Inhibition of Human Steroidogenic Cytochrome P450 c17 by 21-Hydroxypregnenolone and Related Steroid Hormones

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The effects of 21-hydroxypregnenolone and related steroids such as deoxycorticosterone (DOC; 21-hydroxyprogesterone), cortisol, and corticosterone on progesterone 17α-hydroxylase activity by steroidogenic cytochrome P450 c17 (CYP17) were investigated. 21-Hydroxypregnenolone contains a hydroxyl group at C3 in the A cyclic hydrocarbon ring and a double bond at C5 in the B cyclic hydrocarbon ring, whereas DOC, cortisol, and corticosterone contain a ketone group at C3 and a double bond at C4 in the A cyclic hydrocarbon ring. Nonetheless, 21-hydroxypregnenolone exhibited competitive inhibition of progesterone 17α-hydroxylation activity by CYP17 with a Ki value of 36.4 µM. These results suggest that a hydroxyl group at C3 in the A ring and a double bond at C5 in the B ring in steroid hormones are important for the substrate recognition of CYP17.

Key words cytochrome P450 c17; 21-hydroxypregnenolone; 17α-hydroxylation; deoxycorticosterone; corticosterone; progesterone

Cytochrome P450 c17 (CYP17) is found in the endoplasmic reticulum of the adrenal cortex and gonads, and mediates both 17α-hydroxylase and 17,20-lyase reactions of pregnenolone and progesterone, thus being involved in the biosynthesis of glucocorticoids and sex hormones.1) Various steroid hormones are biotransformed by metabolic enzymes including CYP11 and CYP21 as well as CYP17, in adrenal glands (Fig. 1). Deoxycorticosterone (DOC; 21-hydroxyprogesterone), cortisol, and corticosterone are well known to be important in the principal pathways of steroid hormone synthesis.2) 21-Hydroxypregnenolone has been shown to be biotransformed from pregnenolone in human, rat, and sheep adrenal tissue in vitro,3,4) although how 21-hydroxypregnenolone is formed in human tissues or organs remains unknown. 21-Hydroxypregnenolone is detectable in urine of newborn human infants5) and adults,6) and 21-hydroxypregnenolone excretion is highly elevated in 21-hydroxylase deficiency.7) 21-Hydroxypregnenolone contains a hydroxyl group at C3 in the A cyclic hydrocarbon ring and a double bond at C5 in the B cyclic hydrocarbon ring, whereas DOC, cortisol, and corticosterone contain a ketone group at C3 and a double bond at C4 in the A ring. In particular, DOC is identical to 21-hydroxypregnenolone except at C3, C4, and C5 positions. Thus there is a possibility that biotransformed steroid hormones might affect human steroidogenesis in the adrenal gland.

We previously demonstrated that endocrine disrupters such as bisphenol A and nonylphenol exhibited competitive inhibition of progesterone 17α-hydroxylation by human CYP17.8,9) In the present study we investigated the inhibitory effects of 21-hydroxypregnenolone, DOC, cortisol, and corticosterone on catalytic activity of recombinant human CYP17 expressed in Escherichia coli membrane fractions.

MATERIALS AND METHODS

Materials Expression of CYP17 in Escherichia coli and preparation of membrane fractions from the cells were carried

The authors declare no conflict of interest.

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out according to methods described previously.\(^{(10)}\) Progesterone, 21-hydroxypregnenolone, DOC, 17α-hydroxyprogesterone, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Hydrocortisone acetate and testosterone were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Human NADPH-oxidoreductase (POR) was purchased from PanVera LLC (Madison, WI, U.S.A.). All other reagents were of the highest purity commercially available.

**Assay of Progesterone 17α-Hydroxylation Activity** Progesterone 17α-hydroxylation activity was determined by methods described previously\(^{(8,9)}\) with minor modification. Briefly, the membrane fraction containing CYP17 (120 pmol) and POR (210 pmol) was preincubated at 37°C for 3 min, and the mixture was brought to 500 µL; the incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), 10 mM magnesium acetate, 1 mM NADPH, 1 µM (for inhibition by various steroids) or 5–100 µM (for estimation of inhibition constants \([K_i, \text{values}]) progesterone, and 0, 20, 50, or 100 µM steroid hormone. The reaction was started by addition of NADPH; incubation was carried out at 37°C for 10 min. The reaction was terminated by addition of 2 mL of ethyl acetate; 50 µL of 10 µM testosterone was added as internal standard. After ethyl acetate extraction, the organic phase (1.5 mL) was evaporated under nitrogen, the residue was dissolved in 200 µL of the HPLC mobile phase, and 50 µL of the samples were injected into HPLC. The HPLC system consisted of an L-7100 pump, an L-7400 UV-detector (Hitachi, Japan) set at 240 nm, and a D-7500 integrator (Hitachi). A column (4.6×250 mm) packed with Cosmosil 5C18-AR-II (Nacalai Tesque, Kyoto, Japan) was used. The mobile phase consisted of 50% acetonitrile in potassium phosphate buffer (pH 7.4), 10 mM acetate, 1 mM NADPH, 1 µM (for estimation of inhibition constants \([K_i, \text{values}]) progesterone, and 0, 20, 50, or 100 µM steroid hormone. Results are mean or mean±S.D. of 2–5 experiments. Values in parentheses are number of experiments.

