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Dynamics of Plasma Ca and Calcitonin Levels in Stonefish (*Inimicus japonicus*) Administered a High-Ca Solution into the Stomach

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ABSTRACT—In stonefish, changes in plasma total Ca and calcitonin levels were examined after administration of a high-Ca solution into the stomach. Blood was taken successively at 0, 1, 3, 9, 33, and 81 hr from a fine tube cannulated into the aortic bulb. Plasma Ca levels increased acutely at 1 hr and attained the peak after 3–9 hr of the administration. Although plasma calcitonin levels did not exhibit conspicuous changes for 1–3 hr, they began to rise significantly at 33 hr. The plasma Ca level began to decline significantly at 33 hr, although the level was still significantly higher than the initial level. At 33 hr, however, the plasma calcitonin level still continued to increase. At 81 hr, the plasma Ca level had returned to the initial level. At that time, the plasma calcitonin level was also significantly lower than that at 33 hr. These results suggest that, in stonefish, the ultimobranchial gland has the ability to respond physiologically to rises in plasma Ca levels, to secrete calcitonin, and to cease the secretion when the plasma Ca levels return to the initial level.

Key words: plasma calcitonin levels, plasma Ca levels, dynamics, stonefish, high Ca treatment

INTRODUCTION

Stonefish (*Inimiucus japonicus*), which inhabits only seawater, is a typical ichthyophagous species that swallows baits into the stomach. In the processes of digestion, therefore, the Ca levels in the stomach must transiently increase, which may affect the plasma Ca levels.

We have demonstrated so far that calcitonin is a regulating hormone for Ca absorbed from the digestive tract into plasma and that it functions to suppress the excessive increases in plasma Ca levels. In freshwater eels, in the fed-group, in which plasma Ca levels were high, plasma calcitonin levels were also higher than those in the starved-group (Sasayama *et al*., 1996). In goldfish administered a high-Ca solution into the stomach, hypercalcemia occurred. Then, plasma calcitonin levels tended to be higher compared to those in the control (Sasayama *et al*., 1996), suggesting that calcitonin is secreted against hypercalcemia. In a natural environment, however, there must be no goldfish, eating the bait which acutely increases plasma Ca levels. Also in freshwater eels, when hypercalcemia was caused by administration of a high-Ca solution into the stomach, the plasma calcitonin levels were increased after the occurrence of hypercalcemia (Suzuki *et al*., 1999). This result also suggests that calcitonin is secreted when hypercalcemia occurs. In this experiment, however, blood samples were taken by syringes from the tail, where the caudal artery and the caudal vein run. This implies that it is not certain from which blood vessels samples were taken. This is an important point, because calcitonin levels must be different between them. Recently, it was demonstrated that, in freshwater eels, the ultimobranchial gland responds to hypercalcemia caused by direct infusion of a high-Ca solution *via* the pulmonary vein into the gland and secretes calcitonin (Sasayama *et al*., 2002). In all the abovementioned experiments, blood samples were taken only during the first 3 hr. This was the time during which the plasma calcitonin levels just began to rise. Nevertheless, there has been no evidence so far of whether the secretion of calcitonin ceases or not when plasma Ca levels started to decrease from their peak.

In the present study, first, we took blood samples from the aortic bulb, where blood had just passed through the ultimobranchial gland. Furthermore, we clarified how long calcitonin is secreted from the ultimobranchial gland after

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receiving the stimulation of a high-Ca solution into the stomach.

MATERIALS AND METHODS

Stonefish was obtained by breeding of one pair of adults that had been kept in the laboratory. Therefore, the individuals used in the experiments were a litter of fish. Their body weight was 70-90 g (av. 85.2±4.05 g).

The high-Ca solution was made from $CaCl_2$, since calcitonin is secreted in response to ionized Ca. The final Ca concentration was adjusted to 1.25 M. The consommé cube was also dissolved in the solution to assist the absorption of Ca. The reason for determining the Ca concentration and the manual for making the solution have been described previously (Sasayama *et al*., 1996). Fish were slightly anesthetized using seawater-containing 2-phenoxyethanol (1/5,000). The high-Ca solution was administered into the stomach at a rate of 0.5 ml per 100 g body weight by a tube connected with a needle on the syringe.

