EVIDENCE THAT NATURAL VS SYNTHETIC STEROID HORMONES BIND TO PHYSICOCHEMICALLY DISTINCT CELLULAR RECEPTORS

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Natural (corticosterone) vs synthetic (dexamethasone) bound liver cytosols were fractionated on three chromatographic systems. Dexamethasone bound radioactivity eluted in positions different from those that were labelled with corticosterone filled receptors.

The initial step in the mechanism of action of most drugs is belived to consist of the binding of the pharmacological agent with a cellular receptor. This assumes special significance with respect to adrenocortical hormones since a study of their mode of action remains one of the few available models to delineate the organization and expression of the complex eukaryotic genome (1). Two groups of glucocorticoid hormones are currently employed viz : the naturally occurring (corticosterone, cortisol) and the synthetic (triamcinolone, dexamethasone). Although the latter are 10 - 100 fold more active than the former, at equimolar concentrations, it is generally accepted that both sorts of molecules bind to the same cytoplasmic receptor prior to the initiation of the organ specific process. The results presented here constitute a formal proof that these two groups of hormones bind to physicochemically distinct receptor proteins and are intended as a caution to intepretation of data with other hormones viz mineralocorticoids, sex steroids.

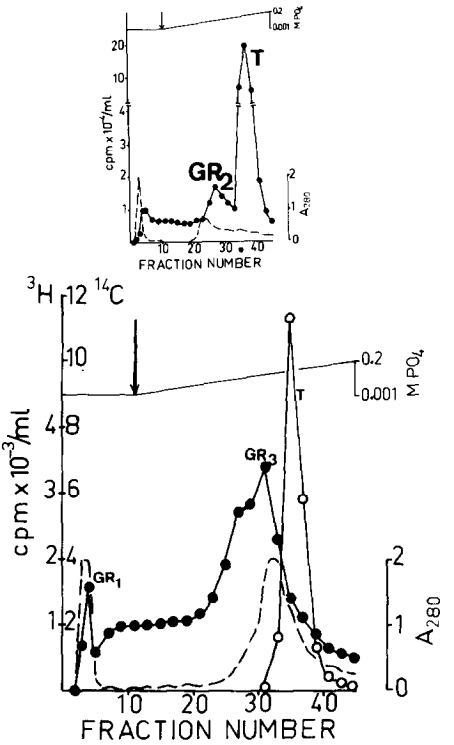

Fig. 1 Discrimination between natural vs synthetic steroid binders in rat liver by ion-exchange chromatography. Male, Wistar rats (150-200 g) were bilaterally adrenalectomized at least 48 h before use, exsanguinated under ether anaesthesia and, after perfusion with the initial buffer by aortic cannulation, liver 105,000 g supernates (4 ml) were incubated (60 min, 4°C) with 10^{-7}M corticosterone (insert) or dexamethasone ; 2 ml blood serum was incubated with 0.25 µCi of 14C-corticosterone. The free radioactivity was removed, separately, by additional incubation in presence of 100 mg ml^{-1} cell of activated charcoal (Sigma C 5260) which was thereafter eliminated by centrifugation (3000 g) and passage through glass wool. The cell sap alone (insert) or with serum was finally loaded on DEAE-cellulose-52 (Whatman) columns (1 x 25 cm) equilibrated with 0.001 M PO_4, pH 7.5. After passage of 60-70 ml of this initial buffer (fraction vol 6-7 ml) protein was eluted (at arrow) with a linear gradient of 60 ml of 0.001 M and 60 ml of 0.2 M sodium phosphate pH 7.5, at a flow rate of 60 ml/h (fraction vol ca. 3 ml). All manipulations were carried out at 4°C. Aliquots (1 ml) were mixed with 10 ml Unisolve (Kochlight) and counted in a Packard Tricarb scintillation spectrometer with corrections for quenching, spilling and background. A_{280} values were determined manually. 1,2, ^{3}H-corticosterone (82 Ci/mM ; batch B12) was a product of Amersham, 1,2, ^{3}H-dexamethasone (15 Ci/mM ; batch 66074) and 4-^{14}C-corticosterone (lot 467 - 248 ; 57 mCi/mM) were purchased from CEA, Saclay. All other chemicals were high purity reagent grade. ------- A_{280} ; ●——● ^{3}H ; O——O ^{14}C.