**Kinetic Analysis** \(K_i\) values were estimated by fitting the inhibition curves to Eq. 1 or Eq. 2, when the inhibition type was competitive or noncompetitive, respectively, as follows:

\[
v = \frac{V_{\text{max}} \cdot S}{K_s (1 + I / K_i) + S}
\]

or

\[
v = \frac{V_{\text{max}}}{1 + I / K_s} \cdot \frac{S}{K_m + S}
\]

where \(v, S, I, V_{\text{max}}, K_m\), and \(K_s\) are the velocity of the metabolite formation and the concentrations of substrate and inhibitor, the maximum velocity of the metabolite formation, and the apparent Michaelis–Menten constant, respectively. These equations were fitted to data by means of a computer program (MULTI)\(^{(11)}\); the fitting evaluation was carried out using Akaike’s information criterion.\(^{(12)}\)

**RESULTS**

The inhibitory effect of steroid hormones (100 µM) on CYP17-mediated progesterone 17α-hydroxylation was investigated (Table 1). Although no marked inhibition was observed for DOC, cortisol, and corticosterone, only 21-hydroxypregnenolone markedly inhibited the activity (81% inhibition).

The inhibitory effect of 21-hydroxypregnenolone on progesterone 17α-hydroxylation by CYP17 was investigated in the concentration range 20–100 µM (Fig. 2). In the absence of 21-hydroxypregnenolone, \(K_m\) and \(V_{\text{max}}\) values for progesterone 17α-hydroxylation by CYP17 were 35.0 µM and 8.82 nmol/min/nmol CYP, respectively; \(V_{\text{max}} / K_m\) value was 0.252 mL/min/nmol CYP. In the presence of increasing 21-hydroxypregnenolone concentrations, a concentration-dependent inhibition of progesterone 17α-hydroxylation was characterized by an increasing \(K_m\) value, whereas \(V_{\text{max}}\) value remained constant. This is consistent with a competitive-type inhibition of progesterone 17α-hydroxylation by 21-hydroxypregnenolone; the \(K_i\) value was calculated to be 36.4±10.0 µM.

**DISCUSSION**

We demonstrated that 21-hydroxypregnenolone, which contains a hydroxyl group at C17 in the A cyclic hydrocarbon ring and a double bond at C4 in the B cyclic hydrocarbon ring, exhibited competitive inhibition of progesterone 17α-hydroxylation activity by CYP17 with a \(K_i\) value of 36.4 µM. In addition, we preliminarily obtained the type I differential spectra of CYP17 binding with 21-hydroxypregnenolone (data not shown). On the other hand, no marked inhibition was observed for DOC, cortisol, and corticosterone, which contain a ketone group at C17 and a double bond at C4 in the A ring. In particular, DOC (21-hydroxyprogesterone) is identical to 21-hydroxypregnenolone except at C3, C4, and C5 positions.
As summarized in Table 2,10,12–15 it has been reported that the IC₅₀ value of abiraterone, which also contains a hydroxyl group at C₃ in the A ring and a double bond at C₅ in the B ring, was 0.072 μM.13) 4-Pregnene-3-one-20β-carboxaldehyde (22-A), which contains a ketone group at C₃ in the A ring and a double bond at C₅ in the B ring, exhibited competitive inhibition of progestosterone 17α-hydroxylation and 17α-hydroxyprogesterone C₁₇,₂₀-lyation by CYP17 with a Kᵢ value of 8.48 and 0.41 μM, respectively.13) Thus it seems that steroids that contain a double bond in the B ring in the CYP17 model inhibit CYP17 activity. Mathieu et al.16) inserted four CYP17 substrates such as pregnenolone, progesterone, and their 17α-hydroxylated forms inside the hamster CYP17 model, performed molecular dynamics simulations on the complexes, and analyzed the resultant trajectories to identify amino acids that interact with substrates. Their results suggest the entry of the substrate into the active site directed by possible interactions or hydrogen bonding to the heme D-ring propionate group, R₉₆ and R₄₄₀ difference in binding of 21-hydroxypregnenolone and DOC droxyprogesterone C₁₇,₂₀-lyation by CYP17 with a substrate concentration, 1)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>17-Hydroxylation</th>
<th>C₁₇,₂₀-Lyation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxycorticosterone (DOC, 21-hydroxyprogesterone)</td>
<td>—</td>
<td>—</td>
<td>Present finding.</td>
</tr>
<tr>
<td>Cortisol</td>
<td>—</td>
<td>—</td>
<td>Present finding.</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>—</td>
<td>—</td>
<td>Present finding.</td>
</tr>
<tr>
<td>21-Hydroxypregnenolone</td>
<td>C 36.4</td>
<td>C 0.41</td>
<td>Present finding.</td>
</tr>
<tr>
<td>4-Pregnene-3-one-20β-carboxaldehyde (22-A)</td>
<td>8.48</td>
<td></td>
<td>Li et al.13)</td>
</tr>
<tr>
<td>Abiraterone</td>
<td>— 0.072</td>
<td>—</td>
<td>Hille et al.10) Substrate concentration: 25 μM</td>
</tr>
<tr>
<td>Ketocanazole</td>
<td>M 39.5</td>
<td>M 3.6</td>
<td>Li et al.13)</td>
</tr>
<tr>
<td>Ketocanazole</td>
<td>— 2.78</td>
<td>—</td>
<td>Hille et al.10) Substrate concentration: 25 μM</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>C 71</td>
<td>—</td>
<td>Niwa et al.15)</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>— 18.99</td>
<td>—</td>
<td>Ye et al.15) Substrate concentration: 1 μM (below Kᵢ)</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>C 61</td>
<td>—</td>
<td>Niwa et al.15)</td>
</tr>
</tbody>
</table>

17α-Hydroxylation and C₁₇,₂₀-lyation activities were determined by using progesterone and 17α-hydroxyprogesterone, respectively, as a substrate. C: competitive inhibition, M: mixed type inhibition. a) The values indicate IC₅₀. b) No inhibition was observed at 100 μM.

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REFERENCES


