

Research Article

Structural Stereochemistry of Androstene Hormones Determines Interactions with Human Androgen, Estrogen, and Glucocorticoid Receptors

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DHEA, 17 α -AED, 17 β -AED, and 17 β -AET exhibit strong biological activity that has been attributed to androgenic, estrogenic, or antiglucocorticoid activity *in vivo* and *in vitro*. This study compared DHEA, 17 α -AED, 17 β -AED, and 17 β -AET for their ability to activate the human AR, ER, and GR and determine the relative androgenicity, estrogenicity, and glucocorticoid activity. The results show that, at the receptor level, these androstene hormones are weak AR and even weaker ER activators. Direct androstene hormone activation of the human AR, ER α , and ER β may not be essential for their biological function. Similarly, these hormones indirectly activated the human GR, only in the presence of high dexamethasone concentrations. These results underscore the major difference between androstene hormone interactions with these nuclear receptors and their biological effects.

1. Introduction

DHEA, an androstene hormone, has been shown to possess a wide range of beneficial biological effects mainly attributed to immune system modulation [1]. DHEA is metabolized into more active metabolites, that is, 17 β -AED and 17 β -AET, as well as testosterone and estradiol [1, 2]. 17 β -AED and 17 β -AET have been reported to prevent the morbidity and mortality of otherwise lethal infections [3, 4], potentiate lymphocyte activation, and counteract the immune suppressive action

of hydrocortisone [5–7], thus leading to beneficial effects in diverse human diseases including resistance to infection, neuroprotection, wound healing, diabetes, hepatic injury, cardiovascular disease, and cancer [8–10].

17 α -AED mediates autophagy of glial and breast cancers and apoptosis of myeloid tumor cells [11–13]. 17 β -AED and 17 α -AED naturally exist in epimeric forms based on whether the hydroxyl group is above (β) or below (α) the Δ^5 cycloperhydrophenanthrene ring. Addition of a hydroxyl group at the C7 position to 17 β -AED results in the formation

of Δ^5 -androstene- 3β , 7β , 17β -triol (17β -AET). The biological activities of 17α -AED, 17β -AED, and 17β -AET have exhibited a structure-activity relationship that depends on the orientation and location of the hydroxyl groups [13].

Androstene hormones (AH) have been shown to promulgate their biological effects in many different animal models including mice, rats, monkeys, and some specific human tissues. Reports have associated the mechanism of action of androstene hormone metabolites with androgen, estrogen, and glucocorticoid receptor activity [14–16]. Adrenal hormones have been shown to activate both androgen and estrogen constructs. In this regard, it has been documented that 17β -AED can activate the AR in prostate tissue in the presence of commonly used antiandrogens [17]. Inhibitors of both the androgen receptor and the estrogen receptors demonstrated that AR and ER β receptors combine to affect gene transcription [18]. Additionally, 17β -AED was recently shown to be a part of an anti-inflammatory mechanism that utilizes the ER β [19]. 17β -AED and 17β -AET have been documented in vitro and in vivo to oppose the action of hydrocortisone indicating that there may be crosstalk with the GR [6, 20, 21].

DHEA has been shown to possess weak androgenicity and estrogenicity [22]. Because 17α -AED, 17β -AED, and 17β -AET are more potent metabolites of DHEA that exhibit strong biological activity that could be attributed to androgenic, estrogenic, or antiglucocorticoid activity in vivo and in vitro, it was advantageous to identify whether or not this is directly mediated by the human ER, AR, and GR at the cellular level. Additionally, androstanediol has been modeled as a chemical with a 3β -hydroxy and a saturated A ring which can act as an estrogen [23]. Consequently, we compared DHEA, 17α -AED, 17β -AED, and 17β -AET in the Indigo Biosciences nuclear receptor assay system for their ability to activate the human AR, ER, and GR and determine the relative androgenicity and estrogenicity of these androstene hormone derivatives.

2. Materials and Methods

2.1. Nuclear Receptor Transactivation Assays. Nuclear receptor transactivation assays were obtained from Indigo Biosciences (State College, PA, USA) and were utilized to assess the activation of human AR, ER β , and ER α receptors. Briefly, stocks of the compounds tested were prepared and diluted in a medium provided by the manufacturer. Cell medium was tested for hormone activity by mass spectrometry (Section 2.3). Frozen reporter cells provided in the assay kit were thawed and compound dilutions were added immediately. Cells were incubated for 24 hours and the activation response was measured on a luminometer (Perkin-Elmer, MA, USA). The cells consisted of non-human mammalian cells engineered by Indigo Biosciences to provide constitutive high-level expression of full length, unmodified human androgen receptor (NR3C4), human estrogen receptor 1 (NR3A1), human estrogen receptor 2 (NR3A2), and full length, human glucocorticoid receptor (NR3C1).

The nonhuman mammalian reporter cells included a luciferase reporter gene functionally linked to a human

nuclear receptor-responsive promoter. The cells are engineered so that only interactions with the human receptor will induce luciferase expression in the treated reporter cells to quantitate nuclear receptor activation. Positive control ligand performance was measured by the manufacturer and provided in the technical manuals thus allowing accurate comparison for assay performance. Additionally, the control ligands of the receptors (testosterone, 17β -estradiol, and dexamethasone) were tested on the same test plates ($n = 3$ to allow statistical analysis) with the androstene hormones and controls.