In earlier studies, by both competition and double labelling, we have shown that glucocorticoid specific receptor GR exists as a polymorphic, heterogeneous unit (2-4) which can be resolved into GR_1 (eluted in 0.001 M PO_4 prewash) and GR_2 (in 0.02 M PO_4 region) with a natural corticoid such as corticosterone (see insert Fig. 1). Conclusive evidence is provided in Fig. 1 that dexamethasone labels two sorts of proteins one of which eluted in the 0.001 M PO_4 prewash and the other in the 0.04 M PO_4 region (where no labelling could be found with a natural corticoid) ; the synthetic steroid does not bind to blood serum transcortin (T) contrary to corticosterone (insert, Fig. 1) and other natural analogues (5). Similar results were obtained when triamcinolone was used in place of dexamethasone. At limiting concentrations (10^{-8}M) labelling in the 0.001 M region was greater with synthetic steroids than their natural counterparts. However, in no instance and at no concentration could we observe radioactivity in 0.02 M PO_4 region with synthetic steroids or the 0.04 M PO_4 region with the natural ones. The specificity of binding was further confirmed by the fact that the various peaks were totally abolished in presence of 100 fold excess of cold, homologous molecule. In cross competition studies, corticosterone did not diminish triamcinolone binding at equimolar (10^{-7}M each) concentrations (and vice versa) although this was totally abolished in presence of a hundred fold excess of the competing, heterologous molecule.

From Fig. 2 it is clear that dexamethasone was bound to a protein eluted in the 113,000 molecular weight region, followed by a broad peak of free steroid. On the other hand, corticosterone (insert Fig. 2) was clearly bound to 3 sorts

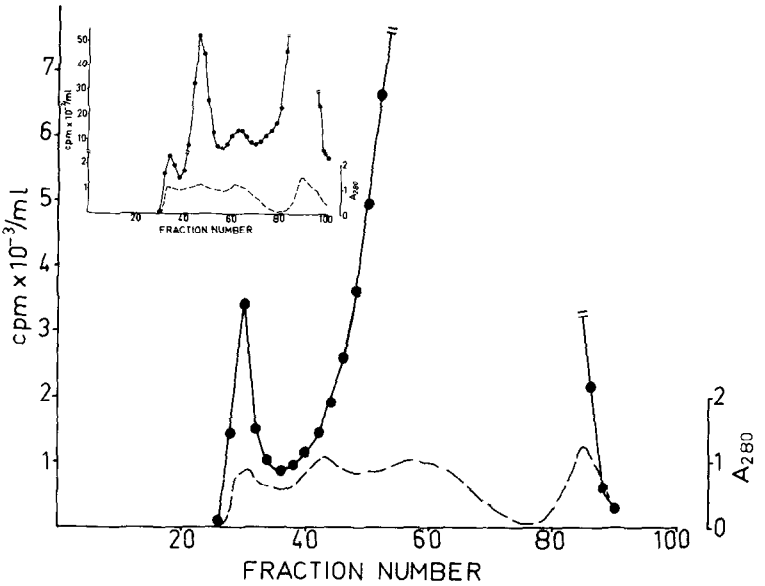

Fig. 2 Discrimination between natural vs synthetic steroid binders by molecular filtration on Sephadex G-200. 2 ml liver cytosol was equilibrated with ^{3}H-corticosterone (insert) or ^{3}H dexamethasone and loaded, without the charcoal treatment, on Sephadex G-200 (Pharmacia) columns (1 x130 cm) equilibrated and eluted with 0.01 M Na phosphate, pH 7.4, containing 0.1 M NaCl. Fractions (1.1 - 1.4 ml) were collected at a flow rate of 10-12 ml at 4°C. All other details as in legend to Fig. 1. --------- A_{280} ; ●——● ^{3}H.

of components with maximum labelling in the 67,000 molecular weight region. In double labelling experiments (not shown), transcortin bound ^{14}C - corticosterone eluted in the 63,000 molecular weight region (2, 4). When cross competition on G-200 was attempted, we obtained conclusions similar to those on DE 52 above.

