Aldosterone Stimulates Epidermal Growth Factor Receptor Expression*

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The steroid hormone aldosterone plays an important role during pathological tissue modifications, similar to cardiovascular or renal fibrosis. The underlying mechanisms for the pathological actions are not understood. Interaction of aldosterone with the epidermal growth factor (EGF) receptor is an attractive hypothesis to explain pathological tissue remodeling elicited by aldosterone, because (i) mineralocorticoids can sensitize cells for EGF, (ii) mineralocorticoid receptor (MR)-antagonists reduce EGFR-mRNA expression, (iii) EGFR itself supports the development of cardiovascular or renal fibrosis, and (iv) signaling elements involved in the pathological action of aldosterone (similar to ERK1/2 or NFKB) are typical downstream modules during EGF signaling. In addition, an interaction of aldosterone and EGF with respect to ERK1/2 activation has been described. Here we show that aldosterone stimulates EGFR expression in renal tissue of adrenalectomized rats and in human renal primary cell cultures. Furthermore, Chinese hamster ovary (CHO) cells normally devoid of MR or GR express EGF receptor after transfection with human MR (CHO-MR cells) but not after transfection with human glucocorticoid receptor (CHO-GR cells). In CHO-MR cells, EGFR-expression is up-regulated by aldosterone and inhibited by spironolactone. CHO-MR cells but not CHO-GR cells respond with ERK1/2 phosphorylation to EGF exposure. The responsiveness to other peptide hormones was virtually not affected. These data suggest that EGFR is an aldosterone-induced protein and is involved in the manifold (patho)biological actions of aldosterone.

Nowadays, it is evident that the importance, especially the pathophysiological importance, of aldosterone and the mineralocorticoid receptor extends beyond the regulation of salt and water homeostasis. Most importantly, it has been demonstrated that aldosterone promotes cardiovascular and renal fibrosis because of tissue remodeling as well as endothelial dysfunction independent of its effects on blood pressure or salt homeostasis (1–5). Several mechanisms have been proposed for the pathological actions of aldosterone, including enhanced collagen and fibronectin synthesis, stimulation of reactive oxygen species formation, decreased nitric oxide availability, and enhanced expression of peptide hormones (e.g. endothelin-1) or their receptors (e.g. AT1-receptor) (2, 6). The pathophysiologically relevant role of aldosterone in pathological effects of support from clinical studies, especially the Randomized Aldactone Evaluation Study and the Eplerenone Post-acute Myocardial Infarction Heart Failure Efficacy and Survival Study. Low doses of mineralocorticoid receptor antagonists led to a dramatic improvement of mortality in patients with severe congestive heart failure or after myocardial infarction (7, 8). Thus, it is clear that aldosterone exerts important non-classical, non-epithelial actions. Unfortunately, the underlying mechanisms of this important action of aldosterone and the mineralocorticoid receptor are only poorly understood.

Classically, aldosterone binds to the cytosolic mineralocorticoid receptor (MR)1 (9), which then translocates to the cell nucleus and acts as a transcription factor. Subsequently, the expression of various proteins like the epithelial sodium channel, the Na+-K+-ATPase, or the serum and glucocorticoid-regulated kinase is modulated (9–14). It has been proposed that the transcription factors NFKB and AP1 as well as the mitogen-activated kinases ERK1/2 are involved in the pathological actions of aldosterone (15–17). However, these signaling pathways are usually associated with growth factors or cytokine receptors rather than with aldosterone signaling. In this respect, it is of interest that steroids, including aldosterone, have the ability to interact with peptide hormone signaling (17). One important peptide signaling "system" with respect to aldosterone is the epidermal growth factor (EGF) and its receptor (EGFR) (17, 18). Pharmacological in vivo studies have shown that mineralocorticoids enhance EGF-induced contraction of arteries (19) and that the MR-antagonist spironolactone reduces the expression of EGFR-mRNA after cerebral ischemia (20). Furthermore, EGFR expression supports fibrosis in cardiovascular and renal tissue (21–23). The importance of the EGFR in mediating pathological effects of heterogeneous signaling systems is supported by its role in endothelin-induced fibrogenic effects (22). Endothelin-induced phosphorylation of the mitogen-activated protein kinase and endothelin-induced increase in collagen I gene activity were completely prevented by an inhibitor of the EGFR kinase. Thus, EGFR