2.2. Preparation of Stock Hormone Solutions. Stock solutions of 17β -AED, 17α -AED, and testosterone (Sigma-Aldrich, St. Louis, MO, USA) were prepared by dissolving the compounds into 100% ethanol to a final 50 mM stock solution concentration; 17β -AET was also a stock solution of 50 mM but was dissolved into 1:1 DMSO/ethanol because of solubility issues. Cyproterone acetate (Sigma-Aldrich) was diluted with 100% ethanol and used at a 10 uM concentration. All stock solutions were diluted to final concentrations using the dilution fluid provided in each kit. All tests were performed with negative controls on the same plate and contained media alone and media containing the same amount of ethanol utilized in the stock solutions. All assay control results were in accordance with the stated technical performance specifications.

2.3. Mass Spectrometry. LC-MS/MS analyses of the steroid hormones were carried out using a Shimadzu Nexera UPLC device coupled (Shimadzu Corp, Kyoto, Japan) to AB Sciex (Foster City, CA, USA) 5500 Hybrid Triple Quadrupole Linear Ion Trap Mass Spectrometer operating in multiple reaction monitoring mode. Nitrogen produced by a high-purity nitrogen generator (PEAK Scientific Instruments Ltd, Chicago, IL, USA) was used as curtain, nebulizer, and collision gases. Unit mass resolution was set in both mass-resolving quadrupole Q1 and Q3. Ionization of the analytes was carried out using an APCI source. Multiple MRM transitions were selected for each analyte to eliminate ambiguity in analyte identification. For all steroids other than AED, 25 microliters of the media was directly injected onto a 2.1×50 mm $2.6 \mu\text{m}$ C18 reverse phase column (Phenomenex) and was separated via a linear gradient of water : methanol 98:2 (Solvent A) to methanol : water 70:30 (Solvent B). Both solvents contained 5 mM ammonium formate with 1% formic acid. Separation of 17α -AED and 17β -AED was carried out using 2.1×150 mm $2.6 \mu\text{m}$ C18 reverse phase column (Phenomenex) and was done using same Solvent A as above and 98:2 methanol : water with 5 mM ammonium formate and 1% formic acid as Solvent B. Steroid hormones were detected using precursor product. MRM pairs are as follows: DHEA (271-213, 271-197), androstenetriol (307-158, 307-254), androstanediol (291-95, 291-159, 291-255), testosterone (289-97, 289-109), androstanedione (287-97, 287-109), and 17β -estradiol (273-107, 273-135, 273-77). Where there were multiple transitions, the dominant peak was used in the analysis.

All analytes demonstrated a minimal limit of detection of at least 0.6 nM.

2.3.1. Cellular Uptake of Androstene Hormones and Normalization of Transactivation Assay Results. It was relevant to determine the relative uptake of each androstene hormone in the Indigo Assay System. Cells and cell medium utilized in the assays were provided by the manufacturer (Indigo Biosciences). The following components were found to be below the limit of detection in the cells and cell medium: DHEA, 17 β -estradiol, androstanedione, testosterone, 17 α -AED, 17 β -AED, and 17 β -AET. LCMS data was utilized as a ratio between the background subtracted signal (area under the curve of the LCMS trace) at time zero and 24 hours for each analyte investigated. The ratios were then utilized to create normalization factors for the cellular uptake of 17 α -AED, 17 β -AED, and 17 β -AET as compared to DHEA. The normalization factors were DHEA: 1, 17 α -AED: 1.71, 17 β -AED: 1.85, and 17 β -AET: 2.40, respectively. Background activity due to cells, media, and vehicle was subtracted before the data were normalized.

2.3.2. Metabolism of Androstene Hormones. The cell medium was analyzed before and after incubation with assay cells. Mass spectroscopy (Section 2.3) was utilized to detect androstanedione, 17 β -AED, 17 α -AED, and 17 β -AET, as well as testosterone and 17 β -estradiol that were expressed in the medium. Approximately 9% of DHEA was metabolized to 17 β -AED after a 24-hour incubation with assay cells. No other DHEA metabolites were detected. Mass spectroscopy did not detect any metabolites of the other androstene hormones in the media after a 24-hour incubation period. The mass spectrometry data show that DHEA was minimally metabolized to 17 β -AED in this cell construct, but not to testosterone or 17 β -estradiol (Section 2.3.2). 17 β -AED, 17 β -AET, and 17 α -AED were not metabolized.

2.4. Statistical Analysis. All statistical analyses were performed using SigmaPlot version 12 (SSI, San Jose, CA, USA). Hormone EC₅₀ level and the estrogen receptor alpha activation statistical analyses were performed with a Student's *t*-test while all other hormone activation statistical comparisons were performed with a one-way ANOVA. *P* value levels < 0.05 were considered significant. Statistics on test groups were done before normalization to cellular uptake and were performed between test groups and controls.

3. Results and Discussion

3.1. Androstene Hormone Structures. The hormones that were used in this study are listed in Figure 1. The structures demonstrate the similarities and unique characteristics of each androstene hormone. The main differences are the orientation of the hydroxyl group at position C17 for 17 α -AED and 17 β -AED, the orientation and position of the hydroxyl group at position C7 for 17 β -AET, and the ketone group at position 17 for DHEA. 17 α -AED and 17 β -AED are chemically identical except for the placement of the hydroxyl group in

relation to (above or below) the Δ^5 cycloperhydrophenanthrene ring. All adrenal hormones in this study, with the exception of DHEA, possess hydroxyl groups in the C3 and C17 position with 17 α -AED having the C17 hydroxyl group in the (α) position. This position at C17 results in remarkable biological actions [11, 13] while the hydroxyl group at C3 was shown not to influence the biological activity [24].

