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Stereoselective and Substrate-Dependent Inhibition of Hepatic Mitochondrial β-Oxidation and Oxidative Phosphorylation by the Non-steroidal Anti-Inflammatory Drugs Ibuprofen, Flurbiprofen, and Ketorolac

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ABSTRACT. Non-steroidal anti-inflammatory drugs (NSAIDs) cause a range of adverse effects, some of which have been associated with perturbances of lipid metabolic pathways. Previous data demonstrating stereoselective formation of the CoA thioester of *R*-ibuprofen in particular were suggestive of possible stereoselective effects on lipid metabolism. Our aim was to characterise the relative stereoselectivity of the effects of ibuprofen, flurbiprofen, and ketorolac (0.01–1.0 mM) on both the β-oxidation of palmitate and oxidative phosphorylation in rat hepatic mitochondria as a means of dissecting prostaglandin related from non-prostaglandin-related events. β-oxidation was inhibited stereoselectively by *R*-ibuprofen ($P = 0.015$), non-stereoselectively by *R*- and *S*-flurbiprofen ($P = 0.002$ and $P = 0.004$, respectively), and was essentially unaffected by either enantiomer of ketorolac. At 0.25 mM, inhibition by *R*-ibuprofen and both flurbiprofen enantiomers was partially reversed by increasing CoA concentrations (0–200 μM). Mitochondrial respiration was moderately inhibited by both enantiomers of ibuprofen and flurbiprofen ($P < 0.01$), but only by high concentrations (≥1 mM) of the enantiomers of ketorolac ($P < 0.01$). Uncoupling of oxidative phosphorylation measured as stimulation of State 4 respiration contributed to these effects. The data support interactions involving both stereoselective CoA-dependent and non-CoA-dependent mechanisms. The plasma drug concentrations required to achieve these effects are not likely to be attained in the majority of patients, although these concentrations are achievable in the gastrointestinal tract and may contribute to the well-known spectrum of adverse effects in this organ. Some patients do experience systemic adverse events which may be mediated by these mechanisms. BIOCHEM PHARMACOL **57**;7:837–844, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. non-steroidal anti-inflammatory drugs; 2-arylpropionic acids; fatty acids; mitochondrial β-oxidation; respiratory function; CoA esters

A number of drugs interfere with the mitochondrial β-oxidation of fatty acids. In particular, NSAIDs§ of the 2-APA chemical class inhibit β-oxidation both *in vitro* and *in vivo* [1–3]. Furthermore, these drugs have been shown to uncouple mitochondrial oxidative phosphorylation [4]. These properties could account for the occasional incidence of adverse hepatic reactions associated with the use of some 2-APAs [1]. The 2-APAs are generally racemic, but the phenomenon of stereoselective formation of *R*-2-APA-CoA thioesters *in vivo* [5–8] has led to the hypothesis that it is the *R*-enantiomers of 2-APAs which interfere with lipid metabolic pathways. However, β-oxidation has been shown to be inhibited by the *S*-enantiomer of ibuprofen [2], leading Freneaux *et al.* to conclude that both a stereoselective CoA-dependent mechanism and a second, non-stereoselective, and non-CoA-dependent mechanism of inhibition were involved.

The aim of this study was to further investigate these interactions in mitochondria from rat hepatocytes with respect to substrate specificity and stereoselectivity using the enantiomers of two 2-APAs (ibuprofen and flurbiprofen) and another NSAID, ketorolac, which have different stereochemical dispositions *in vivo*. There is unidirectional chiral inversion via a CoA thioester intermediate of *R*-ibuprofen to *S*-ibuprofen in rats and humans [8–10]. Flurbiprofen which does not form a CoA intermediate is not inverted in either species [6, 9, 11, 12]. Ketorolac is not

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§ *Abbreviations:* NSAID, non-steroidal anti-inflammatory drug; and 2-APA, 2-arylpropionic acid.

