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CYP3A4 AND CYP3A7-MEDIATED CARBAMAZEPINE 10,11-EPOXIDATION ARE ACTIVATED BY DIFFERENTIAL ENDOGENOUS STEROIDS

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ABSTRACT:

Recently, we reported that several endogenous steroids affect CYP3A4-mediated drug metabolism, using human adult liver microsomes as an enzyme source. Especially, carbamazepine (CBZ) 10,11-epoxidation is activated by androstenedione (AND). In the present studies, we investigated the effects of endogenous steroids on the activity of CBZ 10,11-epoxidation by expressed CYP3A4 and CYP3A7. When expressed CYP3A4 was used as an enzyme source, the addition of AND to the reaction mixture also caused a drastic increase in the activity of CBZ 10,11-epoxidase, and resulted in a change in the kinetics from sigmoid to Michaelis-Menten type. On the other hand, expressed CYP3A7-mediated CBZ 10,11-epoxidation was activated by sulfate conjugate steroids, such as pregnenolone 3-sulfate, 17α-hydroxypregnenolone 3-sulfate, and dehydroepiandrosterone 3-sulfate (DHEA-S), whereas the unconjugated form corresponding to these three steroids did not activate the reaction. Especially, DHEA-S was found to be a potent activator of CBZ 10,11-epoxidation by expressed CYP3A7. The kinetic character of CBZ 10,11-epoxidation by CYP3A7 is Michaelis-Menten type regardless of the presence of DHEA-S. The presence of DHEA-S caused a decrease in K_m and increase in V_{max} for CYP3A7-mediated CBZ 10,11-epoxidation, whereas DHEA-S 16α-hydroxylation was not affected by the coexistence of CBZ. In conclusion, CYP3A4 and CYP3A7-mediated CBZ 10,11-epoxidations are activated by different types of endogenous steroids. This is the first report regarding CYP3A7 cooperativity.

Cytochrome P450s (P450s[1]) comprise a superfamily of enzymes that play important roles in the metabolism of drugs as well as endogenous substrates such as steroids, fatty acids, and prostaglandins (Nelson et al., 1996). The human CYP3A enzymes represent one of the most versatile forms of P450, and have been demonstrated to derive from at least four genes (Beaune et al., 1986; Aoyama et al., 1989; Komori et al., 1989; Domanski et al., 2001). These genes encode four highly related proteins referred to as CYP3A4, CYP3A5, CYP3A7, and CYP3A43. Among these CYP3A enzymes, CYP3A4 is the major form of P450 expressed in adult liver and has been shown to react with large numbers of structurally unrelated chemicals. In contrast, it has been demonstrated that CYP3A7, a member of the human CYP3A family, is the major form of P450 expressed in human fetal liver (Kitada et al., 1985).

Although CYP3A4 and CYP3A7 share nearly 90% base sequence identity (Komori et al., 1989), they exhibit striking functional differences in their catalytic preference for endogenous substrates. For example, CYP3A4 catalyzes testosterone 6β-hydroxylation with high activity, whereas CYP3A7 shows little of this activity. On the other hand, CYP3A7 catalyzes DHEA-S 16α-hydroxylation with high activity, whereas CYP3A4 is mostly unreactive (Kitada et al., 1987; Ohmori et al., 1998). Although only limited data exist about CYP3A7-mediated drug metabolism, the drug metabolic activity of CYP3A7 seems, in general, to be lower than that of CYP3A4 (Ohmori et al., 1998; Pearce et al., 2001; Williams et al., 2002).

Furthermore, several reactions catalyzed by CYP3A4 display non-Michaelis-Menten kinetics, apparently due to allosteric effects, which commonly yields a sigmoid velocity saturation curve. For example, a sigmoid kinetic character has been observed for the metabolism of CBZ (Kerr et al., 1994; Korzekwa et al., 1998), progesterone (Harlow and Halpert, 1998), and testosterone (Ueng et al., 1997; Harlow and Halpert, 1998) by CYP3A4. In addition, it is well known that α-naphthoflavone heterotropically stimulates the metabolism of progesterone, testosterone (Schwab et al., 1988; Harlow and Halpert, 1998), and various other CYP3A substrates (Andersson et al., 1994), providing a change in the kinetic character to the Michaelis-Menten type. Recently, we reported that androgens such as AND, testosterone, and DHEA activate nevirapine 2-, 12-hydroxylations, CBZ 10,11-epoxidation and triazolam 4-hydroxylations, whereas these androgens inhibit erythromycin *N*-demethylation and zonisamide metabolism, using human adult liver microsomes as an enzyme source (Nakamura et al., 2002). Especially, AND is a potent activator for the CBZ 10,11-epoxidation, and AND changes the kinetic character of CBZ 10,11-epoxidation from sigmoid to the Michaelis-Menten type.

[1] Abbreviations used are: P450s, cytochrome P450s; AND, androstendione; DHEA, dehydroepiandrosterone; HPLC, high-performance liquid chromatography; DHEA-S, dehydroepiandrosterone 3-sulfate; EST, estradiol.