3.2. Androstene Hormone Activation of the Human AR. The data demonstrate that both the orientation of the hydroxyl at position C17 and the addition of the hydroxyl at position C7 affected the ability of 17 β -AED, 17 β -AET, and 17 α -AED to activate the human AR construct (Figure 2). The AR construct contains a luciferase reporter gene that is functionally linked to an AR responsive promoter. The luciferase reading is utilized as a surrogate measure for AR binding. The EC₅₀ was calculated for testosterone as the 50% activation point. EC₅₀ values for the androstene hormones and testosterone were calculated (Table 1) and compared utilizing the ratio of the androstene hormone EC₅₀ to testosterone EC₅₀ (relative androgenicity). All of the androstene hormones tested showed a significant (*P* < 0.001) reduced androgenicity when compared to testosterone (Table 1). 17 β -AED had only 1/5th the ability of testosterone to activate the androgen receptor. Changing the orientation of the C17 hydroxyl group on 17 α -AED resulted in a further reduction to 1/60th the activity as compared to testosterone. Addition of the hydroxyl group to the C7 position further reduced the ability to activate the human AR construct to 1/1326th as that of testosterone. The androstene hormone activation of the human AR was rank ordered based on strength of activation. The rank order was 17 β -AED \gg 17 α -AED $\gg\gg$ 17 β -AET (Figure 2). DHEA binding to the AR was excluded from these experiments since its androgenicity has been reported previously [22].

3.3. Androstene Hormone Activation of the Human ER β and ER α Receptors. The data demonstrate that both the orientation of the hydroxyl at position C17 and the addition of the hydroxyl at position C7 affected the ability of DHEA, 17 β -AED, 17 β -AET, and 17 α -AED to activate the human ER β construct (Figure 3). The ER construct contains a luciferase reporter gene that is functionally linked to an ER responsive promoter. The luciferase reading is utilized as a surrogate measure for ER binding. The EC₅₀ was calculated for 17 β -estradiol as the 50% activation point. EC₅₀ values for the androstene hormones and 17 β -estradiol were calculated (Table 2) and compared utilizing the ratio of the androstene hormone EC₅₀ to 17 β -estradiol EC₅₀ (relative estrogenicity). All of the tested androstene hormones demonstrated a significantly (*P* < 0.001) decreased estrogenicity compared to 17 β -estradiol (Table 2). 17 β -AED had only 1/282nd the ability of 17 β -estradiol to activate the ER β . The orientation change of the hydroxyl group at position C17 of 17 β -AED to the (α) position resulted in 17 α -AED possessing 1/7609th the ability of 17 β -estradiol to activate the ER β receptor as 17 β -estradiol. This represents a drastic decrease in estrogenicity from the 17 β -AED epimer. The presence of the hydroxyl group at C7 of 17 β -AET resulted in 1/587th the ability to activate the ER β .

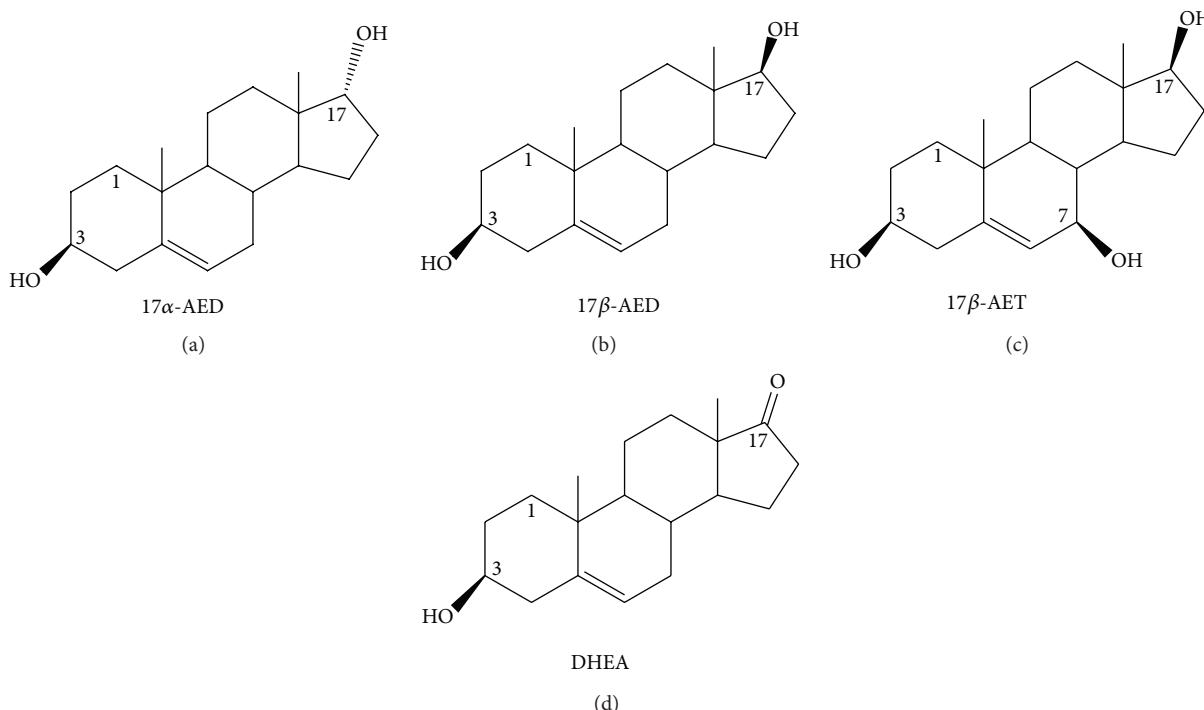


FIGURE 1: The structures of the androstene hormones. The androstene hormones are shown with the Δ^5 cycloperhydrophenanthrene ring. All steroids have a C3 hydroxyl group in the (β) beta position. The C7 hydroxyl group of androstenetriol is in the β -position. The C17 hydroxyl of androstenediol epimers are in either the (α) alpha or (β) beta position.

