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CHIRAL INVERSION OF 2-ARYLPROPIONIC ACID NON-STEROIDAL ANTI-INFLAMMATORY DRUGS—II

RACEMIZATION AND HYDROLYSIS OF (*R*)- AND (*S*)-IBUPROFEN-CoA THIOESTERS

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Abstract—The inversion of 2-arylpropionic acids (2-APAs) has become the subject of much attention. It is a unique reaction specific to this group of drugs. Inversion proceeds via stereoselective activation of the *R*-enantiomer to its CoA thioester whereby it is then racemized and hydrolysed to release free drug. The racemization and hydrolysis processes have been examined in this study using chemically synthesized CoA thioesters of the ibuprofen enantiomers and *in vitro* models employing rat liver homogenate and the mitochondrial and microsomal fractions as the source of the 'racemase' enzymes. Rat liver homogenate mediated the racemization and hydrolysis of both (*R*)- and (*S*)-ibuprofen-CoA thioesters. The rat liver mitochondrial fraction similarly mediated racemization and hydrolysis of both CoA thioesters. There was less racemase activity in the rat liver microsomal fraction and the data indicated that this fraction may contain two hydrolases which act separately on the (*R*)- and (*S*)-ibuprofen-CoA thioesters. The data are further evidence that the stereoselectivity of the CoA synthetase controls the overall stereoselectivity of inversion.

The chiral inversion of 2-arylpropionic acids (2-APAs†) occurs in many species [1], however, the biochemical pathway for inversion is still poorly defined. An hypothesized mechanism of inversion for 2-APAs (Fig. 1; [2]) suggests that the key step is the stereospecific formation of the (*R*)-2-APA-CoA thioester. Subsequent studies have supported this suggestion [3–6].

According to the proposed schema (Fig. 1), the next step is racemization of the (*R*)- and then also the (*S*)-2-APA-CoA thioester. It has been proposed that this is a spontaneous reaction [7] but evidence suggests that it is enzyme catalysed [6, 8, 9]. The final step in the hypothesized mechanism of inversion of 2-APAs is hydrolysis of the ibuprofen-CoA thioesters to release free drug. There are a number of hydrolases which could possibly catalyse this reaction.

The present study was undertaken to further characterize the mechanism of inversion of 2-APAs. The thioesters of (*R*)- and (*S*)-ibuprofen were synthesized, thus by-passing the CoA synthetase step, and data on the racemization and hydrolysis characteristics of rat liver homogenate and subcellular fractions are presented.

MATERIALS AND METHODS

General. Pure (−)-(*R*) and (+)-(*S*)-ibuprofen were obtained from the Boots Company PLC (Nottingham, U.K.). Coenzyme A (sodium salt), trifluoroacetic acid, trifluoroacetic anhydride and thioglycollic acid were purchased from the Sigma Chemical Co. (Poole, U.K.). Solvents were of HPLC grade purchased from Mallincrodt, Australia. Protein concentrations were determined by the method of Lowry *et al.* [10] using bovine serum albumin as a standard.

Assay of ibuprofen enantiomers. The ibuprofen enantiomers were analysed using a stereoselective

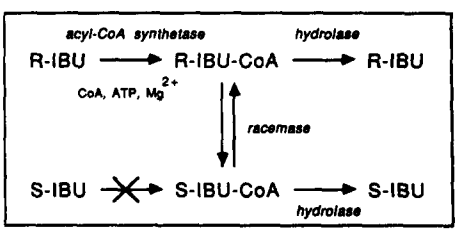

Fig. 1. Mechanism of inversion proposed by Nakamura *et al.* [2]. (*R*)-Ibuprofen is stereospecifically activated to (*R*)-ibuprofen-CoA by an acyl-CoA synthetase. (*R*)-Ibuprofen-CoA is racemized enzymatically to (*S*)-ibuprofen-CoA. The racemase (epimerase) is not enantioselective. The CoA thioesters are subsequently cleaved by a hydrolase to release (*R*)- and (*S*)-ibuprofen. (*S*)-Ibuprofen is not a substrate for the acyl-CoA synthetase.

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† Abbreviations: 2-APAs, 2-arylpropionic acids; CoA, coenzyme A.

