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Glucocorticoid metabolism by 11-β hydroxysteroid dehydrogenase type 2 modulates human mineralocorticoid receptor transactivation activity☆

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Abstract

The mineralocorticoid receptor (MR) binds aldosterone, but also glucocorticoid hormones (corticosterone in rodents, cortisol in humans), which largely prevail in the plasma. To prevent permanent and maximal occupancy of MR by glucocorticoid hormones in aldosterone-target cells, specific effects of aldosterone require metabolism of glucocorticoid hormones into 11-dehydroderivatives by 11-β hydroxysteroid dehydrogenase (11-HSD2). We analyzed the effect of corticosterone or 11-dehydrocorticosterone (11-DHC) on the transactivation activity of the MR, transiently expressed in a new renal cell line expressing 11-HSD2. We show that, because of its metabolism by 11-HSD2, corticosterone is a poor activator of MR transactivation, except at micromolar concentrations, where the enzyme is saturated. We also show that high micromolar concentrations of 11 DHC are required to activate the MR. The weak antagonist property of 11-DHC on aldosterone-induced hMR transactivations is also documented. Such partial agonist activity of 11-DHC is discussed in the light of its positionning in a three-dimensional model of the MR ligand-binding domain.

Keywords: Aldosterone; Cortical collecting duct; Mineralocorticoid selectivity; Mineralocorticoid receptor; 11-β Hydroxysteroid dehydrogenase

1. Introduction

The regulation of renal sodium reabsorption is critical to control extracellular volume, sodium homeostasis and blood pressure. Such regulation involves several hormonal systems, including the mineralocorticoid hormone aldosterone. Aldosterone acts all along the distal parts of the nephron. It binds to the mineralocorticoid receptor (MR), a ligand-dependent transcription factor belonging to the nuclear receptor superfamily [1]. Upon aldosterone-binding, the MR regulates the transcription of several genes, leading to the activation and biosynthesis of the epithelial sodium channel ENaC and the Na/K/ATPase [2]. The MR has the same affinity (10^{-10} to 10^{-9} M) for aldosterone and glucocorticoid hormones [3], which are 100–1000 fold more abundant than aldosterone in the plasma [4]. This should lead to permanent occupancy of the MR by glucocorticoid hormones, and to maximal and permanent sodium reabsorption. The enzyme 11-β hydroxysteroid dehydrogenase type 2 (11-HSD2), which is coexpressed with the MR in the renal cortical collecting duct, ensures mineralocorticoid selectivity by metabolizing glucocorticoid hormones (cortisol in humans, corticosterone in rodents) into inactive 11-dehydro-derivatives (cortisone and 11-dehydrocorticosterone (11-DHC)) [5,6]. The major role of 11-HSD2 in mineralocorticoid selectivity is highlighted by its inactivating mutations identified in the syndrome of apparent mineralocorticoid excess (AME) [7]. Affected children exhibit severe hypertension and hypokaliemia but their plasma levels of renin and aldosterone are very low.

Limited informations are available on the effects of 11-dehydrocorticosteroids produced by 11-HSD2 on MR mediated-sodium transport. It was reported that 11-dehydrocorticosterone could blunt the antinatriuretic effect of aldosterone in rat kidney and in the toad bladder, while 11-dehydrocorticosterone alone was inactive on sodium

Abbreviations: hMR, human mineralocorticoid receptor; 11-HSD2, 11-β hydroxysteroid dehydrogenase type 2; 11-DHC, 11-dehydrocorticosterone; CBX, carbenoxolone

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transport [8,9]. This study addressed the specific question of the activity of 11-dehydro derivatives of glucocorticoid hormones on the transactivation activity of the MR. We evaluated the consequences of glucocorticoid metabolism by 11-HSD2 on human MR transactivation activity in a new renal collecting duct cell line which expresses a tagged 11-HSD2.

2. Materials and methods

2.1. Materials

Cell culture products, media and lipofectamine were from Life Technologies (Cergy-Pontoise, France). The anti-flag-M2 antibody and hormones were from Sigma (St. Quentin-Fallavier, France), and 1,2,6,7-[^{3}H]corticosterone from Amersham (Saclay, France).