1 The abbreviations used are: MR, mineralocorticoid receptor; ADX, adrenalectomized; Aldo, aldosterone; EGF, epidermal growth factor; GR, glucocorticoid receptor; PMA, phorbol 12-myristate 13-acetate; MDCK, Madin-Darby canine kidney cells; PBS, phosphate-buffered saline; Luc, luciferase; ANOVA, analysis of variance; hMR, human MR; hGR, human GR; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.
Aldosterone Induces EGFR

**FIG. 1.** EGFR expression in ADX rats and in human renal cortical cells in primary culture. a, EGFR expression in kidney cortex homogenate of ADX rats and ADX + aldosterone rats after 5 days of treatment was determined by Western blot. Aldosterone application led to enhanced EGFR expression. b, human renal cortical cells in primary culture (RPTEC) turned out positive for MR expression by RT-PCR and by Western blot (c). d, incubation of RPTEC cells with aldosterone led to enhanced EGFR expression. The experiment was performed three times with similar results.

**MATERIALS AND METHODS**

**Animal Study**—Male Munich-Wistar rats weighing between 250 and 350 g (Charles River, Sulzfeld, Germany) underwent adrenalectomy or sham operation under NarcorenR anesthesia using two lumbodorsal incisions. Aldosterone (ADX + ald) (96 μg/100 g body weight and day) or vehicle (ADX) was applied by osmotic mini-pumps (model 2ML1, Alzet, Palo Alto, CA). The adrenalectomized animals were maintained on 0.9% NaCl solution in lieu of drinking water and on a standard rat pellet diet (Walkersville, MD). The adrenalectomized animals were maintained on 0.9% NaCl solution in lieu of drinking water and on a standard rat pellet diet (Walkersville, MD). The adrenalectomized animals were maintained on 0.9% NaCl solution in lieu of drinking water and on a standard rat pellet diet (Walkersville, MD). The completeness of adrenalectomy and the effectiveness of aldosterone application by osmotic mini-pumps were assessed by determination of plasma aldosterone and the plasma Na+/K+ ratio. Plasma aldosterone in ADX animals was 0.06 ± 0.02 and 6.3 ± 0.8 nmol/liter in ADX + aldosterone animals. The Na+/K+ ratio was 27 ± 5 in ADX animals and 54 ± 2 in ADX + aldosterone animals. 5 days after adrenalectomy, the animals were anesthetized with Inactin (120 mg/kg body weight, Byk-Gulden, Konstanz, Germany), the kidneys were removed, and the cortex was homogenized with 20 strokes of a Janke & Kunkel (Staufen, Germany) homogenizer at 4 °C. Protein content was determined according to Lowry (37). Subsequently, equal amounts of protein (60 μg) were subjected to SDS-PAGE and Western blot as described below.

**Cell Culture**—CHO-K1 cells from ATCC (Manassas, VA) were cultivated as described previously (17, 18). Cells were grown in Ham’s F-12 medium supplemented with 10% fetal calf serum at 37 °C and 5% CO2, 24 h prior to the experiments, serum was removed from the media. For the experiments presented, the cells were cultivated either on Petri dishes (BD Biosciences) or in 96-well plates (for pERK1/2-ELISA). Primary cells of human kidney cortex were purchased from Clonetics (Walkersville, MD) and cultured according to the manufacturer's instructions.

**Transfection and Dilution Cloning**—Transfection of the cells was performed with the Qiagen Polyfect reagent (Qiagen, Hilden, Germany) according to the manufacturer's instructions. We used the HER1 expression vector pRKS-HER1 (38), the hMR expression vector pCDNA1-MR, the hGR expression vector pCDNA1-GR (18), and pBabe-puro (39), which carries a puromycin resistance gene (ratio 10:1). Selection of transfected clones was performed with puromycin (5 mg/liter). After isolating clones by limited dilution cloning, their hMR or hGR expression was determined by RT-PCR and their EGFR receptor expression was determined by Western blot analysis.

