Effects of Ca Concentrations in Culture Medium on the Release of Calcitonin from Incubated Ultimobranchial Glands of the Bullfrog, Rana catesbeiana

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ABSTRACT—Calcitonin released from ultimobranchial glands incubated in culture media having different Ca concentrations was determined by rat bioassay. In the first incubation of 30 min, a large quantity of calcitonin was released into various culture media such as normal medium, and high Ca media which have 2 times, 3 times and 6 times Ca concentrations compared to the normal medium. In this term, there was no significant difference in the quantity of calcitonin released into the media among those groups. However, during 48 hr following the first incubation, in the Ca 2 times medium, calcitonin quantity released into the medium was maximum. In the Ca 3 or 6 times medium, the amount of calcitonin released was less than that of Ca 2 times medium. These results imply that in the bullfrog, secretion of calcitonin from ultimobranchial glands in vivo may be induced by a suitable rise in the serum Ca concentration.

INTRODUCTION

Calcitonin is secreted from thyroid glands in mammals or from ultimobranchial glands in non-mammals. It has been well known that in mammals, calcitonin is secreted against the rise of blood Ca levels. In pigs and rabbits, blood calcitonin level is increased when the thyroid gland is perfused with high Ca solution [1-3]. Furthermore, under in vitro condition, calcitonin secretion from the thyroid gland of pigs is accelerated by moderate elevation of Ca concentrations in culture medium [4]. In birds, when the ultimobranchial gland is perfused with high Ca solution, blood calcitonin level is increased [5, 6]. Incubated avian ultimobranchial glands also show the same reactions as in mammals [7, 8]. These facts imply that in higher vertebrates, the rise of blood Ca level is one of the factors which accelerate calcitonin secretion.

On the other hand, it has been reported that in bony fishes such as trouts and eels, rise of blood Ca level does not cause calcitonin secretion from ultimobranchial glands [9, 10]. Therefore, it is suggested that mechanisms which trigger calcitonin secretion may be different between higher vertebrates and lower ones.

In the present study, effects of Ca concentrations in culture medium on calcitonin release from incubated ultimobranchial glands of the bullfrog, Rana catesbeiana, were examined by rat bioassay.

MATERIALS AND METHODS

Male bullfrogs (body weight, 200–300 g) were purchased from commercial source. One pair of ultimobranchial glands found near the glottic sphincter was dissected out carefully under the binocular microscope, and was immediately put into an incubation chamber (Lab-Tek 4804, Nunc Inc.) which contained 1 ml of amphibian Ringer's solutions with different Ca concentrations as described below. The ultimobranchial glands were kept at room temperature for 30 min (the first incubation). Then, the ultimobranchial glands
were transferred into a separate chamber which contained the same kind of incubation medium, and incubated for 24 hr at 25°C (the second incubation). After that, those were again displaced into a distinct chamber with the same kind of the solution, and incubated furthermore for 24 hr at 25°C (the third incubation). Each of the culture media obtained at each incubation time, which included released calcitonin, was lyophilized and frozenly stocked at −50°C until use.

Three sets of incubation chambers were prepared according to the incubation period, such as: 30 min (the first incubation), 24 hr (the second incubation), and next 24 hr (the third incubation). Each set included four culture media having different Ca concentrations as follows: the normal amphibian Ringer’s solution, which served as the standard incubation medium, was composed of NaCl 6.50 g/l, KCl 0.14 g/l, CaCl$_2$ 0.12 g/l, NaHCO$_3$ 0.20 g/l, glucose 1 g/l; the other three were composed of Ringer solutions that contained 2, 3, and 6 times amount of Ca of the standard medium (Ca 2×, Ca 3×, and Ca 6× incubation medium, respectively).

Bullfrog calcitonin released from ultimobranchial glands into incubation medium was detected by rat bioassay [11]. Each of the pooled lyophilized samples which were obtained after incubating 3 pairs of ultimobranchial glands separately, was adjusted to 0.4 ml by saline solution (0.9% NaCl) and administered to a rat. As a control, the saline solution was administered. Furthermore, salmon calcitonin (Novabiochem Inc.) (10, 25 and 100 mU) was administered as a calcitonin standard for comparison with samples. Blood was samples just before medium administration and at 0.5, 1, 2, and 3 hr after. Serum Ca concentrations in rats were determined by atomic absorption spectrophotometry (Hitachi-Zeeman 180–70 type). In the present study, changes in serum Ca levels were exhibited as decline rates from the initial Ca level. Furthermore, the areas which were lower than the initial level were determined to examine the duration of hypocalcemic effect of the culture medium, and were exhibited as serum Ca graphs.

Student’s $t$-test was applied to evaluate the data.

