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ENANTIOSELECTIVE AND DIASTEREOSELECTIVE ASPECTS OF THE OXIDATIVE METABOLISM OF METOPROLOL

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Abstract—Enantio- and diastereoselective aspects of oxidative metabolism of metoprolol (**1**) were examined in the presence of rat liver and human liver microsomes using a pseudoracemate of **1**, made up of equal molar (2*R*)-**1**-d_0 and (2*S*)-**1**-d_2, as substrate. Both O-demethylation and α-hydroxylation showed only slight enantioselectivity, 2*R*/2*S* ratios being 1.18 and 0.93 for these pathways in rat liver microsomes and 1.09 and 0.92 in human liver microsomes. In the presence of the rat liver microsomal fraction, α-hydroxylation yielded predominantly the 1′*R*-hydroxy product, 1′*R*/1′*S* ratio > 12, regardless of the stereochemistry of the side chain. In humans (extensive metabolizers) administered a single 50 mg oral dose of pseudoracemic metoprolol tartrate, urinary α-hydroxymetoprolol (**2**) accounted for 9.3 ± 2.4% of the dose, 2*R*/2*S* ratio 0.85 ± 0.14, and the carboxylic acid metabolite **4**, accounted for 52.7 ± 6.8% of the dose, 2*R*/2*S* ratio 1.15 ± 0.09. The data suggested that preferential O-demethylation of the (2*R*)-enantiomer of **1** could contribute to the 2*S*> 2*R* plasma ratio of metoprolol enantiomers observed in this population.

Metoprolol (**1**) is a β_1-selective aryloxypropanolamine adrenergic antagonist used extensively in the treatment of a variety of cardiovascular disorders. The (2*S*)-enantiomer has significantly greater β_1-adrenergic receptor affinity by >25-fold [1, 2], and there is evidence that the enantiomers are oxidatively metabolized at different rates in humans [3–7]. The main oxidative metabolic pathways are benzylic hydroxylation (α-hydroxylation), O-demethylation, and N-dealkylation (Fig. 1) [3]. In humans, the benzylic alcohol **2** is a relatively minor metabolite (10% of dose), but it is a pharmacologically active one [6]. The O-demethylated metabolite (**3**) undergoes further oxidation to the corresponding carboxylic acid (**4**), which is reported to account for about 65% of the dose in humans [4]. The multi-step N-dealkylation process is the least important quantitatively, with **5** accounting for <10% of the dose [6]. In humans, the oxidative metabolism of metoprolol has been shown to display genetic polymorphism of the debrisoquine type [5, 8–15], and the α-hydroxylation pathway shows a high degree of product stereoselectivity in the presence of rat liver microsomes [16].

In this paper, we report the results of an examination of the enantioselectivity of the major oxidative pathways in rat and human liver microsomes and *in vivo* in extensive metabolizers, and we have examined the diastereoselectivity of the α-hydroxylation process. In addition, we report a stereochemical examination of the urinary metabolites, benzylic alcohol **2** and acid **4**, and unchanged metoprolol in extensive metabolizers. The use of a pseudoracemate of metoprolol, made up of equal molar (2*R*)-**1**-d_0 and (2*S*)-**1**-d_2 [17], facilitated GC–MS§ determination of the enantiomeric metabolites.

MATERIALS AND METHODS

Chemicals and reagents. Pseudoracemic metoprolol tartrate was prepared by dissolving equimolar amounts of the tartrate salts of (2*S*)-metoprolol-d_2 (96.6% $^2H_2$, 94% e.e.) and (2*R*)-metoprolol-d_0 (96.2% e.e.) [17] in water. *N,O*-Bistrimethylsilytrifluoroacetamide (BSTFA) was obtained from the Pierce Chemical Co. (Rockford, IL). (2*R*)-2-Methoxy-2-(trifluoromethyl)phenylacetyl chloride was prepared from the corresponding acid (Aldrich Chemical Co., Milwaukee, WI). Solvents for liquid chromatography were obtained from E. M. Science (Cherry Hill, NJ).

Preparation of rat liver microsomes. Male Sprague–Dawley rats (weighing 140–150 g) were decapitated, and the livers were removed and rinsed with cold 0.01 M phosphate buffer (pH 7.4) containing 1.15% KCl. The livers were then homogenized in 4 vol. of the same buffer using a Potter–Elvehjem teflon pestle homogenizer. The homogenate was centrifuged at 9,000 *g* for 30 min, and the supernatant fraction was recentrifuged at 100,000 *g* for 1 hr. The microsomal pellet was resuspended in the same buffer at a protein concentration of 20–30 mg/mL. The entire operation was performed at 0–4°. Microsomal protein was determined by the method of Lowry *et al.* [18], using bovine serum albumin as standard.