In Experiment I, blood samples were taken 5 times at 0, 1, 3, 9, and 33 hr of the administration of the high-Ca solution. In the control group administered only consommé solution, blood samplings were conducted as well. In Experiment II, blood was taken 4 times, and the sampling time was shifted later to 0, 9, 33, and 81 hr. In both experiments, blood was taken from the aortic bulb *via* a heparinized cannula (PE50 polyethylene tube (Clay Adams, Sparks)). The top of the cannula was connected with a syringe needle (25G Terumo, Tokyo), which was bent for insertion to the aortic bulb (Fig. 1). The inside of the cannula was filled with 0.75% NaCl solution. The end was enclosed with a pin. When blood samples were taken, a syringe was replaced with the pin. The cannula remained implanted in the aortic bulb during the experiments. The operated fish were put individually into cages in which they could not move around although seawater passed freely through the case. The amount of blood samples was 10–20 μl, from which plasma was obtained by centrifuging (11,000 rpm; 5°C; 2 min). Those samples were stocked in a deep freezer (–80°C) until analyses.

The plasma total Ca (ionized Ca + inorganic anion-bound Ca + protein-bound Ca) concentration was determined by a modified method from Gitelman's method (1967) with a kit for human (Wako, Osaka) using a microplate reader (Sasayama *et al*., 1996). The plasma calcitonin level was measured using an enzyme-immunoassay kit (Peninsula Lab., Sochinaz) for salmon calcitonin. We have previously confirmed that a curve plotted by plasma samples of the stonefish calcitonin was paralleled with the standard curve obtained by salmon calcitonin.

A one-way \NOVA, an LSD test, and a Mann-Whitney *U*-test were used to evaluate the numerical data.

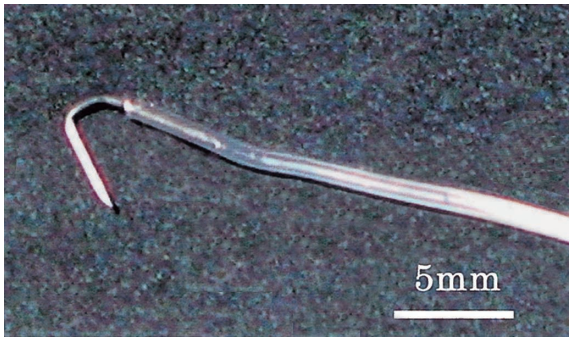

Fig. 1. A photograph showing the cannula using for blood samplings. The top of the cannula is connected with a syringe needle which is bent for insertion to the aortic bulb.

RESULTS

Experiment I Plasma Ca and calcitonin levels until at 33 hr after the administration of a high-Ca solution.

Plasma total Ca levels

Plasma Ca levels significantly increased from the initial level, 2.2±0.25 mM, to 3.5±0.33 (p<0.05) and 4.6±0.48 mM (p<0.01) at 1 and 3 hr, respectively. The Ca level at 3 hr did not change until 9 hrs, and was 4.5±0.49 mM. At 33 hr, however, the plasma Ca level was significantly decreased to 3.2±0.28 mM (p<0.05), although this value was still higher than the initial level (p<0.01). In contrast, in the control group, the initial level of plasma Ca was 2.8±0.33 mM and did not change significantly during the experiment. These results are shown in Fig. 2.

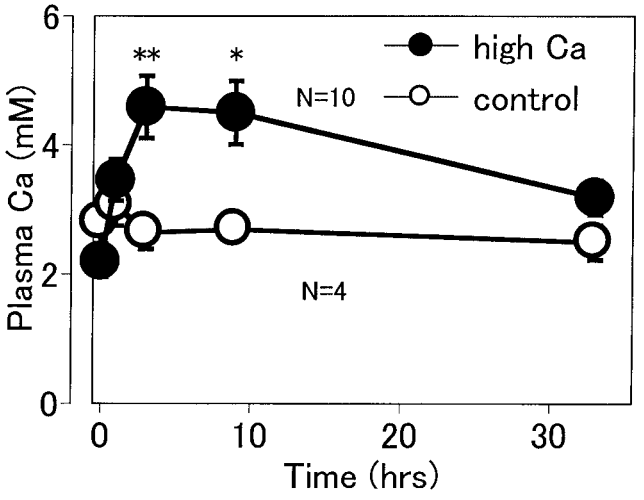

Fig. 2. Plasma Ca levels in stonefish administered a high-Ca solution (high Ca) or a consommé solution (control) into the stomach at 0, 1, 3, 9, and 33 hrs after the treatments. The values are shown as the mean ± SE. Asterisks (*, **) exhibit statistical differences (p<0.05, p<0.01, respectively) from the control values.