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significantly inverted in humans, but is in rats and mice [13–15], and CoA thioester intermediates are probably not formed. In the present study, we examined the effect of the enantiomers of these three NSAIDs on rat hepatic mitochondrial β-oxidation of palmitate and the consequences of changing CoA concentrations on this parameter. The effects of the drugs on measures of oxidative phosphorylation were also studied. The results are discussed with particular reference to the stereochemical disposition of the individual drugs.

MATERIALS AND METHODS

Chemicals and Reagents

S(+)- and *R*(−)-ibuprofen (99% optically pure) and *S*(+)- and *R*(−)-flurbiprofen (95.7% optically pure) were supplied by The Boots Company P/L. *S*(−)-ketorolac (96.4% optically pure) and *R*(+)-ketorolac (>99% optically pure) were supplied by Syntex Laboratories Inc. Reagents and chemicals were obtained from the following sources: methoxyflurane (Penthrane), Abbott Australasia; ATP, CoA lithium salt, L-carnitine.HCl, BSA, Tris hydrochloride and base, and ADP potassium salt, Sigma Chemical Co.; succinic acid and rotenone, ICN Biomedicals; palmitic acid, BDH Chemicals; Carbosorb (alkylamine), Canberra-Packard Instrument Company Inc.; phase combining system (PCS; xylene, 2-ethoxyethanol) and [U-^{14}C] palmitic acid (30.6 GBq/mmol), Amersham Australia; protein assay kit (DC kit II) Bio-Rad Laboratories. All other chemicals and solvents used were of reagent grade.

Animals

Male outbred Wistar rats (270–300 g) were purchased from the Garvan Institute of Medical Research, Darlinghurst, Australia. Rats were allowed food and water *ad lib*. and resided under standard environmental conditions. The study was approved by the Institutional Animal Experimentation Ethics Committee.

Isolation of Rat Liver Mitochondria

Rats were anaesthetised with methoxyflurane (approximately 2 minutes) and the hepatic portal vein catheterised. Following perfusion with ice-cold sucrose solution (0.25 M), the liver was removed and mitochondria isolated according to the method of McGarry *et al.* [16]. Following isolation, mitochondria were suspended in preincubation medium [1] (final pH 8.0) at a concentration of 750 mg liver wet weight/mL, and stored on ice. Aliquots were taken to determine protein content using BSA as a standard with the Bio-Rad DC protein assay kit following the manufacturer's instructions.

β-oxidation of Palmitate

Preincubation medium was used to prepare drug solutions and also for stock solutions of ATP (20 mmol/L), CoA (0.5 mmol/L), and L-carnitine (1.0 mmol/L). Final concentrations of the substrates were: CoA, 50 μmol/L; L-carnitine, 100 μmol/L; and ATP, 2 mmol/L. [U-^{14}C]-palmitate was prepared as a stock (0.8 mmol/L, 0.25 μCi/mL) in a BSA solution (25 mg/mL; 1.16 mmol/L NaOH). The study of the effects of the drugs (0.01–1.0 mmol/L) on the β-oxidation of palmitate was conducted in 25 mL side-arm (stoppered) flasks according to Geneve *et al.* [1]. Reactions were initiated by addition of a 200 μL aliquot of mitochondrial suspension and terminated after 20 min by addition of 1 mL of 1.2N HCl.

The effect of differing CoA concentrations (0–200 μM) on drug inhibition was also examined. Boiled mitochondria exhibited background radioactivity which was approximately 2% of control samples without added drugs (replaced with preincubation medium alone). Vials were counted (Minaxi-β Tri-Carb 4000 LSC scintillation counter; Canberra Packard, Australia) for $^{14}CO_2$, and ^{14}C-labelled acid-soluble β-oxidation products which are primarily ketone bodies [2]. Total recovery of added radiolabel was 91.5% (1.5% as $^{14}CO_2$ and 90% as ^{14}C-labelled acid soluble β-oxidation products). Data for β-oxidation experiments are presented as total acid-soluble counts (dpm) expressed as percentage of control.