Preparation of human liver microsomes. Human

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§ Abbreviations: GC–MS, gas chromatography–mass spectrometry; e.e., enantiomeric excess; and BSTFA, *N,O*-bistrimethylsilyltrifluoroacetamide.

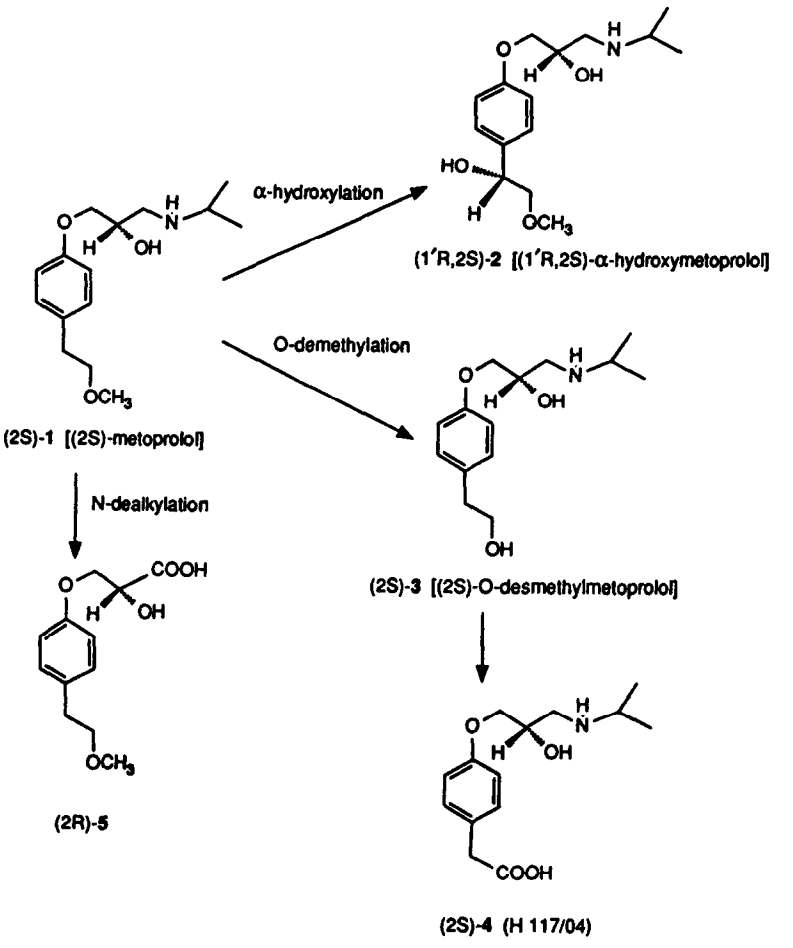

Fig. 1. Metabolism of metoprolol by oxidative pathways. (2*S*)-Metoprolol is shown as an example.

livers were obtained from the human liver bank in the department [19]. Microsomes were prepared from 10 g of the liver tissue. Frozen liver (stored at −70°) was first thawed in distilled water at room temperature and then homogenized in 3 vol. of potassium phosphate buffer, 50 mM, pH 7.4 (0.25 mM sucrose/1 mM EDTA), using a Potter–Elvehjem teflon pestle homogenizer. The homogenate was centrifuged at 9,000 *g* for 30 min, and the supernatant fraction was recentrifuged at 105,000 *g* for 1 hr. The microsomal pellet was resuspended in the homogenization buffer and centrifuged again. The final microsomal pellet was suspended in 7 mL of buffer containing 100 mM potassium phosphate, 20% glycerol and 1 mM EDTA (pH 7.4). All steps in the preparation of microsomes were performed at 0–4°. The protein content was determined by the method of Lowry *et al.* [18].

Microsomal metabolism. The incubation mixtures (3 mL) contained $NADP^+$ (2.3 mg, 1 mM), glucose-6-phosphate (16.92 mg, 20 mM), magnesium chloride (2.85 mg, 10 mM), microsomal protein (3 mg), pseudoracemic metoprolol tartrate (representing 0.201 mg of metoprolol, 250 μM), and glucose-6-phosphate dehydrogenase (5 units) in phosphate buffer (0.2 M, pH 7.4). Incubations were carried out at 37° for 30 min and then terminated by immersing the mixtures in ice.

