

No extra-adrenal aldosterone production in various human cell lines

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Abstract

Extra-adrenal *de-novo* aldosterone (Aldo) production has been described inconsistently. Systematic data based upon state-of-the-art technology including validated controls are sparse. We hypothesized that aldosterone synthase (*CYP11B2*) expression and *de-novo* Aldo production are absent in non-adrenal human cell lines, either immortalized cell lines or commercially available primary cell lines, including peripheral blood mononuclear cells (PBMCs) of individuals without and with primary hyperaldosteronism (PA).

CYP11B2-transfected COS-7 and endogenous *CYP11B2* expressing adrenal H295R cells served as positive controls. Various well-characterized, purchased, immortalized (BeWo, HEK293, HTR-8/SVneo, JEG-3) and primary (HAEC, HLEC, HRGEC, HRMC, HUAEC, HUVEC, PBMC) cell lines as well as self-isolated PBMCs from PA patients (n=5) were incubated with the steroid hormone substrates progesterone, deoxycorticosterone, corticosterone or 18-OH-corticosterone with and without Ang II for 24h to assess *CYP11B2* enzymatic activity. *CYP11B2* expression was analyzed by Real-time PCR and liquid chromatography-mass spectrometry (LC-MS) was used to quantify Aldo production.

Pronounced *CYP11B2* mRNA expression and Aldo production were observed in both positive controls, which followed an incremental time course. Neither substrates alone nor co-incubation with Ang II significantly stimulated *CYP11B2* expression or Aldo production in various immortalized and primary cell lines and PBMCs of PA patients. These results strongly support the absence of a relevant *de-novo* extra-adrenal Aldo production in non-adrenal cells including, blood mononuclear cells irrespective of the absence or presence of autonomous adrenal Aldo production.

Abbreviations:

AGTR1	angiotensin II receptor 1
AGTR2	angiotensin II receptor 2
Aldo	aldosterone
Ang II	angiotensin II
BeWo	human placental cell line
COS-7	fibroblast-like cell lines derived from monkey kidney tissue
CYP11B1	cytochrome P450 steroid 11 beta-hydroxylase
CYP11B2	cytochrome P450 aldosterone synthase
CYP21A2	cytochrome P450 steroid 21-hydroxylase
DOC	Deoxycorticosterone
EtOH	ethanol
HAEC	human aortic endothelial cells
HEK293	human embryonic kidney cells
HLEC	human lymphatic endothelial cells
HRGEC	human renal glomerular endothelial cells
HRMC	human renal mesangial cells
HTR-8/SVneo	human 1 st trimester trophoblasts
HUAEC	human umbilical artery endothelial cells
HUVEC	human umbilical vein endothelial cells
H295R	human adrenal cortical carcinoma cell line
JEG-3	human choriocarcinoma cell line
LC-MS	Liquid chromatography-mass spectrometry
PA	Primary hyperaldosteronism
PBMCs	Peripheral blood mononuclear cells
SRD5A1	5 α -reductase type 1

Introduction

Next to the well-known renal responses, aldosterone (Aldo) has a major impact in non-classical off-target tissues. This leads to the idea of a local extra-adrenal Aldo synthesis. While during pregnancy, Aldo is beneficial for placental growth (Gennari-Moser et al., 2010), it promotes inflammation and fibrosis in vessels and the kidneys. Controversy over local *de-novo* Aldo production in these organs explanatory for its adverse effects has started decades ago, while state-of-the-art technology including validated controls might now enable comprehensive investigations.

Extra-adrenal Aldo production has been postulated based upon data by Casey et al. in 1982 pointing towards the conversion of plasma progesterone to 11-deoxycorticosterone (DOC) in extra-adrenal tissues (including the kidney, aorta, spleen, and several fetal tissues) in pregnant and non-pregnant women and in men (Casey and MacDonald, 1982), though metabolites further downstream of DOC had not been assessed.

Renal cytochrome P450 aldosterone synthase (*CYP11B2*) expression and Aldo production was described in whole kidney tissue, tubular epithelial cells and mesangial cells (Wu et al., 1999), (Xue and Siragy, 2005), (Nishikawa et al., 2005).

In the vasculature, *CYP11B2* expression and Aldo production have been found in mesenteric arteries of healthy (Rudolph et al., 2000), (Takeda et al., 1994), (Takeda et al., 1995b), (Takeda et al., 1995a) and spontaneously hypertensive rats (Takeda et al., 1997), in human umbilical vein endothelial cells (HUVEC) (Takeda et al., 1996), in endothelial and vascular smooth muscle cells of human pulmonary arteries and the aorta of healthy and diseased subjects (Hatakeyama et al., 1994), (Maron et al., 2012), (Matsuzawa et al., 2013), (Alesutan et al., 2017). The level of Aldo production and *CYP11B2* expression observed in endothelial and smooth muscle cells approximated

1/50 of that of adrenal cells (Hatakeyama et al., 1994). Aldo production in HUVECs was responsive to angiotensin II (Ang II), adrenocorticotrophic hormone (ACTH) and potassium (Takeda et al., 1996), and was upregulated in human pulmonary artery endothelial cells in hypoxic conditions (Maron et al., 2014). Interestingly, the classical pathway of *de-novo* Aldo production from cholesterol as substrate was ruled out as no steroidogenic enzymes upstream of CYP11B2 could be detected in endothelial and vascular smooth muscle cells (Hanukoglu, 1992). Consequently, Hatakeyama et al. suspected that the enzyme system responsible for Aldo production in human vascular cells is different from that found in the adrenal cortex and that vascular Aldo may be synthesized from metabolic intermediates which originate from the circulation (Hatakeyama et al., 1996). In clear contrast are findings of absent *CYP11B2* mRNA expression and Aldo biosynthesis in human umbilical veins, and in human pulmonary artery endothelial cells (Ahmad et al., 2004) by a group that used a validated protocol developed to detect very low expression levels of *CYP11B2* in sub-regions of the human brain (Gomez-Sanchez et al., 1997).

In 1999, Takeda et al. described *CYP11B2* expression in peripheral blood mononuclear cells (PBMCs) of patients with idiopathic hyperaldosteronism (Takeda et al., 1999). *CYP11B2* expression was reported to be upregulated in PBMCs of primary hyperaldosteronism (PA) patients as compared to healthy subjects and patients with Aldo-producing adenoma (Miyamori et al., 2000). Later, Miura et al. added that PBMCs of healthy subjects produce Aldo upon Ang II stimulation (Miura et al., 2006). The existence of *de-novo* Aldo production beyond the adrenal glands is still uncertain in most tissues, complicated by issues with respect to control conditions, and despite given methodological improvements over time. A major demand to any study, targeting the proof of absence of a functionally relevant system, is to apply highly sensitive methods.

We therefore hypothesized, that *CYP11B2* expression and *de-novo* Aldo production are absent in non-adrenal human cell lines, either immortalized cell lines or commercially available primary cell lines, including PBMCs of individuals with and without PA. Immortalized cell lines used were: BeWo (human choriocarcinoma cells), HEK293 (human embryonic kidney cells), HTR-8/SVneo (human 1st trimester trophoblasts) and JEG-3 (human choriocarcinoma cells). Purchased primary cell lines used were: HAEC (human aortic endothelial cells), HLEC (human lymphatic endothelial cells), HRGEC (human renal glomerular endothelial cells), HRMC (human renal mesangial cells), HUAEC (human umbilical artery endothelial cells), HUVEC (human umbilical vein endothelial cells), and PBMCs (peripheral blood mononuclear cells).

Specifically, we aimed to assess *CYP11B2* mRNA expression and to measure Aldo production **first** in non-stimulatory conditions and **second** upon Ang II stimulation.

Materials and Methods

Material and cell lines

Cell culture materials were from Techno Plastic Products AG (Trasadingen, Switzerland). Collagen I coated petridishes were from Corning (Milian, Nesselbach, Switzerland), while Poly-L-lysine and fibronectin for cell ware coating were from ScienCell (Chemie Brunschwig, Basel, Switzerland).

BeWo (CCL-98), **HTR-8/SVneo** (CRL-3271), **HAEC** (PCS-100-011), **COS-7** (CRL-1651) and **NCI-H295R** cells (CRL-2128) were purchased from ATCC. The primary cells **HUVEC** (#8000), **HUAEC** (#8010) and **HLEC** (#2500) were from ScienCell (Chemie Brunschwig, Basel, Switzerland).

The primary cells **HRMC** (#4200) and **HRGEC** (#4000) and their corresponding media, MCM (#4201) and ECM (#1001), with the supplements (MsCGS #4252 and ECGS #1052), penicillin/streptomycin (P/S, #0503) and fetal bovine serum (FBS, #0010 and #0025) were obtained from ScienCell (Chemie Brunschwig, Basel, Switzerland). HRMC were cultured on poly-L-lysine, and HRGEC on fibronectin or collagen I coated plates. **HUAEC** cells (#8010) were cultured in ECM (#1001) containing the endothelial cell growth supplements (ECGS #1052) also from ScienCell.

HEK293 (human embryonic kidney cells) #CRL-1573 and **JEG-3** (a human choriocarcinoma cell line) #HTB-36 cells were from ATCC, and their corresponding media DMEM (#41965) and McCoy's (#36600) respectively, were from Gibco. **PBMCs** (4W-270) of six healthy individuals (4 men, 2 women) were purchased from Lonza, Basel, Switzerland. Method of authentication of cells was short tandem repeat analysis for ATCC, immunofluorescence for ScienCell, and QC testing for Lonza. HUVEC, HTR-8/SV neo, and BeWo cells and PBMCs were cultured in RPMI1640 #21875 (with phenol red) and #11835 (without phenol red) from ThermoFisherScientific (Reinach, Switzerland). H295R cells were cultured in DMEM-F12 #11320033 (with phenol red) and #21041 (without phenol red) from ThermoFisherScientific.

HAEC and HLEC cells grew in the Vascular Cell Basal Medium (PCS-100-030) containing the supplements (PCS-100-041) from ATCC. HLEC were cultured on collagen I coated plates.

COS-7 cells were cultured in DMEM #41965 (with phenol red) and #31053 (without phenol red) from Gibco/ThermoFischerScientific.

FBS, P/S, HEPES, ITS+ Premix and sodium pyruvate were from Gibco/ThermoFischerScientific if not otherwise stated.

Fugene E2311(Promega), CYP11B2 plasmid #RC215476 was from Origene, and the pCMV_EV plasmid was a gift. OptiMEM #31985 was from ThermoFisherScientific. All steroid standards for LC-MS analysis were purchased from Cerillant (UK) or Steraloids, Inc. (Newport, RI, USA).

Primary hyperaldosteronism patients and healthy controls

PA patients were recruited at our outpatient clinic of the Department of Nephrology and Hypertension, University Hospital of Bern, Switzerland for the evaluation and treatment of their arterial hypertension. All patients had signs of secondary hypertension due to primary hyperaldosteronism. The diagnosis was made by measuring plasma Aldo and renin levels in lying and standing position. All medication interfering with the renin-angiotensin system was stopped 2 to 3 weeks prior to the measurements. Patients had an elevated Aldo to renin ratio ($ARR > 40$), an elevated plasma Aldo ($PAC \geq 10\text{ng/dL}$ or $\geq 277\text{ pmol/L}$) or a suppressed renin. A confirmation test was conducted in cases where the Aldo level was lower than 20 ng/dL or 555 pmol/L and in absence of spontaneous hypokalemia.

Exclusion criteria were: no signed informed consent, hypertension from another cause with an $AAR < 40$ and a renin level greater than 2.6 ng/L . Medications interfering with the mineralocorticoid receptor (MR) such as spironolactone, eplerenone or finerenone, pregnancy or liver cirrhosis were also exclusion criteria. Detailed characteristics of the patients with PA and of the healthy subjects providing PBMCs are summarized in Table 1. Additional details of the purchased PBMCs of healthy volunteers are shown in Supplementary Figure 1.

Clinical work up of PA patients was done according to standard protocols, full blood was collected, PBMCs were isolated in house and analysis was performed prospectively.

All parts of the studies were approved by the ethics committee of the Canton of Berne, as required for the sample collection according to the Declaration of Helsinki. All patients and participants were only included in the study after signing informed consent.

The provider of the PBMCs from healthy volunteers does not state BMI and BP data.

Table 1.

A) Characteristics of patients with primary aldosteronism

B) Characteristics of healthy subjects providing PBMCs

Treatment of cells

Cells were cultured in their corresponding media with the lowest amount of FBS necessary to guarantee optimal surviving conditions.

Primary, not terminally differentiated cells (HUVEC, HUAEC, HAEC, HLEC, HRGEC, HRMC) were allowed to double maximum 10 times before experiments were performed.

Transfection of COS-7 cells with CYP11B2 plasmid

CYP11B2 (0.5ug/well) and an empty plasmid (0.5ug/well) were mixed with OptiMEM and Fugene. After 15min at RT, the mix was added to cells. Following a 32h incubation period the cells were washed and serum-free, DMEM was added with the steroid hormone substrates progesterone, DOC, corticosterone or 18-OH-corticosterone at a concentration of 10^{-6} M and with or without AngII (10^{-6} M). After

24h, the supernatant was collected for LC-MS analysis and total RNA extraction was performed using the Trizol method.

Real-time PCR

Cells were cultured for 24h in a steroid-free and phenol red-free medium alternative with or without Ang II (10^{-6} M). PBS was the solvent of Ang II and served as the baseline.

Extraction of total RNA was performed using the Trizol method. RNA was reverse transcribed by using oligo dT and random hexamer in the same reaction (PrimeScript RT reagent Kit from TaKaRa). All RT experiments in all cell lines were performed the same way. 50 ng of cDNA was used for Real-time PCR. Assay on demand primers were used for human CYP11B2 (Hs01597732_m1), SRD5A1 (Hs 00971645_g1), CYP21A2 (Hs 00416901_g1), AGTR1 (Hs00258938_m1), AGTR2 (Hs02621316_s1), Cyclophilin A (*PPIA*, 4326316E) and 18S (4310893E). Cyclophilin A and 18S served as endogenous controls. They all were from Applied Biosystems (ThermoFisherScientific, Reinach, Switzerland). GoTaq Probe qPCR Master Mix A6102 was from Promega AG, Dübendorf, Switzerland.

