Glucocorticoid Activity of Adrenal Steroid Precursors in Untreated Patients With Congenital Adrenal Hyperplasia

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Context and Objective: We describe the clinical features and biochemical characteristics of a unique population of severely affected untreated patients with congenital adrenal hyperplasia (CAH) from an Indonesian population with proven cortisol deficiency but without clinical signs of cortisol deficiency. We evaluated the *in vitro* glucocorticoid activity of all relevant adrenal steroid precursors occurring in patients with CAH.

Design: Cross-sectional cohort study and translational research.

Intervention/Main Outcome Measures: Adrenal steroid precursor concentrations before and 60 minutes after ACTH administration to 24 untreated patients with CAH (3 to 46 years) with proven cortisol deficiency (<500 nmol/L post-ACTH) measured by liquid chromatography–tandem mass spectrometry were compared with six control patients (Mann-Whitney *U* test). Glucocorticoid receptor (GR) activation was determined by dual-luciferase assays in human embryonic kidney cells transfected with the GR and exposed to increasing amounts of adrenal steroid precursors for 24 hours.

Results: Blood concentrations of the steroid precursors 11-deoxycortisol (457 nmol/L, $P = 0.003$), 11-deoxycorticosterone (55 nmol/L, $P = 0.003$), 17-hydroxyprogesterone (610 nmol/L, $P < 0.001$), progesterone (29 nmol/L, $P < 0.001$), and 21-deoxycortisol (73 nmol/L) were strongly elevated compared with control subjects. The GR was activated with comparable potency to cortisol by corticosterone and 21-deoxycortisol or with 4 to 100 times lower potency by 11-hydroxyprogesterone, 11-deoxycortisol, aldosterone, 11-deoxycorticosterone, progesterone, and 17-hydroxyprogesterone.

Conclusions: We identified strongly elevated adrenal steroid precursor concentrations in blood from untreated patients with CAH and demonstrated glucocorticoid activity of these adrenal precursors *in vitro*, suggesting a possible role of these precursors in the clinical phenotype of these patients. Further studies are necessary to evaluate the role of these precursors in more detail. (***J Clin Endocrinol Metab*** **104: 5065–5072, 2019)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 6 March 2019. Accepted 9 May 2019.
First Published Online 15 May 2019

Abbreviations: 11OHD, 11-hydroxylase deficiency; 21OHD, 21-hydroxylase deficiency; CAH, congenital adrenal hyperplasia; GR, glucocorticoid receptor; IQR, interquartile range.

Cortisol deficiency is one of the main findings in patients with congenital adrenal hyperplasia (CAH), an autosomal recessive disorder with impaired enzymatic activity in the adrenal cortex. More than 90% of CAH cases are caused by 21-hydroxylase deficiency (21OHD). Another 5% of cases are caused by 11-hydroxylase deficiency (11OHD) (Fig. 1). Due to these enzymatic defects, cortisol production is inadequate, leading to elevated ACTH concentrations and stimulation of the adrenal gland, eventually causing hyperplasia. Adrenal steroid precursors before the enzymatic defect accumulate and are shunted into the androgen synthesis pathway (1). The increased concentrations of adrenal steroid precursors are currently valuable diagnostic markers for CAH (1–3). Patients with CAH are generally treated with synthetic glucocorticoids to substitute for cortisol deficiency to prevent life-threatening adrenal crises and to suppress ACTH levels and consequently the production of steroid precursors and androgens.

In developing countries such as Indonesia, there is a lack of availability of glucocorticoids and limited access to medical care, resulting in a high mortality and morbidity of patients with adrenal insufficiency. Here, we describe the clinical and biochemical features, including the concentrations of all relevant steroid precursors, of an Indonesian cohort of 22 untreated children and adults with severe CAH who survived without glucocorticoid treatment and without signs of cortisol deficiency. We hypothesized that these strongly elevated adrenal precursors may have a stimulating effect on the glucocorticoid receptor (GR), possibly explaining the lack of signs of cortisol deficiency in these patients. Therefore, the glucocorticoid activity of these precursor steroids was assessed by *in vitro* GR transactivation studies.

