Calcitonin treatment and plasma prolactin levels in pregnant and lactating ewes

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Summary. The intravenous injection of a physiological dose of salmon calcitonin (0.5 µg/kg bw) in 5 ewes on day 125 of gestation induced a slight but significant hypo-calcaemia. It had no significant effect on plasma prolactin levels.

Ten days after parturition the same dose of salmon calcitonin given in a similar manner to 5 ewes has no effect on suckling-induced prolactin release, although it significantly decreased plasma calcium concentration.

This demonstrates that short-term changes in plasma calcitonin concentration have no effect on basal plasma prolactin concentration or on suckling-induced prolactin surge in ewes.

Introduction.

One physiological role of the hypocalcaemic hormone, calcitonin (CT), is the protection of the skeleton against excessive demineralization during pregnancy and lactation. This has been demonstrated in rats (Lewis *et al.,* 1971 ; Taylor, Lewis and Balderstone, 1975), goats (Barlet, 1974) and women (Stevenson, Hillyard and MacIntyre, 1979). Among the hormones involved in the regulation of calcium (Ca) metabolism, ovine prolactin (PRL) infused into intact male rats (10 µg per rat per h for 18 h) has been reported to induce a significant increase in plasma Ca concentration (Mahajan, Robinson and Horrobin, 1974). Similarly, ovine PRL injected into intact male rats (1-2 mg per rat daily for 2 days) increased significantly Ca transport across everted jejunal sacs (Mainoya, 1975). More recently, PRL has been shown to stimulate 1α-hydroxylate activity (Spanos *et al.,* 1976 ; Baksi *et al.,* 1978 ; MacIntyre, Brown and Spanos, 1979), thus leading to high plasma levels of 1,25-dihydroxyvitamin D_3, which in turn increase intestinal Ca absorption (Robinson *et al.,* 1982). Pahuja and De Luca (1981) have also

investigated the role of PRL on Ca metabolism in weanling male rats fed a vitamin D-deficient diet from 10 to 16 weeks. These animals were hypocalcaemic and had no detectable 1,25-dihydroxyvitamin D_3 in the plasma. They were then injected with 250 µg of PRL and killed at intervals between 4 and 12 h after injection. PRL had no significant effect on plasma Ca in 4 h, but caused a significant increase within 8 h ; the everted sac method confirmed that intestinal Ca transport was significantly increased from the 4th to the 10th four after PRL treatment, although plasma levels of vitamin D metabolites remained undetectable. Thus, if CT effectively prevents excessive bone loss during periods of stress on Ca metabolism, it is difficult to understand how CT treatment could decrease plasma PRL concentrations in humans (Carman and Wyatt, 1977 ; Isaac et al., 1980) and rats (Olgiati et al., 1982).

In the work reported here, we have studied the influence of a physiological dose of CT on plasma PRL levels in pregnant or lactating ewes.

Material and methods.

Animals and treatments. — Ten primiparous Limousine ewes of known gestation date and weighing 58 ± 4 kg were used in October and November. Eight had a single foetus and two were bearing twins, detected by radiography on day 100 of gestation. To accustom the ewes to handling and to reduce the likelihood of spontaneous prolactin release following stress (Bryant, Linzell and Greenwood, 1970), each ewe was assigned to an individual pen at least 15 days before the experiment. They were fed hay and grain concentrate so the daily intake of each animal was 15 g of Ca and 10 g of inorganic phosphorus. Tap water was freely accessible.

At 9 a.m. on day 125 of gestation, five ewes, one of which was bearing twins, were i.v. injected with 0.25 µg/kg bw of synthetic salmon CT (sCT) (Calsyn, Armour-Montagu ; lot P 5042) dissolved in 0.5 ml 0.9 % NaCl containing 0.01 % bovine serum albumin. This bolus injection was followed by an i.v. infusion of the same dose of the hormone given over a 20-min period. The five control animals received the same volume of solvent (1 ml/kg bw) in the same way.