Mitochondrial Respiration and Oxidative Phosphorylation

The effects of the enantiomers of ibuprofen, flurbiprofen, and ketorolac on mitochondrial respiration were determined using a Clark oxygen electrode (polarising voltage −0.60 volts; Rank Brothers, Botishem) which was calibrated using sodium dithionite. The State 3 respiration rate (in the presence of succinic acid and ADP) and the State 4 respiration rate (following the depletion of ADP) were measured based on the methods of Estabrook [17] and Rickwood *et al.* [18]. Stock solutions of drugs, succinic acid (final concentration 5 mM), and ADP (final concentration 0.18 mM) were prepared as for the β-oxidation studies, but with the preincubation medium containing rotenone (1 nM). Final drug concentrations ranged up to 2.5 mM. Reaction temperature was 30° and 200 μL of mitochondrial suspension was used for reactions.

Lines of best fit were constructed for State 3 and State 4 respiration, their intersection marking the end of State 3. Similarly, the intersection of the line of best fit for substrate alone prior to ADP addition and that of State 3 respiration marked the beginning of State 3. Thus, the vertical distance between the beginning and end of State 3 respiration was used to calculate the molar amount of atomic oxygen consumed and, hence, the corresponding ADP:O ratio.

Data Analysis

Data were analysed using the approach of random effect ANOVA [19]. Three main issues were investigated in the analysis: (1) the relationship between drug concentration and response; (2) the effects of the *R*- and *S*-enantiomers; and (3) the effects of the 3 drugs. The model was Y = P + D + C + M + e, where: Y is the response of a rat mitochondrial preparation P, with drug treatment D, at a drug concentration C. The method of measurement is M and e is the error of measurement. The value of Y is linearly and additively determined by the true mean of a particular rat mitochondrial preparation, plus or minus the effects of drug (D), concentration (C), method (M), and possible interactions between these effects. Specifically, it was assumed that P, D, C, M, and e were normally distributed with mean zero and non-negative variance. It was also assumed that the effect of P was random, reflecting the fact that the rats were randomly selected from a large population, while the effects of D, C, and M were fixed. To test whether the effect of a factor was statistically significant, the parameter of each factor was equated to zero, and the residual mean squares of the new model were compared with the complete model by the F-statistic. Because the study was not a balanced design, the model parameters were estimated and tested using the SAS general linear model procedure.

The Sigmoidal Emax Model [20] and a non-linear least-squares modelling programme (BOOMER) [21] were used to analyse the β-oxidation data.

RESULTS

Effects of NSAID Enantiomers on Mitochondrial β-Oxidation

There was a significant relationship between the concentrations of both *R*- and *S*-ibuprofen and inhibition of the β-oxidation of palmitate by rat hepatic mitochondria when assessed as inhibition of formation of acid-soluble products (Fig. 1a; $P = 0.0001$). *R*-ibuprofen was much more potent than its *S*-antipode when assessed by the general linear models procedure ($P = 0.015$). The effects of *R*- and *S*-flurbiprofen (Fig. 1b) on the oxidation of palmitate were similar ($P = 0.002$, $P = 0.004$, respectively), although inhibition appeared to be maximal at approximately 0.25 mM and then decrease again. While statistically significant regressions were observed for *R*- and *S*-ketorolac ($P = 0.049$, $P = 0.02$, respectively), the slopes of the concentration–response curves were extremely shallow (Fig. 1c), such that both enantiomers were essentially inactive over the range of concentrations studied for the other NSAIDs.

Only the data for *R*-ibuprofen could be fitted using the Emax model. IC_{50} values could not be estimated reliably for *S*-ibuprofen nor for flurbiprofen, reflecting the variability of the data, the uncertainty of the maximum effect of the drugs, and the multifactorial mechanisms in each case. For *R*-ibuprofen, the line of best fit was good ($r^2 = 0.99$) but

