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# Stereoselective Urinary Excretion of Bupivacaine and Its Metabolites During Epidural Infusion

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*ABSTRACT* A sensitive and efficient chiral assay for bupivacaine and its three principal metabolites desbutylbupivacaine, 4′-hydroxybupivacaine, and 3′-hydroxybupivacaine has been applied to urine from five male patients receiving postoperative epidural infusions of *rac*-bupivacaine fentanyl over 60–120 hr. The fraction of the dose of bupivacaine (total dose 840–2093 mg) accounted for in urine was 75 ± 6%. The rate of excretion of bupivacaine enantiomers approximated a steady state after ~30 hr with values of 1.27 ± 0.26 and 0.76 ± 0.13 mg $hr^{-1}$ for (R)- and (S)-enantiomers, respectively. The fraction of the dose of bupivacaine enantiomer excreted unchanged in the urine (fe) varied from 14.3% to 39.1% for (+)-(R)-bupivacaine and 9.2% to 14.0% for (–)-(S)-bupivacaine in the five patients. The rate of excretion of all metabolites also reached a steady state after ~30 hr and the relative amounts of metabolites excreted into urine (fm) suggest bupivacaine is subject to regioselective and stereoselective clearance, which may vary from patient to patient. *Chirality 11:50–55, 1999.* 

*KEY WORDS:* chiral chromatography; bupivacaine enantiomers; bupivacaine metabolites; urine; human

Bupivacaine is a long-acting local anaesthetic widely used in surgery and obstetrics which is marketed as a racemic mixture. Although the biotransformation of bupivacaine in man has been only partially elucidated,[1,2] *N*-desbutylbupivacaine (DBB, also known as 2,6-pipecoloxylidide), 3′-hydroxybupivacaine (3′-OHB), and 4′-hydroxybupivacaine (4′-OHB) have been shown to be major metabolites in rats.[3] Like the parent drug, each of these compounds possesses one chiral center and exists as two enantiomers. The structures of (–)-(S)-bupivacaine and its three metabolites are shown in Figure 1. Measurement of the concentrations of the enantiomers of bupivacaine and its metabolites in biological fluids is necessary to understand pharmacokinetic-pharmacodynamic relationships and toxicity following bupivacaine administration.

Pharmacokinetic studies of the enantiomers of bupivacaine, administered either separately[4] or as the racemate[5] to sheep, showed that total body clearance of the enantiomers was almost exclusively accounted for by hepatic clearance, which was higher for (+)-(R)- than for (–)-(S)-bupivacaine. Only negligible quantities of each enantiomer were excreted into urine and renal clearance was not enantioselective. In humans, plasma concentrations of bupivacaine enantiomers after bolus doses of racemate via a number of different routes of administration were higher for (–)-(S)-bupivacaine,[6–9] possibly due to the higher protein binding of the (S)-enantiomer.[6] During continuous paravertebral infusion of *rac*-bupivacaine for five days, the steady state concentrations of (–)-(S)-bupivacaine were generally higher than those of the (R)-enantiomer, but the difference was not statistically significant.[10,11]

Bupivacaine is significantly more cardiotoxic than other local anaesthetics and has been responsible for a number of fatalities over the years.[12] Evidence from animal studies shows that (+)-(R)-bupivacaine is more cardiotoxic than its antipode,[13–15] which has provided the impetus for the introduction of the single enantiomer form into clinical practice (the chiral switch). A parallel development has already seen the introduction of ropivacaine,[16] the (–)-S-enantiomer of the propyl homologue of bupivacaine, which is claimed to be less cardiotoxic than bupivacaine.[17] Little is known about the relative toxicity of bupivacaine metabolites, but studies in rats and mice suggest that the toxicity of DBB is appreciable.[18,19] Since S-DBB is also a metabolite of ropivacaine in man,[20] there is clearly a need to compare and contrast metabolic profiles and toxicities of the enantiomers of bupivacaine and ropivacaine to understand the consequences of the introduction of single enantiomers into clinical practice.

