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journal or publication title	Zoological Science
volume	25
number	7
page range	739-745
year	2008-07-01
URL	http://hdl.handle.net/2297/14428

doi: 10.2108/zsj.25.739

Prolactin Inhibits Osteoclastic Activity in the Goldfish Scale: A Novel Direct Action of Prolactin in Teleosts

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In teleosts, prolactin is involved in calcium regulation, but its role in scale/bone metabolism is unclear. Using the in-vitro system with goldfish scales developed recently, we explored the effects of teleost prolactin, growth hormone, and somatolactin on osteoclasts and osteoblasts. Addition of prolactin at concentrations of 0.01–100 ng/ml reduced osteoclastic activity, partly via osteoclast apoptosis, after 6–18 h incubation. Conversely, growth hormone and somatolactin at a concentration of 100 ng/ml increased osteoclastic activity after 18 h incubation, indicating the specificity of the inhibitory effect of prolactin on osteoclastic activity. On the other hand, these three hormones promoted osteoblastic activity at concentrations of 10–100 ng/ml. The results from this study are the first demonstration of direct effects of prolactin on scale/bone metabolism and osteoclastic activity in a teleost.

Key words: prolactin, growth hormone, somatolactin, osteoblast, osteoclast, calcium metabolism, scale

INTRODUCTION

The importance of the anterior pituitary hormone prolactin (PRL) in vertebrates is evident from its role in a wide spectrum of functions that include reproduction (or parental behavior), osmoregulation, and immunomodulation (see Bole-Feysot et al., 1998; Sakamoto et al., 2003; Harris et al., 2004). In teleost fishes, PRL also has hypercalcemic effects, mainly by influencing the uptake of calcium from the external environment (Flik et al., 1994; Chakraborti and Mukherjee, 1995; Seale et al., 2003). On the other hand, the role of PRL in calcium turnover in the teleost calcified tissues, bone and scale, remains largely unclear, although PRL influences bone metabolism in mammals by acting directly on osteoblasts (Clément-Lacroix et al., 1999; Coss et al., 2000; Seriwatanachai et al., 2008) and chondrocytes (Zermeño et al., 2006), and through the activation of synovial cell functions (Nagafuchi et al., 1999). In tilapia, bone density was

shown to be increased by in-vivo treatment with ovine PRL (Flik et al., 1986), which binds equally to both growth hormone (GH) receptors and PRL receptors in this species (Prunet and Auperin, 1994).

Teleost scales also contain osteoclasts and osteoblasts (Yamada, 1971; Bereiter-Hahn and Zylberberg, 1993; Yoshikubo et al., 2005; Suzuki et al., 2007), and the scales, rather than the body skeleton, jaws, or otoliths, appear to be an internal calcium reservoir, judging from a ⁴⁵Ca²⁺-labelling study of calcified tissues in goldfish and killifish (Mugiyu and Watabe, 1977). In goldfish scales, the osteoclasts are of the multinucleated, active type that shows tartrate-resistant acid phosphatase (TRAP) staining (Suzuki et al., 2000) and in-situ hybridization with a cathepsin K probe (Azuma et al., 2007). In addition, components of the bone matrix, including type-I collagen (Zylberberg et al., 1992), bone γ -carboxyglutamic acid protein (Nishimoto et al., 1992), osteonectin (Lehane et al., 1999), and hydroxyapatite (Onozato and Watabe, 1979), are present in the scales.

In this context, we explored the direct effects of PRL on osteoclasts and osteoblasts in the scales of mature female goldfish by using a recently developed in-vitro system (Suzuki et al., 2000; Suzuki and Hattori, 2002). In addition,

