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THE STEROID SPECIFICITY OF THE ESTROGEN–RECEPTOR OF HUMAN BREAST CARCINOMA

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SUMMARY

The specificity of the estrogen–receptor in human breast carcinoma was determined by incubating the cytosol fraction with tritiated estradiol-17β alone or in the presence of other steroids. If the steroid competed with the tritiated estradiol-17β for binding sites on the receptor the binding of the estradiol-17β decreased.

The structural requirements of the ligand for binding by the estrogen–receptor of breast carcinoma cytosol are the same as for that of human uterus cytosol. Highest affinity to the receptor is found if the steroid has a phenolic hydroxyl group on C-3 and an alcoholic hydroxyl group on C-17 in the β-configuration. Variations of the functional groups with regard to numbers, position and state of oxidation decrease the affinity for the receptor.

INTRODUCTION

Biochemically, two types of human breast cancer can be distinguished by their affinity for estradiol-17β [1, 2]: One which contains a limited number of saturable estrogen–receptors and which binds relatively more estradiol-17β. The other group binds very little estradiol-17β in a non-specific, non-saturable way. The receptor-binding of estradiol-17β by the former group of breast carcinomas was very similar in its characteristics to that of human uterus [3, 4]. While detailed information on the *in vitro* specificity of the estrogen receptors of the uterus of various species (including rat, rabbit, calf, sow, ewe, mouse, human) is available [Ref. 5 and references therein], relatively little is known about the specificity of the estrogen receptors in human breast carcinoma, and the steroid structure-affinity relationships had not been studied in detail. This is the subject of this report. To determine the specificity of the estrogen–receptor, breast carcinoma cytosol was incubated with tritiated estradiol-17β alone and in the presence of the steroid to be tested. If the steroid competed with the estradiol-17β for binding sites on the receptor the binding of the tritiated estradiol-17β was diminished. This decrease could serve as a measure of the affinity to the estrogen–receptor of the steroid under investigation.

MATERIALS AND METHODS

Tissues

The breast tissues studied (from 11 patients) were part of biopsy specimens sent to the laboratory for frozen sections and subsequent paraffin sections. In some instances mastectomy specimens were obtained. The age and menstrual history for all patients and the pathological report on the biopsy and/or mastectomy were noted. In preliminary tests carcinomas containing specific receptors [2] were selected and used for the investigations.

Steroids

6,7-[^{3}H]-estradiol-17β (S.A. 40 Ci/mmol) was purchased from Radiochemical Centre, Amersham, England.

A complete list of all other steroids used in this study with their systematic names and their source was given in a previous communication [5]. The purity and identity of the steroids was confirmed by infrared spectroscopy in KBr pellets. In addition, 2-hydroxyestriol (estra-1,3,5(10)Trien-2,3,16α,17β-Tetrol, Mann Research Laboratories, New York) was tested.

Preparation of tissue fractions and incubation

The fresh tissue was homogenized in Tris buffer pH 8·0 and the soluble tissue fraction isolated as described [5]. Aliquots of the cytosol were diluted 1 to 10 with buffer and 1 ml portions incubated with 2×10^{-13} mol tritiated estradiol-17β. After 30 min incubation at 25°C the reaction mixture was cooled in ice water. One millilitre of a suspension of dextran-coated charcoal in Tris buffer was added. After briefly mixing the contents the mixture was kept in ice-water for 20 min after which time the charcoal was spun down. The

supernatant was poured directly into counting vials, 10 ml Bray scintillator was added and the radioactivity measured.

For inhibition experiments 50, 100, 200, 1000, 5000, 10000 pg of non-radioactive estradiol-17β or of other steroid was added to the reaction mixture together with the tritiated estradiol-17β and incubated as described.

The incubation mixture containing tritiated estradiol-17β only was used as a control (= 100%). This control value was obtained in 5 replicates with each run. Incubations in the presence of non-radioactive estradiol-17β and the steroids under test were done in triplicate for each of the five concentrations.

The results were expressed as bound radioactivity divided by the radioactivity in the incubation medium (bound d.p.m./medium d.p.m.). These values were plotted over the logarithm of the total (tritiated + non-radioactive) steroid concentration (pg) present in the incubation medium.

