DIFFERENTIAL BINDING TO RENAL RECEPTOR SUBPOPULATIONS AS AN EXPLANATION OF MINERALOCORTICOID AGONIST ACTION

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1. Introduction

It is currently accepted that binding of steroid-receptors constitutes the first step whereby a number of hormone dependant processes are initiated in the appropriate target tissue. Although with the usual approach of competitive binding and Scatchard analysis a unitary (K_D (37°C) 5×10^{-10} M) site [1] or a 10^{-9} M site [2] has been reported, we have recently provided exclusive chromatographic evidence for the existence of a polymorphic mineralocorticoid (aldosterone) specific receptor (MR) present in the target tissue kidney [3], an organ also endowed with glucocorticoid (corticosterone) specific receptors (GR) in common with the liver [4]. In mammals, ion regulation is principally accomplished by aldosterone, but deoxycorticosterone 18-hydroxy-deoxycorticosterone also play some role, the latter especially during pathogenesis and these, by Scatchard analysis and competition, are presumably bound to the same unitary receptor though with decreasing affinity according to physiological plasma concentration [1,2]. Evidence presented in this report reveals that binding of the two agonists of the mineralocorticoid occurs to units chromatographically distinct from those that exhibit aldosterone binding. Thus, contrary to mere differences in the affinity and in the in vivo concentrations, mineralocorticoid agonist action may proceed via differential binding to physicochemically distinct protein entities.

2. Materials, methods and results

As previously reported [5] mineralocorticoid (aldosterone) specific receptors (MR) eluted as a more abundant species (MR_1) in the 0.001 M PO_4 region followed by a less abundant species (MR_2) at 0.06 M NaCl (insert fig.1a); this second peak was totally wanting when desoxycorticosterone (DOC) or its 18-hydroxy analogue (18-OH-DOC) even at 10^{-7} M (required to saturate the component in 0.001 M prewash) was employed in place of 10^{-8} M aldosterone (fig.1a, 1b). Under these conditions, prolonged incubation (3 h) of the cell sap–steroid mixtures or addition of the steroid to crude homogenates, prior to ultracentrifugation, in order to attempt ligand protection by the specific substrate did not increase binding upon subsequent chromatography. Rather, DOC appeared consistently and largely bound to some components still retained by DE-52 after 0.2 M NaCl and which coeluted with [^{14}C]corticosterone bound to serum transcortin.

To this end, 0–0.2 M PO_4 gradients were employed in order to obtain total elution from the DE-52 column in place of the NaCl gradients [5] (where ten-fold higher concentrations are required for the elution of the same component). Data in fig.2 clearly show that even at 10^{-7} M concentration, no radioactivity was found in neither the 0.006 M PO_4 region with either DOC (fig.2) or 18-OH-DOC (not shown) where aldosterone bound MR_2 receptors are usually eluted [3], nor the 0.02 M PO_4 region where glucocorticoid specific GR receptors elute from the kidney or rat [4,5] and even human liver [6] cytosols; although labelled with most natural steroids, the macromolecules in the 0.001 M PO_4 region from both organs appear to be adapted to hormone-specific functions [4] Rather, DOC (but not 18-DOC) was bound mostly to some component in the 0.04–0.06 M PO_4 region (MR_3) which coeluted with transcortin (T) clearly

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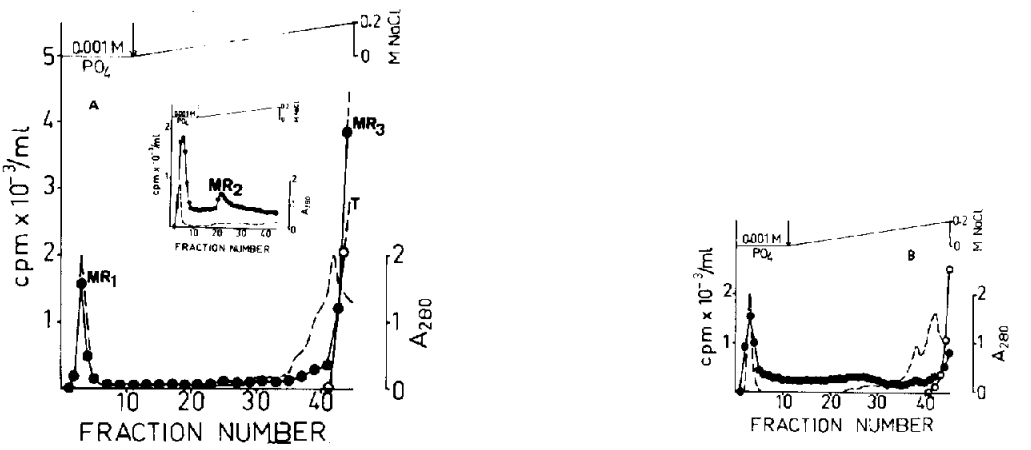
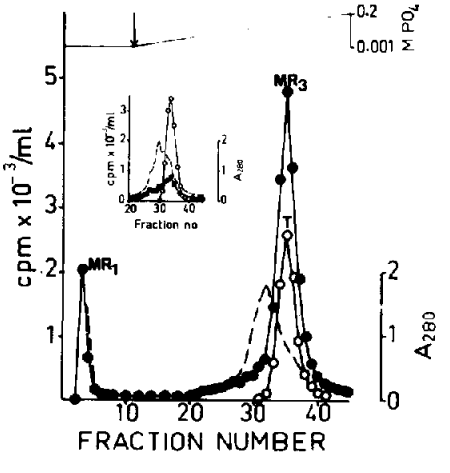