Subjects and Methods

Patients

A total of 22 untreated patients with CAH (age 3 to 46 years) from the Center for Biomedical Research, Faculty of Medicine Diponegoro University, Semarang, Indonesia, were available for biochemical clinical and biochemical evaluation. The study was approved by the local ethical committee of Diponegoro University. Oral and written informed consent was obtained after full explanation of the purpose and nature of all procedures. Data were collected on type of CAH, karyotype, sex, mutation analysis, signs of salt wasting, and episodes of severe stress in each subject's medical history (critical illness, trauma, surgery) by the local pediatric endocrinologist (A.U.).

We included the biochemical data from two untreated patients with CAH from the Netherlands. Six healthy individuals in whom an ACTH test was performed for different indications were used as controls.

Biochemical analysis

The standard ACTH stimulation test was performed in all patients to determine adrenal functioning by measuring steroid concentrations before and after ACTH administration. Synacthen (0.25 mg; Sigma Tau

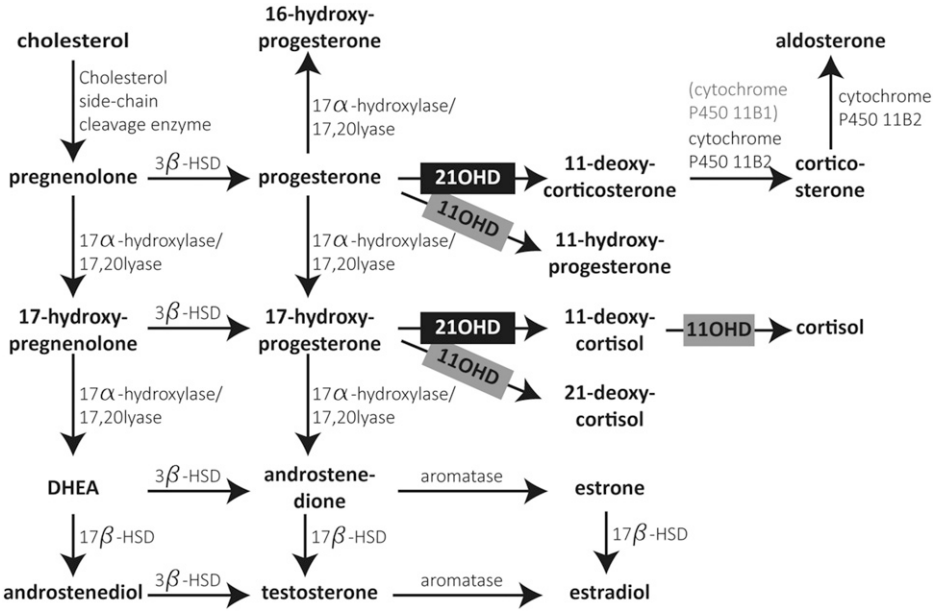

Figure 1. Adrenal steroidogenesis. Cholesterol is converted to aldosterone, cortisol, and androgens in the adrenal cortex via several enzymatic steps. 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase.

BV, Utrecht, Netherlands) was injected intravenously; blood draws for steroid analysis were performed before and 60 minutes after the injection. None of the patients received glucocorticoids at the time of biochemical analysis. Serum before and after ACTH administration was analyzed with liquid chromatography–tandem mass spectrometry to determine cortisol, corticosterone, 11-deoxycortisol, 11-deoxycorticosterone, 17-hydroxyprogesterone, progesterone, and 21-deoxycortisol concentrations.

In vitro transactivation study

Cell culture

Human embryonic kidney cells (HEK293) were grown as a monolayer culture in DMEM with 4.5 g/L glucose with L-glutamine (Lonza, Leusden, Netherlands) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Landsmeer, Netherlands) and 1% antibiotics (penicillin-streptomycin 10,000 U/mL; Gibco). Cells were cultured at 37°C in a humidified 95% air/5% CO_2 atmosphere and passaged when confluent.

In vitro *dual-luciferase transactivation assays*

Human GR transactivation was measured using dual-luciferase transactivation assays (Promega, Leiden, Netherlands) in which pcDNA6-V5/Hisb-hGR, MMTV-luc, and pRL-TK vectors were used as described elsewhere (4).