2.2. Plasmids and constructs

The epitope-tagged 11-HSD2 expression plasmid was constructed by inserting by PCR the flag-M2 sequence (MDYKDDDD) at the C-terminus of the human 11-HSD2 cDNA [10]. The pFC31Luc and the pchMR plasmids, contains the MMTV promoter driving the luciferase gene [11] and the entire human mineralocorticoid receptor (hMR) coding sequence, respectively [12]. The pSVβ plasmid encoding for the β-galactosidase was used as an internal transfection control.

2.3. Cell culture, transfection and establisment of a stable cell line

Cultured media were medium A: HAMF12-DMEM (1:1), 14 mM $NaHCO_3$, 20 mM HEPES, 2 mM glutamine. Medium B: medium A supplemented with 10^4 U/ml penicillin/streptomycin, 5 μg/ml insulin, 50 nM dexamethasone, 5 μg/ml transferrine, 60 nM sodium selenite, 1 nM triiodothyronine, 10 ng/ml epidermal growth factor (EGF) and 2% fetal calf serum. Medium C: medium A supplemented with 5 μg/ml insulin, 5 μg/ml transferrine, 60 nM sodium selenite, 10 ng/ml EGF and 2% charcoal-treated fetal calf serum.

The RCCD1 renal cell line [13] was routinely cultured in medium B. One day before transfection, cells were trypsinized and replated in six-well plates at the density of 2.5×10^5 cells per well in the medium B. Four hours before transfection, cells were incubated in medium A and transfected with 1 μg pcDNA3-HSD2-flag or 1 μg pcDNA3 as control and with 5 μl lipofectamine according to the recommendations of the manufacturer. Fourty-eight hours after transfection, the medium was removed and cells were washed with PBS and incubated with the medium B supplemented with 400 μg/ml of geneticin, for selection. About 45 clones were isolated and tested for enzyme activity and Western blotting. Cells lines were cultured in the medium B in the presence of geneticin (200 μg/ml). Clone 18 (C18 cells) was selected for further experiments.

2.4. Western blotting and immunoprecipitation

RCCD1 or C18 cells were lysed in buffer containing 50 mM Tris–HCl, 150 mM NaCl, 1% nonidet 40, 2 mM EGTA, 0.1% SDS, pH 7.5 and proteases inhibitor cocktail (Sigma) and processed for Western blot using the anti-flag-M2 antibody (1:2000 dilution). For immunoprecipitation, cell lysates (500 μg protein) where incubated with the anti-flag-M2 antibody coupled to agarose beads at 4 °C for 1 h. After gel electrophoresis and transfer onto a PDVF membrane, 11-HSD2 was detected using the anti-HSD2 antibody [14] at 1:6000. The secondary antibody, a goat anti-rabbit IgG-horseradish peroxydase, was used at 1:10,000. Immunoreactivity was detected by using ECL chemiluminescent substrate from Amersham.

2.5. Measurement of 11-HSD2 activity

RCCD1 or C18 cells were seeded in 24-well trays (1.5×10^5 cells per well) and cultured for 48 h in medium C. After incubation of cells at 37 °C with tritiated corticosterone (1,2,6,7-^{3}H corticosterone), 10 μl samples of supernatant were collected and 90 μl of HPLC mobile phase (methanol/H_2O 1:1) containing 10^{-4} M unlabeled corticosterone and 11-dehydrocorticosterone as internal standards was added. HPLC analysis was performed as previously described [15]. The amount of steroid metabolite generated per well was standardized for the amount of protein, determined by the Bradford method [16] after homogenization of cells in the lysis buffer. Results are expressed as femtomoles of 11-dehydrocorticosterone produced per microgram protein and per 10 min incubation time (fmol/μg/10 min) or per total incubation time.

2.6. hMR transactivation assays

One day before transfection, C18 cells were trypsinized and replated in six-well plates at the density of 4×10^6 cells per well in medium B. Cells were transfected 24 h later. For each well, 8.5 μl of lipofectamine, 0.5 μg of the pchMR, 1 μg of pFC31Luc and 0.35 μg of the pSVβ were mixed in 1200 μl of medium A. Samples were handled according to the manufacturer's recommendations. Sixteen hours later, the medium was removed and cells were washed with PBS and further incubated for 24 h in medium C in the presence of hMR-ligand to be tested. Cells were washed in PBS, lysed as previously described [17] and assayed for the luciferase and β-galactosidase activities. To standardize for transfection efficiency, the relative light units obtained in the luciferase assay were divided by the optical density obtained in the β-galactosidase assay. All measurements were performed in triplicate.