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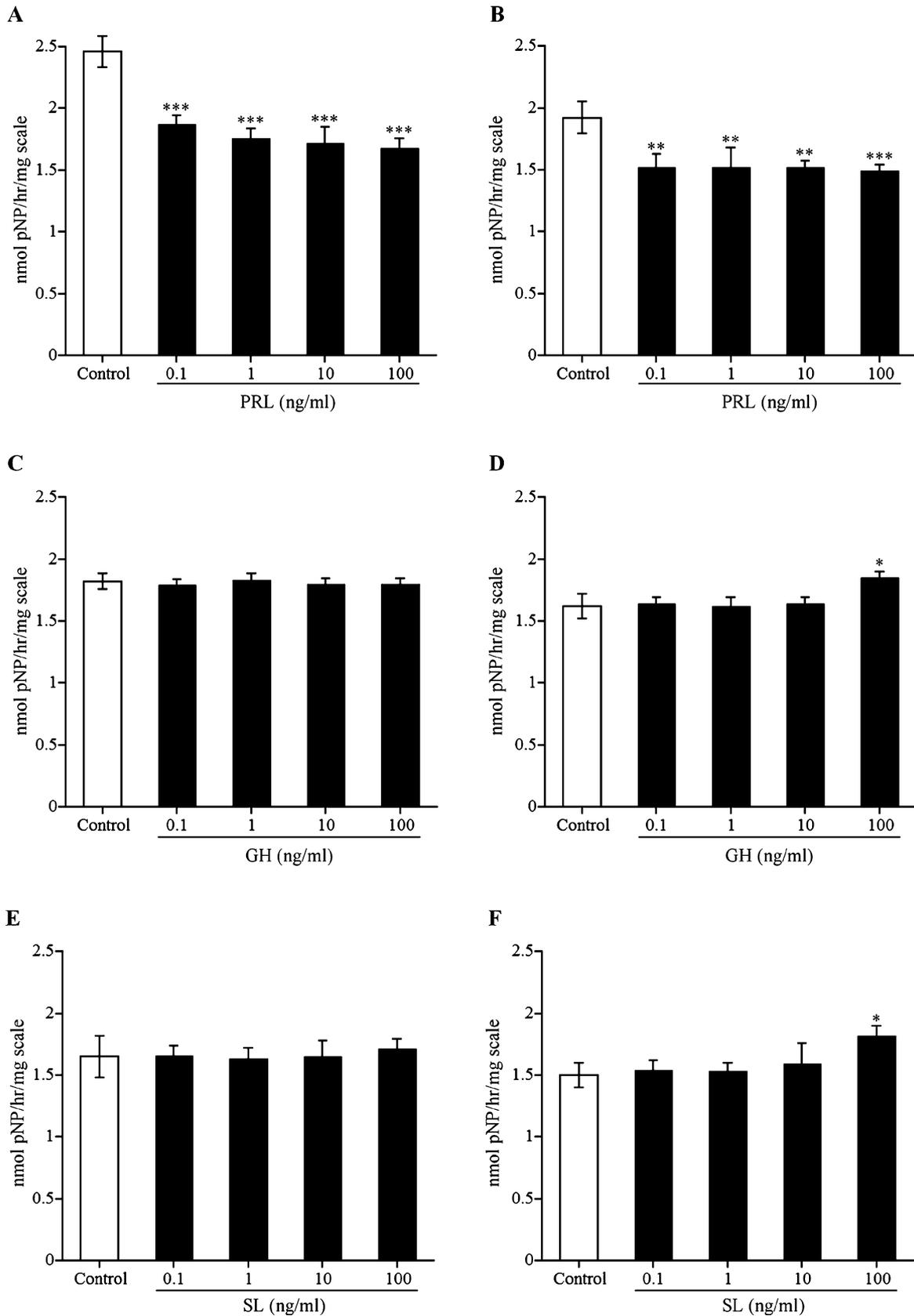


Fig. 1. Effects of PRL, GH, and SL (0.1 to 100 ng/ml) on TRAP activity in cultured goldfish scales after 6 h (A, C, E) and 18 h (B, D, F) of incubation. Values are mean \pm SEM (N=8). *, **, and *** indicate significant differences at $P < 0.05$, 0.01, and 0.001, respectively, from the values in the control scales (ANOVA with Dunnett's post-hoc test).

we compared the actions of PRL with those of GH and somatotactin (SL), other members of a hormone family sharing a common ancestral gene with PRL in teleosts (Rand-Weaver et al., 1993). Here we report that PRL acts specifically on goldfish scales to reduce osteoclastic activity and promote osteoblastic activity. These findings indicate for the first time that PRL plays a direct role in the regulation of scale/bone metabolism in teleosts.

MATERIALS AND METHODS

Animals

A previous study using goldfish (Suzuki et al., 2000; Suzuki and Hattori, 2002) indicated that the sensitivity for calcemic hormones was highest in mature females. Therefore, mature female goldfish (*Carassius auratus*, 30–50 g in weight) were purchased from Higashikawa Fish Farm (Yamatokoriyama, Japan) as previously described (Suzuki et al., 2000). As needed, goldfish were anesthetized in tricaine methanesulfonate. All procedures were approved by the Okayama University Committee in accordance with national guidelines.

Primary scale culture

As previously described (Suzuki and Hattori, 2002), scales were removed from goldfish and incubated at 15°C in Eagle's modified minimum essential medium (MEM; ICN Biomedicals Inc., OH, USA) containing a 1% penicillin-streptomycin mixture (ICN Biomedicals, Inc., OH, USA) with or without the addition of chum salmon PRL, GH, or SL. These salmonid hormones (Kawauchi et al., 1986; Yasuda et al., 1986; Rand-Weaver, 1993) exhibit approximately 70% amino-acid identities to the goldfish counterparts (Chan et al., 1996; Law et al., 1996; Cheng et al., 1997) and high specificities to their respective receptors in fishes (Prunet and Auperin, 1994; Tse et al., 2000; Lee et al., 2001; Fukada et al., 2005). For each comparison between treatment and control groups, scales were collected from a single fish. Scales were fixed for 2 h in cold 10% formalin in 0.05 M cacodylate buffer (pH 7.4), rinsed and kept in 0.05 M cacodylate buffer at 4°C until the analyses.

Assays of osteoclastic and osteoblastic activities

In our system, the activities of both osteoclasts and osteoblasts were detected with TRAP and alkaline phosphatase (ALP) as respective markers (Suzuki and Hattori, 2002), as similarly utilized for determination of the effects of particular hormones on osteoclasts and osteoblasts in mammals (Veas, 1988; Noda et al., 2005). We detected the respective enzyme activity from individual scales by transferring each scale into a well of a 96-well microplate for incubation.