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It is considered that a spacious active site in CYP3A4, which can accommodate more than one substrate molecule at a time, produces homotropic and/or heterotropic cooperativity. Several kinetic analyses based on the hypothesis of multi-binding sites at the active site of CYP3A4 have been reported (Korzekwa et al., 1998; Domanski et al., 2000; Shou et al., 2001). However, a similar examination of CYP3A7 has not yet been reported. The purpose of this study is to clarify whether or not there are differential effects of several endogenous steroids on CBZ 10,11-epoxidation by expressed CYP3A4 and CYP3A7. This is the first report that examines CYP3A7 cooperativity.

Materials and Methods

Materials. Microsomes prepared from baculovirus-infected insect cells (SUPERSOMES) expressing human CYP3A4 and CYP3A7 (coexpressed with human NADPH-cytochrome P450 reductase and cytochrome b_5) were purchased from Gentest (Woburn, MA). CBZ and CBZ 10,11-epoxide were obtained from Novartis Pharma Co. (Tokyo, Japan). *N,N*-dimethyzonisamide were provided by Dainippon Pharmaceutical Co. (Osaka, Japan). Pregnenolone (5-pregnen-3β-ol-20-one), pregnenolone 3-sulfate (5-pregnen-3β-ol-20-one 3-sulfate), progesterone (4-pregnene-3, 20-dione), *d*-aldosterone (4-pregnen-18-al-11β, 21-diol-3, 20-dione), cortisol (11β, 17α, 21-trihydroxypregn-4-ene-3, 20-dione), DHEA (5-androsten-3β-ol-17-one), DHEA 3-sulfate (5-androsten-3β-ol-17-one 3-sulfate), AND (4-androstene-3, 17-dione), testosterone (4-androsten-17β-ol-3-one), estrone (1, 3, 5[10]-estratrien-3-ol-17-one), β-estradiol (1, 3, 5[10]-estratriene-3, 17β-diol), 11β-hydroxyandrosterone (5α-androstane-3α, 11β-diol-17-one), and Sulfatase type H-1 (from helix pomatia) were purchased from Sigma-Aldrich (St. Louis, MO). 17α-Hydroxypregnenolone (5-pregnen-3β, 17-diol-20-one), 17α-hydroxypregnenolone 3-sulfate (5-pregnen-3β, 17-diol-20-one 3-sulfate), and 17α-hydroxyprogesterone (4-pregnen-17-ol-3, 20-dione) were purchased from Steraloids Co. (Wilton, NH). All other chemicals and solvents used were of the highest grade or analytical grade commercially available.

Assay of CBZ 10,11- Epoxidase Activity. The reaction mixtures contained 100 mM potassium phosphate, pH 7.4, 0.1 mM EDTA, an NADPH-generating system (0.33 mM $NADP^+$, 0.1 U of glucose 6-phosphate dehydrogenase, 8 mM glucose 6-phosphate, and 6 mM $MgCl_2$), a methanolic solution of substrate (100 μM), and a steroid as an effector in a final volume of 0.5 ml. The content of CYP3A enzyme was 10 pmol for CYP3A4 and 15 pmol for CYP3A7. The final concentration of methanol in the reaction mixture was 2%. The reactions were started by the addition of the NADPH-generating system and were conducted for 20 (CYP3A4) or 60 min (CYP3A7) at 37° with shaking. The reactions were linear up to 30 min catalyzed by CYP3A4 and 60 min when catalyzed by CYP3A7. The reactions were stopped by adding 5 ml of extract [chloroform/ethanol, 10/1 (v/v)], and 10 μl of internal standard (20 μg/ml of *N,N*-dimethylzonisamide in chloroform) was added. After centrifugation (3,000 rpm, 10 min), the organic phase was evaporated at 40°. The residue was dissolved in 100 μl of HPLC mobile phase, and 40 μl was injected into an HPLC. The mobile phase consisted of methanol/acetonitrile/water (3/1/7, v/v/v). HPLC system consisted of an L-6000 pump (Hitachi, Tokyo, Japan), a Hitachi L-4200 UV absorbance detector (monitoring at 220 nm), a Hitachi D-2500 chromatointegrator, a Hitachi C-5000 LC controller, and a Purecil column (5 μm, 4.6 $\times$ 150 mm; Waters, Milford, MA) preceded by a precolumn (5 μm, 4.6 $\times$ 50 mm). The mobile phase was delivered at a flow rate of 1 ml/min at 35°. Under these conditions, the retention times of CBZ 10,11-epoxide and the internal standard were 12 and 15 min, respectively.