2.7. Immunofluorescence studies

C18 cells were grown on glass coverslip and fixed with methanol for 10 min at −20 °C before immunolabeling. The antibody against human 11-HSD2 (1/500) was laid over cells for 2 h at room temperature. Following several washes with phosphate buffer, the secondary antibody (goat anti-rabbit coupled to the fluorochrome CY3 (from Jackson)) was incubated at 1/200, 1 h at room temperature. After washes with phosphate buffer, slides were mounted using Vectashield (Vector) and examined under confocal microscope (TCS 4D, Leica).

2.8. Ligand docking within the hMR ligand-binding domain

11-Dehydrocorticosterone and corticosterone were docked in the homology model of the hMR ligand-binding domain generated from the human progesterone receptor crystal structure according to the method previously described [12]. Complexes were energy minimized in 2000 steps with the Discover-InsightII package (Molecular Simulation Inc., San Diego, CA), using the Newton procedure.

3. Results and discussion

3.1. C18 cells: a novel cell line expressing the human 11-HSD2

Most cell lines derived from the renal collecting duct usually loose 11-HSD2 as well as MR or their expression is only at low level. We have established a new renal cell line (C18 cells) expressing the human 11-HSD2 fused to a flag epitope. The RCCD1, chosen as parental cell line, is a highly differentiated cell line originating from the rat collecting duct; however this cell line has also lost MR and HSD2 expression [13]. In this study, several stable cell lines, transfected with a tagged 11-HSD2, were obtained. Among them, the clone 18 (C18 cells) was chosen for further characterization. Western blot analysis of C18 cells lysates (using the anti-flag-M2 antibody) revealed a single band whose intensity increased with the amount of lysate loaded onto the gel (Fig. 1A; lanes 2–4). This band corresponds to a molecular weight of ∼40 kDa, a size in good agreement with that of 11-HSD2 [10]. No band was detected with RCCD1 cells lysate (Fig. 1A; lane 1). C18 cells lysates were immunoprecipitated by the anti-flag-M2 antibody coupled to agarose beads and tested for 11-HSD2 using the human anti-11-HSD2 antibody. Fig. 1B shows the presence of 11-HSD2 in the cells lysate and in the anti-flag-M2 precipitated fraction (Fig. 1B; lanes 1 and 2) but not in the washes (Fig. 1B; lanes 3 and 4). Confocal immunofluorescence using the human anti-11-HSD2 antibody (Fig. 2A and B) showed that C18 cells exhibit a strong fluorescence over the cytoplasm, with some punctuated nuclear fluorescence, which may correspond to the cytoplasmic layer covering the nuclei. In contrast, no detectable 11-HSD2 immunofluorescence signal was apparent in RCCD1 cells (Fig. 2C), consistent with Western blotting and immunoprecipitation results (not shown).