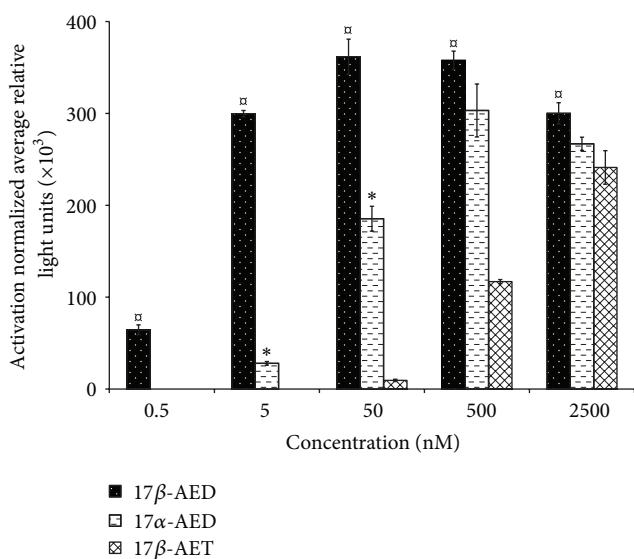


FIGURE 2: Androstene hormone activation of the human androgen receptor. Reporter cells were treated with androstene hormone metabolites ($n = 3$), incubated for 24 hours, and then assayed for luciferase activity. Androstene hormone activity was normalized to cellular uptake (Section 2.3.1). Error bars, ± 1 SD. Statistical significance, $P < 0.001$ from all less activating androstene hormone metabolites (\blacktriangle), $P < 0.001$ from less activating androstene hormone metabolites (*).

TABLE 1: Relative androgenicity of androstene hormones.

Hormone	EC_{50} (nM)	Androgenicity	P value
Testosterone	0.35	1	1
Δ^5 -Androstene-3 β , 17 β -diol	1.8	1/5	<0.001
Δ^5 -Androstene-3 β , 17 α -diol	21	1/60	<0.001
Δ^5 -Androstene-3 β , 7 β , 17 β -triol	464	1/1326	<0.001

The EC_{50} was calculated as the 50% activation point. The androgenicity is the testosterone EC_{50} divided by the androstene hormone EC_{50} . This data does not normalize the cellular uptake of androstene hormones.

DHEA, with a ketone group in the C17 position, possessed 1/3543rd the ability to activate the ER β .

The rank order of androstene hormone activation on the human ER β receptor can be displayed as follows: 17 β -AED > 17 β -AET > DHEA > 17 α -AED (Figure 3). These androstene hormones also specifically activated the human ER β receptor and demonstrated the crucial effect of the (β) C17 hydroxyl group. 17 β -AED and 17 α -AED activated the ER β receptor 2 and 3 orders of magnitude lower, respectively, than 17 β -estradiol (Table 2). The 17 β -AED demonstrated an estrogenicity of 1/1176 when assayed on the human ER α . Activation of the ER α receptor by 17 β -AED did not become apparent until the concentration reached 25 nM (Figure 4) which was 3 orders of magnitude lower than 17 β -estradiol, further demonstrating the weak estrogenicity displayed by these hormones at the level of the ERs. Finally, it should be

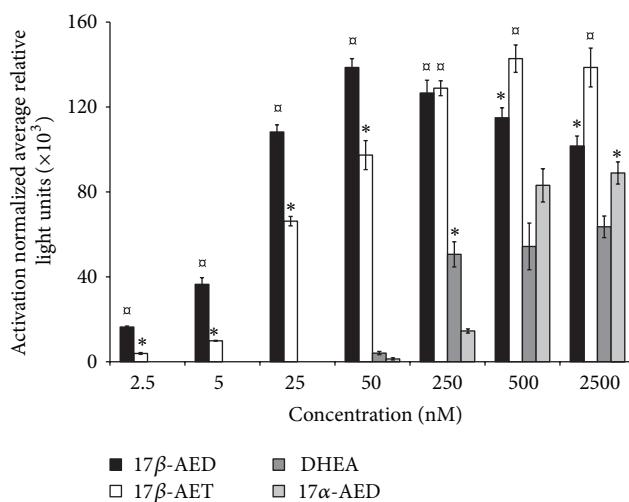


FIGURE 3: Androstene hormone activation of the human estrogen receptor beta. Reporter cells were treated with androstene hormones ($n = 3$), incubated for 24 hours, and then assayed for luciferase activity. Androstene hormone activity was normalized to cellular uptake (Section 2.3.1). Error bars, ± 1 SD. Statistical significance, $P < 0.001$ (☒) versus androstene hormone metabolites in the same treatment group, $P < 0.001$ (*) versus lower reacting androstene hormones in the same treatment group.

TABLE 2: Relative estrogenicity of androstene hormones.