Assay of DHEA-S 16α-Hydroxylation Activity. The same reaction mixture used to assay CBZ metabolism was prepared except that 100 μM DHEA-S was used as the substrate. The content of CYP3A7 was 10 pmol. The final concentration of methanol in the reaction mixture was 2%. The reactions were started by the addition of the NADPH-generating system and were conducted for 30 min at 37° with shaking. The reaction was linear up to 30 min when catalyzed by CYP3A7. The reactions were stopped on boiling (10 min), and, after cooling, 1 ml of 2 M acetate buffer (pH 5.2) and 500 units of sulfatase were added. After overnight incubation at 37°, 100 μl of 0.1 mM 11β-hydroxyandrosterone was added as an internal standard. Then 16α-hydroxyDHEA was extracted with 5 ml of ethyl acetate. After centrifugation (3,000 rpm, 10 min), 4 ml of the organic phase was evaporated at 40°. Then, using dansyl hydrazine as a prelabeling reagent (Kawasaki et al., 1981), 16α-hydroxyDHEA was measured by HPLC with fluorescence detection. The HPLC system consisting of an L-7100 pump (Hitachi), a Hitachi L-7480 fluorescence detector (monitored at 330 nm ex and 495 nm em), a D-7500 integrator, and an Inertsil SIL column (5 μm, 4.6 $\times$ 250 mm, GL Sciences Tokyo, Japan). The mobile phase, which consisted of dichloromethane/ethanol (100/1, v/v), was delivered at a flow rate of 1 ml/min at room temperature. Under these conditions, the retention times of 16α-hydroxyDHEA and the internal standard were 17 and 22 min, respectively.

Assay of AND 6β-Hydroxylation Activity. The assay and HPLC conditions for AND 6β-hydroxylation were the same as those for the CBZ 10,11-epoxidation assay except that AND was used as the substrate and the incubation time was 5 min. The reaction was linear up to 5 min when catalyzed by CYP3A4.

Mathematical Derivation and Analysis. Kinetic parameters for CBZ 10,11-epoxidation and AND 6β-hydroxylation by CYP3A4 were determined by the modified two-site equation ($V_{max1} = 0$) (Korzekwa et al., 1998; Domanski et al., 2000):

$$V = (V_{max2}\, S^2/K_{m1}\, K_{m2})/(1 + S/K_{m1} + S^2/K_{m1}\, K_{m2})$$

In this model, the binding of substrate to site-1 facilitates binding to site-2, the sole site responsible for product formation.

Parameters for DHEA-S 16α-hydroxylation and CBZ 10,11-epoxidation by CYP3A7 were determined from the Michaelis-Menten equation.

The values were adjusted by iteration of the calculation until the best data fit was obtained using the Levenberg-Marquardt (Marquardt, 1963) nonlinear least-squares algorithm by the pro Fit program version 5.5 (QuantumSoft, Zurich, Switzerland).

Results

Effects of Various Endogenous Steroids on CBZ 10,11-Epoxidation by Expressed CYP3A4 and CYP3A7. To investigate the effects of various endogenous steroids on CBZ 10,11-epoxidation by CYP3A4 and CYP3A7, endogenous steroids or α-naphthoflavone as a reference effector for CYP3A enzymes were added to the reaction mixtures. As shown in Fig. 1A, when CYP3A4 was used as an enzyme source, androgens such as AND, DHEA, and testosterone activated CBZ 10,11-epoxidation more than 1.5-fold. In particular, AND activated this activity by about 3-fold. On the other hand, as shown in Fig. 1B, when CYP3A7 was used as an enzyme source, AND and sulfate-conjugated steroids such as DHEA-S, pregnenolone-S, and 17α-hydroxypregnenolone-S activated CBZ 10,11-epoxidation more than 1.5-fold. In particular, pregnenolone-S, and DHEA-S activated this activity about 3-fold.

The effects of steroid concentration on CBZ 10,11-epoxidation by CYP3A4 and CYP3A7 were investigated. Table 1 shows the changes in CBZ 10,11-epoxidation activities with increasing concentrations of AND, estradiol (EST), and DHEA-S. The activity of CBZ 10,11-epoxidation by CYP3A4 increased in a concentration-dependent manner for AND, whereas EST and DHEA-S maintained control activity. On the other hand, The activity of CBZ 10,11-epoxidation by CYP3A7 increased in a concentration-dependent manner for AND and DHEA-S, whereas it decreased in a concentration-dependent manner for EST.

Kinetic Analysis of the Effects of Endogenous Steroids on CBZ 10,11-Epoxidation by Expressed CYP3A4 and CYP3A7. The substrate-velocity curve and corresponding Eadie-Hofstee plots for CBZ 10,11-epoxidation by expressed CYP3A4 and CYP3A7 are shown in Fig. 2. The Eadie-Hofstee plots in Fig. 2A demonstrate that the kinetic character of CBZ 10,11-epoxidation by expressed CYP3A4 is sigmoid in the absence of steroid, indicating that more than one substrate may