Plasma calcitonin levels

Plasma calcitonin levels changed slightly from the initial level, 440.2±43.78 pg/ml, to 684.8±136.44 and 631.9±136.45 pg/ml at 1 and 3 hr, respectively. At 9 hr, however, the level began to rise and was 878.5±210.49 pg/ml. At 33 hr, the plasma calcitonin level still continued to rise and attained 1993.3±411.06 pg/ml (p<0.05). In the control group, the initial level of the plasma calcitonin was 541.8±129.85 pg/ml and did not changed significantly during the experiment. These results are shown in Fig. 3.

Experiment II Plasma Ca and calcitonin levels until at 81 hr after the administration of a high-Ca solution.

Plasma total Ca levels

Changes in plasma Ca levels until at 33 hr after the administration were quite similar to the results of Experiment

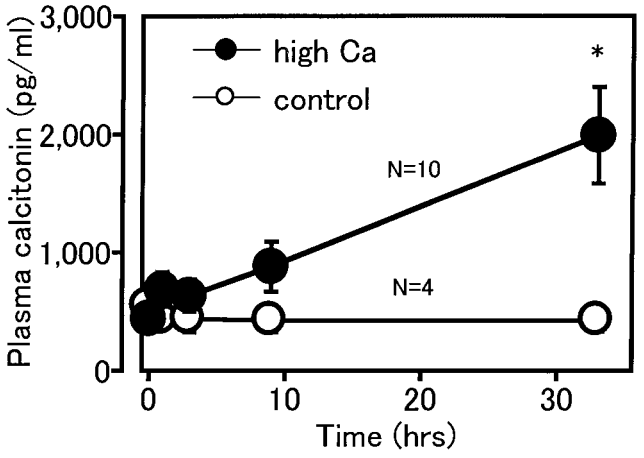

Fig. 3. Plasma calcitonin levels in stonefish administered a high-Ca solution (high Ca) or a consommé solution (control) into the stomach at 0, 1 ,3, 9, and 33 hr after the treatments. The values are shown as the mean ± SE. Asterisk (*) indicates a statistical difference (p<0.05) from the control values.

I. Although the initial level was 3.0±0.11 mM, it was significantly increased to 4.8±0.33 mM at 9 hr (p<0.01), followed by a slight decrease 3.8±0.20 mM at 33 hr which was still significantly higher than the initial level (p<0.05). At 81 hr, however, the plasma Ca levels had declined further to 3.1±0.19 mM, which was not significantly different from the initial level. These results are shown in Fig. 4.

Plasma calcitonin levels

Also in the plasma calcitonin levels, the changes until at 33 hr after the administration were very similar to those observed in Experiment I. The plasma calcitonin levels were 320.7±18.41 pg/ml and 685.2±95.44 pg/ml at 0 and 9 hr, respectively. At 33 hr, the level continued to rise and was 3221.8±755.54 pg/ml (p<0.01). At 81 hr, however, the plasma calcitonin level had decreased significantly to about a half of the value at 33 hr and was 1508.9±326.21 pg/ml (p<0.01). These results are shown in Fig. 4.