H295R and COS-7 cells transfected with CYP11B2 were used as positive controls. Results are displayed as ct values. Amplification cycle number was 50 and assays were performed in triplicate.

7500 Fast Real-time PCR and Quant Studio 1 machine were used both for all cell lines assessed. They were from Applied Biosystems (Thermo-Fisher-Scientific, Reinach, Switzerland).

Liquid chromatography–mass spectrometry (LC-MS)

Cells were cultured for 24h in a steroid-free and phenol red-free medium alternative with the steroid hormone substrates progesterone, DOC, corticosterone or 18-OH-corticosterone at a concentration of 10^{-6} M and with or without Ang II (10^{-6}). EtOH was the solvent of the substrates and served as the baseline. Reasons for phenol red-free medium were to exclude stimulatory conditions and interference of phenol red with the LC-MS equipment. After 24h cell supernatant was collected, centrifuged, aliquoted and stored at -20°C until LC-MS analysis.

For the LC-MS analysis, 500 μL cell aliquots were spiked with 38 μL internal standard mix and steroids subsequently extracted using solid-phase extraction on an OasisPrime HLB 96-well plate according to the protocol previously published (Andrieu et al., 2022). The LC-MS system consists of a Vanquish UHPLC (equipped with an ACQUITY UPLC HSS T3 Column, 100\AA , $1.8\ \mu\text{m}$, $1\ \text{mm} \times 100\ \text{mm}$; Waters, Switzerland) coupled to a Q Exactive Orbitrap Plus (both from Thermo-Fisher-Scientific, Reinach, Switzerland). Separation was achieved using gradient elution over 17 minutes using water and methanol (mobile phase B) both supplemented with 0.1 % formic acid (all Sigma-Aldrich, Buchs, Switzerland) as mobile phases. The separation of steroid metabolites was achieved through the following elution gradient (at a constant flow of $0.15\ \text{mL}/\text{min}$): 0–0.5 min 1% B, 0.5–1 min linear gradient to 1–46% B, 1–4 min 46%, 4–12 min linear gradient 46–73% B, 12–12.5-min linear gradient 73–99% B, 12.5–14.5 min 99% B, 14.5–15-min linear gradient to 1% B, and 15–17 min 1% B. All LC-MS grade solvents required for analysis were from BioSolve (Switzerland).

Data analysis was performed using TraceFinder 4.1 (Thermo-Fisher-Scientific, Reinach, Switzerland).

Steroid hormone concentrations are displayed in nmol/L . The lower limit of accurate quantification (LLOQ) was $0.085\ \text{nmol}/\text{L}$ for Aldo, $0.705\ \text{nmol}/\text{L}$ for corticosterone,

0.476 nmol/L for progesterone and 0.092 nmol/L for DOC. 18-OH-Corticosterone was detected in the mass channel of corticosterone (m/z 347.2217), its elution time confirmed from timepoint 0h cell aliquots and it was quantified relative to the calibration curve of corticosterone.

For each batch of LC-MS analysis the same positive control H295R cells + AngII was used as internal control. The steroid hormone concentrations after 24h were compared to the initial baseline steroid hormone concentrations at timepoint 0h. Assays were performed in triplicate, except for HAEC and HRMC cells. HAEC and HRMC assays were performed only once due to material limits.

Statistical methods

Three independent cell culture experiments were performed per cell line, except for HAEC and HRMC. Due to a delivery bottleneck, the experiments with HAEC and HRMC cells were performed only once. PBMC experiments were done 6x with healthy subjects and 5x with PA patients.

Data in tables and figures are presented as mean \pm SEM. An unpaired parametric T-test was used to compare two parameters with each other.

Significance was assigned at $p < 0.05$. *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$. $p > 0.05$ as ns = not significant. NA = not assessed. ND = not detected.

All statistical analyses were performed using GraphPad PRISM version 9 (PRISM, USA).

Results

mRNA expression of CYP11B2

JEG-3, HTR-8/SV neo, BeWo, HUVEC, HUAEC, HAEC, HLEC, HRGEC, HRMC, HEK293, PBMCs, H295R and COS-7/CYP11B2 cells were cultured as described above. RNA was isolated and real-time PCR was performed to detect mRNA levels of *CYP11B2*. No expression of *CYP11B2* could be detected in JEG-3, HTR-8/SV neo, BeWo, HUVEC, HUAEC, HAEC, HLEC, HRGEC, HRMC, HEK293 cells and in PBMCs of healthy subjects and PA patients (ct values > 35, 50 cycles). In the positive control H295R cells, the baseline *CYP11B2* expression levels were ~ ct 34, and dropped upon Ang II stimulation to ~ ct 26 as expected. COS-7 cells overexpressing *CYP11B2* showed *CYP11B2* ct values of ~ 15 independent of Ang II stimulation (Table 2).

Table 2 mRNA expression of CYP11B2

mRNA expression of AGTR1 and AGTR2

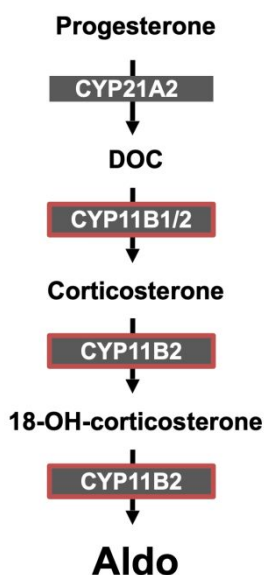
JEG-3, HTR-8/SV neo, BeWo, HUVEC, HUAEC, HAEC, HLEC, HRGEC, HRMC, HEK293, H295R and COS-7/CYP11B2 cells were cultured as described above. RNA was isolated and real-time PCR was performed to detect mRNA levels of *AGTR1* and *AGTR2*. Results are shown in Supplementary Table 5.

Production of *de-novo* steroid hormones from the substrates progesterone, DOC, corticosterone and 18-OH-corticosterone

Supernatant from cell experiments were collected and steroid hormone production assessed with a high-resolution LC-MS-based method. Most results are shown in absolute values, nmol/L (mean ± SEM), represented in tables. The concentration of each metabolite at timepoint 0h is compared to its concentration at time point 24h. p-

values are displayed directly next to the metabolites. NA = not assessed, ND = not detected.

Pathway of Aldo production



Graphical presentation of the absolute values of the steroid hormone metabolites in CYP11B2 transfected COS-7 cells supplemented with the steroid hormone substrates DOC or corticosterone

COS-7 cells overexpressing *CYP11B2* converted the substrate DOC to corticosterone, 18-OH-corticosterone and Aldo and these cells metabolized the substrate corticosterone to 18-OH-corticosterone and Aldo.

Figure 1

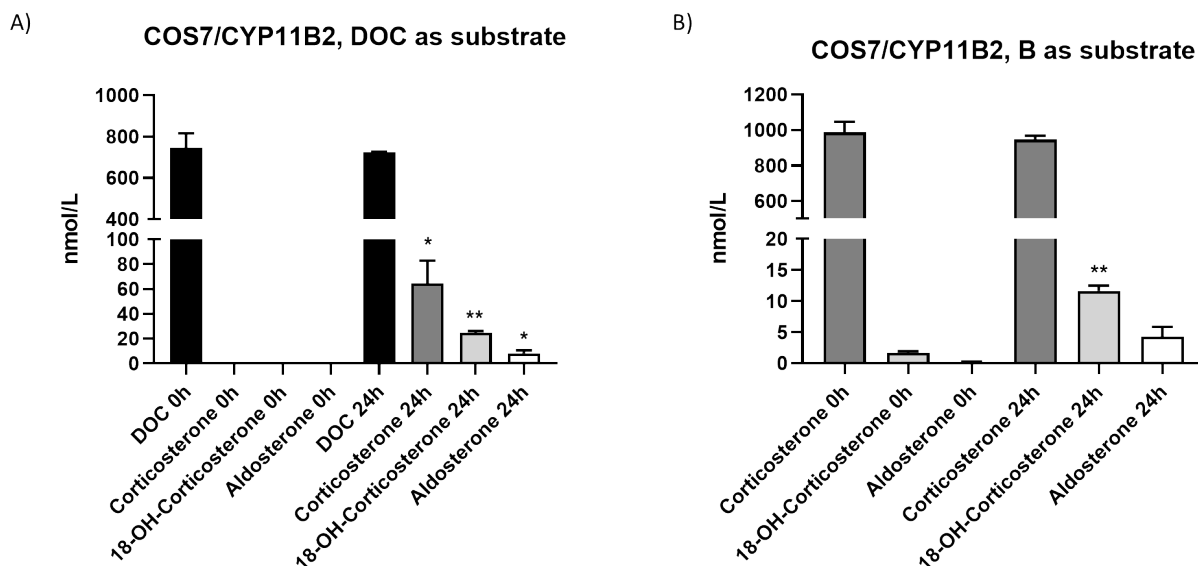


Figure 1 legend

A) Conversion of DOC to corticosterone, 18-OH-corticosterone and Aldo in COS-7 cells transfected with CYP11B2. T-test; DOC 0h vs. DOC 24h: ns, $p=0.713$; corticosterone 0h vs. corticosterone 24h: *, $p=0.038$; 18-OH-corticosterone 0h vs. 18-OH-corticosterone 24h: **, $p=0.002$; Aldo 0h vs. Aldo 24h: *, $p=0.034$.

B) Conversion of corticosterone to 18-OH-corticosterone and Aldo in COS-7 cells transfected with CYP11B2. T-test; corticosterone 0h vs. corticosterone 24h: ns, $p=0.471$; 18-OH-corticosterone 0h vs. 18-OH-corticosterone 24h: **, $p=0.004$; Aldo 0h vs. Aldo 24h: ns, $p=0.066$.

Time points 0h and 24h are shown. Steroid hormone data are displayed in nmol/L. $n=3$, unpaired parametric T-test.

Absolute values of the steroid hormone metabolites in COS-7 cells transfected with CYP11B2 and supplemented with the steroid hormone substrates progesterone, DOC, corticosterone and 18-OH-corticosterone without (A) and with (B) Ang II

Table 3

Absolute values of the steroid hormone metabolites in H295R cells supplemented with the steroid hormone substrates progesterone, DOC, corticosterone and 18-OH-corticosterone without (A) and with (B) Ang II

Table 4**Placental cell lines**

Absolute values of the steroid hormone metabolites in JEG-3, BeWo and HTR-8/SVneo cells supplemented with the steroid hormone substrates progesterone, DOC, corticosterone and 18-OH-corticosterone without (A) and with Ang II (B)

Table 5**Endothelial cell lines**

Absolute values of the steroid hormone metabolites in HUVEC, HUAEC, HAEC, HRGEC and HLEC cells supplemented with the

steroid hormone substrates progesterone, DOC, corticosterone and 18-OH-corticosterone without (A) and with (B) Ang II

Table 6

Renal cell lines

Absolute values of the steroid hormone metabolites in HRMC and HEK293 cells supplemented with the steroid hormone substrates progesterone, DOC, corticosterone and 18-OH-corticosterone without (A) and with (B) Ang II

Table 7

PBMCs

Absolute values of the steroid hormone metabolites in PBMCs of healthy subjects and of PA patients supplemented with the substrates progesterone, DOC, corticosterone, and 18-OH-corticosterone without (A) and with (B) Ang II

Table 8

Data of PBMCs are additionally shown as dot plots in Supplementary Figures 3-6.

Aldosterone production in all assessed cell lines and primary cells

Aldo production in COS-7 cells transfected with CYP11B2 and supplemented with the substrate progesterone or 18-OH-corticosterone was 0.0 ± 0.0 nmol/L after 24h no matter if stimulated with Ang II or not. With DOC as substrate, Aldo production was 8.4 ± 1.6 nmol/L (no AngII) and 7.6 ± 0.7 nmol/L (+ AngII) after 24h. With corticosterone as substrate, Aldo production was 4.3 ± 1.1 nmol/L (no AngII) and 4.0 ± 0.9 nmol/L (+ AngII) respectively.

In H295R cells, Aldo baseline levels significantly increased upon Ang II stimulation with all substrates used (24h values; substrate progesterone: Aldo 0.4 ± 0.2 nmol/L (no Ang II), 2.0 ± 0.3 nmol/L (+ AngII); substrate DOC: Aldo 0.6 ± 0.2 nmol/L (no Ang II), 2.4 ± 0.2 nmol/L (+ Ang II); substrate corticosterone: Aldo 0.6 ± 0.2 nmol/L (no Ang II), 2.1 ± 0.7 nmol/L (+ AngII); substrate 18-OH-corticosterone: Aldo 0.8 ± 0.2 nmol/L (no Ang II), 1.1 ± 0.0 nmol/L (+ AngII)).

In JEG-3, BeWo, HTR-8/SVneo, HUVEC, HUAEC, HAEC, HRGEC, HLEC, HRMC, HEK293 cells, and in PBMCs of healthy subjects and PA patients no significant Aldo production could be detected in all conditions, except for the substrate 18-OH-corticosterone. In 18-OH-corticosterone supplemented JEG-3 and in all the other cell models (BeWo, HTR-8/SVneo, HUVEC, HUAEC, HAEC, HRGEC, HLEC, HRMC, HEK293, PBMCs of healthy subjects and PA patients) where 18-OH-corticosterone was used as substrate, there were low detectable baseline Aldo levels at the 0h time points and after 24h. As 24h levels were not significantly higher compared to the baseline levels, we assume no *de-novo* production, but contribute these peak detections to contaminants in the 18-OH-corticosterone steroid standard stock.

