EXPERIMENTAL STUDY

Changes in uterine ornithine decarboxylase activity and steroid receptor levels during decidualization in the rat induced by CDRI-85/287

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Abstract

CDRI-85/287 is an anti-estrogen and interferes with decidualization in the rat uterus. In this study, uterine estrogen receptor (ER) and progesterone receptor (PR) levels were determined during the inhibition of decidualization. The effect of 85/287 on uterine ornithine decarboxylate (ODC) activity (a marker enzyme for decidualization) was also studied, using immature ovariectomized rats divided into four different groups: control, 2.5 mg/kg 85/287 only; 1 µg estradiol only; and 85/287 + estradiol. Pseudopregnant rats were administered 85/287 (2.5 mg/kg p.o.) on day 3 post-coitum. Deciduoma was induced in one of the uterine horns on day 4 and animals were autopsied 18 h post-traumatization. Both ERs and PRs showed an increase in traumatized horns compared with non-traumatized. In the 85/287-treated uterus, there was a reduction in cytosolic ERs in both traumatized horns. However, nuclear ER and PR levels increased in both horns under the influence of 85/287. Similarly, in a tamoxifen (0.03 mg/kg)-treated group a decline was noticed in cytosolic ER with a mild increase in nuclear PR. Total ER content, expressed per 100 µg DNA, showed a decline in 85/287- or tamoxifen-treated rats. However, no significant alterations were observed in total PR levels in non-traumatized horns. In an immature rat model, 85/287 caused a significant (> 50%) reduction in estradiol-induced ODC activity. These findings suggest that the decidualization inhibitory activity of 85/287 may be attributed to inhibition of certain timed biochemical events and genomic/non-genomic actions of estrogens in the rat uterus.

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Introduction

The specific response to an artificial stimulus involves extensive reorganization of endometrial cell populations in the progesterone-dominated uterus which mimics gestation, but without feto-placental formation and wherein pseudopregnancy is prolonged into decidualization. Estrogen opens an implantation window, but predisposes the uterus to cellular transformation, namely decidualization.

The decidual cell response consists of several phases involving increase in vascular permeability, histamine release, prostaglandin synthesis (1–3) and ornithine decarboxylase (ODC) activity associated with the growth (4). The continuous progesterone action appears to be the result of estrogenic modulation of this action resulting in decidualization. The balance between the two can be distorted by disturbing either of the hormones or the two receptor systems (estrogen and progesterone) (5). Triphenylethylene derivatives like clomiphene and tamoxifen are reported to inhibit the decidual cell response at the time of induction treatment

by inhibition of at least one of the actions of estrogen in the uterus (6).

Compound CDRI-85/287 is virtually devoid of agonistic activity, but is a potent anti-estrogen and anti-implantation agent (7), and has also been reported to inhibit decidualization (8) in the rat. The present studies were aimed at elucidating the effect of this novel molecule on uterine steroid receptors during the inhibition of decidualization.

Materials and methods

Chemicals

All chemicals used in this study were purchased from Sigma Chemical Company, St Louis, MO, USA or were of analytical grade generally available commercially. [^{14}C]Ornithine hydrochloride (specific activity 56 mCi/mmol), [2,4,6,7- ^3H]estradiol (specific activity 113.4 Ci/mmol) and [1,2,6,7- ^3H]progesterone (specific activity 96 Ci/mmol) were obtained from Amersham International, Amersham, Bucks, UK.

Animals

Immature (40–50 g) and adult (180–200 g) female rats (Sprague–Dawley strain) maintained under uniform husbandry conditions (temperature $24 \pm 1^\circ\text{C}$) with free access to pelleted food and water were used in this study.

Immature rats were bilaterally ovariectomized under ether anesthesia and after a rest period of 1 week divided into four different groups each comprising six rats. Animals of Group I were maintained as control (fed water only). Rats from Group II were administered the compound 85/287 at its contraceptive dose (2.5 mg/kg p.o.) as a gum acacia aqueous suspension. Animals of Group III were given (s.c.) estradiol-17 β (1 $\mu\text{g}/\text{rat}$) and rats of Group IV were administered the same dose of estradiol 18 h after the anti-estrogen administration. Animals of all groups were autopsied 5 h after estradiol administration.

