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Authors: Sasayama, Yuichi, Takei, Yoshio, Hasegawa, Sanaé, and Suzuki, Daisuke

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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

Yuichi Sasayama[1*], Yoshio Takei[2], Sanaé Hasegawa[2] and Daisuke Suzuki[1]

[1]*Noto Marine Laboratory, Faculty of Science, Kanazawa University, Ishikawa 927-0553, Japan*
[2]*Ocean Research Institute, the University of Tokyo, Tokyo 164-8639, Japan*

ABSTRACT—In eels, a $CaCl_2$ solution was infused into the pneumatic duct vein. Plasma Ca levels were significantly increased during 3 hr and were followed by significant raises in plasma calcitonin levels. These results strongly suggest that, in eels, direct raises in blood Ca levels by infusion of a high-Ca solution *via* blood vessels can accelerate the secretion of calcitonin from the ultimobranchial gland.

Key words: calcitonin, blood Ca, ultimobranchial glands, eels

INTRODUCTION

Calcitonin is a peptide hormone that is secreted from the C-cells of the thyroid gland in mammals or from the ultimobranchial gland in most vertebrates. In mammals, this hormone suppresses the activity of osteoclasts and decreases the mobilization of Ca from bones. As a result, blood Ca levels decline, and the hardness of bones is maintained (Azria, 1989).

On the other hand, except for anuran amphibians (Robertson 1971, 1988), the function of calcitonin is not clear in lower vertebrates (Chan *et al*., 1968; Hayslet *et al*., 1971; Sasayama, 1999). In the past, however, we suggested that, in chum salmon, calcitonin might work at early stages of body growth because the ultimobranchial gland in the fry before hatching was already immunostained with anti-calcitonin antiserum (Sasayama *et al*., 1989).

Recently, it was determined that plasma Ca levels and calcitonin levels in normally fed eels were significantly higher than those in starved eels (Sasayama *et al*., 1996). When a high-Ca solution or saline solution was infused into the stomach in goldfish, plasma Ca levels were significantly elevated only in the high-Ca group (Sasayama *et al*., 1996). In addition, the number of individuals with a high level of plasma calcitonin was significantly larger in the high-Ca group than in the saline group. Furthermore, we repeated a similar experiment in eels, in which plasma calcitonin levels in individuals administered a high-Ca solution into the stomach were significantly raised slightly after the elevation of plasma Ca levels (Suzuki *et al*., 1999). This result also demonstrates that, in teleosts, the ultimobranchial gland secretes calcitonin when blood Ca levels are raised. Taking these all results into consideration, it is strongly suggested that, in teleosts, calcitonin plays a physiological role on daily occurrences such as suppressing transitory hypercalcemia arising from feeding.

In teleosts, however, all evidence that we have submitted so far has been obtained by infusing Ca into the digestive tract. It is known that, in mammals, some gut hormones affect the secretion of calcitonin on the processes of digestion (Azria, 1989; Person *et al*., 1988; Sethi *et al*., 1983). For example, gastrin, CCK, glucagons, and secretin all accelerate the secretion of calcitonin. Therefore, when a high-Ca solution was infused into the stomach, it was not clear whether some gut hormones brought about the secretion of calcitonin from the ultimobranchial gland or the elevation of blood Ca levels did so directly.

The purpose of this study is to determine whether or not, in eels, raising blood Ca levels by infusing a high-Ca solution into the blood stream can accelerate the secretion of calcitonin from the ultimobranchial gland.

MATERIALS AND METHODS

Japanese eels (*Anguilla japonica*) were used. In eels, the ultimobranchial gland is located on the transverse septum that separates the heart from the visceral mass. Consequently, it is necessary to find a blood stream toward the transverse septum after bathing the ultimobranchial gland with a high-Ca solution. In this study, we selected the pneumatic duct vein, which immediately flows to the sinus venosus of the heart *via* the transverse septum from the swim bladder. A canula was set in the pneumatic duct vein to infuse the high Ca-solution. Another canula was put in the arterial

*Corresponding author: Tel. +81-768-74-1151;
FAX. +81-768-74-1644.
E-mail: sasayama@suzu2.suzu.or.jp

bulb for collecting blood.