Relevant negative controls were included to confirm that the HEK293 cell line did not contain relevant amounts of endogenous steroids or endogenous steroid receptors. HEK293 cells were seeded at 40,000 cells per well in 24-well plates. Transient transfection was performed after 24 hours using 0.2 μg pcDNA6-V5/Hisb-hGR, 0.3 μg MMTV-luc, and 0.01 μg pRL-TK per well and 1 μL TransIT-LT1 transfection reagent (Mirus, Ochten, Netherlands) according to the manufacturer's protocol. Cells were treated for 24 hours with one of the steroids [progesterone, 17-hydroxypregnenolone, pregnenolone (Sigma-Aldrich, Zwijndrecht, Netherlands) or cortisol, aldosterone, corticosterone, 11-deoxycortisol, 11-deoxycorticosterone, 17-hydroxyprogesterone, 11β-hydroxyprogesterone, 16-hydroxyprogesterone, 21-deoxycortisol (Steraloids, Newport, RI)] 2 days after transfection. Steroid solutions were prepared in ethanol (200 times concentrated) and diluted 1:200 in culture medium prior to treatment. Thereafter, firefly and renilla luciferase activity was measured on a Fluoroskan FL luminometer (Thermo Scientific) according to the manufacturer's protocol (Promega), and firefly/renilla ratios were calculated. Each concentration was measured in triplicate.

As controls, HEK293 cells were transfected with the MMTV-luc and pRL-TK vector but not with the pcDNA6-V5/Hisb-hGR and treated with 10^{-4} M cortisol. HEK293 cells were transfected with all the vectors but were not treated with steroid (medium with 0.5% ethanol). Neither of these approaches resulted in transactivation activity.

Statistical analyses

GraphPad Prism software version 5.0 for Windows and SPSS Statistics 22 (SPSS Inc., Chicago, IL) were used to analyze adrenal steroid concentrations. Normality was assessed, and median steroid concentration and interquartile range (IQR; Q1 to Q3) were calculated. Patients with 21OHD and 11OHD were compared with the control group, and distributions were compared using the Mann-Whitney *U* test; $P < 0.05$ was considered significant. GraphPad Prism was used to calculate dose-response curves using nonlinear regression. The EC_{50} its 95% CI were also determined. Relative functional sensitivity of the GR to the different steroids was calculated as $EC_{50\ cortisol}/EC_{50\ test\ steroid} \times 100\%$, in which cortisol was set as 100%.

Results

Clinical characteristics of untreated patients with CAH

Clinical characteristics of the 22 included Indonesian patients with CAH are shown in Table 1. There were 17 patients with 21OHD and five patients with 11OHD. All patients with 11OHD were from the same extended family. The patients with 21OHD were from 13 families. One patient with 21OHD had a sibling who died at the age of 50 days, but there is no medical record of this sibling. No other deaths have been reported among siblings of the included patients.

Most patients (n = 14) had never been treated with glucocorticoids. Eight patients had been treated in the past but had been off treatment of at least 2 years at the time of biochemical analysis and were therefore considered as untreated. In all patients, the diagnosis of CAH was confirmed by mutation analysis. Episodes of severe illness while untreated in their medical history were reported in 13 of 22 patients (Table 1). In none of these episodes were patients treated with stress dosages of glucocorticoids. Five patients reported hospital admissions because of salt wasting crisis or because of episodes of vomiting and/or seizures.