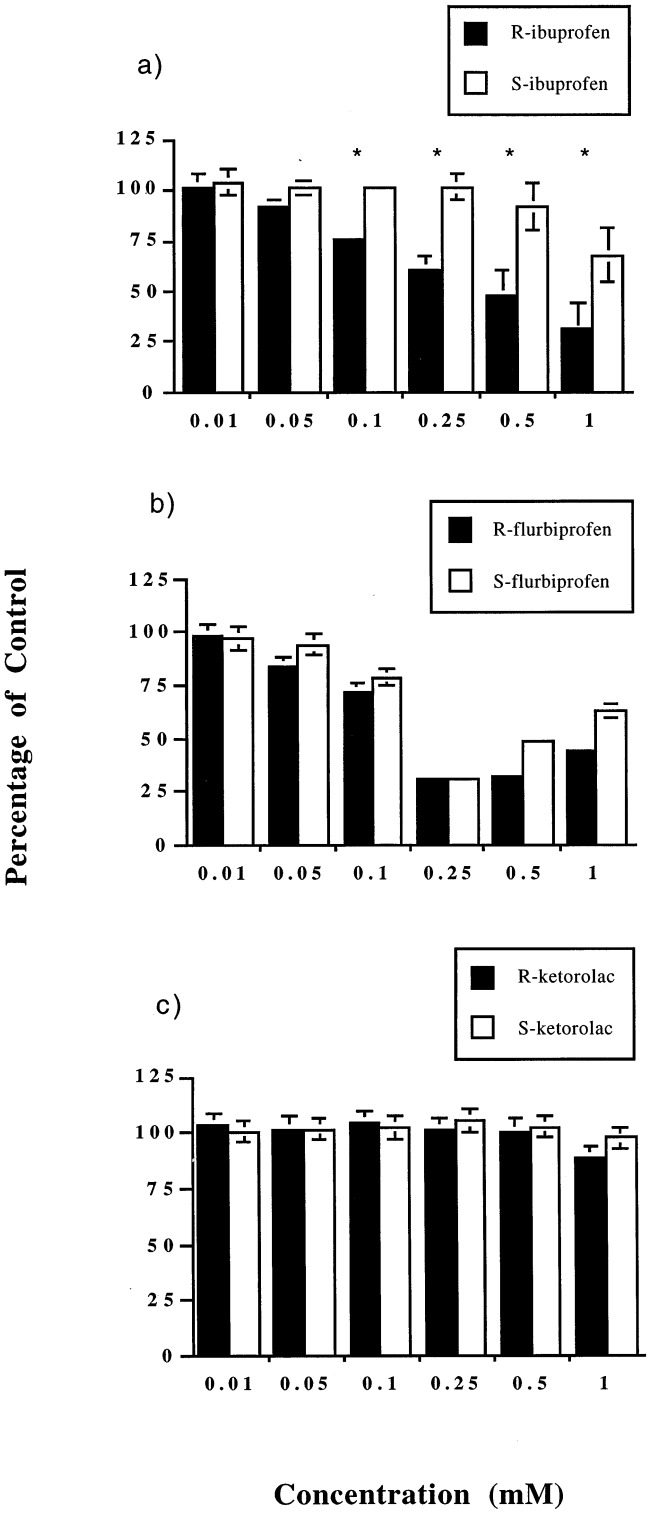


FIG. 1. Effect of an increasing concentration of each of the pairs of enantiomers of (a) ibuprofen, (b) flurbiprofen, and (c) ketorolac on the β-oxidation of ^{14}C-palmitate in rat hepatic mitochondria as assessed by inhibition of formation of acid-soluble products (primarily ketone bodies). Data (mean ± SD, N = 4–6) are expressed as a percentage of the control, i.e. in the absence of added drug (replaced with preincubation medium). *denotes statistically significant stereoselectivity of effect ($P < 0.01$).

