Increased prostaglandin e2 has a positive correlation with plasma calcium during goldfish reproduction

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Increased Prostaglandin E_2 Has a Positive Correlation with Plasma Calcium during Goldfish Reproduction

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We recently demonstrated that prostaglandin E_2 (PG_2) increases osteoclastic activity and induces bone resorption in both *in vitro* and *in vivo* experiments using goldfish. In the fish reproductive period, the plasma calcium (Ca) level in female teleosts increases remarkably to make vitellogenin, which is a major component of egg protein and a Ca–binding protein. In this period, however, there is no reported relationship between PGE_2 and Ca metabolism in fish. To clarify the Ca metabolism in fish reproduction, we examined plasma PGE_2 and Ca levels and measured tartrate–resistant acid phosphatase (TRAP) activities as an indicator of osteoclastic activity in goldfish. Plasma PGE_2 levels in the reproductive stage significantly increased as compared with those in non–reproductive stages. Also, both plasma Ca and TRAP increased in the reproductive stage. Significant positive correlations were recognized between plasma Ca and the gonad somatic index (r=0.81, p<0.001), plasma Ca and plasma PGE_2 levels (r=0.635, p<0.05), and plasma Ca and plasma TRAP activities (r=0.584, p<0.05) from the analysis using samples of both reproductive and non–reproductive stages. Taking these data into consideration, we suggested that PGE_2 acts on osteoclasts and increases plasma Ca as a result of osteoclastic bone resorption, and we concluded that PGE_2 is an important hormone in Ca metabolism during fish reproduction.

Key words: goldfish, plasma Ca, Prostaglandin E_2, reproduction, TRAP

INTRODUCTION

Prostaglandin E_2 (PGE_2) functions to bone metabolism and is an important hormone in bone and promoter of osteoclastogenesis (Kaji *et al*., 1996; Gardner, 2007; Kaneko *et al*., 2007). The bone–resorbing activity of mature osteoclasts in osteoblast–containing mouse bone cell cultures was increased by PGE_2, although it did not affect osteoclast–like cell formation in osteoblast–free mouse spleen cell cultures (Kaji *et al*., 1996). Therefore, we focused on fish scales that coexist with both osteoclasts and osteoblasts (Bereiter–Hahn and Zylberberg, 1993; Suzuki *et al*., 2000; Yoshikubo *et al*., 2005; Suzuki *et al*., 2007; Suzuki *et al*., 2008; Suzuki *et al*., 2011; Yano *et al*., 2013). Using the goldfish scale *in vitro* assay system, we recently demonstrated that PGE_2 acts on osteoblasts and then increases the osteoclastic activity in the scales of goldfish as it does in the bone of mammals (Omori *et al*., 2012). In addition, the intraperitoneal injection of PGE_2 into goldfish induced hypercalcemia (Omori *et al*., 2012).

In the reproductive period, the plasma calcium (Ca) level in female teleosts increases remarkably (Watts *et al*., 1975; Yamauchi *et al*., 1978; Norberg *et al*., 1989; Suzuki *et al*., 2004). This Ca is bound to vitellogenin, which is a major component of egg protein and the calcium–binding protein (Tinsley, 1985; Kwon *et al*., 1993). In this period, PGE_2 synthesized in the ovaries functions to cause ovulation in fish (for a review, see Takahashi *et al*., 2013). As van Anholt *et al*. (2003) reported that PGE_2 in the blood may serve some physiological roles in fish, PGE_2 secreted from the ovaries might influence plasma Ca in fish. However, there has been no reported relationship between PGE_2 and Ca metabolism during the fish reproductive period.

To clarify the Ca metabolism in fish reproduction, we examined plasma PGE_2 and Ca levels and measured tartrate–resistant acid phosphatase (TRAP) activities as an indicator of osteoclastic activity in goldfish.

We concluded that PGE_2 is an important hormone in Ca metabolism during fish reproduction.

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MATERIALS AND METHODS

Animals

Female goldfish (n=14, 49.16±3.77 g) were purchased from a commercial source (Higashikawa Fish Farm, Yamatokoriyama, Japan) and used in the present study. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Kanazawa University.