Progesterone metabolism and SRD5A1 mRNA expression

Progesterone levels decreased during the 24h incubation period in JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells, and in PBMCs of healthy subjects and PA patients, however no relevant DOC, corticosterone, 18-OH-corticosterone, or Aldo levels could be detected. As progesterone metabolism was suspected to occur, 5 α -reductase (*SRD5A1*) expression as well as the prominent formation of the progesterone metabolites: 6 α / β -hydroxyprogesterone, 20 α -hydroxyprogesterone, 11 α -hydroxyprogesterone, 5 α / β -dihydroprogesterone, allopregnanolone/isopregnanolone and 6 α -hydroxypregnanolone could be confirmed in JEG-3, BeWo, HTR-8/SVneo, HRMC, and HEK293 cells, and in PBMCs of healthy subjects and PA patients by Real-time PCR and LC-MS analysis, respectively. Detailed results showing *SRD5A1* ct values and absolute values of progesterone metabolites in nmol/L are shown in supplementary data (**Supplementary Table 1 and Supplementary Tables 2-4**).

Supplementary Figure 2 shows an assumed progesterone metabolism pathway in JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells, and in PBMCs of healthy subjects and hyperaldosteronism patients.

mRNA expression of CYP21A2 in cells with active progesterone metabolism

CYP21A2 is the steroidogenic enzyme which converts progesterone to DOC in the adrenal glands. However, no DOC, and no metabolites down-stream of DOC (corticosterone, 18-OH-corticosterone and Aldo) were found in JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells, and in PBMCs of healthy subjects and PA patients after supplementation with progesterone. This results therefore questions the presence of CYP21A2 in these cell lines and the assessment of *CYP21A2* expression levels were additionally investigated. JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells

and the positive control H295R cells expressed significant levels of *CYP21A2*. No *CYP21A2* expression was however found in PBMCs of both cohorts and in HLEC cells. Cyclophilin A served as the endogenous control.

Supplementary Table 1

Discussion

Neither significant *CYP11B2* mRNA expression nor *de-novo* Aldo production from classical substrates was identified in various well-characterized, purchased, immortalized and primary human cell lines including mononuclear cells of healthy subjects and of patients suffering from PA with and without Ang II stimulation using a highly sensitive analytical method. The possibility that Aldo can be produced from mineralocorticoid intermediate steroid hormones downstream of progesterone was ruled out by supplying all assessed cells with DOC, corticosterone or 18-OH-corticosterone as steroid hormone substrates.

AngII, a known stimulator of the RAAS, was unable to boost *CYP11B2* expression and Aldo production in all the cells assessed. The positive controls (H295R cells and COS-7 cells overexpressing *CYP11B2*) expressed *CYP11B2* mRNA and produced Aldo. In H295R cells, Aldo production was stimulated up to 5-fold by AngII, as expected. In BeWo, JEG-3, HTR-8/SVneo, HEK293, and HRMC cells, PBMCs and PBMCs of PA patients, progesterone levels decreased over time, but no classic downstream mineralocorticoid metabolites such as DOC, corticosterone, 18-OH-corticosterone or Aldo were detected. Progesterone metabolism such as it exists in many cells and organs (Lobo, 1999), (Klossner et al., 2021) was suspected to occur and could be confirmed. Even though JEG-3, BeWo, HTR-8/SVneo, HRMC and HEK293 cells express *CYP21A2*, they favor the conversion of progesterone to the downstream progesterone metabolites rather to DOC. Biological effects of the

identified progesterone metabolites in off-target tissues are conceivable (Klossner et al., 2021).

In the positive controls, H295R cells and COS-7/CYP11B2, 18-OH-corticosterone seems to be a suboptimal substrate for the CYP11B2 whose conversion to Aldo was only marginal and not inducible by Ang II as compared to the substrates progesterone, DOC and corticosterone. The very low expression of Ang II receptors in COS-7 could additionally explain this minor response. Findings of Reddish and Guengerich (Reddish and Guengerich, 2019), that a higher enzyme concentration, more substrate and more time is needed for the reaction 18-OH-corticosterone-Aldo to occur, supports this assumption. The low Aldo levels found in several cell lines supplemented with high concentrations of 18-OH-corticosterone did not increase significantly with time or Ang II stimulation and therefore no Aldo was produced. A potential cross-talk between 18-OH-corticosterone and Aldo could be ruled out as for the MS analysis, as Aldo was detected in negative ion mode at m/z 359.1864 at 5.47 min and 18-OH-corticosterone in positive mode at m/z 363.2166 at 6.64 min.

In any case, if such small Aldo concentrations hypothetically would be active, they would compete against a 1000x higher systemic concentration of cortisol – a steroid hormone with access to the MR in 11 β -hydroxysteroid dehydrogenase 2-lacking off-target tissues (Ackermann et al., 2022).

Findings in line with ours:

In line with our results, no *CYP11B2* mRNA expression and Aldo biosynthesis was detected by the group of Gomez-Sanchez in three different human vascular endothelial cell lines, not even after stimulation with Ang II (Ahmad et al., 2004).

Findings not in line with ours:

Many research groups detected and published extra-adrenal *CYP11B2* expression and Aldo production in whole kidney tissue and/or renal cells (Wu et al., 1999), (Xue and

Siragy, 2005). (Nishikawa et al., 2005); in vessels and/or endothelial cells (Takeda et al., 1994), (Takeda et al., 1995b) (Takeda et al., 1997), (Takeda et al., 1996), (Hatakeyama et al., 1994), (Rudolph et al., 2000), (Maron et al., 2012), (Maron et al., 2014).

We assume, that the Aldo concentration found in several tissues comes from the adrenal glands and is not locally produced in these tissues or is erroneously detected. As Aldo sequestration was found in the brain (Gomez-Sanchez et al., 2010), it needs to be addressed, if adrenal Aldo can be stored, accumulated and released in off-target tissues.

The strength of this study is the analysis of different steroid hormone metabolites with high resolution LC-MS. Utilizing H295R cells as a control cell line endogenously expressing functional CYP11B2 and the COS-7 cells transfected with the CYP11B2 plasmid supports our methodology. For our PCR analysis, amplification cycle number was 50, which is higher as in most studies performed and permits the detection of very low *CYP11B2* mRNA expression levels. Possible steroidogenic acute regulatory protein independent mechanisms were ruled out by adding steroid hormone substrates such as progesterone, DOC, corticosterone and 18-OH-corticosterone.

Limitations of our study:

This study investigated ex vivo Aldo production in primary or immortalized, purchased human cell lines and self-isolated PBMCs of PA patients in 2D-culture conditions and can therefore not be extrapolated 1:1 to in vivo conditions and tissues in which de-novo Aldo production has been reported. As we did not analyze Aldo production in cells of all tissues mentioned to de-novo synthesize Aldo in literature, we might have missed analyzing extra-adrenal tissues producing Aldo. But as Gomez-Sanchez et al. have correctly explained, there cannot be significant extra-adrenal Aldo production as in adrenalectomized animals no significant Aldo production was

detectable, the amount of Aldo produced outside the adrenals is minimal and not clinically relevant (Ahmad et al., 2004). As some of the purchased primary cells and cell lines are from one single individual, gender, age-related, or intra-individual variability in these cells is possible. Other, not yet characterized CYP11B2 substrates, cofactors or stimulators are conceivable. Most of the assessed cell lines only marginally express AGTR1 and/or AGTR2 and therefore would not be expected to significantly respond to Ang II. *In vivo* studies investigating organ specific *de-novo* Aldo production are complex and complicated by the systemic distribution of Aldo. Our protocol with incubation times of 24h does not cover rapid mRNA changes or steroid hormone conversions. But if rapid mRNA changes were missed, steroid hormones would not be missed as they are stable for a long time once released.

To summarize, no significant *CYP11B2* mRNA expression and no Aldo production could be detected in human vascular endothelial cell lines (HUVEC, HUAEC, HAEC, HRGEC), lymphatic endothelial cells (HLEC), in trophoblasts (BeWo, JEG-3, HTR-8/SV neo), in kidney cells (HEK293, HRMC) and in human peripheral blood mononuclear cells (PBMC) of healthy subjects and PA patients. If there is Aldo production in these cells, it is below detection limits of the LC-MS method and presumably not of clinical relevance.

We conclude that high circulating Aldo levels observed in PA patients are not due to Aldo production in PBMCs, nor due to autocrine/paracrine Aldo production in Aldo off-target tissues.

Declaration of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure Captions

For all figures: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, $p > 0.05$ as ns = not significant

Supplementary data

In a separate file

Table 1 A)

Patient ID	1	2	3	4	5
Age years	68	63	51	65	62
Gender	M	M	F	M	M
BP mmHg	150/80	165/100	141/89	145/82	169/105
BMI kg/m ²	30.2	31.6	21.5	27	25
Crea Umol/L	55	104	64	80	93
eGFR ml/min/1.73m ²	102	100	96	89	76
Renin ng/L	<1.2	<1.2	<1.2	<1.2	<1.2
ARR pmol/ng	205	133	1000	250	967
Aldo pmol/L	354	236	1240	299	1160

BP: blood pressure, BMI: body mass index, Crea: creatinine, eGFR: estimated glomerular filtration rate, ARR: aldosterone to renin ratio, Aldo: aldosterone.

Table 1 B)

Healthy subjects ID	1	2	3	4	5	6
Age years	47	21	43	23	21	44
Gender	M	M	F	M	F	M

Table 2

mRNA expression of CYP11B2 shown as ct values

	ct CYP11B2 no Ang II	ct CYP11B2 + Ang II
JEG-3	>35	>35
HTR-8/SV neo	>35	>35
BeWo	>35	>35
HUVEC	>35	>35
HUAEC	>35	>35
HAEC	>35	>35
HLEC	>35	>35
HRGEC	>35	>35
HRMC	>35	>35
HEK293	>35	>35
PBMCs healthy subjects	>35	>35
PBMCs PA patients	>35	>35
H295R	34	26
COS-7 + CYP11B2 plasmid	15	15
COS-7 + empty plasmid	>35	>35

**Absolute values of the steroid hormone metabolites
of the CYP11B2 transfected COS-7 cells**

A)

Progesterone as substrate		nmol/L		
	time	mean ± SEM	p-value	
Progesterone	0h	754.2 ± 135.1	0.313	ns
DOC	0h	0.0 ± 0.0	0.269	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns
Progesterone	24h	567.4 ± 35.0		
DOC	24h	3.2 ± 2.1		
Corticosterone	24h	0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0		

DOC as substrate

DOC as substrate		nmol/L		
	time	mean ± SEM	p-value	
DOC	0h	745.8 ± 49.8	0.713	ns
Corticosterone	0h	0.0 ± 0.0	0.038	*
18-OH-corticosterone	0h	0.0 ± 0.0	0.002	**
Aldosterone	0h	0.0 ± 0.0	0.034	*
DOC	24h	724.7 ± 1.4		
Corticosterone	24h	64.6 ± 13.0		
18-OH-corticosterone	24h	24.8 ± 1.1		
Aldosterone	24h	8.4 ± 1.6		

corticosterone as substrate

corticosterone as substrate		nmol/L		
	time	mean ± SEM	p-value	
Corticosterone	0h	986.2 ± 42.8	0.471	ns
18-OH-corticosterone	0h	1.7 ± 0.2	0.004	**
Aldosterone	0h	0.1 ± 0.1	0.066	ns
Corticosterone	24h	946.4 ± 14.4		
18-OH-corticosterone	24h	11.6 ± 0.6		
Aldosterone	24h	4.3 ± 1.1		

18-OH-corticosterone as substrate

18-OH-corticosterone as substrate		nmol/L		
	time	mean ± SEM	p-value	
18-OH-corticosterone	0h	1037.0 ± 179.5	0.651	ns
Aldosterone	0h	0.5 ± 0.5	0.423	ns
18-OH-corticosterone	24h	868.2 ± 265.5		
Aldosterone	24h	0.0 ± 0.0		

B)

Progesterone + Ang II as substrate		nmol/L		
	time	mean ± SEM	p-value	
Progesterone	0h	642.3 ± 130.4	0.9759	ns
DOC	0h	2.1 ± 2.1	0.7213	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns
Progesterone	24h	637.8 ± 12.4		
DOC	24h	3.9 ± 3.9		
Corticosterone	24h	0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0		

DOC + Ang. II as substrate

DOC + Ang. II as substrate		nmol/L		
	time	mean ± SEM	p-value	
DOC	0h	739.5 ± 90.3	0.635	ns
Corticosterone	0h	0.0 ± 0.0	0.009	**
18-OH-corticosterone	0h	0.0 ± 0.0	0.004	**
Aldosterone	0h	0.0 ± 0.0	0.008	**
DOC	24h	675.0 ± 73.4		
Corticosterone	24h	60.6 ± 5.8		
18-OH-corticosterone	24h	22.2 ± 1.4		
Aldosterone	24h	7.6 ± 0.7		

corticosterone + Ang II as substrate

corticosterone + Ang II as substrate		nmol/L		
	time	mean ± SEM	p-value	
Corticosterone	0h	962.8 ± 60.0	0.443	ns
18-OH-corticosterone	0h	1.8 ± 0.0	0.995	ns
Aldosterone	0h	0.0 ± 0.0	0.999	ns
Corticosterone	24h	903.4 ± 23.4		
18-OH-corticosterone	24h	14.8 ± 4.7		
Aldosterone	24h	4.0 ± 0.9		

18-OH-corticosterone + Ang II as substrate

18-OH-corticosterone + Ang II as substrate		nmol/L		
	time	mean ± SEM	p-value	
18-OH-corticosterone	0h	1068.0 ± 113.7	0.989	ns
Aldosterone	0h	0.3 ± 0.3	>0.999	ns
18-OH-corticosterone	24h	1001.0 ± 371.5		
Aldosterone	24h	0.0 ± 0.0		

**Absolute values of the steroid hormone metabolites
of the adrenal cells H295R**

A)