HPLC assay which is based on the preparation of the diastereomeric (*S*)-2-octyl esters [11].

Synthesis of (R) *and* (S)*-ibuprofen-CoA.* (*R*)- and (*S*)-ibuprofen-CoA thioesters were synthesized on a small scale using the method of Chase and Tubbs [12]. The method involved the following:

(i) Preparation of ibuprofen-thioglycollate. (*R*)- or (*S*)-ibuprofen (2 g) was dissolved in trifluoroacetic acid (7.5 mL) and trifluoroacetic anhydride (1.25 mL) was added. After 1 hr at room temperature redistilled thioglycollic acid (0.7 mL) was added, and 30 min later water (1 mL) was added. The mixture was evaporated and the oily residue was left overnight in a dessicator under vacuum over NaOH. Addition of hexane (5 × 20 mL) did not result in crystallization as is usually observed with thioglycollates of fatty acids prepared in this manner. The ibuprofen-thioglycollate was used without further attempts at purification by crystallization.

(ii) Preparation of ibuprofen-CoA thioester. (*R*)- or (*S*)-ibuprofen thioglycollate (70 μL) was combined with *t*-butanol (0.72 mL), water (0.64 mL) and NaOH (0.2 mL; 1 M). CoA (20 mg) was dissolved in *t*-butanol (0.5 mL) and water (0.5 mL). These two preparations were mixed and the pH adjusted to 8.5 with NaOH (1 M). After 45 min at 30° the solution was acidified to pH 3 with $HClO_4$ (10%) and the *t*-butanol was evaporated with nitrogen. The pH was then adjusted to 1–2 with $HClO_4$ solution and the mixture extracted with ether (6 × 2–3 mL) and cooled. Ether was evaporated with nitrogen and NaCl was added to induce precipitation. The precipitate was centrifuged for 3 min and the pellet was resuspended in water (1 mL) at a final pH of 6–7 adjusted with Tris base (1 M).

(*R*)- and (*S*)-ibuprofen-CoA thioesters were quantitated using a spectrophotometric assay based on the detection of free CoA by its reaction with Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)]. An aliquot of synthesized thioester (5 μL) was added to a cuvette containing Tris buffer (980 μL; 0.1 M pH 8.1) and Ellman's reagent (12.5 μL; 10 mM dissolved in H_2O). The cuvette was placed in the spectrophotometer (416 nm) and partially purified rat brain acyl-CoA thioesterase (5 μL; courtesy of Dr Michael Edwards, University of New South Wales, Sydney) was added to catalyse the reaction. The hydrolysis of (*R*)- and (*S*)-ibuprofen-CoA was complete in 2 min as indicated by no further release of free CoA. The concentration of the CoA thioester formed was calculated from the relationship, $C = \Delta A/\varepsilon$ where C is the concentration of the CoA thioester, ΔA is the absorbance change and ε is the molar extinction coefficient (13,600; [13]). The enantiomeric purity of the CoA esters was determined by hydrolysing the CoA thioesters with NaOH solution (100 μL, 2 N, 30 min at room temperature) and measuring the enantiomers of ibuprofen by HPLC.

Preparation of rat liver homogenate. Animal studies were approved by the Garvan Institute of Medical Research Animal Ethics Committee. Male Wistar strain rats (200–250 g) were purchased from the Garvan Institute (Australia). Animals were allowed free access to food and water. Following ether anaesthesia the liver was flushed with cold saline via the portal vein and excised immediately. The liver was homogenized in 5 volumes of buffer (50 mM Tris–HCl pH 7.7, 0.25 M sucrose and 50 mM $MgCl_2$, 4°) using a mechanically driven Teflon–glass homogenizer (10 vertical passes). Cellular debris was separated by centrifugation (15 min, 4°, 2000 *g*).

Preparation of the rat liver mitochondrial fraction. A male Wistar rat (250 g) was anaesthetized with ether, the liver was flushed with saline via the portal vein and removed immediately. The liver was homogenized (1 g tissue : 9 mL buffer; 50 mM Tris–HCl, pH 7.4, 0.25 M sucrose, 4°) using a mechanically driven Teflon–glass homogenizer (10 vertical passes) and the homogenate was centrifuged (10 min, 4°, 2500 *g*). The resulting supernatant was further centrifuged (10 min, 4°, 10,000 *g*). The supernatant was discarded and the pellet was gently resuspended in buffer (20 mL). This suspension was centrifuged (10 min, 4°, 10,000 *g*) and the supernatant was discarded. This procedure was repeated twice. The final mitochondrial pellet was resuspended in buffer (1 mL buffer : 5 g original tissue).