Table 1. Clinical Characteristics of Untreated Patients With CAH

No.	Age, y	CAH Type	Karyo-type	Sex	Mutation	CAH Subtype	Renin Level, mU/L	Treatment With Glucocorticoids	History of Severe Stress While Untreated
I1	28	11OHD	46XX	F	c.799G>A, c.799G>A		9.7	Never	Genital surgery
I2	19	11OHD	46XX	F	c.799G>A, c.799G>A		<3	Never	No
I3	5	11OHD	46XX	M	c.799G>A, c.799G>A		<3	Never	No
I4	13	11OHD	46XX	M	c.799G>A, c.799G>A		7.9	Never	No
I5	3	11OHD	46XX	U	c.799G>A, c.799G>A		5.9	Never	No
I6 (S1)	19	21OHD	46XX	F	p.Ile172Asn, p.Ile172Asn	SV	38	Treated at age 8–16 y	No
I7 (S1)	23	21OHD	46XX	F	p.Ile172Asn, p.Ile172Asn	SV	99	Treated at age 13–20 y	No
I8	21	21OHD	46XX	F	p.Arg356Trp, p.Arg356Trp	SW	630	Treated at age 10–19 y	Genital surgery
I9 (S2)	9	21OHD	46XX	F	intron splice, p.Arg356Trp	SW	190	Treated at age 5–7 y	Repeated hospital admissions for salt wasting crises
I10 (S2)	4	21OHD	46XX	F	intron splice, p.Arg356Trp	SW	500	Treated at age 0–2 y	Repeated hospital admissions for salt wasting crises
I11	13	21OHD	46XX	F	p.Arg356Trp, p.Arg356Trp	SV	140	Treated since childhood	Genital surgery
I12	46	21OHD	46XX	M	p.Trp20,[a] p.Ile172Asn	SV	57	Never	Severe gastritis (2x) typhoid fever
I13 (S3)	15	21OHD	46XX	M	Intron splice, intron splice	SV	140	Never	No
I14 (S3)	32	21OHD	46XX	M	intron splice, intron splice	SV	61	Never	Genital surgery
I15	13	21OHD	46XX	F	p.Arg356Trp, p.Arg356Trp	SV	94	Treated for 1 y at age 5 y	Genital surgery, tonsillectomy, dengue hemorrhagic fever, typhoid fever
I16	15	21OHD	46XX	M	p.Arg356Trp, deletion of exon 1–3	SW	100	Never	Repeated hospital admissions for vomiting and seizures
I17	14	21OHD	46XX	M	p.Ile386del, p.Arg356Trp	SW	84	Never	Repeated hospital admissions for seizures
I18	14	21OHD	46XX	F	p.Pro30Leu, p.Pro30Leu	SV	57	Treated at age 4–10 y	Genital surgery
I19	10	21OHD	46XX	M	p.Gln196,[a] p.Arg356Trp	SW	280	Never	Hospital admission for vomiting, dengue hemorrhagic fever
I20 (S4)	19	21OHD	46XX	F	p.Ile172Asn, deletion of exon 1–6	SV	170	Never	No
I21	3	21OHD	46XX	F	p.Ile172Asn, p.Arg356Trp	SV	360	Never	No
I22 (S4)	21	21OHD	46XX	F	Deletion of exon 1–6, NB no second mutation[b]	SV	189	Never	Tonsillectomy, dengue hemorrhagic fever
D1	0	21OHD	46XY	M	Deletion of exon 1–3, p.Ile172Asn	SW	290	Treatment started after ACTH stimulation test	NA
D2	0	21OHD	46XY	M	Deletion of exon 1–7, p.Pro30Leu	SW	530	Treatment started after ACTH stimulation test	NA

All patients with 11OHD belong to the same extended family. S1, S2, S3, and S4 indicate sibling pairs.

Abbreviations: D, untreated patients from Dutch cohort; F, female; I, untreated patients from the Indonesian cohort; M, male; NA, not applicable; SV, simple virilizing; SW, salt wasting; U, undefined.

[a]These patients also had a cluster of mutations in the CYP21A2 promotor region in one allele, possibly resulting in a more severe phenotype.

[b]This patient had very high 17OHP levels (>400 nmol/L), and. although no second mutations have been found, it is very likely that this patient belongs to the same genotype group (B) as her sister (#I20).

Biochemical evaluation of serum adrenal steroid precursors

The biochemical results of the study population are shown in Fig. 2. In control subjects, cortisol concentrations increased adequately after ACTH administration (>500 nmol/L) (5–7), and all other measured steroids remained low, with a two- to fivefold increase, as expected. In contrast, in our study population cortisol concentrations remained low after ACTH administration, with median concentrations of 73 nmol/L (IQR, 64 to 129) in the patients with 21OHD and 180 nmol/L (IQR, 159 to 214) in the patients with 11OHD, confirming severe cortisol deficiency. We did not find significant differences in cortisol concentrations before and after ACTH administration, suggesting that the adrenal gland is already maximally stimulated in the unstimulated condition. Adrenal steroid precursors before the enzymatic block were significantly elevated before and after ACTH stimulation, also without any difference before and after ACTH stimulation.

