Vol. 109, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 16, 1982 Pages 291-296

PARADOXICAL NATURE OF ESTROGEN AGONIST AND ANTAGONIST BINDING IN RAT LIVER

M. K. Agarwal

CNRS, 15 rue de l'Ecole de Médecine, 75270 Paris Cedex 06, France.

Received September 28, 1982

SUMMARY: Whereas Tamoxifen exerts potent antiestrogenic action in ER dependent breast cancer, it was largely without effect on rat liver gluconeogenesis which could be dramatically diminished by estrogens and androgens. Although estradiol was preferentially bound to an ER_4 component that coeluted with CBG from DE-52 columns, ^3H-tamoxifen labelled the ER_3 moiety that was clearly distinct from transcortin. Similarly, testosterone was bound to the AR_4 entity but R-1881 was eluted in the AR_3 region. All these ER and AR populations were furthermore distinct from liver GR. These, for the first time, demonstrate polymorphic nature of AR and ER and suggest that agonist and antagonist actions may be expressed via separate populations of the receptor, contrary to the established, classical view that dictates competitive antagonism between them for the one and the same site.

INTRODUCTION: All five major groups of steroid hormones bind their specific, high affinity, low capacity, cytoplasmic receptors whose translocation to the nucleus is believed to initiate tissue specific, hormone responsive, processes (1-3). Liver is endowed not only with the GR (3), but also an ER (4) and an AR (5,6), as well as 'intracellular transcortin' (7). Earlier studies from this laboratory have established that gonadal hormones hamper liver gluconeogenesis (5,6) but do not influence other liver enzymes. What would be the influence of antiestrogens under these conditions? Tamoxifen (1-(4-β-dimethylaminoethoxyphenyl)1,2-diphenylbut-1-ene), commonly called Nolvadex, is a nonsteroidal antiestrogen currently employed as the only specific medical therapy in human breast cancer (2). Here, the physiological action of this drug in the liver was investigated in relation to the binding of tritiated tamoxifen, procured through special synthesis, to different liver vectors.

Abbreviations: AR = androgen receptor; CBG = corticoid binding globulin = T = transcortin; ER = estrogen receptor; GR = glucocorticoid receptor; MR = mineralocorticoid receptor; SBG = sex steroid binding globulin; TA = triamcinolone acetonide.

0006-291X/82/210291-06$1.00/0

MATERIALS AND METHODS: Male, Wistar rats (150-200 g) were bilaterally adrenalectomized 2-3 days prior to use and maintained on 1% NaCl and pellet food ad libitum. Animals were sacrificed under ether anesthesia, liver was perfused with the initial buffer according to the experimental procedure, and the cell sap obtained by centrifugation at 105,000 g. Blood was allowed to clot at 37°C (1 h), then at 4°C (90 min), and finally centrifuged at 3000 g.

For kinetics of association, 0.5 ml cytosol (in 0.01 M Tris-HCl, pH 7.4) was incubated (60 min 4°C) with the desired tritiated hormone alone or in presence of an excess of the homologous cold steroid. Free steroids were thereafter removed by the addition of 0.5 ml activated charcoal (50 mg/ml), further incubation (10 min 4°C), and centrifugation (3000 g). Aliquots of 0.5 ml were mixed with 10 ml Unisolve (Kochlight, U.K.) and counted in a Packard Scintillation Spectrometer equipped for quench and background correction.

For chromatography, liver cytosol in the initial buffer was equilibrated with the tritiated steroid of choice, charcoal treated, and passed through glass wool prior to passage through the gel.Undiluted serum was treated similarly Further details are given in legends and have been published (8,10,11).

Liver glycogen levels were determined by the classical procedure where 5-hydroxymethylfurfural, generated from glycogen in presence of hot, concentrated H_2SO_4, is qunatitated spectrophotometrically against a glucose standard (5,6).