Preparation of the rat liver microsomal fraction. A male Wistar rat (250 g) was anaesthetized with ether, the liver was flushed with saline and removed immediately. The liver was homogenized (1 g tissue : 3 mL buffer; 20 mM Tris–HCl, pH 7.4, 1.15% KCl, 1 mM EDTA, 4°) using a mechanically driven Teflon–glass homogenizer (10 vertical passes). The homogenate was centrifuged (10 min, 4°, 2500 *g*) to remove cellular debris. The supernatant was centrifuged (15 min, 4°, 10,000 *g*) to remove the mitochondrial pellet and further centrifuged (1 hr, 4°, 105,000 *g*) to precipitate the microsomal pellet. This pellet was resuspended in the above buffer (20 mL) without KCl and centrifuged (1 hr, 4°, 105,000 *g*). This procedure was repeated and the final pellet was resuspended in Tris–HCl buffer. A similar protein concentration (7.0 mg/mL) to that used for the mitochondrial fraction (7.3 mg/mL) was used so that the enzyme activities could be compared.

Incubation conditions. The incubation mixture contained Tris–HCl (50 mM, pH 7.7, 100 μL) either (*R*)- or (*S*)-ibuprofen-CoA and aliquots (100 μL) of either rat liver homogenate (25 mg/mL protein), mitochondrial fraction (7.3 mg/mL protein) or microsomal fraction (7.0 mg/mL protein). These samples were all placed in a shaking water bath maintained at 37°. Rat liver homogenate incubated with the thioesters was monitored for 4 hr while the mitochondrial and microsomal fractions incubated with the thioesters were monitored for 60 min. On removal from the water bath samples were treated in two ways: (i) acidified (0.3 mL, 3 N HCl), extracted (10 mL hexane, 15 min) and assayed for (*R*)- and (*S*)-ibuprofen, and (ii) subjected to alkaline hydrolysis (100 μL, 2 N NaOH, 30 min at room temperature) followed by acidification (0.3 mL, 3 N HCl) and extraction (10 mL hexane, 15 min). The concentrations of drug in these samples represent the total recovery of (*R*)- and (*S*)-ibuprofen.

RESULTS

CoA thioesters

The recovery of CoA thioester following synthesis based on CoA was only 10% for both thioesters. The