RESULTS

In contrast to uterus which due to its size permits a large number of tests to be performed on a single specimen, tissue samples of breast carcinoma are in most cases quite small. The investigation of the influence on the binding of tritiated estradiol-17β by competitive inhibition was limited, therefore, to a few steroids and non-radioactive estradiol-17β per individual tissue. In addition, binding of estradiol-17β by breast carcinoma varies greatly from one specimen to the other. These factors combine to make it difficult to collect comparable data on the specificity of the estrogen–receptor in breast carcinoma cytosol. To overcome these problems as far as possible only tissues with similar binding affinities for estradiol-17β were grouped together when the influence of steroid structure on receptor-binding was studied.

The reduction in the binding of tritiated estradiol-17β by the presence of increasing amounts of non-radioactive estradiol-17β was used to demonstrate specific, saturable receptors in the tissues. The addition of 50 pg of non-radioactive estradiol-17β decreased the binding of tritiated estradiol-17β on average to 67% of the control value. The steep decrease continued when 100 pg (to 52% of control) or 200 pg (to 38% of control) were added. The binding continued to decrease on a gentler slope (to 21% of the control in the presence of 1000 pg non-radioactive estradiol-17β). Saturation was reached at 5000 pg (decrease to 12% of control); further addition of non-radioactive estradiol-17β did not significantly decrease the binding of tritiated estradiol-17β (10% of control with 10,000 pg).

The affinity of the other steroids to the breast cancer cytosol receptor was tested by measuring their influence on the binding of tritiated estradiol-17β in the same way. The following steroids (in amounts up to 10,000 pg) did not significantly influence the binding of tritiated estradiol-17β; progesterone; dehydroepiandrosterone; 5β-androstan-3α,17β-diol; 5α-androstan-3α,17β-diol; 5α-androstan-3β,17β-diol; androst-4-ene-3α,17β-diol; 2-hydroxy-3-deoxyestradiol-17β; 16,17-epiestriol; 1-methylestradiol-17β; estra-5-ene-17β-ol-3-one; 16-hydroxyestrone; 3-deoxyestrone; 2-hydroxyestriol.

The following compounds had the same or nearly the same effects as non-radioactive estradiol-17β on the binding of tritiated estradiol-17β; 18-norestradiol-17β, 2-methylestradiol-17β, 17α-ethynylestradiol-17β and hexestrol. All steroids in this group have an aromatic ring A, a phenolic hydroxyl group on carbon-3 and an alcoholic hydroxyl group on carbon-17 in the β-configuration.

Two more groups can be distinguished [5]: One includes estrone, estriol, 16-epiestriol, 17α-estradiol, 16α-estradiol and 17-epiestriol which, if present in amounts of 100 pg, reduces estradiol binding to about 80% of the control and at the 1000 pg level reduce the binding to about 45% of the control. The common features of these steroids are the aromatic ring A, the phenolic hydroxyl group at carbon-3 and an oxygen function in ring D.

The steroids of the other group had an effect on the binding of (60 pg) tritiated estradiol-17β only at considerably higher concentrations (1000 pg or more). These steroids are 16-oxoestradiol-17β, 1-estradiol, 17β-estradiol-3-glucosiduronate, estradiol-17β-glucosiduronate, 19-nortestosterone, 3-deoxyestradiol-17β, 17-deoxyestrone and 17β-methoxyestradiol. In the presence of 5000 pg of these steroids–estradiol binding was reduced to about 70% of the control and 10,000 pg reduced the estradiol binding to about 55%.

As it was not possible to investigate the affinity of the various steroids for the estrogen–receptor in a single tumor specimen, it was necessary to determine the steroid structure–receptor affinity relationships in a series of test runs including a number of carcinoma specimens which did not all have the same affinity for estradiol-17β. By careful examination of results for individual tumors like those shown on Fig. 1 (i.e. complete inhibition curves based on 7 different steroid concentrations) supplemented by results in which inhibition of estradiol-17β binding was determined for many steroids but at only a single concentration, it was found that the ability of a steroid to compete with estradiol-17β for receptor sites in human breast carcinoma cytosol decreased in the following order (non-

