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TISSUE DEPENDENT RECEPTOR BINDING OF 9α FLUOROHYDROCORTISONE

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SUMMARY: Organ specific differences were observed when tritiated 9α fluorocortisol saturated tissue cytosol was fractionated on three different systems of chromatography. The results are discussed in terms of a complex, heterogeneous receptor system whose various subpopulations exhibit qualitative differences that could be related to target-specific physiological functions.

INTRODUCTION: Current models of corticoid hormone action are based upon the preamble that in an initial step the steroid binds its specific cytoplasmic receptor with subsequent translocation of the complex to nuclear acceptor sites. Mammalian heart is drawing increasing attention as a target particularly well suited to unravel corticosteroid action, and receptors for glucocorticoids (1) as well as estrogens (2) have already been reported. Ionotropic effects as well as increase in RNA polymerase activity make myocardium a candidate for mineralocorticoid type of receptors as well, though such sites have hitherto not been detected, due possibly to low receptor levels in this organ (1). Fluorination of cortisol results in a derivative with gluco- and mineralo- corticoid properties that are more potent than their natural counterparts (3,4). Tritiated 9α fluorocortisol, procured through special synthesis, was therefore used to observe the behaviour of steroid receptors in cell cytoplasm of three different tissues in the rat in relation to binding to rat serum transcortin.

Abbreviations used are: MR = Mineralocorticoid-specific receptor; GR = Glucocorticoid-specific receptor; CBG = corticoid-binding globulin; 9α F = 9α fluorohydrocortisone

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METHODS: Male, Wistar rats (150-200 g) were bilaterally adrenalectomized 2-3 days prior to use in all cases. Food and 1% NaCl were available at all times after surgery.

Animals were sacrificed by exsanguination under ether anaesthesia, the desired organ was perfused with the initial buffer, according to the chromatographic procedure intended, and homogenised in an equal volume of the same buffer. The cell sap was obtained by centrifugation at 105,000 g (50 min).

Blood, obtained by aortic cannulation, was allowed to clot at 37°C (30 min), then at 4°C (60-90 min), and finally centrifuged at 3000 g (10 min) to obtain serum.

Details of chromatographic procedure have been published earlier (5-8). Further details of the experimental design and specifications have been provided in the appropriate figure legend.

RESULTS AND DISCUSSION: Results in Fig. 1 demonstrate 9α F binding to a component in the low ionic (0.001 M phosphate, pH 7.5) prewash followed by another in the 0.06 M phosphate region (GR_4) that coeluted with blood serum transcortin (T) in this double labelled chromatography on cellulose-DEAE-52. In other experiments, the first peak could be diminished by either cold corticosterone or spironolactone revealing that this was the region for both the GR_1 and the MR_1 components of the gluco- and the mineralo- receptor, respectively. Peak 2 was totally unaffected by nonradioactive spironolactone and would appear to constitute an entity of the gluco-specific receptor (GR). Although the CBG-associated 9α F also eluted in this T region (not shown), no such GR moiety was observed in either the hepatic (Fig. 1b) or renal (not shown) cytosol despite the fact that liver synthesizes and stores large quantities of this serum carrier. Rather, 9α F was bound to a GR_3 component (in 0.04 M PO_4) in the liver (Fig. 1b) and to an MR_2 component of the mineralocorticoid-specific receptor (MR) in the kidney (not shown). Thus, despite comparable saturation characterstics (Fig. 2) and low levels of the binder concentration in the heart (Fig. 2), physical resolution of the cytoplasmic receptor clearly demonstrated organ specifc differences. This further strengthens that saturation and competition are not adequate in elucidating

