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Serum Concentrations of Ionized Calcium and Calcitonin under Basal and Hypercalcemic States in Oophorectomized Rats

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ABSTRACT. It is very important to measure serum ionized calcium (iCa) accurately in the evaluation of calcitonin (CT) secretion in patients with abnormal Ca metabolism. To evaluate CT secretion in relation to iCa concentration, a newly developed calcium ion analyzer which simultaneously measured the pH level was used. In oophorectomized rats (deficiency of E_2), regardless of normal iCa level, a low basal CT concentration was found. However, normal capacity of CT secretion in response to hypercalcemic stimulation was observed. Therefore, this low basal CT level might be dependent not on the iCa level but on other factor.

Key words : ionized calcium — oophorectomy — calcitonin

It is well known that free (ionized) calcium has biological activity in the permeability of cellular membranes, excitation of neuromuscles, blood coagulation, activation of enzymes and other physiological functions. However, calcium circulates in plasma in three forms; ionized, protein-bound and complex-bound. Therefore, to understand the calcium metabolism, it is important to measure accurately the ionized calcium concentration.

There exists the evidence on bone loss associated with aging in female, especially after menopause or oophorectomy. Studies in human indicate that age and sex influence calcitonin secretion.[1] Calcitonin has the effect to decrease bone resorption by inhibiting the activity of osteoclast and osteocyte, and produces hypocalcemia and hypophosphatemia.

In this study, a newly developed flow-through electrode[2] was used to measure ionized calcium concentration in control and oophorectomized rats under basal and hypercalcemic states. In addition, calcitonin secretion in response to calcium challenge was studied.

MATERIALS AND METHODS

Female C.D. rats (Charles River Breeding Lab. Inc., Mass.), weighing from 180 to 200 g, were used for this study. They were fed on a diet of calcium 1.2%, phosphorus 0.86% and vitamin D 4.3 IU/g. The experiments were conducted after an over-night (16-18 hrs) fasting. Rats were divided into 2 groups; (1) the control group received Sham treatment, and (2) three weeks

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prior to the experiment, the oophorectomized rats group was operated on. Each group contained 10 rats.

Calcium chloride (1 mg calcium per 100 g rat) was administered intravenously in the jugular vein within 30 sec. Blood samples were taken at 0 and 5 min after the initiation of infusion. The samples were analyzed for total calcium (TCa), ionized calcium (iCa), phosphorus (P), calcitonin (CT), albumin, and estradiol (E_2) concentrations. The iCa to TCa ratios in baseline and post-calcium load were also calculated. The increases in TCa, iCa and CT concentrations (ΔTCa, ΔiCa and ΔCT) after hypercalcemic stimulation were determined. Furthermore, as an index of CT secretion, the ΔCT to ΔTCa ratios (ΔCT/ΔTCa) and ΔCT to ΔiCa ratios (ΔCT/ΔiCa) were obtained. The increase in P concentration (ΔP) after Ca infusion was also calculated. Biochemical parameters were measured as follows; TCa by a Corning Calcium Analyzer Model 940 (Corning Scientific Instrument, Mass.), iCa by a Model SERA-250 Blood Ion Analyzer (Horiba Ltd., Kyoto), and P and albumin by an assay kit (Pierce, IL, and Sigma Chemical Co., MO, respectively). E_2 levels were determined by the radioimmunoassay (RIA) kit (NMS Pharmaceuticals Inc., CA). CT was assayed with RIA using human CT as a standard and labelled hormone, and rabbit antihuman CT serum for an antibody. The assay was done in a non-equilibrium system. Bound and free fractions were separated by the double antibody method. In this assay system, sensitivity was 20 pg/ml. Calculation of the corrected Ca level was done by Payne's formula[3]; [Corrected Ca value = TCa concentration (mg/dl)-serum albumin concentration (g/dl) + 4.0]. The results of all experiments were expressed as the mean ± S.D. Statistical analysis between two groups (control vs. oophorectomy) was done by Student's t test.

RESULTS

Table 1 shows the body weight, E_2 and albumin concentrations in control and oophorectomized rats. As for body weight, the oophorectomized group showed a significantly higher value, when compared with the control group ($p < 0.001$). The E_2 concentration in oophorectomized rats was significantly lower than that in control rats ($p < 0.001$). However, no significant difference in the albumin level was observed.

TABLE 1. Background of two groups; control (Sham operation) and oophorectomized rats. Significantly high body weight and low E_2 level were observed in oophorectomized rats. Mean ± S.D. (N=10). Control vs. oophorectomy (*$p<0.001$).

