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1,25-Dihydroxyvitamin D_3 injections into rat fetuses : effects on fetal plasma calcium, plasma phosphate and mineral content

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Summary. The *in vivo* effects of 1,25-$(OH)_2D_3$ were assessed using fetuses from normal and thyroparathyroidectomized (TPTX) pregnant rats. 21.5-day old decapitated fetuses from TPTX mothers exhibited lowered basal plasma calcium, elevated basal plasma phosphate and an increased percentage of total ash compared to intact littermates. In decapitated fetuses from normal mothers, neither plasma calcium nor plasma phosphate was changed. Subcutaneous injection of 1 μg of 1,25-$(OH)_2D_3$/kg of body weight to 19.5-day old fetuses (intact or deprived of their parathyroid glands by decapitation) from TPTX mothers induced a marked rise in plasma calcium levels (2.01 and 3.66 mg/dl, respectively) 48 h later. Little change occurred in fetuses from normal mothers (1.06 mg/dl in decapitated and no change in intacts). A decrease in plasma phosphate levels was observed with the same dose in both decapitated and intact fetuses from TPTX mothers ($-$ 1.39 and $-$ 0.65 mg/dl, respectively), while no modification was found in fetuses from normal females. Therefore, the hypersensitivity of fetuses from TPTX mothers to 1,25-$(OH)_2D_3$ was unrelated to the development of the fetal hyperparathyroidism secondary to maternal TPTX. The percentage of ash was unchanged in decapitated fetuses from TPTX mothers and was increased in intact littermates after 1,25-$(OH)_2D_3$ treatment. However, these values for total ash may represent alterations in bones and/or soft tissues.

Introduction.

Few investigations have been done on the *in vivo* effects of 1,25-dihydroxyvitamin D_3(1,25-$(OH)_2D_3$) administration to the fetus. In sheep fetuses one month before term, the injection of 1α-$(OH)D_3$ induced a rise in both plasma calcium and plasma phosphate levels (Barlet *et al.*, 1978). It is already known that fetal bones from thyroparathyroidectomized (TPTX) pregnant rats are more sensitive to the *in vitro* effects of 1,25-$(OH)_2D_3$ (evaluated by ^{45}Ca-release in the medium) than fetal bones normal mothers (Rebut-Bonneton *et al.*, 1983). Thus, using both normal and TPTX pregnant rats, we have compared the effects of fetal 1,25-$(OH)_2D_3$ administration on plasma calcium, plasma phosphate and mineral content in the fetus. Moreover, some fetuses were deprived of their parathyroid glands by decapitation *in utero* to evaluate the role of the well-known fetal hyperparathyroidism secondary to maternal hypocalcemia which occurs in TPTX rats (Garel and Geloso-Meyer, 1971).

Material and methods.

Animals. — Pregnant Wistar rats were fed a commercial diet (UAR 103 ; Usine d'Alimentation Rationnelle, Villemoisson/Orge) containing 0.92 % calcium, 0.92 % phosphorus, 0.15 % magnesium and 4 000 IU/kg of vitamin D ; water was given *ad libitum.* Gestational age was calculated from the estimated time of ovulation. A female rat was caged with a male between 17.00 and 09.00 h, and ovulation was assumed to occur at 01.00 h. Pregnant animals were detected by palpation 12.5 days later ; parturition normally takes place in our colony after 22 days.

Experimental procedures. — Pregnant females were TPTX by surgery at 12.5 days of pregnancy under light ether anaesthesia. At 19.5 days of pregnancy, fetuses from TPTX or normal females were decapitated *in utero* under light ether anaesthesia, according to Jost (1947), and received at the same time a subcutaneous injection of 0.1 or 1.0 µg of 1,25-$(OH)_2D_3$/kg of body weight ; untreated fetuses from one other group of normal or TPTX mothers were used as controls. Mothers and fetuses were killed at 21.5 days of pregnancy. Fetuses were excised one by one from the well-ventilated mother under light ether anaesthesia, leaving placental circulation intact. Fetal blood was drawn by section of the axillary vessels with heparinized pasteur pipettes. At the end of the experiment, the maternal blood was removed by intracardiac puncture. The fetuses were then weighed and mineralized at 600 °C for 16 h.

