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# Effects of Ultimobranchialectomy on the Mineral Balances of the Plasma and Bile in the Stingray (Elasmobranchii)

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**ABSTRACT**—Effects of ultimobranchialectomy (UBX) on plasma and bile minerals were examined in the stingray *Dasyatis akajei* (Elasmobranchii). Plasma urea and glucose concentrations were also measured as references. At 1 week after the operation, plasma CT level in the UBX group was significantly lower than that in the sham-operated (SHAM) group. However, there was no significant difference in plasma Ca level between UBX and SHAM groups at 1 week. On the other hand, bile Ca concentration in the UBX group was significantly lower than that in the SHAM group. Bile K and Cl levels, and bile volumes in the UBX group were significantly higher than those in the SHAM group. These results suggests that in the stingray, CT may function to control bile mineral concentrations.

## INTRODUCTION

Calcitonin (CT), a 32-amino acid peptide hormone, is secreted from C-cells of the thyroid glands in mammals and from parenchymal cells of the ultimobranchial glands in non-mammals [4]. It is well known that in mammals, this hormone functions to suppress the activity of osteoclasts, resulting in hypocalcemia and the mineralization of bones [14].

It has been reported that cartilaginous fish, as well as other vertebrates, possess ultimobranchial glands (UBG), and that administration of shark ultimobranchial extract (UBE) to rats induces hypocalcemia, as does homologous CT [4].

Recently, we reported that the stingray *Dasyatis akajei* possesses a pair of large UBG which contains abundant CT [21]. Furthermore, administration of synthetic stingray CT induces hypocalcemia in rats, similar to the effect of UBE from shark [19]. Thus, it is clear that cartilaginous fish has the ability to produce CT which can affect mineral balances in mammals, although the stingray itself does not possess bones. On the other hand, in mammals, bile has been shown to contain higher levels of Ca than serum [25]. Therefore, the gall-bladder is regarded as an important Ca excretory organ. In addition, it has been reported that in thyro-parathyroidectomized rats, CT remarkably accelerates Ca excretion *via* bile [24]. In cartilaginous fish, the gall-bladder also contains bile in which the Ca level is 3–4 times higher than in serum [1, 2]. In the present study, to elucidate the physiological role of CT in cartilaginous fish, the effects of ultimobranchialectomy (UBX) on plasma and bile mineral balances in the stingray were examined.

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## MATERIALS AND METHODS

Seventeen stingray *Dasyatis akajei*, ranging 240–2,800 g in body weight and caught in Toyama Bay during the period from May to July 1993 were used. Prior to experiments, fish were acclimatized in an aquarium (120 cm×60 cm×45 cm) for 1 week after collection. UBX was then performed according to the following procedures. Fish were anesthetized with tricaine methanesulfonate in chilled seawater (4°C) at a dilution of 1/10,000. As the stingray possesses one pair of UBG just dorsal to the heart, an incision was made in the portion ventral to the heart. The heart was then pushed to the left side with a cotton, and the UBG of the right side was removed. The heart was next pushed to the right side, and the UBG of the left side was also removed. Thereafter, the incision was sutured with surgical thread. The same procedure was performed in the sham-operated (SHAM), but the UBGs were not removed. To allow recovery from anesthesia, fish were kept in physiological saline for elasmobranchus (380.0 mM NaCl, 6.9 mM KCl, 3.0 mM $CaCl_2$, 2.3 mM $MgCl_2$, 2.4 mM $NaHCO_3$ and 483.0 mM $NH_2CONH_2$) for 20 min, and then returned to normal seawater. During the experimental period of one week, seawater was exchanged at least once a day to prevent accumulation of $NH_4^+$. Nine individuals (4 males and 5 females) were used for the UBX group and 8 individuals (5 males and 3 females) for the SHAM group. One week after the operation, both groups were anesthetized again, and blood was collected from the heart with heparinized syringes. Blood was centrifuged at 25,000×g for 10 min at 4°C, and hematocrit values were measured. The plasma samples were immediately frozen and kept at −50°C until analysis. In parallel with this procedure, bile was also taken from the gall-bladder with syringes, and the volume was examined.

A competitive inhibition enzyme-linked immunosorbent assay (ELISA) was used for measurement of plasma CT level in the stingray according to the method of Robertson [15, 16].