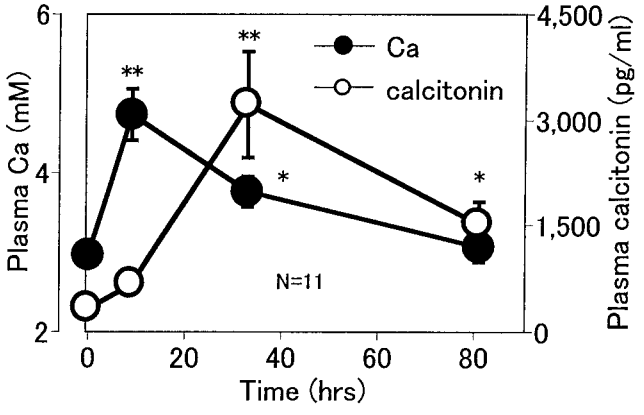

Fig. 4. Plasma Ca and calcitonin levels in stonefish administered a high-Ca solution into the stomach at 0, 9, 33, and 81 hr after the treatment. The values are shown as the mean ± SE. Asterisks (*, **) indicate statistical differences (p<0.05, p<0.01, respectively) from the initial value.

DISCUSSION

In the previous studies using freshwater eels, we determined calcitonin levels in the blood mingled of arterial blood and venous blood (Sasayama *et al*., 1996; Suzuki *et al*., 1999). The values were in the extent of 0–1100 pg/ml. In again freshwater eels, however, in the blood taken from the aortic bulb, where blood had just passed through the ultimobranchial gland, the calcitonin levels were 1900-14000 pg/ml. This difference suggests that calcitonin in blood may be degraded by some catabolic enzymes during circulating in the body. Therefore, in the present study, blood was collected from the aortic bulb.

The present results demonstrate that, also in stonefish, the ultimobranchial glands have the ability to secrete calcitonin against the excessive increases of plasma Ca levels, as recognized in goldfish and eels (Sasayama *et al*., 1996; Suzuki *et al*., 1999). In the phase of changes in plasma Ca and calcitonin levels, however, there were some differences. Although the plasma Ca levels responded quickly to the administration of a high-Ca solution and rose at 1 hr, the plasma calcitonin levels began to increase after the plasma Ca levels had increased. This fact suggests that the ultimobranchial gland responds accurately to the increases of plasma Ca levels. At 33 hr, however, the plasma calcitonin levels continued to increase even more from the level at 9 hr in spite of the fact that the plasma Ca levels had begun to decline at 33 hr. These phenomena suggest two possibilities. The first possibility is that it may be overshoot of the secretion of calcitonin for the increases of plasma Ca levels. The second one is that calcitonin may be overflowed in the plasma, as consumption of calcitonin must have been decreased in correspondence to the declines in plasma Ca levels. It had been reported that, in brown trout (*Salmo trutta*), calcitonin in the plasma continues to be consumed according to the amount of Ca contained in environmental water, even if the plasma Ca levels look stable (Oughterson *et al*., 1995). At 81 hr, however, the plasma calcitonin level declined from the peak when the plasma Ca levels returned to the initial level. This is the first finding that demonstrates the dynamics of calcitonin secretion corresponding to a series of the rises and decreases in plasma Ca levels in teleosts. Also in stonefish inhabiting a natural environment, similar changes may occur in plasma Ca and calcitonin levels when the fish swallow small fishes into the stomach.

REFERENCES

Gitelman HJ (1967) An improved automated procedure for the determination of calcium in biological specimens. Anal Biochem 18: 521–531

Oughterson SM, Mounoz-Chapuli R, De Andres V, Lawson R, Heath S, Davies DH (1995) The effects of calcitonin on serum calcium levels in immature brown trout, *Salmo trutta*. Gen

Comp Endocr 97: 42–48
Sasayama Y (1999) Hormonal control of Ca homeostasis in lower vertebrates: considering the evolution. Zool Sci 16: 857–869
Sasayama Y, Abe I, Suzuki N, Hayakawa T (1996) Plasma calcium and calcitonin levels at food intake in eels and goldfish. Zool Sci 13: 731–735
Sasayama Y, Takei Y, Hasegawa S, Suzuki D (2002) Direct raises in blood Ca levels by infusing a high-Ca solution into the blood stream accelerate the secretion of calcitonin from the ultimobranchial gland in eels. Zool Sci 19: 1039–1043
Suzuki N, Suzuki D, Sasayama Y, Srivastav AK, Kambegawa A, Asahina K (1999) Plasma calcium and calcitonin levels in eels fed a high calcium solution or transferred to seawater. Gen Comp Endocr 114: 324–329

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