Analysis. — After mineralization, the ash was dissolved in 3N-HCl after its weight had been determined. Calcium and potassium were estimated by flame photometry (Eppendorf, FCM 6341). Inorganic phosphorus was determined by the method of Chen *et al.* (1956). The data were presented as means ± SEM and the significance of differences between treated and control fetuses were evaluated using Student's t-test.

Results.

1. *Effects of 1,25-$(OH)_2D_3$ injections on fetal plasma calcium and phosphate levels.*

Fetuses from normal pregnant rats. — The decapitation of fetuses at 19.5 days of pregnancy had no effect on fetal plasma calcium and phosphate levels at 21.5 days of pregnancy (fig. 1). Fetal injection of 1 µg of 1,25-$(OH)_2D_3$/kg of body weight had no effect on body weight in either intact or decapitated fetuses 48 h later. An increase in plasma calcium level occurred only in decapitated fetuses (12.37 ± 0.38 *vs* 11.31 ± 0.25 mg/dl in untreated fetuses ; $P < 0.05$), whereas plasma phosphate levels were unchanged in both intact and decapitated fetuses (fig. 1).

Fetuses from TPTX pregnant rats. — Plasma calcium levels at term were decreased in intact fetuses from TPTX mothers compared to those observed in intact fetuses from normal females (8.60 ± 0.23 *vs* 11.14 ± 0.21 mg/dl, respectively ; $P < 0.001$), whereas plasma phosphates were increased (13.28 ± 0.45 *vs* 11.27 ± 0.21 mg/dl, respectively ; $P < 0.01$). These data are in agreement with

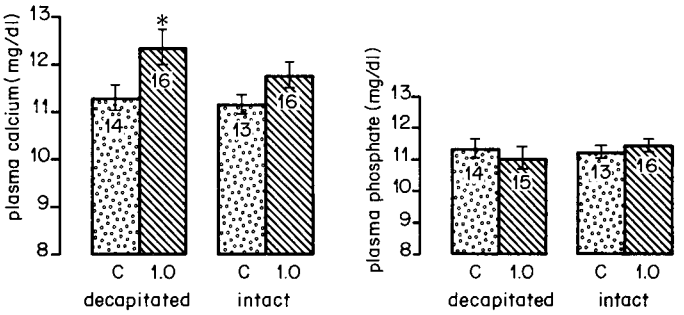

FIG. 1. — *Effects of 1,25-$(OH)_2D_3$ injection on plasma calcium and plasma phosphate levels in intact or decapitated fetuses from normal pregnant rats.*
1 µg of 1,25-$(OH)_2D_3$/kg of body weight was given subcutaneously at 19.5 days of pregnancy and the effects were studied 48 h later.
▭: control fetuses (vehicle only) ; ▨: treated fetuses.
Means ± SEM and the number of animals ; * : $P < 0.05$ from controls.

previously published results (Garel and Geloso-Meyer, 1971 ; Garel, Gilbert and Besnard, 1981). Lowered plasma calcium (− 2.37 mg/dl ; $P < 0.001$) and increased plasma phosphate (+ 1.38 mg/dl ; $P < 0.05$) were observed in decapitated fetuses compared to intact littermates (fig. 2). Plasma calcium levels were sharply increased in both intact and decapitated fetuses after the administration of 1 µg of 1,25-$(OH)_2D_3$/kg of body weight, but the rise was greater in decapitated than in intact fetuses (10.61 ± 0.26 *vs* 8.60 ± 0.23 mg/dl in intact, $P < 0.001$; 9.89 ± 0.42 *vs* 6.23 ± 0.31 mg/dl in decapitated, $P < 0.001$). For the same dose of the vitamin D_3 metabolite, the plasma phosphate level was slightly decreased but the difference was only significant for decapitated fetuses (fig. 2).