**Western Blot Analysis**—Cells were washed three times with ice-cold phosphate-buffered saline (PBS) and lysed in ice-cold Triton X-100 lysis buffer (50 mm Tris-HCl, pH 7.5, 100 mm NaCl, 5 mm EDTA, 200 μg sodium orthovanadate, 0.1 mm phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 40 μg/liter bestatin, 2 μg/liter aprotinin, 1% Triton X-100) or RIPA buffer for 25 min at 4 °C. Cells lysates were matched for protein content, separated by SDS-PAGE, and transferred to a nitrocellulose membrane. Subsequently, membranes were blotted with either rabbit anti-phospho-ERK1/2 antibody, rabbit anti-ERK1/2 antibody (1:1000, New England Biolabs, Beverly, MA), rabbit anti-EGFR-antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), or goat anti-MR-antibody (1:1000). The bound primary antibody was visualized using horseradish peroxidase-conjugated secondary IgG (1:10,000–25,000) and the ECL system (Amersham Biosciences).

**Immunofluorescence**—Cells were fixed with 4% formaldehyde (15 min), washed three times with PBS, and permeabilized with 0.5% Triton X-100. Subsequently, cells were incubated for 10 min in 1% SDS/PBS followed by 10 min of 100 mmol/liter glycine/PBS and finally for 20 min in 10% serum, 1% bovine serum albumin/PBS. After 60-min incubation with anti-EGFR, the cells were washed three times with PBS and incubated for 45 min with anti-rabbit-Cy3 secondary antibody. After three further washes, the cells were transferred to the stage of an inverted microscope (Zeiss IM 35) and fluorescence images were taken using an ICCD camera (Hamamatsu, Herrsching, Germany).

**Quantification of ERK1/2 Phosphorylation by ELISA**—Quantification of ERK1/2 phosphorylation was done by pERK1/2-ELISA according to Versteeg et al. (40) with minor modifications described previously (17). Cells were seeded in 96-well plates (Maxisorp, Nunc) and serum-starved for 24 h prior to the experiment. After stimulation as indicated...
FIG. 2. EGFR expression in CHO cells transfected with hMR. a, successful transfection of CHO cells with human MR (CHO-MR) was evaluated by RT-PCR. Subsequently, EGFR expression in CHO-MR cells and in cells transfected with the empty plasmid (CHO-pcDNA) was determined by Western blot. CHO cells transfected with human EGFR (CHO-HER1) (17) were used as positive control. b, to confirm MR-induced EGFR expression, two additional cell clones were analyzed. c, none of the cell clones transfected with the empty plasmid EGFR was detectable.

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in the text, the cells were fixed with 4% formaldehyde in PBS and washed three times with PBS containing 0.1% Triton X-100. Cells were blocked with 10% fetal calf serum in PBS/Triton X-100 for 1 h and incubated overnight with the primary antibody (1:1000). After three washes, cells were incubated with the secondary antibody (peroxidase-conjugated mouse anti-rabbit antibody, dilution 1:10000) in PBS/Triton X-100 with 5% bovine serum albumin for 1 h at room temperature and washed three times with PBS/Triton X-100 for 5 min and twice with PBS. Subsequently, the cells were incubated with 50 µl of a solution containing 0.4 mg/ml O-phenylenediamine, 11.8 mg/ml NaH₂PO₄, 7.3 mg/ml citric acid, and 0.015% H₂O₂, 15 min at room temperature in the dark. The resulting signal was detected at 490 nm with a multiwell multilabel counter (Victor², Wallac, Turku, Finland). Finally, protein content in the wells was determined with a trypan blue solution (17).

**RT-PCR, PCR, and Sequencing**—RNA was isolated using the TRIzol reagent (Invitrogen), and genomic DNA was isolated using standard techniques (41). RT-PCR was performed using the SuperScript™ One-Step RT-PCR kit from Invitrogen. PCR was performed using the TapPCR kit from Qiagen. Sequencing was carried out by MWG-Biotech AG (Ebersberg, Germany). The following primers were used: MR forward 1, 5'-ACACTGTTCTGGCACTCTT-3'; MR reverse 1, 5'-AAAGGT-TCATCGTTGTTCCG-3' (annealing temperature, 57 °C, 35 cycles, product size 190 bp); MR forward 2, 5'-ATCCAGATTGGCATCCTGAAG-3'; MR reverse 2, 5'-CCATAATGGCATCCTGAAG-3' (annealing temperature 57 °C, 35 cycles, product size 244 bp); GR forward 1, 5'-TCCCTTCCCCACTGACAGAC-3'; and GR reverse 1, 5'-CATCATTCTTGACAGCAT-3' (annealing temperature 55 °C, 35 cycles, product size 371 bp).