2,4,6,7,^{3}H-estradiol (108 Ci/m mol; batch 54), 1,2,6,7,^{3}H-testosterone (88 Ci/m mol, batch 30), 1,2,^{3}H-corticosterone (82 Ci/m mol, batch B12), and 4-^{14}C-corticosterone (52 mCi/m mol, batch 12) were purchased from Amersham, U.K. ^{3}H-R-1881 (17-α-methyltrienolone) (87 Ci/m mol, lot 1022-128) was obtained from New England Nuclear.^{3}H-tamoxifen (19.5 Ci/m mol) and the corresponding cold material were gifts from ICI, U.K. Radiochemical purity in all cases exceeded 98%. All other steroids were procured from Sigma.

RESULTS AND DISCUSSION: Data in table 1 establish that as much as 20 mg of tamoxifen, alone, did not significantly influence either the basal level of liver glycogen, or TA induced gluconeogenesis. Tamoxifen was only weakly effective in this regard in presence of 2 mg estradiol which, in 20 mg doses, clearly exerted significant antigluconeogenic action; the effect of testosterone was similar to that of estradiol (not shown). Induction of several other enzymes was not influenced under these conditions (5,6). Thus, the known anti-

TABLE 1. Influence of Estrogen Agonists and Antagonists on Liver Glycogen.

Treatment	Glycogen (mg %) - TA	+ TA	Statistics vs Control
Control	11.5 ± 2.5	25.5 ± 2.1	
Estradiol 2 mg	12.2 ± 2.9	23.5 ± 4.0	N.S. (=not significant)
20 mg	2.4 ± 0.3	9.4 ± 1.2	$p < 0.005$ (- TA) and $p < .01$ (+ TA)
Tamoxifen 20 mg	9.6 ± 2.0	23.9 ± 2.8	N.S.
Tamoxifen 20 mg + Estradiol 2 mg	7.6 ± 2.1	18.8 ± 1.8	$p < 0.01$ in both cases

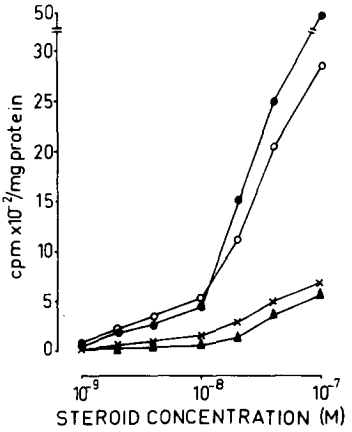

Figure 1. KINETICS OF STEROID BINDING TO LIVER VECTORS.

Liver cytosol was incubated, in triplicate, in presence of the indicated amount of radioactive steroid alone, or in presence of one hundredfold excess of homologous, unlabelled molecule to account for nonspecific binding which was subtracted from receptor bound hormone.

^{3}H-R-1881 (O); ^{3}H-Estradiol (●); ^{3}H-Tamoxifen (X); ^{3}H-Testosterone (▲).

estrogenic action of tamoxifen in breast cancer could not be duplicated on physiological functions innate to the liver.

Data in Fig. 1 show that ^{3}H-tamoxifen and ^{3}H-testosterone were bound to nearly the same extent in the liver and that this was far less than the binding of either ^{3}H-estradiol or of ^{3}H-R-1881 which is considered to be an ideal androgen receptor ligand (9) and thus suggests the presence of AR in the liver. Do these materials bind to distinct populations of the receptor in each class?

Data in Fig. 2 show that whereas ^{3}H-estradiol bound ER_4 component eluted in 0.06 M phosphate (Fig. 2a), the position where transcortin also elutes, tamoxifen was bound to an ER_3 population eluted in 0.04 M phosphate region and thus clearly distinct from CBG in the double labelled study (Fig. 2b). An ER_1 component was equally evident with both materials on DE-52 columns.