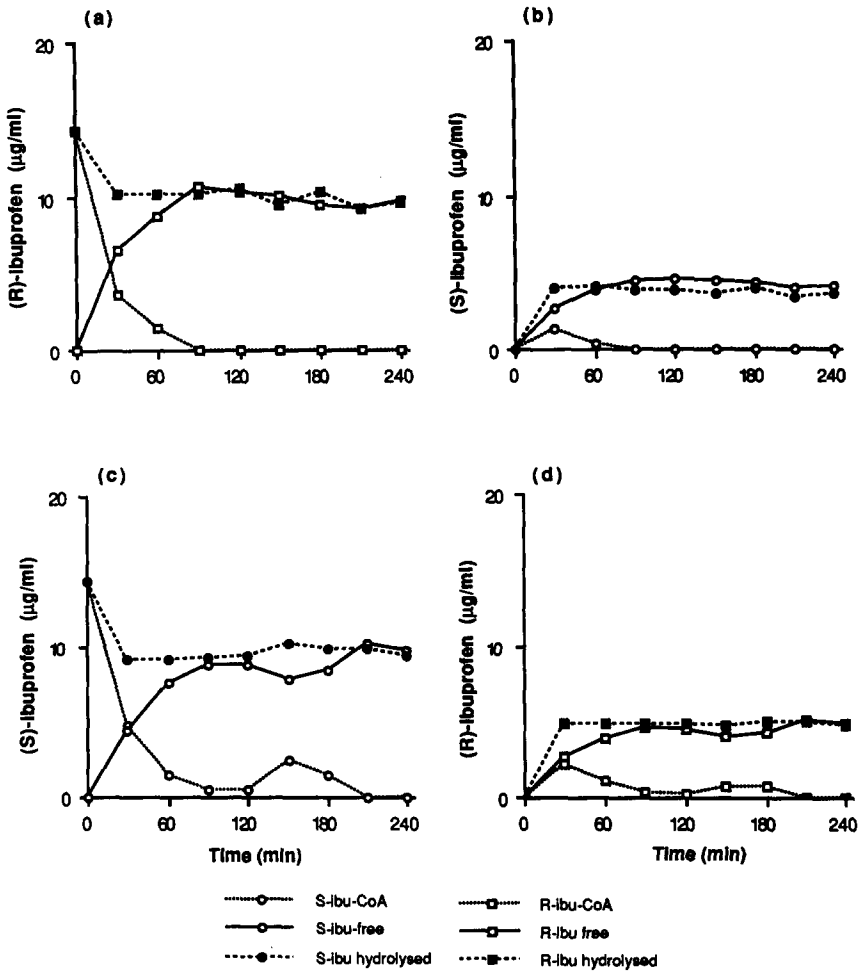

Fig. 2. Incubation of (*R*)-ibuprofen-CoA (15 μg/mL; a, b) and (*S*)- ibuprofen-CoA (15 μg/mL; c, d) with rat liver homogenate (25 mg/mL protein). Profiles (a) and (d) are the (*R*)-ibuprofen concentrations only and profiles (b) and (c) are the (*S*)-ibuprofen concentrations only. Free concentrations are those prior to alkaline hydrolysis of the samples. Hydrolysed concentrations are those after alkaline hydrolysis and represent the total drug present in either the *R*- or *S*-configuration at that time point. (*R*)- and (*S*)-ibuprofen-CoA concentrations are determined by subtraction of free from hydrolysed concentrations. Each time point is the mean of duplicate samples.

chemical purity of (*R*)- and (*S*)-ibuprofen-CoA was 85 and 83%, respectively, as determined following hydrolysis and measurement of the released CoA. There was no evidence that the partially purified rat brain acyl-CoA thioesterase was stereoselective in its action on the thioesters. The enantiomeric impurity following hydrolysis was 20.9 ± 3.7% for both enantiomers.

Rat liver homogenate

(*R*)-Ibuprofen-CoA was rapidly hydrolysed by rat liver homogenate with appearance of (*R*)-ibuprofen (Fig. 2a). There was also racemization of (*R*)-ibuprofen-CoA to (*S*)-ibuprofen-CoA and subsequent release of (*S*)-ibuprofen (Fig. 2b). The concentrations of the enantiomers determined after hydrolysis of the samples indicated that the amount of loss of (*R*)-ibuprofen was equal to the amount of (*S*)-ibuprofen formed.

A similar pattern was observed for (*S*)-ibuprofen-CoA. (*S*)-Ibuprofen-CoA racemized to give (*R*)-ibuprofen-CoA (Fig. 2d) and both thioesters were hydrolysed as evidenced by the appearance of (*R*)- and (*S*)-ibuprofen (Fig. 2c and d). The rate of hydrolysis and racemization appeared to be similar for both (*R*)- and (*S*)-ibuprofen-CoA, although the data did not allow a quantitative estimate of the rate constants.

Mitochondrial fraction

The mitochondrial fraction also catalysed racemization and hydrolysis of both (*R*)- and (*S*)-ibuprofen-CoA, giving profiles similar to those found for rat liver homogenate. (*R*)-Ibuprofen-CoA thioester was rapidly racemized as evidenced by the appearance of (*S*)-ibuprofen-CoA, and hydrolysed as evidenced by the formation of (*R*)- and (*S*)-ibuprofen (Fig. 3a and b).