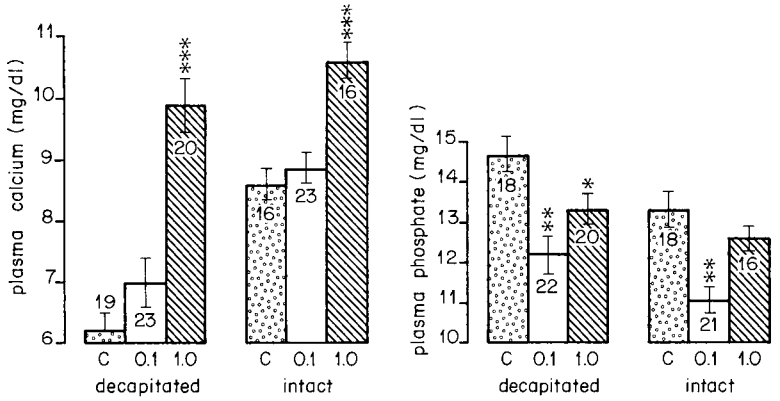

FIG. 2. — *Effects of 1,25-$(OH)_2D_3$ injection on plasma calcium and plasma phosphate levels in intact or decapitated fetuses from TPTX pregnant rats.*
Both doses of 1,25-$(OH)_2D_3$ were given subcutaneously at 19.5 days of pregnancy and the effects were studied 48 h later.
▭: control fetuses ; ▭: 0.1 µg of 1,25-$(OH)_2D_3$/kg of body weight ; ▨: 1 µg of 1,25-$(OH)_2D_3$/kg of body weight.
Means : ± SEM and the number of animals.
* : $P < 0.05$ from controls ; ** : $P < 0.01$ from controls ; *** : $P < 0.001$ from controls.

The injection of a lower dose (0.1 µg/kg of body weight) failed to modify plasma calcium levels in either intact or decapitated fetuses (fig. 2). In contrast, a decrease in plasma phosphate was observed in both groups of fetuses (fig. 2) (12.18 ± 0.43 *vs* 14.67 ± 0.41 mg/dl in untreated decapitated fetuses, $P < 0.01$; 11.06 ± 0.32 *vs* 13.28 ± 0.45 mg/dl in untreated intact fetuses, $P < 0.01$).

2. *Mineral content of fetuses from TPTX mothers injected with 1,25-$(OH)_2D_3$.*

Fetal weight was unchanged in decapitated and intact fetuses 2 days after injection of 1 µg of 1,25-$(OH)_2D_3$/kg of body weight (fig. 3). The percentage of

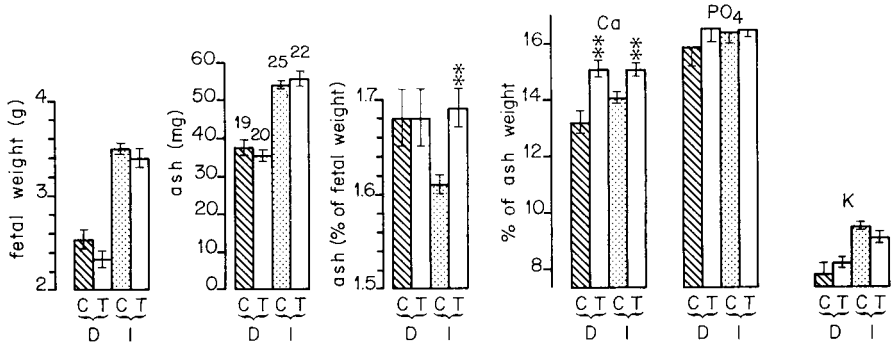

FIG. 3. — *Effects of 1,25-$(OH)_2D_3$ injection on mineral accumulation in intact* (I) *or decapitated* (D) *fetuses from TPTX pregnant rats.*
1 µg of 1,25-$(OH)_2D_3$/kg of body weight was given subcutaneously at 19.5 days of pregnancy and the effects were studied 48 h later.
C : control decapitated (▨) or intact (▩) fetuses.
T : treated decapitated (▭) or intact (▭) fetuses.
Means ± SEM and the number of animals.
**: $P < 0.01$ from controls.

ash was slightly increased in untreated decapitated fetuses compared to untreated intact fetuses (+ 4 % ; $P < 0.05$), and their relative potassium content was reduced (fig. 3). A 5 % increase in the percentage of ash in intact fetuses was observed after injection of 1 µg of 1,25-$(OH)_2D_3$/kg of body weight ; this value was then similar to that found in untreated decapitated fetuses. The percentage of ash was unchanged in decapitated fetuses treated with the vitamin D_3 metabolite. The relative calcium content of ash from treated-intact or treated-decapitated fetuses was slightly increased, while the percentage of ash phosphorus and potassium was unchanged in these animals. All the above parameters were unaltered in both decapitated and intact fetuses 48 h after injection of 0.1 µg of 1,25-$(OH)_2D_3$/kg of body weight (data not shown).