Extraction and derivatization of metabolites. The microsomal incubation mixtures were made alkaline by the addition of 1 mL of aqueous 0.5 M Na_3PO_4 (pH 12.0) after addition of propranolol (12 μg) as internal standard to each. Aliquots of 2 M phosgene in toluene (2 × 30 μL) were added, and the mixtures were vortexed for 4 min. The metabolites were then extracted using a mixture of 3 mL of diethyl ether and 2 mL of dichloromethane. After centrifugation, the organic phase was collected and the aqueous phase extracted for a second time. The combined organic extracts were transferred to a Reacti-vial and evaporated to dryness using a stream of dry nitrogen. For mass spectral determination, the residue was allowed to react with 50 μL of BSTFA at 60° for 15 min. After cooling, 50 μL of ethyl acetate was added and 1 μL of this solution was used in the GC–MS analysis. For HPLC analysis the evaporated sample (before derivatization with BSTFA) was flushed with argon, and dry benzene (100 μL) and pyridine (5 μL) were added. After mixing the contents, (2*R*)-2-methoxy-2-(trifluoromethyl)-phenylacetyl chloride [20] (5 μL) was added, and the

mixture was maintained at room temperature for 2 hr to complete the derivatization. Samples were then evaporated to dryness with a nitrogen stream at 45°, the residue was diluted with ether (3 mL) and washed with NaOH solution (0.1 M, 250 μL) and then with H_2O (250 μL). The ether layer was evaporated to dryness, and the residue was dissolved in 200 μL of mobile phase and then subjected to HPLC analysis.

Disposition of pseudoracemic metoprolol in men. Urinary metabolites were examined from eight male volunteers, who were extensive metabolizers of debrisoquine [8, 9], at the Royal Hallamshire Hospital in Sheffield, England. The study was approved by the Royal Hallamshire Ethics Committee. Each volunteer received a single dose of pseudoracemic metoprolol tartrate (50 mg) in 100 mL of water after an overnight fast. Urine was collected for the 0–12 hr and 12–24 hr time periods. Urinary metoprolol (**1**), α-hydroxymetoprolol (**2**) and acid **4** were determined by HPLC from urinary aliquots by the method of Lennard [21].

For HPLC determination of **2** and metoprolol (**1**), methylene chloride extracts of alkalinized urine samples were evaporated and dissolved in mobile phase, water:acetonitrile (88:12), containing 1% triethylamine which was adjusted to pH 3 with ascorbic acid. A C_{18} reversed phase column with a precolumn was used. Detection was determined fluorometrically with excitation at 193 nm and no emission filter [21].

Determination of acid **4** was done by HPLC using urinary aliquots diluted with the mobile phase of 0.05 M sodium dihydrogen phosphate:methanol (98:2), pH 5.5, adjusted with sodium hydroxide. Detection was done fluorometrically, excitation at 193 nm, without an emission filter [21].

For determination of enantiomeric composition of the metabolites and metoprolol, 1-mL samples of urine were extracted and derivatizations were performed as described for the microsomal samples above.

GC–MS analysis. The GC–MS spectra were obtained on an HP5710 gas chromatograph interfaced with a VG7070H mass spectrometer and equipped with a VG2050 data system. The column was a J&W DB-5 fused silica capillary column of 30 m × 0.32 mm i.d. and 0.25 mm film thickness. Chromatographic parameters were: carrier gas, helium; vent flow rate, 60 mL/min; column head pressure, 15 psi; injector temperature, 250°, and the temperature program, 200° for 1 min and then increased to 280° at 10°/min. Mass spectral conditions were: source temperature, 200°; dwell time, 50 msec; and ionizing voltage, 70 eV.

HPLC, GC–MS analysis of α-hydroxylated metabolite 2 diastereomers. The HPLC analysis was performed with an LKB 2150 pump, equipped with a Waters 484 tuneable absorbance detector (226 nm) and a Spectra-Physics 4100 computing integrator. The diastereomeric components of the derivatized metabolite **6**, were resolved by HPLC using two columns connected in series: Ultrasphere ODS, 5 μm, 4.6 mm × 15 cm (Beckman) and Microsorb C_{18}, 4.6 mm × 10 cm (Rainin), as described previously [16]. Elution was performed isocratically with a mixture of CH_3CN:CH_3OH:phosphate buffer (0.01 M, pH 7.0)::20:47:33, at a flow rate of 1 mL/min. A 20-μL quantity of the sample solution was injected for each analysis, and the eluant for the peaks arriving at retention times 18.28 and 19.54 min was collected separately. Collected fractions from two runs were combined and concentrated to one-third of the original volume using a nitrogen stream. Then, methylene chloride (2 mL) was added, and the supernatant fraction was washed with water (1 mL). The methylene chloride layer was then evaporated to complete dryness and the residue was reconstituted in 200 μL of methylene chloride. GC–MS analysis was carried out on 1–2 μL of this solution. Ions at m/z 291 and m/z 293 were monitored. The retention time was 9 min 6 sec.