Studies in pseudopregnant animals were carried out by adopting the procedure reported earlier (7). In short, coeval females were co-caged overnight with adult males of proven fertility and the day vaginal smears showed the presence of spermatozoa was considered as day 1 post-coitum (p.c.). Mated animals were divided into different groups and their fallopian tubes ligated on day 2 p.c. Animals were administered 85/287 (2.5 mg/kg; Group II), tamoxifen (0.03 mg/kg; Group III) (8) and water (Group I; control) on day 3 p.c. On the morning of day 4, unilateral decidualization was induced by administering gradually 0.05 ml peanut oil intraluminally through a 27 gauge needle inserted near the uterotubal junction on day 5 p.c. and rats were killed 6 and 18 h post-traumatization. Uterine tissue was freed of fat and the adhering mesentery, weighed in a torsion balance, cut into pieces and used for enzyme/receptor assays.

Enzyme assay

ODC levels were measured by the modified (9) method described by Kaye *et al.* (10) and Kobayashi *et al.* (11). In short, the method involved the use of substrate (L- ^{14}C)ornithine hydrochloride and Tris buffer, trapping of CO_2 evolved and termination of the reaction by citric acid. Radioactivity was assayed in a scintillation counter, and after quench correction was expressed as c.p.m./mg protein (12).

Receptor assay

Tissue of known weight was washed with chilled saline followed by 10 mM Tris–HCl buffer pH 7.4 containing 12 mM monothioglycerol, 3 mM MgCl_2 10% glycerol and 1 μM cortisol (TMMG buffer). Homogenate was prepared (250 mg/ml) in TMMG buffer by an ultraturax tissue homogenizer, then centrifuged at 800 g in a refrigerated centrifuge for 15 min. After separating the nuclear fraction, the pellet was kept cold ($0-4^\circ\text{C}$)

and the supernatant ultracentrifuged at 105 000 g for 60 min at $0-4^\circ\text{C}$. Cytosolic and nuclear ERs (ER_c and ER_n) and PRs (PR_c and PR_n) were measured by the method of Martel & Psychoyos (13) with certain modifications as described by Gupta *et al.* (14).

Measurement of cytosolic and nuclear receptors

Prior to assay, cytosol was treated with dextran-coated charcoal (DCC) (0.5% charcoal and 0.05% dextran in TMMG buffer) to remove any endogenous steroid, and ER and PR were estimated in nuclear and cytosolic fractions as described earlier. [^3H]Estradiol and [^3H]progesterone were used as radioligand for ER and PR assays respectively. Bound and free steroids were separated by DCC and bound fractions were counted in a scintillation counter. An aliquot of cytosolic and nuclear fractions was taken for estimation of protein and DNA by Lowry's (12) and Burton's methods (15) respectively.

Specific binding sites were calculated by subtracting the non-specific binding sites (i.e. in the presence of unlabelled diethylstilbestrol (DES) or progesterone) from the total binding sites (i.e. without unlabelled DES or progesterone). Cytosolic receptors were expressed as fmol/mg protein and nuclear receptors as fmol/100 μg DNA. Values are mean $\pm$ S.D. of five observations. Level of significance was analysed by Student's *t*-test.

Results

Enzyme levels

Results in Table 1 show that estradiol administration to ovariectomized rats almost doubled the enzyme levels (86%) and simultaneous administration of the compound 85/287 antagonized this increase bringing down (32%) the levels to almost control values. The compound *per se* did not increase these levels compared with control. Since the antagonism of estradiol-induced ODC activity was evident in this experiment, measurement of these levels was taken up during decidualization.

ER and PR levels

As demonstrated in Figs 1 and 2 there was an increase in ER_c and ER_n and PR_n content in the traumatized

Table 1 ODC activity in immature ovariectomized rats treated with estradiol and 85/287. Data are mean of three observations.

Treatment	ODC activity
	(c.p.m./mg protein per min of [^{14}C]CO ₂ recovered)
Vehicle	628
Estradiol (1 $\mu\text{g}/\text{rat}$ for 3 days)	1166
85/287 (2.5 mg/kg for 3 days)	537
Estradiol + 85/287	785

Figure 1 ER_c, ER_n and total ER levels (means $\pm$ S.D.) in non-traumatized and traumatized horns of the rat uterus. Rats were treated with 85/287 or tamoxifen (Tam) as described in Materials and Methods. • not significant; * $P < 0.001$; ** $P < 0.005$ vs respective controls.