Hormone	EC ₅₀ (nM)	Estrogenicity	P value
17β-Estradiol	0.046	1	1
Δ ⁵ -Androstene-3β, 17β-diol	13	1/282	<0.001
Δ ⁵ -Androstene-3β, 7β, 17β-triol	27	1/587	<0.001
DHEA	163	1/3543	<0.001
Δ ⁵ -Androstene-3β, 17α-diol	350	1/7609	<0.001

The EC₅₀ was calculated as the 50% activation point. The estrogenicity is the 17β-estradiol EC₅₀ divided by the androstene hormone EC₅₀. This data does not normalize the cellular uptake of androstene hormones.

noted that the androstene hormones only weakly activated the AR and were even weaker activators of the human ERs.

3.4. Androstene Hormones Activation of the Human Glucocorticoid Receptor (GR). 17β-AED and especially 17β-AET are known to produce significantly affected glucocorticoid activity in vivo [6, 20, 21]. Therefore, the human GR construct response to 17β-AED, 17β-AET, DHEA, and 17α-AED alone and in combination with dexamethasone was evaluated. The results showed that dexamethasone alone activated the human GR while 17β-AED, 17β-AET, DHEA, and 17α-AED alone were negative at all concentrations tested (Figure 5). The human GR was then tested for activity with androstene hormones in the presence of dexamethasone. Unexpectedly, at suprapharmacological levels of dexamethasone there was a considerable activation of the human GR by 1μM of each of the androstene hormones, which was greater than with dexamethasone alone with a higher activity when dexamethasone concentration was increased from 333 pM to 1000 pM (Figure 6).

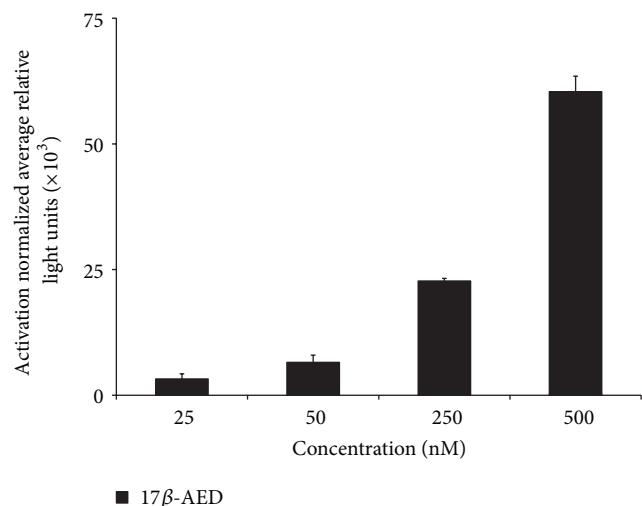


FIGURE 4: 17β-AED activation of the human estrogen receptor alpha. Reporter cells were treated with 17β-AED ($n = 3$), incubated for 24 hours, and then assayed for luciferase activity. 17β-AED activity was normalized to cellular uptake (Section 2.3.1), error bars, ± 1 SD.

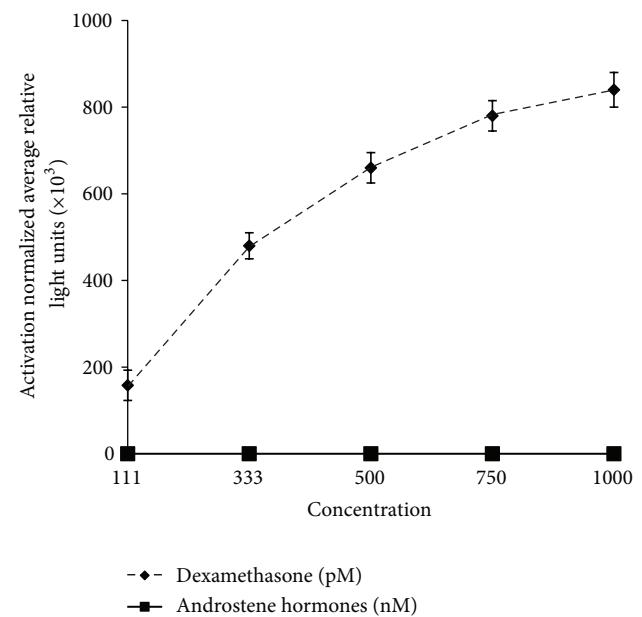


FIGURE 5: Dexamethasone or androstene hormone activation of the human glucocorticoid receptor. Reporter cells were treated with dexamethasone or androstene hormones alone ($n = 3$), incubated for 24 hours, and then assayed for luciferase activity. Androstene hormones are DHEA, 17β-AED, 17α-AED, and 17β-AET. Error bars, ± 1 SD.

The rank order of activation of the dexamethasone-bound human GR in the presence of the androstene hormones is as follows: 17β-AET > 17β-AED > 17α-AED > DHEA. These data demonstrate that the C7 hydroxyl present in 17β-AET produced the strongest activation of the dexamethasone-bound human GR. The (β) C17 hydroxyl of 17β-AED produced a stronger activation than did the (α) C17 hydroxyl of 17α-AED. Thus, while 17β-AED and 17α-AED produced