For TRAP activity, scales were incubated at 20°C for 60 min in 200 μ l of 100 mM sodium acetate buffer, pH 5.3, containing 20 mM tartrate and 10 mM para-nitrophenyl-phosphate. For ALP activity, the buffer was 100 mM Tris-HCl, pH 9.5, containing 1 mM MgCl₂ and 0.1 mM ZnCl₂. Color development was quantified by absorption at 405 nm.

Analyses of apoptotic osteoclasts

To examine the possible involvement of apoptosis in the inhibition of osteoclastic activity by PRL (see Figs. 1 and 2), osteoclasts were induced by the autotransplantation of scales in goldfish (our unpublished results). The collected scales were intramuscularly autotransplanted, and the fish were kept in fresh water containing antibiotic (Green F Gold, Sanei Co. Ltd., Tokyo, Japan) for 7 days. Thereafter, the transplanted scales were removed, cut into halves, and cultured as described above with or without salmon PRL (10 ng/ml). After 6 h incubation, the scale halves were fixed as above, and TRAP staining was performed by the methods of Cole and Walters (1987). After TRAP staining, DNA fragmentation associated

with apoptosis was detected by the TUNEL method of Gavrieli et al. (1992) using an In Situ Cell Death Detection Kit (Roche, Tokyo, Japan; Takahashi et al., 2006a, b, 2007). The TRAP-stained samples were washed twice in 100 mM Tris-HCl buffer, pH 7.6, containing 150 mM sodium chloride and 0.1% Tween 20 (TBST), and fixed in methanol at -20°C for \geq 24 h. To rehydrate the samples following methanol fixation, each scale was washed in TBST four times at room temperature for 15 min. After microwave irradiation, the samples were transferred to TUNEL buffer (25 mM Tris-HCl, pH 7.6, containing 200 mM sodium cacodylate, 5 mM cobalt chloride and 0.25% bovine serum albumin), and incubated overnight at 4°C. After washing in TUNEL buffer for 30 min at room temperature, the scales were incubated at 37°C for 4 h with TdT and fluorescein-labeled dUTP. The reaction was terminated by transferring the scales to TBST for 15 min and mounted on a glass slide with a coverslip. The specimens were examined with a fluorescence microscope (EFD2A with a 100-W Hg light source; Nikon, Tokyo, Japan) equipped with a chilled CCD camera (600CL, Pixera Co., Los Gatos, CA, USA). The excitation, dichroic, and emission filters were the EX 420–490, DM 510, and BA 520, respectively. The omission of TdT gave completely negative results. The ratio of TUNEL-positive osteoclasts per total osteoclasts was quantified at 200X magnification by image analysis (Studio 3.0, Pixera Co., Los Gatos, CA, USA). Five to seven osteoclasts were examined for each scale piece (N=11).

RESULTS

Effects of PRL, GH, and SL on osteoclastic activity in scales

Fig. 1 shows the effect of PRL, GH, and SL on scale TRAP activity as an osteoclastic marker in primary culture. PRL at concentrations of 0.1–100 ng/ml reduced TRAP activity by ~20% after 6 h and 18 h incubation. Even at 10 pg/ml, PRL was effective in reducing osteoclastic activity after 6 h (Fig. 2). In contrast, GH and SL increased TRAP activity by ~10% only at a concentration of 100 ng/ml after 18 h incubation; there was no significant effect after 6 h different from that by PRL. Increased concentrations of each

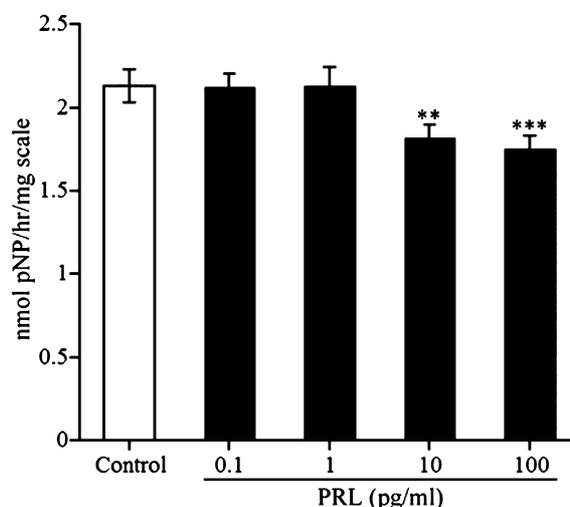


Fig. 2. Effects of PRL (0.1 to 100 pg/ml) on TRAP activity in cultured goldfish scales after 6 h of incubation. Values are mean \pm SEM (N=8). ** and *** indicate statistically significant differences at $P < 0.01$ and 0.001 , respectively, from the values in the control scales (ANOVA with Dunnett's post-hoc test).

