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Comparative Study on the Effects of Estradiol and Estriol on Pituitary Prolactin Secretion and Mammary Gland DNA Synthesis of Rats in Relation to Their Role in Mammary Tumorigenesis*

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Abstract—*The effects of estriol on pituitary prolactin secretion and mammary gland DNA synthesis, both being primary factors for mammary tumorigenesis, were compared with those of estradiol in female rats, as a possible step to evaluate the role of estriol in mammary tumorigenesis. Ovariectomized female Sprague–Dawley rats were given daily s.c. injections of* 5 *and* 50 μg *of estradiol-17β or estriol for* 3 days, *or received s.c. implantation of cholesterol pellets containing estradiol or estriol at the ratio of* 1 : 10 *for* 48 *and* 72 hr. *While daily injections of* 50 μg *estriol increased serum prolactin levels, the effect was less than for estradiol, although the difference was not statistically significant. Furthermore, estriol injection did not promote the* in vitro *incorporation of* [^{3}H] *thymidine into mammary gland DNA, whereas it was elevated by estradiol. On the other hand, the administration of estriol in a pellet was effective on both factors to a similar extent as for estradiol. The results indicate that the different estrogenic effects of estradiol and estriol are mainly ascribed to the difference in the period of effective distal retention of the hormones and that a protective effect of estriol itself on breast cancer is unlikely, if it is present constantly in the circulation.*

INTRODUCTION

ESTRIOL is often considered to be a weak estrogen [1] and to act protectively on the development and progression of human breast cancer [2]. Moreover, Lemon *et al.* [3] and MacMahon *et al.* [4] claimed that a higher urinary estriol quotient (estriol/estrone + estradiol) or estriol proportion (estriol/estrone + estradiol + estriol) is associated with a lower risk of developing breast cancer. On the other hand, the hypothesis that estriol modulates or opposes the action of estrone and estradiol has been challenged by several lines of data [5, 6]. It has also been shown that estriol is as active as estradiol on human breast cancer [7] and mouse mammary tumor [8]. Recently, Clark *et al.* [9] have reported that less effectiveness of estriol is dependent upon the short nuclear retention time of receptor–estriol complexes, and that estriol is a potent estrogen without antagonistic properties when it is present in the circulation continuously and receptor–estriol is elevated and maintained.

While estradiol participates in the development and progression of both normal and neoplastic mammary glands by acting directly on mammary cells and indirectly on the pituitary to promote prolactin secretion [10], the information concerning the role of estriol in this process is scanty.

In this paper, we have compared the effects of estradiol and estriol on pituitary prolactin secretion and mammary gland DNA synthesis, both being primary factors for mammary tumorigenesis [11, 12], as a possible step to evaluate the role of estriol in mammary tumorigenesis.

MATERIALS AND METHODS

Animals

Sprague–Dawley female rats were used. They were kept in an animal room that was

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air-conditioned (24±0.5°C and 65–70% r.h.) and artificially illuminated (14 hr light from 5:00 a.m. to 7:00 p.m.) and provided with a commercial diet and tap water *ad libitum*. At 10 weeks of age, all rats except intact controls were bilaterally ovariectomized under light ether anesthesia.

Administration of estradiol and estriol

In Experiment 1, rats were given daily s.c. injections of 5 or 50 μg estradiol-17β or estriol (Sigma Chem. Co., St Louis, Missouri, U.S.A.) for 3 days beginning 1 week after ovariectomy. The steroids were dissolved in ethanol and diluted with olive oil to make the final concentration of 10%, and each dose of hormone was in 0.1 ml. Intact and ovariectomized controls received vehicle only. On the morning following the last injection, when rats given 50 μg of each estrogen showed clear estrous vaginal smears, all rats were killed. Intact controls were killed on the morning of estrus at a similar age.

In Experiment 2, rats received s.c. implants of cholesterol pellets (5 mm diameter, 2 mm thickness and 50 mg weight) containing estradiol or estriol at a concentration of 10% after 1 week of ovariectomy. Intact and ovariectomized controls were given pellets of cholesterol only. Rats were killed on the morning after 48 and 72 hr of pellet implantation, when all experimental rats showed proestrous and estrous vaginal smears, respectively.

In both experiments, rats were weighed and killed by decapitation at about 10:00 a.m. and blood was drawn from the trunk. Serum was frozen and kept at −20°C for assay of prolactin.

Serum prolactin level

Serum prolactin level was assayed by radioimmunoassay as an index of the rate of pituitary secretion using the kit provided by NIAMDD, NIH, U.S.A.