In providing long-duration pain relief after abdominal surgery, bupivacaine is commonly administered by continuous epidural infusion or intercostal neural blockade to

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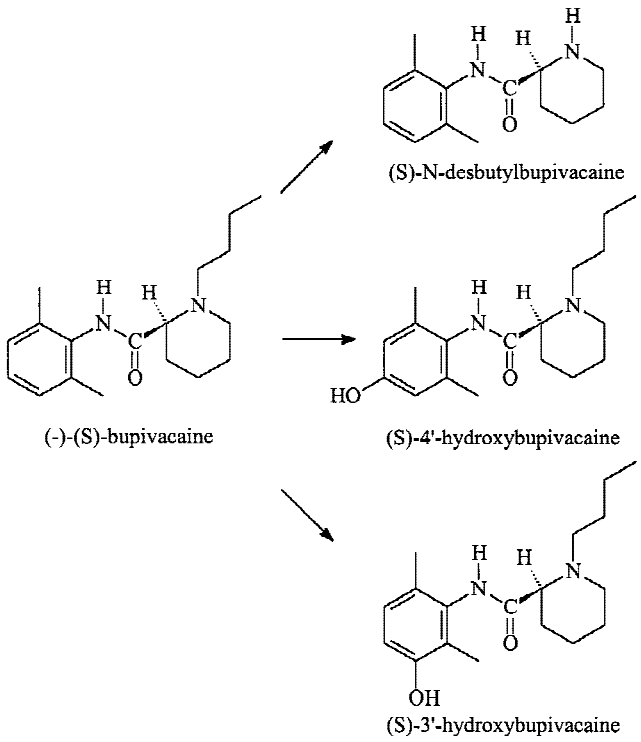


**Fig. 1.** Structure of (–)-(S)-bupivacaine and three of its principal metabolites.

control postoperative pain.[1] Patients are treated with bupivacaine for long periods of time, during which they are potentially exposed to high concentrations of bupivacaine and its metabolites in the systemic circulation. The purpose of this study was to investigate the stereoselectivity of urinary excretion of bupivacaine and its metabolites during long-term continuous postoperative epidural infusion of racemic drug as a preliminary to a more comprehensive pharmacokinetic study involving chiral analysis of drugs in blood.

## EXPERIMENTAL

### *Materials*

*rac*-Bupivacaine hydrochloride [(RS)-1-butyl-2′,6′-pipecoloxylidide hydrochloride] was purchased from Sigma Chemical Company (St. Louis, MO). (+)-(R)- and (–)-(S)-bupivacaine (enantiomeric excess >99%) were donated by Chiroscience, Cambridge, UK. Racemic 3′-OHB, 4′-OHB, and DBB, kindly donated by Professor John Caldwell, Imperial College, London, UK, were chromatographically pure. Ropivacaine [(–)-(S)-1-propyl-2′,6′-pipecoloxylidide hydrochloride, enantiomerically pure by chiral HPLC] was obtained from Astra Pain Control AB, Södertälje, Sweden. Diethylamine, propan-2-ol, and *n*-hexane were from BDH Chemicals Ltd. (Poole, UK). All other reagents were of analytical grade. Water for HPLC was distilled and passed through a reverse osmosis Milli-Q Reagent Water System.

