Effect of Gestagen on Mitosis in the Cultured Mammary Glands of Mice

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(Received August 8, 1982)

Abstract Fragments of mammary glands from mid-pregnant mice were cultured in Medium 199 containing insulin ($1\text{ }\mu\text{g/ml}$), prolactin ($5\text{ }\mu\text{g/ml}$), and progesterone (200 nM) or its derivatives. The frequency of mitosis was assessed in alveolar cells after treatment with colchicine for 3 hr. The mitotic index was increased at 24 hr after the beginning of the culture with progesterone. 20α -Hydroxy-4-pregnen-3-one, one of the metabolites of progesterone in the mammary glands, showed stimulatory effect on the mitosis, while 5α -pregnane-3, 20-dione, one of the metabolites of progesterone in mammary tumors but not in the normal mammary glands, had no significant effects. These results suggested that both progesterone and 20α -hydroxy-4-pregnen-3-one are mitogenic for the mouse mammary glands during pregnancy.

Jpn. J. Zootech. Sci., 54 (2): 106-109, 1983

Progesterone was metabolized to 20α -hydroxy-4-pregnen-3-one in the mammary glands^{1,2)}, and to 5α -reduced compounds, such as 5α -pregnane-3,20-dione, in the mammary tumors^{2,3)} of mice and rats. The metabolism of steroid hormones in their target organs is recognized as an important phenomenon for regulating the potency of the hormones and for diversifying its actions⁴⁾. Although the accumulation of considerable amounts of information on hormonal control of mammary growth, the role of progesterone in this tissue is not clarified yet⁵⁾. The present study was conducted to elucidate the direct effect of progesterone, 20α -hydroxy-4-pregnen-3-one, and 5α -pregnane-3, 20-dione on the mitosis of the mammary glands of mid-pregnant mice by using the fragment culture technique, which was first reported by ELIAS⁶⁾ and developed by several groups including our laboratory⁷⁾.

Materials and Methods

Inbred KA strain mice maintained in our laboratory were used for the experiment on day 7 or 8 of gestation. The mammary glands were exposed by stripping the skin under pentobarbital anesthesia (30 mg per animal). After trimming off adhering tissues, approximately 45 fragments of 1 mm square each were excised from the second thoracic mammary gland laying between the internal and external thoracic arteries.

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The tissues were occasionally moistened with Medium 199 during the operation. Three to 5 fragments were randomly distributed into a culture dish, which contained 3 ml of Medium 199. Regimen of Medium 199 with Hanks' salt was modified by diluting 9 ml of 10-times concentrated M 199 (Difco Laboratories, Detroit, Mich., U.S.A.) with 91 ml of 0.2% sodium bicarbonate which had been autoclaved. Bovine insulin (Ultralente, Novo Industri A/S, Denmark) was dissolved in the medium to achieve the final concentration of 1 $\mu\text{g/ml}$ (0.12 U/ml). One mg of ovine prolactin (NH-P-S8) was dissolved in 0.1 ml of 0.1 mM sodium hydroxide and diluted to 5 $\mu\text{g/ml}$ with the medium. These solutions were filtered through membrane filters (pore size; 0.45 μm , Millipore Corp., Bedford, Mass., U.S.A.) prior to use. Steroids obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.) were dissolved in ethanol and used after dilution with the medium. The final concentration of ethanol did not exceed 0.5% (v/v). All the medium was supplemented with Penicillin G (50 U/ml). Incubation was performed at 37°C under an atmosphere of O₂:CO₂ (95:5). Colchicine 2 μg in 0.1 ml of the medium was added into a culture dish 3 hr before termination of the culture to stop mitoses at the metaphase. Each fragment was fixed in Bouin's fixative. Serial paraffin sections of 7 μm thickness were stained with hematoxylin and eosin. Mitotic index was expressed as the number of cells at the metaphase per 1,000 alveolar cells. The observation was performed on randomly chosen four sections, each consisted at least 1,000 alveolar cells. Results were analyzed for significance by Student's *t* test.

Results and Discussion

In order to investigate the effect of progesterone on mitosis, fragments of the mammary gland were cultured in medium containing insulin (1 $\mu\text{g/ml}$), prolactin (5 $\mu\text{g/ml}$), and 200 nM (63 ng/ml) of progesterone for 5, 10, 24, 48, 72, and 96 hr. The results are shown in Fig. 1. In the presence of progesterone, the mitotic index was increased at 24 hr after the beginning of culture ($P < 0.01$ against control at 24 hr), and then decreased to the control level. This concentration of progesterone is comparable to the level in blood during mid-pregnancy⁸, and an increase of progesterone beyond this concentration depressed the effect (data not shown). The decline of the mitosis after 24 hr of culture is consistent with the study of STOCKDALE and TOPPER⁹, in which they discussed that the mammary gland contain a limited number of cells which produce different daughter cell type after division and these daughter cells do not repeatedly divided during culture.