Progesterone as substrate		nmol/L		
	time	mean ± SEM	p-value	
Progesterone	0h	952.9 ± 23.5	< 0.0001	***
DOC	0h	0 ± 0	0.032	*
Corticosterone	0h	0 ± 0	0.012	*
18-OH-corticosterone	0h	NA		
Aldosterone	0h	0 ± 0	0.062	ns
Progesterone	24h	0.4 ± 0.2		
DOC	24h	56.3 ± 17.4		
Corticosterone	24h	4.5 ± 1.0		
18-OH-corticosterone	24h	NA		
Aldosterone	24h	0.4 ± 0.2		

DOC as substrate

DOC as substrate		nmol/L		
	time	mean ± SEM	p-value	
DOC	0h	1133.0 ± 177.7	0.004	**
Corticosterone	0h	0.3 ± 0.2	0.006	**
18-OH-corticosterone	0h	NA		
Aldosterone	0h	0 ± 0	0.08	ns
Professor AJL Clark	#####	91561720		
Corticosterone	24h	6.6 ± 1.2		
18-OH-corticosterone	24h	NA		
Aldosterone	24h	0.6 ± 0.2		

corticosterone as substrate

corticosterone as substrate		nmol/L		
	time	mean ± SEM	p-value	
Corticosterone	0h	1594.0 ± 186.2	0.405	ns
18-OH-corticosterone	0h	NA		
Aldosterone	0h	0.0 ± 0.0	0.083	ns
Corticosterone	24h	1377.0 ± 141.8		
18-OH-corticosterone	24h	NA		
Aldosterone	24h	0.6 ± 0.2		

18-OH-corticosterone as substrate

18-OH-corticosterone as substrate		nmol/L		
	time	mean ± SEM	p-value	
18-OH-corticosterone	0h	911.7 ± 69.9	0.342	ns
Aldosterone	0h	0.5 ± 0.1	0.166	ns
18-OH-corticosterone	24h	820.9 ± 47.0		
Aldosterone	24h	0.8 ± 0.2		

B)

Progesterone + Ang II as substrate		nmol/L		
	time	mean ± SEM	p-value	
Progesterone	0h	910.6 ± 93.2	0.001	**
DOC	0h	0 ± 0	0.022	*
Corticosterone	0h	0 ± 0	0.009	**
18-OH-corticosterone	0h	NA		
Aldosterone	0h	0 ± 0	0.003	**
Progesterone	24h	0.5 ± 0.1		
DOC	24h	119.9 ± 32.9		
Corticosterone	24h	43.0 ± 8.9		
18-OH-corticosterone	24h	NA		
Aldosterone	24h	2.0 ± 0.3		

DOC + Ang. II as substrate

DOC + Ang. II as substrate		nmol/L		
	time	mean ± SEM	p-value	
DOC	0h	1031.0 ± 114.2	0.002	**
Corticosterone	0h	0.4 ± 0.3	0.003	**
18-OH-corticosterone	0h	NA		
Aldosterone	0h	0 ± 0	< 0.0001	***
DOC	24h	139.3 ± 28.4		
Corticosterone	24h	51.3 ± 8.1		
18-OH-corticosterone	24h	NA		
Aldosterone	24h	2.4 ± 0.2		

corticosterone + Ang II as substrate

corticosterone + Ang II as substrate		nmol/L		
	time	mean ± SEM	p-value	
Corticosterone	0h	1746.0 ± 295.2	0.263	ns
18-OH-corticosterone	0h	NA		
Aldosterone	0h	0.0 ± 0.0	0.0001	**
Corticosterone	24h	1324.0 ± 136.2		
18-OH-corticosterone	24h	NA		
Aldosterone	24h	2.1 ± 0.7		

18-OH-corticosterone + Ang II as substrate

18-OH-corticosterone + Ang II as substrate		nmol/L		
	time	mean ± SEM	p-value	
18-OH-corticosterone	0h	888.8 ± 24.3	0.333	ns
Aldosterone	0h	0.3 ± 0.2	0.008	**
18-OH-corticosterone	24h	792.2 ± 84.4		
Aldosterone	24h	1.1 ± 0.0		

NA: not assessed

**Absolute values of the steroid hormone metabolites
of the placental cells JEG-3, BeWo, HTR-8/Svneo**

A)

Progesterone as substrate	time	JEG-3			BeWo			HTR-8/SVneo		
		nmol/L			nmol/L			nmol/L		
		mean ± SEM	p-value		mean ± SEM	p-value		mean ± SEM	p-value	
Progesterone	0h	1339.0 ± 132.6	0.189	ns	803.4 ± 57.1	0.236	ns	1123.0 ± 144.9	0.061	ns
DOC	0h	0.0 ± 0.0	0.177	ns	0.0 ± 0.0	0.012	*	0.0 ± 0.0	>0.999	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Progesterone	24h	1090.0 ± 85.70			540.9 ± 179.7			724.2 ± 51.5		
DOC	24h	0.2 ± 0.1			0.1 ± 0.0			0.0 ± 0.0		
Corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0		

DOC as substrate	time	JEG-3			BeWo			HTR-8/SVneo		
		nmol/L			nmol/L			nmol/L		
		mean ± SEM	p-value		mean ± SEM	p-value		mean ± SEM	p-value	
DOC	0h	1460.0 ± 163.0	0.029	*	792.2 ± 75.0	0.228	ns	1304.0 ± 245.5	0.131	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.2 ± 0.4	0.937	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
DOC	24h	1141.0 ± 24.5			639.0 ± 77.3			1001.0 ± 129.9		
Corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.2 ± 0.3		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0		

corticosterone as substrate	time	JEG-3			BeWo			HTR-8/SVneo		
		nmol/L			nmol/L			nmol/L		
		mean ± SEM	p-value		mean ± SEM	p-value		mean ± SEM	p-value	
Corticosterone	0h	1532.0 ± 443.0	0.874	ns	1076.0 ± 90.4	0.959	ns	1727.0 ± 204.2	0.18	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	2.1 ± 0.3	0.664	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.1 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Corticosterone	24h	1483.0 ± 246.3			1065.0 ± 162.4			1536.0 ± 34.7		
18-OH-corticosterone	24h	0.0 ± 0.0			1.9 ± 0.1			0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0		

18-OH-corticosterone as substrate	time	JEG-3			BeWo			HTR-8/SVneo		
		nmol/L			nmol/L			nmol/L		
		mean ± SEM	p-value		mean ± SEM	p-value		mean ± SEM	p-value	
18-OH-corticosterone	0h	1285.0 ± 253.3	0.241	ns	827.9 ± 162.5	0.613	ns	966.2 ± 207.9	0.540	ns
Aldosterone	0h	0.5 ± 0.1	0.107	ns	0.2 ± 0.2	0.901	ns	0.5 ± 0.2	0.600	ns
18-OH-corticosterone	24h	1047.0 ± 160.3			707.7 ± 147.6			851.8 ± 211.6		
Aldosterone	24h	0.4 ± 0.1			0.2 ± 0.1			0.4 ± 0.2		

B)

Progesterone + Ang II as substrate		JEG-3				BeWo				HTR-8/SVneo	
	time	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	
		mean ± SEM			mean ± SEM			mean ± SEM			
Progesterone	0h	1252.0 ± 120.7	0.111	ns	709.7 ± 105.8	0.512	ns	1227.0 ± 122.2	0.0373	*	
DOC	0h	0.0 ± 0.0	0.374	ns	0.0 ± 0.0	0.016	*	0.0 ± 0.0	>0.999	ns	
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	
Progesterone	24h	943.3 ± 91.7			563.8 ± 172.9			762.1 ± 89.5			
DOC	24h	0.1 ± 0.1			0.1 ± 0.0			0.0 ± 0.0			
Corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0			
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0			
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0			

DOC + Ang. II as substrate		JEG-3				BeWo				HTR-8/SVneo	
	time	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	
		mean ± SEM			mean ± SEM			mean ± SEM			
DOC	0h	1375.0 ± 249.1	0.258	ns	923.1 ± 49.6	0.157	ns	1364.0 ± 176.8	0.116	ns	
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.3 ± 0.4	0.447	ns	
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	
DOC	24h	1144.0 ± 173.1			598.3 ± 180.3			1050.0 ± 207.4			
Corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.1 ± 0.1			
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0			
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0			

corticosterone + Ang II as substrate		JEG-3				BeWo				HTR-8/SVneo	
	time	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	
		mean ± SEM			mean ± SEM			mean ± SEM			
Corticosterone	0h	1778.0 ± 244.3	0.095	ns	955.2 ± 97.2	0.211	ns	1595.0 ± 270.8	0.870	ns	
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	1.7 ± 0.1	0.413	ns	0.0 ± 0.0	>0.9999	ns	
Aldosterone	0h	0.1 ± 0.1	0.618	ns	0.0 ± 0.0	>0.999	ns	0.1 ± 0.1	>0.9999	ns	
Corticosterone	24h	1384.0 ± 195.9			777.3 ± 69.6			1656.0 ± 545.1			
18-OH-corticosterone	24h	0.0 ± 0.0			1.4 ± 0.3			0.0 ± 0.0			
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0			

18-OH-corticosterone + Ang II as substrate		JEG-3				BeWo				HTR-8/SVneo	
	time	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	
		mean ± SEM			mean ± SEM			mean ± SEM			
18-OH-corticosterone	0h	1197.0 ± 132.0	0.043	*	712.1 ± 118.9	0.338	ns	927.7 ± 274.0	0.394	ns	
Aldosterone	0h	0.5 ± 0.0	0.104	ns	0.2 ± 0.2	0.694	ns	0.5 ± 0.1	0.917	ns	
18-OH-corticosterone	24h	944.1 ± 70.5			571.3 ± 51.4			756.3 ± 146.7			
Aldosterone	24h	0.4 ± 0.1			0.3 ± 0.2			0.5 ± 0.2			

**Absolute values of the steroid hormone metabolites
of the endothelial cells HUVEC, HUAEC, HAEC, HRGEC and HLEC**

A)

Progesterone as substrate	time	HUVEC			HUAEC			HAEC			HRGEC			HLEC		
		nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value
		mean ± SEM			mean ± SEM			mean ± SEM			mean ± SEM			mean ± SEM		
Progesterone	0h	680.4 ± 54.7	0.656	ns	671.9 ± 42.6	0.843	ns	1100			864.0 ± 109.9	0.133	ns	850.1 ± 53.1	0.163	ns
DOC	0h	0.0 ± 0.0	>0.999	ns	0.6 ± 0.5	0.481	ns	0.0			2.6 ± 2.6	0.916	ns	4.1 ± 4.1	0.981	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	<0.0001	***	0.0			0.0 ± 0.0	0.116	ns	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Progesterone	24h	644.6 ± 50.5			695.2 ± 101.6			910.8			646.7 ± 35.3			1137.0 ± 159.5		
DOC	24h	0.0 ± 0.0			0.2 ± 0.1			0.1			2.2 ± 2.2			4.3 ± 4.2		
Corticosterone	24h	0.0 ± 0.0			4.8 ± 0.2			6.2			2.4 ± 1.2			0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			0.0 ± 0.0		

DOC as substrate	time	HUVEC			HUAEC			HAEC			HRGEC			HLEC		
		nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value
		mean ± SEM			mean ± SEM			mean ± SEM			mean ± SEM			mean ± SEM		
DOC	0h	1028.0 ± 75.3	0.627	ns	824.0 ± 60.3	0.803	ns	1108			737.2 ± 118.0	0.347	ns	992.5 ± 247.1	0.780	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	0.169	ns	2.4			0.0 ± 0.0	<0.0001	***	0.1 ± 0.1	0.374	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	0.120	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.1 ± 0.1	0.374	ns	0.0			0.1 ± 0.1	>0.999	ns	0.0 ± 0.0	>0.999	ns
DOC	24h	905.8 ± 219.0			889.8 ± 238.8			992.3			561.0 ± 115.8			1079.0 ± 149.8		
Corticosterone	24h	0.0 ± 0.0			11.9 ± 7.1			5.6			3.5 ± 0.0			0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			1.2 ± 0.6		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			0.0 ± 0.0		

corticosterone as substrate	time	HUVEC			HUAEC			HAEC			HRGEC			HLEC		
		nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value
		mean ± SEM			mean ± SEM			mean ± SEM			mean ± SEM			mean ± SEM		
Corticosterone	0h	903.6 ± 49.6	0.331	ns	760.5 ± 103.4	0.324	ns	1197.6			1171.0 ± 137.2	0.850	ns	1405.0 ± 496.9	0.786	ns
18-OH-corticosterone	0h	2.0 ± 0.2	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	2.5 ± 1.3	0.108	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Corticosterone	24h	996.4 ± 67.9			936.8 ± 118.1			1220.6			1129.0 ± 153.7			1586.0 ± 371.3		
18-OH-corticosterone	24h	1.8 ± 0.1			0.0 ± 0.0			0.0			0.0 ± 0.0			5.6 ± 0.8		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			0.0 ± 0.0		

18-OH-corticosterone as substrate	time	HUVEC			HUAEC			HAEC			HRGEC			HLEC		
		nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value	nmol/L		p-value
		mean ± SEM			mean ± SEM			mean ± SEM			mean ± SEM			mean ± SEM		
18-OH-corticosterone	0h	663.9 ± 128.1	0.719	ns	1005.0 ± 102.7	0.238	ns	968.6			1152.0 ± 58.3	0.101	ns	1214.0 ± 423.1	0.780	ns
Aldosterone	0h	0.3 ± 0.1	0.284	ns	0.4 ± 0.1	0.733	ns	0.6			0.3 ± 0.1	0.797	ns	0.6 ± 0.2	0.666	ns
18-OH-corticosterone	24h	566.3 ± 90.5			1313.0 ± 197.0			1063.5			1287.0 ± 24.4			1026.0 ± 465.1		
Aldosterone	24h	0.4 ± 0.1			0.3 ± 0.0			0.9			0.3 ± 0.0			0.7 ± 0.2		

B)