Fig.1. Ion exchange separation of rat kidney mineralocorticoid binding proteins by NaCl gradients. Male, Wistar rats (150–200 g) were bilaterally adrenalectomized at least 48 h before use, exsanguinated under ether anesthesia and perfused with the initial buffer by aortic cannulation. Blood serum (2 ml) was incubated with 0.2 μCi of [^{14}C]corticosterone for 60 min at 4°C. The 105 000 g supernatant fraction (5 ml) of rat kidney was also equilibrated for 60 min at 4°C with 10^{-7} M of either [^{3}H]deoxycorticosterone (DOC) or [^{3}H]18-hydroxy-deoxycorticosterone (18-OH-DOC) or 10^{-8} M [^{3}H]aldosterone. Free radioactivity was removed by further incubation (10 min) in presence of activated charcoal (Sigma C-5260), centrifugation at 3000 g (10 min) and passage through glass wool, successively. The cytosol and serum were finally mixed and layered on DEAE-52 (1 × 25 cm) column, prepared, packed and eluted as before [3–6]. After passage of 60–70 ml of the initial buffer (fraction volume 6–7 ml) elution was begun (at arrow) by a linear gradient between 60 ml each of 0.001 M PO_4, pH 7.5 (initial buffer) and this buffer containing 0.2 M NaCl, at a flow rate of 60 ml/h at 4°C (fraction vol. approximately 3 ml). Aliquots of 1 ml were mixed with 10 ml Unisolve (Kochlight) and counted in a Packard Tricarb scintillation spectrometer with corrections for quenching, spilling and background [3–6]; A_{280} values were recorded manually (– – – –). [1,2^{3}H]aldosterone (46 Ci/mM; batch B 12) and [1,2^{3}H]18-hydroxy deoxycorticosterone (51 Ci/mM; batch 2) and [4-^{14}C]corticosterone (52 mCi/mM, batch 9) were products of Amersham; [1,2^{3}H]deoxycorticosterone was purchased from CEA, France; all other chemicals were high purity reagent grade. DEAE-52 was purchased from Whatman (England) and Sephadex G-200 from Pharmacia (Sweden). The experiment with 10^{-8} M aldosterone (insert) was done without serum (as before, [5]) (•)^{3}H; (○)^{14}C.



(fig.2) but which could not be revealed with 18-OH-DOC that has a high affinity for the serum binder. In parallel experiments, serum bound DOC > 18-DOC > aldosterone also peaked in this 0.04–0.06 M PO_4 region from the DE-52 column. Thus, DOC binding (fig.2) would probably represent association with another moiety. This latter possibility appeared very real since DOC binding components could not be found in the high phosphate region from the liver

Fig.2. Ion exchange separation of deoxycorticosterone binder in rat kidney. All details as in legend to fig.1 except that kidney cytosol (5 ml) and serum (2 ml) were equilibrated with 10^{-7} M [^{3}H]deoxycorticosterone and 0.2 μCi of [4-^{14}C] corticosterone, respectively, and eluted with a gradient between 0.001 M and 0.2 M PO_4, pH 7.5. For liver cytosol (insert), under exactly the same conditions as kidney, only the relevant portion is shown. (•)^{3}H; (○)^{14}C; (– – –) A_{280}.

which synthesizes and may trap (despite prolonged perfusion in vitro) far larger quantities of transcortin than kidney (see insert fig.2).