GC–MS determination of metoprolol metabolites. Extracted metabolites and standards, derivatized sequentially with phosgene and BSTFA, were determined by the GC–MS method of Hoffmann *et al.* [22]. The ions m/z 336 (metabolite-d_0) and m/z 338 (metabolite-d_2) were monitored in the selected ion mode. The retention times were 7.02 min for propranolol, and 9.41 and 10.48 min for *O*-demethyl- and α-hydroxymetoprolol metabolites **3** and **2** respectively. Standard curves (correlation coefficient >0.98) were constructed from boiled microsomal suspensions of 2–10 μg of these metabolites using propranolol (12 μg) as the internal standard. Repeated determinations of **3** and **2** from simple samples varied less than 5%.

RESULTS

To examine the enantioselectivity of the oxidative metabolic processes, pseudoracemic metoprolol, made up of equal molar (2*R*)-**1**-d_0 and (2*S*)-**1**-d_2, was used in all experiments. The two a.m.u. difference in masses of the metabolites arising from individual enantiomers allowed their facile determination by GC–MS. Metabolites **2** and **3** from (2*S*)-metoprolol-d_2 [(2*S*)-**1**] afford the m/z 338 fragment ion, and metabolites from (2*R*)-metoprolol-d_0[2*R*)-**1**] afford the m/z 336 fragment ion. These ions arise from loss of a methyl group from the trimethylsilyl ether of the oxazolidinone derivative of **3** and from loss of the terminal methoxymethylene group from the trimethylsilyl ether of the oxazolidine derivative of **2** [22].

Enantioselectivity data on the formation of **2** and **3** in the presence of rat and human liver microsomes are summarized in Tables 1 and 2 respectively. In the presence of rat liver microsomes, enantioselectivity was noted in both the O-demethylation and α-hydroxylation processes, and the enantiomeric preference in these two processes was chirally opposite, but slight. The *R*/*S* ratio for the formation of **3** was 1.18, favoring the metabolism of the (2*R*)-enantiomer and the *R*/*S* ratio was 0.93 for the formation of **2**, slightly favoring metabolism of the (2*S*)-enantiomer. Under these conditions, the α-hydroxylation of **1** proceeded 30% faster than did the O-demethylation process. In the five human liver microsomal preparations, O-demethylation predominated over α-hydroxylation by *ca.* 7 to 12-fold, and only

Table 1. Stereoselectivity data on the rat liver microsomal metabolism of pseudoracemic metoprolol

Metabolite	Enantioselectivity 2*R*2/*S* Ratio of **1**	Amount in incubation mixture (μg)
O-Demethylmetoprolol (**3**)	1.18 ± 0.04	17.6 ± 1.8
α-Hydroxymetoprolol (**2**)	0.93 ± 0.01	23.7 ± 0.6
1′*R* products (1′*R*,2*R*-**2** and 1′*R*,2*S*-**2**)	0.90 ± 0.01	22.0 ± 0.7
1′*S* products (1*S*,2*R*-**2** and 1′*S*,2*S*-**2**)	1.42 ± 0.10	1.73 ± 0.24

Pseudoracemic metoprolol was incubated (200 μg/3 mL incubation) at 37° for 30 min with microsomal protein (1 mg/mL). Values are means ± SD (N = 3).

Table 2. Enantioselectivity data on the human liver microsomal metabolism of pseudoracemic metoprolol

	α-Hydroxylation (**2**)		O-Demethylation (**3**)		**3**/**2**
Human liver ID	Amount (μg)	2*R*/2*S* Ratio	Amount (μg)	2*R*/2*S* Ratio	Ratio
HL 101	1.06 ± 0.04	0.97 ± 0.02	7.69 ± 0.63	1.19 ± 0.05	7.3
HL 107	0.91 ± 0.22	0.90 ± 0.02	8.88 ± 2.27	1.11 ± 0.01	9.8
HL 109	0.36 ± 0.01	0.93 ± 0.01	4.31 ± 0.13	0.90 ± 0.01	12.0
HL 110	1.61 ± 0.11	0.87 ± 0.03	13.75 ± 1.19	1.22 ± 0.08	8.5
HL 111	0.67 ± 0.01	0.87 ± 0.01	6.50 ± 0.16	1.05 ± 0.01	9.7
Mean ± SD	0.97 ± 0.47	0.91 ± 0.04	8.23 ± 3.0	1.09 ± 0.12	9.5 ± 1.0

Pseudoracemic metoprolol was incubated (200 μg/3 mL incubation) at 37° for 30 min, with microsomal protein (1 mg/mL). Values are means ± SD (N = 3).

slight enantioselectivity was observed for these processes. In most samples, however, the *R*/*S* ratio was >1.0 for the formation of **3**, and the *R*/*S* ratio was always ≤ 1.0 for α-hydroxylation (Table 2).