### *Chromatography*

The HPLC system consisted of a Shimadzu solvent delivery system (Model LC-AS10), a Rheodyne injector (Model 7725) fitted with a 50 µl fixed loop, a Shimadzu UV detector (Model SPD-10A) set at 210 nm, and an SRI model 8600-2000 peak simple II data system as integrator. The analytical column (Chrom Tech, Sweden, AGP:50.4) was of stainless steel packed with $\alpha_1$-acid glycoprotein bonded to a silica support (150 × 4 mm, 5 µm). A guard column (10 × 3.0 mm) of the same material was included in the system. The mobile phase was prepared by mixing an aqueous solution containing sodium dihydrogen phosphate (8 mM) and sodium chloride (0.1 M) with propan-2-ol and diethylamine (96.4:3:0.6, v/v/v). The solution was adjusted to pH 7.04 ± 0.01 with 50% phosphoric acid, filtered through a 0.45 µm filter, and degassed for 30 s with a sonicator before use. The mobile phase was delivered isocratically at 0.9 ml $min^{-1}$. Under these conditions baseline resolution of all stereoisomers was obtained (Fig. 2). The (+)-(R) and (–)-(S) enantiomers of bupivacaine eluted at 37.1 and 47.5 min, respectively, in the same order as previously reported by Hermansson.[21] The peaks due to DBB at 6.8 and 17.7 min were assigned by comparison with those found in urine from a patient on ropivacaine (Naropin, Astra Pharmaceuticals). Of the four peaks observed in ropivacaine urine samples, only one matched the retention time of one of the DBB peaks, which was therefore assigned to that of S-DBB. Assignment of the peaks due to the enantiomers of the hydroxylated metabolites of bupivacaine at 8.1 and 10.1 min for 4′-OHB and 13.1 and 15.5 min for 3′-OHB was based on the assumption that they eluted in the same order as the (R)- and (S)-enantiomers of bupivacaine and DBB. There was no interference with any of the peaks from endogenous urine components nor from fentanyl or other medications administered to the patients at the time of surgery.

### *Sample Preparation*

Urine (1 ml) was made alkaline with 0.1 ml sodium carbonate solution (1 M, pH 10) and extracted with 6.5 ml of *n*-hexane-propan-2-ol (5:1) by rotating on a tumble mixer at 28 rpm for 10 min. After centrifugation at 3600 rpm for 20 min, 5 ml of the organic layer was evaporated to dryness at ambient temperature using a Savant Speed Vac concentrator Model SVC-200H equipped with refrigerated vapour traps RVT 4104 and VP 100 and a two-stage Savant pump. Extracts were reconstituted in 210 µl of mobile phase and 50 µl aliquots analysed by HPLC. Urine samples containing concentrations exceeding the calibration range were reanalysed after dilution with isotonic saline.

### *Assay Validation*

The calibration curves for all stereoisomers were linear ($r^2 > 0.99$) in the range 5–1000 ng $ml^{-1}$ with intercepts not significantly different from zero. The lower limits of detection (signal-to-noise ratio of 3:1) for the various stereoisomers varied with their retention times but were all less than 5 ng $ml^{-1}$. Coefficients of variation for intra-day precision based on analysis of five urine samples each spiked at five concentrations over the concentration range 5–1000 ng $ml^{-1}$ ranged from 4% at 1000 ng $ml^{-1}$ to 10% at 5 ng $ml^{-1}$ for all stereoisomers. Determination of inter-day precision by analysis on three separate days produced similar values.