The eels were divided into two groups. In one group, 2 mM $CaCl_2$ solution was infused at a rate of 0.3 ml/hr by an infusion pump. This concentration and rate were determined by considering the quantity of blood in eels and the results of the previous experiments (Sasayama *et al*., 1996; Suzuki *et al*., 1999). We intended to take blood successively at 0, 0.5, 1, and 3 hr after infusion of the high-Ca solution into the heparinized syringe. When the solution was actually infused to 6 individuals, however, most eels died as soon as infusion started. We could collect data from only 2 surviving individuals. One survived for 3 hr, while the other one died after 1 hr. Therefore, the $CaCl_2$ concentration was decreased to 0.5 mM, and the infusion rate was changed to 0.4 ml/hr. Seven individuals were exposed to these conditions. Four of them survived for 3 hr, and 3 died after 1 hr. Consequently, the $CaCl_2$ concentration was again decreased to 0.3 mM, and the infusion rate was readjusted to 0.3 ml/hr. Two individuals were exposed to these conditions, but both died after 1 hr. Therefore, we judged that direct infusion of $CaCl_2$ solution to the sinus venosus affected the heart muscle toxically, although the solution passed through the transverse septum from the pneumatic duct vein. In this study, as a result, 3 kinds of $CaCl_2$ concentrations were tried, and data of 11 individuals were obtained.

On the other hand, as a control group, we infused 1.0 mM or 0.6 mM NaCl solution in 4 individuals and 1 individual, respectively, when considering the chloride concentrations of $CaCl_2$. Because no eels died in this experiment, we could obtain data from 5 individuals.

Plasma was preserved in a deep-freezer until examination for analyzing Ca and calcitonin levels. Total Ca levels in plasma were determined using a microplate reader (Sasayama *et al*., 1996) by a modified method of Gitelman (1967). Plasma calcitonin levels were measured by a sandwich method of ELISA (Sasayama *et al*., 1996).

For determining ionic Ca levels and Na levels in plasma, a large quantity of blood was taken at the final sampling at 3 hr. In samples taken at other times as well, plasma Na levels were determined when the blood volume was adequate to measure it. Furthermore, we checked the hematocrit value at every sampling time to determine whether the effects of blood samplings were different or not between the $CaCl_2$ group and the NaCl group.

The significance of changes in data was statistically examined using the Friedman-*test*. In the $CaCl_2$ group, we separately assayed the data from individuals from which blood samples could be taken until 3 hr and 1hr after infusion. The average body weights of the $CaCl_2$ group and the NaCl group at the initial level were 189.2±2.02 g and 184.0±6.26 g, respectively. There was no significant difference between them.

RESULTS

Plasma Ca levels

All 3 kinds of $CaCl_2$ solutions brought about raises in plasma Ca levels (Table 1). Although there were 6 times more differences in the Ca concentrations among those solutions infused, the rates of raises in plasma Ca levels did not correlate directly with the Ca concentrations of the solu-

Table 1. Individual values in plasma Ca levels (mM) in eels infused with a high-Ca solution. Ca concentrations (mM) in the solutions infused and the infusion rates are also cited in this table.

No.	0 hr	0.5 hr	1 hr	3 hr	(mM)	ml/hr
1	3.12	6.44	11.00	–	2.00	0.3
2	2.40	3.77	4.32	7.53	2.00	0.3
3	2.79	6.71	8.23	–	0.50	0.4
4	2.25	5.11	7.06	8.68	0.50	0.4
5	4.82	8.33	9.46	–	0.50	0.4
6	2.59	5.29	7.06	9.38	0.50	0.4
7	3.02	6.19	5.36	9.56	0.50	0.4
8	2.77	3.99	4.89	9.01	0.50	0.4
9	3.22	7.44	9.06	–	0.50	0.4
10	2.40	5.18	5.71	–	0.30	0.3
11	1.92	2.64	5.39	–	0.30	0.3
av.±SE	2.85± 0.23	5.55± 0.51	7.05± 0.65	8.83± 0.36		