The α-hydroxylation of metoprolol generates a new chiral center in each of the enantiomers of the drug, thus yielding diastereomers of **2**. The rates of formation of each of the diastereomers were determined by HPLC separation, followed by GC–MS analysis for determination of the enantioselectivity. The diastereomeric components (1′*R*,2*R*)-**2**-d_0 and (1′*R*,2*S*)-**2**-d_2 were separated from (1′*S*,2*R*)-**2**-d_0 and (1′*S*,2*S*)-**2**-d_2 by HPLC analysis of the (2*R*)-2-methoxy-2-(trifluoromethyl)phenylacetyl esters of the alcohol at the 1′ position (**6**), after formation of the oxazolidinone derivative of the amino alcohol of the side chain (Fig. 2). The eluents from the HPLC were collected, and the deuterated and non-deuterated components were determined by GC–MS. In this way, (1′*R*,2*R*)-**2**-d_0 and (1′*R*,2*S*)-**2**-d_2 were determined in one GC–MS run, and (1′*S*,2*R*)-**2**-d_0 and (1′*S*,2*S*)-**2**-d_2 were determined in another. The monitored fragment ions *m*/*z* 291 and 293 arise from methoxystyrenes **7** formed by the thermal decomposition of **6** (Fig. 2) [16]. This combination of HPLC and GC–MS methods in conjunction with stable isotope methodology allowed acquisition of complete stereochemical data on the formation of **2**.

Data on product stereoselectivity [23], the generation of a new chiral center in a metabolic reaction, for α-hydroxylation in rat liver microsomes appear in Table 1. Significantly more 1′*R* hydroxylation than 1′*S* hydroxylation occurred in the enantiomers of metoprolol, the 1′*R*/1′*S* ratio being >12 (22.0/1.73). This ratio is the sum of the 1′*R*-products [(1′*R*,2*R*)-**2** and (1′*R*,2*S*)-**2**] to the sum of the 1′*S*-products [(1′*S*,2*R*)-**2** and (1′*S*2*S*)-**2**]. However, only moderate enantioselectivity in the benzylic oxidation of metoprolol was noted, i.e. difference in the rate of α-hydroxylation of (2*R*)-**1** vs (2*S*)-**1**. Interestingly, an opposite chiral preference for the substrate metoprolol enantiomers was observed in the formation of 1′*R* and 1′**S** α-hydroxyl metabolites (**2**), as noted previously [16]. Little enantioselectivity was observed for formation of 1′*R* diastereoisomers, 2*R*/2*S* ratio ≈ 0.9, but (2*R*)-metoprolol was oxidized more rapidly to form the 1′*S* diastereoisomers, 2*R*/2*S* ratio 1.4. Because in the human liver microsomal system formation of only small amounts of α-hydroxy metabolite **2** were observed, about one-tenth the amount of **3** (**3**/**2** ratio 9.5, Table 2), product stereoselectivity was not determined.

Enantioselectivity and product stereoselectivity of the metabolism of metoprolol were examined *in vivo* in humans after a single oral dose of pseudoracemic metoprolol tartrate (50 mg) to eight extensive metabolizers. From the 0–24 hr urine the amounts and stereochemistry of unchanged metoprolol (**1**), α-hydroxymetoprolol (**2**), and acid **4** were determined (Table 3). Unchanged metoprolol (**1**) accounted for 2.8 ± 1.5% of the dose, mean ± SD, 2*R*/2*S* ratio 0.73 ± 0.16; α-hydroxymetoprolol (**2**) accounted for 9.3 ± 2.4% of the dose, mean ± SD, 2*R*/2*S* ratio 0.85 ± 0.14; and the major metabolite, acid **4**, accounted for >50% of the dose, 2*R*/2*S* ratio 1.15 ± 0.09. Quantitatively, the recovery of the *ca.* > 50% of the dose was similar to that observed by McGourty *et al.* [11] in extensive metabolizers (54–77%, after 100 mg metoprolol tartrate orally) and the distribution of metabolites acid **4** and α-hydroxymetoprolol (**2**) was similar [52.7 vs 57.6% for **4**, and 9.3 vs 9.3% for **2**]. The metoprolol enantioselectivity for these urinary metabolites was