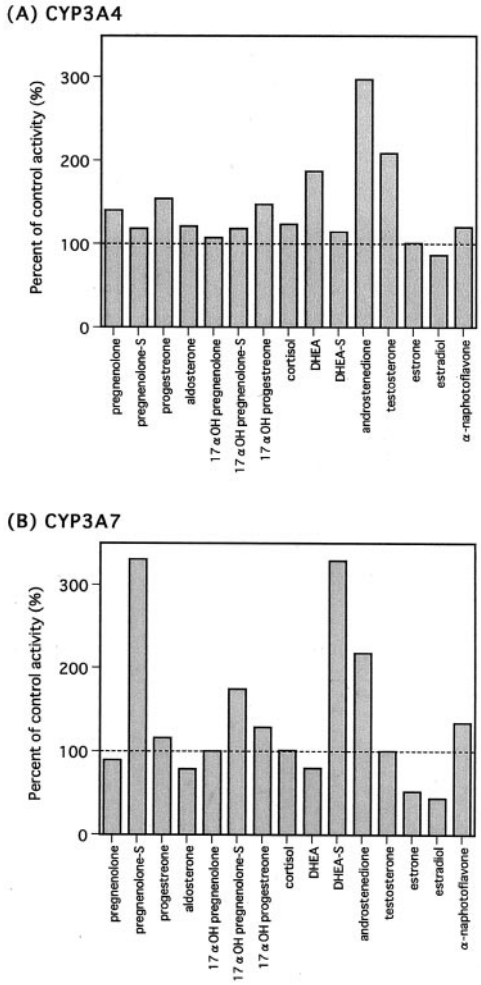

FIG. 1. *Effects of endogenous steroids and α-naphthoflavone on CBZ 10,11-epoxidation by expressed CYP3A4 (A) and CYP3A7 (B).*

The concentrations of endogenous steroids, α-naphthoflavone, and CBZ were 100 μM. Basal activities of CYP3A4 and CYP3A7 were 3.360 and 0.077 nmol/nmol P450/min, respectively.

be binding to the active site. The sigmoid curve changed to hyperbolic upon the addition of AND or EST. Moreover, AND caused a marked increase in the rate of metabolism, and this stimulation was more remarkable at low substrate concentrations. On the other hand, DHEA-S hardly affected the kinetic character of CBZ 10,11-epoxidation by expressed CYP3A4. The kinetic parameters were calculated by the modified two-site equation for CBZ 10,11-epoxidation by expressed CYP3A4. As shown in Table 2, the addition of AND and EST caused a remarkable decrease in the K_{m1}. Although the addition of DHEA-S caused hardly any change in K_{m2} and V_{max2}, the addition of AND caused a decrease in K_{m2} and an increase in V_{max2}, and the addition of EST caused an increase in K_{m2}. The intrinsic clearance (V_{max2}/K_{m2}) was increased by about 2-fold by the addition of AND, and decreased by about 1/3 by the addition of EST, compared with the absence of steroids.

As shown in Fig. 2B, the activity of CYP3A7-mediated CBZ 10,11-epoxidation was increased by the addition of AND and DHEA-S, whereas the activity was inhibited by the addition of EST. The Eadie-Hofstee plots demonstrate that the kinetic character of CBZ 10,11-epoxidation by expressed CYP3A7 is hyperbolic in both the absence and presence of steroids. The kinetic parameters were calculated from the Michaelis-Menten equation for CYP3A7. As shown in

TABLE 1

Concentration-dependent effects of endogenous steroids on CBZ 10,11-epoxidation by expressed CYP3A4 and CYP3A7

The concentration of substrate was 100 μM.

Added Steroids	CYP3A4	CYP3A7
μM	*nmol/nmol P450/min (%)*	*nmol/nmol P450/min (%)*
none		
0	3.275 (100.0)	0.062 (100.0)
AND		
25	6.229 (190.2)	0.104 (167.6)
50	8.944 (273.1)	0.127 (205.7)
100	9.769 (298.3)	0.134 (216.5)
200	9.320 (284.6)	0.159 (256.6)
EST		
25	3.249 (99.2)	0.032 (51.8)
50	3.017 (92.1)	0.023 (37.5)
100	2.857 (87.2)	0.023 (36.7)
200	3.085 (94.2)	0.021 (33.8)
DHEA-S		
25	3.190 (97.4)	0.155 (251.4)
50	2.774 (84.7)	0.184 (296.9)
100	3.163 (108.5)	0.194 (330.9)
200	3.414 (104.3)	0.215 (348.1)

the Table 2, the addition of steroids caused a decrease in the K_m value. And the addition of DHEA-S caused slightly increased V_{max}, whereas the addition of EST caused a decrease in V_{max}. The intrinsic clearance was increased by over 2-fold by the addition of AND and DHEA-S, whereas the value was decreased by 1/3 by the addition of EST.

Kinetic Analysis of the Effects of CBZ on AND 6β-hydroxylation by Expressed CYP3A4, and on DHEA-S 16α-hydroxylation by Expressed CYP3A7. Next, we investigated the effect of CBZ on AND 6β-hydroxylation by expressed CYP3A4, and on DHEA-S 16α-hydroxylation by expressed CYP3A7, because CBZ 10,11-epoxidation by expressed CYP3A4 and CYP3A7 was magnitude activated by the addition of AND and DHEA-S, respectively. The substrate-velocity curve and corresponding Eadie-Hofstee plots for AND 6β-hydroxylation by expressed CYP3A4 are shown in Fig. 3. The kinetics of AND 6β-hydroxylation also showed a sigmoid character. It was observed that the rate of AND 6β-hydroxylation decreased upon the addition of CBZ. However, the sigmoid kinetics were not altered by the addition of CBZ. Table 3 shows the kinetic parameters of AND 6β-hydroxylation by expressed CYP3A4. The addition of CBZ caused a decrease in the of V_{max2} value.

