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Effect of Ovariectomy on Circulating Calcitonin Levels in the Rat (41029)[1]

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Abstract. The relationship between the reproductive system and calcitonin secretion was investigated in the rat. Plasma levels of calcitonin were measured in normal and ovariectomized rats at basal levels and during calcium challenge. Sensitivity of the normal and operated animals to exogenous calcitonin administration was also studied. Ovariectomy leads to a transient fall in both CT levels and in the secretion of the hormone in response to a calcium challenge. However, a greater sensitivity of ovariectomized animals to salmon calcitonin is maintained after the return to normal of both the basal level of the hormone and of its secretion in response to a calcium challenge. Estrogen substitutive therapy does not restore CT levels and antagonizes the action of exogenously administered CT in ovariectomized rats. These results suggest the existence of a direct or indirect effect of ovarian factors, other than estrogens, on CT secretion.

Several lines of investigation favour the existence of a link between calcitonin (CT) secretion and reproductive hormones such as: (a) differences in basal levels of the hormone in relation to sex for example higher levels of CT in adult female versus male rats (1) and the reverse in humans (2–4). (b) increased levels of immunoreactive CT in plasma during gestation in sheep (5, 6), human (7), and rat (8). The hypothesis of the existence of a connection between sexual hormones and CT has received some support by our recent demonstration of a fall in CT levels and a decrease in CT secretion after calcium challenge in ovariectomized rats (9). In the present work possible relationship between the ovary, estrogens, and CT in the rat have been more precisely investigated.

Materials and Methods. *Animals.* Female wistar CF rats aged 3 months (250 g body wt) were randomly distributed in two equal groups—ovariectomized and controls—. Twenty animals per experience were used in the calcium and CT challenge experiments and 100 animals to measure basal CT level of estrogens treated or untreated animals. During the experimental period the animals were fed *ad libitum* and had free access to tap water. Blood samples were obtained via the retroorbital sinus using heparin as anticoagulant. Plasma was separated at 4° and immediately used for estimation of calcium by atomic absorption. Aliquots were frozen until assayed for CT.

Operation. Ovaries were removed by a retroperitoneal approach via a bilateral dorsal incision under ether anesthesia in 3-month-old female rats. Sham-operated animals were subjected to bilateral dorsal incision under anesthesia.

Radioimmunoassay of calcitonin. CT was estimated by a sensitive assay for human CT which shows a total cross-reaction with murine CT (10, 11). In brief 50 μl of rat plasma was incubated in the presence of antiserum against human CT (HCT) at a final dilution of 1/250,000 in phosphate-buffered albumin to a final volume of 400 μl for 6 days; then 100 μl of ^{125}I-HCT was added and incubation continued for another 5 days. Free and bound hormone were separated by dextran charcoal absorption. Standard curves were performed using synthetic HCT and protein effects were controlled by the addition of affinity-stripped plasma (50 μl per tube) (12).

Estrogens treatment. Animals received subcutaneously 1.25 μg/day of estradiol benzoate (OB) during 14 days. The untreated animals received the vehicle alone.

Calcium challenge. Six weeks after abla-

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tion of ovaries 10 ovariectomized (OVX) and 10 control (CON) animals received 1.38 mg of Ca/100 g body wt (as calcium gluconate) intraperitoneally and blood samples were obtained at 0, 15, 30, and 60 min after injection. Blood levels of calcium and CT were measured in each animal.

Calcitonin challenge. Thirteen days and two months after operation 10 ovariectomized animals and 10 controls received ip 1.6 U of synthetic salmon calcitonin (SCT) (Sandoz). The same experiment was performed 14 days after operation on 20 OVX and 20 CON, half of the animals had been treated to estrogens during 14 days. Blood samples were obtained by retroorbital puncture 0, 1, 3, and 6 hr after injection and plasma calcium levels were estimated.

Statistical treatment of the data. Differences between basal levels of CT were tested by a nonparametric test (Wilcoxon test). CT levels in the calcium challenge experiment and calcium levels were compared using the *t* test.

Results. *Plasma CT levels.* Seven days after operation median level of CT of ovariectomized animals was decreased to undetectable level ($P < 0.01$) as compared to controls. Estrogen-treated controls had a lower median CT level as compared to untreated controls ($P < 0.01$). No difference was observed between treated and untreated ovariectomized animals (Fig. 1).

Calcium challenge. Six weeks after ablation of the ovaries, neither the induced hypercalcemia nor the levels of CT were significantly different from controls ($P > 0.05$) (Fig. 2).