In patients with 21OHD, 17-hydroxyprogesterone and progesterone concentrations were elevated (median, 610 nmol/L; IQR, 509 to 762; $P < 0.001$ and median, 29 nmol/L; IQR, 20 to 43, $P < 0.001$, respectively) compared with control subjects (median, 4.8 nmol/L; IQR, 2.7 to 5.6 and median, 1.0 nmol/L; IQR, 0.5 to 1.1, respectively). Concentrations of 21-deoxycortisol were also elevated (median, 73 nmol/L; IQR, 46 to 112) in contrast to control subjects, in whom 21-deoxycortisol was not detectable.

In patients with 11OHD, blood 11-deoxycortisol and 11-deoxycorticosterone concentrations were significantly elevated, with median concentrations of 457 nmol/L (IQR, 364 to 612; $P = 0.003$) and 55 nmol/L (IQR, 25 to 119; $P = 0.003$), respectively, compared with control subjects (median, 3.2 nmol/L; IQR, 1.9 to 5.9 and median, 0.5 nmol/L; IQR, 0.2 to 0.8 nmol/L, respectively) (IQR, 55 to 78 nmol/L).

GR transactivation in human embryonic kidney cells

To determine the potency of steroid precursors for GR transactivation, we performed dual-luciferase assays, which allow for the quantification of GR-induced