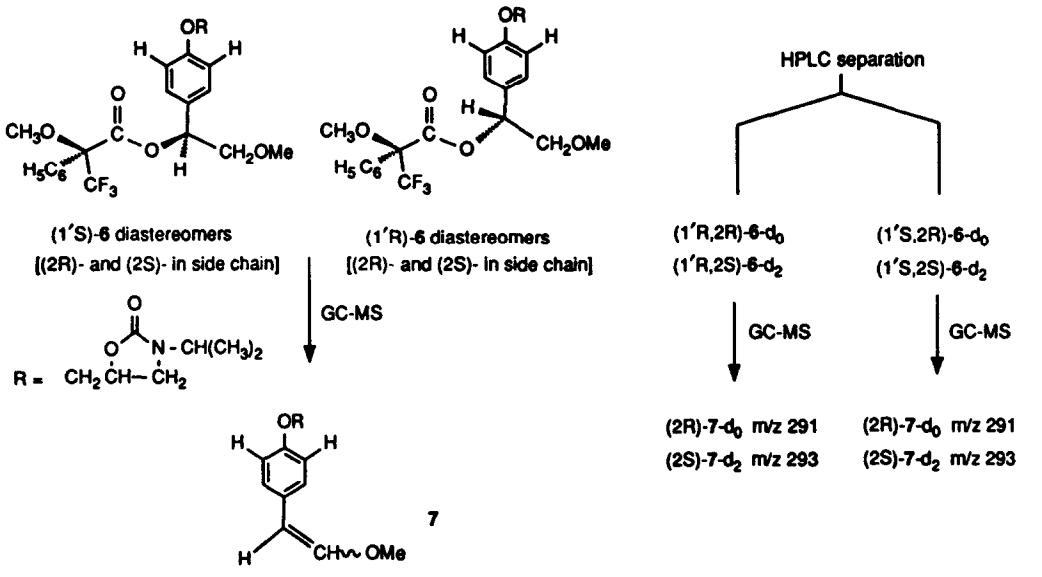

Fig. 2. Separation of diastereomeric components of **6** (Mosher esters of **2**) via HPLC, followed by GC–MS of HPLC eluents.

Table 3. Quantitation and enantioselectivity of human 24-hr urinary metoprolol and metabolites after a single dose of pseudoracemic metoprolol tartrate

Subject	1 (Metoprolol) % Dose	1 (Metoprolol) 2R/2S	2 % Dose	2 2R/2S	Acid 4 % Dose	Acid 4 2R/2S
1	2.4	0.62	12.3	0.82	55.8	1.16
2	3.5	0.59	10.3	0.75	40.7	1.10
3	4.1	0.71	8.0	0.95	53.0	1.25
4	2.8	0.71	10.0	0.79	49.3	1.25
5	1.8	0.63	8.7	0.71	55.0	1.17
6	5.2	1.07	4.4	1.13	50.0	0.98
7	2.1	0.81	10.2	0.91	64.8	1.11
8	0.2	0.67	10.7	0.71	52.9	1.15
Mean ± SD	2.8 ± 1.5	0.73 ± 0.16	9.3 ± 2.4	0.85 ± 0.14	52.7 ± 6.8	1.15 ± 0.09

Pseudoracemic metoprolol tartrate (50 mg) was administered as a solution to eight subjects. Urinary metoprolol (**1**), α-hydroxymetoprolol (**2**), and acid **4** were determined from aliquots of the 24-hr urine. Values are means of duplicate determinations.

2*R*/2*S* ratio 0.85 for α-hydroxymetoprolol (**2**) and 1.15 for acid **4**, respectively, ratios that are different from each other.

Product stereoselectivity data for urinary **2** appear in Table 4. Formation of a new 1′*R* chiral center in **2** was approximately three times greater than the 1′*S*-diastereomer, with the 1′*R*,2*S*-diastereomer of **2** being the metabolite found in slightly greater amount than the 1′*R*, 2*R*-diastereomer. A summary of the enantioselective and diastereoselective results appears in Fig. 3.

DISCUSSION

Our results for the rat liver and human liver microsomal oxidation of metoprolol are in general agreement with previous data on α-hydroxylation and O-demethylation [5, 24, 25]. The rate of α-hydroxylation exceeded O-demethylation by about 30% in the rat liver microsomal preparation. However, in the human liver microsomal preparations, O-demethylation exceeded α-hydroxylation by about 7 to 12-fold. There are enantioselectivity similarities between the two microsomal systems: both showed very little or no enantioselectivity in the α-hydroxylation process, 2*R*/2*S* ratios being 0.93 and 0.87–0.97, respectively, and only slight $R > S$ enantioselectivity for O-demethylation, 2*R*/2*S* ratio being 1.18 in the rat liver microsomes and ~1.10 in the human liver microsomes.