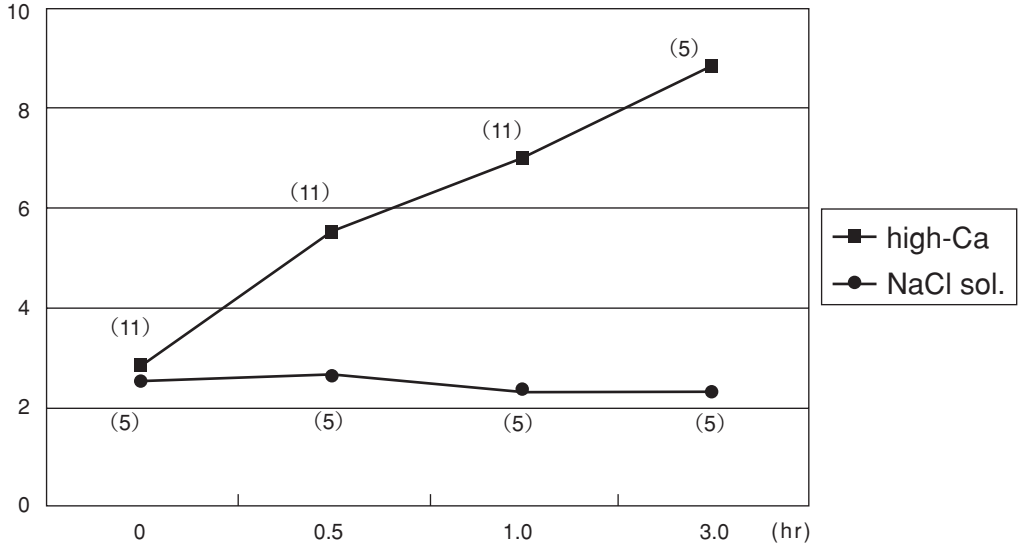

Fig. 1. Changes in plasma Ca levels (mM) in eels infused with a high-Ca solution or NaCl solution. The number in parenthesis is the number of fish examined.

tions. Therefore, the average value in plasma Ca at each sampling time was calculated by putting the data together. The results indicated that the plasma Ca value was 2.9±0.23 mM just before the infusion of $CaCl_2$ solutions, 5.6±0.51 mM at 0.5 hr after infusion, 7.1±0.65 mM at 1.0 hr after infusion, and 8.8±0.36 mM at 3.0 hr after infusion (Table 1) (Fig. 1). The final value was 3 times higher than the initial value. These changes in plasma Ca levels were statistically significant (p<0.01).

In the NaCl group, the initial value of plasma Ca was 2.5±0.19 mM, which was not significantly different from that in the $CaCl_2$ group. After the infusion of the NaCl solution, plasma Ca levels were 2.6±0.12 mM at 0.5 hr, 2.3±0.15 mM at 1.0 hr, and 2.3±0.17 mM at 3 hr. These changes were not statistically significant (Fig. 1) (Table 2).

Plasma calcitonin levels

Calcitonin values were rather variable in the initial levels from individual to individual in either group (Tables 3 and 4). Plasma calcitonin levels were not correlated with either plasma Ca levels or the $CaCl_2$ concentrations infused as well. In Fig. 2, changes in plasma calcitonin levels during 3 hr in the high-Ca group are shown individually by converting each initial value to r1.0. Plasma calcitonin levels began to increase at 0.5 o 1 hr after infusion in most individuals and ascended to higher levels at 3 hr. These changes were statistically significant (p<0.01).

On the other hand, changes in plasma calcitonin levels in the NaCl group are also shown in Fig. 3 as they are for the $CaCl_2$ group. There was no significant change during 3 hr.

Ionic Ca levels in plasma

Ionic Ca values in the $CaCl_2$ group could be determined in 3 individuals that survived for 3 hr. The value was

Table 2. Individual values in plasma Ca levels (mM) in eels infused with a NaCl solution.

No.	0 hr	0.5 hr	1.0 hr	3.0 hr
1	1.85	2.50	1.82	1.87
2	2.64	2.32	2.15	2.19
3	2.69	3.02	2.42	2.82
4	2.52	2.57	2.64	2.52
5	2.97	2.77	2.59	2.15
av.±SE	2.50±0.19	2.64±0.12	2.32±0.15	2.29±0.17

Table 3. Individual values in plasma calcitonin levels (pg/ml) in eels infused with a high-Ca solution.