Parturition occurred on day 145 of gestation. The birth weight of the 12 lambs, born alive and in good condition, was 3.3 ± 0.4 kg. Ten days later at 10 p.m. the lambs were removed from their dams and placed in a room so that the ewes could neither see nor hear them. The next morning at 9 a.m. the ewes were i.v. injected with sCT (0.25 µg/kg bw), followed by 25-min i.v. infusion of the same dose of the hormone. The five control ewes received the same volume of solvent (1 ml/kg bw) in the same way. The lambs were put with their dams at 9 : 10 a.m. to suckle for 10 min and were removed again from the ewes during the experimental period.

Each injection and infusion was given through a catheter inserted in the right jugular vein. Serial blood samples (2 ml each) were taken through a catheter in the left jugular vein. Both catheters were implanted the day before each experiment. After the blood was centrifuged, the plasma was frozen until analysis.

Analysis. — Plasma prolactin (Kann, 1971) was measured by double antibody radioimmunoassay using NIH PS6 as a standard. Sensitivity was 0.3 ng/ml of plasma. The inter and intra-assay coefficients of variation were 8 and 6 %, respectively.

Plasma Ca concentration was measured by atomic absorption spectrophotometry (Perkin Elmer 400). The results were expressed as the mean ± SEM. The statistical significance of differences measured between the two groups of animals was calculated using the Mann-Whitney U-test.

Results.

In pregnant (fig. 1) as in lactating (fig. 3) ewes, CT treatment induced a slight but significant decrease in plasma Ca level that was not observed in the control animals.
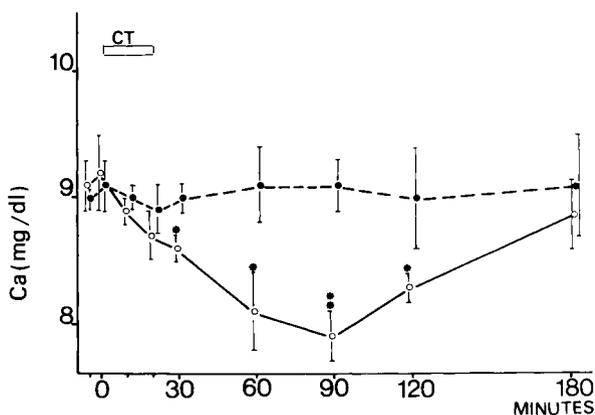

FIG. 1. — *Plasma calcium* (Ca) *concentrations in 5 pregnant ewes treated with calcitonin* (CT : solid line) *and in control ewes* (dashed line) mean ± SEM ; * P < 0.05) ; ** P < 0.01 : comparison between the two groups of ewes).
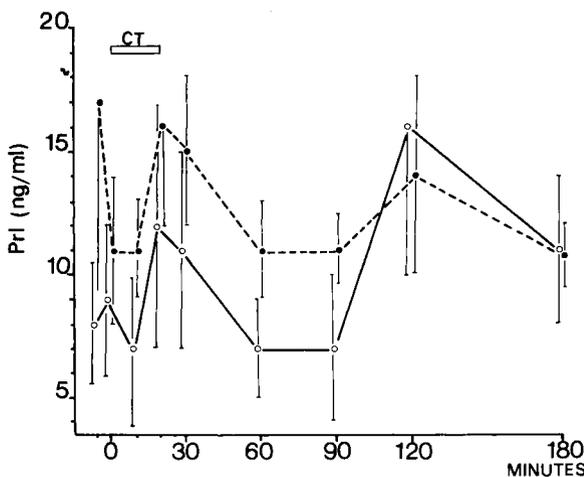

FIG. 2. — *Plasma prolactin* (PRL) *concentrations in 5 pregnant ewes treated with calcitonin* (CT : solid line) *and in 5 control ewes* (dashed line) (mean ± SEM).

Before the experiment, plasma PRL concentrations measured in the 2 ewes bearing twins were 10.7 ng/ml and 12.1 ng/ml, respectively. They were not different from those measured in the 8 ewes bearing a single foetus (11.8 $\pm$ 3.9 ng/ml).

No significant variation in plasma PRL concentration (mean value : 10.4 $\pm$ 4.9 ng/ml for both groups of pregnant ewes) was observed during the experimental period during pregnancy. CT treatment had no significant effect on these animals (fig. 2).