Injection of exogenous calcitonin. Thirteen days after operation, injection of SCT lowered plasma calcium levels more in OVX than in controls (Fig. 3a) and the higher sensitivity of OVX rats to exogenous CT persisted 2 months after the operation (Fig. 3b). Hypocalcemia was maximal 3 hr after injection, 13 days after operation while it persisted for 6 hr at 2 months. The same experiment performed on estrogen-treated and -untreated animals 14 days after operation (Fig. 4) showed a smaller sensitivity of estrogen-treated animals as compared to untreated animals in the same state, see legend of Fig. 4 for results of statistical tests.

Discussion. *1. Ovariectomy and calcitonin levels.* Our results fully confirm our precedent experiment, i.e., the fall of plasma CT levels after ovariectomy in the rat. However, this fall appears to be transient as CT levels return to normal within 1 month. More so, the decreased secretion of CT in response to a calcium challenge observed 7 days after ovariectomy (9) is no more detectable when basal CT levels have returned to normal. The transient fall in CT levels after ovariectomy is not corrected by

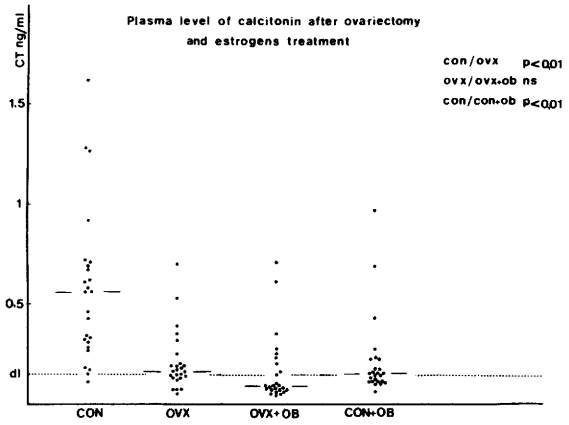

FIG. 1. Plasma CT levels 7 days after ovariectomy, half of the animals were treated with estradiol benzoate (OB) 1.25 μg/day during the experimental period (ovariectomized animals, OVX; controls, CON).

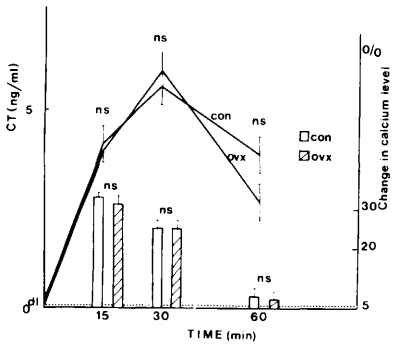

FIG. 2. Changes in plasma concentration of calcitonin (CT) and percentage increase of plasma calcium levels following calcium load 6 weeks after ovariectomy. (Animals were identified before injection and bled at appropriate times.)

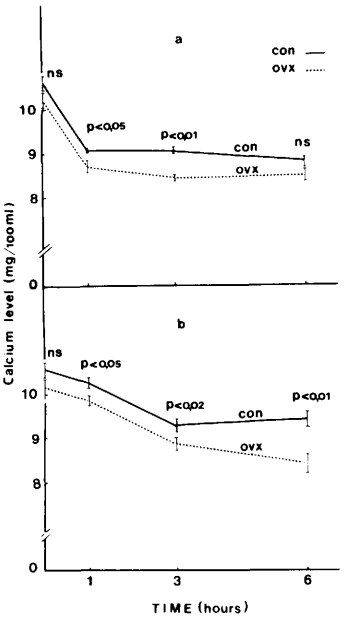

FIG. 3. Effect of exogenous CT on plasma calcium levels of ovariectomized (OVX) and control (CON) animals 13 days (a) and 2 months (b) after ovariectomy.

injecting estrogens, implying that the fall in CT levels is probably not due to a lack of this steroid. Furthermore estrogens at the dose level and schedule used depress CT levels in the control animals.

2. Ovariectomy and plasma calcium levels. During the 2-month period, we have not observed a significant fall in plasma calcium in the ovariectomized rat, whereas a decrease in plasma calcium has been reported after 3 months (13). Fluctuations in CT levels during the course of this study did not affect calcium plasma levels. Whether the maintenance of normal calcium levels is due to a compensatory increase in circulating PTH is being studied. An elevated plasma calcium level is observed in estrogen-treated animals, confirming the transient rise noted by Cruess and Hong in ovariectomized estrogen-treated animals (13).

3. Sensitivity to exogenous CT. The hypocalcemic action of CT appears to be enhanced after ovariectomy, since exogenous hormone was found to be more potent than in control animals. This increased sensitivity is furthermore maintained after the secretion of CT has returned to normal, implying that the higher sensitivity of castrated animals is not the consequence of the lowered levels of endogenous CT. The delay in the maximum fall in blood calcium in the 2-month-older animals is probably due to a decrease of sensitivity to CT with age. The increased hypocalcemic activity of exogenous CT is probably related to the lack of estrogens in the ovariectomized rats as administration of estrogens to these animals decreased the hypocalcemic effect of exogenous calcitonin. Furthermore even in the intact animal estrogens tend to antagonize this effect of CT. Analogous observation has been reported by Shai and Wallach and Kaplan *et al.* in the intact (14) or in the castrated male rat (15).