Figure 4 shows the substrate-velocity curve and corresponding Eadie-Hofstee plots for DHEA-S 16α-hydroxylation by expressed CYP3A7. The kinetic character of DHEA-S 16α-hydroxylation was hyperbolic in both the absence and presence of CBZ. CBZ had no substantial effect on DHEA-S 16α-hydroxylation by CYP3A7. As shown in Table 4, the kinetic parameters of this reaction are hardly changed by the addition of DHEA-S.

Discussion

The cooperative binding effects associated with CYP3A4 substrates are well documented in several previous articles, and it has been widely accepted that these phenomenon may arise from the binding of multiple molecules to CYP3A4, either within the active site (Korzekwa et al., 1998; Shou et al., 1999; Domanski et al., 2000) or at separate distant locations on the enzyme (Schwab et al., 1988; Ueng et al., 1997). However, there is no information about cooperativity at CYP3A7, which is the fetus-peculiar CYP3A enzyme. The purpose of this study is to clarify whether the effects of several endogenous steroids on CBZ 10,11-epoxidation by expressed CYP3A7 are similar to those observed for CYP3A4.

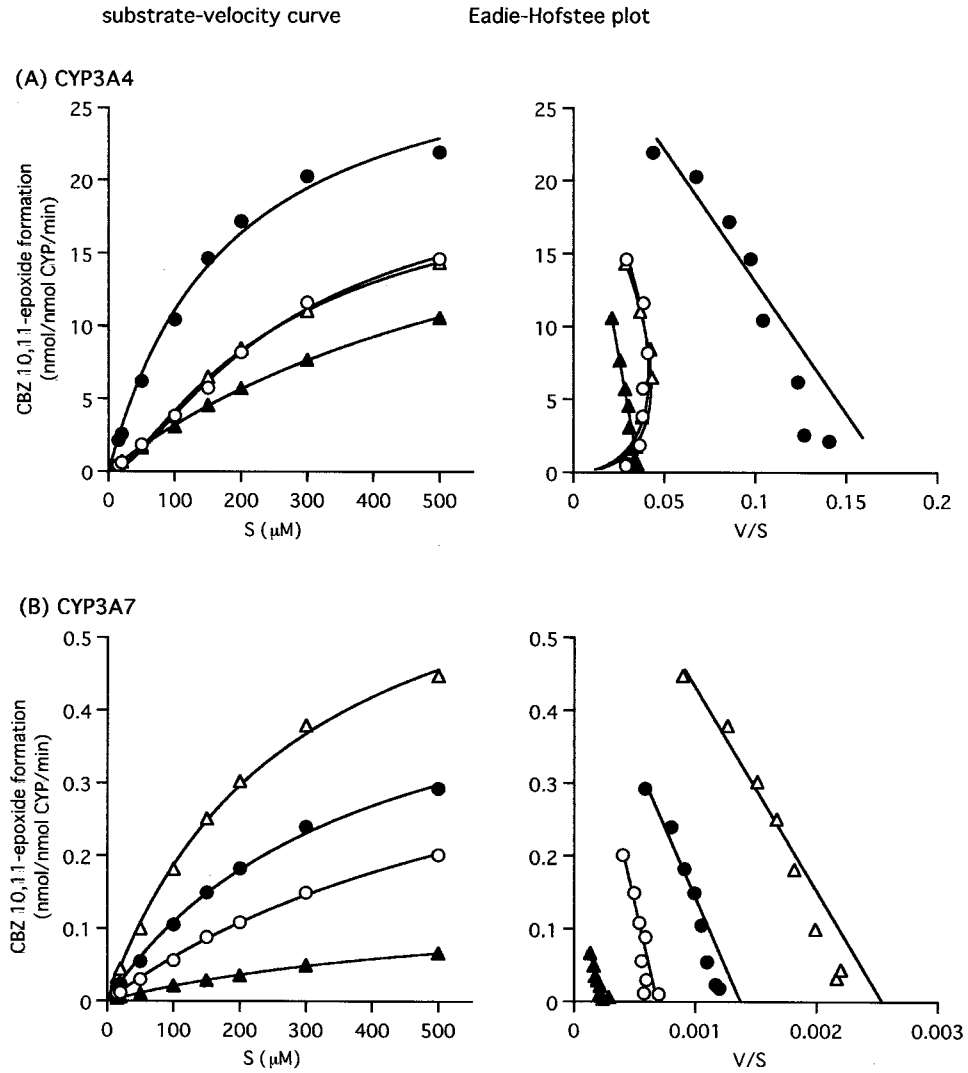

FIG. 2. *Substrate-velocity curve and Eadie-Hofstee plots of CBZ 10,11-epoxidation by expressed CYP3A4 (A) and CYP3A7 (B).*

The reactions were performed in the absence or presence of 100 μM effectors. Lines were drawn using data analyzed by the modified two-site equation for CYP3A4, and by the Michaelis-Menten equation for CYP3A7. ○, CBZ only; ●, plus AND; ▲, plus EST; △, plus DHEA-S.

