1,25-Dihydroxyvitamin D3 induces in vivo the decidualization of rat endometrial cells*

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Summary. Intraluminal injection of female rats at Day 5 of pseudopregnancy with 10–500 ng 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) significantly increased the uterine weight and induced decidual reaction. This effect was observed as early as the 3rd day after 1,25-(OH)2D3 injection. It was detectable only in the injected left horn and not in the non-injected right horn. A 500 ng dose of 25-(OH)D3 had no such effect. The present in-vivo results suggest that 1,25-(OH)2D3 may play a physiological role in endometrial cell differentiation into decidual cells, a crucial step in the process of blastocyst implantation.

Keywords: 1,25-Dihydroxyvitamin D3; uterus; endometrial cells; decidualization; fertility; rat

Introduction

Recent work suggests that vitamin D, and especially its hormonal form 1,25-dihydroxyvitamin D (1,25-(OH)2D3), plays a significant role in female reproductive functions. Mating success, fertility and litter size are reduced in rats fed a vitamin D-deficient diet (Halloran & De Luca, 1980; Kwiecinski et al., 1989) and can be restored by chronic administration of vitamin D3 or low doses of 1,25-(OH)2D3 (100 ng/day) to vitamin D-deficient females (Kwiecinski et al., 1989). These effects do not appear to be mediated by changes in calcium metabolism, but could result from a direct vitamin D regulatory effect on the organs involved in reproduction. The uterus appears to be a likely candidate for such a regulatory action, as this organ contains specific receptors for 1,25-(OH)2D3 (Walters, 1981; Levy et al., 1984), and as isolated endometrial cells possess a 25-(OH)D3–24-hydroxylase activity (Acker et al., 1982) and are responsive to 1,25-(OH)2D3 (Lieberherr et al., 1984). While these observations suggest that the uterus is a target organ for the hormonal form of vitamin D, nothing is known of its mechanism of action.

Uterine weight increases dramatically during decidualization, due to hyperplasia and hyperproliferation of endometrial cells (Finn, 1977), and endometrial cells differentiate into enlarged decidual cells with large nuclei and increased chromatin surface, some of these cells being binucleate (Kennedy, 1986). Similar events can be induced in the rat uterus by local artificial stimuli, provided the stimulus is given when the uterus is most sensitive to deciduoma induction, at Day 5 of pseudopregnancy (De Feo, 1963). The effects of these stimuli can best be observed 5 days after intraluminal injection (De Feo, 1963). In the present work, we examined the possible role of 1,25-(OH)2D3 during early pregnancy, and more precisely on the decidual reaction, a process associated with ovum implantation. To do so, vitamin D3 metabolites were injected directly into the uterus of pregnant rats at the time when endometrium is most sensitive to factors inducing the decidual reaction (De Feo, 1963; Kennedy, 1980).

Materials and Methods

Substances used. 25-Hydroxyvitamin D3 (25-(OH)D3) and 1,25-(OH)2D3 were kindly provided by Roussel Laboratories (Paris, France) and Roche Laboratories (Basel, Switzerland) respectively.

*Reprint requests to Dr M. Garabédian.
**Animals.** Female Wistar rats, 2–3 months old, obtained from Lessieux (Bray-Lu, France), were maintained on a normal diet (Extra-Labo, Provins, France) and housed in a room with controlled illumination (14-h light and 10-h darkness). Female rats were caged overnight with males. The oviducts of those rats having spermatozoa in vaginal smears on the next day were sectioned 2–4 h later. Vitamin D₃ metabolites or solvent were injected into the lumina of the uterine horns on the afternoon of the 4th day following the initial surgery.

**Intrauterine injections.** At 4 days after oviduct section, animals were anaesthetized with ether and the uterus exposed via laparotomy. Vitamin D₃ metabolites or solvents were injected into the uterine lumen with a 1-ml plastic syringe and a 16/5 mm needle (Terumo, Haasnode, Belgium) via the upper part of the left uterine horn. Care was taken not to alter the uterine wall, as it is well known that local injury of the uterine epithelium can by itself induce decidualization (De Feo, 1967; Finn, 1977). The caudal end of the uterus was not ligatured to avoid a stress-induced decidualization.