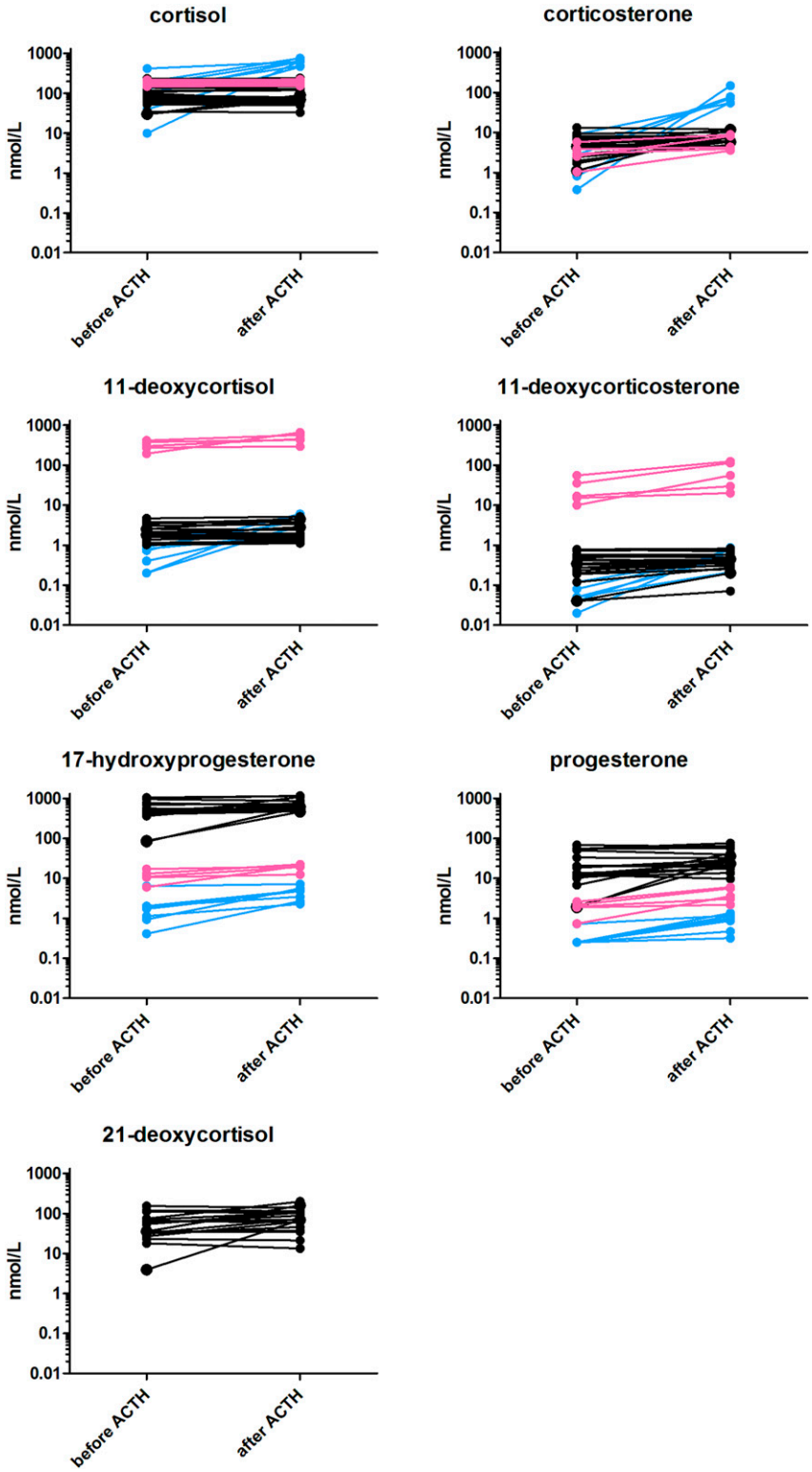

Figure 2. Steroid concentrations in untreated patients with CAH. Blood adrenal steroid concentrations were determined before and 60 min after ACTH administration. Black indicates 21-hydroxylase–deficient patients, pink indicates 11-hydroxylase–deficient patients, and blue indicates control.

gene expression (Table 2; Fig. 3). We found an EC_{50} of 11 nM (95% CI, 5.9 to 20) for cortisol, which is used as a reference steroid. Similar potencies were found for corticosterone (EC_{50}, 17 nM; 95% CI, 8.9 to 32) and 21-deoxycortisol (EC_{50}, 22 nM; 95% CI, 12 to 42) because the CIs overlap. 11-Hydroxyprogesterone, 11-deoxycortisol, and aldosterone exhibited somewhat higher EC_{50} values (47, 71, and 111 nM, respectively). Exposure to 11-deoxycorticosterone (EC_{50}, 725 nM), progesterone (EC_{50}, 1147 nM), and 17-hydroxyprogesterone (EC_{50}, 1668 nM) also resulted in GR transactivation, although these EC_{50} values were at least 65 times higher than that of cortisol. GR transactivation was observed only at a very high concentration (100,000 nM) of 16-hydroxyprogesterone, whereas no GR transactivation was found for pregnenolone or 17-hydroxypregnenolone.

Discussion

Here, we describe a unique group of untreated patients with 21OHD and untreated patients with 11OHD with CAH with biochemically confirmed severe cortisol deficiency. None of these patients had clinical signs of cortisol deficiency, and more than half of these patients reported a history of severe stress situations, such as surgery or severe infectious diseases, but they recovered without glucocorticoid stress dosing. We were able to measure multiple adrenal steroid precursors in these untreated patients with CAH before and after ACTH stimulation. As expected from their enzymatic defect, in untreated patients with 21OHD, 17-hydroxyprogesterone (127 times higher), progesterone (29 times higher), and 21-deoxycortisol (measurable only in 21OHD) concentrations were significantly increased compared with control subjects. Furthermore, in untreated patients with 11OHD, we found 11-deoxycortisol (457 times higher) and 11-deoxycorticosterone (55 times higher) to be the most important accumulating steroid precursors. We hypothesized and confirmed in the current study that these strongly elevated adrenal precursor concentrations may have a stimulating effect on the GR.