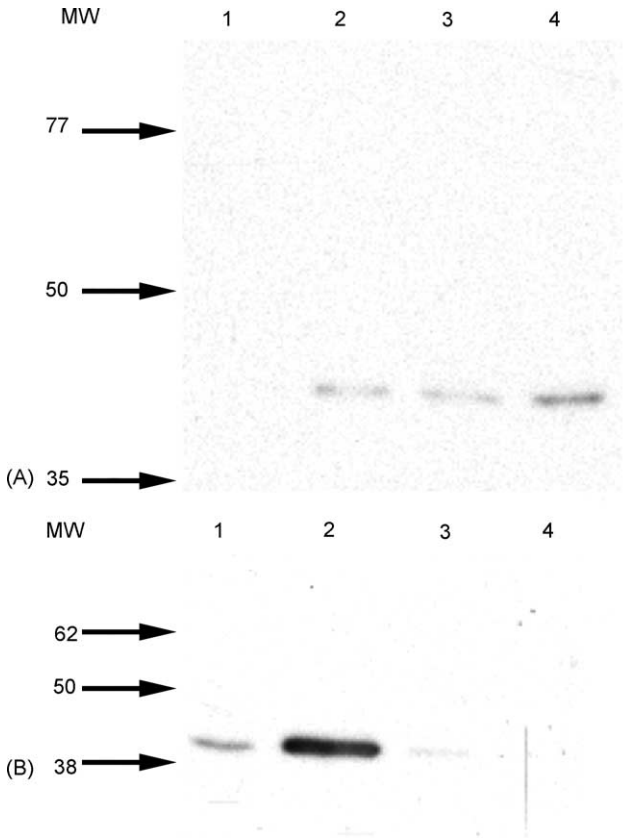

Fig. 1. Immunodetection of 11-HSD2 in C18 cells. Panel A: Western blot analysis of 11-HSD2 in RCCD1 (75 μg cell lysate per lane, lane 1) and in C18 cells (40, 50 and 75 μg cell lysate per lane, in lanes 2, 3 and 4) probed with the anti-flag-M2 antibody. A 40 kDa protein corresponding to the 11-HSD2-flag was detected in C18 cells. Panel B: C18 cells lysate was immunoprecipitated with the anti-flag antibody and 11-HSD2 was detected with the anti-11-HSD2 antibody. Lane 1: C18 cells lysate before immunoprecipitation, lane 2: cell fractions immunoprecipitated with the anti-flag antibody; lanes 3 and 4 correspond to washes of cell fraction after immunoprecipitation. Detection of the protein by the human anti-11-HSD2 antibody shows that a 40 kDa protein was immunoprecipitated.

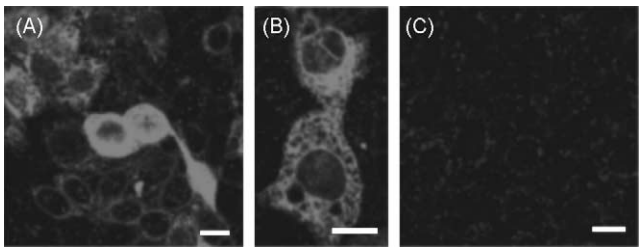

Fig. 2. Immunoflorescence staining of C18 cells and RCCD1 cells with the human anti-11-HSD2 antibody. C18 cells (Panels A and B) exhibit a strong fluorescence over cytoplasm while the parental cell line RCCD1 (Panel C) is negative. Bar: 10 μm.

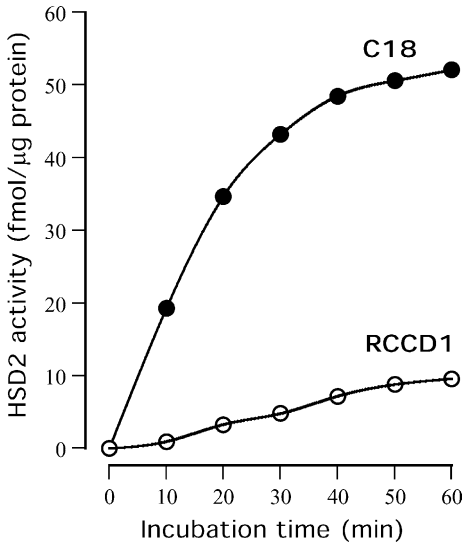

Fig. 3. A representative experiment of kinetics of 11-HSD2 activity in RCCD1 and C18 cells. Cells were incubated at 37 °C with 10^{-8} M [^{3}H]corticosterone for 0–60 min and the rate of transformation of the substrate was analyzed by HPLC. 11-HSD2 activity is measured by the amount of [^{3}H]11-dehydrocorticosterone formed.