Measurement of plasma PGE_2, Ca, and TRAP levels of female goldfish in reproductive and non–reproductive stages

Goldfish in both the reproductive (March) (n=8) and non–reproductive (August) (n=6) stages were anesthetized with ethyl 3–aminobenzoate methanesulfonic acid salt (Sigma–Aldrich Inc., MO, USA). After weighing, the gonad somatic index (GSI) (%) was calculated. A blood sample was then collected from the dorsal aorta using a heparinized syringe. After centrifugation at 15,000 rpm for 3 mm, the plasma was immediately frozen and kept at –80°C until use. The plasma total Ca (mg/100 ml) and PGE_2 (pg/ml) levels were determined using specific assay kits (Ca: Calcium E test; PGE_2: PGE_2–ELISA kit, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

The plasma TRAP level was measured using 2 μl of plasma from each goldfish. TRAP activities were measured using an acid tartrate buffer (a 20 mM tartrate in a 0.1 M sodium acetate buffer (pH 5.3)). An aliquot of 100 μl of 20 mM para–nitrophenyl phosphate in an acid tartrate buffer was added to each well in a 96–well microplate. This plate was then incubated at 20°C for 30 min while being shaken. After incubation, the reaction was stopped by adding 50 μl of a 3 N NaOH–20 mM EDTA solution, and the absorbance was then measured at 405 nm. The absorbance was converted into the amount of produced para–nitrophenol (pNP) using a standard curve for pNP.

Statistical analysis

All results are expressed as the means ± SE. Statistical significance was assessed by Student's t–test. Simple correlation coefficients were calculated to assess the relationship among GSI values, plasma PGE_2 levels, plasma Ca levels, and plasma TRAP activities. The statistical significance of the correlation was evaluated using the method of Snedecor and Cochran (1980). In all cases, the selected significance level was $p<0.05$.

RESULTS

Changes in GSI, plasma PGE_2 levels, Ca levels, and TRAP activities of female goldfish in reproductive and non–reproductive stages

There was a significant difference in the values of GSI between goldfish in March and August (Fig. 1). In addition, the plasma PGE_2 levels, Ca levels, and TRAP activities of female goldfish in March were significantly higher than those in August (Figs. 2, 3, and 4).

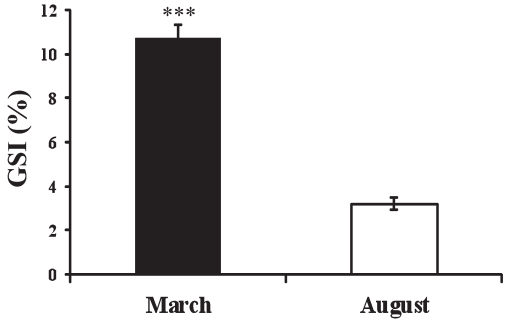

Fig. 1. GSI values of female goldfish in the reproductive (March) and non–reproductive (August) stages. *** indicates a statistically significant difference at $p<0.001$ in the values of the reproductive and non–reproductive stages.

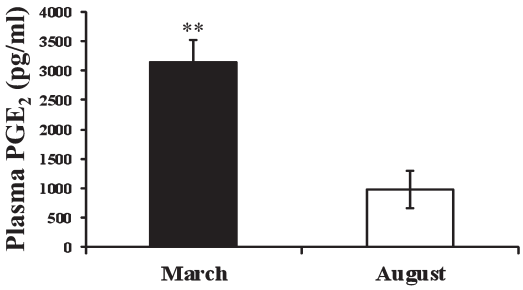

Fig. 2. Plasma PGE_2 values of female goldfish in the reproductive (March) and non–reproductive (August) stages. ** indicates a statistically significant difference at $p<0.01$ in the values of the reproductive and non–reproductive stages.

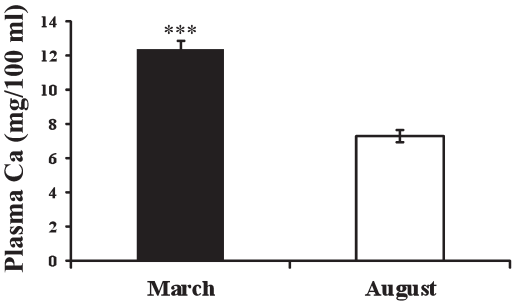

Fig. 3. Plasma Ca values of female goldfish in the reproductive (March) and non–reproductive (August) stages. *** indicates a statistically significant difference at $p<0.001$ in the values of the reproductive and non–reproductive stages.