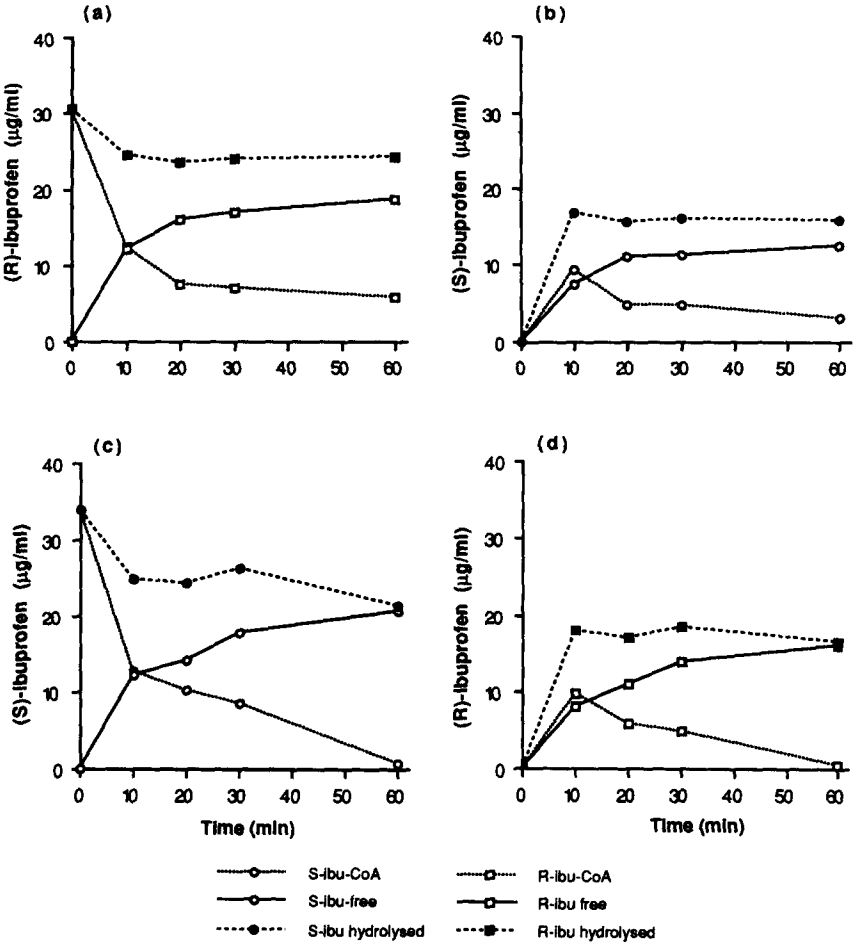

Fig. 3. Incubation of (*R*)-ibuprofen-CoA (40 μg/mL; a, b) and (*S*)-ibuprofen-CoA (40 μg/mL; c, d) with the rat liver mitochondrial fraction (7.3 mg/mL protein). Profiles (a) and (d) are the (*R*)-ibuprofen concentrations only and profile (b) and (c) are the (*S*)-ibuprofen concentrations only. Free concentrations are those prior to alkaline hydrolysis of the samples. Hydrolysed concentrations are those after alkaline hydrolysis and represent the total drug present in either the *R*- or *S*-configuration at that time point. (*R*)- and (*S*)-ibuprofen-CoA concentrations are determined by subtraction of free from hydrolysed concentrations. Each time point is the mean of duplicate samples.

A similar profile was observed for (*S*)-ibuprofen-CoA. (*S*)-Ibuprofen-CoA was racemized and hydrolysed when incubated with the mitochondrial fraction (Fig. 3c and d) as indicated by the appearance of (*R*)-ibuprofen-CoA with subsequent hydrolysis to (*R*)-ibuprofen (Fig. 3d) and (*S*)-ibuprofen hydrolysed from the starting (*S*)-ibuprofen-CoA (Fig. 3c).

Table 1. Enantiomer ratios of ibuprofen as CoA thioesters or as free ibuprofen following incubation of either (*R*)- or (*S*)-ibuprofen-CoA with the rat liver mitochondrial fraction

	(*R*)-Ibuprofen-CoA		(*S*)-Ibuprofen-CoA	
Time (min)	*S*/*R* CoA	*S*/*R* Free	*R*/*S* CoA	*R*/*S* Free
10	0.76	0.61	0.77	0.67
20	0.62	0.69	0.58	0.78
30	0.70	0.67	0.56	0.77
60	0.56	0.67	0.63	0.77

Again, the combined processes of racemization and hydrolysis apparently were not stereoselective.