4. Ovariectomy and postmenopausal osteoporosis. The higher incidence of osteoporosis in postmenopausal women has been ascribed to a lack of estrogens. We have recently reported that CT levels in postmenopausal osteoporotic patients were lower than in normal age- and sex-matched subjects (16). The present results point to the possible existence of other ovarian factors, which could promote a decrease in endogenous CT levels and thus play a separate role in the genesis of postmenopausal osteoporosis. However, in rats, females have higher levels of CT than males (1), while in humans the reverse has been reported (2–4), it remains to be shown that the same type of link between ovarian

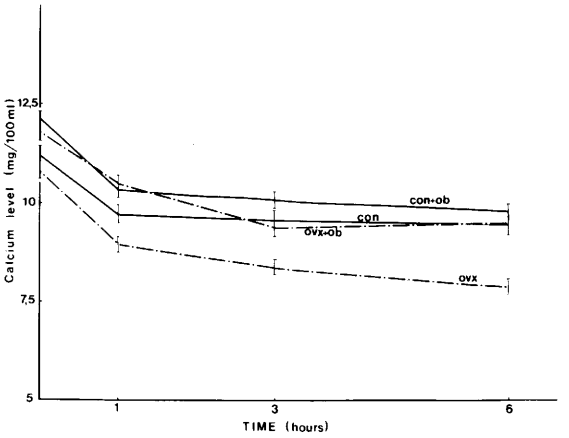

FIG. 4. Effect of exogenous CT on plasma calcium levels of ovariectomized (OVX) and control (CON) animals treated or untreated with OB during 14 days.

	0	1 hr	3 hr	6 hr
CON/OVX	ns	$P < 0.02$	$P < 0.01$	$P < 0.001$
CON/CON OB	$P < 0.01$	ns	ns	ns
OVX/OVX OB	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$
CON OB/OVX OB	ns	ns	$P < 0.05$	ns
CON/OVX OB	ns	$P < 0.05$	ns	ns

function and CT secretion we observe in rats exists in humans.

Though our results do confirm the existence of a link which may be direct (ovarian factors other than estrogens) or indirect (via hypothalamic or hypophyseal hormones) with the ovary, the transient nature of the decrease in calcitonin secretion raises the question of the mechanisms involved both in the acute fall of CT after ovariectomy and in the restoration of a normal CT level.

1. Roos, B. A., Cooper, C. W., Frelinger, A. L., and Deftos, L. J., Endocrinology **103**, 2180 (1978).
2. Heath, H., and Sizemore, G. W., J. Clin. Invest. **60**, 1135 (1977).
3. Parthemore J. G., and Deftos, L. J., J. Clin. Endocrinol. Metabol. **47**, 184 (1978).
4. Hillyard C. J., Stevenson, J. C., and MacIntyre, I., Lancet **1**, 961 (1978).
5. Garel, J. M., Care, A. D., and Barlet, J. P., J. Endocrinol. **62**, 497 (1974).
6. Garel, J. M., Savajol, H., and Barlet, J. P., Biol. Neonate **28**, 207 (1976).
7. Samaan, N. A., Wigoda, C. and Castillo, S. G., *in* "Endocrinology," Proc. 4th Int. Symp., London 1973 (S. Taylor ed.), p. 362. Heinemann, London (1974).
8. Garel, J. M., and Jullienne, A., J. Endocrinol. **75**, 373 (1977).
9. Cressent, M., Bouizar, Z., Pidoux, E., Moukhtar, M. S., and Milhaud, G., C.R. Acad. Sci. D **289**, 501 (1979).
10. Milhaud, G., Tharaud, D., Jullienne, A., and Moukhtar, M. S., *in* "Endocrinology", Proc. 3rd Int. Symp., London, 1971 (S. Taylor ed.), p. 380. Heinemann, London (1972).
11. Moukhtar, M. S., Tharaud, D., Jullienne, A., Raulais, D., Calmettes, C., and Milhaud, G. Experientia **29**, 552 (1973).
12. Moukhtar, M. S., Jullienne, A., Tharaud, D., Taboulet, J., and Milhaud, G., C.R. Acad. Sci. D **276**, 3445 (1973).
13. Cruess, R. L., and Hong, K. C., Endocrinology **104**, 1188 (1979).
14. Shai, F., and Wallach, S., Endocrinology **93**, 1044 (1973).
15. Kaplan, E. L., Singh, M., and Arnaud, C. D., Curr. T., Surg. Res. **2**, 167 (1970).
16. Milhaud, G., Benezech-Lefèvre, M., and Moukhtar, M. S., Biomed. **29**, 272 (1978).

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