Plasma PRL concentrations in the ten lactating ewes increased from 21 $\pm$ 12 ng/ml 10 min before suckling to 838 $\pm$ 137 ng/ml (P < 0.01) 10 min after suckling had ended. Plasma PRL concentration in CT-treated ewes was never significantly different from that measured in the control animals (fig. 4).
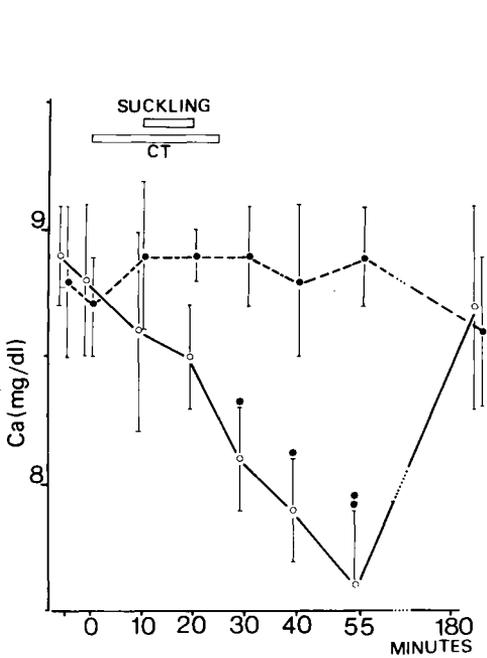
FIG. 3. — *Plasma calcium* (Ca) *concentrations in 5 calcitonin* (CT)-*treated* (solid line) *lactating ewes and in 5 control ewes* (dashed line) (mean ± SEM ; * P <0.05, ** P < 0.01 : comparison between the two groups of ewes).
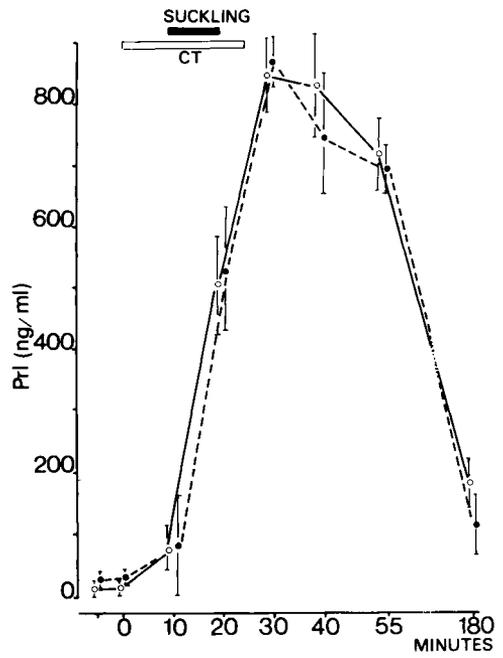
FIG. 4. — *Influence of suckling on plasma prolactin* (Prl) *concentrations in 5 calcitonin* (CT)-*treated* (solid line) *lactating ewes and in 5 control ewes* (dashed line) (mean ± SEM).

Discussion.

Conflicting results have been reported concerning the influence of CT on plasma PRL in humans. Fifteen micrograms of sCT given subcutaneously in the forearm of 9 psychotic patients decreased plasma PRL levels on the following day (Carman and Wyatt, 1977). Similarly, sCT (0.5 µg/kg bw) infused into the brachial

vein of 9 healthy volunteers and 4 hyperprolactinemic patients decreased basal plasma PRL levels in both groups. sCT also decreased the rise in plasma PRL concentration observed in 5 healthy subjects following i.v. injection of thyro-tropin-releasing hormone (TRH ; 5 µg/kg bw) (Isaac et al., 1980). On the contrary, Stevenson et al. (1977) reported that serum PRL levels remained unchanged when measured basally 10 min and 4, 8 and 24 h after subcutaneous injection of 1 mg of human CT in seven volunteers. In 5 normal subjects, 21 µg of sCT i.v. infused for 60 min were unable to induce a significant variation in basal plasma PRL levels, but decreased the rise in plasma PRL following TRH injection (Ceda et al., 1982).