TABLE 2

Kinetic parameters of CBZ 10,11-epoxidation by expressed CYP3A4 and CYP3A7

Substrate concentrations were 15 to 500 μM. The reaction was performed in the absence or presence of 100 μM effectors. K_{m1}, K_{m2}, and V_{max2} were calculated by a modified two-site equation ($V_{max1} = 0$) $V = (V_{max2}\ S^2/K_{m1}\ K_{m2})/(1 + S/K_{m1}\ K_{m2})$ (Korzekwa et al., 1998) K_m and V_{max} were calculated from the Michaelis-Menten equation $V = V_{max}/(1 + (K_m/S))$.

	CYP3A4				CYP3A7		
	K_{m1} (apparent)	K_{m2} (apparent)	V_{max2}	V_{max2}/K_{m2}	K_m (apparent)	V_{max}	V_{max}/K_m
	μM		*nmol/nmol P450/min*		*μM*	*nmol/nmol P450/min*	
CBZ only	104.8	248.3	23.64	0.0952	720.9	0.50	0.0007
Plus AND	12.8	139.5	29.03	0.20806	382.8	0.53	0.0014
Plus EST	2.1	666.9	24.76	0.03713	616.7	0.15	0.0002
Plus DHEA-S	80.9	246.4	22.59	0.0917	280.1	0.71	0.0025

Recently, we reported that androgens such as AND, testosterone, and DHEA activate various types of CYP3A4-mediated drug metabolism, such as nevirapine 2-, 12-hydroxylations, CBZ 10,11-epoxidation and triazolam 4-hydroxylations, using human adult liver microsomes as an enzyme source. In the present study, using expressed CYP3A4 as enzyme source, similar results were obtained. That is, the same androgens activated CBZ 10,11-epoxidation by expressed CYP3A4. On the other hand, interestingly, CYP3A7-mediated CBZ 10,11-epoxidation was activated by position three sulfate conjugated steroids such as pregnenolone-S, 17α-hydroxypregnenolone-S, and DHEA-S. The position three sulfate seems to play an important role in the activation of this reaction, because the unconjugated forms of these three steroids did not activate the reaction.

The kinetic analysis showed that the sigmoidal kinetic pattern of

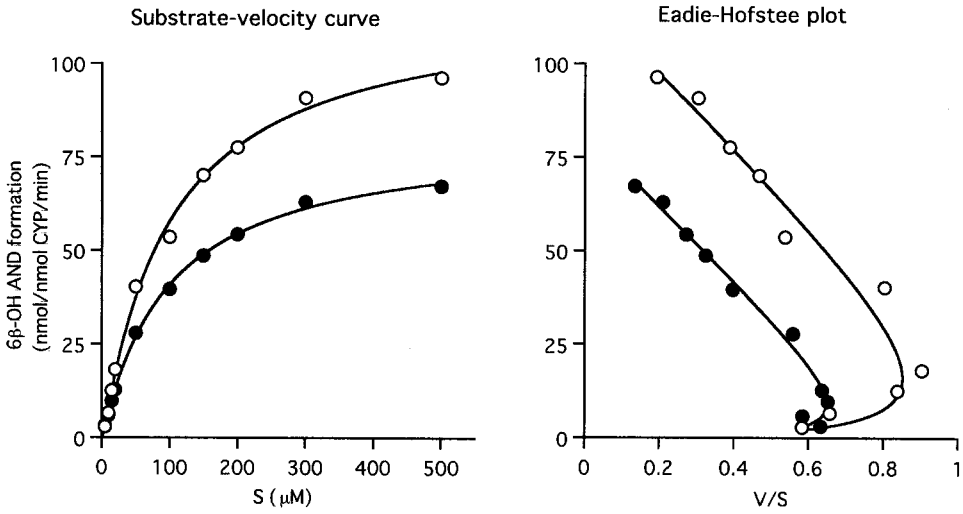

FIG. 3. *Substrate-velocity curve and Eadie-Hofstee plots of AND 6β-hydroxylation by expressed CYP3A4.*

The reactions were performed in the absence or presence of 100 μM CBZ. Lines were drawn using data analyzed by the modified two-site equation. ○, AND only; ●, plus CBZ.

TABLE 3

Kinetic parameters of AND 6β-hydroxylation by expressed CYP3A4

Substrate concentrations were 5 to 500 μM. The reaction was performed in the absence or presence of 100 μM CBZ.

	K_{m1} (apparent)	K_{m2} (apparent)	V_{max2}	V_{max2}/K_{m2}
	μM		*nmol/nmol P450/min*	
AND only	3.5	100.3	117.49	1.17139
Plus CBZ	2.0	97.8	81.37	0.83200

TABLE 4

Kinetic parameters of DHEA-S 16α-hydroxylation by expressed CYP3A7

Substrate concentrations used were 10 to 500 μM. The reaction was performed in the absence or presence of 100 μM CBZ.