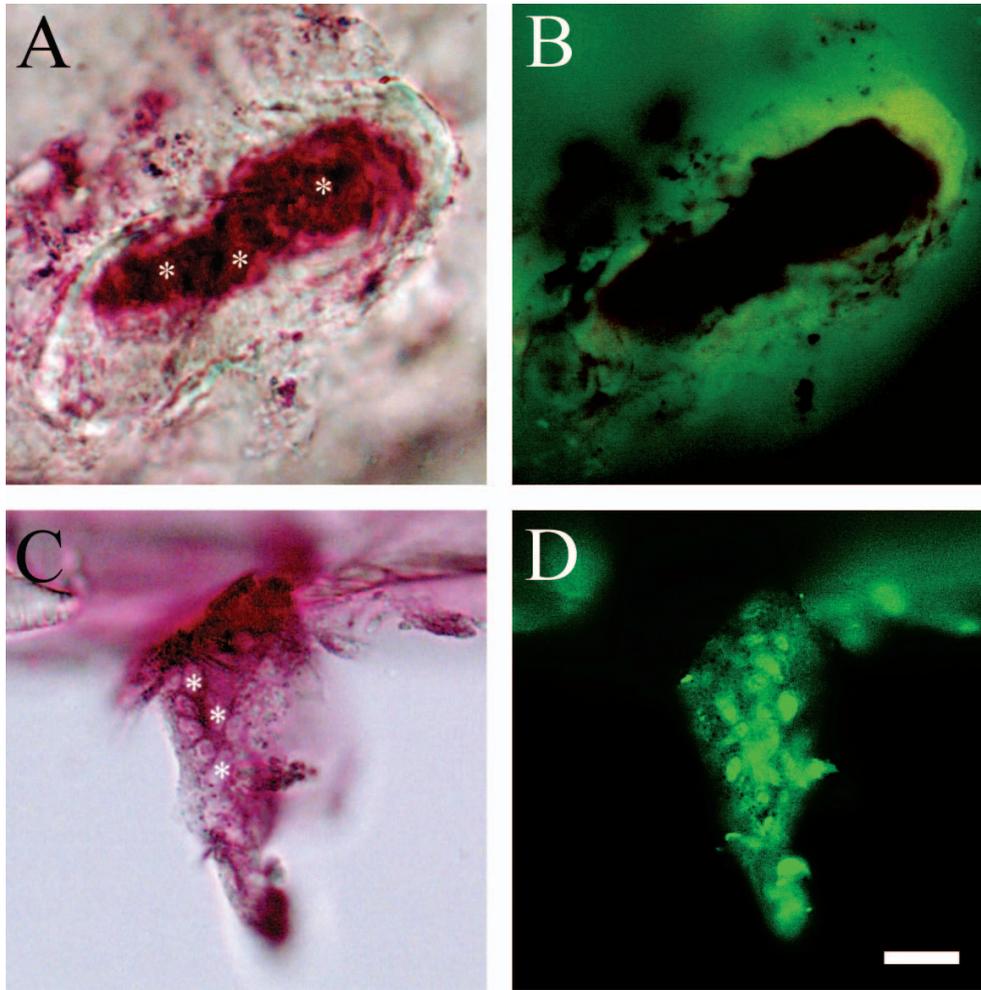


Fig. 3. Stained whole-mounted goldfish scales showing apoptosis labeled by TUNEL (fluorescence; **B, D**) of a TRAP-positive osteoclast (reddish-stained cell; **A, C**). TUNEL-positive multinuclei are evident in the osteoclast adjacent to the surface of a scale treated with PRL (**C, D**), whereas the osteoclast is not labeled by TUNEL in the control scale (**A, B**). Representative results are shown; no significant effect of PRL treatment was detected in the proportion of apoptotic osteoclasts compared with that seen in controls. Asterisks indicate the multinuclei typical of osteoclasts. Scale bar=20 μ m.

hormone resulted in greater effects.

The multinuclei of some TRAP-stained osteoclasts in the scales were labeled with TUNEL to detect DNA breaks. No significant effect was seen on the proportion of these apoptotic osteoclasts by treatment with PRL ($37 \pm 10\%$) compared with that seen in controls ($30 \pm 9\%$; $P=0.9$; $N=11$) (Fig. 3).

Effects of PRL, GH and SL on osteoblastic activity in the scales

Fig. 4 shows the effects of PRL, GH, and SL on ALP activity as an osteoblastic marker. All the hormones promoted the activity by $\sim 30\%$; SL was potent also at a concentration of 10 ng/ml. Increased concentrations of each hormone resulted in greater effects.