The effects of 5 α -pregnane-3,20-dione and 20 α -hydroxy-4-pregnen-3-one were also evaluated from the mitotic index of mammary alveolar cells which had been cultured as a fragment in the presence of insulin, prolactin, and 200 nM of each steroid. A similar pattern as in the case of progesterone was observed in the culture with 20 α -hydroxy-4-pregnen-3-one, but this steroid had a prolonged effect on mitosis than progesterone itself. Even after 96 hr of cultivation, 20 α -hydroxy-4-pregnen-3-one was still effective on mitosis ($P < 0.01$ at 48 and 96 hr, and $P < 0.05$ at 72 hr against

Fig. 1. Effect of various gestagen on mitotic indices of cultured mammary glands of mice. Fragments of the mammary glands were cultured at 37°C in Medium 199 containing insulin (1 μ g/ml), prolactin (5 μ g/ml), and 200 nM progesterone (●), 20 α -hydroxy-4-pregnen-3-one (▲), or 5 α -pregnane-3,20-dione (■) in an O₂:CO₂ (95:5) atmosphere. Colchicine 2 μ g was added 3 hr before the termination of culture. The points and bars represents means $\pm$ S. E. (n=4). The shaded area indicates mean $\pm$ S. E. (n=4) of the control group in which the tissues were cultured with insulin and prolactin.

control). Since 20 α -hydroxysteroid dehydrogenase was involved in interconversion between progesterone and 20 α -hydroxy-4-pregnen-3-one¹⁰⁾, the prolonged effect of this steroid on mitosis could be explained by its conversion to progesterone during the culture. However, it is not clarified from this experiment that which steroid has the mitogenic activity on the mammary gland.

On the other hand, 5 α -pregnane-3, 20-dione had no effect on mitosis in this tissue, since the curve was similar to that obtained from the culture without any steroid. There are several reports on the physiological importance of 5 α -reduction of progesterone. In chick oviducts, progesterone was metabolized to 5 α -pregnane-3, 20-dione and this metabolite was more active in avidin synthesis than progesterone itself¹¹⁾. NUTI and KARAVOLAS¹²⁾ have also shown the significance of 5 α -reductase in the neuroendocrine tissues for the feedback effects of progesterone on gonadotropin secretion. On the contrary, in addition to the previous work which showed the absence of 5 α -reductase activity in the normal mammary glands¹³⁾, the present study provides evidence that 5 α -reduction of progesterone is not involved in the mitogenic effect of progesterone in the mammary glands.

The authors are indebted to Toichiro KONDO in this laboratory for his assistance during this experiment.

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マウス培養乳腺の細胞分裂に対するゲスターゲンの効果

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プロゲステロンは、マウス乳腺で 20α -ヒドロキシ-4-プレグネン-3-オンに代謝されるが、乳癌では主に 5α -プレグナン-3,20-ジオンに代謝される。本研究は、乳腺の細胞分裂に対するプロゲステロンおよびその代謝産物の効果を調べるために、乳腺の器官培養を用いておこなったものである。用いた乳腺は、妊娠中期の雌マウス (KA 系) より得た。1 mm 角の乳腺組織片をインスリン ($1 \mu\text{g}/\text{ml}$)、プロラクチン ($5 \mu\text{g}/\text{ml}$) および各種のゲスターゲン (200 nM) を含む M199 合成培養液中で、温度 37°C 、95% 酸素 5% 炭酸ガスの気相のもとで培養した。培養終了 3 時間前にコルヒチン ($2 \mu\text{g}$) を添加し、細胞分裂を停止させ、乳腺細胞に分裂中期像の現われる

頻度を計数した。その結果、プロゲステロンを加えて培養したものの方がインスリンとプロラクチンのみを含んだ培養液中で培養した乳腺よりも、培養開始 24 時間後に分裂の頻度が上昇していた。 5α -プレグナン-3,20-ジオンにはこのような効果は認められなかったが、 20α -ヒドロキシ-4-プレグネン-3-オンは 24 時間後ではプロゲステロンと同程度の分裂促進効果があり、この効果は培養開始 96 時間まで持続していた。以上の結果は、妊娠中の乳腺の細胞分裂にはプロゲステロンおよび、 20α -ヒドロキシ-4-プレグネン-3-オンが関与している可能性を示唆している。

日畜会報, 54 (2): 106-109, 1983