Vitamin D metabolites were injected in 0.1 ml PBS-G (0.1 M-phosphate in 0.154 M-NaCl containing 0.1% gelatin) with 2.5% ethanol. The solution of vitamin D₃ metabolites in PBS-G was prepared immediately before each injection. Each metabolite was dissolved in ethanol (0.5 mM), added to PBS-G (2.5% final ethanol concentration), and injected into the uterus 15 min later. To determine the amount of vitamin D metabolite actually injected to the animal, similar preparations were made using tritiated 1,25-(OH)₂D₃ instead of 1,25-(OH)₂D₃, and injected into counting vials for radioactivity determination. Over 80% of the metabolite added to the solvent was actually injected using this technique. Control rats received 0.1 ml PBS-G solvent alone. In some additional experiments, vitamin D₃ metabolites were injected in 0.1 ml absolute ethanol; 100% of the metabolite added to the solvent was actually injected.

**Tissue preparations.** Animals were killed 3 or 5 days after the intrauterine injection. The left and right uterine horns were removed, cleaned of fatty tissue, weighed and placed in Bouin’s fluid. The entire tissue was embedded in paraffin wax and cut in a series of transverse sections (7 μm). Each 20th section was fixed and stained by the Masson trichrome technique. Histological examination of the uterus was carried out by two independent observers. Uterine horns considered positive for decidual reactions were those exhibiting decidual cells on more than 5 successive stained uterine sections (corresponding to 0.6 mm uterus length).

**Statistical analysis.** The weight difference between the left and right horns was analysed by Student’s paired t test. Differences between vitamin D-treated and solvent-injected horns were analysed by the Student’s unpaired t test. Differences between the percentage of decidual reaction in rats injected with vitamin D and rats injected with solvent only were tested by χ² test.

**Results**

A single intraluminal injection of 1,25-(OH)₂D₃ into the rat uterus, at Day 5 of pseudopregnancy, had a significant effect on uterine weight 5 days later (Fig. 1a), as well as on the appearance of a decidual reaction (Figs 1b and 2). The weight of uterine horns injected with 1,25-(OH)₂D₃ was 3–4-fold higher than that of uterine horns injected with PBS-G solvent; a decidual reaction was observed in 75–100% of the horns injected with 1,25-(OH)₂D₃, but in only 15% of those injected with PBS-G solvent. The 1,25-(OH)₂D₃ effect was significant at doses from 50 to 500 ng (P < 0.001) and was detectable as early as the 3rd day after 1,25-(OH)₂D₃ injection (Fig. 3). It was found in the injected left horn but not in the uninjected right horn; the difference between left and right horns of the same animals was statistically significant with a 10 ng dose of 1,25-(OH)₂D₃, and was maximum with 50 ng (Figs 1a, b).

The hormonal precursor, 25-(OH)D₃, produced no such effect at a dose of 500 ng, 10 times the maximal active dose of 1,25-(OH)₂D₃.

As solvent may have a direct effect on the appearance of a decidual reaction (De Feo, 1967; Kennedy, 1980, 1986), experiments were repeated using ethanol as solvent. With this vehicle, the 1,25-(OH)₂D₃ action on uterus was uncoupled: the hormone did not significantly increase uterine weight, but did significantly increase the percentage of uterine horns with decidual reaction, 3 days after intraluminal injections of 100 ng or 500 ng hormone (Fig. 3).

**Discussion**

In the rodent uterus, natural (ovo-implantation) but also artificial stimuli can induce the appearance of the decidual tissue which supports the development of embryo before the placenta becomes
Fig. 1. Effects of single intraluminal injections of 1,25-(OH)2D3, 25-(OH)D3, or PBS-G solvent (control group) on (a) uterine weight and (b) decidualization of female rats on Day 5 of pseudopregnancy. Effects were analysed 5 days after the injection. Values are mean (± s.e.m. in (a)) for the number of rats indicated in parentheses. *P = 0.03; **P < 0.01; ***P < 0.001 compared with control group.

