PHYSIOLOGICAL ACTIVITY AND RECEPTOR BINDING OF 9∝FLUOROHYDROCORTISONE

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Received August 5, 1977

SUMMARY

Hepatic gluconeogenic stimulation by 9∝fluorocortisol was associated with saturation of GR_1 and GR_3 entities of the glucocorticoid specific receptor (GR), even in presence of spironolactone; renal glycogen levels were not altered. Binding to MR_1 and MR_2 components of the mineralocorticoid specific receptor (MR) in the kidney persisted even in presence of 100 fold excess of nonradioactive corticosterone although this was totally abolished by cold equimolar spironolactone. These data suggest that this fluorinated derivative may be particularly appropriate in studying organ specific responses.

The anti-inflammatory influence and liver gluconeogensis by natural glucocorticoid hormones can be enhanced by 9-∝-fluorination of the prednisolone molecule (triamcinolone); halogenation of cortisol yields a derivative possessing powerful gluco- and mineralo- corticoid properties (1,2). In recent years, binding of a steroid to its specific, cytoplasmic receptor is said to constitute the first step whereby tissue specific gene modulation is eventually accomplished (2,3). In the past, steroid-receptor association was limited to displacement by cold 9-∝-fluorocortisol of ^{3}H-mineralo- or ^{3}H-gluco- corticoid-receptor complex. Studies of this sort had led to the generally accepted concept of a unitary cellular receptor that was eventually found to be polymorphic and heterogeneous when studied by various procedures of physico-chemical fractionation (3). The present report analyzes the receptor binding in relation to physiological activity of ^{3}H-9-∝-fluorocortisol which is not commercially available and which

Data in table 1 show that liver gluconeogenesis could be

ISSN 0006-291X

Table 1: COMPARATIVE STUDIES ON RENAL AND HEPATIC GLUCONEOGENESIS

	Glycogen (mg%)✦	
	Liver	Kidney
Water control	6.56 ± 1.57 (5)	1.11 ± 0.07 (10)
Cortisol (10 mg)	13.34 ± 1.20 (4)	
Triamcinolone acetonide:		
1 mg	18.60 ± 3.50 (5)	
5 mg	18.88 ± 1.82 (5)	1.08 ± 0.10 (10)
9α fluorohydrocortisone:		
1 mg	14.06 ± 1.55 (5)	
5 mg	24.63 ± 2.46 (5)	0.83 ± 0.07 (5)

✦ Male Wistar rats (150-200 g) were bilaterally adrenalectomized 3-5 days prior to use and maintained on laboratory food and 1% NaCl ad libitum. They were injected with the test steroid 4 h prior to sacrifice and the glycogen levels were determined by a colorimetric procedure based on the formation 5-hydroxymethylfurfural as previously described (8). Glycogen levels are expressed as mg/100 mg tissue. All steroids were obtained from Sigma.

Each value is the mean ± the standard error of the number of determinations shown in parantheses.

induced significantly ($p<0.01$) by cortisol, triamcinolone acetonide (TA), and 9-α-fluorocortisol (9αF). However, 10 mg cortisol induced less than 1 mg TA ≈ 1mg 9αF. Rather surprisingly,

whereas 5 mg TA was not more effective than 1 mg TA, 5 mg 9αF was clearly the most potent gluconeogenic steroid. The reason for these differences are not understood. Data in this table also show that kidney glycogen levels could not be increased by any of the steroids tested. This is a further caution against extrapolation *in vivo* of results obtained with slices *in vitro* where renal gluconeogenesis, as tested by precursor utilization or enzyme induction, could be altered under selected experimental conditions not involving 9αF (4,5). Thus, although we could confirm the previously observed (1) potentiation in liver gluconeogenic activity by 9-α-fluorination of cortisol, this was not the case in kidney which appears to have a separate mechanism (4,5) for regulation of glycogen metabolism. No attempts were made to asses the mineralocorticoid activity of 9αF since this has already been done elsewhere (2).

Attention was next directed to the binding of 9αF to cellular gluco- and mineralo- type of receptors. In view of our recent observations on receptor multiplicity (3), rather than analyzing the saturation characteristics previously attempted (2) we chose to proceed with physical separation of the steroid-receptor complex. Data in Fig. 1 show that 10^{-8} M 9αF was bound to both the MR_1 and the MR_2 moieties of the mineralocorticoid receptor (MR), even in presence of 10^{-6} M of cold corticosterone (the natural glucocorticoid in the rat). Specificity of binding was further confirmed by the fact that no bound 9αF could be observed when chromatography was attempted in presence of only 10^{-8} M of an MR antagonist spironolactone, and that both MR_1 and MR_2 were still just as evident in presence of an equimolar (10^{-8} M) nonradioactive aldosterone. Thus, 9-α-fluorohydrocortisol would appear to possess greater affinity for renal

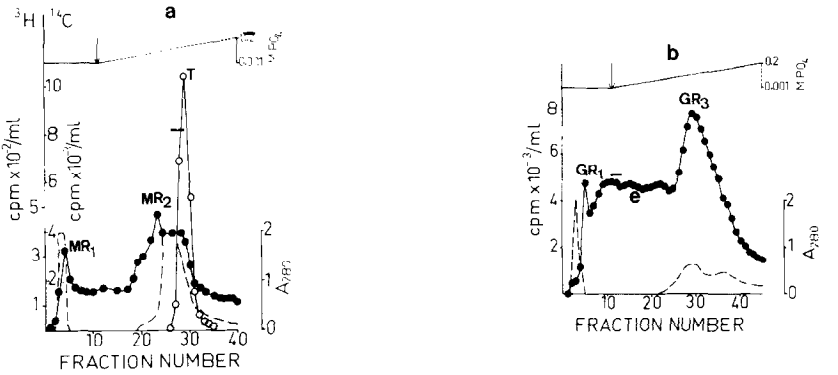

Fig. 1. Analysis of 9-α-fluorohydrocortisone binding by ion-exchange chromatography.