	Control	Oophorectomy
Body weight (g)	245±10	275±18*
E_2 (pg/ml)	107±34	61±10*
Albumin (g/dl)	3.20±0.20	2.97±0.21

Fig. 1 shows the changes in TCa and iCa concentrations, and iCa/TCa ratio after Ca infusion. No significant difference in the TCa level was observed between control and oophorectomized rats in both basal and hypercalcemic states. On the other hand, significantly lower level of iCa was observed in the

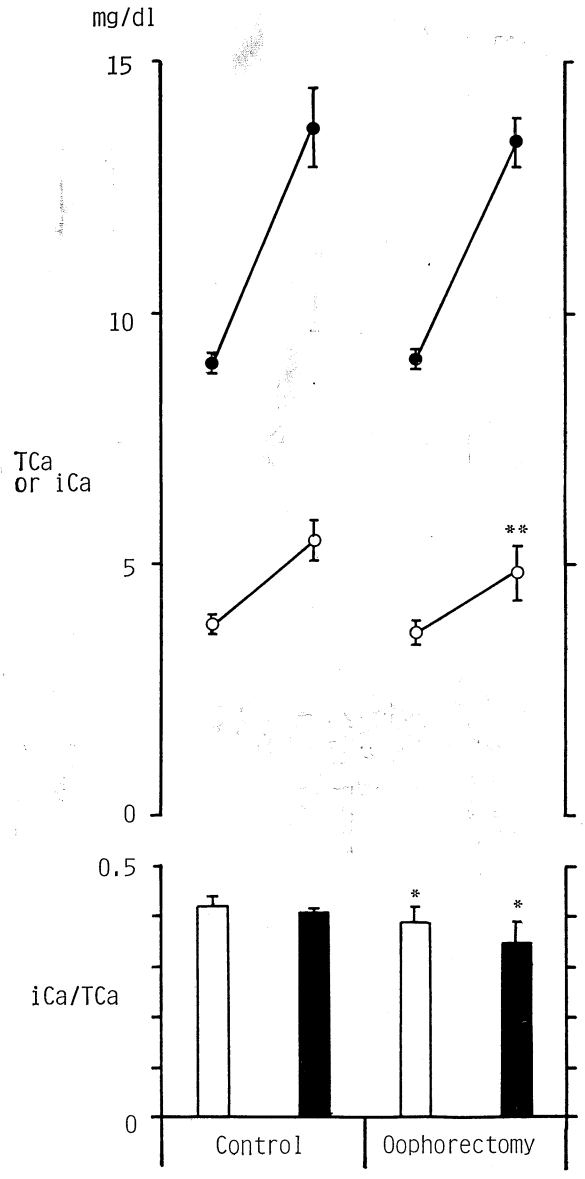

Fig. 1. Plasma TCa, iCa and iCa/TCa responses to Ca infusion in control and oophorectomized rats. In oophorectomized rats, iCa concentration after Ca infusion and iCa/TCa value in both basal and hypercalcemic states was significantly low.
Mean ± S.D. (N=10). Control vs. oophorectomy (*$p<0.01$, and **0.02).

oophorectomized rats after Ca infusion ($p < 0.02$). Significantly lower iCa/TCa ratio under both basal and hypercalcemic states was also demonstrated in oophorectomized rats ($p < 0.01$).