Ca, Mg, K and Na levels were determined by atomic absorption spectrophotometry (180–70 type; Hitachi-Zeeman; Hitachi Co. Ltd., Tokyo). Inorganic phosphate level was measured by the method of Fiske and Subbarow [7]. Urease-indophenol [20] and murotase-glucoseoxidase methods [13] were used for measurement of urea and

glucose, respectively. Cl level was measured with a chloridemeter (C-50 type, Jookoo Sangyo Co., Ltd.,Tokyo). Data were analyzed by Student's *t*-test.

## RESULTS

Plasma CT level in the UBX group (457.6±77.8 ng/ml) was significantly lower than that in the SHAM group (1321.2 ±319.0 ng/ml) 1 week after the operation ($P<0.05$) (Fig. 1).

Plasma mineral, urea and glucose concentrations and hematocrit values in both groups 1 week after the operation are shown in Table 1. There were no significant differences in values of any of these parameters between the UBX and SHAM groups.

The results regarding bile minerals and bile volume are shown in Table 2. Bile Ca concentration in the UBX group was significantly lower than that in the SHAM group ($P<0.05$). In contrast, bile K and Cl concentrations in the UBX group were significantly higher than those in the SHAM group ($P<0.05$). There was no significant differences in bile levels of other minerals between the two groups. Bile volume in the UBX group was significantly ($P<0.05$) greater than that in the SHAM group.

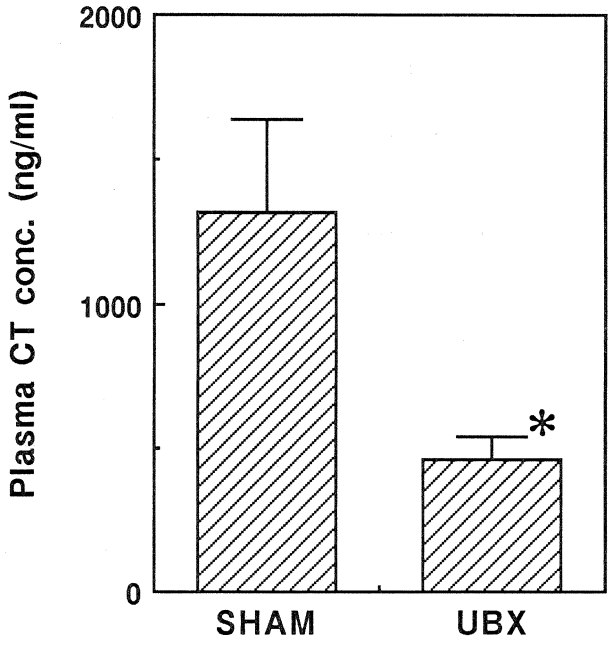


FIG. 1. Plasma calcitonin levels in the ultimobranchialectomized group (UBX) and the sham-operated group (SHAM) in the stingray. The numbers of stingrays used were 9 in UBX and 8 in SHAM. Asterisk (*) indicates significant difference from the value for SHAM. $P<0.05$.

## DISCUSSION

At 1 week after the operation, plasma CT level in the UBX group was significantly lower than that in the SHAM group. However, the CT value in the UBX group was not decreased to near zero, but to about one-third of the level in the SHAM group. This leads us to consider that certain extra-ultimobranchial source(s) of CT may be present in the stingray. In the lizards [8] and salamander [12], such extra-ultimobranchial sources were demonstrated. On the other hand, Idler *et al.* [10] reported that in the skate *Raja erinacea*, hypophysectomy or interrenalectomy does not bring about significant changes in the glycogen levels in the liver when compared to sham-operated controls. It is possible that in cartilaginous fish, turnover rate of the hormone itself may be slower than in other vertebrates. On the other hand, it has been known that in lower vertebrates such as some teleosts and amphibians, plasma CT levels are so high (ng/ml order), when compared to those in mammals (pg/ml order) [15, 26]. In addition, also in shark, *Triakis semifasciata*, plasma CT level is ranged in ng/ml order [9]. Therefore, it may be possible that in these animals, all CT molecules present in blood do not function as a hormone. To clarify this problem, it may be helpful to determine the level of CT receptor in lower vertebrates, or to examine the effect of removal of all CT molecules by adding antibody against CT into blood.