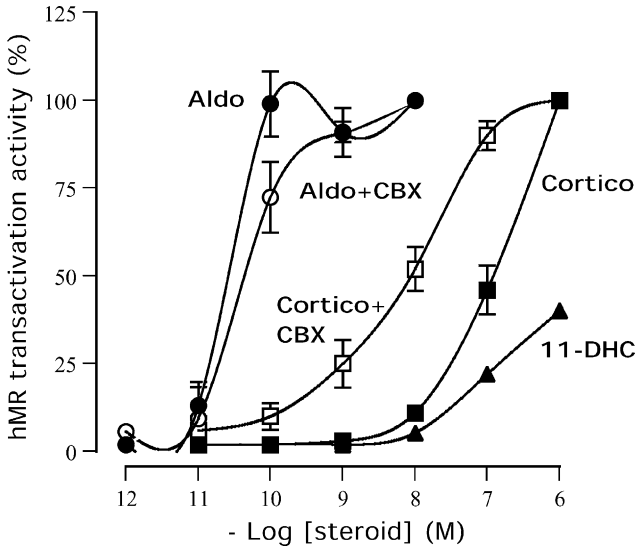

Fig. 4. Transactivation activity of the human mineralocorticoid receptor (hMR) transiently expressed in C18 cells in response to various steroids. C18 cells were transiently transfected with pchMR, pFC31Luc as a reporter plasmid, and pSVβ as an internal control and incubated for 24 h with various concentrations of aldosterone (Aldo) (close circles), corticorticosterone (cortico, close squares), 11-dehydrocorticosterone (11-DHC; close triangles), aldosterone plus CBX (open circles), or corticosterone plus CBX (open squares). The results are expressed as luciferase activity (corrected by β-galactosidase activity). Each point is the mean ± S.E. of two to six independent experiments, with three determinations in each experiment. The luciferase activity has been normalized to the aldosterone-induced MR activity at the plateau, or to the corticosterone-induced hMR activity measured at 10^{-6} M.

The addition of the flag sequence to 11-HSD2 did not modify the enzyme activity, as tested in RCCD1 cells transiently transfected with the cDNA of the human 11-HSD2 or of the human 11-HSD2-flag (not shown). The activity of the 11-HSD2-flag fusion protein expressed in C18 cells was measured by analyzing the capacity of these cells to metabolize [^{3}H]corticosterone (10^{-8} M) into [^{3}H]11-dehydrocorticosterone. Fig. 3 shows that in C18 cells, the rate of reaction was almost linear over the first 30 min of incubation allowing to determine conditions of initial velocity. In RCCD1 cells, a very low endogenous activity was found, at a level about 10 times lower than in C18 cells. C18 cells express 11-HSD2 at a level close to that of native cells in vivo. Indeed, after 10 min incubation with 10^{-8} M [^{3}H]corticosterone, the enzyme activity of C18 cells is 20 fmol/µg protein while 11-HSD2 activity of rat renal collecting duct is 10 fmol/3 mm tubular length/10 min [18], i.e. 27 fmol/µg protein/10 min. From these results, it appears that C18 cells represent a good model to investigate consequences of glucocorticoid hormone metabolism on hMR transactivation function in a cellular environment resembling the native collecting duct.

3.2. Transactivation activity of hMR transiently expressed in C18 cells in response to various steroids

C18 cells were transiently transfected with an expression vector of the hMR and a reporter gene to test the efficiency of corticosteroids to activate the hMR transactivation function in the absence or in the presence of carbenoxolone (CBX), an inhibitor of 11-HSD2. Results are illustrated in Fig. 4. As previously described [3,17,19], aldosterone is very efficient on hMR transactivation activity since 50% of the steroid-induced hMR activity (ED_{50}) was observed at 5×10^{-11} M. The addition of CBX did not modify the aldosterone dose–response curve. High concentrations of corticosterone were required to activate the hMR in C18 cells. The ED_{50} value (10^{-7} M) was higher than that observed in COS-7 cells devoid of 11-HSD2 [3,17,19,20]. Addition of CBX shifted the corticosterone-dose–response curve towards lower concentrations (ED_{50}: 10^{-8} M). This suggests that corticosterone is metabolized in C18 cells, thus reducing the effective corticosterone concentration. However, at 10^{-6} M, we observed that the corticosterone-induced hMR activity was the same in the presence or absence of CBX, suggesting a limited conversion of corticosterone into 11-dehydrocorticosterone, consistent with the kinetics of saturation of the enzyme [18]. The ability of 11-dehydrocorticosterone (the main metabolite of corticosterone produced by 11-HSD2 in kidney cells) to activate the hMR was extremely low, inducing at 10^{-6} M ∼30% of the maximum aldosterone-induced hMR activity. To examine whether 11-dehydrocorticosterone could inhibit the aldosterone-induced hMR transactivation, C18 cells transiently transfected with the hMR cDNA were incubated with 10^{-9} M aldosterone in the presence of increasing concentrations of 11-dehydrocorticosterone. As shown in

