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SPECIFIC CELL ADHESION TO ESTRADIOL-DERIVATIZED AGAROSE BEADS

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SUMMARY

Estrogen-target cells in suspension adhere to agarose beads derivatized with estradiol. The interaction is shown to be specific and saturable by appropriate control tests.

INTRODUCTION

While the major evidence is unquestionably in support of the prominence of the intracellular receptor in the steroid action mechanism, many examples have been given of steroid–cell interaction which cannot be readily fitted in this general mechanism. In particular, evidence has been provided for the occurrence of specific binding sites for steroid hormones in the plasmamembrane of steroid-target cells [for review, see 1]. Such evidence has been brought about by both cell-free and intact cell systems; among the latter, an affinity-binding approach using protein-linked fluorescent estrogens appeared to be ideally suited for binding studies to the cell surface more than disrupting procedures [2, 3]. In fact, this experimental system has demonstrated that estrogen-target cells are equipped with a structural, estradiol-binding component fully integrated in the fluid mosaic of the plasmamembrane, which thus represents a level of steroid–cell interaction additional to the classical receptor [2, 3].

The occurrence of surface binding sites for estrogen has been further investigated by an affinity binding approach in the reverse direction; that is, a study was carried out on the specific interaction of estrogen-target cells with an insoluble support derivatized with estrogen. In this approach, already exploited in a variety of systems [4, 5], the cells are evaluated according to their affinity for a solid support coated with a biospecific reagent.

EXPERIMENTAL AND RESULTS

In the devised experiment, the tool was beads of agarose coated with a multichain copolymer of lysine and alanine to which either diethylstilbestrol or estradiol derivatives were covalently attached (kindly provided by Dr G. A. Puca, Institute of General Pathology, University of Naples, Italy) [6, 7]. These estrogen-containing agarose derivatives possess chemical stability and display high affinity for specific estrogen binding proteins; they have been successfully employed to purify estrogen receptors by affinity chromatography [8].

The retained reactivity of estradiol determinants on agarose beads for binding proteins is well demonstrated by the specific interaction of estradiol antibodies by immunofluorescence technique (Fig. 1). When intact target cells in suspension, like human breast cancer cells, are incubated with the estradiol-coated beads, the free floating cells may be seen to stick to the beads (Figs 2 a–d). In contrast, no cells could be seen to adhere to the supports derivatized with diethylstilbestrol.

DISCUSSION

The cell adhesion to estradiol-derivatized beads may easily be reproduced, provided that care is taken in handling cells, since excessive shaking or rapid flow could remove them from the beads. Several sets of control experiments have confirmed that this adhesion reflects the specific interaction of the estradiol determinants of the beads with some specific constituents of the cell plasma membrane, thus excluding the possibility of a non-specific event (Tables 1 and 2).

First of all, cells did not bind to unsubstituted beads; moreover, the presence of non-steroidal determinants on the beads was ineffectual; lastly, no

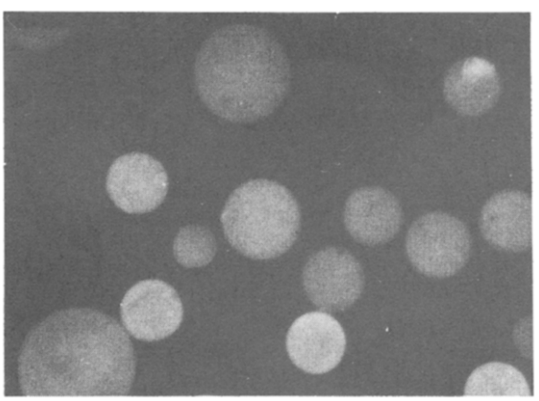

Fig. 1. Estradiol determinants on the derivatized beads are displayed by immunofluorescence technique using specific estradiol antibodies.