Lastly (Fig. 3), dexamethasone bound components eluted in the 0.18 M and 0.4 M regions when chromatography on sephadex A-25 was attempted, based both on charge and molecular weight. Transcortin bound corticosterone eluted in the 0.6 M KCl region, under these conditions. In separate experiments, when liver

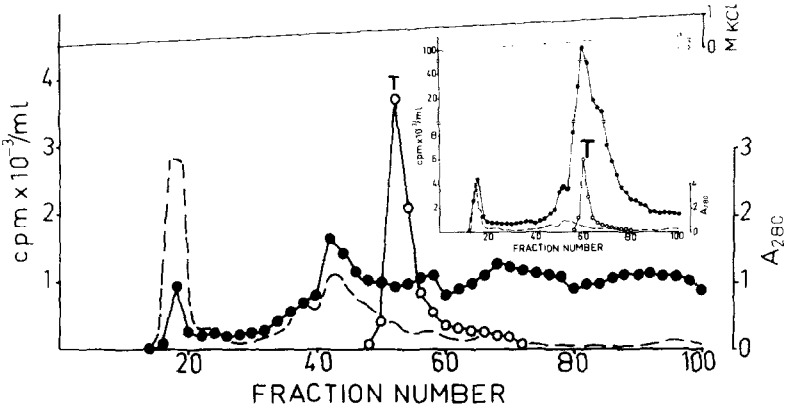

Fig. 3 Analysis of natural vs synthetic steroid binders by chromatography on DEAE-Sephadex A-25 columns. 4 ml each of liver cytosol were incubated with 10^{-7}M of either ^{3}H-corticosterone or to ^{3}H-dexamethasone ; 4 ml blood serum were incubated with 0.5 µCi ^{14}C-corticosterone. After charcoal treatment, separately, each liver cytosol was mixed with 2 ml serum and loaded on the A-25 (Pharmacia) column (90 x 16 cm) equilibrated with 0.05 M Tris-HCl, pH 7.5, and eluted with a linear gradient between 150 ml each of 0 or 1M KCl in this initial buffer. Fractions (3 ml) were collected at a flow rate of 8-10 ml/h. All other details as in legend to Fig. 1. -------- A_{280} ; ●——● ^{3}H; ○——○ ^{14}C.

homogenate was equilibrated with corticosterone, in place of dexamethasone, the peak in the 0.4 M KCl region was reduced to a mere hump of a much larger peak coeluting with transcortin bound corticosterone. It has previously been shown that presence of KCl, required for elution from A-50 gels, greatly diminishes the clarity of elution profiles obtained with phosphate gradients on DE-52 (4).

The results described here demonstrate convincingly that proteins carrying synthetic steroids migrate in positions different from those that carry natural corticoids, in three chromatographic systems. This may explain the disparity in saturation characteristics observed during cross competition in earlier studies (6). It has also been observed that the

synthetic steroids bind nonspecifically to receptors far more easily than natural corticoids (7). There is a common tendency to equate the dexamethasone (5) or triamcinolone (8) or corticosterone (9) binders as the glucocorticoid receptor. It is obvious that one must at all costs avoid the temptation to draw universal inferences from a limited set of measurements ; workers with cell cultures need be especially careful since natural steroids are mostly inactive in this system whereas dexamethasone can evoke certain responses (5). It can not still be said whether the various profiles observed here are modified forms of a common unit that secondarily assumes a certain conformation dictated by the structure of the steroid, or whether they are actually different proteins. The former possibility appears more logical since the animal does not come in contact with synthetic steroids during either ontogeny or phylogeny. Purification of the receptor is required to answer this unequivocally. In any event, physiological response to the glucocorticoid hormones would appear to proceed via saturation of a subpopulation of the polymorphic receptor that had hitherto been deemed unitary through studies only by association-dissociation, competition and Scatchard analysis.

REFERENCES

1. Smith, R.G., Irmain, C.A., Bultham, V.C. and O'Malley, B.W. Nature, 253 : 271 (1975)
2. Agarwal, M.K., Nature, 254 : 623 (1975)
3. Agarwal, M.K., FEBS Letters, 62 : 25 (1976)
4. Agarwal, M.K., Biochem. J., 154 : 567 (1976)
5. Lippman, M. and Thompson, G.B., J. Steroid Biochem., 5 : 461 (1974)
6. De Kloet, R.E. and McEwen, B.S., Biochim. Biophys. Acta, 421 : 124-132 (1976)
7. Agarwal, M.K., FEBS Letters (in press)
8. Mayer, M. , Kaiser, N., Milholland, R.J. and Rosen, F., J. Biol. Chem., 249 : 5236 (1974)
9. Beato, M. and Feigelson, P., J. Biol. Chem., 247 : 7890 (1972)
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