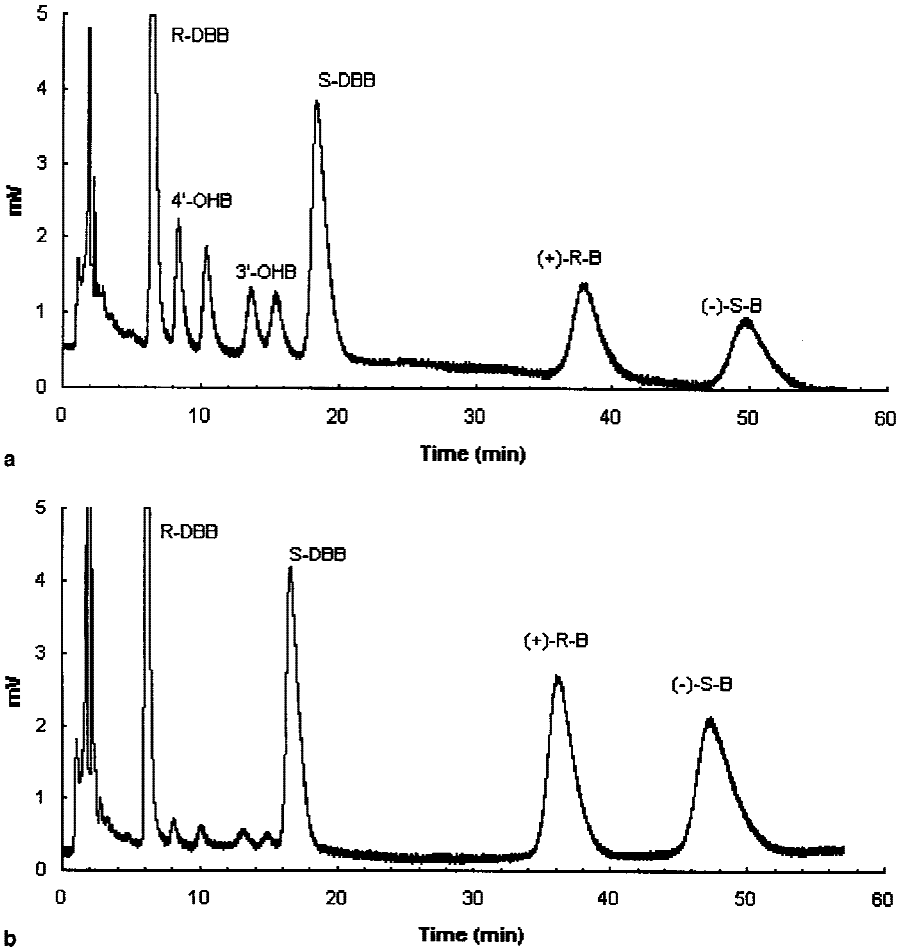


**Fig. 2.** HPLC profile of (**a**) urine spiked with racemic samples of bupivacaine, DBB (1 μg/ml), 3′-OHB, and 4′-OHB (0.5 μg/ml), and (**b**) a urine sample from patient C 18 hr following initiation of epidural bupivacaine-fentanyl infusion.

Recoveries from spiked urine over the range 5–1000 ng $ml^{-1}$ were >89% for all stereoisomers.

### *Clinical Study*

Urine was collected from five male patients (age, 55–78 years; weight, 59–85 kg) receiving postoperative long-term epidural *rac*-bupivacaine fentanyl infusions (Biomed, Auckland, New Zealand). Details of the five patients including their status according to the American Society of Anesthesiologists (ASA) classification and previous chronic drug therapy, are given in Table 1. The results of kidney and liver function tests on the patients were not significantly outside the normal reference ranges. The study was approved by the Southern Regional Health Authority Ethics Committee (Otago) and written informed consent was obtained from all patients. Patients A–C underwent gastrectomy, whereas patients D and E had abdominal aortic aneurism repair. The premedication for all patients was midazolam 7.5 mg followed by induction with thiopentone. The patients were paralysed with vecuronium (0.1 mg $kg^{-1}$) given i.v. to facilitate tracheal intubation. General anaesthesia was maintained with isoflurane and oxygen and air. Patients received a bolus dose of *rac*-bupivacaine (15–20 ml of 0.5% v/v) followed by continuous epidural infusion of *rac*-bupivacaine fentanyl (0.125% v/v bupivacaine, 2 μg $ml^{-1}$ fentanyl at a rate of 8–14 ml $h^{-1}$) over 60–120 hr with occasional bolus doses such that the total dose of bupivacaine in the five patients ranged from 840 to 2093 mg (Table 2). Cumulative urine samples were collected at intervals of 5.5 hr during the infusion and then every 4 hr postinfusion for a further 24 hr. Urine samples were stored at −84°C prior to assay.

### *Analysis of Data*

The fraction of the dose of bupivacaine accounted for in urine was calculated as the sum of the amounts of all enantiomers of bupivacaine and its three principal metabo-

**TABLE 1. Details of patients undergoing major surgery with ASA status and previous chronic drug therapy**

| Patient | Age | Weight (kg) | ASA status | Previous drug therapy |
|---|---|---|---|---|
| A | 76 | 59 | 3 | ranitidine, frusemide, salbutamol |
| B | 65 | 65 | 2 | salbutamol, beclomethasone |
| C | 65 | 79 | 2 | dipyridamole, warfarin |
| D | 55 | 65 | 2 | prednisolone, mesalazine |
| E | 78 | 85 | 3 | aspirin, cimetidine, frusemide, paracetamol, dextropropoxyphene |