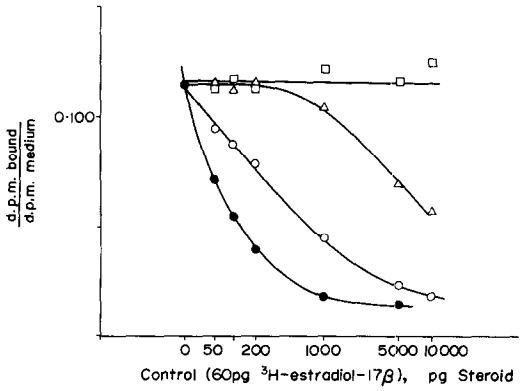

Fig. 1. Inhibition of the binding of tritiated estradiol-17β by other steroids. On the ordinate binding is expressed as d.p.m. bound/d.p.m. medium, where d.p.m. bound is the radioactivity in the charcoal supernatant (receptor-bound radioactivity) and d.p.m. medium is the total radioactivity in the incubation medium at the beginning of the experiment. On the abscissa the logarithm of the sum of tritiated estradiol-17β and non-radioactive steroid is plotted. Point 0 (=control) shows the binding of 60 pg tritiated estradiol-17β in the absence of non-radioactive steroid. The steroids shown represent the four groups, i.e. no effect on estradiol-17β binding (2-hydroxy-3-deoxyestradiol-17β □——□), influence only at high concentrations (16-oxo-estradiol-17β, △——△), considerable competition for estradiol-17β receptors (16α-estradiol, ○——○) and estradiol-17β-like activity (estradiol-17β ●—— ●).

steroids in brackets): estradiol-17β = 17α-ethynylestradiol-17β (=diethylstilbestrol)($\geqslant$ hexestrol) > 2-methylestradiol-17β $\geqslant$ 18-norestradiol-17β > 16-epiestriol $\lesseqgtr$ 17-epiestriol $\geqslant$ 16α-estradiol $\geqslant$ 17α-estradiol $\geqslant$ estriol > estrone > 17β-methoxyestradiol > 16-oxoestradiol-17β = estradiol-17β-glucosiduronate > 17β-estradiol-3-glucosiduronate > 3-deoxyestradiol-17β > 17-deoxyestrone > 16-hydroxyestrone, 3-deoxyestrone, 16,17-epiestriol and all the other steroids that have no influence at all on the estradiol-17β binding. This order of affinity may not be the same for all breast carcinoma cytosols: In one instance (carcinoma PAR) estriol competed more efficiently for the estrogen receptors than 16-epiestriol, while the reverse order was commonly found. Another example is 17-epiestriol which in one instance (WIM) was as active as estradiol-17β in reducing the binding of tritiated estradiol-17β to the receptor while in others it ranked with 16-epiestriol or below.

DISCUSSION

The relative binding affinity of a few steroids to human breast carcinoma cytosol was studied by Korenman and Dukes [6]. The rank order of affinity in one of their patients was estradiol-17β > 16-epiestriol > estrone > estriol > 17α-estradiol. This order is in close agreement with the one reported here. However, in our experience estriol was in most cases a more potent competitor than estrone; in only one carcinoma with very high binding activity estrone and estriol had the same affinity. In addition, the variety of steroids tested for their influence on the binding of estradiol-17β by human breast carcinoma cytosol is considerably greater in the present report. Comparison of the receptors in human uterus and human breast carcinoma shows that their specificity for steroids is very similar if not identical [5]. The most important requirement for receptor-binding is the phenolic hydroxyl group on C-3. If it was missing (e.g. 3-deoxyestrone), substituted (e.g. 17β-estradiol-3-glucosiduronate), in a different position (2-hydroxy-3-deoxyestradiol-17β) or if it was an alcoholic hydroxyl group rather than a phenolic hydroxyl group (e.g. 5α-androstanediol-3α, 17β) the affinity of the steroid towards the receptor was largely or entirely abolished. Introduction of a methyl group on C-1 also led to a loss of affinity towards the receptor while a methyl group on C-2 had no effect. A second requirement for receptor binding is an oxygen function on ring D but it is not essential. While optimal binding was associated with a 17β-hydroxyl group (e.g. estradiol-17β), neither the position of the group (e.g. estradiol-16α) nor the configuration (e.g. estradiol-17α), nor its state of oxidation (e.g. estrone) were critical, i.e. considerable receptor binding was still possible. Additional oxygen functions on ring D decreased the affinity of the steroid to the receptor to a varying degree (e.g. estriol, 16-oxoestradiol-17β). Presence or absence of the angular methyl group on C-13 had no influence on the binding. The steroid structure–receptor affinity relationship was discussed in more detail before [5].

It was found earlier that the association constant of the estradiol–receptor complex and the number of binding sites were of the same order of magnitude for both human uterine tissue cytosol and human breast carcinoma cytosol [2]. As furthermore the specificity of the receptors in the two tissues is very similar with regard to steroid structure, it is suggested that the estrogen receptors of both uterus and breast carcinoma cytosol may be closely related.

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