The administration of sCT either i.v. (2.5 or 10 µg/kg bw) or into the lateral cerebral ventricles (2.5 or 25 ng/rat) of unanaesthetized male rats decreases the plasma PRL levels in these animals. However, such injections are completely ineffective in rats with median eminence lesions (Olgiati et al., 1981). The effects of intracerebroventricular (25 ng/rat) or i.v. injections (10 µg/kg bw) of sCT on PRL secretion were also determined in intact or ovariectomized, oestrogen-treated female rats. sCT injections 9 days after oestrogen treatment did not significantly modify PRL plasma levels in intact rats. In ovariectomized, oestrogen-primed animals sCT given intracerebroventricularly or i.v. did not modify the afternoon surge of PRL secretion when injected during the surge. However, suckling-induced PRL secretion was inhibited when sCT was given 30 or 60 min previously (Olgiati et al., 1982).

PRL release in response to suckling in lactating rats appears to be under several hypothalamic controls, including TRH (Grosvenor and Mena, 1967 ; Mattheij et al., 1982). The abrupt surge of PRL in lactating ewes following stimulation of the mammary gland during milking or nursing is due to a secretory reflex originating in the teat nerve endings ; denervation of the mammary gland suppresses the PRL surge induced by milking or nursing. Although i.v. injections of TRH can mimic this PRL surge observed during milking, the putative role of TRH in PRL secretion during mammary stimulation has not been demonstrated in ewes (Kann et al., 1977). Since Ca specifically stimulates PRL synthesis and messenger RNA sequences in GH_3 cells, it could be the intracellular mediator of the action of hypophysiotropic hormones such as TRH (White, Bauerle and Bancroft, 1981). This would explain the apparent discrepancy of the results obtained in lactating rats (Olgiati et al., 1982) and ewes (fig. 4).

We infused approximately 1 ng of sCT per min and per kg of body weight in our animals (0.5 µg/kg bw given in 20-25 min to pregnant or lactating ewes weighing 50-60 kg, respectively). This dose was a physiological dose since the rate of CT secretion measured in sheep is 3-4 ng/min/kg bw (Garel, Care and Barlet, 1974). Such a dose of CT induced a slight but significant decrease in plasma Ca concentrations in pregnant (fig. 1) as well as in lactating (fig. 3) ewes. Although this CT treatment had no significant on plasma PRL levels (figs. 2, 4), in vitro studies (MacLeod et al., 1980) have shown that a decrease in ionic Ca can inhibit the release of this hormone by pituitary cells in culture medium. However, in the adult male rat, PRL has no function in the short-term regulation of Ca homeostasis (Mattheij, Sterrenberg and Swarts, 1980). Intravenous infusion

of EDTA does not affect the suckling-induced rise of plasma PRL levels in female rats, while the same dose of EDTA given intraperitoneally completely blocks this rise (Mattheij et al., 1982).

In conclusion, although several works inducate that PRL plays a role in the regulation of Ca homeostasis during pregnancy and lactation, CT, in our experimental conditions, had no significant effect either on basal plasma PRL levels in pregnant ewes or on the suckling-induced rise of PRL in lactating ewes.

Résumé.   La calcitonine exogène ne modifie pas la prolactinémie de la brebis gestante ou allaitante.

L'injection intraveineuse d'une dose physiologique de calcitonine de saumon (0,5 µg/kg PV, en 20 min) à 5 brebis au 125e j de gestation induit une baisse légère mais significative de la calcémie sans modifier la prolactinémie de ces animaux.

Dix jours après la mise bas, une même dose de calcitonine, injectée dans des conditions analogues, n'a eu aucun effet significatif sur l'hyperprolactinémie induite par la tétée des agneaux chez ces brebis, bien qu'elle diminua significativement la calcémie de ces animaux.

Ces résultats démontrent que, chez la Brebis, des variations de courte durée de la calcitoninémie n'ont aucun effet sur la prolactinémie basale ni sur l'hyperprolactinémie induite par la tétée.

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