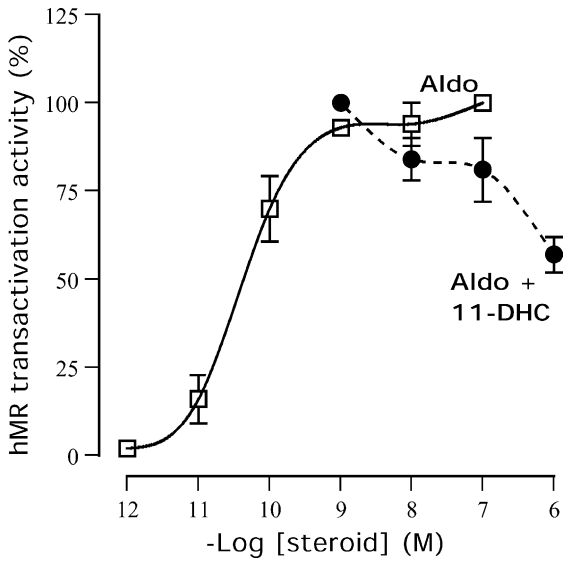

Fig. 5. Effect of 11-dehydrocorticosterone on the aldosterone-induced hMR activity. Transactivation activity of hMR transiently expressed in C18 cells was measured in response to aldosterone (10^{-12} to 10^{-7} M), or to 10^{-9} M aldosterone plus 11-dehydrocorticosterone (10^{-9} to 10^{-6} M). Results are the mean ± S.E. of five independent experiments. Results are expressed as in Fig. 4.

Fig. 5, 11-dehydrocorticosterone has a limited capacity to inhibit aldosterone-mediated response, since 50% inhibition was observed with 10^{-6} M 11-dehydrocorticosterone. Since 11-dehydrocorticosterone concentrations required to activate or inhibit aldosterone-induced hMR activity are high (micromolar range), it is unlikely that circulating levels of 11-dehydrocorticosterone (30–50 nM) [21] can interfere significantly with MR transactivation function in vivo in physiological situations. However this may be important when glucocorticoid plasma levels are elevated (hypercorticism, stress situations . . .). Our results also explain why aldosterone action was blunted in the presence of 11-dehydrocorticosterone in the toad bladder [8], since very high concentrations of 11-dehydrocorticosterone (10 μM) were used in this study, i.e. in conditions where an antagonistic effect of 11-dehydrocorticosterone on aldosterone-MR transactivation does occur.

3.3. Docking of corticosterone and 11-dehydrocorticosterone in the ligand-binding domain of the hMR

In order to understand how 11-dehydrocorticosterone can act as a partial agonist, we docked 11-dehydrocorticosterone and corticosterone within the ligand-binding pocket of the previously described homology model of the hMR ligand-binding domain [22]. Fig. 6 shows that corticosterone adopts a position close to that previously described for aldosterone [12,22]. The corticosterone C3-ketone group is anchored to glutamine 776 and arginine 817 through two strong hydrogen bonds and the C21-hydroxyl group establishes a hydrogen bond with asparagine 770 (2.7 Å). The C11-hydroxyl group of corticosterone is in very close contact with alanine 773 (3.3 Å). Because of the horizontal orientation of its C11-ketone function, 11-dehydrocorticosterone cannot adopt the same orientation. Positioning of 11-dehydrocorticosterone results in ligand rotation around the oxygen at the C3-position for accommodation. Such rotation increases the distance between the C21-hydroxyl group and asparagine 770 (3.3 Å) (Fig. 6). The interaction between the asparagine 770 and the 21-hydroxyl group of corticosteroids is of crucial importance for the stabilization of the active hMR conformation