It has been reported that in bullfrog tadpoles, serum Ca

TABLE 1. Plasma mineral, urea and glucose concentrations (mM) and hematocrit values (%) in the ultimobranchialectomized (UBX) and the sham-operated (SHAM) groups in the stingray

| | Hem | Ca | Mg | Pi | Na | Cl | Urea | Glucose |
|---|---|---|---|---|---|---|---|---|
| UBX | 21.10±1.49 | 3.55±0.16 | 1.46±0.13 | 1.20±0.11 | 263.9±9.5 | 249.3±7.6 | 125.0±2.6 | 2.2±0.28 |
| SHAM | 24.80±1.05 | 3.53±0.15 | 1.63±0.37 | 1.25±0.17 | 271.7±3.5 | 248.9±3.8 | 131.4±2.3 | 2.0±0.27 |

The number of stingrays used are 9 for UBX group and 8 for SHAM group. The values show mean±SE. Hem: Hematocrit value.

TABLE 2. Bile mineral concentrations (mM) and bile volume (ml/Kg BW) in the ultimobranchialectomized (UBX) and sham-operated (SHAM) groups in the stingray

| | Ca | Mg | K | Na | Cl | Bile volume |
|---|---|---|---|---|---|---|
| UBX | 16.25±1.18* | 7.42±0.47 | 8.00±0.63* | 244.5±4.03 | 84.7±9.3* | 1.7±0.11* |
| SHAM | 20.38±0.96 | 8.71±0.65 | 5.86±0.73 | 243.9±6.88 | 53.8±7.0 | 1.2±0.21 |

The numbers of stingrays used are 9 for UBX group and 8 for SHAM group. The values show mean±SE. Asterisk (*) indicates significant difference from the SHAM level.

levels are increased significantly at 1 week after UBX when kept in Ca-rich water [18]. It was predicted that similar change may occur also in the stingray. However, there was no significant difference in the plasma Ca levels between UBX and SHAM groups at 1 week after the operation. CT is known to affect the ionic type of Ca, but not the protein-bound type of Ca [6]. It is known that in cartilaginous fish, the percentage of plasma ionic Ca is so high (about 80%) when compared to the value (50%) of most other vertebrates [5]. In the stingray, plasma Ca level could not be increased after UBX, because plasma was already saturated with ionic Ca. On the other hand, Chan and Wong [3] reported that in the lip-shark *Hemiscyllium plagiosum*, plasma Ca levels were more precisely regulated than Na, Cl and Mg levels when transferred to 70% or 35% seawater. It is possible that in the stingray, other mechanisms for regulation of plasma Ca level began to work immediately after UBX.

On the other hand, cartilarginous fish does not have "chondroclasts" which correspond to osteoclasts as a target for CT in bony vertebrates. Therefore, it is possible that in cartilaginous fish, CT plays some roles in Ca homeostasis by mechanism different from that on bones.

In higher vertebrates, the gall-bladder is an important Ca excretory organ [1, 2, 24, 25]. In hagfish, it is reported that Ca contained in the bile is 12 times higher than the level in plasma [17]. Also in amphibians, Ca contents in the gall-bladder are higher than those in other soft tissues [22]. In the present study, the bile Ca concentration in the UBX group was significantly lower than that in the SHAM group. In the UBX group, Ca excretion *via* bile might be inhibited due to the lack of UBG. On the other hand, bile volume was greater in the UBX group than in the SHAM group. Therefore, in the UBX group, the fish might compensate for Ca excretion by increasing the bile volume. Consequently, bile $Cl^-$ and $K^+$ which are usually reabsorbed with water in gall-bladder, might be increased [11, 23].

The present results suggest that in cartilaginous fish, the gall-bladder may be one of the important targets for CT. This function of CT has been recognized so far only in mammals. Therefore, this action of CT could be ascended to at least this animal group. In addition, it seems of interest to consider from the physiological point of view that the CT action is not related to the presence of bones or cartilages.

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## REFERENCES

1 Boyer JL, Schwarz J, Smith N (1976) Biliary secretion in elasmobranchs. I Bile collection and composition. Am J Physiol 230: 970–973

2 Boyer JL, Schwarz J, Smith N (1976) Biliary secretion in elasmobranchs. II Hepatic uptake and biliary excretion of organic anions. Am J Physiol 230: 974–981

3 Chan DKO, Wong TM (1977) Physiological adjustments to dilution of the external medium in the lip-shark, *Hemiscyllium plagiosum* (Bennett). J Exp Zool 200: 71–84

4 Copp DH, Brooks CE, Low BS, Newsome F, O'dor RK, Parkes, CO, Walker V, Watts, EG (1970) Calcitonin and ultimobranchial function in lower vertebrates. In "Calcitonin" Ed by S Taylor, Heinemann, London, pp 281–294