DISCUSSION

In the present study, we demonstrated for the first time that PRL inhibits osteoclastic activities. Conversely, GH and SL increased TRAP activities, indicating the specificity of the

suppressive action of PRL in the osteoclasts. Furthermore, this in-vitro effect of PRL on goldfish scales occurred at a very low concentration (10 pg/ml), which was within the range of plasma PRL concentrations (~ 0.1 –10 ng/ml) in teleosts, including the goldfish (Wong et al., 2002). Such a direct and specific effect of PRL has not been demonstrated before in teleosts, although PRL has been recognized to act on various tissues (see Sakamoto et al., 2003). Recently, some effects of PRL on cultured gill epithelia were reported, but the specificities of these effects were unclear (Kelly and Wood, 2003; Zhou et al., 2003, 2004). In-vivo sodium-retention bioassays, involving hypophysectomies and hormonal injections of fishes, have been used to test the bioactivity of PRLs in teleosts (Grau et al., 1984; Hasegawa et al., 1986; Suzuki et al., 1991; Jackson et al., 2000); however, except for transfection studies on PRL receptor cDNAs, our scale TRAP assay is the only in-vitro bioassay specific for teleost PRLs, one that can be completed in several hours.

The inhibitory effect of PRL on osteoclastic activity seems to be mediated in part through osteoclast apoptosis,

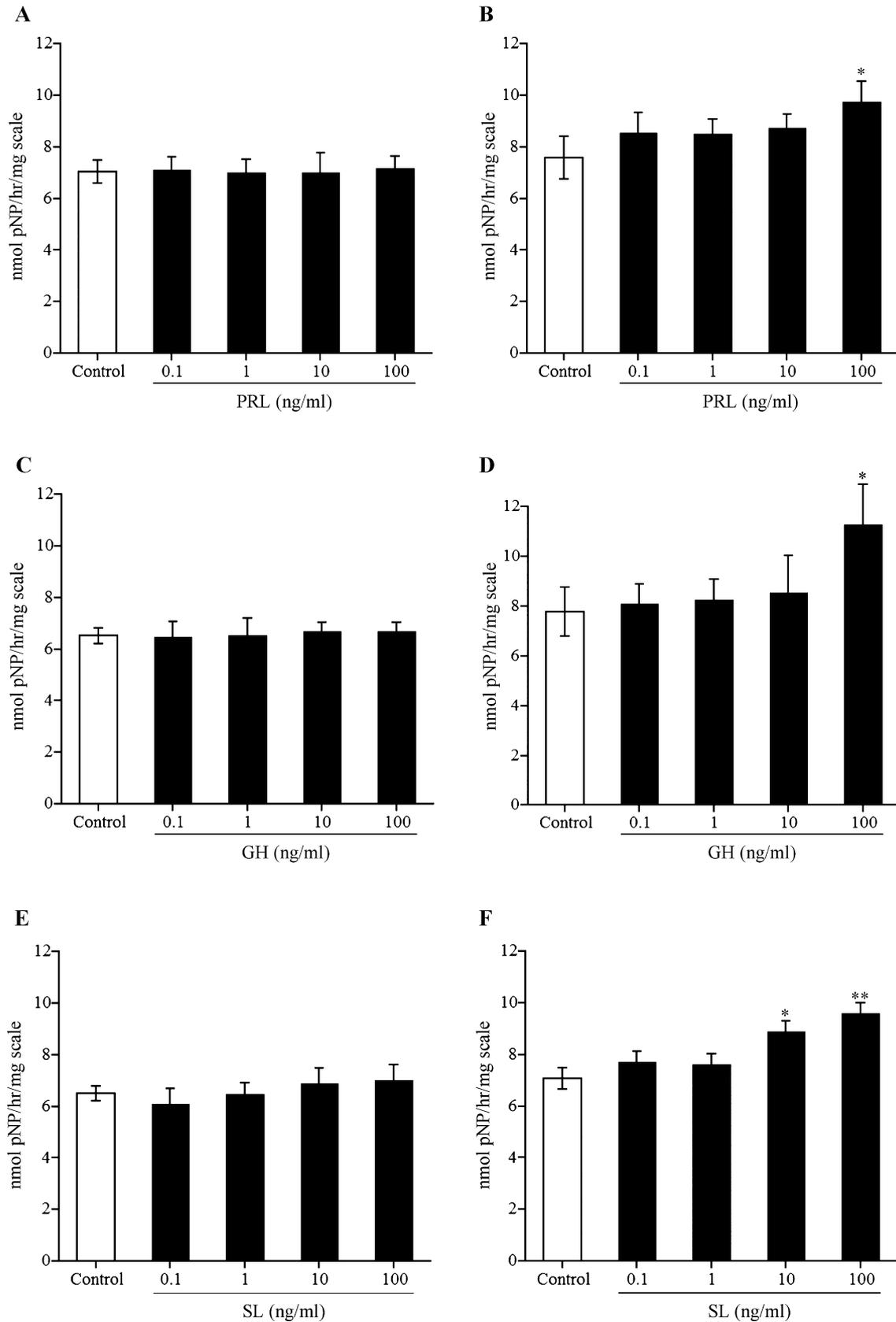


Fig. 4. Effects of PRL, GH, and SL (0.1 to 100 ng/ml) on ALP activity in cultured goldfish scales after 6 h (A, C, E) and 18 h (B, D, F) of incubation. Values are mean \pm SEM (N=8). * and ** indicate statistically significant differences at $P < 0.05$ and 0.01 , respectively, from the values in the control scales (ANOVA with Dunnett's post-hoc test).

since we observed apoptotic nuclei in TRAP-stained osteoclasts after PRL treatment. However, no statistically significant induction was detected, possibly due to the loss of TRAP activity at advanced apoptotic stages. Although further morphological characterization by transmission electron microscopy appears to be necessary, PRL was also shown to stimulate apoptosis in newt spermatogonia and rat luteal tissues (Kiya et al., 1998; Abe, 2004). Indeed, a large number of the effects of PRL reported throughout the vertebrates are directly associated with apoptosis and/or cell proliferation (Sakamoto and McCormick, 2006). The mechanisms by which PRL induces apoptosis are presently unclear and offer fertile ground for further investigation.

