i.e., icv injection) had a negative impact on food intake at lower doses, where a response could be seen after administration of PrRP at 5 ng/g body mass (Fig. 2B). At 10 ng/g body mass, the negative impact of PrRP on food intake was maximal, as increasing the dose to 25 ng/g body mass had no further effect.
Hypothalamus PrRP mRNA expression in response to food deprivation and feeding. Postprandial PrRP mRNA expression in the hypothalamus after a single feeding event differed between fed and food-deprived fish. In fish fed daily, PrRP mRNA expression increased after feeding, reaching a maximum at 6 h, which was significantly elevated relative to time 0 (Fig. 3A). In fish that were food deprived for 7 days before feeding a single meal, no variations in PrRP mRNA expression in the hypothalamus were found (Fig. 3B). In goldfish deprived of food for 3 days, no significant increase in hypothalamic PrRP mRNA expression was observed. However, after 7 days of food deprivation, a significant increase in PrRP mRNA expression was found (Fig. 4).

PrRP administration and pituitary PRL mRNA expression. Intraperitoneal injection of PrRP at doses consistent with those found to reduce food intake (i.e., 25 ng/g ip) resulted in significantly elevated pituitary PRL mRNA levels (Fig. 5, A and B). In the time course studied, data indicated that pituitary PRL mRNA expression did not significantly elevate above preinjection expression levels until 8 h after administration. Administration of 250 ng/g ip resulted in a significant elevation in pituitary PRL mRNA expression but not significantly different from that caused by the 25 ng/g ip injection.

PrRP and PRL mRNA expression, serum osmolality, and food intake in fish acclimatized to ion-poor water. In fish acclimated to ion-poor water, PrRP mRNA expression in the hypothalamus and pituitary PRL mRNA expression significantly increased (Fig. 6B). As an overall indicator of hydromineral balance, serum osmolality significantly decreased in fish acclimatized to ion-poor conditions (Fig. 6A), indicating that elevated PrRP and PRL mRNA levels were in accord with greater demands on hypersomoregulatory mechanisms. Although an elevation in PrRP may be expected to elicit a reduction in food intake, as intraperitoneal and intracerebroventricular PrRP administration reduced food intake in our feeding experiments, a significant increase in food intake occurred in goldfish acclimatized to ion-poor conditions, as saline-injected fish consumed more food than saline-injected fish acclimatized to regular water (Fig. 7). Furthermore, administration of PrRP in fish acclimatized to ion-poor conditions did not significantly reduce food intake at comparable doses (i.e., 25 ng/g body mass, see Fig. 7). At the highest doses tested (250 ng/g body mass), food intake was significantly reduced in fish acclimatized to both conditions. But at this high dose, food intake in fish acclimatized to ion-poor conditions was significantly greater than that found in fish held in regular water (Fig. 7). Feeding diets rich in salt to fish acclimatized to ion-poor water allowed us to dissect this apparent paradox further, as PrRP administration in these fish at a dose of 25 ng/g body mass, once again, significantly reduced food intake (Fig. 8). This was not the case in fish acclimatized to ion poor water and fed a regular (control) diet.
Discussion

Results from cloning, sequence analysis, and expression analysis of goldfish prepro-PrRP are consistent with previous observations (25) that reported PrRP expression (using Southern blot analysis) within discrete brain regions with the exception of the olfactory bulbs. By Northern blot analysis of total RNA, our cDNA probe detected a single band, suggesting that the gene product produces a single transcript (Fig. 1B). Next, we hypothesized, on the basis of widespread brain distribution and hypothalamic presence (18, 25, 36; see Results), that PrRP in bony fish would be involved in the regulation of appetite, as has been observed in mammals and aves (6, 13, 14, 26, 30, 31). Our findings support this hypothesis, as PrRP administration had dose-dependent, negative effects on food intake in goldfish. These observations are in line with the anorexogenic effects of PrRP in mammals but contrast with the effects seen in aves, where PrRP has been reported to stimulate appetite (30, 31).