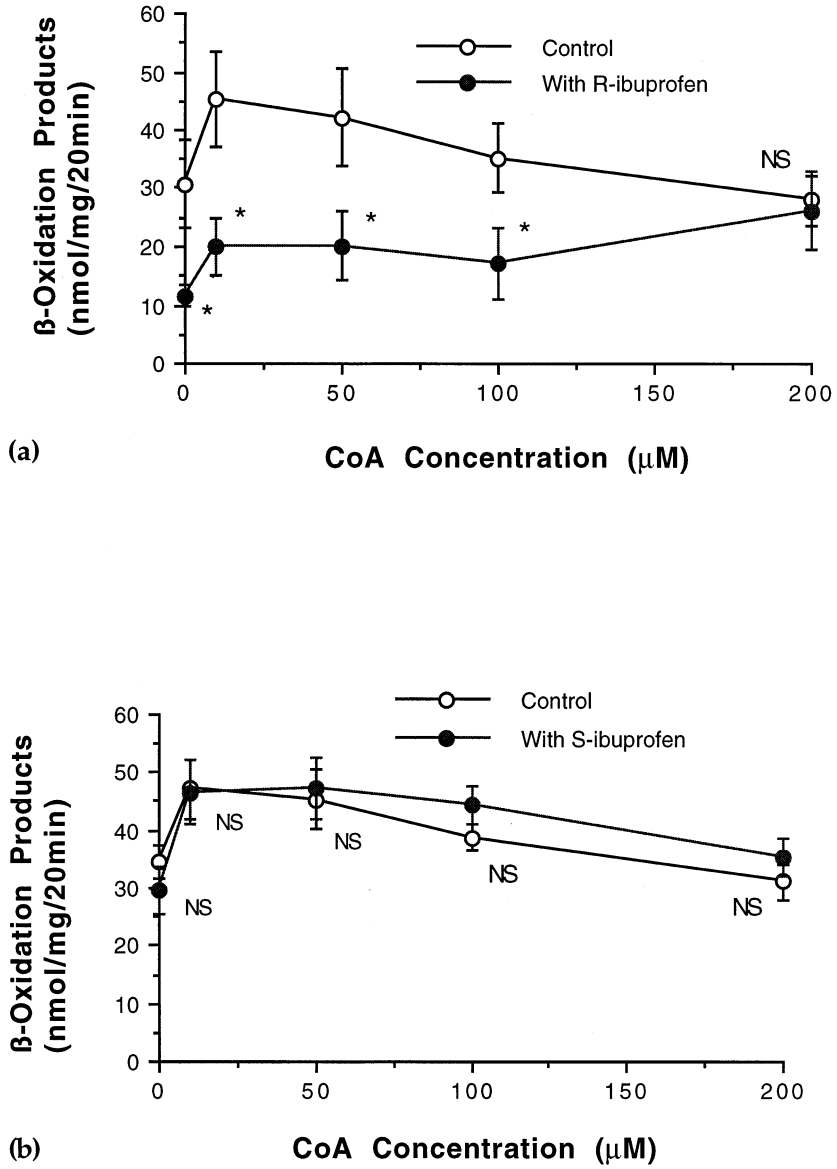


FIG. 2. Effect of increasing concentrations of added CoA (0–200 μM) on the inhibition of β-oxidation of ^{14}C-palmitate in rat hepatic mitochondria by (a) *R*-ibuprofen, (b) *S*-ibuprofen, (c) *R*-flurbiprofen, and (d) *S*-flurbiprofen (0.25 mM). Statistically significant differences between control and drug samples at each concentration of CoA are indicated (*$P < 0.01$, †$P < 0.05$, NS = no significant difference).

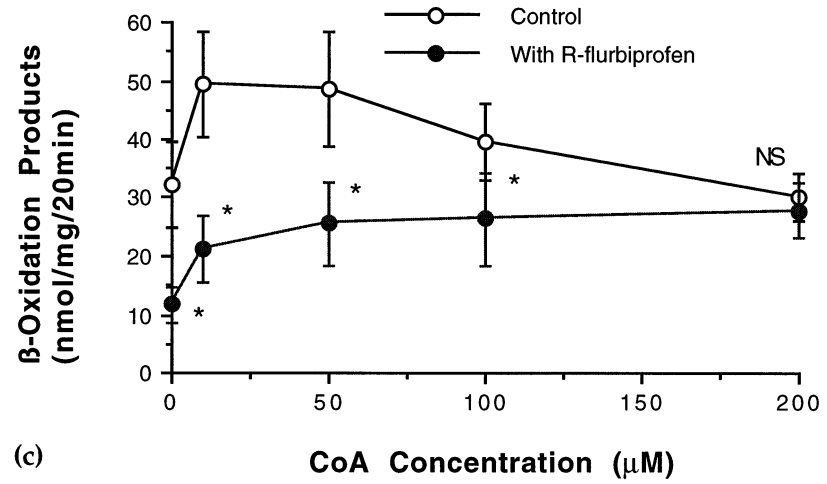
the variability was high as indicated by the variability of the estimated IC_{50} (mean ± SD = 0.37 ± 0.22 mM). The relative order of potency for inhibition of β-oxidation was approximately: *R*-flurbiprofen = *S*-flurbiprofen ≥ *R*-ibuprofen ≫ *S*-ibuprofen > *R*-ketorolac = *S*-ketorolac. Effects on $^{14}CO_2$ generation, which accounted for only about 2% of total radioactivity, were much more variable, but generally were similar (data not shown) to those for the acid-soluble products.