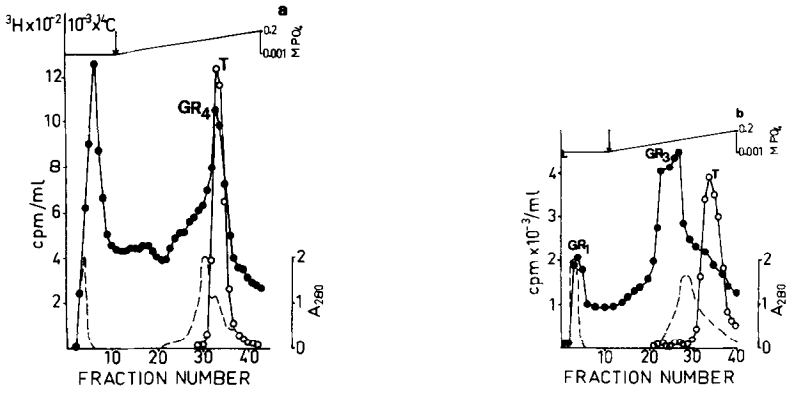


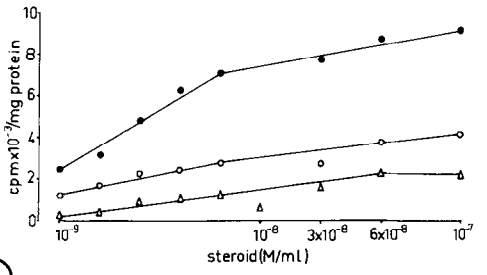
Fig. 1. Ion exchange separation of 9α fluorocortisol binders.

4 ml cardiac (a) or liver (b) cytosol (105,000 g) was incubated (60 min) with 3 x 10^{-8} M of ^{3}H-9-α-fluorohydrocortisone; 2 ml blood serum transcortin was equilibrated with 0.25 µCi of ^{14}C-corticosterone. The free radioactivity was removed, separately, by additional incubation (10 min) in presence of 100 mg/ml cell sap of activated charcoal (Sigma C-5260) which was thereafter eliminated by centrifugation (3000 g) and passage through glass wool. Cytosol and sera were mixed and applied onto a DEAE-cellulose-52 (Whatman) column (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 PO_4, pH 7.5, at a flow rate of 60 ml/h (fraction vol ca. 3 ml). All manipulations were carried out at 2-4°C. Aliquots (1 ml) were mixed with 10 ml Scintix (Isotec,Versailles, France) and counted in a Packard Tricarb Scintillation Spectrometer equipped with corrections for quenching, background and spilling. Recovery exceeded 98% of the applied load. A_{280} values were determined manually. Male, Swiss, albino rats, adrenalectomized 3-5 days prior to use, were employed throughout. 1,2,^{3}H-9α-fluorohydrocortisone (26.7 Ci/mM) was kidly synthesized and purified (> 97%) by Drs. R. Philibert and J. P. Raynaud, Roussel UCLAF, Romainville, France to whom we are indebted. 4-^{14}C-corticosterone (52 mCi/mM; batch 10) was a product of Amersham, G.B. All other chemicals were Reagent grade from Merck.

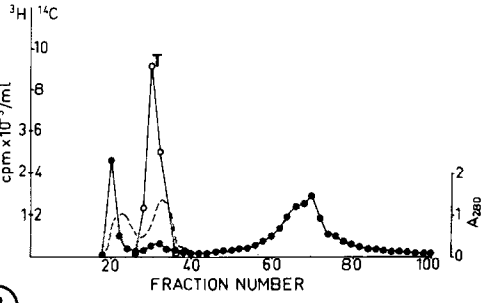
----------A_{280}; ●——● ^{3}H; ○——○ ^{14}C.

the molecular nature of the cellular vector.

Data in Fig. 3 indicate that all cardiac binders appear to elute in the 113,000 dalton molecular weight range from the Sephadex G-200 column, though clearly distinct from CBG in this double labelled study. Similar results were obtained with both



(2)



(3)

Fig. 2. Saturation characterstics of tissue 9α fluorohydrocortisone binding.