Steroid hormone action is initiated by binding of the hormone to its receptor. The hormone–receptor complex subsequently translocates to the nucleus, where it binds to hormone response elements in the regulatory region of target gene promoters, initiating transactivation (8). Several studies have been performed evaluating the binding of steroids to the GR and mineralocorticoid receptor and/or the nuclear translocation of the receptor complex, but only a few studies report on GR transactivation (9–14), mainly focusing on aldosterone and cortisol, showing that cortisol has higher potency for GR transactivation than aldosterone (10, 11, 13, 14). It is well established that adrenal steroids have steroid hormone cross-reactivity, as explained by the high degree of homology of the DNA binding domain of the GR and the mineralocorticoid receptor (15). A previous study from our group showed that 21-deoxycortisol, 17-hydroxyprogesterone, and progesterone activate the GR (9). In studying the potency to activate the GR for more than 10 adrenal steroid precursors relative to cortisol, we found that 21-deoxycortisol and corticosterone had similar potency to activate the GR. Also, 11-hydroxyprogesterone, 11-deoxycortisol, aldosterone 11-deoxycorticosterone, progesterone, and 17-hydroxyprogesterone showed a potency to activate the GR, but this was 4 to 10 times

Table 2. Relative Potency of Steroids to Activate the GR

	EC_{50} (95% CI), nM	Relative Potency to GR, %
Cortisol	11 (5.9–20)	100
Corticosterone	17 (8.9–32)	64
21-Deoxycortisol	22 (12–42)	49
11-Hydroxyprogesterone	47 (30–73)	23
11-Deoxycortisol	71 (56–92)	15
Aldosterone	111 (72–171)	9.8
11-Deoxycorticosterone	725 (422–1246)	1.5
Progesterone	1147 (664–1981)	0.95
17-Hydroxyprogesterone	1668 (1300–2140)	0.65
16-Hydroxyprogesterone	NC[a]	
Pregnenolone	NA	
17-Hydroxypregnenolone	NA	

Data of the transactivation assays in human embryonic kidney cells were used to calculate dose-response curves using nonlinear regression. EC_{50} values could be calculated when a complete dose-response curve was available, including a concentration in which maximum transactivation was reached (plateau phase).

Abbreviations: NA, not applicable; NC, not calculable.

[a]Some transactivation occurred at 100,000 nM, but no maximum transactivation was reached with the concentrations tested.

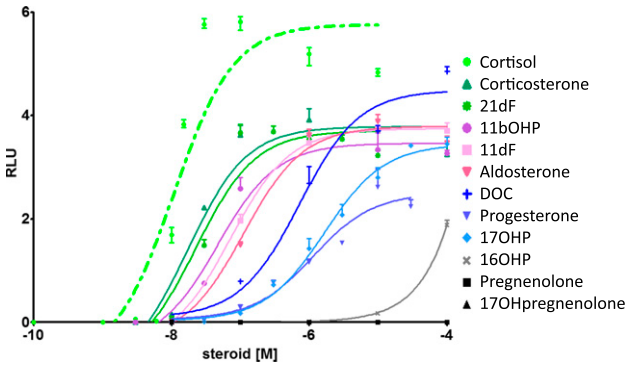

Figure 3. Glucocorticoid receptor transactivation by different adrenal steroids. Glucocorticoid receptor transactivation as measured by a dual-luciferase assay in human embryonic kidney cells that were exposed to increasing amounts of steroids for 24 h. Cortisol was used as a reference. All concentrations were measured in triplicate; mean and range are depicted. 11β-OHP 11b β-hydroxyprogesterone; 11df, 11-deoxycortisol; 16OHP, 16-hydroxyprogesterone; 17OHP, 17-hydroxyprogesterone; 21df, 21-deoxycortisol; DOC, 11-deoxycorticosterone.

and 65 times, respectively, lower compared with cortisol.

Comparison of the transactivation data with the molecular structure of the adrenal steroid precursors shows that dehydrogenation of the steroid precursors by 3β-hydroxysteroid dehydrogenase is a prerequisite to enable a steroid to activate the GR because pregnenolone and 17-hydroxypregnenolone, which are not dehydrogenated, were not able to cause GR transactivation *in vitro*. We also observed that dehydrogenation alone (progesterone) or combined with 21-hydroxylation (11-deoxycorticosterone) or 17-hydroxylation (17-hydroxyprogesterone) resulted in relatively low potency to activate the GR. The combination of dehydrogenation and hydroxylations of position 11 and 21 (corticosterone), position 11 and 17 (21-deoxycortisol), or position 17 and 21 (11-deoxycortisol) increased the potency to activate the GR because EC_{50} values were similar or seven times higher than that of cortisol. The most potent GR activation precursor steroids are 11β-hydroxylated steroids. This confirms the hypothesis of Hellal-Levy *et al.* (11) and Rousseau *et al.* (16) that hydroxylation at position 11 enhances glucocorticoid activity.