Mammary gland DNA synthesis

Mammary gland DNA synthesis was estimated by the *in vitro* incorporation of [^{3}H] thymidine into mammary gland DNA. Bilateral inguinal fat pads containing parenchyma were sliced with a micro tissue slicer (Hotta Rika Inc., Tokyo, Japan), and about 100 mg of slices were incubated for 2 hr at 37°C in 4 ml of Medium 199 (Difco Labs., Detroit, Michigan, U.S.A.), containing 10 μCi [^{3}H] thymidine (5 Ci/mmole; Radiochemical Centre, Amersham, England) under a constant gasflow with 95% O_2 and 5% CO_2. After incubation, mammary gland DNA was extracted and [^{3}H] thymidine incorporated was counted with a scintillation counter and expressed in terms of dpm μg DNA. All procedures were the same as described previously [13].

Statistics

Significant differences between groups in all parameters were evaluated by Duncan's multiple range test.

RESULTS

There were little differences between groups in body weight at sacrifice in both Experiments 1 and 2.

Experiment 1

Serum prolactin level. The results of serum prolactin level in each group are shown in Fig. 1. While daily injections of 5 μg of each estrogen had only slight effect, daily dose of 50 μg increased serum prolactin levels significantly for both estrogens. The level in the 50 μg estriol group, which was comparable to that in the intact controls at estrus, was apparently lower than that in the 50 μg estradiol group, although the difference was not statistically significant owing to the large variation in the values of the 50 μg estradiol group.

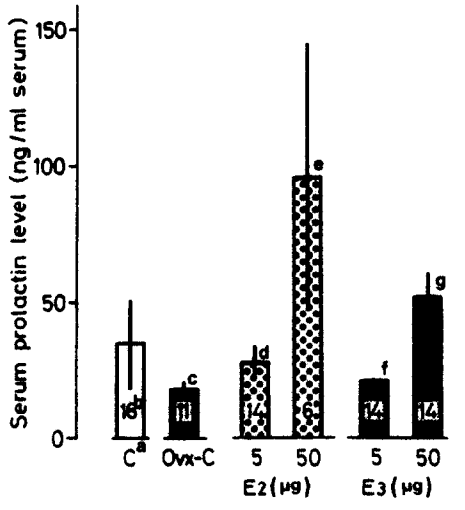

Fig. 1. *Effects of s.c. injections of estradiol-17β (E_2) or estriol (E_3) on serum prolactin levels in ovariectomized (Ovx) female rats (Experiment* 1*) (Means±S.E.M.).* [a]*See text for details of treatments.* [b]*Number of rats used. Significance of difference: c, f/g:* $P<0.01$. *c, d/e:* $P<0.05$.

Mammary gland DNA synthesis. [^{3}H] Thymidine incorporation into mammary DNA as an index of mammary gland DNA synthesis was extremely higher in the 50 μg estradiol group than in all other groups, the differences among which were not statistically significant (Fig. 2). The results indicate

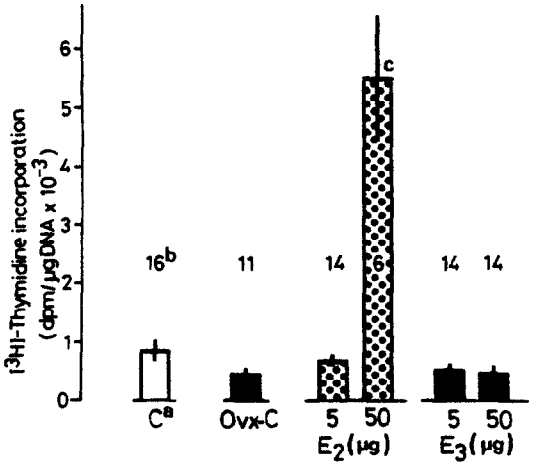

Fig. 2. Effects of s.c. injections of estradiol-17β (E_2) or estriol (E_3) on mammary gland DNA synthesis in ovariectomized (Ovx) female rats (Experiment 1) (Means $\pm$S.E.M.). [a]See text for details of treatments. [b]Number of rats used. [c]Significantly different from all other groups at $P<0.01$.

that the subcutaneous injections of estriol had no effect on mammary gland DNA synthesis even at daily dose of 50 μg.

Experiment 2

Serum prolactin level. As shown in Fig. 3,

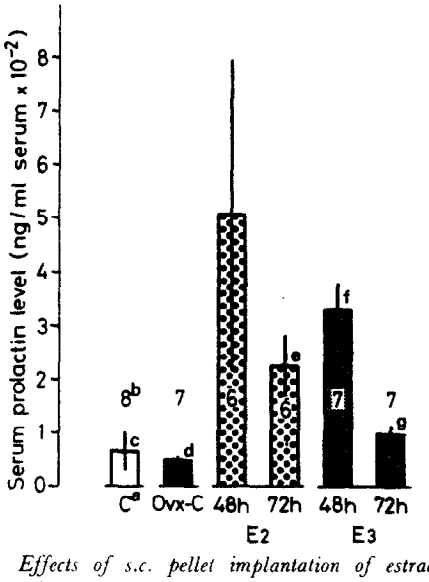

Fig. 3. Effects of s.c. pellet implantation of estradiol-17β (E_2) or estriol (E_3) on serum prolactin levels in ovariectomized (Ovx) female rats (Experiment 2) (Means $\pm$S.E.M.). [a]See text for details of treatments. [b]Number of rats used. Significance of difference: c,d,g/f: d/e: $P<0.01$. *c/e: d/g:* $P<0.05$.