Periprandial variations in mRNA expression of several appetite-regulating factors have been described in fish (for a review, see Ref. 35). In the current study, we observed a postprandial increase in hypothalamic PrRP mRNA expression. This is in accord with postprandial elevations in brain mRNA expression of other anorexigenic factors in fishes such as cholecystokinin (21) and cocaine- and amphetamine-regulated transcript (34). Conversely, under conditions of negative energy balance (i.e., 7 days of food deprivation) refed goldfish did not exhibit a postprandial increase in PrRP mRNA expression (Fig. 3B). These data indicate that when goldfish are food deprived, PrRP does not possess the same anorectic properties as when goldfish are in a state of positive energy balance. This response would seem intuitively adaptive, allowing fish to eat more to compensate for a period of food shortage/absence. However, in our interpretation of the postprandial changes in hypothalamic PrRP mRNA expression after a single feeding event in 7-day food deprived fish, we must also take into account changes in hypothalamic PrRP mRNA expression that occur in response to food deprivation itself. That is, goldfish that are food deprived for a period of 7 days exhibit a significant increase in hypothalamic PrRP mRNA expression (Fig. 4). This response appears atypical compared with other appetite-regulating neuropeptides in fish under similar conditions. For example, brain mRNA expression of neuropeptide Y, a potent appetite stimulant in goldfish, increased under conditions of food deprivation (19). In contrast, cocaine-amphetamine-regulated transcript, which has been demonstrated to reduce food intake in goldfish, exhibits a reduction in brain mRNA expression in response to food deprivation (34). However, although this is the first report on piscine PrRP gene expression in response to food deprivation, other studies have reported an elevation in circulating and pituitary PRL levels in fasted fish (37). Taken together, these observations suggest that PrRP may be involved in negatively regulating food intake in fish only in the short term and that increased PrRP mRNA expression after longer periods of food deprivation may be linked to other physiological processes. Peptide factors involved in the regulation of appetite and body weight can have short-term or long-term actions (28). The latter regulate body weight, whereas factors with short-term actions regulate the initiation and termination of feeding events. In mammals, PrRP...
has been suggested to exert its effect on energy homeostasis only in the short to medium term (7). Overall, our feeding studies and observations of PrRP mRNA expression in response to nutritional manipulation in goldfish are in general agreement with these views.

Our second objective was to address a potential role for PrRP in the maintenance of hydromineral balance in goldfish. To initiate this objective, we first established a correlate between PrRP and the activity of lactotrophs in goldfish by examining temporal alterations in pituitary PRL mRNA expression after PrRP administration. Previous studies have demonstrated that a single intraperitoneal injection of PrRP has significant and potent effects on PRL release in fish (18). Furthermore, PrRP administration not only increases PRL release but elevates pituitary PRL mRNA expression (23). In goldfish, a single intraperitoneal injection of PrRP also resulted in a significant increase in pituitary PRL mRNA expression (Fig. 5). These data support the idea that in goldfish, PrRP stimulates the secretion and synthesis of pituitary PRL, as it does in other fish species (18, 23, 27).

In bony fishes, PRL is often referred to as a “freshwater-adapting” hormone. Typically, circulating PRL levels in fish are higher in a hyposmotic environment (e.g., FW) than in a hyperosmotic environment (e.g., seawater). However, the ionic composition of FW can vary considerably under natural conditions, and an ion-poor FW environment will necessitate an increased involvement of endocrine factors such as PRL that regulate hyperosmoregulatory mechanisms. In a relatively ion-poor environment, goldfish serum osmolality decreased, whereas both hypothalamic PrRP mRNA expression and pituitary PRL mRNA expression increased. These observations support our hypothesis that PrRP participates in the regulation of hydromineral balance in goldfish (i.e., specifically hyperosmoregulatory mechanisms) as they are in general accord with the currently accepted role of PRL as an osmoregulatory hormone in fish (for a review, see Ref. 16). Furthermore, they
parallel previous observations that reported higher brain PrRP and pituitary PRL mRNA expression in a euryhaline fish species acclimated to a hypoosmotic environment relative to a hyperosmotic environment (24).

Our final objective was to examine whether the involvement of PrRP in appetite regulation and osmoregulation interacted in a physiologically relevant manner. Initial studies indicated that in goldfish 1) PrRP has anorexigenic properties and 2) PrRP is involved in hyperosmoregulation. These results seem inconsistent because increased PrRP in the CNS of fish acclimated to ion-poor FW may be expected to result in a potentially maladaptive reduction in food intake. This reduction in food intake would restrict the acquisition of dietary minerals as well as dietary energy. To address this paradox, we reexamined the effect of PrRP administration on food intake in fish acclimated to ion-poor FW. In fish acclimated to ion-poor FW, doses of PrRP that significantly reduced food intake in fish held in normal FW conditions did not reduce food intake (Fig. 7). This indicates that the anorexigenic actions of PrRP are diminished under conditions where osmoregulatory homeostasis requires a greater involvement of the PrRP-PRL system in maintaining hydromineral balance. Therefore, it would appear that we can observe a pattern of dual physiological function emerging that, in goldfish, is dependent on systemic demand for overall homeostatic control. Currently, there is no evidence that PrRP responds to hydromineral imbalance in mammals as hypothalamic PrRP and UHR1/GPR10 mRNA did not respond to salt loading in rats (12). Regardless, it seems of more importance to consider that vertebrate appetite, growth, and development is controlled by both internal factors (including the CNS, endocrine and neuroendocrine systems), as well as environmental factors. In goldfish, an environmentally induced chronic elevation of CNS PrRP may have necessitated a reorganization of mechanisms previously sensitive to the mediation of anorectic actions in favor of those that are less sensitive to anorectic actions and instead favor osmoregulatory and/or energy acquisition needs. The exact nature of these mechanisms will require further investigation but could include PrRP receptor redistribu-
ion-poor FW, appear to diminish the appetite-reducing effects of exogenously administered PrRP. These observations provide an impetus for further studies that consider how environmental conditions may alter the appetite-regulating effects of endocrine factors in the fish CNS.

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