Fig. 2 shows the changes in P concentration and the ΔP values after Ca

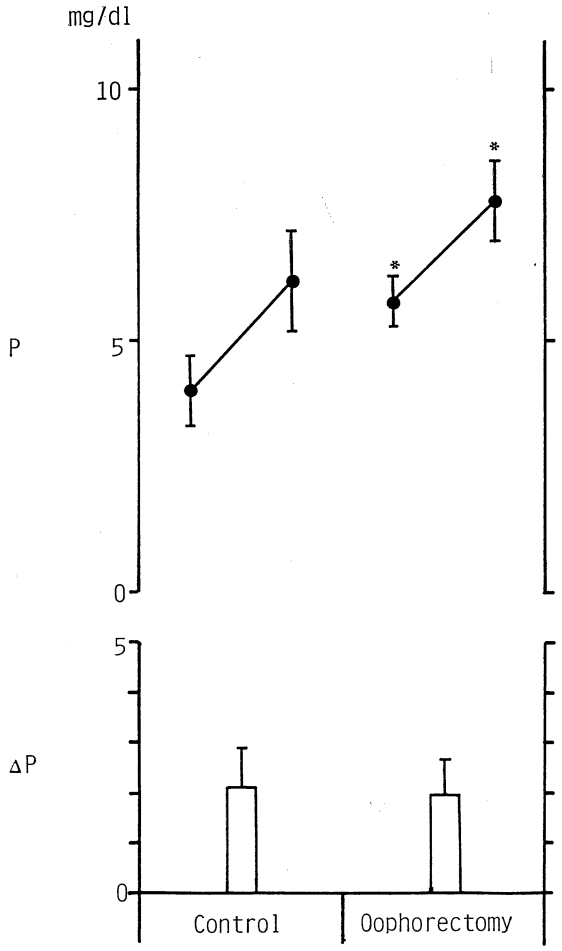

Fig. 2. Plasma P and ΔP responses to Ca infusion in control and oophorectomized rats. In oophorectomized rats, P concentration was significantly high in both basal and hypercalcemic states.
Mean $\pm$ S.D. (N=10). Control vs. oophorectomy (*$p<0.001$).

infusion. Significantly higher P concentration was obtained in oophorectomized rats before and after Ca infusion ($p < 0.001$). On the other hand, no significant difference of ΔCa was observed.

Fig. 3 shows the changes in CT concentration after Ca infusion. Before infusion, significantly lower CT concentration was observed in the oophorectomized rats ($p < 0.01$). On the other hand, after Ca infusion, no significant difference of CT level between the control and oophorectomized rats was observed.

Fig. 4 shows the changes in ΔTCa, ΔiCa, ΔCT, ΔCT/ΔTCa and ΔCT/ΔiCa values after Ca infusion. No significant differences in ΔTCa, ΔiCa, ΔCT, ΔCT/ΔTCa and ΔCT/ΔiCa were observed between the control and oophorectomized rats.

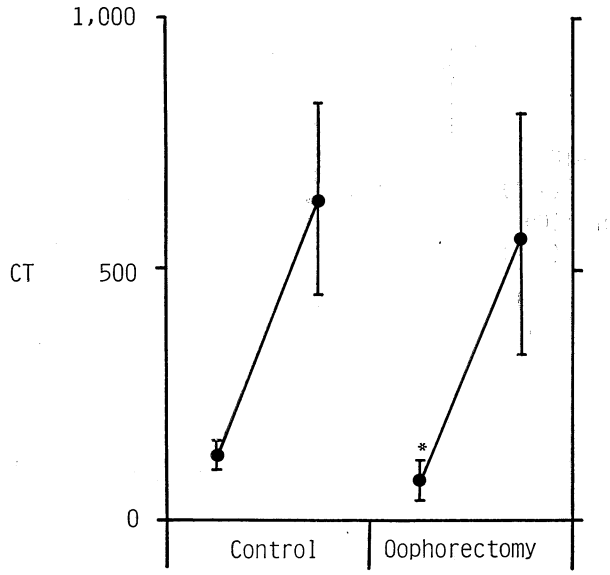

Fig. 3. Plasma CT responses to Ca infusion in control and oophorectomized rats. In oophorectomized rats, basal CT concentration was significantly low. Mean ± S.D. (N=10). Control vs. oophorectomy (*p<0.01).

Table 2 shows the result of measured TCa, corrected Ca and measured iCa in the basal state. The corrected Ca concentration was significantly higher in oophorectomized rats, when compared with the control group ($p < 0.01$). However, as for the measured TCa and the measured iCa, no significant differences were found between control and oophorectomized rats.

TABLE 2. Concentration of measured TCa, corrected Ca and measured iCa in control and oophorectomized rats. Corrected Ca value was falsely high in oophorectomized rats. Mean ± S.D. (N=10). Control vs. oophorectomy (*p<0.01).

	Control	Oophorectomy
Measured TCa (mg/dl)	9.03±0.15	9.12±0.22
Corrected Ca (mg/dl)	9.83±0.15	10.15±0.22*
Measured iCa (mg/dl)	3.85±0.15	3.63±0.25

DISCUSSION

The decreased bone mass that occurs with age in human, especially post-menopausal females, is a complex phenomenon. Although many factors contribute to this bone loss, increased bone resorption seems to be a common pathogenetic mechanism. As one of causes of bones loss, the age-related calcitonin deficiency has been demonstrated.[1,4] Furthermore, it is well known that a deficiency of E_2 production, *e.g.* oophorectomy, causes bone loss. Therefore, in order to clarify