**TABLE 2. Urinary excretion of the enantiomers of bupivacaine as percentages of the enantiomer dose**

| Patient | Total Dose (mg) | Parameter | Percentage R(+) | Percentage S(–) | SI[c] |
|---|---|---|---|---|---|
| A | 839.9 | fe[a] | 39.1 | 9.2 | 4.3 |
| | | fe*[b] | 43.2 | 16.0 | 2.7 |
| B | 844.5 | fe | 24.0 | 12.2 | 2.0 |
| | | fe* | 16.6 | 8.0 | 2.1 |
| C | 1162 | fe | 22.0 | 14.0 | 1.6 |
| | | fe* | 23.6 | 13.0 | 1.8 |
| D | 2093 | fe | 14.3 | 13.6 | 1.1 |
| | | fe* | 11.3 | 10.9 | 1.0 |
| E | 1326 | fe | 15.8 | 12.1 | 1.3 |
| | | fe* | 16.6 | 12.7 | 1.3 |

[a]fe = cumulative amount of enantiomer in urine ÷ dose of enantiomer.
[b]fe* = rate of excretion of enantiomer at steady state ÷ rate of infusion of enantiomer.
[c]SI, stereoselective index.

lites excreted in urine divided by the total dose. The fraction of each bupivacaine enantiomer excreted unchanged (fe) was calculated using two equations: fe = cumulative amount of enantiomer in urine/dose of enantiomer; fe* = rate of excretion of enantiomer at steady state/rate of infusion of enantiomer. The fraction of bupivacaine excreted as a particular metabolite (fm) was calculated as fm = cumulative amount of metabolite/dose of enantiomer. The fraction of the dose of (+)-(R)-bupivacaine excreted as (R)-enantiomers or of (–)-(S)-bupivacaine excreted as (S)-enantiomers was calculated as the sum of fe and fm for all the (R)- or (S)-enantiomers, respectively. The ratio of the higher value of fe, fe*, or fm to the lower value for a given stereoisomer is referred to as the stereoselective index (SI). Stereoselectivity for a given pharmacokinetic parameter is considered important if SI is > 1.2.[22] Steady state was established by visual inspection, and the rate of excretion at steady state was the mean of the rates for urine samples collected on the plateau. The half-lives of the terminal phase of excretion were calculated by plotting the log of excretion rate against time for the postinfusion period.

## RESULTS

The fraction of the dose of bupivacaine accounted for in urine varied from 67.9 to 81.7% in the five patients. The cumulative mass of the (R)-enantiomers excreted in urine accounted for 79 ± 6% of the enantiomer dose compared with 71 ± 8% for the (S)-enantiomers. The rate of excretion of all stereoisomers attained steady state after ~30 hr. The time profiles of the rate of excretion of all metabolites (four pairs of enantiomers) in one of the patients are shown in Figure 3.

Values of fe in the five patients are shown in Table 2 and varied from 14.3% to 39.1% for (+)-(R)-bupivacaine and 9.2% to 14.0% for (–)-(S)-bupivacaine. Values of fe* are also given in Table 2 and agreed well with those of fe in all patients. Except for one patient, the SI were greater than 1.2. The steady-state rates of excretion of all stereoisomers are given in Table 3 and values of fm are given in Table 4. Dealkylation appears to be the predominant route of metabolism of bupivacaine in patients C, D, and E, whereas hydroxylation appears to be the predominant route in patients A and B. Excretion appears to be stereoselective for all metabolites except for DBB when dealkylation is the major route of metabolism. The half-lives for the terminal phase of excretion of (+)-(R)- and (–)-(S)-bupivacaine were 10.1 and 10.3 hr, respectively, and values for the bupivacaine metabolites were similar, ranging from 9.4 hr for R-4′-OHB to 10.3 hr for R-DBB.