	K_m (apparent)	V_{max}	V_{max}/K_m
	μM	*nmol/nmol P450/min*	
DHEA-S only	17.0	6.60	0.3882
Plus CBZ	21.5	6.05	0.2814

CBZ 10,11-epoxidation by CYP3A4 changes to the Michaelis-Menten type by the addition of AND and EST. When the analysis was performed using the modified two-site equation, the addition of these steroids resulted in a decrease in the K_{m1} for CBZ 10,11-epoxidation, as compared with that obtained in the absence of steroids. Figure 5A shows the kinetic scheme for the effect of endogenous steroids on CYP3A4-mediated CBZ 10,11-epoxidation. The high affinity for site 1 of these steroids seems to be the reason for the change from sigmoid to Michaelis-Menten type kinetics. Namely, when a steroid binds to site 1 with high affinity, CBZ can be metabolized even if CBZ does not bind to site 1. Thus, only one site (site 2) is associated with CBZ metabolism, and the kinetic pattern changes to the Michaelis-Menten type. In fact, the K_{m1} of AND is remarkably smaller than that of CBZ (3.5 versus 104.8 μM). If the binding of AND to site 1 causes changes in the conformation of site 2 and/or an interaction between the substrates take place in the large substrate pocket of CYP3A4, they could be the cause of these phenomena.

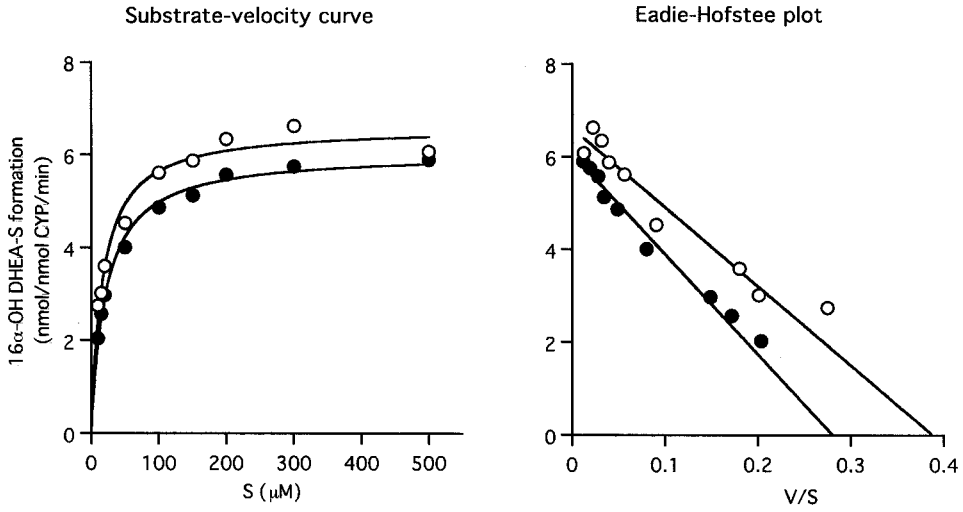

FIG. 4. *Substrate-Velocity curve and Eadie-Hofstee plots of DHEA-S 16α-hydroxylation by expressed CYP3A7.*

The reactions were performed in the absence or presence of 100 μM CBZ. Lines were drawn using data analyzed by the Michaelis-Menten equation. ○, DHEA-S only; ●, plus CBZ.