The α-hydroxylation process, as reflected in the urinary α-hydroxylation metabolite in men, preferentially generates a new 1′*R* chiral center over a new 1′*S* chiral center by 3-fold (1′*R*/1′*S* ratio), regardless of the chirality of the propanolamine side chain. The stereochemistry of benzylic hydroxylation processes are reported to be very substrate dependent, e.g. varying from an *R*/*S* ratio of >20 for

Table 4. Enantioselectivity and product stereoselectivity data on urinary **2** from eight extensive metabolizers

Subject	Enantioselectivity ratio* 2*R*/2*S*	Product stereoselectivity ratio† 1′*R*-**2**/1′*S*-**2**	Enantioselectivity of 1′*R* hydroxylation‡ Metoprolol 2*R*/2*S* ratio	Enantioselectivity of 1′*S* hydroxylation§ Metoprolol 2*R*/2*S* ratio
1	0.82	3.41	0.80	0.87
2	0.75	3.91	0.74	0.78
3	0.95	3.60	0.93	1.025
4	0.79	3.20	0.79	0.79
5	0.71	2.91	0.69	0.76
6	1.13	2.17	1.16	1.05
7	0.91	3.10	0.91	0.94
8	0.71	2.71	0.71	0.73
Mean ± SD	0.85 ± 0.14	3.12 ± 0.54	0.84 ± 0.16	0.87 ± 0.12

Values are means of triplicate determinations of single samples.
* [(1′*R*,2*R*)-**2** + (1′*S*,2*R*)-**2**]/[(1′*R*,2*S*)-**2** + (1′*S*,2*S*)-**2**].
† [(1′*R*,2*R*)-**2** + (1′*R*,2*S*)-**2**]/[(1′*S*,2*R*)-**2** + (1′*S*,2*S*)-**2**].
‡ [(1′*R*,2*R*-**2**)]/[(1′*R*,2*S*-**2**)].
§ [(1′*S*,2*R*-**2**)]/[(1′*S*,2*S*-**2**)].