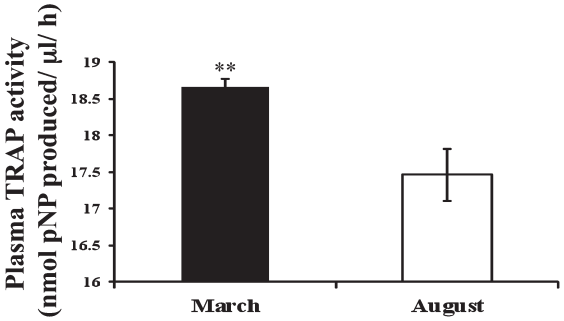

Fig. 4. Plasma TRAP activity (nmol pNP produced/μl/h) values of female goldfish in the reproductive (March) and non–reproductive (August) stages. ** indicates a statistically significant difference at $p<0.01$ in the values of the reproductive and non–reproductive stages.

Correlation among GSI, plasma PGE_2 levels, Ca levels, and TRAP activities

The results of correlation among GSI, plasma PGE_2 levels, Ca levels, and TRAP activities are indicated in Table 1. Significant positive correlations were recognized between GSI and plasma PGE_2 (r=0.790, p<0.001), GSI and plasma Ca (r=0.813, p<0.001), and GSI and plasma TRAP (r=0.631, p<0.05) from the analysis using samples of both reproductive and non–reproductive stages.

Table 1. Correlation among GSI, plasma Ca levels, PGE_2 levels, and TRAP activities (n=14).

	r values	p values
GSI vs Plasma PGE_2	r=0.790	p=0.0007
GSI vs Plasma Ca	r=0.813	p=0.0004
GSI vs Plasma TRAP	r=0.631	p=0.015
Plasma Ca vs Plasma PG_{E2}	r=0.635	p=0.014
Plasma Ca vs Plasma TRAP	r=0.584	p=0.028
Plasma PGE_2 vs Plasma TRAP	r=0.514	p=0.058

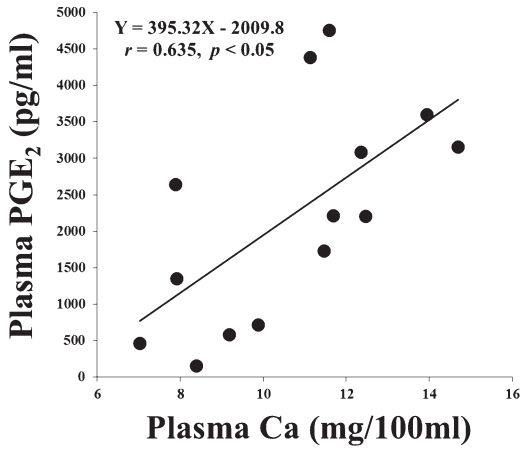

Fig. 5. Relationship between PGE_2 (pg/ml) and Ca (mg/100 ml) in the plasma of goldfish in the reproductive (March) and non–reproductive (August) stages.

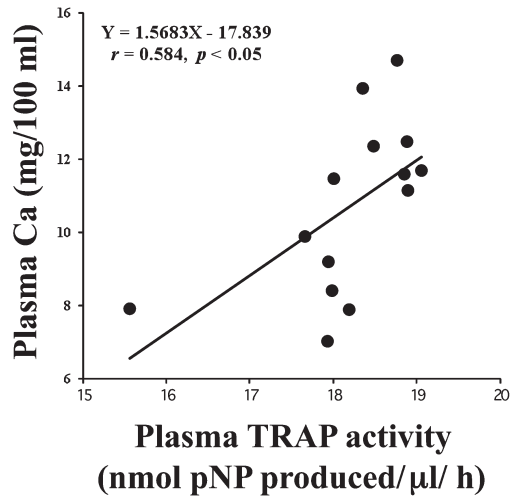

Fig. 6. Relationship between Ca (mg/100 ml) and TRAP activities (nmol pNP produced/μl/h) in the plasma of goldfish in the reproductive (March) and non–reproductive (August) stages.