**EGFR Promoter Activity Determination**—The pER-1-Luc construct containing firefly luciferase under the control of the EGFR promoter was kindly provided by Dr. A. Johnson (National Institutes of Health) (30). Cells were transfected with pER-1-Luc and the secretory alkaline phosphatase 2 construct (constitutively expressing secretory alkaline phosphatase to determine transfection efficiency). Luciferase activity was determined using the Steady-Glo luciferase assay system (Promega). Luciferase activity was always corrected for secretory alkaline phosphatase 2 activity.

**Materials**—U0126, tyrphostin AG 1478, and the protease inhibitors were purchased from Calbiochem. Unless stated otherwise, all of the other materials were obtained from Sigma. Control Ringer solution was composed of (mM): 130 NaCl, 5.4 KCl, 1.0 CaCl₂, 1.0 MgCl₂, 1.0 NaH₂PO₄, 10 HEPES, and 5 glucose, pH 7.4, at 37 °C plus the respective vehicles (ethanol or Me₂SO ≤ 1%).

**Statistics**—The data are presented as mean values ± S.E. Significance of difference was tested by paired or unpaired Student’s t test or ANOVA as applicable. Differences were considered significant if p < 0.05. Cells from at least two different passages were used for each experimental series. n represents the number of cell or tissue culture dishes investigated.

**RESULTS AND DISCUSSION**

First, we tested whether aldosterone application to adenocarcinomatized rats affects EGFR expression. For this purpose, we analyzed kidney cortex homogenates. Fig. 1a shows that the application of aldosterone resulted in an enhanced expression of EGFR. The serum concentration of aldosterone (6.3 ± 0.8 nmol/liter) was in the high physiological to low pathophysiological range of aldosterone but was one to two orders of magnitude below the concentration range of glucocorticoids. Our results show that aldosterone stimulates EGFR expression in vivo, most probably via the MR. Of course, the in vivo data presented here are not complete and future studies will have to investigate in detail different tissues and compare the effects of aldosterone to those of dexamethasone (a GR agonist) and spironolactone (a MR antagonist). Studies from other groups have already provided evidence for a stimulatory action of aldosterone on EGFR expression (19, 20). A general up-regulation of protein expression can be excluded because (i) equal amounts of total protein were loaded for each sample and (ii) because we have shown in a previous study (36) that total cortical NHE3 expression is not affected by the same maneuver. In a next step, we used human kidney cortex cells to test whether there is a direct effect of aldosterone on human cells. RT-PCR (Fig. 1b) and Western blot analysis (Fig. 1c) provided evidence for the expression of MR in the cells used. Application of aldosterone in a similar concentration range as in the in vivo study led to an increased EGFR expression (Fig. 1d). Thus, the in vivo data could be reproduced in a primary cell culture of human origin.

Because our data indicated that aldosterone stimulates EGFR expression via the MR, we turned our experimental focus to a heterologous expression system of CHO cells transfected with hMR or hGR. This system has the advantage of being well defined and the disadvantage of not representing differentiated tissue. However, for our purposes the advantages clearly outweigh the disadvantages. Fig. 2a shows that CHO cells, which do not express EGFR or MR and are not responsive to EGFR under control conditions (17), do express EGFR after stable transfection with human MR (CHO-MR cells) but not after transfection with the empty plasmid (pcDNA1) when kept in serum-containing media. This result was reproduced in three independently isolated CHO-MR cell clones and several mock-transfected clones (Fig. 2, b and c). The expression of EGFR could be also detected by immunofluorescence (Fig. 2d), and there was no major difference in EGFR distribution when comparing cells transfected with human EGFR1 (17) or with human MR. Thus, not only estrogen receptors and thyroid hormone receptors affect EGFR expression as mentioned under Introduction but also the mineralocorticoid receptor.