Although testosterone was eluted in the same position as estradiol (not shown), ^{3}H-R-1881 eluted in the AR_3 region which was therefore comparable to ER_3 and which did not coincide with CBG at all (Fig. 2c). These ER_3 and AR_3 moieties are furthermore distinct from liver GR_2 entity in the 0.02 M PO_4 region (Fig. 2d). The elution position of SBG under these conditions remains

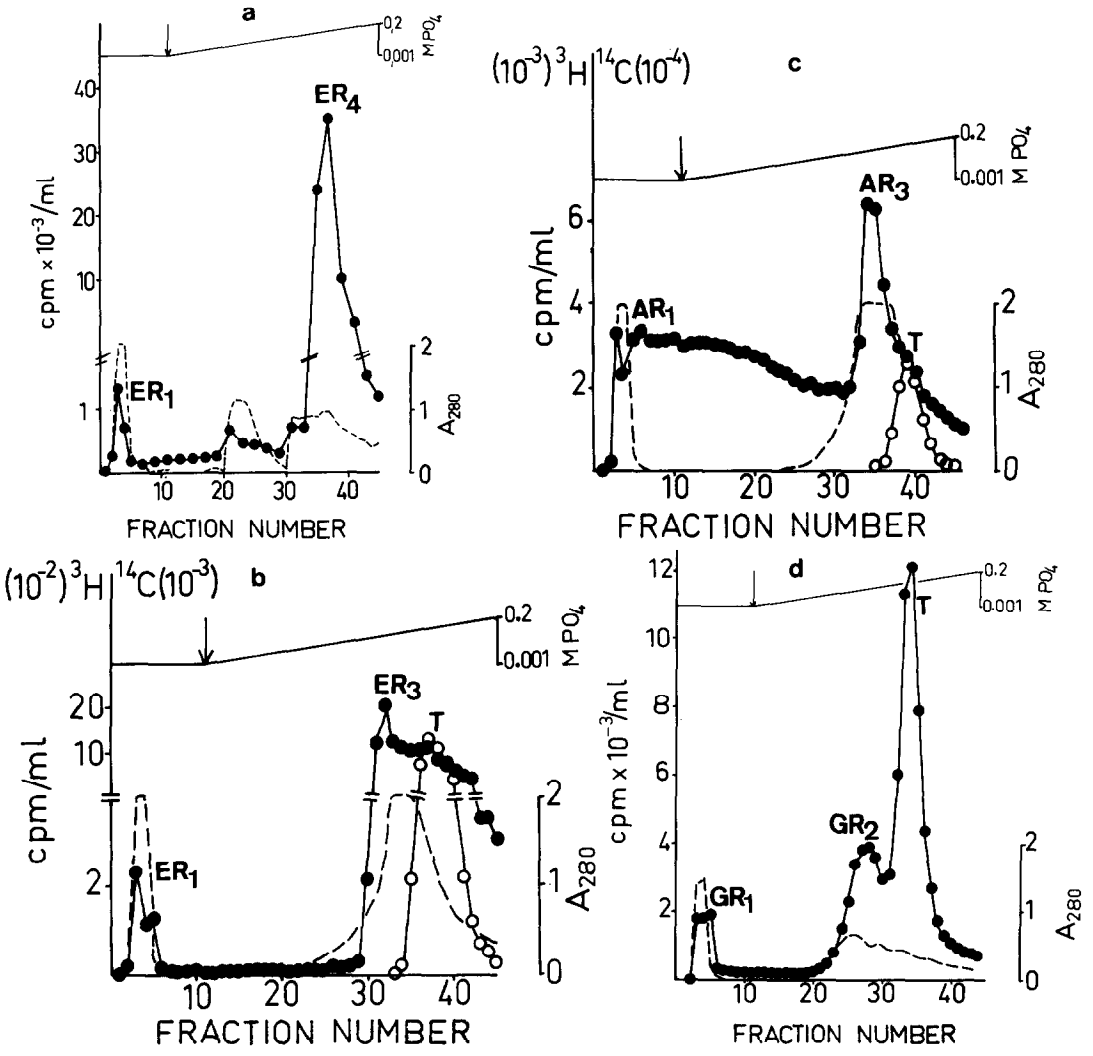

Figure 2. ION EXCHANGE SEPARATION OF VARIOUS RECEPTORS IN RAT LIVER.