The ratio of the ibuprofen enantiomers following incubation of (*R*)- or (*S*)-ibuprofen-CoA with the mitochondrial fraction (Table 1) did not change significantly over the 60 min time course of the study for either the CoA thioesters (*S*-CoA/*R*-CoA and *R*-CoA/*S*-CoA) or for the free enantiomer concentrations after hydrolysis ((*S*)-ibuprofen/(*R*)-ibuprofen and (*R*)-ibuprofen/(*S*)-ibuprofen).

Microsomal fraction

(*R*)-Ibuprofen-CoA was rapidly hydrolysed by the microsomal fraction as observed by the release of (*R*)-ibuprofen (Fig. 4a). Rapid racemization was also evident by the immediate appearance of (*S*)-ibuprofen-CoA (Fig. 4b). The (*S*)-ibuprofen-CoA thus formed was acted upon quite slowly by the hydrolase to gradually release free (*S*)-ibuprofen (Fig. 4b).

(*S*)-Ibuprofen-CoA was also racemized and hydro-

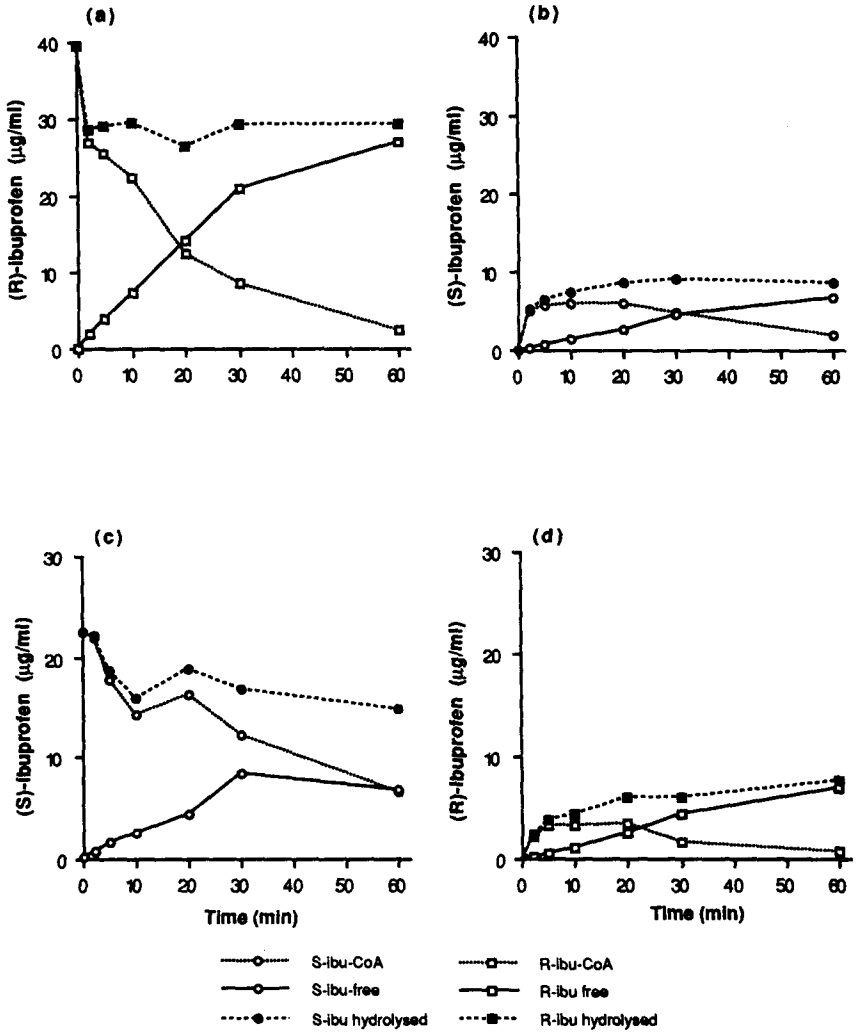

Fig. 4. Incubation of (*R*)-ibuprofen-CoA (40 μg/mL; a, b) and (*S*)- ibuprofen-CoA (40 μg/mL; c, d) with the rat liver microsomal fraction (7.0 mg/mL protein). Profiles (a) and (d) are the (*R*)-ibuprofen concentrations only and profile (b) and (c) are the (*S*)-ibuprofen concentrations only. Free concentrations are those prior to alkaline hydrolysis of the samples and indicate the activity of the enzymes in the rat liver microsomal fraction. Hydrolysed concentrations are those after alkaline hydrolysis and represent the total drug present in either the *R*- or *S*-configuration at that time point. (*R*)- and (*S*)-ibuprofen-CoA concentrations are determined by subtraction of free from hydrolysed concentrations. Each time point is the mean of duplicate samples.