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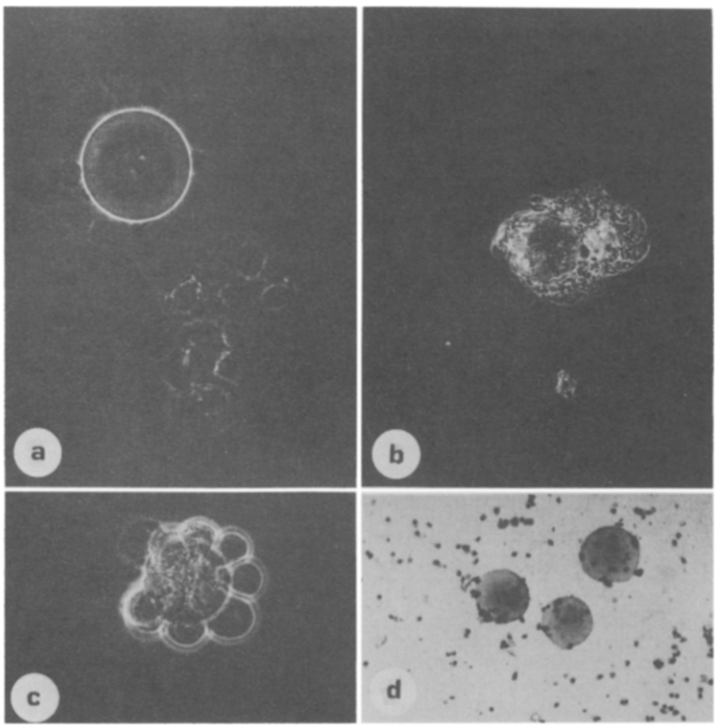

Fig. 2. (a) Two estradiol-coated beads appear to be stuck with adherent cells at different focus plane by phase contrast. (b) The cell coat realizes the cohesion of two derivatized beads; phase contrast. (c) Nomarsky light microscopy shows an evident cell rosette centered on the bead. (d) The cell adhesion to beads is also clearly evident in preparations stained with toluidine blue.

cell adhesion could be seen when the estradiol determinants on the beads had been previously obliterated by specific estradiol antibodies. Just as meaningful as the evidence for the unique role of estradiol determinants, is the selectivity of the interaction restricted to estrogen-target cells. It is worth noting that neither several non-target cell lines nor non-epithelial cells contaminating our cell preparations from breast cancer, never showed any significative adhesion to beads.

The steroid specificity of the observed interaction was determined by applying the principle of ligand competition by the same or other classes of steroid hormones (Table 2). The most effective inhibition was achieved by pre-incubating the cells with protein-linked estrogen analogs; free estradiol was able to elicit a substantial suppression of the phenomenon only if added in large amount to the incubation mixture. This result suggests either a very ephemeral interaction of free estradiol with the plasmamembrane or that the surface binding sites recognize the hormone better in a macromolecular protein-carried state. In contrast with the inhibition elicited by natural estrogen, the cell adhesion of estradiol-agarose did not seem to be affected by both the synthetic estrogen, diethylstilbestrol, and the anti-estrogen, tamoxifen, even when they were present in large amount in the incubation medium. The lack of inhibition by diethylstilbestrol is well correlated with the lack of interaction of cells with beads derivatized with the same non-steroidal estrogen and with the inefficiency of non-steroidal steroid analogs in preventing the binding of the parent steroid to membrane binding sites [1–3]. Lastly, it is worth stressing that the phenomenon was essentially uninfluenced by the pres-

Table 1. Control experiments: to exclude non-specific adhesion of cells to beads

Cells do not bind to unsubstituted agarose beads.
Cells do not stick on DES-derivatized beads.
No cell adhesion is observed when the estradiol groups on beads have been blocked by specific estradiol antibodies.
Non-epithelial cell types (Erytrocytes, lymphocytes, macrophages, polymorphonucleates, adipocytes etc.) do not show any significative adhesion.
Non-specific binding of cells to agarose beads is not involved in this affinity cell binding system.

Table 2. Control experiments: to determine saturability and steroid specificity

E_2-17-HS-BSA and E_2-6-CMO-BSA give the most effective inhibition.
E_2 gives a similar degree of inhibition when added in excess to the E_2-derivatized beads.
Diethylstilbestrol and tamoxifen do not affect the cell adhesion to beads.
Testosterone and progesterone, both in native and protein-linked forms, do not inhibit the cell binding.
The cell membrane component responsible for the binding of estrogen is specific for the native hormone and saturable.

ence of other steroids such as testosterone and progesterone, used either as native molecules or linked to bovine serum albumin into macromolecular complexes like the estrogenic ones.

In conclusion, this experiment suggests, as the previous ones, that target cells expose on the surface specific recognizing sites for steroid hormones; no direct study of the binding affinity has been possible in such a system, which can just exclude non-specific interactions. What biological role could be assigned to these steroid-binding sites on the cell plasmamembrane is quite an open matter.

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