## DISCUSSION

This study represents the first example of a clinical study in which bupivacaine and three of its principal metabolites are analyzed simultaneously. Previous studies of bupivacaine metabolism have been limited to assay of bupivacaine, DBB, and 4′-OHB.[23–26] Although limited to urinary excretion, our results confirm that N-dealkylation and ring hydroxylation are principal routes of metabolism of bupivacaine in man and that excretion of DBB, 3′-OHB, and 4′-OHB accounts for most of the dose administered via continuous epidural infusion. Urinary excretion of all metabolites reached steady state, tending to refute previous suggestions that metabolites may accumulate during long-term administration of bupivacaine.[24]

The time to steady state for excretion of bupivacaine into urine of ~30 hr is consistent with values of the time to steady state in blood found in other studies involving long-term continuous epidural infusion of *rac*-bupivacaine.[10,11,26] Burm et al.[27] found systemic absorption of bupivacaine during epidural administration to 12 surgical patients occurred via two parallel first-order processes with a half-life of 6 hr for the slow one. On this basis they suggested a steady-state blood concentration would be reached after 24 hr. This shorter time is probably a reflection of the younger age of the patients studied by Burm et al. (32 ± 10 years) compared to our patients (68 ± 8 years).

With regard to the stereoselectivity of bupivacaine urinary excretion, our results show that the fraction of (+)-(R)-bupivacaine excreted unchanged in urine is greater than that of (–)-(S)-bupivacaine in all patients. Given that this situation pertains to steady-state excretion, the difference indicates that the ratio of renal to total clearance of (+)-(R)-bupivacaine is greater than that of its antipode. In terms of the enantiomeric ratio in blood, Mather et al.[7] found the ratio of (+)-(R)- to (–)-(S)-bupivacaine in 12 patients receiving bolus injections of *rac*-bupivacaine at eight hourly intervals was 0.74 ± 0.11. In this case, the difference in blood levels was ascribed to a greater clearance and larger volume of distribution of (+)-(R)-bupivacaine.

The rates of excretion of the enantiomers of the three principal metabolites of bupivacaine reached steady state after ~30 hr and the relative rates of excretion of the (R)- and (S)-enantiomers at steady state and values of fm suggest regioselectivity and stereoselectivity are important in the clearance of bupivacaine. The different metabolic profiles observed in our patients suggest bupivacaine metabolism may be mediated by different amounts of microsomal enzymes such as cytochrome P450 isoforms, each with