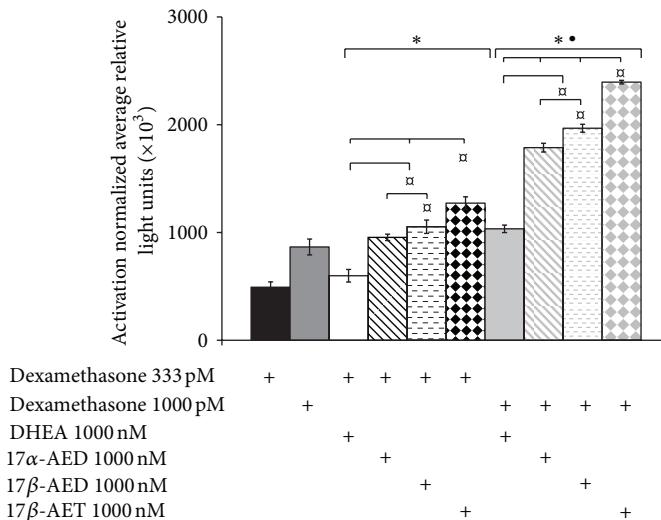


FIGURE 6: Androstene hormones and dexamethasone activation of the human GR. Reporter cells were treated with dexamethasone alone or a combination of dexamethasone and androstene hormone, incubated for 24 hours, and then assayed for luciferase activity ($n = 3$). Androstene hormone activity was normalized to cellular uptake (Section 2.3.1). Error bars, ± 1 SD. Statistical significance, P is at least < 0.05 from other androstene hormone metabolites within the dexamethasone concentration treatment group (☒), P is at least < 0.05 versus control (*), P is at least < 0.05 from 333 pM dexamethasone treatment group (•).

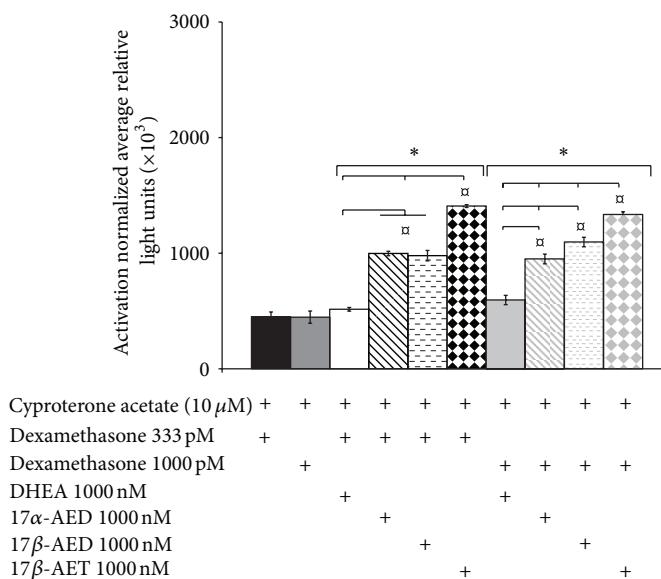


FIGURE 7: Androstene hormone and dexamethasone activation of the human GR in the presence of cyproterone acetate. Reporter cells were treated with dexamethasone and cyproterone acetate alone or androstene hormones with dexamethasone and cyproterone acetate ($n = 3$), incubated for 24 hours, and then assayed for luciferase activity. Androstene hormone activity was normalized to cellular uptake (Section 2.3.1). Error bars, ± 1 SD. Statistical significance, P is at least < 0.05 from other androstene hormone metabolites within the treatment group (☒), $P < 0.05$ from controls (*).

unique activation of the dexamethasone-bound human GR, the effect of the C17 hydroxyl group conformation was less apparent. DHEA, which has the keto group in the C17 position, possessed the least ability to activate the dexamethasone-bound human GR.

We next tested these effects with the dexamethasone inhibitor, cyproterone acetate, to see if we could reduce or eliminate the dexamethasone and androstene hormone

activation of the human GR. Cyproterone acetate was selected as the inhibitor because of its unique glucocorticoid receptor inhibiting properties [25]. Cyproterone effectively inhibited the activation of the human GR by dexamethasone (Figure 7). Cyproterone acetate at a concentration of 10 μ M was tested in the presence of the androstene hormones alone (1.0 μ M) and there was no activation detected (data not shown). Androstene hormones, however, in the presence of

ciproterone and dexamethasone exhibited different levels of activation that were significantly increased above the dexamethasone/ciproterone alone controls (Figure 7). These results are of particular clinical significance because it demonstrates that high-dose dexamethasone alters the human GR to interact with other biologically active hormones at the receptor level. Importantly, dexamethasone is known to cause adverse effects in humans [26].

The rank order of activation of the dexamethasone/ciproterone acetate-bound human GR in the presence of these androstene hormones is as follows: 17 β -AET > 17 β -AED > 17 α -AED > DHEA. This rank order of activation on the inhibited human GR was the same as that of the uninhibited human GR indicating that the interaction of the androstene hormones and the dexamethasone/ciproterone-bound human GR was not disrupted. Since ciproterone acetate is a passive inhibitor of the human GR and opposes dexamethasone through an overlapping steroid scaffold mechanism, this suggests that the androstene hormone activation is mediated by an interaction that occurs outside the dexamethasone/ciproterone acetate-bound complex [25]. Additionally, the presence of dexamethasone-bound human GR is required to observe activation by the androstene hormones while ciproterone acetate alone does not mediate this effect. Together these data suggest an indirect activation of the ligand-bound human GR by 17 β -AET, 17 β -AED, 17 α -AED, and DHEA.