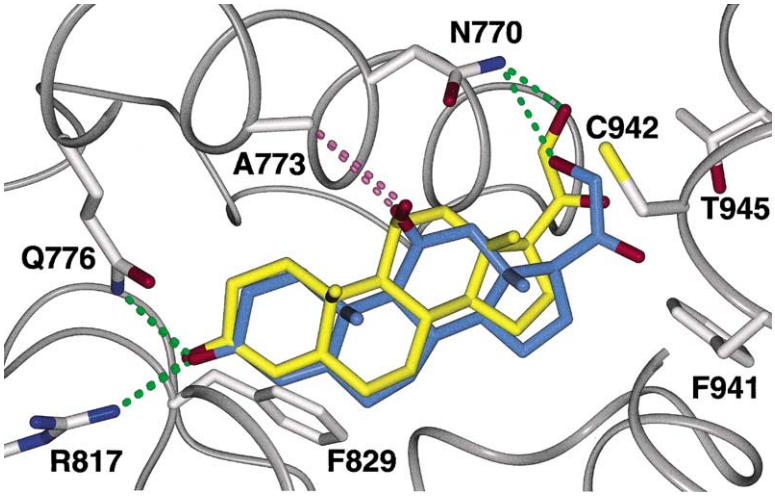

Fig. 6. Corticosterone and 11-dehydrocorticosterone docking within the hMR ligand-binding pocket. The hMR-LBD backbone is drawn as grey ribbons and selected residues side chains in close contact with the ligands are depicted. Corticosterone is colored in yellow and 11-dehydrocorticosterone in blue with their oxygen atoms in red. The hydrogen bonds are depicted in green and the close contact between alanine 773 and the C11-oxygen atom of the ligand is shown as purple dots. The figure was producted with Dino (Philippsen A (2001) Dino: Visualizing structural biology. http://www.bioz.unibas.ch/~xray/dino.

state and for inducing its transactivation function [12,17]. Steroids unable to establish this contact, such as progesterone and spirolactones, display antagonist properties although they bind to the hMR with the same affinity as aldosterone. Furthermore it has been reported that the hMR activity in response to 21-hydroxylated steroids was highly dependent on the C11 substituent [17]. Thus, the weak hydrogen bond between asparagine 770 and the 21-hydroxyl group of 11 dehydrocorticosterone explains the low hMR activity in response to 11-dehydrocorticosterone and is compatible with the partial agonist property of this compound reported here.

In conclusion, this study shows that 11-HSD2 modulates hMR transactivation activity by two complementary ways: 11-HSD2 reduces the amount of corticosterone available for the hMR transactivation within the limits of enzyme activity, i.e. below 10^{-7} M corticosterone concentration. 11-HSD2 produces 11-dehydrocorticosterone which is per se a low agonist for MR transactivation; but can antagonize aldosterone-induced MR transactivation when present at micromolar concentrations. It also brings further mechanistic insights on the interactions between the hMR ligand-binding domain and corticosteroids which are important to determine MR agonist/antagonist properties of steroids.

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References

[1] D.J. Mangelsdorf, C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, et al., The nuclear receptor superfamily: the second decade, Cell 83 (1995) 835–839.

[2] F. Verrey, Early aldosterone action: toward filling the gap between transcription and transport, Am. J. Physiol. 277 (1999) F319–F327.

[3] J.L. Arriza, R.B. Simerly, L.W. Swanson, R.M. Evans, The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response, Neuron 1 (1988) 887–900.

[4] N. Farman, M.E. Rafestin-Oblin, Multiple aspects of mineralocorticoid selectivity, Am. J. Physiol. Renal. Physiol. 280 (2001) F181–F192.

[5] C.R. Edwards, P.M. Stewart, D. Burt, L. Brett, M.A. McIntyre, W.S. Sutanto, E.R. de Kloet, C. Monder, Localisation of 11 beta-hydroxysteroid dehydrogenase–tissue specific protector of the mineralocorticoid receptor, Lancet 2 (1988) 986–989.

[6] J.W. Funder, P.T. Pearce, R. Smith, A.I. Smith, Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated, Science 242 (1988) 583–585.

[7] P.C. White, T. Mune, A.K. Agarwal, 11 Beta-hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess, Endocr. Rev. 18 (1997) 135–156.