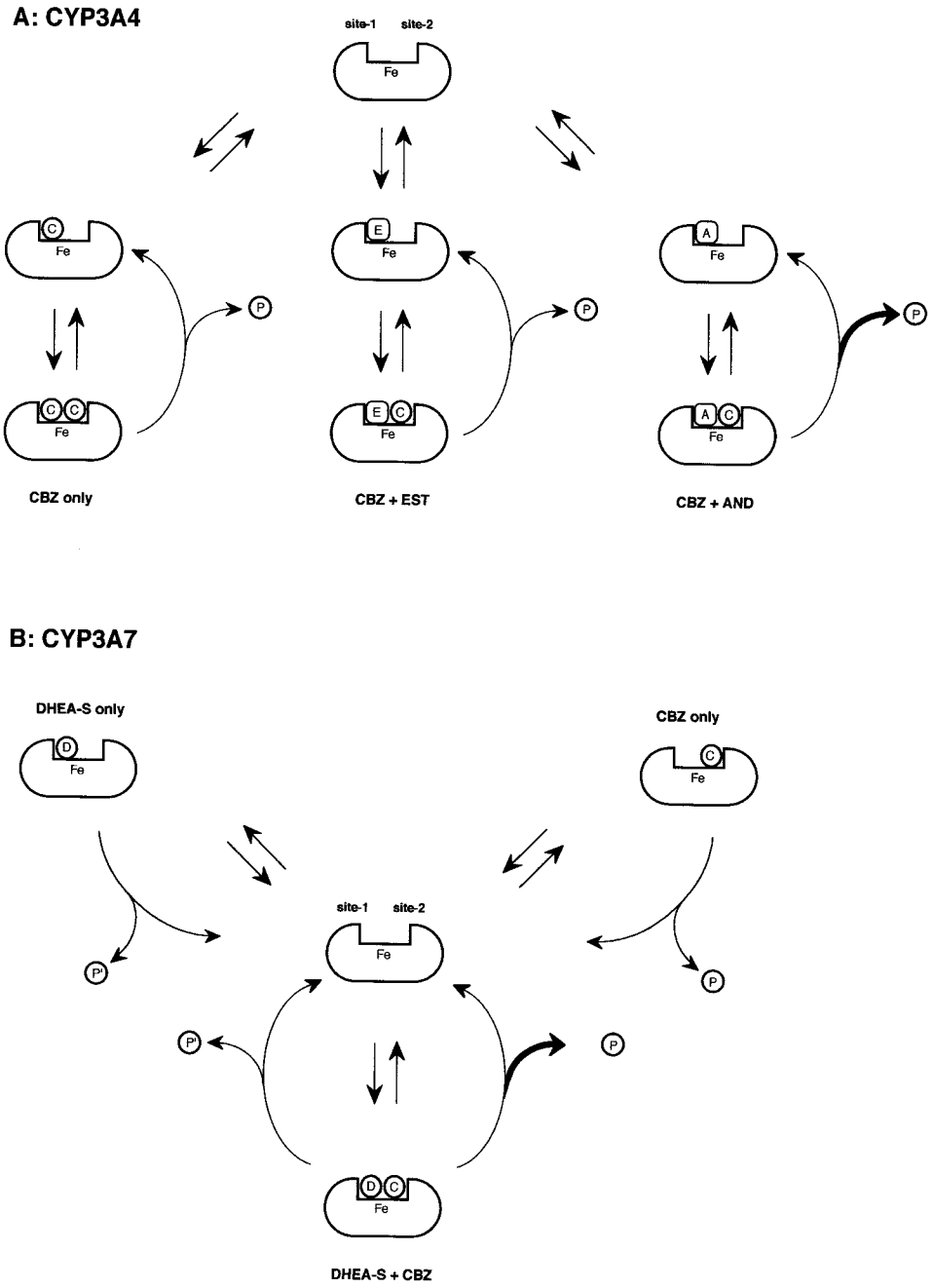

FIG. 5. *Kinetic scheme for the effect of endogenous steroids on CYP3A4-mediated CBZ 10,11-epoxidation (A) and the effect of DHEA-S on CYP3A7-mediated CBZ 10,11-epoxidation (B).*

C, CBZ; A, AND; E, EST; D, DHEA-S; P, 10,11-epoxi CBZ; P′, 16α-hydroxy DHEA-S.

On the other hand, CBZ 10,11-epoxidation by CYP3A7 was recognized as Michaelis-Menten type in both the presence and absence of steroids. In addition, the DHEA-S 16α-hydroxylation by CYP3A7 also showed Michaelis-Menten type kinetics, and CBZ and DHEA-S did not inhibit the metabolism of one another. These results indicate that CBZ and DHEA-S are metabolized at separated sites on CYP3A7. Figure 5B shows the kinetic scheme for CBZ 10,11-epoxidation and DHEA-S 16α-hydroxylation by CYP3A7. As in the case of AND for CYP3A4, a conformational change of site 2 induced by DHEA-S and/or an interaction between DHEA-S and CBZ in the active site of CYP3A7 may be the cause of these phenomena.

DHEA-S exists at high density in the fetal and neonatal periods (France, 1971; Kojima et al., 1981), and plays an important role in the maintenance of pregnancy and the growth of the fetus (Schuetz and Guzelian, 1993). Since DHEA-S activates CYP3A7-mediated CBZ 10,11-epoxidation, there is a possibility that DHEA-S affects drug-metabolism in fetuses and neonates period in vivo. Although only limited data exist about CYP3A7-mediated drug metabolism, the drug metabolism activity of CYP3A7 is generally lower than that of CYP3A4 in vitro (Ohmori et al., 1998; Pearce et al., 2001; Williams et al., 2002). However, the results of an in vitro reaction system, which contains a single substrate and enzyme, may or may not reflect drug metabolism activity in vivo. For example, while the activity of CBZ 10,11-epoxidation by CYP3A7 was lower than that of CYP3A4

in vitro, the elimination half-life of CBZ was reported about 24 h at neonatal period (Singh et al., 1996). It is no inferiority in that of the adult. The reason for this might be not only the effect of the amount of CYP3A7 in the neonatal period, but also the effect of DHEA-S on CYP3A7 activity in vivo.

In addition, we demonstrated in a previous study that the effects of endogenous steroids on CYP3A4-mediated drug metabolism depend on the combination of drugs and steroids used (Nakamura et al., 2002). Therefore, the effect of endogenous steroids (especially DHEA-S) on CYP3A7-mediated drug metabolism might also be not uniform. Some kinds of drug metabolism may be inhibited by the existence of DHEA-S. For safe medication during the neonatal period, further detailed investigations of the effects of DHEA-S on CYP3A7-mediated drug metabolism are required.

In conclusion, CYP3A4- and CYP3A7-mediated CBZ 10,11-epoxidation are activated by different types of endogenous steroids. Especially, DHEA-S is potent activator of CBZ 10,11-epoxidation by expressed CYP3A7. The fact that DHEA-S and CYP3A7 exist at very high levels in the fetal and early neonatal periods indicates that DHEA-S may affect drug metabolism in these periods.

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