Progesterone + Ang II as substrate		HUVEC			HUAEC			HAEC			HRGEC			HLEC		
time	nmol/L	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value
		Progesterone	0h		856.3 ± 91.2	0.135		ns	613.2 ± 47.9		0.907	ns		902.7		
DOC	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	0.049	*	0.0			0.0 ± 0.0	0.43	ns	0.0 ± 0.0	>0.999	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	0.042	*	0.0			0.0 ± 0.0	<0.0001	***	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.1 ± 0.1	0.374	ns	0.0			0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Progesterone	24h	673.8 ± 34.9			604.4 ± 51.9			1036.4			856.9 ± 148.2			894.0 ± 70.1		
DOC	24h	0.0 ± 0.0			0.1 ± 0.0			0.2			0.1 ± 0.0			0.0 ± 0.0		
Corticosterone	24h	0.0 ± 0.0			7.7 ± 2.6			3.4			3.3 ± 0.1			0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			0.0 ± 0.0		

DOC + Ang. II as substrate		HUVEC			HUAEC			HAEC			HRGEC			HLEC		
time	nmol/L	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value
		DOC	0h		1098.0 ± 169.5	0.132		ns	872.4 ± 179.8		0.779	ns		940.5		
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	0.0003	**	2.5			0.0 ± 0.0	0.0003	**	0.0 ± 0.0	0.374	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	1.5 ± 1.5	0.529	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
DOC	24h	770.7 ± 37.5			811.4 ± 94.3			1026.9			874.0 ± 186.5			1461.0 ± 520.0		
Corticosterone	24h	0.0 ± 0.0			5.1 ± 0.4			3.6			3.4 ± 0.3			0.3 ± 0.3		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			2.8 ± 1.3		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			0.0 ± 0.0		

corticosterone + Ang II as substrate		HUVEC			HUAEC			HAEC			HRGEC			HLEC		
time	nmol/L	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value
		Corticosterone	0h		1020.0 ± 155.7	0.727		ns	1009.0 ± 121.1		0.765	ns		1179.4		
18-OH-corticosterone	0h	2.1 ± 0.3	0.383	ns	0.0 ± 0.0	>0.999	ns	0.0			0.0 ± 0.0	>0.999	ns	1.7 ± 0.7	0.139	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.1 ± 0.1	0.374	ns	0.0			0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Corticosterone	24h	961.6 ± 15.5			948.8 ± 146.2			1126.9			937.0 ± 35.3			1718.0 ± 251.8		
18-OH-corticosterone	24h	1.7 ± 0.2			0.0 ± 0.0			0.0			0.0 ± 0.0			8.4 ± 3.5		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0			0.0			0.0 ± 0.0			0.0 ± 0.0		

18-OH-corticosterone + Ang II as substrate		HUVEC			HUAEC			HAEC			HRGEC			HLEC		
time	nmol/L	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value	mean ± SEM		p-value
		18-OH-corticosterone	0h		881.3 ± 44.8	0.135		ns	1069.0 ± 132.4		0.177	ns		982.1		
Aldosterone	0h	0.4 ± 0.1	0.662	ns	0.3 ± 0.1	0.942	ns	0.7			0.3 ± 0.1	0.941	ns	0.3 ± 0.0	0.007	**
18-OH-corticosterone	24h	671.0 ± 103.3			1377.0 ± 52.4			884.5			1241.0 ± 70.7			803.6 ± 48.2		
Aldosterone	24h	0.3 ± 0.1			0.3 ± 0.1			0.9			0.3 ± 0.1			0.6 ± 0.0		

**Absolute values of the steroid hormone metabolites
of the renal cells HRMC and HEK293**

A)

Progesterone as substrate		HRMC		HEK293	
	time	nmol/L		nmol/L	
		mean ± SEM	p-value	mean ± SEM	p-value
Progesterone	0h	1076.0		1101.0 ± 241.7	0.421 ns
DOC	0h	0.0		0.0 ± 0.0	0.213 ns
Corticosterone	0h	0.0		0.0 ± 0.0	>0.999 ns
18-OH-corticosterone	0h	0.0		0.0 ± 0.0	>0.999 ns
Aldosterone	0h	0.0		0.0 ± 0.0	>0.999 ns
Progesterone	24h	1008.0		799.8 ± 232.7	
DOC	24h	0.2		0.1 ± 0.0	
Corticosterone	24h	5.2		0.0 ± 0.0	
18-OH-corticosterone	24h	0.00		0.0 ± 0.0	
Aldosterone	24h	0.00		0.0 ± 0.0	

DOC as substrate		HRMC		HEK293	
	time	nmol/L		nmol/L	
		mean ± SEM	p-value	mean ± SEM	p-value
DOC	0h	1188.0		1237.0 ± 255.4	0.132 ns
Corticosterone	0h	0.0		0.0 ± 0.0	0.163 ns
18-OH-corticosterone	0h	0.0		0.2 ± 0.2	0.400 ns
Aldosterone	0h	0.0		0.0 ± 0.0	>0.999 ns
DOC	24h	860.0		687.8 ± 137.5	
Corticosterone	24h	4.8		0.5 ± 0.3	
18-OH-corticosterone	24h	0.0		0.0 ± 0.0	
Aldosterone	24h	0.0		0.0 ± 0.0	

corticosterone as substrate		HRMC		HEK293	
	time	nmol/L		nmol/L	
		mean ± SEM	p-value	mean ± SEM	p-value
Corticosterone	0h	1267.7		2093.0 ± 343.1	0.469 ns
18-OH-corticosterone	0h	0.0		2.8 ± 0.1	0.236 ns
Aldosterone	0h	0.0		0.0 ± 0.0	>0.999 ns
Corticosterone	24h	1411.3		1738.0 ± 282.8	
18-OH-corticosterone	24h	0.0		2.6 ± 0.1	
Aldosterone	24h	0.0		0.0 ± 0.0	

18-OH-corticosterone as substrate		HRMC		HEK293	
	time	nmol/L		nmol/L	
		mean ± SEM	p-value	mean ± SEM	p-value
18-OH-corticosterone	0h	796.4		901.9 ± 59.9	0.025 *
Aldosterone	0h	0.2		0.4 ± 0.1	0.903 ns
18-OH-corticosterone	24h	839.5		606.9 ± 58.9	
Aldosterone	24h	0.4		0.4 ± 0.2	

B)

Progesterone + Ang II as substrate		HRMC			HEK293		
	time	nmol/L		nmol/L			
		mean ± SEM	p-value	mean ± SEM	p-value		
Progesterone	0h	968.0		1367.0 ± 220.8	0.309	ns	
DOC	0h	0.0		0.0 ± 0.0	0.258	ns	
Corticosterone	0h	0.0		0.0 ± 0.0	>0.999	ns	
18-OH-corticosterone	0h	0.0		0.0 ± 0.0	>0.999	ns	
Aldosterone	0h	0.0		0.0 ± 0.0	>0.999	ns	
Progesterone	24h	676.0		1011.0 ± 210.9			
DOC	24h	0.2		0.1 ± 0.1			
Corticosterone	24h	5.1		0.0 ± 0.0			
18-OH-corticosterone	24h	0.0		0.0 ± 0.0			
Aldosterone	24h	0.0		0.0 ± 0.0			

DOC + Ang. II as substrate		HRMC			HEK293		
	time	nmol/L		nmol/L			
		mean ± SEM	p-value	mean ± SEM	p-value		
DOC	0h	1192.0		1193.0 ± 229.2	0.400	ns	
Corticosterone	0h	0.0		0.2 ± 0.2	0.801	ns	
18-OH-corticosterone	0h	0.0		2.4 ± 2.4	0.376	ns	
Aldosterone	0h	0.0		0.0 ± 0.0	>0.999	ns	
DOC	24h	852.0		945.6 ± 129.0			
Corticosterone	24h	4.6		0.1 ± 0.1			
18-OH-corticosterone	24h	0.0		0.0 ± 0.0			
Aldosterone	24h	0.0		0.0 ± 0.0			

corticosterone + Ang II as substrate		HRMC			HEK293		
	time	nmol/L		nmol/L			
		mean ± SEM	p-value	mean ± SEM	p-value		
Corticosterone	0h	1430.8		1535.0 ± 448.9	0.861	ns	
18-OH-corticosterone	0h	0.0		2.2 ± 0.3	0.854	ns	
Aldosterone	0h	0.0		0.0 ± 0.0	>0.999	ns	
Corticosterone	24h	1170.3		1688.0 ± 687.9			
18-OH-corticosterone	24h	0.0		2.2 ± 0.4			
Aldosterone	24h	0.0		0.0 ± 0.0			

18-OH-corticosterone + Ang II as substrate		HRMC			HEK293		
	time	nmol/L		nmol/L			
		mean ± SEM	p-value	mean ± SEM	p-value		
18-OH-corticosterone	0h	808.7		853.1 ± 81.3	0.188	ns	
Aldosterone	0h	0.4		0.4 ± 0.3	0.368	ns	
18-OH-corticosterone	24h	651.9		699.6 ± 52.4			
Aldosterone	24h	0.2		0.1 ± 0.1			

0

**Absolute values of the steroid hormone metabolites
of the PBMCs from healthy subjects and PA patients**

A)

Progesterone as substrate	time	PBMC no PA			PBMC with PA		
		nmol/L			nmol/L		
		mean ± SEM	p-value		mean ± SEM	p-value	
Progesterone	0h	924.7 ± 58.2	0.044	*	1072.0 ± 98.5	0.533	ns
DOC	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	0.676	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Progesterone	24h	721.1 ± 66.7			995.0 ± 65.0		
DOC	24h	0.0 ± 0.0			0.1 ± 0.1		
Corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0		

DOC as substrate	time	PBMC no PA			PBMC with PA		
		nmol/L			nmol/L		
		mean ± SEM	p-value		mean ± SEM	p-value	
DOC	0h	748.3 ± 166.7	0.744	ns	1010.0 ± 61.6	0.414	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.4 ± 0.4	0.911	ns	0.0 ± 0.0	>0.999	ns
DOC	24h	715.2 ± 174.8			932.3 ± 66.2		
Corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0		
Aldosterone	24h	0.4 ± 0.4			0.0 ± 0.0		

corticosterone as substrate	time	PBMC no PA			PBMC with PA		
		nmol/L			nmol/L		
		mean ± SEM	p-value		mean ± SEM	p-value	
Corticosterone	0h	813.5 ± 86.4	0.629	ns	1077.0 ± 52.5	0.732	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	1.8 ± 0.5	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Corticosterone	24h	859.2 ± 207.5			1105.0 ± 60.9		
18-OH-corticosterone	24h	0.0 ± 0.0			1.9 ± 0.5		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0		

18-OH-corticosterone as substrate	time	PBMC no PA			PBMC with PA		
		nmol/L			nmol/L		
		mean ± SEM	p-value		mean ± SEM	p-value	
18-OH-corticosterone	0h	798.0 ± 63.2	0.075	ns	864.8 ± 60.5	0.573	ns
Aldosterone	0h	0.4 ± 0.0	0.266	ns	0.1 ± 0.1	0.932	ns
18-OH-corticosterone	24h	623.2 ± 61.2			810.6 ± 69.6		
Aldosterone	24h	0.4 ± 0.0			0.1 ± 0.1		

B)

Progesterone + Ang II as substrate		PBMC no PA			PBMC with PA		
	time	nmol/L		p-value	nmol/L		
		mean ± SEM			mean ± SEM	p-value	
Progesterone	0h	986.9 ± 62.2	0.018	*	1019.0 ± 101.9	0.182	ns
DOC	0h	0.0 ± 0.0	>0.999	ns	0.3 ± 0.2	0.141	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Progesterone	24h	754.2 ± 53.8			866.7 ± 21.2		
DOC	24h	0.0 ± 0.0			0.0 ± 0.0		
Corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0		

DOC + Ang. II as substrate		PBMC no PA			PBMC with PA		
	time	nmol/L		p-value	nmol/L		
		mean ± SEM			mean ± SEM	p-value	
DOC	0h	882.9 ± 67.1	0.017	*	967.5 ± 75.6	0.748	ns
Corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
DOC	24h	664.0 ± 175.0			936.2 ± 55.9		
Corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0		
18-OH-corticosterone	24h	0.0 ± 0.0			0.0 ± 0.0		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0		

corticosterone + Ang II as substrate		PBMC no PA			PBMC with PA		
	time	nmol/L		p-value	nmol/L		
		mean ± SEM			mean ± SEM	p-value	
Corticosterone	0h	833.3 ± 305.0	0.511	ns	1128.0 ± 34.0	0.537	ns
18-OH-corticosterone	0h	0.0 ± 0.0	>0.999	ns	2.2 ± 0.1	0.738	ns
Aldosterone	0h	0.0 ± 0.0	>0.999	ns	0.0 ± 0.0	>0.999	ns
Corticosterone	24h	934.8 ± 199.9			1177.0 ± 66.9		
18-OH-corticosterone	24h	0.0 ± 0.0			2.3 ± 0.1		
Aldosterone	24h	0.0 ± 0.0			0.0 ± 0.0		

18-OH-corticosterone + Ang II as substrate		PBMC no PA			PBMC with PA		
	time	nmol/L		p-value	nmol/L		
		mean ± SEM			mean ± SEM	p-value	
18-OH-corticosterone	0h	774.6 ± 34.1	0.001	**	868.8 ± 60.3	0.757	ns
Aldosterone	0h	0.4 ± 0.0	0.007	**	0.1 ± 0.1	0.908	ns
18-OH-corticosterone	24h	515.5 ± 40.7			898.3 ± 69.6		
Aldosterone	24h	0.3 ± 0.0			0.1 ± 0.1		

Figure 1

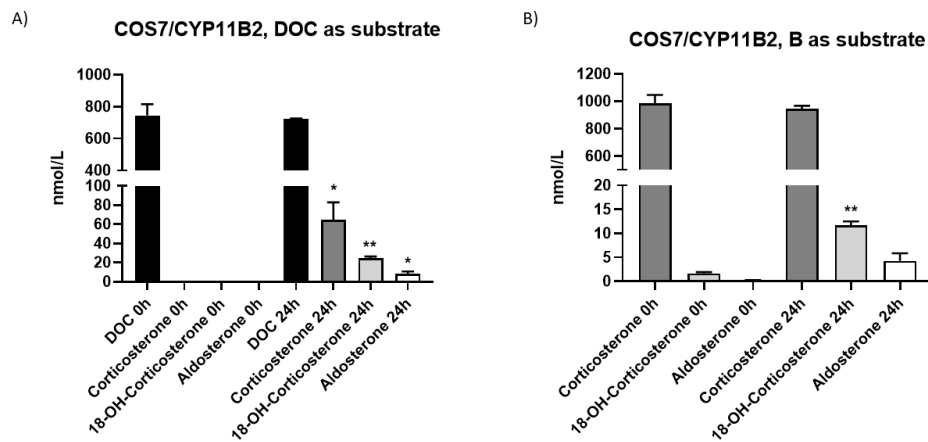


Figure 1

338x190mm (96 x 96 DPI)

Supplementary Data to:

No extra-adrenal aldosterone production in various human cell lines

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Short title: Extra-adrenal aldosterone production

Keywords: CYP11B2, aldosterone, primary hyperaldosteronism, progesterone, cell lines

Methods

Real-time PCR

Cells were cultured for 24h in a steroid-free and phenol red-free medium alternative with or without Ang II (10^{-6} M). PBS was the solvent of Ang II and served as the baseline.