0.5 ml aliquots of the organ cytosol were incubated (60 min; 4°C) with the designated concentration of the tritiated steroid. Equilibrium was disturbed by the addition of 0.5 ml of a 100 mg/ml solution of the activated charcoal in 0.01 M Tris-HCl, pH 7.5. Samples were vortexed, allowed to stand for 10 min, and finally centrifuged (5000 g, 5 min). 200 µl aliquots were mixed with 10 ml Scintix and counted as in legend to Fig. 1. Protein was determined by the Biuret method.

●——● Liver; ○——○ Kidney; △——△ Heart.

Fig. 3. Separation of heart 9α fluorocortisol binders based on molecular weights.

2 ml myocardium cytosol was equilibrated (60 min) with 3 x 10^{-8} M tritiated 9α fluorocortisol; 2 ml blood serum was incubated with 0.25 µCi of ^{14}C-corticosterone. Free radioactivity was removed, separately, by charcoal treatment in all cases. Cytosol and serum were mixed and loaded onto Sephadex G-200 (Pharmacia) columns (1 x 130 cm) equilibrated and eluted with 0.01 M phosphate pH 7.4, containing 0.1 M NaCl. Fractions (ca 1.3 ml) were collected at a flow rate of 10-12 ml/h at 4° C. For other details see legend to Fig. 1 and (5-8).

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

renal and hepatic cytosols (not shown). Separation on Sephadex-DEAE-A-25 columns, based both on charge and molecular weight, revealed a clear difference between transcortin and cardiac cytoplasmic receptors. However, the cytosol binder eluted as a single peak due possibly to the formation of monomers in presence of KCl in the buffer.

These results confirm the existence of multiplicity in the

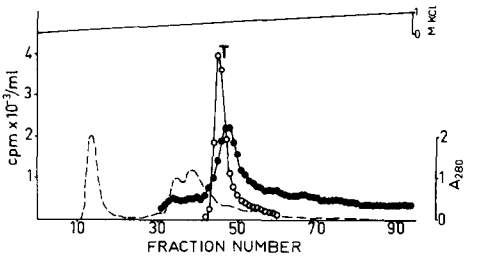


Fig. 4. Resolution of 9α fluorocortisol binders based both on molecular weight and charge.

4 ml cardiac cytosol was equilibrated with 3 x 10^{-8} M tritiated 9α fluorohydrocortisone; 2 ml blood serum was incubated with 0.25 μCi of ^{14}C-corticosterone. Reaction was stopped after 60 min by addition of charcoal (100 mg/ml cytosol or serum) which was thereafter removed as in Figure legends above. Cytosol-serum mixture was loaded onto a DEAE-Sephadex A-25 column (Pharmacia, 1·6 x 90 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 contained in this initial buffer. Fractions of 3 ml were collected at a flow rate of 8-10 ml/h and processed for determination of radioactivity and protein as above. For further details see (5-8).

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

nature of cytoplasmic vectors for corticosteroids and furthermore reveal some tissue dependent differences. Potent pharmacological action of 9αF in liver and kidney can be associated with preferential labelling of selected GR and MR moieties (Fig. 1). The effects of corticoids on cardiac function are complex and numerous and it has been suggested that steroid concentration may determine the type of effect observed under a given set of conditions (1). Although myocardial cytosol is endowed with sites similar to the other two organs in the low ionic prewash from DE-52, binding of 9αF to CBG like entities is unique to the heart since it was not observed in either the liver or the kidney. These are reminiscent of earlier observations (6) with natural mineralocorticoid analogues. Caution must also be exercised in separation methods used

since these components exhibit comparable molecular weights (Fig. 3), but are usually distinguishable by ion exchange (Figs. 1,4). The binding of 9α F to transcortin deserves special mention in view of the fact that other synthetic, fluorinated derivatives (dexamethasone, triamcinolone) exhibit only very limited binding to the serum carrier (7). Thus, lack of CBG binding to the latter two steroids would not appear to be the result of the presence of a fluorine atom in the steroid molecule. Collectively, tritiated 9α fluorocortisol may provide a unique tool to probe the manner in which the occupancy of selected, organ specific receptor components and of CBG may eventually lead to physiological modulation in the appropriate target tissue.

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