In the case of osteoblastic activities, significant stimulating effects of PRL, GH, and SL were observed at a concentration of 100 ng/ml, whereas SL increased the activities also at 10 ng/ml. GH can bind the receptor for SL, which plays a role in calcium metabolism (Kaneko, 1996), albeit with an 8-fold lower affinity than SL (Fukada et al., 2005). The osteoblastic activities of GH (and PRL) might be mediated through the SL receptors. Altogether, these inductions, as well as those of ALP, by GH and SL might be non-specific/general actions of these cytokine hormones, whereas the inhibition of TRAP activity by PRL should be unique.

Our in-vitro study has demonstrated the first direct effects of PRL on scale/bone metabolism in a mature female fish. The contribution of these relatively modest changes in scale/bone metabolism to plasma calcium homeostasis appears to be counterintuitive and minor, since PRL is known to induce "hyper" calcemia in teleosts in vivo, mainly via the gill, even in fresh water, where calcium availability is limited (Wendelaar Bonga, 1997). On the other hand, as we observed for calcitonin (Suzuki et al., 2000), PRL may inhibit the excess degradation of bone tissue by osteoclasts in female goldfish, since plasma PRL levels usually increase during the reproductive period (see Sakamoto et al., 2003). During the reproductive period, estrogen stimulates bone degradation for the synthesis of vitellogenin, a Ca-binding protein (Suzuki and Hattori, 2002; Suzuki et al., 2004). Thus, PRL may act on calcium deposition into scales/bone independently of calcium homeostasis in teleosts.

ACKNOWLEDGMENTS

This study was supported in part by grants to T. S. (Grants-in-Aid for Scientific Research (C) Nos. 17570049 and 19570057 from JSPS) and to N. S. (Grant-in-Aid for Scientific Research (C) No. 18500375 from JSPS; Ground-based Research Announcement for Space Utilization by the Japan Space Forum). H. T. was supported by research fellowships from the Japan Society for the Promotion of Science for young scientists (No. 192156). We thank Dr. Jason P. Breves for critical reading of the manuscript.

REFERENCES

Abe S (2004) Hormonal control of meiosis initiation in the testis from Japanese newt, *Cynops pyrrhogaster*. *Zoolog Sci* 21: 691–704
 Azuma K, Kobayashi M, Nakamura M, Suzuki N, Yashima S, Iwamuro S, Ikegame M, Yamamoto T, Hattori A (2007) Two osteoclastic markers expressed in multinucleate osteoclasts of goldfish scales. *Biochem Biophys Res Commun* 362: 594–600
 Bereiter-Hahn J, Zylberberg L (1993) Regeneration of teleost fish scale. *Comp Biochem Physiol* 105: 625–641

Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA (1998) Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev* 19: 225–268
 Chakraborti P, Mukherjee D (1995) Effects of prolactin and fish pituitary extract on plasma calcium levels in common carp, *Cyprinus carpio*. *Gen Comp Endocrinol* 97: 320–326
 Chan YH, Cheng KW, Yu KL, Chan KM (1996) Identification of two prolactin cDNA sequences from a goldfish pituitary cDNA library. *Biochim Biophys Acta* 1307: 8–12
 Cheng KW, Chan YH, Chen YD, Yu KL, Chan KM (1997) Sequence of a cDNA clone encoding a novel somatolactin in goldfish, *Carassius auratus*. *Biochem Biophys Res Commun* 232: 282–287
 Clément-Lacroix P, et al. (1999) Osteoblasts are a new target for prolactin: analysis of bone formation in prolactin receptor knockout mice. *Endocrinology* 140: 96–105
 Cole AA, Walters LM (1987) Tartrate-resistant acid phosphatase in bone and cartilage following decalcification and cold-embedding in plastic. *J Histochem Cytochem* 35: 203–206
 Coss D, Yang L, Kuo CB, Xu X, Luben RA, Walker AM (2000) Effects of prolactin on osteoblast alkaline phosphatase and bone formation in the developing rat. *Am J Physiol Endocrinol Metab* 279: E1216–E1225
 Flik G, Fenwick JC, Kolar Z, Mayer-Gostan N, Wendelaar Bonga SE (1986) Effects of ovine prolactin on calcium uptake and distribution in *Oreochromis mossambicus*. *Am J Physiol* 250: R161–R166
 Flik G, Rentier-Delrue F, Wendelaar Bonga SE (1994) Calcitropic effects of recombinant prolactins in *Oreochromis mossambicus*. *Am J Physiol Regul Integr Comp Physiol* 266: R1302–R1308
 Fukada H, Ozaki Y, Pierce AL, Adachi S, Yamauchi K, Hara A, Swanson P, Dickhoff WW (2005) Identification of the salmon somatolactin receptor, a new member of the cytokine receptor family. *Endocrinology* 146: 2354–2361
 Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 119: 493–501
 Grau EG, Prunet P, Gross T, Nishioka RS, Bern HA (1984) Bioassay for salmon prolactin using hypophysectomized *Fundulus heteroclitus*. *Gen Comp Endocrinol* 53: 78–85
 Harris J, Stanford PM, Oakes SR, Ormandy CJ (2004) Prolactin and the prolactin receptor: new targets of an old hormone. *Ann Med* 36: 414–425
 Hasegawa S, Hirano T, Kawauchi H (1986) Sodium-retaining activity of chum salmon prolactin in some euryhaline teleosts. *Gen Comp Endocrinol* 63: 309–317
 Jackson LF, Swanson P, Duan C, Fruchtman S, Sullivan CV (2000) Purification, characterization, and bioassay of prolactin and growth hormone from temperate basses, genus *Morone*. *Gen Comp Endocrinol* 117: 138–150
 Kaneko T (1996) Cell biology of somatolactin. *Int Rev Cytol* 169: 1–24
 Kawauchi H, Moriyama S, Yasuda A, Yamaguchi K, Shirahata K, Kubota J, Hirano T (1986) Isolation and characterization of chum salmon growth hormone. *Arch Biochem Biophys* 244: 542–552
 Kelly SP, Wood CM (2003) Dilute culture media as an environmental or physiological simulant in cultured gill epithelia from freshwater rainbow trout. *In Vitro Cell Dev Biol Anim* 39: 21–28
 Kiya T, Endo T, Goto T, Yamamoto H, Ito E, Kudo R, Behrman HR (1998) Apoptosis and PCNA expression induced by prolactin in structural involution of the rat corpus luteum. *J Endocrinol Invest* 21: 276–283
 Law MS, Cheng KW, Fung TK, Chan YH, Yu KL, Chan KM (1996) Isolation and characterization of two distinct growth hormone cDNAs from the goldfish, *Carassius auratus*. *Arch Biochem Bio-*