![Graph A](image1.png)

**Fig. 1.** Time courses (0–3 hr) of serum Ca concentration fall (delta % from the initial levels) in rats after administrations of the first incubation (30 min) medium (A) and the second incubation (24 hr) medium (B) and the third incubation (24 hr) medium (C). Vertical bar shows mean±SE. Each symbol means normal Ca medium (▲), Ca 2 times medium (●), Ca 3 times medium (■), Ca 6 times medium (★) and saline solution (○). The numbers in parentheses mean number of rats used. Significantly different from the value of normal Ca medium: * $P<0.05$, ** $P<0.005$, *** $P<0.001$. 
RESULTS

When incubation media were administered to rats, serum Ca levels were decreased. Decline patterns by the first, the second and the third incubation media having different Ca concentrations are exhibited in Figure 1A, B, C, respectively. In Figure 2, decline patterns are shown when salmon calcitonin (10, 25 and 100 mU) was administered as a calcitonin standard.

In Figure 3A, areas (cm²) declined by the administration of normal Ca Ringer (1×), Ca 2 times medium (2×), Ca 3 times medium (3×), Ca 6 times medium (6×), salmon calcitonin (sCT: 10, 25 and 100 mU) and saline solution as a control, which were lower than the initial Ca level during 0–3 hr in each of the incubation time (the first culture, the second culture and the third culture), are shown in histograms. Among the areas made by administration of 4 kinds of the first incubation media, there was no significant difference, when
they were compared after the subtraction of the area by the saline administration. The calcitonin released in this incubation time was in large quantities for its short term. Those amounts were approximately compared to 30–72% of salmon calcitonin 10 mU judging from the area. However, among the second incubation media, the area made by Ca 2× medium was the largest (P<0.001 to the normal Ringer), which corresponded to 118% of salmon calcitonin 25 mU. The area by Ca 3× medium was also significantly larger than that by saline control (P<0.05). When the third incubation media were administered, the area made by Ca 2× medium was also the largest (P<0.005 to the normal Ringer), which was comparable with 87% of salmon calcitonin 25 mU. Furthermore, the areas by Ca 3× and Ca 6× media were also larger than that by the saline control (P<0.05). In Figure 3B, the total areas, which include all areas declined during 48.5 hr, are exhibited in histograms. The area made by Ca 2× medium was 1.9 times larger than the normal Ringer. The area made by Ca 3× medium was 1.4 times larger than the normal Ringer. The area by Ca 6× was only 1.1 times larger.

**DISCUSSION**

It has been reported that in the incubated thyroid gland of the pig, secretion of calcitonin into the medium was concentrated in the first 15 min period [4]. In the present study, it was known that also in the bullfrog, release of large quantity of calcitonin from the incubated ultimobranchial gland occurred in the first 30 min incubation time. It has been reported that in the leopard frog, *Rana pipiens*, ultimobranchial glands are innervated, and that the secretion of calcitonin is suppressed by the nervous system [12]. Therefore, it is possible that also in the bullfrog, under *in vitro* condition, suppressive control of the nervous system for ultimobranchial secretion was eliminated. We reported previously that in the ultimobranchiectomized bullfrog tadpoles, the serum Ca concentration of the environmental water was about 3 times that of the serum Ca level (the average value of the serum Ca before the treatment was 7.4 mg/100 ml). Final serum Ca value was 14.3 mg/100 ml which was 1.9 times that of the initial value. However, the serum Ca levels of the sham-operated group did not show any increases. This fact suggests that in bullfrog tadpoles, the ultimobranchial glands can secrete calcitonin effectively against at least 2 times rise of the serum Ca concentrations. Furthermore, when 0.5 ml of extremely high Ca water (200 mg/100 ml) was infused in the intestine of the ultimobranchiectomized bullfrog tadpoles, the serum Ca concentrations was raised 1.6 times that of the sham-control group at 24 hr later [14]. These observations suggest that in the ultimobranchiectomized bullfrog tadpoles, serum Ca level does not elevate more than twice of the normal level at least for a short term, even if any treatments are done. Therefore, also in the adult bullfrog, serum Ca level may not elevate more than twice of the normal level. In mammals, it has been known that secretion rate of calcitonin is directly and linearly related to the actual increment in plasma Ca level [1–3]. On the other hand, ultimobranchial glands of teleosts do not respond to rises of serum Ca level [9, 10]. In the present study using adult bullfrog, it was the Ca 2× medium that was most effective on releasing calcitonin from incubated ultimobranchial glands. Therefore, calcitonin secretion from ultimobranchial glands may be related to the rise of serum calcium levels, to some extent in bullfrogs. The Ca 3× medium and Ca 6× medium were also effective, but less potent than Ca 2× medium. In anuran amphibians, delicate control system of calcitonin secretion as in mammals may not have been established.

**REFERENCES**

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