serum prolactin levels 48 hr after pellet implantation increased considerably in both the estradiol and estriol groups, but the former was higher than the latter. Prolactin levels declined after 72 hr in both groups. The level was again rather lower in the estriol group than in the estradiol group, although the difference was not statistically significant.

Mammary gland DNA synthesis. Figure 4 pre-

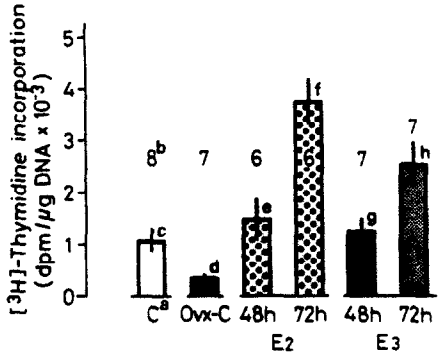

Fig. 4. Effects of s.c. pellet implantation of estradiol-17β (E_2) or estriol (E_3) on mammary gland DNA synthesis in ovariectomized (Ovx) female rats (Experiment 2) Means $\pm$S.E.M.). [a]See text for details of treatments. [b]Number of rats used. Significance of difference: c,d/f,h; e/f: $P<0.01$. *d/c,e,g; g/h:* $P<0.05$.

sents [^{3}H] thymidine incorporation into mammary DNA. The incorporations 48 hr after implantation of both estradiol and estriol were significantly higher than that in ovariectomized controls and comparable to that in the intact controls at estrus. The incorporation further increased and exceeded the level of the controls after 72 hr. The rate of increase in [^{3}H] thymidine incorporation after pellet implantation was not significantly different between the estradiol group and the estriol group, although it was rather lower in the latter.

DISCUSSION

In the present study, estriol when injected s.c. was less effective than estradiol in the effects on pituitary prolactin secretion, although the difference was not statistically significant. Estriol injection did not promote the mammary gland DNA synthesis, whereas it was elevated by estradiol. By contrast, with pellet implantation, estriol stimulated both factors to the levels comparable to estradiol. Anderson *et al.* [14] reported the failure of estriol to remain long enough in the uteri of immature rats; the nuclear receptor–estradiol

complex and the receptor–estriol complex were equivalent between 1 and 3 hr after estrogen injection and were associated with a similar increase in uterine weight. However, the receptor–estriol complex declined to that of the saline-injected control levels, corresponding to a loss of uterotropic effects at 6 hr after injection. Lippman *et al.* [7, 15] found in hormone-responsive human breast cancer that estradiol and estriol bind to an equal number of sites when saturating concentrations are used, while the dissociation constant is apparently lower for estradiol than for estriol and that estriol also stimulates the progression of tumors in culture. Thus, the difference between estradiol and estriol in the effects on each factor observed in the present study could be attributable to the difference in the route of administration. When estrogens were injected daily at 24-hr intervals, the period of effective retention would be much shorter for estriol than for estradiol. Meanwhile, both estradiol and estriol would act on the target tissues continuously in a similar manner when administered as pellets. In this respect, estriol, when given by repeated injections, was found to increase uterine weight of rats to the same extent as when estradiol was injected repeatedly [16, 17] or when these estrogens were given as pellets [9]. It has recently been claimed that the hypothesis that a low urinary estriol ratio is related to the etiology of breast cancer is unlikely, because many extraneous factors affect both estrogen excretion and the estriol ratio [18, 19]. The present findings also indicate from another point of view that the protective effects of estriol itself on breast cancer is unlikely, if it is present constantly in the circulation; its effects on pituitary prolactin secretion and mammary DNA synthesis, both being primary factors for mammary tumorigenesis [11, 12], are similar to those of estradiol, when each steroid was administered in a pellet.

Serum prolactin levels were lower at 72 hr than at 48 hr after pellet implantation in both groups, although the difference in the estradiol group was not statistically significant. This may partly be due to the refractoriness of the pituitary with the advance of time to the stimulating effects of extremely high dose of estrogen [20]. Alternatively, more estrogen discharged from the pellet at 48 hr than at 72 hr would be another probable explanation, since the rate of hormone release from the pellet is known to decrease exponentially with time after implantation.

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