4. Conclusion

This report indicates that the position of the hydroxyl group at C17 and/or the addition of the hydroxyl group at position C7 significantly affected the ability of 17 β -AET, 17 β -AED, 17 α -AED, and DHEA to interact with the human estrogen, androgen, and ligand-bound glucocorticoid receptors. 17 β -AET, 17 β -AED, 17 α -AED, and DHEA were shown to interact either directly or indirectly with the human AR, ER, and GR. Importantly, 17 β -AET, 17 β -AED, 17 α -AED, and DHEA were shown to possess weak androgenicity and even weaker estrogenicity at the receptor level. Clinically, this is beneficial because the biological effects can be realized without unwanted androgenic or estrogenic effects.

In a stark contrast to the minimal receptor activation of AR, ER, and GR, these same androstene hormones produce striking biological effects *in vitro* and *in vivo* which have been attributed to activity with the AR, ER, or GR. Clearly, these effects may not be mediated by the direct androstene hormone interaction with the human AR, ER α , ER β , and GR. Indeed, the biological mechanism, may not require AR or ER to achieve significant effects [27, 28]. Furthermore, the interaction with dexamethasone is indirect, occurs at high doses, and is not abolished by ciproterone acetate. Taken together, the data shows that interactions of 17 β -AET, 17 β -AED, 17 α -AED, and DHEA with the human AR, ER α , ER β , and GR are directed by the structure activity of these androstene hormones with minimal androgenic, estrogenic, or glucocorticoid effects and accentuates the need to further uncover the implied yet unidentified main mechanism(s) of action of these important adrenal hormones.

Abbreviations

17 β -AED:	Δ^5 -androstene-3 β , 17 β -diol
17 α -AED:	Δ^5 -androstene-3 β , 17 α -diol
17 β -AET:	Δ^5 -androstene-3 β , 7 β , 17 β -triol
Androstene hormones:	17 β -AED, 17 α -AED, 17 β -AET, DHEA
Androstene hormone metabolites:	17 β -AED, 17 α -AED, 17 β -AET
DHEA:	Dehydroepiandrosterone
AR:	Androgen receptor
ER α :	Estrogen receptor alpha
ER β :	Estrogen receptor beta
GR:	Glucocorticoid receptor
(α):	Alpha position
(β):	Beta position.

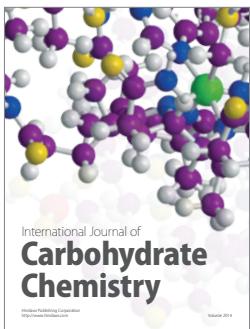
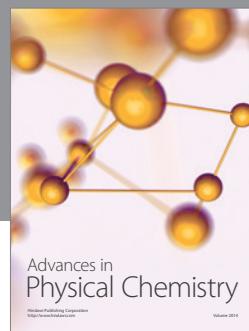
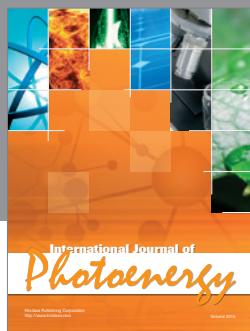
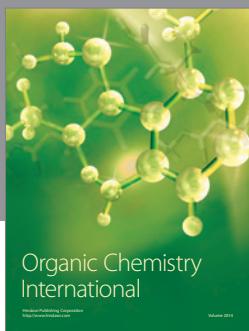
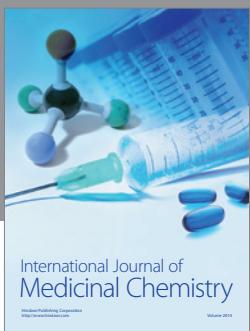
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References

- [1] J. S. Dillon, "Dehydroepiandrosterone, dehydroepiandrosterone sulfate and related steroids: their role in inflammatory, allergic and immunological disorders," *Current Drug Targets*, vol. 4, no. 3, pp. 377–385, 2005.
- [2] R. Oberbeck and P. Kobbe, "DEhydroepiandrosterone (DHEA): a steroid with multiple effects. Is there any possible option in the treatment of critical illness?" *Current Medicinal Chemistry*, vol. 17, no. 11, pp. 1039–1047, 2010.
- [3] R. M. Loria and D. A. Padgett, "Androstenediol regulates systemic resistance against lethal infections in mice," *Annals of the New York Academy of Sciences*, vol. 685, pp. 293–295, 1993.
- [4] R. M. Loria and D. A. Padgett, "Mobilization of cutaneous immunity for systemic protection against infections," *Annals of the New York Academy of Sciences*, vol. 650, pp. 363–366, 1992.
- [5] R. M. Loria, D. A. Padgett, and P. N. Huynh, "Regulation of the immune response by dehydroepiandrosterone and its metabolites," *Journal of Endocrinology*, vol. 150, pp. S209–S220, 1996.