[8] A.S. Brem, D.J. Morris, Interactions between glucocorticoids and mineralocorticoids in the regulation of renal electrolyte transport, Mol. Cell Endocrinol. 97 (1993) C1–C5.

[9] D.J. Morris, G.W. Souness, A.S. Brem, M.E. Oblin, Interactions of mineralocorticoids and glucocorticoids in epithelial target tissues, Kidney Int. 57 (2000) 1370–1373.

[10] A.L. Albiston, R.E. Smith, V.R. Obeyesekere, Z.S. Krozowski, Cloning of the 11 beta HSD type II enzyme from human kidney, Endocr. Res. 21 (1995) 399–409.

[11] F. Gouilleux, B. Sola, B. Couette, H. Richard-Foy, Cooperation between structural elements in hormono-regulated transcription from the mouse mammary tumor virus promoter, Nucleic Acids Res. 19 (1991) 1563–1569.

[12] J. Fagart, J.M. Wurtz, A. Souque, C. Hellal-Levy, D. Moras, M.E. Rafestin-Oblin, Antagonism in the human mineralocorticoid receptor, EMBO J. 17 (1998) 3317–3325.

[13] M. Blot-Chabaud, M. Laplace, F. Cluzeaud, C. Capurro, R. Cassingena, A. Vandewalle, N. Farman, J.P. Bonvalet, Characteristics of a rat cortical collecting duct cell line that maintains high transepithelial resistance, Kidney Int. 50 (1996) 367–376.

[14] R.W. Brown, K.E. Chapman, Y. Kotelevtsev, J.L. Yau, R.S. Lindsay, L. Brett, C. Leckie, P. Murad, V. Lyons, J.J. Mullins, C.R. Edwards, J.R. Seckl, Cloning and production of antisera to human placental 11 beta-hydroxysteroid dehydrogenase type 2, Biochem. J. 313 (1996) 1007–1017.

[15] S. Kenouch, N. Alfaidy, J.P. Bonvalet, N. Farman, Expression of 11 beta-OHSD along the nephron of mammals and humans, Steroids 59 (1994) 100–104.

[16] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.

[17] C. Hellal-Levy, B. Couette, J. Fagart, A. Souque, C. Gomez-Sanchez, M. Rafestin-Oblin, Specific hydroxylations determine selective corticosteroid recognition by human glucocorticoid and mineralocorticoid receptors, FEBS Lett. 464 (1999) 9–13.

[18] N. Alfaidy, M. Blot-Chabaud, D. Robic, S. Kenouch, R. Bourbouze, J.P. Bonvalet, N. Farman, Characteristics and regulation of 11 beta-hydroxysteroid dehydrogenase of proximal and distal nephron, Biochim. Biophys. Acta 1243 (1995) 461–468.

[19] M. Lombes, S. Kenouch, A. Souque, N. Farman, M.E. Rafestin-Oblin, The mineralocorticoid receptor discriminates aldosterone from glucocorticoids independently of the 11 beta-hydroxysteroid dehydrogenase, Endocrinology 135 (1994) 834–840.

[20] R. Rupprecht, J.L. Arriza, D. Spengler, J.M. Reul, R.M. Evans, F. Holsboer, K. Damm, Transactivation and synergistic properties of the mineralocorticoid receptor: relationship to the glucocorticoid receptor, Mol. Endocrinol. 7 (1993) 597–603.

[21] G. Morineau, A. Boudi, A. Barka, M. Gourmelen, F. Degeilh, N. Hardy, A. al-Halnak, H. Soliman, J.P. Gosling, R. Julien, J.L. Brerault, P. Boudou, P. Aubert, J.M. Villette, A. Pruna, H. Galons, J. Fiet, Radioimmunoassay of cortisone in serum, urine, and saliva to assess the status of the cortisol-cortisone shuttle, Clin. Chem. 43 (1997) 1397–1407.

[22] G. Auzou, J. Fagart, A. Souque, C. Hellal-Levy, J.M. Wurtz, D. Moras, M.E. Rafestin-Oblin, A single amino acid mutation of ala-773 in the mineralocorticoid receptor confers agonist properties to 11beta-substituted spirolactones, Mol. Pharmacol. 58 (2000) 684–691.