Effect of Added CoA Concentration on Inhibition of β-Oxidation by 2-APAs

The data from the CoA studies were complicated by an overall effect of CoA itself on the degree of inhibition of β-oxidation. Addition of CoA (10 μM) initially increased overall oxidation in control samples (Fig. 2 a, b, c, and d) as might be anticipated. Thereafter, there was a general trend to a decrease in oxidation between approximately 50–200 μM CoA. Oxidation was less in the presence of *R*-ibuprofen and *R*- and *S*-flurbiprofen at 0.25 mM as shown previously. However, in the presence of inhibitors, there was a gradual convergence with control values as CoA was increased, such that at 200 μM CoA there was no significant difference between control and drug groups. This convergence indicated a general reversal of inhibition relative to control with increasing CoA.

Effects of NSAIDs on Mitochondrial Respiration

In the absence of drug, the mean respiratory control ratio ranged from 4.3–5.4. This compared favourably with those



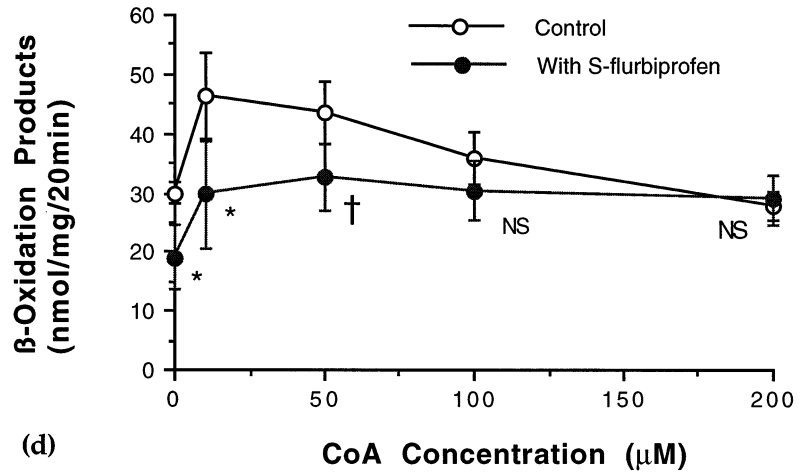


FIG. 2. Continued.

reported for functional rat liver mitochondria of 4.0–6.0 [18]. Similarly, mean control ADP:O ratios ranged from 1.47–1.65 compared to the literature value of 1.6–2.0 [18]. However, variation in this value is possible in response to differing reaction conditions. Rotenone has been reported to decrease the ADP:O ratio with succinate as the substrate to 1.46 ± 0.04 [22]. Furthermore, a degree of intrinsic error in the ratios exists, since protonophores were not included in the present studies to allow correction for proton leakage. There was also some subjectivity in line fitting for State 3 and State 4 respiration.

Mitochondrial ADP:O ratios were inhibited significantly by *R*- and *S*-ibuprofen, and *R*- and *S*-flurbiprofen (Table 1). Both enantiomers of ibuprofen and flurbiprofen also stimulated State 4 respiration, indicating an uncoupling of oxidative phosphorylation. Flurbiprofen appeared the more potent and there was no prominent stereoselectivity evident for either drug, although there was a modest significant difference between *R*- and *S*-flurbiprofen regressions (State 3, $P < 0.01$; State 4, $P < 0.05$). Regression analysis for ketorolac showed significant effects, however, the concentrations required to achieve these effects were 10-fold higher than for the 2-APAs.