- [6] R. M. Loria, "Antiglucocorticoid function of androstenetriol," *Psychoneuroendocrinology*, vol. 22, supplement 1, pp. S103–S108, 1997.
- [7] D. A. Padgett, R. M. Loria, and J. F. Sheridan, "Endocrine regulation of the immune response to influenza virus infection with a metabolite of DHEA-androstenediol," *Journal of Neuroimmunology*, vol. 78, no. 1-2, pp. 203–211, 1997.
- [8] F. Svec and J. R. Porter, "The actions of exogenous dehydroepiandrosterone in experimental animals and humans," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 218, no. 3, pp. 174–191, 1998.
- [9] C. D. Santos, M. P. A. Toldo, F. H. Santello, M. D. V. Filipin, V. Brazão, and J. C. do Prado Júnior, "Dehydroepiandrosterone increases resistance to experimental infection by *Trypanosoma cruzi*," *Veterinary Parasitology*, vol. 153, pp. 238–243, 2008.
- [10] V. R. Feeser, N. B. Menke, K. R. Ward, R. M. Loria, and R. F. Diegelmann, "Androstenediol reverses steroid-inhibited wound healing: biomedical hypothesis article," *Wound Repair and Regeneration*, vol. 17, no. 5, pp. 758–761, 2009.
- [11] P. Huynh and R. Loria, "Contrasting effects of alpha- and beta-androstenediol on oncogenic myeloid cell lines *in vitro*," *Journal of Leukocyte Biology*, vol. 62, no. 2, pp. 258–2267, 1997.
- [12] M. R. Graf, W. Jia, and R. M. Loria, "The neuro-steroid, 3β androstene 17α diol exhibits potent cytotoxic effects on human malignant glioma and lymphoma cells through different programmed cell death pathways," *British Journal of Cancer*, vol. 97, no. 5, pp. 619–627, 2007.
- [13] M. R. Graf, W. Jia, M. L. Lewbart, and R. M. Loria, "The anti-tumor effects of androstene steroids exhibit a strict structure-activity relationship dependent upon the orientation of the hydroxyl group on carbon-17," *Chemical Biology and Drug Design*, vol. 74, no. 6, pp. 625–629, 2009.
- [14] H. Miyamoto, S. Yeh, H. Lardy, E. Messing, and C. Chang, " $\delta 5$ -Androstenediol is a natural hormone with androgenic activity in human prostate cancer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 19, pp. 11083–11088, 1998.
- [15] R. Hackenberg, I. Turgetto, A. Filmer, and K. D. Schulz, "Estrogen and androgen receptor mediated stimulation and inhibition of proliferation by androst-5-ene- $3\beta,17\beta$ -diol in human mammary cancer cells," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 46, no. 5, pp. 597–603, 1993.
- [16] C. C. Head, M. J. Farrow, J. F. Sheridan, and D. A. Padgett, "Androstenediol reduces the anti-inflammatory effects of restraint stress during wound healing," *Brain, Behavior, and Immunity*, vol. 20, no. 6, pp. 590–596, 2006.
- [17] P. Marwah, A. Marwah, H. A. Lardy, H. Miyamoto, and C. Chang, "C19-Steroids as androgen receptor modulators: design, discovery, and structure-activity relationship of new steroidal androgen receptor antagonists," *Bioorganic and Medicinal Chemistry*, vol. 14, no. 17, pp. 5933–5947, 2006.
- [18] J. T. Arnold, X. Liu, J. D. Allen, H. Le, K. K. McFann, and M. R. Blackman, "Androgen receptor or estrogen receptor- β blockade alters DHEA-, DHT-, and E2-induced proliferation and PSA production in human prostate cancer cells," *Prostate*, vol. 67, no. 11, pp. 1152–1162, 2007.
- [19] K. Saijo, J. G. Collier, A. C. Li, J. A. Katzenellenbogen, and C. K. Glass, "An ADIOL-ER β -CtBP transrepression pathway negatively regulates microglia-mediated inflammation," *Cell*, vol. 145, no. 4, pp. 584–595, 2011.
- [20] D. A. Padgett and R. M. Loria, "*In vitro* potentiation of lymphocyte activation by dehydroepiandrosterone, androstenediol, and androstenetriol," *The Journal of Immunology*, vol. 153, no. 4, pp. 1544–1552, 1994.
- [21] R. M. Loria and D. A. Padgett, "Androstenediol regulates systemic resistance against lethal infections in mice," *Archives of Virology*, vol. 127, no. 1–4, pp. 103–115, 1992.
- [22] F. Chen, K. Knecht, E. Birzin et al., "Direct agonist/antagonist functions of dehydroepiandrosterone," *Endocrinology*, vol. 146, no. 11, pp. 4568–4576, 2005.
- [23] M. E. Baker, "Co-evolution of steroidogenic and steroid-inactivating enzymes and adrenal and sex steroid receptors," *Molecular and Cellular Endocrinology*, vol. 215, no. 1–2, pp. 55–62, 2004.
- [24] R. M. Loria and M. R. Graf, "17 α -androstenediol-mediated oncophagy of tumor cells by different mechanisms is determined by the target tumor," *Annals of the New York Academy of Sciences*, vol. 1262, no. 1, pp. 127–133, 2012.
- [25] C. Honer, K. Nam, C. Fink et al., "Glucocorticoid receptor antagonism by cyproterone acetate and RU486," *Molecular Pharmacology*, vol. 63, no. 5, pp. 1012–1020, 2003.
- [26] K. Matsumoto, H. Yamasaki, S. Akazawa et al., "High-dose but not low-dose dexamethasone impairs glucose tolerance by inducing compensatory failure of pancreatic β -cells in normal men," *Journal of Clinical Endocrinology and Metabolism*, vol. 81, no. 7, pp. 2621–2626, 1996.
- [27] P. N. Huynh, W. H. Carter, and R. M. Loria, "17 Alpha androstenediol inhibition of breast tumor cell proliferation in estrogen receptor-positive and -negative cell lines," *Cancer Detection and Prevention*, vol. 24, no. 5, pp. 435–444, 2000.
- [28] R. Loria, M. Graf, W. Jia, and T. Powell, "5-androstene $3\beta,17\alpha$ diol induces autophagic cell death of human breast cancer cells and enhances radiation cytotoxicity," *Journal of Cancer Therapeutics and Research*, vol. 1, no. 1, 25 pages, 2012.




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