To evaluate adrenal function in clinical practice, a clinical threshold of 500 nmol cortisol/L blood after ACTH administration is widely used, although lower thresholds have been proposed (17, 18). All patients in our cohort had stimulated cortisol concentrations far below this threshold. However, cortisol production was not completely blocked, possibly explaining the lack of signs of cortisol deficiency. In addition, the strongly increased concentrations of the 11-hydroxylated steroid precursors 21-deoxycortisol and 11-hydroxyprogesterone in untreated patients with 21OHD, which are able to activate the GR, might contribute to cortisol activity in these untreated patients. Our results suggest that 17-hydroxyprogesterone and progesterone might also contribute because we found increased concentrations in patients with 21OHD and in patients with 11OHD, although the potential to activate the GR is lower. Furthermore, in patients with 11OHD, 11-deoxycortisol and 11-deoxycorticosterone might compensate for the cortisol deficiency because these steroids had good GR activation potency.

Our study has some limitations. In some of the reported stress situations, there may have been an underlying adrenal crisis because some episodes were reported as salt wasting crises or severe vomiting and/or seizures. Still, it is remarkable that all patients recovered from these episodes without administration of glucocorticoid medication. Because neonatal screening is not implemented in Indonesia, the prevalence of patients with CAH is probably higher than the current number of patients diagnosed with CAH, which might have led to the selection of our patient group. Especially male patients with CAH are expected to be underdiagnosed either because they have no complaints or because they have died within the first weeks of life due to a salt wasting crisis.

In our transactivation experiments, only one cell line was used, and only the final step in GR activation was studied. However, our results are consistent with previously published findings. We are confident that the transactivation we detected was a result of the interaction between the added steroids and the GR because we included relevant control experiments where no transactivation was found. We have studied the effect of single-steroid precursors on the GR. Future research involving experiments with coincubation with the different steroid precursors would provide valuable additional information. Because of the *in vitro* nature of the experiments, we cannot draw conclusions on the clinical consequences of the concentrations of the adrenal steroid precursors found in patients with CAH. Further research is necessary to translate the *in vitro* results to clinical practice.

In summary, we describe a unique cohort of untreated patients with 21OHD and untreated patients with 11OHD with CAH with proven cortisol deficiency. We showed that these patients have accumulated adrenal steroid precursors that can activate the GR, especially 21-deoxycortisol, 11-hydroxyprogesterone, 11-deoxycortisol, and 17-hydroxyprogesterone. Further research should focus on establishing cut-off values for cortisol and the contribution of adrenal steroid concentrations in patients with CAH.

Acknowledgments

We thank Joop Heuvel for assistance in the *in vitro* cell culture experiments; Professor Wiebke Arlt, University of Birmingham, for carefully reading our manuscript and giving valuable advice; and all patients who participated in this study.

Financial Support: This work was partially funded by Faculty of Medicine Grant, Diponegoro University, Semarang, Indonesia (PNBP FK.T.A. 2016 number 3817/UN7.3.4/PG/2016) (to A.U).

Author Contributions: M.E., K.J.P.K., A.U., S.M.H.F., A.E.v.H., P.N.S., F.C.S., and H.L.C.v.d.G. conceived and designed the study. K.J.P.K. and A.U. acquired the clinical data. S.O.A. performed the biochemical analysis. M.E. performed the cell culture experiments. M.E., K.J.P.K., and A.U. analyzed and interpreted the data. All authors were involved in drafting or critically revising the manuscript, and all authors gave final approval of the version to be published. All authors agreed to be accountable for all aspects of the work.

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Disclosure Summary: The authors have nothing to disclose.

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