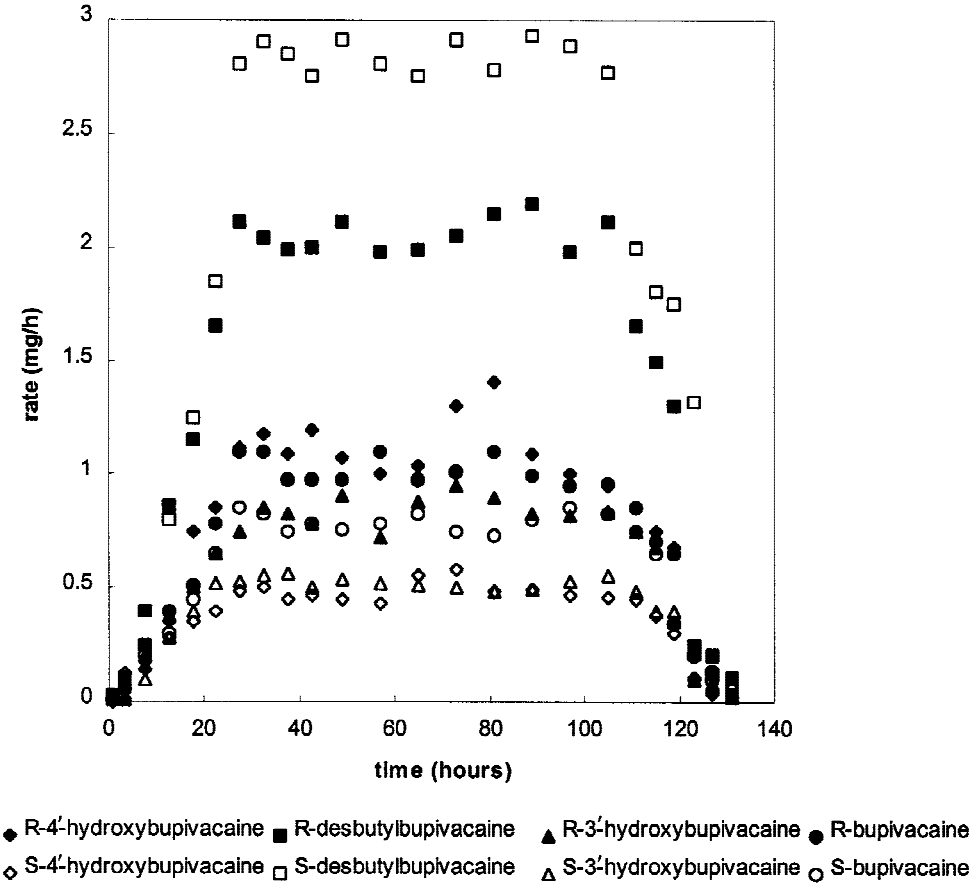


**Fig. 3.** Time profile of rate of excretion of R-bupivacaine and its metabolites (filled symbols) and S-bupivacaine and its metabolites (open symbols) for patient E.

**TABLE 3. Rates of excretion of the enantiomers of bupivacaine and its metabolites into the urine at steady state***

| | Patient | | | | |
|---|---|---|---|---|---|
| Compound | A | B | C | D | E |
| R-DBB | 0.64 ± 0.13 | 1.38 ± 0.28 | 2.72 ± 0.26 | 2.41 ± 0.25 | 2.10 ± 0.07 |
| S-DBB | 0.23 ± 0.05 | 0.37 ± 0.06 | 3.75 ± 0.29 | 2.91 ± 0.27 | 2.84 ± 0.07 |
| R-3′-OHB | 0.24 ± 0.14 | 0.26 ± 0.02 | 0.20 ± 0.04 | 1.38 ± 0.40 | 0.84 ± 0.07 |
| S-3′-OHB | 1.48 ± 0.62 | 2.03 ± 0.53 | 0.59 ± 0.07 | 0.74 ± 0.10 | 0.52 ± 0.02 |
| R-4′-OHB | 1.00 ± 0.33 | 0.87 ± 0.09 | 0.72 ± 0.15 | 1.66 ± 0.12 | 1.12 ± 0.13 |
| S-4′-OHB | 0.56 ± 0.14 | 0.40 ± 0.07 | 0.49 ± 0.02 | 0.71 ± 0.08 | 0.48 ± 0.04 |
| (+)-(R)-B | 1.65 ± 0.32 | 1.25 ± 0.26 | 1.47 ± 0.24 | 0.99 ± 0.13 | 1.01 ± 0.05 |
| (−)-(S)-B | 0.63 ± 0.32 | 0.62 ± 0.11 | 0.81 ± 0.08 | 0.96 ± 0.12 | 0.80 ± 0.04 |

*Data are mean ± SD for rate of excretion at steady state ($n$ = 6–20); rates are given in mg $hr^{-1}$.

**TABLE 4. Urinary excretion of the enantiomers of the three metabolites of bupivacaine as percentages of the enantiomer dose**

| | desbutylbupivacaine (DBB) | | | 3′-hydroxybupivacaine (3′-OHB) | | | 4′-hydroxybupivacaine (4′-OHB) | | |
|---|---|---|---|---|---|---|---|---|---|
| Patient | R | S | SI[a] | R | S | SI | R | S | SI |
| A | 13.8 | 4.8 | 2.8 | 4.9 | 33.9 | 6.8 | 22.7 | 9.4 | 2.3 |
| B | 24.4 | 6.2 | 3.9 | 4.3 | 39.9 | 9.1 | 17.2 | 8.0 | 2.1 |
| C | 43.0 | 52.0 | 1.2 | 3.3 | 9.5 | 2.8 | 12.0 | 7.8 | 1.5 |
| D | 30.9 | 37.3 | 1.2 | 18.1 | 12.9 | 1.4 | 23.1 | 10.2 | 2.3 |
| E | 33.4 | 43.4 | 1.3 | 14.2 | 8.5 | 1.7 | 18.3 | 7.3 | 2.5 |

[a]SI, stereoselective index.

different substrate stereoselectivities. We hope that future chiral studies of in vitro metabolism of bupivacaine by phenotyped human liver samples will elucidate these differences and whether they are clinically important.

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