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*Fig. 3. Modulation of EGFR expression in CHO-MR cells.* a, the mineralocorticoid receptor antagonist spironolactone reduces EGFR expression. b, cultivation of CHO-MR cells for 72 h in serum-free media abolished EGFR expression. When serum or aldosterone was added for the last 48 h, EGFR expression was restored. Aldosterone clearly stimulates EGFR expression, although to a lesser extent than serum. c, the effect of aldosterone on EGFR expression is concentration-dependent and can be antagonized by spironolactone. All of the experiments were performed at least three times with similar results.
The negative RT-PCR results for MR obtained with CHO-mock cells could be interpreted in the way that the primers used did not correspond to the CHO-MR sequence, which is currently unknown, and therefore endogenous MR was not detected. To rule out this possibility, we used the MR primer pair 1 (see "Materials and Methods"), which spans a region on exon 1 of the hMR (42) for genomic PCR. Fig. 2e shows the positive signal obtained as well as the sequence identity with hMR, indicating that our primers were suitable to detect CHO-MR. These data support our hypothesis that endogenous MR expression in CHO cells is neglectable. Stable transfection of hMR may lead to overexpression, and it cannot be ruled out completely that overexpressed MR exhibits a different transactivation profile than endogenous MR. The animal studies and the data on human cells in primary culture (Fig. 1) argue against this possibility. To strengthen our hypothesis of MR-mediated EGFR expression regulation, we tested the effect of aldosterone in another cell type, MDCK-C11 cells (43), originally derived from canine-collecting duct. Although MDCK cells have been widely used to study effects of mineralocorticoids, the expression of MR in these cells has not been investigated. We performed RT-PCR with RNA from MDCK-C11 cells using the MR primer pair 2, which spans intron B of hMR (42). The expected product obtained from RNA has a size of 244 bp. Fig. 2f shows that we obtained a product of the expected size which has 95% homology to the human MR. Thus, MDCK-C11 cells express endogenous MR spontaneously. Finally, we incubated the cells for 72 h with aldosterone and determined EGFR expression. As shown in Fig. 2f, 10 nM aldosterone stimulated EGFR expression. These data provide further support for our hypothesis of MR-stimulated EGFR expression. In addition, Fig. 2g confirms the lack of expression of MR in CHO-wild-type or CHO-mock cells.

Application of the clinically used MR antagonist spironolactone clearly reduced EGFR expression in CHO-MR cells as shown in Fig. 3a. Removal of serum for 72 h reduced the expression of EGFR below the detection limit (Fig. 3b). However, when serum was added for the last 48 h of the incubation period, EGFR expression was restored (Fig. 3b). Similarly, the addition of aldosterone under serum-free conditions restored EGFR expression (Fig. 3c). However, aldosterone was less efficient than serum. This can be easily explained by additional serum factors stimulating EGFR expression. The action of aldosterone on EGFR expression was concentration-dependent (Fig. 3c) and could be inhibited by spironolactone. These data conclusively show that EGFR expression can be triggered by the ligand-receptor pair aldosterone/MR at relevant aldosterone concentrations.

To test whether the induced EGFR is functional, i.e., displays intact downstream signaling, we determined EGF-induced ERK1/2 phosphorylation. As shown in Fig. 2a, mock-transfected CHO cells did not respond with enhanced ERK1/2 phosphorylation during application of either EGF or aldosterone. As expected, the cells responded to the positive control (phorbol ester PMA that activates protein kinase C). b, CHO-MR cells, which express EGFR (see Figs. 2 and 3), respond with enhanced ERK1/2 phosphorylation during application of EGF and show a mild response to aldosterone. e, expression of ERK1/2 in CHO-MR cells was not different when compared with CHO-mock cells. d, all of the three CHO-MR cell clones responded to EGF (1 μg/liter EGF, n = 12, *p < 0.05) to a similar extend. e, the effect of EGF was concentration-dependent. Half-maximal activation (EC50) occurred at 1.1 ± 0.2 μg/liter EGF. f, the effect of 1 μg/liter EGF (n = 12, *p < 0.05) was prevented by inhibition of the kinase MEK1/2 (10 μmol/liter U0126, n = 9) or by inhibition of the EGFR kinase (100 nmol/liter tyrphostin AG1478, n = 9). These data show that CHO-MR cells express an intact EGF-to-ERK1/2 signaling cascade.
of the three CHO-MR cell clones after 5 min of aldosterone application (Fig. 4b). At present, the mechanisms underlying this effect are not known. A sole increase in EGFR expression can probably not explain this phenomenon, because EGFR-transfected cells respond to aldosterone only in the presence of EGF (17). Most probably, the MR is required for the observed effect. This interesting observation is currently under investigation. To rule out the possibility that enhanced EGF-induced ERK1/2 phosphorylation is because of enhanced ERK1/2 expression, we compared the expression in mock- and MR-transfected cells (Fig. 4c) but did not detect significant differences.