Table 2. Enantiomer ratios of ibuprofen as CoA thioesters or as free ibuprofen following incubation of either (*R*)- or (*S*)-ibuprofen-CoA with the rat liver microsomal fraction

	(*R*)-Ibuprofen-CoA		(*S*)-Ibuprofen-CoA	
Time (min)	*S*/*R* CoA	*S*/*R* Free	*R*/*S* CoA	*R*/*S* Free
2	0.19	0.18	0.10	1.3
5	0.22	0.19	0.19	0.57
10	0.27	0.18	0.23	0.70
20	0.47	0.19	0.21	0.98
30	0.56	0.21	0.13	0.95
60	0.76	0.25	0.10	0.82

lysed by the microsomal fraction as evidenced by the disappearance of (*S*)-ibuprofen-CoA (Fig. 4c) and appearance of (*R*)-ibuprofen-CoA (Fig. 4d). It appeared that the rate of hydrolysis of (*S*)-ibuprofen-CoA was slower in comparison to the rate of hydrolysis of (*R*)-ibuprofen-CoA by the microsomal fraction.

In contrast to the enantiomeric ratios for the mitochondrial fraction, there was a difference in the ratios of (*R*)-ibuprofen-CoA and (*S*)-ibuprofen-CoA thioesters depending on whether the starting material was (*R*)- or (*S*)-ibuprofen-CoA (Table 2). Also, the ratio of the free enantiomer concentrations

was markedly different depending on which one of the ibuprofen thioesters was incubated with the microsomal fraction (Table 2).

DISCUSSION

Data in this study were corrected for the enantiomeric impurity of the synthesized CoA thioesters. This "impurity" was partly apparent and was brought about by racemization during alkaline hydrolysis of the CoA thioesters (8.0 ± 1.2%, [4]), but was also real (12%) and was due to racemization during chemical synthesis. We used alkaline hydrolytic conditions because this is a standard procedure, but primarily because we had no direct assay for the diastereomeric ibuprofen-CoA thioesters. In retrospect, hydrolysis using the partially purified rat brain acyl-CoA thioesterase would have been a useful approach as it hydrolysed both CoA thioesters with equal efficiency. This enzyme deserves further investigation in this respect. The correction of the data for the enantiomeric "impurity" was an adjustment which we believe did not affect the conclusions based on these data.

Chiral inversion is a process which has been demonstrated for many 2-APAs *in vivo* [1, 14] and in rat hepatocytes [15]. The enzyme(s) responsible for the activation of 2-APAs to their CoA thioesters has received increasing attention [2–5] because this is apparently the step controlling the overall stereoselectivity of chiral inversion (Fig. 1). We have previously demonstrated that (*R*)-ibuprofen was stereospecifically inverted by rat liver homogenate preparations [4] in agreement with Nakamura *et al.* [2]. Other workers [8] observed no inversion in similar preparations for reasons which remain unclear. We have previously noted the potential difficulties with such preparations where a multi-enzyme system is involved, in particular the disruption of the normal sequence of ordered intracellular events brought about by tissue homogenization [4]. The data indicated that there was no significant overall stereoselectivity in the combined steps of racemization and hydrolysis (Fig. 2). The present data thus reaffirm the key role of the acyl-CoA synthetase in the overall stereoselectivity of inversion.

Knadler and Hall [5] recently reported that CoA thioesters of (*R*)-ibuprofen and (*R*)-fenoprofen were racemized and hydrolysed by rat liver microsomal and mitochondrial preparations, a finding also observed here, and amplifying and reaffirming the original report by Nakamura *et al.* [2]. Again, inversion by subcellular preparations has not been universally demonstrated [8] for unknown reasons.