No.	0 hr	0.5 hr	1.0 hr	3.0 hr
1	1501.7	2774.9	3300.4	–
2	1523.1	4283.5	5702.6	11186.3
3	7150.4	7571.6	12084.0	–
4	977.6	862.8	1488.8	3948.0
5	374.0	541.0	633.3	–
6	3005.8	4172.3	4494.6	20351.0
7	3615.6	4315.3	8498.9	27876.3
8	1736.7	1893.7	5080.5	6666.4
9	386.3	425.2	1484.1	–
10	436.7	417.4	1743.0	–
11	278.2	1145.0	3017.2	–
av.±SE	1907.8± 620.13	2582.1± 689.92	4320.7± 1041.38	14005.6± 4447.63

Table 4. Individual values in plasma calcitonin levels (pg/ml) in eels infused with a NaCl solution.

No.	0 hr	0.5 hr	1.0 hr	3.0 hr
1	1337.8	1555.6	1168.5	1141.7
2	868.8	889.9	914.0	1262.3
3	2145.4	1303.0	824.7	552.6
4	2353.5	4714.7	4965.8	3577.5
5	1599.2	679.2	334.6	276.4
av.±SE	1660.9± 269.26	1828.5± 737.65	1641.5± 841.99	1362.1± 583.12

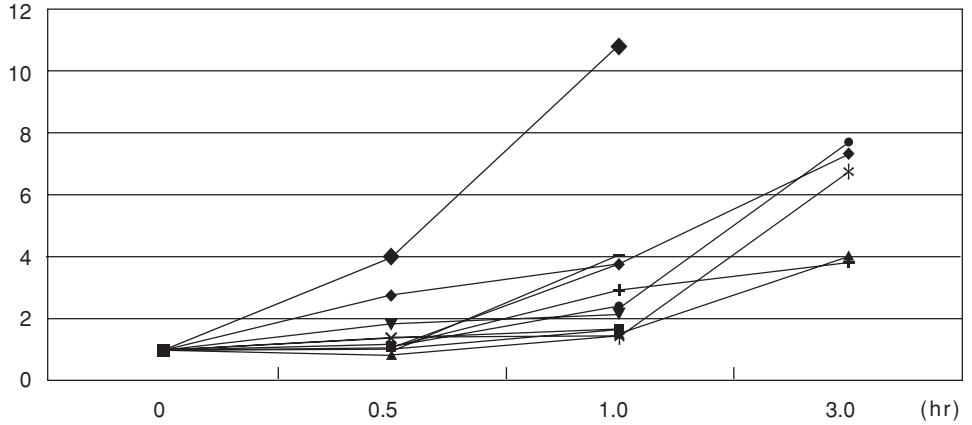

Fig. 2. Individual changes in plasma calcitonin levels in eels infused with a high-Ca solution when converting each initial value 1.0.

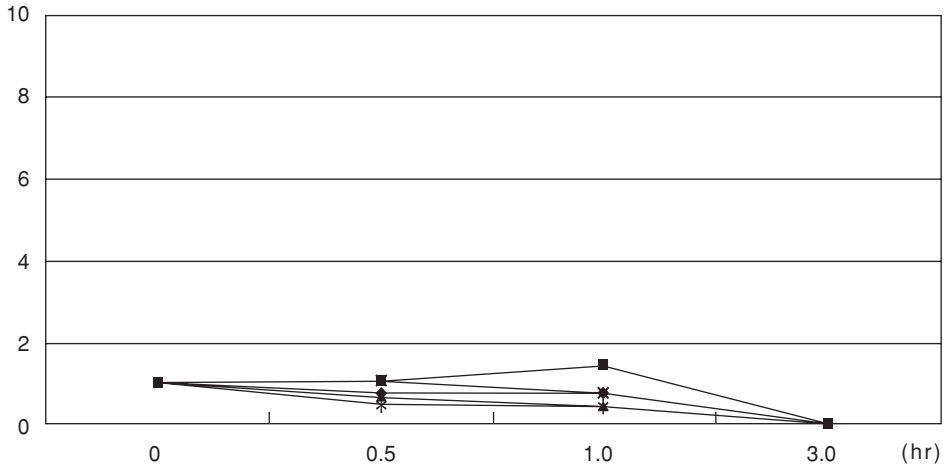

Fig. 3. Individual changes in plasma calcitonin levels in eels infused with a NaCl solution when converting each initial value 1.0.