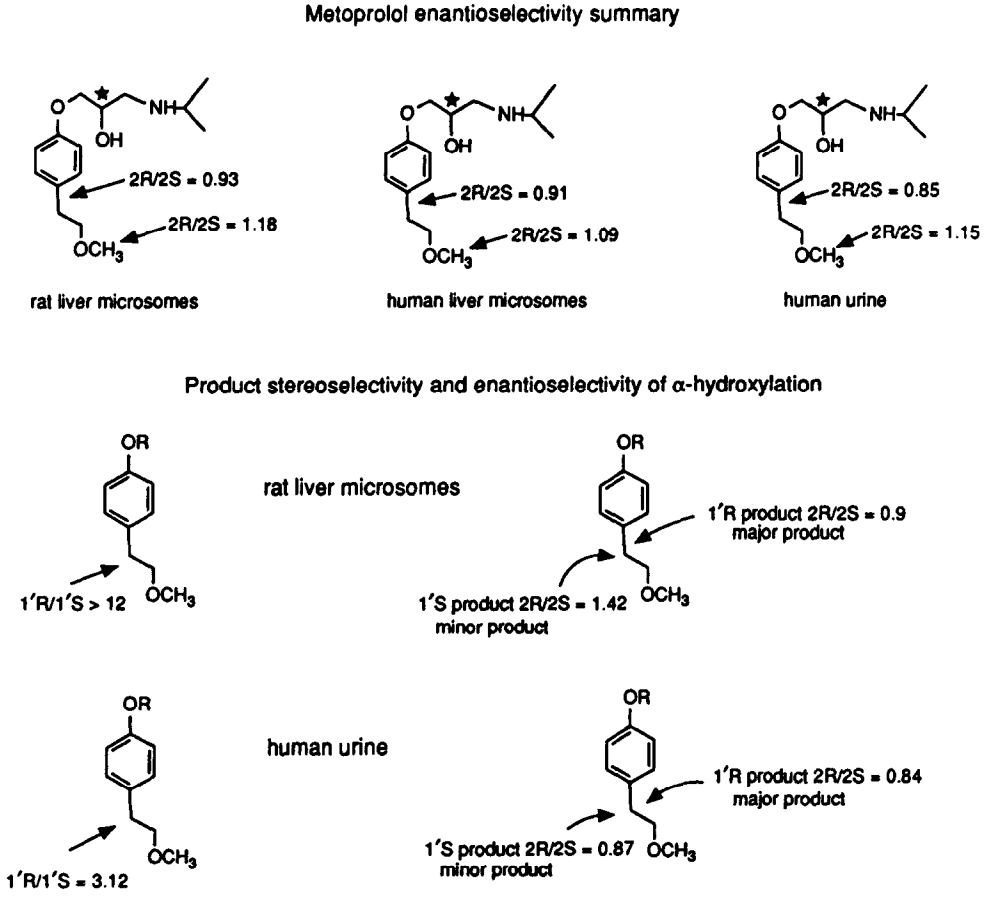

Fig. 3. Summary of stereoselective aspects of metoprolol metabolism.

benzylic hydroxylation of debrisoquine by extensive metabolizers, as measured as urinary metabolites [26–28], to rat liver microsomal oxidation of ethylbenzene where the *R*/*S* ratio is *ca.* 1.0 [29]. Clearly, the interaction of substrate with the P_{450} enzymes is heavily dependent on differential alignment of the important functional groups of the substrate and the enzyme. With small structural changes, differ-

ent energies of activation for the enantio- and diastereoselective oxidative processes would be expected.

The enantioselectivity of α-hydroxylation was slight both in rat and human liver microsomal preparations. The enantioselectivity of the microsomal α-hydroxylation process remained slight, even when the two diastereomeric products were examined individually (1′*R* products vs 1′*S* products). The enantioselective 2*R*/2*S* ratio for α-hydroxylation was 0.87–0.97 in all five of the human liver microsomal samples. The data obtained from the *in vivo* experiments in men are in agreement and seem to reflect the expected 2*R*/2*S* plasma concentration ratio of metoprolol enantiomers, where the 2*R*/2*S* ratio <1 has been observed [12]. *In vivo*, the enantiomeric ratio for the 1′*R* product [(1′*R*,2*R*)-**2** vs (1′*R*,2*S*)-**2**] showed essentially the same enantiomeric ratio as was determined for the sum of the diastereomeric products [(1′*R*,2*R*)-**2** + (1′*R*,2*S*)-**2**] vs [(2*S*,2*R*)-**2** + (1′*S*,2*S*)-**2**], as expected, because about three-fourths of the products have the 1′*R* stereochemistry. The 1′-*S* products also showed nearly the same 2*R*/2*S* ratio, approximately 0.85 (Table 4). We conclude that because the chiral center in the propanolamine side chain is remote from the catalytic site of benzylic hydroxylation, it has almost no effect on enantioselectivity.

The stereoselectivity of O-demethylation process observed from urinary metabolites *in vitro* ultimately may be reflected in acid **4**, even though subsequent conversion of the primary alcohol to the carboxylic acid must occur. Because no urinary alcohol was noted, the conversion of **3** to acid **4** is believed to be nearly quantitative. The observed enantioselectivity 2*R*/2*S* ratio >1 would seem to indicate that this oxidative process has significant enantioselectivity for (2*R*)-enantiomer of metoprolol, because the *R*/*S* concentration ratio of metoprolol enantiomers in plasma is reported to be less than 1.0 [12]. The observed slight 2*S* > 2*R* enantioselectivity for the O-demethylation of metoprolol enantiomers in human liver microsomal preparations is consistent with this speculation.

Recent results suggesting two enzymes are involved in these oxidations [25], the debrisoquine-related P_{450} enzyme associated with α-hydroxylation and an additional cytochrome P450 enzyme responsible for human liver microsomal O-demethylation, mitigate against over-interpretation of the observed metoprolol enantioselectivity data. The observed enantioselectivity may be a composite number arising from different enantiomeric contributions in these two processes and perhaps others. The enantioselectivities may be different for the high K_m-low capacity debrisoquine-related enzyme P_{450} and for the low K_m-high capacity debrisoquine-unrelated oxidative process [24]. Possible enantioselectivity in the metabolic processes following the first O-demethylation step could also influence the results, but this possibility seems less likely because no subsequent intermediates or secondary metabolites, e.g. aldehyde or alcohol, were observed *in vivo*.

In summary, our data clearly indicate a difference in enantioselectivity for the α-hydroxylation and O-demethylation processes in the rat and human liver microsomal preparations, and *in vivo* in men (Fig. 3). The diastereoselectivity of the α-hydroxylation process affords both possible products, with products with the new chiral center 1′*R* predominating. The stereochemical observations *in vivo* are consistent with the stereochemistry of the O-demethylation process being at least partly responsible for the difference in plasma levels of (2*R*)- and (2*S*)-metoprolol in humans.

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