93. Histochemical Localization of Adenylate Cyclase in Hormonally Sensitized Rat Endometrium

Effect of Application of Deciduogenic Stimulus

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In rodents, decidual cell reaction (DCR) occurs in response to blastocysts or artificial stimuli applied to the endometrium sensitized by an appropriate regimen of hormonal treatment. Evidence has recently accumulated suggesting the involvement of prostaglandins (PGs) and cyclic adenosine-3',5'-monophosphate (cAMP) in the incidence and development of DCR. Uterine concentrations of these compounds have been reported to increase soon after application of deciduogenic stimuli. However, little is known on the mechanisms by which these compounds have their effects on the endometrium. Previous studies have suggested that PGs are produced by epithelial cells in the rat endometrium. Since histological methods using 5'-adenyl-imidodiphosphate (AMP-PNP) as a substrate for demonstrating adenylate cyclase (AC) have been well-established, a histochemical analysis of localization of AC and the possible effect of deciduogenic stimulus on it was conducted in hormonally sensitized rat endometrium.

Materials and methods. Seven groups of female rats (60 to 90 days of age) of the T strain used in the present experiments were maintained in a temperature- and light-controlled room (lights on from 0500 to 1900 h). The rats were ovariectomized on the day of vaginal estrus and given 3 daily injections of 3 mg progesterone (P) in 0.1 ml sesame oil commencing on the day after operation. On the last day of the P injection period, they received a single injection of 0.1 µg estradiol-17β (E2) in 0.05 ml oil. One of the 7 groups of rats was sacrificed 16 hr after the E2 injection. The remaining groups were subjected to either endometrial traumatization (3 groups) or intraluminal instillation of 0.1 ml sesame oil (3 groups) as deciduogenic stimulus in the right uterine horn, 16 hr after the E2 injection. The two groups sacrificed 96 hr after the stimulus were given a further treatment with P for 3 consecutive post-stimulation days. The number of rats examined was 3 in each group.

At autopsy, the uteri were removed immediately, frozen in liquid N₂ and stored at −80°C until use. Frozen sections cut with a cryostat-microtome at 10 µm were fixed with 1% ice-cold glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.4) for 10 min. After rinse with the same buffer for a few min, sections were incubated in a modified medium of Howell and Whitfield: 80 mM tris-maleate buffer (pH 7.4) added with 8% sucrose, 4 mM lead nitrate, 4 mM magnesium sulfate, 2 mM theophylline and 0.2 mM AMP-PNP (P-L Biochem. Inc., Milwaukee, WI) as a substrate. In some experiments, PG-E2

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(0.1 mg/ml, Funakoshi Pharmaceutical Co., Japan) was added to the incubation medium. The incubation was carried out at 30°C for 30 min in a water bath. Two types of control experiments were conducted. In the first control, the sections were incubated similarly but in a medium without the nucleotide or with 5 mM alloxan (Sigma Chemical Co., St. Louis, MO), an AC inhibitor. In the second control, the sections were boiled for 10 min before incubation. Following incubation, all sections were washed in D.W. and then treated with an aqueous solution of 2% yellow ammonium sulfide for 2 min. The sections were rinsed with D.W. and mounted in glycerine jelly.

Results and discussion. In ovariectomized rats given a 3-day treatment with P and a single injection of E2, incubation of uterine sections in the standard medium with AMP-PNP produced a positive AC reaction in the luminal and glandular epithelia in the endometrium (Fig. 1). The colored product
occurred mainly along the plasma membrane of epithelial cells, whereas the reaction was negative in endometrial stromal cells and smooth muscle cells in the myometrium. In uterine blood vessels, endothelial cells showed a positive reaction, seemingly stronger than did epithelial cells. Control sections incubated in the standard medium without substrate or with alloxan showed hardly any reaction. No reaction product was formed in sections boiled before incubation (Fig. 2).

In ovariectomized rats given deciduogenic stimulation following the 3-day hormone treatment, regardless of the type of stimulus, the uterine sections incubated in the standard medium exhibited the AC reaction very similar both in distribution and in intensity to the reaction in rats given no deciduogenic stimuli (Fig. 3). In endometrial stromal cells of the rats sacrificed 5 min or 5 hr after application of the stimulus, the AC reaction was negative. Similar localization of AC in the rat endometrium was demonstrated at the ultrastructural level using adenosine triphosphate as a substrate of AC. Thus, deciduogenic stimulation failed to provide any definite histochemical evidence for an increase in AC activity in the uterus. Although an increase in uterine cAMP concentration following deciduogenic stimulation has been demonstrated biochemically, it is likely that endometrial stromal cells are not involved in the increase. In ovariectomized rats given a 7-day treatment with P and E\textsubscript{2} and sacrificed 96 h after application of endometrial stimulation, uteri invariably formed decidualomas in response to endometrial traumatization or oil-instillation. Interestingly, decidual cells and vascular endothelial cells showed a positive AC reaction, while luminal and glandular epithelial cells none at all (Fig. 4). Abundant reaction product was deposited mainly along the plasma membrane of decidual cells. The AC reaction in the decidualized endometrium appeared to be similar in localization to that of alkaline phosphatase in deciduomata induced in the mouse and the rat (unpublished). The role of AC in decidual cell function remains to be studied.

On the basis of several lines of evidence, a hypothesis was put forward that deciduogenic stimuli increase uterine concentrations of PGs, particularly of E-series, and this in turn rises intracellular cAMP concentrations by activating AC, thereby promoting endometrial function to initiate decidualization.

In the present study, however, PGE-2 added to the incubation medium brought about no distinct changes in localization and magnitude of the AC reaction in sections of the uteri removed from ovariectomized rats given a standard regimen of hormone treatment (Fig. 5). Accordingly, it appears likely that the effect of PGE-2 on endometrial stromal cells is not mediated by cAMP. This may account for, at least in part, the lack of deciduomal response to the intrauterine administration of cAMP or its analogue. However, since AC is present in the membrane of vascular endothelial cells, the possibility cannot be ruled out that the increase in endometrial vascular permeability observed prior to decidualization is mediated by cAMP as has been suggested by Kennedy.

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**References**

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