6.3±0.38 mM, a level that corresponded to 69–76% of the total Ca levels. On the other hand, ionic Ca levels in the NaCl group were determined in 4 individuals. The value was 1.5±0.09 mM at 3 hr, a level that also corresponded to 61–73% of the total Ca levels. Notwithstanding the fact that the value in the NaCl group was conspicuously lower than that in the $CaCl_2$ group, the rates of ionic Ca values to total Ca values were very similar in both groups.

Plasma Na levels

In the $CaCl_2$ group, the initial level of plasma Na in 8 individuals was 150.3±2.15 mM. In the NaCl group, the initial level in 4 individuals was 152.8±1.89 mM. There was no significant difference between the 2 groups. At 3 hr after the infusion of either solution, however, plasma Na levels in 4 individuals in the $CaCl_2$ group were slightly decreased to 144.8±2.50 mM; on the other hand, in 4 individuals in the NaCl group, it tended to increase to 160.8±2.63 mM. There was a significant difference between the 2 groups ($p<0.05$). In the NaCl group, therefore, NaCl infusion might affect plasma Na levels.

Hematocrit value

Although the initial value of the hematocrit in the $CaCl_2$ group was 30.5±1.71, its level was acutely decreased to 17.0±2.28 at 3 hr after infusion. This decline was significant ($p<0.05$). In the NaCl group, the initial value was 28.8±2.89, a level which was not significantly different from that in the $CaCl_2$ group. At 3 hr after infusion, the initial level in the NaCl group was also acutely decreased to 21.2±2.52 ($p<0.01$). There was no significant difference in the values at 3 hr between the 2 groups.

DISCUSSION

The present results clearly demonstrate that 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 the previous experiment using eels that had been infused a $CaCl_2$ solution into the stomach, plasma Ca concentrations were increased from 2.63 mM of the initial value to 6.20 mM at 0.5 hr, 7.45 mM at 1 hr, and 8.50 mM at 3 hr (Suzuki *et al*., 1999). These values were very similar to the results obtained in this study. On the other hand, in the previous experiment, plasma calcitonin levels were increased from 30 pg/ml of the initial value to 205.6 pg/ml at 0.5 hr, 697.5 pg/ml at 1 hr, and 1118.2 pg/ml at 3 hr. In contrast, the average values in this study were 1,907.8 pg/ml at 0 hr, 2,582.1 pg/ml at 0.5 hr, 4,320.7 pg/ml at 1 hr, and 14,005.6 pg/ml at 3 hr. The absolute values of plasma calcitonin were undoubtedly higher in the present experiment. The difference in the values must be due to the differences in the site from which blood samples were taken. In the previous experiment, blood was sampled at the caudal artery; on the other hand, in this study, blood just perfused from the ultimobranchial gland was taken at the arterial bulb.

In this study, as mentioned above, the rates of raises in plasma Ca levels were similar to those in the case of the infusion of a high-Ca solution into the stomach (Suzuki *et al*., 1999). In that experiment, no eels died. Therefore, the raise in plasma Ca levels itself does not seem to be the cause of death of fish. On the other hand, the rate of ionic Ca to total Ca was similar between the high-Ca group and the NaCl group. This fact indicates that ionic Ca infused into the blood stream was immediately combined with some proteins at the same rate as that in the NaCl group. It may be important for a living body to keep the rate of ionic Ca/total Ca constant. However, it is also a fact that the absolute quantity of ionic Ca was very high in the high-Ca group compared to that in the NaCl group. Therefore, an excessive number of Ca ions in the blood stream might result in toxicity. When plasma Ca levels are raised *via* the digestive tract, there may be unknown systems preventing the heart from stopping beating. In our previous experiment, therefore, we should determine the ionic Ca level in plasma. Recently, we

knew that, in humans, hypercalcemia harms the function of the heart (Yang *et al*., 1997; Demers *et al*., 1998).

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