In still other experiments, components of comparable mol. wt. bind all three steroids resulting in essentially similar elution profiles (fig.3a–c). Binding to the heavier (113 000) mol. wt. component (H) exhibited the order aldosterone > DOC > 18-OH-DOC; the lower (67 000) mol. wt. component (L) was maximally revealed with DOC > aldosterone > 18-DOC (reminiscent of large quantities of DOC binding on DE-52 above) and, at least in case of aldosterone, coeluted with serum transcortin. In separate experiments, serum bound DOC < aldosterone < 18-OH-DOC eluted in this position from the G-200 column (not shown). Separation was less clear when chromatography was attempted on Sephadex A-25 columns based both on molecular weight and charge (not shown).

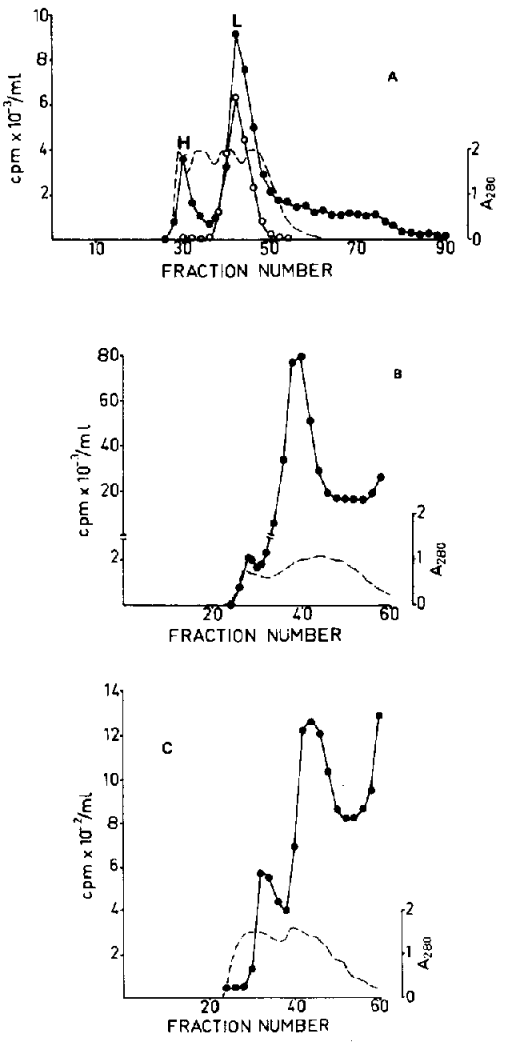


Fig.3. Molecular filtration of rat kidney mineralocorticoid binding proteins. 2 ml kidney cytosol was incubated with 10^{-7} M of either [^{3}H]aldosterone, [^{3}H]DOC, or [^{3}H]18-OH-DOC and layered, without charcoal pretreatment, on to Sephadex G-200 columns (1 × 30 cm) packed, equilibrated and eluted with 0.01 M PO_4, pH 7.4 containing 0.1 M NaCl; further details as before [4].

Mineralocorticoid interaction with receptors [1,2] by Scatchard analysis (with relative affinities: aldosterone > DOC > 18-OH-DOC) does not reveal whether binding occurs to a unitary receptor endowed with a flexible site capable of stereospecific adjustments dictated by the steroid structure in question or brings into account the possible presence of physicochemically distinct 'isoreceptors', 'proreceptors', and 'inhibitors'. Pioneering evidence is provided here to substantiate the thesis that agonist (and by extrapolation antagonist) action may proceed via preferential activation or inactivation of a subspecies within any one class of receptor population since both MR and GR exist in a polymorphic state [3–6] and since 18-OH-DOC binds only to the 0.001 M component (with only minimal association to GR). Moreover, agonist (DOC but not 18-OH-DOC action may also proceed via association with another moiety (MR_3) that (along with MR_2) is clearly lacking in the liver (having no physiological role), that coelutes with intra- or extra-cellular transcortin (T), and that cannot be distinguished by separation based on molecular weights alone. Indeed, it has recently been suggested by autoradiography that a type III corticosterone binder may have some role in kidney specific physiology although from saturation characteristics (just as chromatographic profile of MR_3 here) it was previously confused with transcortin [1]. Thus, intracellular regulation may call into action qualitative variations in a number of parameters contrary to mere quantitative adjustments attempted by Scatchard analysis at equilibrium.

Acknowledgments

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