Extraction of total RNA was performed using the Trizol method. RNA was reverse transcribed by using Oligo dT and random hexamer in the same reaction (PrimeScript RT reagent Kit from TaKaRa). All RT experiments in all cell lines were performed the same way. 50 ng of cDNA was used for Real-time PCR. Assay on demand primers were used for human CYP11B2 (Hs01597732_m1), SRD5A1 (Hs 00971645_g1), CYP21A2 (Hs 00416901_g1), AGTR1 (Hs00258938_m1), AGTR2 (Hs02621316_s1), Cyclophilin A (*PPIA*, 4326316E) and 18S (4310893E). Cyclophilin A and 18S served as endogenous controls. They all were from Applied Biosystems (ThermoFisherScientific, Reinach, Switzerland). GoTaq Probe qPCR Master Mix A6102 was from Promega AG, Dübendorf, Switzerland.

H295R and COS-7 cells transfected with CYP11B2 were used as positive controls. Results are displayed as ct values. Amplification cycle number was 50 and assays were performed in triplicate.

7500 Fast Real-time PCR and Quant Studio 1 machine were used both for all cell lines assessed. They were from Applied Biosystems (Thermo-Fisher-Scientific, Reinach, Switzerland).

Liquid chromatography–mass spectrometry (LC-MS)

Cells were cultured for 24h in a steroid-free and phenol red-free medium alternative with the steroid hormone substrates progesterone, DOC, corticosterone or 18-OH-corticosterone at a concentration of 10^{-6} M and with or without Ang II (10^{-6}). EtOH was the solvent of the substrates and served as the baseline. Reasons for phenol red-

free medium were to exclude stimulatory conditions and interference of phenol red with the LC-MS equipment. After 24h cell supernatant was collected, centrifuged, aliquoted and stored at -20°C until LC-MS analysis.

For the LC-MS analysis, 500 µL cell aliquots were spiked with 38 µL internal standard mix and steroids subsequently extracted using solid-phase extraction on an OasisPrime HLB 96-well plate according to the protocol previously published (Andrieu et al., 2022). The LC-MS system consists of a Vanquish UHPLC (equipped with an ACQUITY UPLC HSS T3 Column, 100Å, 1.8 µm, 1 mm X 100 mm; Waters, Switzerland) coupled to a Q Exactive Orbitrap Plus (both from Thermo-Fisher-Scientific, Reinach, Switzerland). Separation was achieved using gradient elution over 17 minutes using water and methanol (mobile phase B) both supplemented with 0.1 % formic acid (all Sigma-Aldrich, Buchs, Switzerland) as mobile phases. The separation of steroid metabolites was achieved through the following elution gradient (at a constant flow of 0.15 mL/min): 0–0.5 min 1% B, 0.5–1 min linear gradient to 1–46% B, 1–4 min 46%, 4–12 min linear gradient 46–73% B, 12–12.5-min linear gradient 73–99% B, 12.5–14.5 min 99% B, 14.5–15-min linear gradient to 1% B, and 15–17 min 1% B. All LC-MS grade solvents required for analysis were from BioSolve (Switzerland).

Data analysis was performed using TraceFinder 4.1 (Thermo-Fisher-Scientific, Reinach, Switzerland).

Steroid hormone concentrations are displayed in nmol/L. The lower limit of accurate quantification (LLOQ) was 0.085 nmol/L for Aldo, 0.705 nmol/L for corticosterone, 0.476 nmol/L for progesterone and 0.092 nmol/L for DOC. 18-OH-Corticosterone was detected in the mass channel of corticosterone (m/z 347.2217), its elution time confirmed from timepoint 0h cell aliquots and it was quantified relative to the calibration curve of corticosterone.

For each batch of LC-MS analysis the same positive control H295R cells + AngII was used as internal control. The steroid hormone concentrations after 24h were compared to the initial baseline steroid hormone concentrations at timepoint 0h. Assays were performed in triplicate, except for HAEC and HRMC cells. HAEC and HRMC assays were performed only once due to material limits.

Primary hyperaldosteronism patients and healthy controls

Details of the purchased PBMCs of healthy volunteers are shown in Supplementary Figure 1.

Supplementary Figure 1

Cell Inventory

4W-270 - Human Peripheral Blood Mononuclear Cells (hPBMC)
24.11.2020

Material	Cell Type	Plant	Batch	Stock	Donor ID	Age	Sex	Race	Blood Type	Smoke	HIV/HCV/HBV	CMV	Viability [%]	Cell Count [in Million]
4W-270	hPBMC, 10M cells	US	3038013	34	11714	47	M	A	B+	No	Pass	Positive	95.0	17.0
4W-270	hPBMC, 10M cells	US	3038016	46	18424	21	M	C	A+	No	Pass	Negative	90.0	18.0
4W-270	hPBMC, 10M cells	US	3038019	16	18061	43	F	UNK	O+	No	Pass	Negative	90.0	14.0
4W-270	hPBMC, 10M cells	US	3038099	59	15211	23	M	C	B+	No	Pass	Positive	90.0	15.0
4W-270	hPBMC, 10M cells	US	3041652	20	20932	21	F	C	A+	Yes	Pass	Negative	95.0	13.0
4W-270	hPBMC, 10M cells	US	3041690	225	21600	44	M	C	O+	No	Pass	Negative	96.0	16.0

Cryopreserved ampule of Mononuclear Cell (MNC) rich cells from leukapheresis are depleted of RBCs and platelets.
Count and viability is determined using AO/PI. Cells are collected from healthy donors following IRB protocols. Manufactured by Allicells®

Results

Progesterone metabolism and SRD5A1 mRNA expression

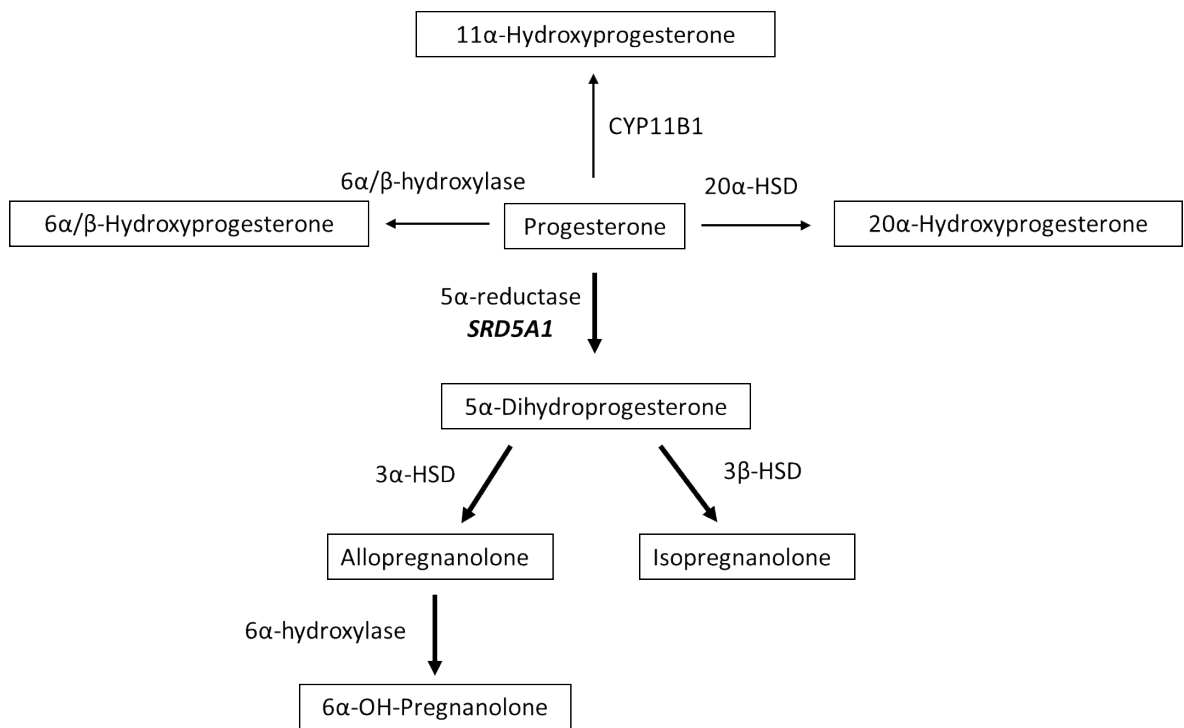
Progesterone levels decreased during the 24h incubation period in JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells, and in PBMCs of healthy subjects and PA patients, but no relevant DOC, corticosterone, 18-OH-corticosterone, and Aldo levels

could be detected. As progesterone metabolism was suspected to occur, *5 α -reductase* (*SRD5A1*) expression as well as the prominent formation of the progesterone metabolites *6 α / β -hydroxyprogesterone*, *20 α -hydroxyprogesterone*, *11 α -hydroxyprogesterone*, *5 α / β -dihydroprogesterone*, *allopregnanolone* and *6 α -hydroxypregnanolone* could be confirmed in JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells, and in PBMCs of healthy subjects and PA patients by Real-time PCR and high resolution LC-MS-based methods, respectively. As isopregnanolone was only produced in HRMC, it is not displayed in all other tables. Detailed results showing *SRD5A1* ct values and absolute values of progesterone metabolites in nmol/L are shown in supplementary data (**Supplementary Table 1 and Supplementary Tables 2-4**). LC-MS results are shown in absolute values nmol/L (mean \pm SEM). The concentrations of the substrate progesterone at time point 0h and 24h are shown. The concentrations of all other progesterone metabolites are displayed as 24h values minus 0h values which reflects their true production. An unpaired parametric T-test was used to assess significance between 0h and 24h progesterone values. ND = not detected.

Supplementary Figure 2 shows an assumed progesterone metabolism pathway in placental and renal cells, and in PBMCs of healthy subjects and PA patients.

Supplementary Table 1: mRNA expression of *SRD5A1* and *CYP21A2* shown as ct values

Supplementary Figure 2



Supplementary Figure 2 legend: Assumed progesterone metabolism pathway in JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells, and in PBMCs of healthy subjects and hyperaldosteronism patients.

mRNA expression of CYP21A2 in cells with active progesterone metabolism

CYP21A2 is the steroidogenic enzyme which converts progesterone to DOC in the adrenal glands. As no DOC, and no metabolites down-stream of DOC (corticosterone, 18-OH-corticosterone and Aldo) were found in JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells, and in PBMCs of healthy subjects and PA patients after supplementation with progesterone, the presence of CYP21A2 needed to be assessed. JEG-3, BeWo, HTR-8/SVneo, HRMC, HEK293 cells and the positive control H295R cells expressed significant levels of *CYP21A2*. No *CYP21A2* expression was however found in PBMCs of both cohorts and in HLEC. Cyclophilin A served as the endogenous control.

Supplementary Table 1

mRNA expression of AGTR1 and AGTR2

JEG-3, HTR-8/SV neo, BeWo, HUVEC, HUAEC, HAEC, HLEC, HRGEC, HRMC, HEK293, H295R and COS-7/CYP11B2 cells were cultured as described above. RNA was isolated and real-time PCR was performed to detect mRNA levels of *AGTR1* and *AGTR2*. Results are shown in Supplementary Table 5.

Supplementary Table 5

Progesterone metabolism measured by LC-MS

The prominent formation of the progesterone metabolites: 6 α / β -hydroxyprogesterone, 20 α -hydroxyprogesterone, 11 α -hydroxyprogesterone, 5 α / β -dihydroprogesterone, allopregnanolone/isopregnanolone and 6 α -hydroxypregnanolone could be confirmed in JEG-3, BeWo, HTR-8/SVneo, HRMC, and HEK293 cells, and in PBMCs of healthy subjects and PA patients by LC-MS analysis.