4 ml cytosol in 0.001 M phosphate, pH 7.5, was incubated with 10^{-7} M of either ^{3}H-estradiol (a), ^{3}H-tamoxifen (b), ^{3}H-testosterone (c), or ^{3}H-corticosterone (d). 2 ml serum was similarly incubated with 0.5 µCi of ^{14}C-corticosterone. All samples were charcoal treated (see methods) and loaded onto a column (1.0 x 25 cm) of DEAE-cellulose-52 (Whatman) equilibrated with 0.001 M phosphate. After an initial prewash, protein was eluted by a linear gradient (begun at the arrow in figures) between 0.001 M and 0.2 M phosphate, pH 7.5 (60 ml each). 1 ml samples were processed for radioactivity and the absorbance was recorded manually. For Figs b,c, serum was mixed with liver cytosol just before chromatography. For further details see (8,10,11).

---------- A_{280}; ●——● ^{3}H; ○——○ ^{14}C

unknown although adult rat plasma is devoid of specific estrogen binding activity (7).

Data in Fig. 3 confirm receptor polymorphism on Ultrogel-ACA-44 columns, based on molecular weights, and show that estradiol (Fig. 3a) eluted in posi-

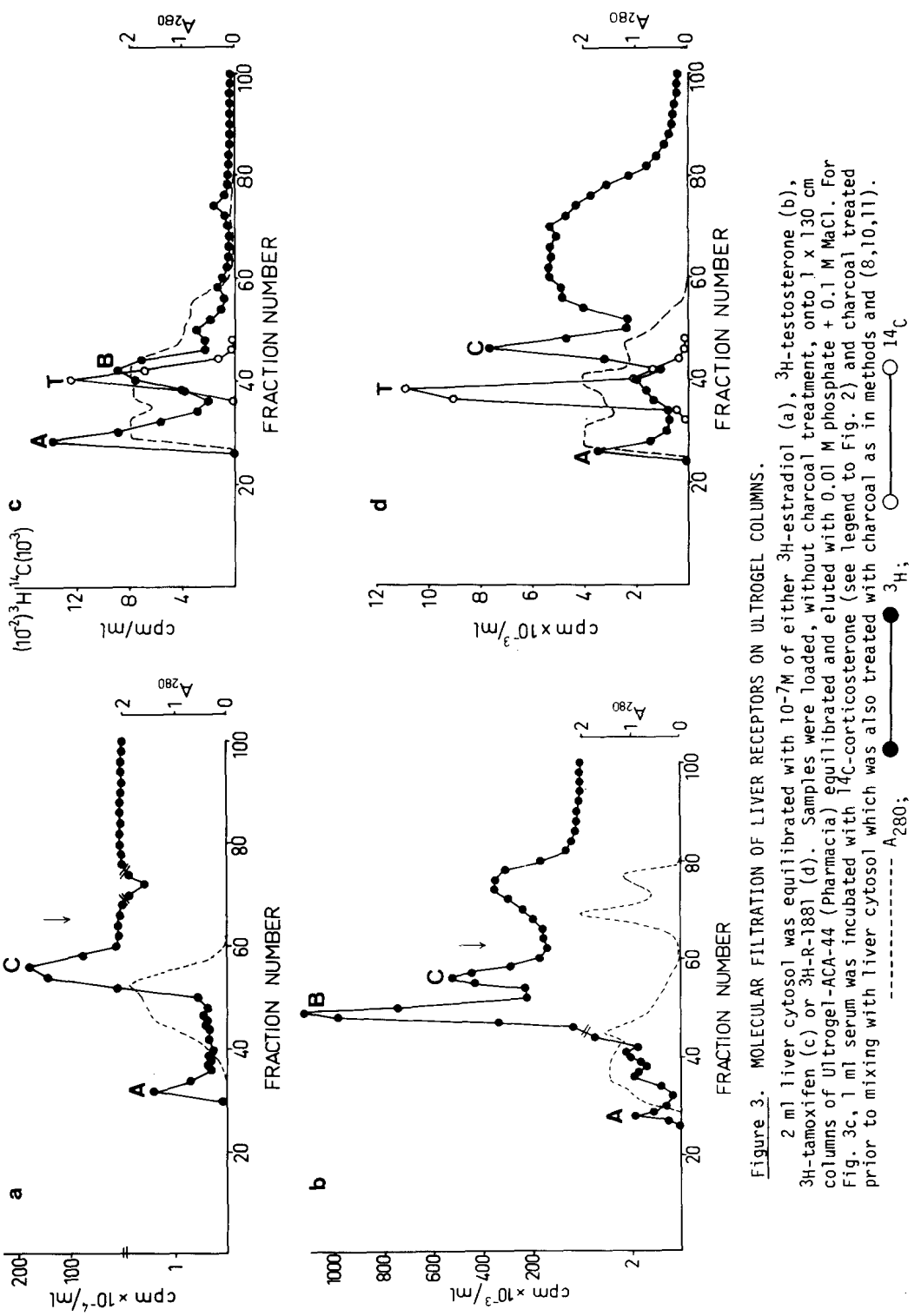

Figure 3. MOLECULAR FILTRATION OF LIVER RECEPTORS ON ULTROGEL COLUMNS.

2 ml liver cytosol was equilibrated with 10^{-7}M of either ^{3}H-estradiol (a), ^{3}H-testosterone (b), ^{3}H-tamoxifen (c) or ^{3}H-R-1881 (d). Samples were loaded, without charcoal treatment, onto 1 x 130 cm columns of Ultrogel-ACA-44 (Pharmacia) equilibrated and eluted with 0.01 M phosphate + 0.1 M MaCl. For Fig. 3c, 1 ml serum was incubated with ^{14}C-corticosterone (see legend to Fig. 2) and charcoal treated prior to mixing with liver cytosol which was also treated with charcoal as in methods and (8,10,11).

tions A and C whereas testosterone was resolved into an additional B peak intermediate between A and C components (Fig. 3b). Although peak A was also observed, ^{3}H-tamoxifen further labelled the component B that overlapped with transcortin (T) in the double labelled study, but did not label the estradiol bound component C (Fig. 3c). ^{3}H-R-1881 eluted in positions A and C with no indication of B, as with estradiol (Fig. 3d). Thus, receptor polymorphism was againt evident and confirmed results in Fig. 2, as well as those observed earlier with GR and MR (3,8).