As shown in Fig. 4d, all of the three CHO-MR clones reacted similarly to EGF. The reaction to 1 μmol/liter PMA (a positive control for ERK1/2 phosphorylation) was similar in transfected and non-transfected cells (Fig. 4d). EGF-induced ERK1/2 phosphorylation was concentration-dependent with an EC_{50} of 1.1 ± 0.2 μg/liter (Fig. 4e). Finally, EGF-induced ERK1/2 phosphorylation was prevented by specific blockade of the EGFR kinase using tyrphostin AG1478 (Fig. 4f) or by specific blockade of MEK1/2 kinases, which ultimately phosphorylate ERK1/2 using U0126. Thus, MR and aldosterone induced the expression of a functional EGFR with intact downstream signaling cascade. EGF responsiveness of CHO cells could also be achieved by transient MR transfection (48 h after transient transfection with MR, application of 1 μg/liter EGF for 5 min stimulated ERK1/2 phosphorylation to 300 ± 30% of control (p < 0.05, n = 6); mock transfection was without effect). These data rule out the remote possibility of artifacts due to cell clone selection by limited dilution cloning.

To test whether the effects observed are specific for hMR or can be also achieved by hGR, CHO cells were stably transfected with hGR (Fig. 5a). Subsequently, three cell clones were randomly chosen and analyzed for EGFR expression. As shown in Fig. 5b, no EGFR expression could be detected. In addition, we determined the EGF responsiveness of the cells with respect to ERK1/2 phosphorylation. Although all of the three clones responded to 1 μg/liter PMA, none of them responded to EGF (Fig. 5c). These data show that the stimulation of EGFR expression is specific for MR compared with GR. Thus, modulation of EGFR expression may contribute to the specificity of MR
action over GR. To date, most proteins that are induced by MR can also be induced by GR. Further support for the differential effects of MR and GR on EGFR expression is provided by the EGFR promoter assay (Fig. 6a). Transient transfection with hMR certainly does not exert a consistent stimulatory effect in any of the equal amount of hGR plasmid did not enhance EGFR promoter activity (Fig. 6a). These results support the hypothesis of the EGFR as a MR- but not GR-induced protein. A more detailed analysis of the interaction between MR and the EGFR promoter will be subject of future studies.

Finally, we tested whether hMR transfection leads to a more general up-regulation of peptide hormone signaling. For this purpose, we investigated the effects of three other hormones, namely angiotensin II, endothelin-1, and vasopressin on ERK1/2-phosphorylation. Fig. 6b shows that none of these three hormones exerted a consistent stimulatory effect in any of the three CHO-MR cell clones. Thus, although we cannot exclude that besides EGFR the signaling of another uninvestigated mediator is up-regulated, transfection with hMR certainly does not induce a general nonspecific up-regulation of cell signaling.

Our results suggest that the mineralocorticoid hormone aldosterone participates via the mineralocorticoid receptor in the regulation of EGFR expression. Thus, EGFR seems to be an aldosterone-induced protein. Possibly, our data can help to explain pharmacological studies, which suggest a link between aldosterone application to animals and EGFR expression (19, 20, 44). As described above, various cellular signaling pathways are involved in the regulation of EGFR expression and further studies are needed to determine the mechanism(s) employed by aldosterone/GR. At present, it is not known whether MR binds to the EGFR promoter. However, MR could also be linked indirectly to EGFR expression, for example, via activation of API (15). The promotion of EGFR expression can also affect signaling of other hormones that interact with the EGFR by a mechanism called transactivation (17). Transactivation of the EGFR is used by a variety of mediators with potential pathophysiological relevance similar to angiotensin II or endothelin (45). Thus, aldosterone enhances the expression of a molecular target (EGFR) involved in various signaling networks and may indirectly influence the action of a broad spectrum of signaling pathways. This way our data also help to explain the interaction of aldosterone with peptide hormone and cytokine signaling (6). Of course, this still is a hypothesis that needs to be validated in future studies. We think that the interaction of aldosterone/GR with EGFR is of importance especially with respect to aldosterone as a mediator of progressive cardiovascular and renal dysfunction because EGFR may translate elevated aldosterone concentrations, for example, into tubulo-interstitial fibrosis, cardiac fibrosis, or reduced vascular compliance (2, 3, 6, 16). Our next effort will be to determine pathophysiological consequences of aldosterone-induced EGFR expression on the cellular level. These include the production of reactive oxygen species and synthesis of collagen or fibronectin. Ultimately, the role of EGFR in aldosterone-induced tissue damage has to be tested in vivo.

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