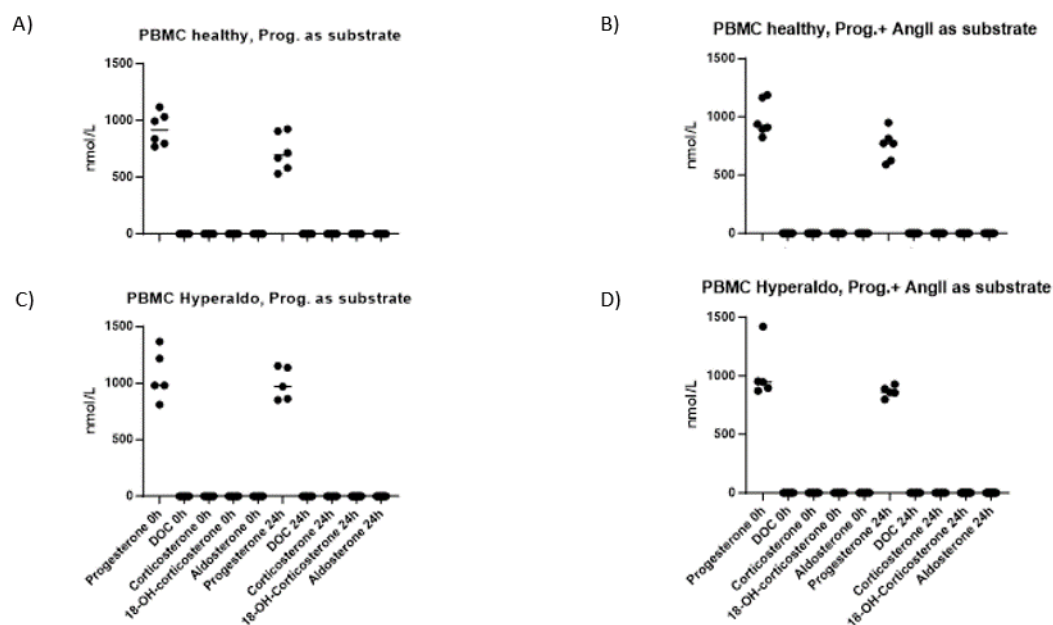
Supplementary Table 2

Supplementary Table 3

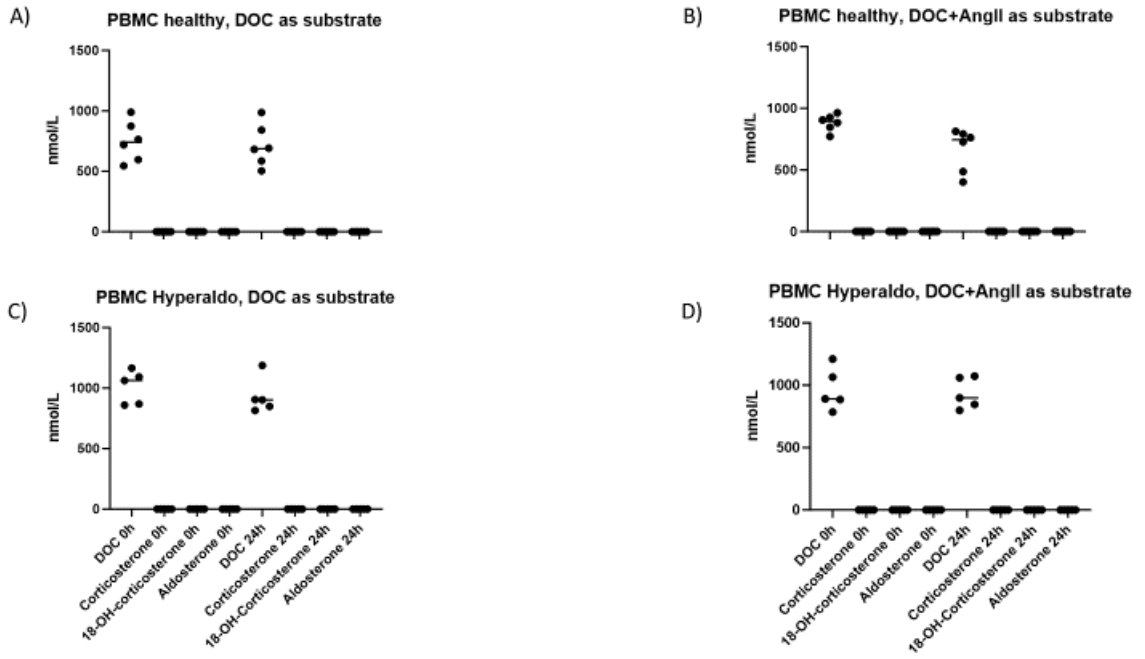
Supplementary Table 4

Absolute values of the steroid hormone metabolites in PBMCs of healthy subjects and of PA patients supplemented with the substrates progesterone (Supplementary Figure 3), DOC (Supplementary Figure 4), corticosterone (Supplementary Figure 5), and 18-OH-corticosterone (Supplementary Figure 6) without (A, C) and with (B, D) Ang II shown as dot plots

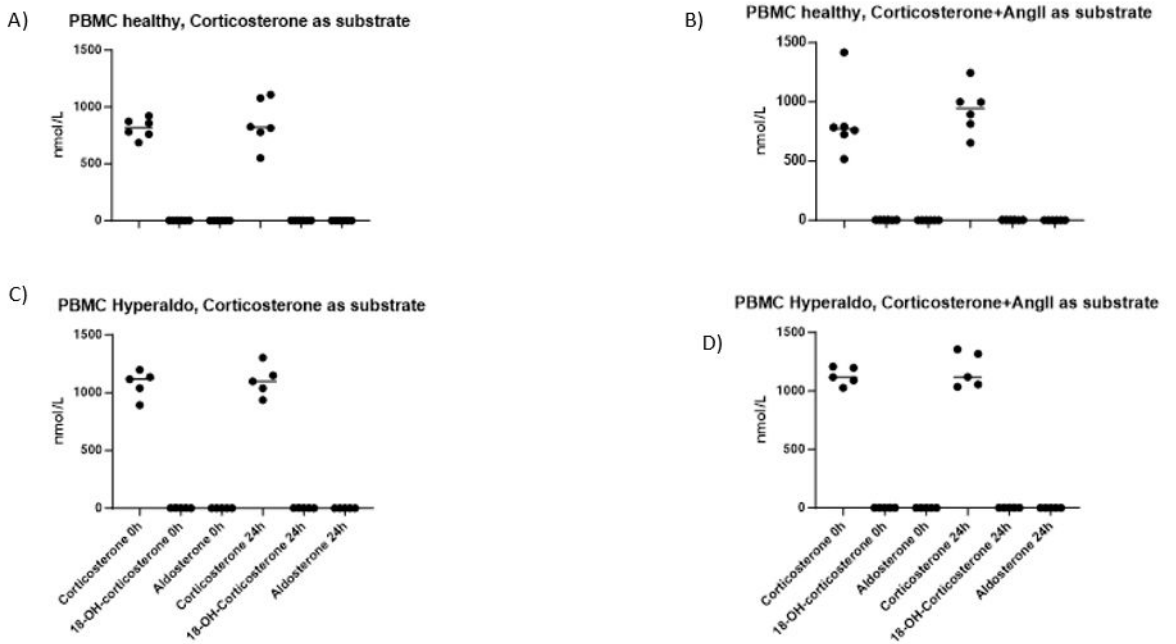
SupplementaryFigure 3



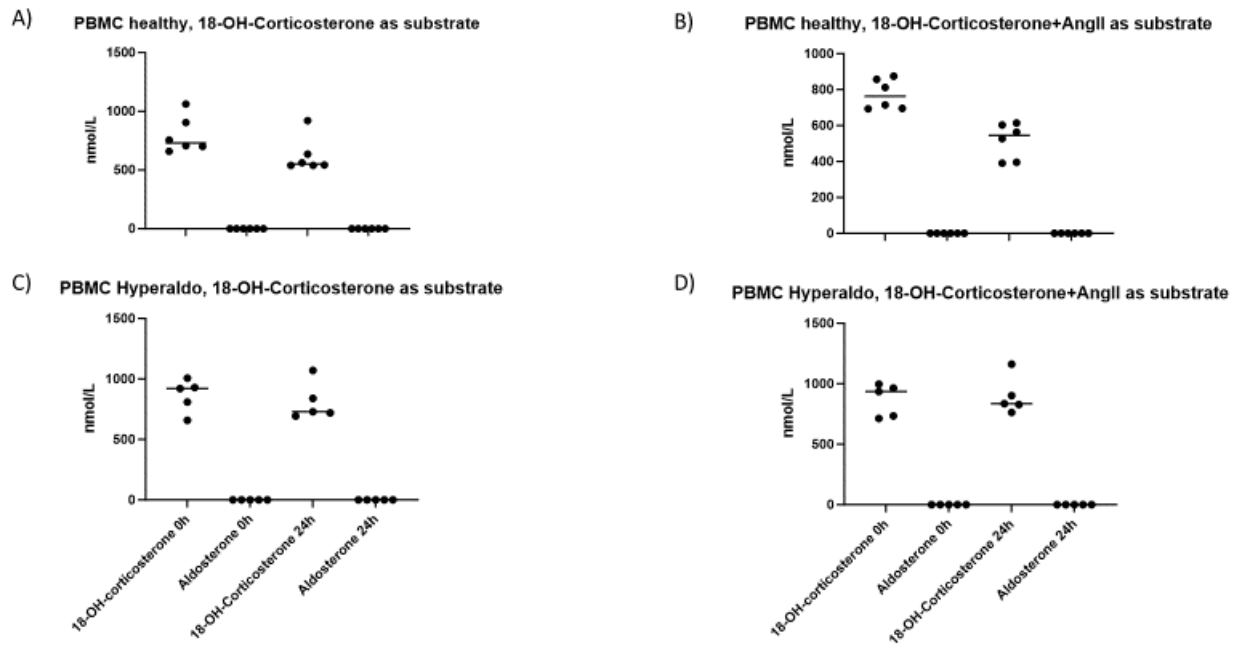
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 1

Cell Inventory

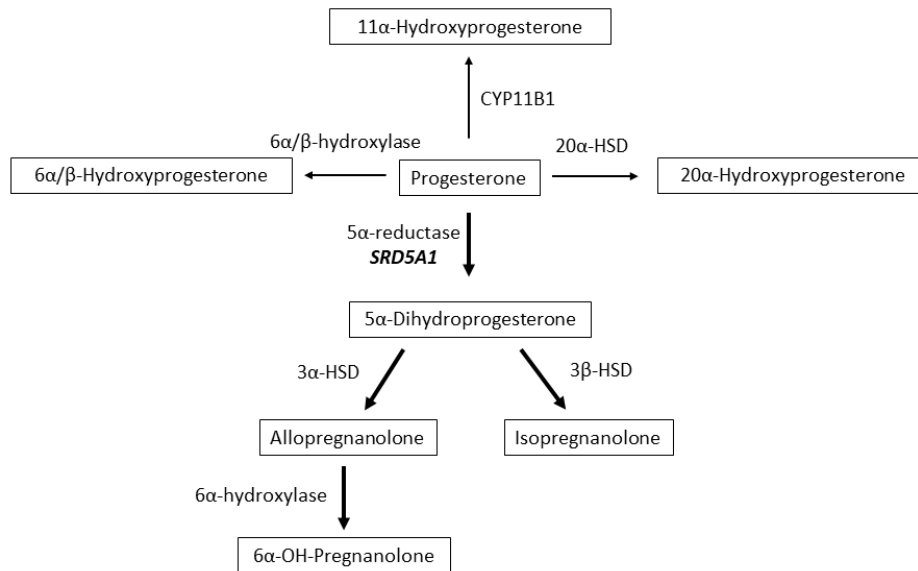
4W-270 - Human Peripheral Blood Mononuclear Cells (hPBMC)
24.11.2020

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4W-270	hPBMC, 10M cells	US	3038099	59	15211	23	M	C	B+	No	Pass	Positive	90.0	15.0
4W-270	hPBMC, 10M cells	US	3041652	20	20932	21	F	C	A+	Yes	Pass	Negative	95.0	13.0
4W-270	hPBMC, 10M cells	US	3041690	225	21600	44	M	C	O+	No	Pass	Negative	96.0	16.0

Cryopreserved ampule of Mononuclear Cell (MNC) rich cells from leukapheresis are depleted of RBCs and platelets.
count and viability is determined using AO/PI. Cells are collected from healthy donors following IRB protocols. Manufactured by AllCells®

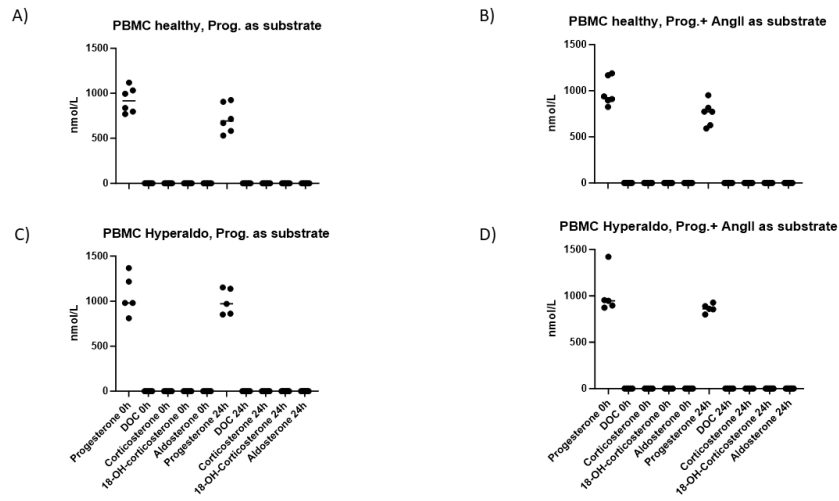
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Supplementary Figure 2 Putative progesterone metabolism pathway in placental and renal cells, and in PBMCs of healthy subjects and PA patients



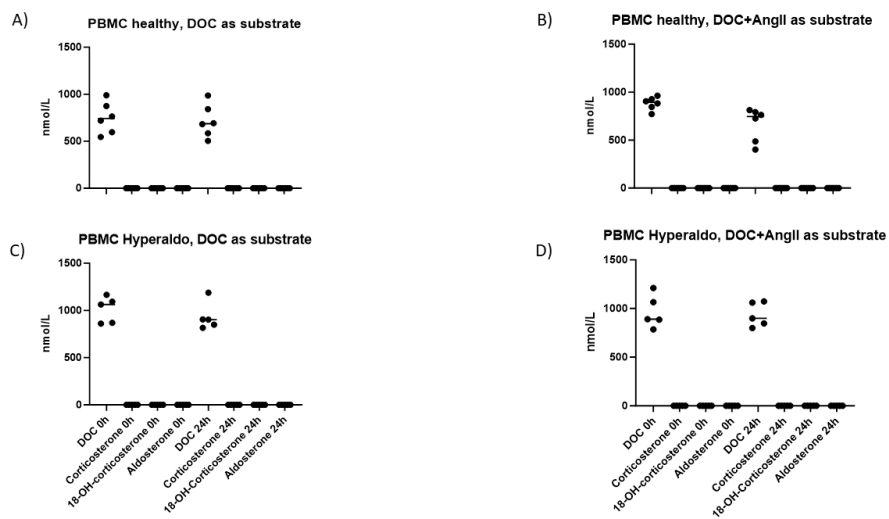
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Supplementary Figure 3