- phys 330: 19–23
- Lee LT, Nong G, Chan YH, Tse DL, Cheng CH (2001) Molecular cloning of a teleost growth hormone receptor and its functional interaction with human growth hormone. *Gene* 270: 121–129
- Lehane DB, Mckie N, Russell RGG, Henderson IW (1999) Cloning of a fragment of the osteonectin gene from goldfish, *Carassius auratus*: its expression and potential regulation by estrogen. *Gen Comp Endocrinol* 114: 80–87
- Mugiya Y, Watabe N (1977) Studies on fish scale formation and resorption II: effect of estradiol on calcium homeostasis and skeletal tissue resorption in the goldfish, *Carassius auratus*, and the killifish, *Fundulus heteroclitus*. *Comp Biochem Physiol* 57A: 197–202
- Nagafuchi H, Suzuki N, Kaneko A, Asai T, Sakane T (1999) Prolactin locally produced by synovium infiltrating T lymphocytes induces excessive synovial cell functions in patients with rheumatoid arthritis. *J Rheumatol* 26: 1890–1900
- Nishimoto SK, Araki N, Robinson FD, Waite JH (1992) Discovery of bone γ -carboxyglutamic acid protein in mineralized scales. *J Biol Chem* 267: 11600–11605
- Noda T, Tokuda H, Yoshida M, Yasuda E, Hanai Y, Takai S, Kozawa O (2005) Possible involvement of phosphatidylinositol 3-kinase/Akt pathway in insulin-like growth factor-I-induced alkaline phosphatase activity in osteoblasts. *Horm Metab Res* 37: 270–274
- Onozato H, Watabe N (1979) Studies on fish scale formation and resorption. III. Fine structure and calcification of the fibrillary plates of the scales in *Carassius auratus* (Cypriniformes: Cyprinidae). *Cell Tissue Res* 201: 409–422
- Prunet P, Auperin B (1994) Prolactin receptor. In "Fish Physiology, Vol 13" Ed by NM Sherwood, CL Hew, Academic Press, San Diego, pp 367–391
- Rand-Weaver M, Kawauchi H, Ono M (1993) Evolution of the structure of the growth hormone and prolactin family. In "The Endocrinology of Growth, Development, and Metabolism in Vertebrates" Ed by MP Schreibman, CG Scanes, PKT Pang, Academic Press, New York, pp 13–42
- Sakamoto T, McCormick SD (2006) Prolactin and growth hormone in fish osmoregulation. *Gen Comp Endocrinol* 147: 24–30
- Sakamoto T, Fujimoto M, Ando M (2003) Fishy tales of prolactin-releasing peptide. *Int Rev Cytol* 225: 91–130
- Seale AP, Richman NH 3rd, Hirano T, Cooke I, Grau EG (2003) Cell volume increase and extracellular Ca^{2+} are needed for hyposmotically induced prolactin release in tilapia. *Am J Physiol Cell Physiol* 284: C1280–C1289
- Seriwatanachai D, Thongchote K, Charoenphandhu N, Pandaranandaka J, Tudpor K, Teerapornpantakit J, Suthiphongchai T, Krishnamra N (2008) Prolactin directly enhances bone turnover by raising osteoblast-expressed receptor activator of nuclear factor kappaB ligand/osteoprotegerin ratio. *Bone* 42: 535–546
- Suzuki N, Hattori A (2002) Melatonin suppresses osteoclastic and osteoblastic activities in the scales of goldfish. *J Pineal Res* 33: 253–258
- Suzuki N, Suzuki T, Kurokawa T (2000) Suppression of osteoclastic activities by calcitonin in the scales of goldfish (freshwater teleost) and nibbler fish (seawater teleost). *Peptides* 21: 115–124
- Suzuki N, Yamamoto K, Sasayama Y, Suzuki T, Kurokawa T, Kambe-gawa A, Srivastav AK, Hayashi S, Kikuyama S (2004) Possible direct induction by estrogen of calcitonin secretion from ultimobranchial cells in the goldfish. *Gen Comp Endocrinol* 138: 121–127
- Suzuki N, Kitamura K, Nemoto T, Shimizu N, Wada S, Kondo T, Tabata MJ, Sodeyama F, Ijiri K, Hattori A (2007) Effect of vibration on osteoblastic and osteoclastic activities: analysis of bone metabolism using goldfish scale as a model for bone. *Adv Space Res* 40: 1711–1721
- Suzuki R, Yasuda A, Kondo J, Kawauchi H, Hirano T (1991) Isolation and characterization of Japanese eel prolactins. *Gen Comp Endocrinol* 81: 391–402
- Takahashi H, Sakamoto T, Narita K (2006a) Cell proliferation and apoptosis in the anterior intestine of an amphibious, euryhaline mudskipper (*Periophthalmus modestus*). *J Comp Physiol B* 176: 463–468
- Takahashi H, Takahashi A, Sakamoto T (2006b) *In vivo* effects of thyroid hormone, corticosteroids and prolactin on cell proliferation and apoptosis in the anterior intestine of the euryhaline mudskipper (*Periophthalmus modestus*). *Life Sci* 79: 1873–1880
- Takahashi H, Prunet P, Kitahashi T, Kajimura S, Hirano T, Grau EG, Sakamoto T (2007) Prolactin receptor and proliferating/apoptotic cells in esophagus of the Mozambique tilapia (*Oreochromis mossambicus*) in fresh water and in seawater. *Gen Comp Endocrinol* 152: 326–331
- Tse DL, Chow BK, Chan CB, Lee LT, Cheng CH (2000) Molecular cloning and expression studies of a prolactin receptor in goldfish (*Carassius auratus*). *Life Sci* 66: 593–605
- Vaes G (1988) Cellular biology and biochemical mechanism of bone resorption. *Clin Orthop* 231: 239–271
- Wendelaar Bonga SE (1997) The stress response in fish. *Physiol Rev* 77: 591–625
- Wong AO, Cheung HY, Lee EK, Chan KM, Cheng CH (2002) Production of recombinant goldfish prolactin and its applications in radioreceptor binding assay and radioimmunoassay. *Gen Comp Endocrinol* 126: 75–89
- Yamada J (1971) A fine structural aspect of the development of scales in the chum salmon fry. *Bull Jpn Soc Sci Fish* 37: 18–29
- Yasuda A, Itoh H, Kawauchi H (1986) Primary structure of chum salmon prolactins: occurrence of highly conserved regions. *Arch Biochem Biophys* 244: 528–541
- Yoshikubo H, Suzuki N, Takemura K, Hoso M, Yashima S, Iwamura S, Takagi Y, Tabata MJ, Hattori A (2005) Osteoblastic activity and estrogenic response in the regenerating scale of goldfish, a good model of osteogenesis. *Life Sci* 76: 2699–2709
- Zermeño C, Guzmán-Morales J, Macotela Y, Nava G, López-Barrera F, Kouri JB, Lavalle C, de la Escalera GM, Clapp C (2006) Prolactin inhibits the apoptosis of chondrocytes induced by serum starvation. *J Endocrinol* 189: R1–R8
- Zhou B, Kelly SP, Ianowski JP, Wood CM (2003) Effects of cortisol and prolactin on Na^+ and Cl^- transport in cultured branchial epithelia from FW rainbow trout. *Am J Physiol Regul Integr Comp Physiol* 285: R1305–R1316
- Zhou B, Kelly SP, Wood CM (2004) Response of developing cultured freshwater gill epithelia to gradual apical media dilution and hormone supplementation. *J Exp Zool A* 301: 867–881
- Zylberberg L, Bonaventure J, Cohen-Solal L, Hartmann DJ, Bereiterhahn J (1992) Organization and characterization of fibrillar collagens in fish scales *in situ* and *in vitro*. *J Cell Sci* 103: 273–285

(Received April 4, 2008 / Accepted April 27, 2008)