Functional (Krehbiel, 1937; Shelesnyak, 1957; Psychoyos, 1974; Finn, 1977; Kennedy, 1980, 1986; Kleinkfeld & O'Shea, 1983). The present experiments were designed to determine whether 1,25-(OH)2D3 could have such an effect, as this hormonal form of vitamin D3 appears to be implicated in the regulation of reproduction (Kwiecinski et al., 1989) and to act directly on the uterus (Walters, 1981; Levy et al., 1984; Lieberherr et al., 1984).

Indeed, 1,25-(OH)2D3 elicited a marked decidual response when injected directly into the uterine lumen at the time of its maximal sensitivity to factors inducing the decidual reaction (De Feo, 1963; Kennedy, 1980). The lack of uterine changes following local administration of 25-(OH)D3, the 1,25-(OH)2D3 precursor, suggests that uterine cells respond specifically to 1,25-(OH)2D3. When injected in PBS-G solvent, which is itself an artificial stimulus of the decidual reaction (Kennedy, 1986), 1,25-(OH)2D3 induced cell differentiation and increased uterine weight as early as 3 days after intraluminal administration. When ethanol was used as solvent, endometrial cells were partly destroyed, but 1,25-(OH)2D3 still induced significant decidual reaction, although this reaction was not accompanied by increased uterine weight. These observations suggest that 1,25-(OH)2D3 specifically influences cell differentiation of the uterus, as it does that of circulating monocytes (Tinkler et al., 1980), skin (Kurokit, 1985), mammary glands (Mezzetti et al., 1987), and
Fig. 2. Appearance under the light microscope of typical decidual cells in a left uterine horn injected with 1,25-(OH)2D3 (a), and of the non-decidualized contralateral non-injected horn of the same animal (b). × 400.

several cancer cell lines (Abe et al., 1981; Miyaura et al., 1981; Tanaka et al., 1982; Dodd et al., 1983).

The 1,25-(OH)2D3 doses required to obtain a maximal effect (50 ng) or a half-maximal effect (25 ng) on uterine decidualization were lower than those used in vivo to demonstrate effects on other functions unrelated to calcium metabolism, such as brain enzyme activities (Sonnenberg et al., 1986), insulin secretion (Kadowaki & Norman, 1985; Tanaka et al., 1986) or, most important, to restore the fertility of vitamin D-deficient female rats (Kwiecinski et al., 1989).

Strikingly, 1,25-(OH)2D3 affected the injected horn but not the uninjected contralateral horn; the endometrium responded to local 1,25-(OH)2D3 and not to 1,25-(OH)2D3 transferred into the general circulation after its luminal injection. Decidualization of the endometrium is known to require tissue injury and close contact between the endometrial epithelium and the blastocyst (De Feo, 1967; Finn, 1977). It can also be caused by locally administered chemical signals, including prostaglandins (Sananes et al., 1976; Kennedy, 1986) and, as we have now shown, 1,25-(OH)2D3; however, the physiological significance of this observation remains to be delineated. The 1,25-(OH)2D3 produced in the kidney and delivered to the tissues via the circulation is responsible for most of the currently known physiological functions of vitamin D (Brommage & De Luca, 1985; Kumar, 1986), but some of the newly described actions of 1,25-(OH)2D3 may involve local production of this metabolite, as in the auto or paracrine systems described in the immune system (Rigby, 1988), in the skin (Bikle et al., 1986; Holick et al., 1987), and in the aortic endothelium (Merke et al., 1989). Our results are in agreement with the hypothesis that decidualization of the uterus may
Fig. 3. Effects of 1,25-(OH)2D3 on (a) uterine weight and (b) decidualization, 3 or 5 days after single intraluminal injections in PBS-G solvent or ethanol. The numbers of rats in each group are indicated in parentheses. **P < 0.01; ***P < 0.001 compared with control.

be similarly regulated locally by 1,25-(OH)2D3. Whether local 1,25-(OH)2D3 is produced by the blastocyst or by the uterus itself remains unknown.

In conclusion, the normal reproductive function of female rats has been shown to be vitamin D-dependent (Halloran & De Luca, 1980; Kwiecinski et al., 1989); the present in-vivo results suggest that part of this dependence could be due to 1,25-(OH)2D3 inducing endometrial cell differentiation into decidual cells, a crucial step in the process of blastocyst implantation.

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References


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