As a result of having paid attention to the relations with plasma Ca, we discovered a significant positive relationship between plasma Ca and PGE_2 (r=0.635, p<0.05) (Fig. 5), and between plasma Ca and TRAP (r=0.584, p<0.05) (Fig. 6).

As PGE_2 levels increased, plasma TRAP activities tended to rise (r=0.514, p=0.058).

DISCUSSION

The present study is the first to demonstrate that PGE_2 is related to Ca metabolism in fish reproduction. Corresponding to increased GSI, plasma PGE_2 levels, Ca levels, and TRAP activities rose. In addition, significant correlations between plasma Ca and PGE_2 and between Ca and TRAP were observed. Because TRAP is known as an osteoclast–specific marker (for a review, see Vaes, 1988), the increased PGE_2 in the March fish activated osteoclasts and promoted osteoclastic bone resorption. As described in the introduction, we recently demonstrated that PGE_2 acts on osteoblasts and increases the osteoclastic activity in the scales of goldfish as it does in the bone of mammals (Omori *et al.*, 2012). In an *in vivo* experiment, furthermore, hypercalcemia was induced as a result of osteoclastic bone resorption after an intraperitoneal injection of PGE_2 into goldfish (Omori *et al.*, 2012). Taking these results into consideration together with the present study, we have concluded that PGE_2 acts as a calcemic hormone in fish reproduction.

In the present study, the highest correlation between GSI and plasma Ca was recognized. We think that several hormones, with the exception of PGE_2, are involved in Ca metabolism during fish reproduction. The candidate for this hypercalcemic hormone is estrogen. In female teleosts, estrogen enhances the synthesis of vitellogenin, which is a major component of egg protein and a Ca–binding protein (Tinsley, 1985; Kwon *et al.*, 1993). At the same time, estrogen promotes Ca resorption from the scales by activating osteoclasts (Persson *et al.*, 1995; Suzuki *et al.*, 2000; Suzuki and Hattori, 2003; Suzuki *et al.*, 2009). Consequently, plasma vitellogenin and Ca levels increase corresponding to the increase in estrogen level (Norberg *et al.*, 1989). PGE_2 is closely related to ovulation (late stage of fish reproduction) (for a review, see Takahashi *et al.*, 2013), suggesting that in the early stage of fish reproduction, estrogen acts as a hypercalcemic hormone, and then PGE_2 plays roles in both ovulation and Ca metabolism.

On the other hand, we previously demonstrated that a hypocalcemic hormone, calcitonin, acts on scales and inhibits osteoclastic activity using an *in vitro* scale assay system with goldfish (Suzuki *et al.*, 2000). As estrogen activates osteoclasts in some teleosts in both *in vivo* and *in vitro* experiments (Persson *et al.*, 1995; Suzuki *et al.*, 2000; Suzuki and Hattori, 2002; Suzuki and Hattori, 2003; Suzuki *et al.*, 2009), a counteraction may

exist between calcitonin and estrogen in osteoclasts of the scale. Using the *in vitro* scale assay system, the increased osteoclastic activity with estrogen was actually suppressed by calcitonin in goldfish (Suzuki *et al*., 2000). Furthermore, our previous study demonstrated the interaction between calcitonin and estrogen. In the ultimobranchial gland, which is the secretion organ of calcitonin, estrogen receptors were detected by estrogen–specific binding assay and immunohistochemical analysis in goldfish (Suzuki *et al*., 2004). Also, three types of estrogen receptors were detected in the ultimobranchial gland of goldfish (Suzuki *et al*., 2004). Moreover, just after injecting estrogen into goldfish, plasma calcitonin level increased before the rise of plasma Ca (Suzuki *et al*., 2004). Considering from our present data, we strongly suggested that PGE_2 affects other calcemic hormones in fish reproduction. Thus, in the future, we will examine the interaction among calcemic hormones, such as PGE_2, calcitonin, and estrogen, and elucidate the mechanism of teleost bone metabolism during the reproductive stages.

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