5 Dacke CG (1979) Calcium regulation in the chondrichthyes. In "Calciumn Regulation in Sub-mammalian Vertebrates", Academic Press, London, pp 96–98

6 Deftos LJ (1989) Exogenous calcitonin. In "The Calcitonins" Ed by M Azria, Karger, New York, pp 67–132

7 Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66: 375–400

8 Galan Galan F, Rogers RM, Girgis SI, Arnett TR, Ravazzola M, Orci L, MacIntyre I (1981) Immunochemical characterization and distribution of calcitonin in the lizard. Acta Endocrinol 97: 427–432

9 Glowacki J, O'sullivan J, Miller M, Wilkie DW, Deftos LJ (1985) Calcitonin produces hypocalcemia in leopard sharks. Endocrinology 116: 827–829

10 Idler DR, O'Halloran MJ, Horne DA (1969) Interrenalectomy and hypophysectomy in relation to liver glycogen levels in the skate (*Raja erinacea*). Gen Comp Endocrinol 13: 303–306

11 Kutz K, Paumgartner G, Egger G, Preisic R (1974) A comparison between the bile acid concentration and inorganic cation-anion difference in dog bile. Gastroenterology 67: 135–141

12 Matsuda K, Sasayama Y, Oguro C, Kambegawa A (1989) Calcitonin-immunoreactive cells found in the extra-ultimobranchial area of the salamander, *Hynobius nigrescens*, during larval development. Zool Sci 6: 611–614

13 Miwa I, Okuda J, Maeda K, Okuda G (1972) Mutarotase effect on colorimetric determination of blood glucose with $\beta$-D-glucose oxidase. Clin Chim Acta 37: 538–540

14 Reginster JY (1993) Calcitonin for prevention and treatment of osteoporosis. Am J Med 95 (Suppl 5A): 44–47

15 Robertson DR (1981) A competive inhibition enzyme-linked immunosorbent assay for frog calcitonin. Gen Comp Endocrinol 45: 12–20

16 Robertson DR (1986) Calcitonin In "Methods of Enzymatic Analysis Vol 9" Ed by HU Bergmeyer, Veriag-Chemie, Weinheim, pp 497–508

17 Robertson JD (1974) Osmotic and ionic regulation in cyclostomes In "Chemical Zoology" Ed by M Florkin and BT Scheer, Academic Press, New York, pp 149–193

18 Sasayama Y, Oguro C (1976) Effects of ultimobranchialectomy on calcium and sodium concentrations of serum and coelomic fluid in bullfrog tadpoles under high calcium and high sodium environment. Comp Biochem Physiol 55A: 35–37

19 Sasayama Y, Suzuki N, Oguro C, Takei Y, Takahashi A, Watanabe TX, Nakajima K, Sakakibara S (1992) Calcitonin of the stingray: comparison of the hypocalcemic activity with other calcitonins. Gen Comp Endocrinol 86: 269–274

20 Searcy RL, Foreman JA, Ketz A, Reardon J (1967) A new automated method for urea nitrogen analysis. Am J Clin Pathol 47: 677–681

21 Takei Y, Takahashi A, Watanabe TX, Nakajima K, Sakakibara S, Sasayama Y, Suzuki N, Oguro C (1991) New calcitonin isolated from the ray, *Dasyatis akajei*. Biol Bull 180: 485–488

22 Uchida K, Ohtani Y, Sasayama Y, Nambu H, Yoshizawa H, Akabane S, Suzuki K, Suzuki N (1993) Levels of calcium in the

skin of some amphibians and possible evolutionary implications. Zool Sci 10: 819–826

23 Wheeler HO, Ramos OL (1960) Determinants of the flow and composition of bile in the unanesthetized dog during constant infusions of sodium taurocholate. J Clin Invest 39: 161–170

24 Yamaguchi M, Yamamoto T (1979) Effects of various calcitonins on calcium concentrations in the bile and serum of thyroparathyroidectomized rats. Chem Pharm Bull 27: 1671–1674

25 Yamaguchi M, Yamamoto T, Hasegawa A (1979) Physiological significance of calcium excretion into the bile of rats. Chem Pharm Bull 27: 3137–3139

26 Yamauchi H, Orimo H, Yamauchi K, Takano K, Takahashi H (1978) Increased calcitonin levels during ovarian development in the eel, *Anguilla japonica*. Gen Comp Endocrinol 36: 526–529