4 ml kidney (a) or liver (b) cytosol (105,000 g) was incubated (60 min) with 10^{-8} M ^{3}H-9-α-fluorocortisol in presence, respectively, of 10^{-6} M nonradioactive corticosterone or 10^{-8} M spironolactone; 2 ml blood serum 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 centrifeugation (3000 g) and passage through glass wool. Renal cytosol with serum, or liver cytosol alone, was 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 PO4, 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 Unisolve (Koch Light, G.B.) 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. 1,2, ^{3}H-9-α-fluorohydrocortisone (specific activity 26.7 Ci/mM) was kindly synthesized and purified (> 97%) by Drs. R. Philibert and J. P. Raynaud of Roussel UCLAF, Romainville, France. 4-^{14}C-corticosterone (52 mCi/mM; batch 10) was a product of Amersham, G.B. All other chemicals were high purity Reagent grade from Merck.

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

mineralocorticoid receptor (MR) than the natural hormone. It is also evident that these MR moieties are not to be confused with rat serum transcortin (T) that eluted in a separate position (Fig. 1a.).

To analyze 9αF binding to GR, liver cytosol was used and data in Fig. 1b show GR_1 and GR_3 labelled even in presence of

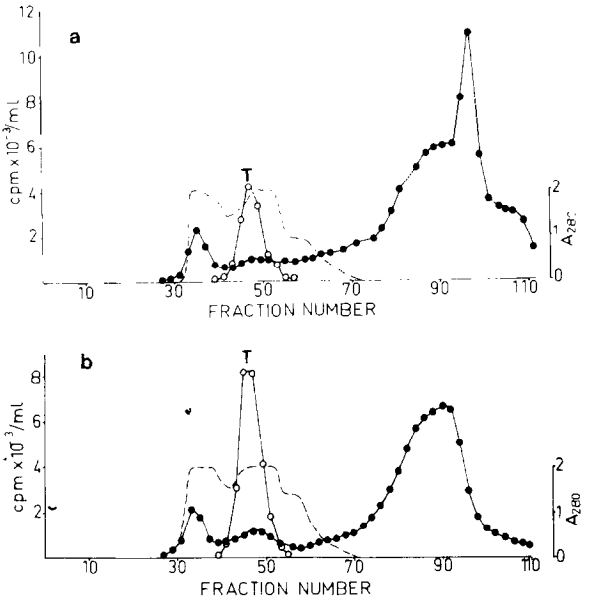

Fig. 2. Molecular weight filtration of 9-α-fluorocortisol binders. 2 ml kidney (a) or liver (b) cytosol was equilibrated (60 min) with 3 x 10^{-8} M of ^{3}H-9-α-fluorohydrocortisone; 2 ml blood serum was incubated with 0.25 μCi of ^{14}C-corticosterone. Free radioactivity was removed, separately, by 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 sodium 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 6,7).

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

spironolactone (that had inhibited renal MR binding in Fig. 1a.). In still other studies (not shown), 10 fold greater cold corticosterone diminished 9αF binding to liver cytosol but did not eliminate it. This is in keeping with the fact that synthetic steroids possess greater affinity for GR_1 and GR_3 than natural analogues which mostly label GR_2 and GR_4 entities (3,7).

To assess molecular nature, kidney or hepatic cytosol 9αF mixtures were analyzed on Sephadex G-200 columns equilibrated at physiological pH and tonicity. Data in Fig. 2 show that in both

cases bound radioactivity was eluted in a region that in other studies (3), corresponds to 113,000 daltons, followed by a free peak. Thus, distinction between GR and MR was not possible by separation based solely on molecular weights, in keeping with the observation that they may possess comparable monomer sizes contributing to the erroneous impression of a unitary receptor population. Again, these receptor entities were clearly distinct from rat serum transcortin (T) during double labelled chromatography (Fig. 2.) as previously observed (Fig. 1.). Absence of low molecular weight (67,000) entities may be related to greater potency of synthetic derivatives.

Results described here show that control of gluconeogenesis in liver may not be subject to same restraints as in the kidney. Even hepatic gluconeogenesis appears regulated at different loci hence the difference between TA and 9αF in this parameter. Since TA-receptor complex also eluted in GR_1 and GR_3 positions (7), as with 9αF in Fig. 1, the latter may possess greater affinity for the receptor or the acceptor. It remains to be seen whether the level at which 9αF begins to saturate renal GR corresponds to gluconeogenic stimulation in this organ although this would be an extremely difficult task to accomplish in vivo. In a bifunctional organ like the kidney 9αF preferentially labelled MR in keeping with strong mineralocorticoid action of this fluorinated derivative (2). Finally, results in Fig. 1a suggest that binding of 9αF to MR would make it a better marker than natural analogues to study renal mineralocorticoid receptor.

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9. This work was supported by grants from DGRST, CNRS (A1 03. 1917) and the INSERM (CL 76.5.001.4). Drs. R. Philibert and J.P. Raynaud of Roussel UCLAF, Romainville, France, kindly synthesized ^{3}H-9-α-fluorohydrocortisone used in this study. Thanks are due to Rebecca Horrell for preparing the typescript.