Discussion.

Our results clearly demonstrate that fetuses from TPTX pregnant rats were more sensitive to the *in vivo* action of 1,25-$(OH)_2D_3$ than fetuses from normal mothers. A rise of 2.01 mg/dl in plasma calcium was observed after

administration of 1 μg of 1,25-$(OH)_2D_3$/kg of body weight in intact fetuses from TPTX females, but no change occurred in intact fetuses from normal females. The same phenomenon was found in decapitated fetuses from both groups of pregnant rats (3.66 mg/dl and 1.06 mg/dl for fetuses from TPTX and normal females, respectively). With the same dose, our results on plasma phosphate also showed a greater sensitivity to the vitamin D_3 metabolite of fetuses from TPTX mothers (− 1.39 and − 0.65 mg/dl in decapitated and intact fetuses, respectively) ; plasma phosphates were unchanged in both decapitated and intact fetuses from normal females after injection of 1 μg of 1,25-$(OH)_2D_3$/kg. A greater drop in plasma phosphate level was observed with the lower dose of 1,25-$(OH)_2D_3$ (0.1 μg/kg) in fetuses from TPTX mothers, while no effect on fetal plasma calcium was found.

The results suggest that 1,25-$(OH)_2D_3$ might be involved in the control of fetal plasma calcium ; however, the dose used was high. One month before term, sheep fetuses from normal ewes appeared more sensitive to vitamin D_3 metabolites since 0.1 μg of 1α-$(OH)D_3$/fetus elicited an increase in both plasma calcium and plasma phosphate levels that lasted 144 h (Barlet *et al.,* 1978).

Our results are in agreement with the *in vitro* effects of 1,25-$(OH)_2D_3$ on bone ^{45}Ca release which showed a greater response in fetuses from TPTX rats than in normals (Rebut-Bonneton, Garel and Delbarre, 1983). This hypersensitivity was unrelated to the hyperparathyroid state secondary to maternal TPTX that occurred in these fetuses since fetal response to 1,25-$(OH)_2D_3$ injections was not reduced in fetuses deprived of parathyroid glands by decapitation. However, the hypersensitivity might be due to the reduction in bone size secondary to the high weight loss observed in fetuses from TPTX females. The more marked effects of 1,25-$(OH)_2D_3$ in decapitated than in intact fetuses (ΔCa : 3.66 and 2.01 mg/dl, respectively ; ΔPO_4 : − 1.39 and − 0.65 mg/dl, respectively) might be explained by a lower plasma 1,25-$(OH)_2D_3$ level in the former than in the latter ; it is well known that blood 1,25-$(OH)_2D_3$ levels in adults are under the control of the parathyroid glands (Garabédian *et al.,* 1972). The hypothesis of reduced blood levels of 1,25-$(OH)_2D_3$ in fetuses from TPTX mothers might also be the cause of their hypersensitivity to this vitamin D_3 metabolite.

Our experiments provide indirect arguments in favor of the fetal hyperparathyroidism occurring in fetuses from TPTX pregnant rats because decapitation of 19.5-day old fetuses from these mothers induced at term a sharp decrease in plasma calcium level (− 2.37 mg/dl) and an increase in plasma phosphate (1.39 mg/dl). In contrast, both plasma calcium and phosphate levels were unchanged in decapitated fetuses from normal females. These data suggest that fetal parathyroid glands played a more important role in the control of fetal plasma calcium in TPTX than in normal rats. In normal rats, the fact that the plasma calcium levels in 21.5-day old fetuses decapitated 2 days before did not decrease confirms previously published data by Pic (1973) ; it is known that plasma calcium levels at term are reduced only if decapitation occurs before 19.5 days of pregnancy (Pic, 1973). However, there is some indirect evidence that fetal parathyroid glands are involved in the control of fetal plasma calcium

just before term (Pic, 1973 ; Garel, 1970, 1975). These findings were substantiated by the detection of immunoreactive parathyroid hormone in the plasma of rat fetuses during the 3 days before term (Thomas, Anast and Forte, 1981). The fact that decapitation of rat fetuses at 19.5 days of pregnancy had no effect on plasma calcium levels 2 days later is actually not understood. Different hypotheses might explain this observation ; for example, fetal decapitation at 19.5 days of pregnancy might have suppressed not only the source of parathyroid hormone but also a mechanism that, from this stage of pregnancy, could compensate the hypercalcemic effect of parathyroid hormone.