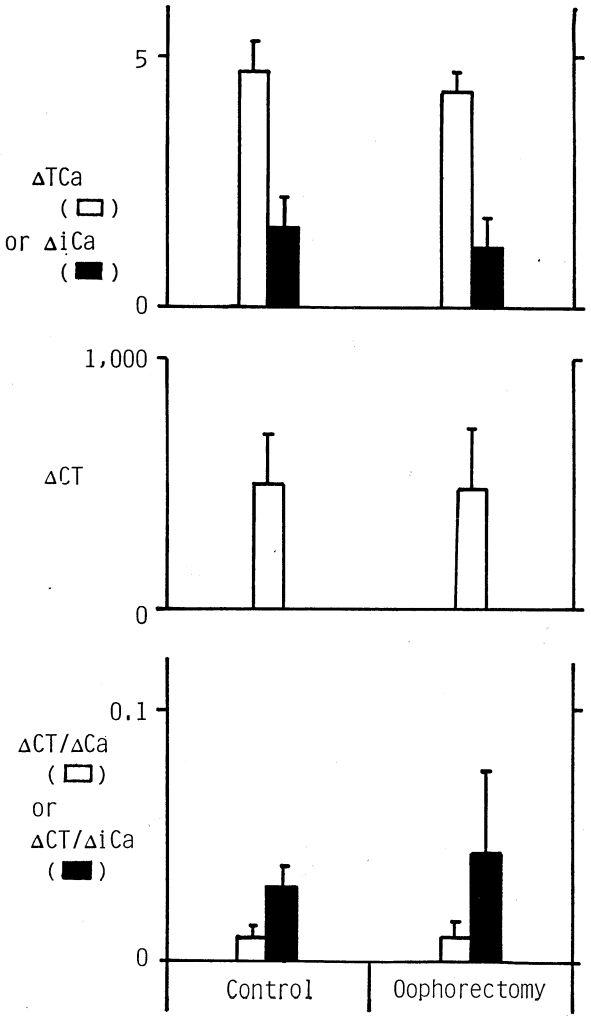

Fig. 4. Values of ⊿TCa, ⊿iCa, ⊿CT, ⊿CT/⊿TCa and ⊿CT /⊿iCa in responses to Ca infusion in control and oophorectomized rats. No significant changes of ⊿TCa, ⊿iCa, ⊿CT, ⊿CT/⊿TCa and ⊿CT/⊿iCa values could be observed between two groups.
Mean ± S.D. (N=10).

the pathogenesis of the bone loss in oophoretomy, it is important to know the secretion of CT, which is the inhibitory hormone of bone resorption. In the present study, relationship between TCa or iCa concentration and CT secretion in oophorectomized rats was investigated.

SERA-250, calcium ion analyzer, used in this study is able to measure simultaneously iCa and pH in whole blood, plasma and serum. In this machine, a unique flow-through type is applied to minimize carry-over and clogging. So far, with conventional electrode methods for iCa, the operation is time-consuming and troublesome, and the data reliability is somewhat questionable to evaluate true iCa value. Since iCa concentration has a close relation with pH, and is

changing together with pH, it is important to obtain the corrected iCa by measuring iCa and pH simultaneously. Therefore, SERA-250, being able to obtain iCa level corrected automatically for pH, is a reliable unit. We found by using SERA-250 that the TCa concentration after Ca infusion and corrected Ca value, calculated from Payne's formula, did not reflect a true iCa concentration. Furthermore, significantly low fraction of iCa to TCa, being concomitant with significantly high P levels, was observed in oophorectomized rats. These data suggested that iCa concentration might be influenced not only by albumin level but also by other factors such as phosphorus level which could form calcium-phosphorus complex.[5]

In a deficiency of E_2 (oophorectomized rats) basal CT concentration was low. Despite a normal basal iCa concentration, the low basal CT concentration indicates relative deficient secretion of this hormone, and might lead to the occurrence of osteoporosis. This possibility was also supported by the fact that P concentrations in oophorectomy in basal and hypercalcemic state were significantly higher than those in control. This elevation of P concentration might be suggesting the occurrence of bone resorption in oophorectomized rats. However, the secretion of CT after Ca infusion was normal, when it was assessed as $\Delta CT/\Delta iCa$. This result is different from previous paper.[4] One of these differences might be concerned with the measurement of not iCa but TCa in such reports. Thus, it could be shown that the low basal CT level in E_2 deficient state was due to other causes different to the iCa level. Since osteoporosis is a heterogeneous disease, it is unlikely that any single factor such as CT deficiency can be considered as its pathogenetic basis. Extensive studies would be necessary to further evaluate whether relative CT deficiency in oophorectomy is related to bone loss.

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