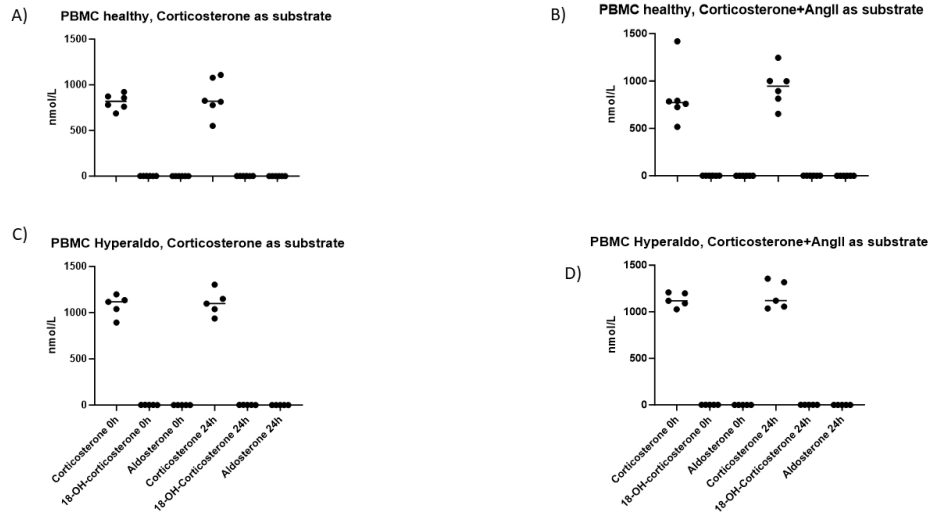
338x190mm (96 x 96 DPI)

Supplementary Figure 4



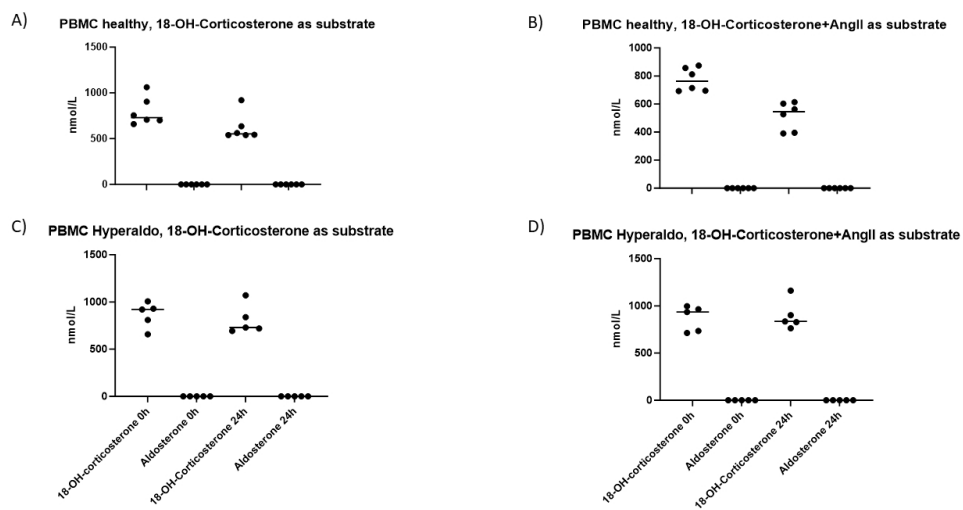
338x190mm (96 x 96 DPI)

Supplementary Figure 5



338x190mm (96 x 96 DPI)

Supplementary Figure 6



338x190mm (96 x 96 DPI)

Supplementary Table 1

mRNA expression of SRD5A1 shown as ct values

	ct SRD5A1 no Ang II	ct SRD5A1 + Ang II
JEG-3	25.3	25.2
BeWo	26.3	26.7
HTR-8/SV neo	24.2	23.3
HRMC	23.8	23.5
HEK293	23.5	23.5
PBMCs healthy subjects	29.8	30.7
PBMCs PA patients	29.7	28.9
HLEC	28.0	28.0
H295R	23.9	23.9

mRNA expression of CYP21A2 shown as ct values

	ct CYP21A2 no Ang II	ct CYP21A2 + Ang II
JEG-3	31.6	31.6
BeWo	30.6	31.5
HTR-8/SV neo	34.0	32.0
HRMC	30.0	29.8
HEK293	30.7	30.5
PBMCs healthy subjects	> 35	> 35
PBMCs PA patients	> 35	> 35
HLEC	> 35	> 35
H295R	20.1	20.3

Absolute values of the progesterone metabolites of the placental cells JEG-3, BeWo, HTR-8/SVneo

A)

Progesterone as substrate	time	JEG-3			BeWo	
		nmol/L	nmol/L	p-value	nmol/L	p-value
		mean ± SEM			mean ± SEM	
Progesterone	0h	1339.0 ± 132.6	0.189	ns	803.4 ± 57.1	0.236
Progesterone	24h	1090.0 ± 85.7			540.9 ± 179.7	
6α-Hydroxyprogesterone	24h	3.2 ± 0.4			1.0 ± 0.5	
6β-Hydroxyprogesterone	24h	9.7 ± 1.3			ND	
20α-OH Progesterone	24h	10.2 ± 0.7			19.8 ± 6.4	
20β-OH Progesterone	24h	10.0 ± 1.45			ND	
11α-Hydroxyprogesterone	24h	2.1 ± 0.6			2.5 ± 0.3	
5α/β-Dihydroprogesterone	24h	25.0 ± 3.1			42.2 ± 11.7	
Allopregnanolone	24h	ND			1.8 ± 1.6	
6α-OH-Pregnanolone	24h	16.2 ± 2.3			34.1 ± 5.4	

B)

Progesterone + Ang II as substrate	time	JEG-3			BeWo	
		nmol/L	nmol/L	p-value	nmol/L	p-value
		mean ± SEM			mean ± SEM	
Progesterone	0h	1252.0 ± 120.7	0.111	ns	709.7 ± 105.8	0.512
Progesterone	24h	943.3 ± 91.7			563.8 ± 172.9	
6α-Hydroxyprogesterone	24h	3.0 ± 0.4			0.9 ± 0.3	
6β-Hydroxyprogesterone	24h	9.4 ± 1.5			ND	
20α-OH Progesterone	24h	8.8 ± 0.1			23.6 ± 9.0	
20β-OH Progesterone	24h	8.1 ± 0.4			ND	
11α-Hydroxyprogesterone	24h	1.8 ± 0.6			2.6 ± 0.2	
5α/β-Dihydroprogesterone	24h	25.1 ± 1.7			39.0 ± 11.1	
Allopregnanolone	24h	ND			1.6 ± 1.4	
6α-OH-Pregnanolone	24h	14.1 ± 0.7			33.3 ± 8.2	

HTR-8/SVneo			
nmol/L			
mean \pm SEM		p-value	
ns	709.7 \pm 105.8	0.512	ns
	563.8 \pm 172.9		
	0.9 \pm 0.3		
	ND		
	23.6 \pm 9.0		
	ND		
	2.6 \pm 0.2		
	39.0 \pm 11.1		
	1.6 \pm 1.4		
	33.3 \pm 8.2		

HTR-8/SVneo			
nmol/L			
mean \pm SEM		p-value	
ns	1227.0 \pm 122.2	0.037	*
	762.1 \pm 89.5		
	ND		
	ND		
	4.3 \pm 0.1		
	ND		
	ND		
	124.5 \pm 21.3		
	17.7 \pm 17.3		
	40.3 \pm 7.6		

Absolute values of the progesterone metabolites of the renal cells HRMC and HEK293

A)

Progesterone as substrate	time	HRMC		HEK293	
		nmol/L mean ± SEM	p-value	nmol/L mean ± SEM	p-value
Progesterone	0h	1076.0		1101 ± 241.7	0.421
Progesterone	24h	1008.0		799.8 ± 232.7	
6α-Hydroxyprogesterone	24h	2.0		4.3 ± 0.7	
6β-Hydroxyprogesterone	24h	9.9		14.2 ± 1.8	
20α-OH Progesterone	24h	82.9		12.0 ± 2.6	
20β-OH Progesterone	24h	23.3		15.0 ± 2.2	
11α-Hydroxyprogesterone	24h	1.9		9.4 ± 1.6	
5α/β-Dihydroprogesterone	24h	174.1		74.8 ± 4.9	
Allopregnanolone	24h	45.4		4.6 ± 1.2	
Isopregnanolone	24h	135.0		ND	
6α-OH-Pregnanolone	24h	27.9		27.1 ± 5.5	

B)

Progesterone + Ang II as substrate	time	HRMC		HEK293	
		nmol/L mean ± SEM	p-value	nmol/L mean ± SEM	p-value
Progesterone	0h	968.0		1367 ± 220.8	0.309
Progesterone	24h	676.0		1011 ± 210.9	
6α-Hydroxyprogesterone	24h	2.1		5.6 ± 1.3	
6β-Hydroxyprogesterone	24h	9.4		18.7 ± 3.7	
20α-OH Progesterone	24h	75.8		15.8 ± 3.4	
20β-OH Progesterone	24h	21.2		25.2 ± 5.9	
11α-Hydroxyprogesterone	24h	1.8		11.6 ± 1.9	
5α/β-Dihydroprogesterone	24h	181.3		95.9 ± 10.5	
Allopregnanolone	24h	47.8		6.0 ± 0.5	
Isopregnanolone	24h	147.2		ND	
6α-OH-Pregnanolone	24h	38.5		26.6 ± 2.1	

ns

ns

Absolute values of the progesterone metabolites of the PBMCs from healthy subjects and PA patient

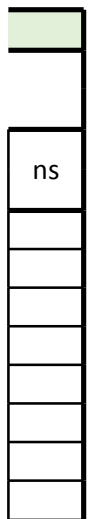
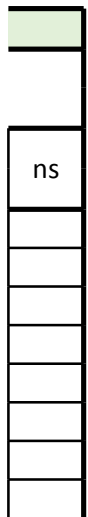
A)

Progesterone as substrate	time	PBMC no PA			PBMC with PA	
		nmol/L mean ± SEM	p-value	ns	nmol/L mean ± SEM	p-value
Progesterone	0h	1048.0 ± 36.7	0.058	ns	1072.0 ± 98.5	0.533
Progesterone	24h	848.3 ± 66.4			995.0 ± 65.0	
6α-Hydroxyprogesterone	24h	0.6 ± 0.3			0.9 ± 0.1	
6β-Hydroxyprogesterone	24h	5.8 ± 2.0			7.5 ± 0.5	
20α-OH Progesterone	24h	3.8 ± 3.5			11.0 ± 5.6	
20β-OH Progesterone	24h	ND			1.4 ± 3.7	
11α-Hydroxyprogesterone	24h	ND			0.2 ± 0.0	
5α/β-Dihydroprogesterone	24h	59.3 ± 33.4			62.9 ± 15.6	
Allopregnanolone	24h	3.9 ± 1.7			ND	
6α-OH-Pregnanolone	24h	ND			ND	

B)

Progesterone + Ang II as substrate	time	PBMC no PA			PBMC with PA	
		nmol/L mean ± SEM	p-value	ns	nmol/L mean ± SEM	p-value
Progesterone	0h	1083.0 ± 94.7	0.072	ns	1019.0 ± 101.9	0.182
Progesterone	24h	722 ± 114.9			866.7 ± 21.2	
6α-Hydroxyprogesterone	24h	0.5 ± 0.2			0.9 ± 0.1	
6β-Hydroxyprogesterone	24h	3.4 ± 1.5			6.3 ± 0.4	
20α-OH Progesterone	24h	1.4 ± 1.3			8.8 ± 4.4	
20β-OH Progesterone	24h	ND			ND	
11α-Hydroxyprogesterone	24h	ND			0.1 ± 0.0	
5α/β-Dihydroprogesterone	24h	38.5 ± 20.0			58.1 ± 11.6	
Allopregnanolone	24h	ND			ND	
6α-OH-Pregnanolone	24h	ND			ND	

nts



Supplementary Table 5

mRNA expression of AGTR1 and AGTR2

cycle number: 50

Cell line	Condition	ct AGTR1	ct AGTR2
JEG-3	PBS	Undet	33.470
JEG-3	Ang II 10-6M	Undet	33.900
HTR-8/SV neo	PBS	35.985	Undet
HTR-8/SV neo	Ang II 10-6M	38.333	44.895
BeWo	PBS	Undet	35.264
BeWo	Ang II 10-6M	Undet	30.643
HUVEC	PBS	Undet	35.494
HUVEC	Ang II 10-6M	Undet	37.279
HUAEC	PBS	Undet	Undet
HUAEC	Ang II 10-6M	Undet	35.179
HAEC	PBS	38.501	32.895
HAEC	Ang II 10-6M	Undet	32.263
HLEC	PBS	Undet	31.687
HLEC	Ang II 10-6M	Undet	Undet
HRGEC	PBS	39.368	36.250
HRGEC	Ang II 10-6M	Undet	35.185
HRMC	PBS	34.473	33.892
HRMC	Ang II 10-6M	34.303	33.018
HEK293	PBS	34.444	33.026
HEK293	Ang II 10-6M	34.288	33.004
H295R	PBS	27.816	36.166
H295R	Ang II 10-6M	28.791	35.608
COS-7 + CYP11B2 plasmid	PBS	Undet	38.056
COS-7 + CYP11B2 plasmid	Ang II 10-6M	Undet	38.250

Undet: undetected