The rat liver mitochondrial fraction in the present study racemized and hydrolysed both ibuprofen-CoA thioesters to a similar extent as shown by the *S*/*R* ratios of the thioesters and of the free enantiomers. In contrast, (*R*)- and (*S*)-ibuprofen-CoA were differentially hydrolysed by the microsomal fraction, and racemization of the thioesters was less efficient. These observations are again a reaffirmation of previous data with regards to the microsomal fraction where racemization did not proceed very efficiently [2, 5]. The data may indicate that in the rat liver microsomal fraction there are two hydrolases, each stereoselective for one of the ibuprofen-CoA thioesters. This is a conclusion recently supported by data on the effect of clofibrate on the chiral inversion of (*R*)-ibuprofen [16].

The present study has provided additional information on the characteristics of the steps in the inversion pathway. The data are further evidence that it is the stereoselectivity of the acyl-CoA synthetase which primarily accounts for the stereoselectivity of inversion. Additionally, there is an uneven distribution of racemase/hydrolase activities in the rat liver mitochondrial and microsomal fractions, and it appears that an additional contribution to the overall enantioselectivity of inversion may be differential hydrolysis rates of the epimeric CoA thioesters.

REFERENCES

1. Hutt AJ and Caldwell J, The metabolic chiral inversion of 2-arylpropionic acids. A novel route with pharmacological consequences. *J Pharm Pharmacol* **35**: 693–704, 1983.
2. Nakamura Y, Yamaguchi T, Takahashi S, Hashimoto S, Iwatani K and Nakagawa Y, Optical isomerization mechanism of *R*(−)- hydratropic acid derivatives. *Pharmacobiodynamics* **4**: S-1, 1981.
3. Knights KM, Drew R and Meffin PJ, Enantiospecific formation of fenoprofen coenzyme A thioesters *in vitro*. *Biochem Pharmacol* **37**: 3539–3542, 1988.
4. Knihinicki RD, Williams KM and Day RO, Chiral inversion of 2- arylpropionic acid non-steriodal anti-inflammatory drugs 1. *In vitro* studies of ibuprofen and flurbiprofen. *Biochem Pharmacol* **38**: 4389–4395, 1989.
5. Knadler MP and Hall SD, Stereoselective arylpropionyl-CoA thioester formation *in vitro*. *Chirality* **2**: 67–73, 1990.
6. Chen C, Chen T and Shieh W, Metabolic stereoisomeric inversion of 2-arylpropionic acids. On the mechanism of ibuprofen epimerization in rats. *Biochim Biophys Acta* **1033**: 1–6, 1990.
7. Caldwell J, Hutt AJ and Fournel-Gigleux S, The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. *Biochem Pharmacol* **37**: 105–114, 1988.
8. Mayer JM, Bartolucci C, Maitre J and Testa B, Metabolic chiral inversion of anti-inflammatory 2-arylpropionates: lack of reaction in liver homogenates, and study of methine proton acidity. *Xenobiotica* **18**: 533–543, 1988.
9. Stabler SP, Marcell PD and Allen RH, Isolation and characterisation of DL-methylmalonyl-coenzyme A racemase from rat liver. *Arch Biochem Biophys* **241**: 252–264, 1985.
10. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
11. Lee EDJ, Williams KM, Graham GG, Day RO and Champion GD, Liquid chromatographic determination and plasma concentration time profile of optical isomers of ibuprofen in humans. *J Pharm Sci* **73**: 1542–1544, 1984.
12. Chase JFA and Tubbs PK, Specific inhibition of mitochondrial fatty acid oxidation by 2-bromopalmitate and its coenzyme A and carnitine esters. *Biochem J* **129**: 55–56, 1972.
13. Ellman GL, Tissue sulfhydryl groups. *Arch Biochem Biophys* **82**: 70–77, 1959.
14. Williams KM, Enantiomers in arthritic disorders. *Pharmacol Ther* **46**: 273–295, 1990.
15. Muller S, Mayer JM, Etter J and Testa B, Metabolic

chiral inversion of ibuprofen in isolated rat hepatocytes. *Chirality* **2**: 74–78, 1990.
16. Knights KM, Addinall TF and Roberts BJ, Enhanced chiral inversion of *R*-ibuprofen in liver from rats treated with clofibric acid. *Biochem Pharmacol* **41**: 1775–1777, 1991.