Figure 2 PR_c, PR_n and total PR levels (means $\pm$ S.D.) in non-traumatized and traumatized horns of the rat uterus. Rats were treated with 85/287 or tamoxifen (Tam) as described in Material and Methods. • not significant; * $P < 0.001$; ** $P < 0.005$; *** $P < 0.02$ vs respective controls.

uterine horn compared with non-traumatized. In 85/287-treated rat uterus, ER_c showed a decline, both in traumatized (control 290.9 ± 18.6 ; treated 11.59 ± 12.3) and non-traumatized (control 260.7 ± 25.8 ; treated 123.6 ± 10.1) horns. ER_n, however, showed an increase in traumatized (control 100.56 ± 20.3 ; treated 156.7 ± 15.0) compared with non-traumatized (control 58.3 ± 12.7 ; treated 72.4 ± 12.5) horns.

A decrease was also noted in PR_c levels, but changes were less significant compared with ER_c in both traumatized (control 158.4 ± 15.2 ; treated 131.0 ± 12.8) and non-traumatized (control 101.6 ± 8.5 ; treated 96.4 ± 14.3) horns.

In the non-traumatized horn, PR_n showed a decline (control 44.4 ± 6.3 ; treated 29.5 ± 5.0) and in the traumatized horn an increase was noted (control 30.5 ± 8.4 ; treated 52.8 ± 10.8). Similarly, in the tamoxifen-treated rat uterus, both in traumatized and non-traumatized horns, a decline was noted in ER_c and a mild increase in PR_n. The total ER content (ER_c + ER_n) showed a decline in 85/287- or tamoxifen-treated rats in both traumatized (control 7.9 ± 1.6 ; 85/287-treated 1.1 ± 0.2 ; tamoxifen-treated 1.9 ± 0.4) and non-traumatized (control 2.5 ± 0.8 ; 85/287-treated 1.4 ± 0.1 ; tamoxifen-treated 1.4 ± 0.5) horns (values are expressed as pmol/100 µg DNA). However, no significant alterations were noticed in total PR levels in the non-traumatized horn.

Discussion

Decidualization is characterized by hypertrophy, hyperplasia and differentiation of the uterine stromal cells, which depends on early estrogen/progesterone priming followed by obligatory progesterone support (16). Since the decidualization is associated with tissue growth, ODC activity is associated with the rate of growth (17), its activity increasing under conditions of cell proliferation and differentiation (18, 19).

Estrogens are known to elevate ODC activity, which is antagonized under the influence of anti-estrogens like tamoxifen and monohydroxy tamoxifen (6) as well as with 85/287 (present studies) as tested in ovariectomized rat models. But there are reports that some anti-estrogens display full agonistic activity with respect to inducing ODC activity comparable to that induced by estradiol (9), where the higher efficacy of tamoxifen as an agonist was quite evident to much higher levels even using 1/80th the dose of that of 85/287. In this context there have been reports on the anti-estrogen-induced ODC activity resulting in temporal shifts mediated by different dosing regimens (9). The inhibition of increases in ODC levels by 85/287 may be attributed to its anti-estrogenic potency (7) leading to inhibition of initiation of decidual cell reaction (DCR) events (20), probably by the elimination of progesterone supply at the time of induction of DCR (21).

In the mechanism of DCR action, the progesterone

action is a central event (22) which might lead to a reduction in the formation and activation of receptor complex/reduction in receptor synthesis resulting in the decline of receptor concentration. In the repression of decidual reaction by triphenylethylenes, two of the genomic effects may plausibly be involved, i.e. synthesis of PRs and/or synthesis of polypeptide growth factors and other proteins (6). A decline in PR_c was noted in both traumatized and non-traumatized uterine horns in our studies, presumably because of antagonism of estrogen action, since the synthesis is mainly regulated by estrogen (23, 24). In this context it may be noted that clomiphene does not react with receptors but diminishes receptors, by one of the actions of estrogen on the uterus (6) and/or a disturbance in the balance of estrogen and progesterone as decidualization appears to be the result of estrogenic modulation and continual progesterone action. Moreover, estrogen action stimulates the PRs in rat (23) but reduction in total PRs may reflect the inhibition of action (14).

Triphenylethylene anti-estrogens bind to ERs (25) leading to a partial/incomplete activation of them. A decline in ER_c, ER_n and total ER in both traumatized and non-traumatized horns of treated rats was noted in our studies; the dilution effect of ER concentration in the traumatized horn may be attributed to tissue changes. However, the compound in general interferes with the replenishment process due to its anti-estrogenic action or inhibition of estradiol-induced transcription activation as in the case of clomiphene and nafoxidene (26, 27).

These findings suggest that the DCR inhibitory activity of 85/287 in rat uterus may be attributed to the inhibition of certain timed biochemical events (8) and also asynchrony of ERs and PRs which include both non-genomic and genomic responses to estradiol action.

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