The percentage of total ash was 9 % less in intact fetuses from TPTX mothers (1.61 % *vs* 1.76 % in fetuses from normal control mothers, unpublished results), but a 4 % increase occurred in fetuses deprived of parathyroid glands by decapitation (fig. 3). All these data provide new arguments in favor of the hyperparathyroidism occurring in fetuses from TPTX pregnant rats ; an increase in fetal basal bone ^{45}Ca release *in vitro* has already been reported (Garel, Rebut-Bonneton and Delbarre, 1980). Moreover, the percentage of ash in intact fetuses treated with 1,25-$(OH)_2D_3$ became similar to that found in decapitated fetuses, and the relative content of their ash calcium was slightly increased. This result suggests that the vitamin D_3 metabolite had impaired the development of the fetal hyperparathyroid state in TPTX mothers. The lack of 1,25-$(OH)_2D_3$ effect in decapitated fetuses and the slight increase in the percentage of ash shown in intact fetuses seem at variance with the hypothesis that 1,25-$(OH)_2D_3$ acts on fetal bone by increasing resorption to elevate plasma calcium. However, the values we obtained for total ash may represent alterations in bone and/or soft tissues.

The hypophosphatemic effect of 1,25-$(OH)_2D_3$ was only observed in fetuses from TPTX mothers in which basal plasma phosphate levels were elevated. Such findings have already been described in adult rats (Garabédian *et al.,* 1976). This effect also occurred at a dose (0.1 µg/kg) that was not hypercalcemic. As in adults (Garabédian *et al.,* 1976), this effect did not require the presence of the thyroparathyroid glands. The mechanism by which 1,25-$(OH)_2D_3$ modulates plasma phosphate is actually not understood.

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Résumé. *Effets de l'injection de 1,25-$(OH)_2D_3$ sur la calcémie, la phosphatémie et le contenu minéral du fœtus de rat.*

Nous avons étudié les effets *in vivo* du 1,25-$(OH)_2D_3$ en utilisant des fœtus de rattes normales ou thyro-parathyroïdectomisées (TPTX). L'injection sous-cutanée de 1,25-$(OH)_2D_3$ à raison de 1 µg/kg de poids corporel à des fœtus de mères TPTX âgés de

19,5 jours (privés de leurs parathyroïdes par décapitation ou laissés intacts) provoque une forte augmentation de la calcémie 48 h plus tard : 3,66 et 2,01 mg/dl, respectivement. Au contraire, chez les fœtus de mères normales la calcémie est peu modifiée (1,06 mg/dl chez les décapités ; pas de changement chez les intacts). Ce même traitement diminue la phosphatémie à la fois chez les fœtus décapités et entiers de mères TPTX (− 1,39 et − 0,65 mg/dl, respectivement), par contre la phosphatémie des fœtus de mères normales est inchangée. Ainsi, la sensibilité accrue au 1,25-$(OH)_2D_3$ du fœtus de rattes TPTX ne semble pas liée au développement de l'hyperparathyroïdisme fœtal consécutif à l'hypoparathyroïdisme maternel. L'effet hypercalcémiant du métabolite de la vitamine D_3 pourrait-être le résultat d'une action au niveau de l'os fœtal plus marquée chez le fœtus de mères TPTX en raison de la taille réduite de l'os. Cette hypothèse semble apparemment en contradiction avec l'observation, lors du traitement au 1,25-$(OH)_2D_3$, d'une absence de modification du pourcentage de cendres chez le fœtus décapité, et d'une augmentation de ce dernier chez le fœtus intact ; cependant nos valeurs qui portent sur les cendres totales représentent à la fois des modifications au niveau de l'os et/ou des tissus mous.

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