Collectively, binding of tamoxifen to components distinct from those that are saturated by estradiol correlates well with the lack of influence of this molecule in the liver whereas it exerts potent antiestrogenic action in the breast where physicochemical analysis of ER has never been attempted. Thus, it is conceptually wrong to try to understand receptor function merely by competition of agonists and antagonists for the one and the same vector. If receptor multiplicity were absent, the minute amounts of agonists in circulation would be incapable of exerting any physiological action in vivo.

REFERENCES

(1) E. J. Ariens and A. J. Beld, Biochem. Pharmacol., 26: 913 (1977).
(2) V. C. Jordan in "Antihormones", M. K. Agarwal (ed), p 253, Elsevier/North Holland, 1979.
(3) M. K. Agarwal, FEBS Letters, 85: 1 (1978).
(4) W. deBoer, L. Snippe, G. Ab and M. Gruber, Endocrinology, 110: 1217 (1982).
(5) M. K. Agarwal, G. Lazar and S. Sekiya, Biochem. Biophys. Res. Comm., 79: 499 (1977).
(6) M. K. Agarwal and F. Coupry, FEBS Letters, 82: 172 (1977).
(7) P. C. Beers and W. Rosner, J. Steroid Biochem., 8: 251 (1977).
(8) M. K. Agarwal (ed) "Hormone Antagonists", Walter de Gruyter, 1982.
(9) C. Barne, Steroids, 27: 497 (1976).
(10) M. K. Agarwal (ed) "Multiple Molecular Forms of Steroid Hormone Receptors", Elsevier/North Holland, 1977.
(11) M. K. Agarwal (ed) "Proteases and Hormones", Elsevier/North Holland, 1979.
(12) These studies were aided by grants from the CNRS (AI 03 1917) and the UER Broussais Hotel Dieu. Thanks are due to ICI (U.K.) for kindly providing the tamoxifen preparations and the corresponding technical data. Some of these studies were presented at the 12th International Congress of Biochemistry, Perth, August 1982 and at the VI International Congress on Hormonal Steroids, Jerusalem, September 1982.