DISCUSSION

The stereoselective inhibition of β-oxidation displayed by *R*-ibuprofen was consistent with previous data [1, 2], supporting the conclusion that a CoA-dependent mechanism was involved, although the degree of inhibition was rather greater in the present study. However, *S*-ibuprofen also inhibited β-oxidation, albeit less potently, while both enantiomers of flurbiprofen were inhibitory. This suggested that a non-CoA-dependent process was also involved,

TABLE 1. Effect of NSAIDs on mitochondrial respiration

Drug	N	Conc. (mM)	State 3 nmol O_2/mg/min mean ± SD	*P*	State 4 nmol O_2/mg/min mean ± SD	*P*	ADP:O mean ± SD	*P*
R-ibuprofen	4	control	94.6 ± 12.6		23.7 ± 2.6		1.51 ± 0.06	
	4	0.10	92.6 ± 9.7	NS	30.3 ± 2.9	<0.01	1.34 ± 0.09	<0.01
	3	0.25	85.4 ± 3.1	NS	33.6 ± 1.9	<0.01	1.26 ± 0.15	<0.01
	3	0.50	83.9 ± 2.0	NS	45.8 ± 3.4	<0.01	1.15 ± 0.13	<0.01
S-ibuprofen	5	control	97.5 ± 6.6		23.2 ± 0.9		1.49 ± 0.09	
	3	0.10	92.5 ± 7.9	NS	26.6 ± 0.3	NS	1.42 ± 0.06	NS
	3	0.25	96.1 ± 3.0	NS	33.6 ± 2.3	<0.01	1.30 ± 0.04	<0.01
	3	0.50	97.6 ± 6.1	NS	46.5 ± 2.6	<0.01	1.14 ± 0.04	<0.01
	3	1.00	79.5 ± 7.5	<0.01	57.0 ± 6.9	<0.01	0.83 ± 0.04	<0.01
R-flurbiprofen	3	control	119.3 ± 12.0		22.2 ± 2.3		1.61 ± 0.05	
	3	0.05	103.5 ± 5.8	NS	26.1 ± 2.2	NS	1.67 ± 0.00	NS
	3	0.10	96.9 ± 6.7	<0.01	30.3 ± 3.6	<0.01	1.57 ± 0.13	NS
	3	0.25	81.3 ± 6.5	<0.01	50.7 ± 4.2	<0.01	1.20 ± 0.09	<0.01
S-flurbiprofen	4	control	100.3 ± 3.7		19.9 ± 0.7		1.65 ± 0.06	
	3	0.025	90.1 ± 7.3	NS	21.2 ± 1.9	NS	1.63 ± 0.15	NS
	3	0.05	95.6 ± 3.7	NS	24.4 ± 0.3	<0.01	1.42 ± 0.05	<0.01
	3	0.10	88.2 ± 4.9	<0.05	28.9 ± 0.2	<0.01	1.34 ± 0.08	<0.01
	3	0.25	93.7 ± 5.7	NS	40.5 ± 1.8	<0.01	1.24 ± 0.06	<0.01
R-ketorolac	4	control	105.7 ± 10.9		21.3 ± 1.5		1.52 ± 0.04	
	3	0.10	99.7 ± 3.2	NS	23.5 ± 3.8	NS	1.56 ± 0.05	NS
	3	0.25	98.0 ± 3.8	NS	22.4 ± 2.0	NS	1.51 ± 0.07	NS
	3	0.50	100.7 ± 4.7	NS	23.8 ± 1.4	NS	1.47 ± 0.04	NS
	3	1.00	84.0 ± 5.5	<0.01	26.8 ± 1.5	<0.01	1.41 ± 0.11	NS
	3	2.50	65.1 ± 3.6	<0.01	33.8 ± 1.9	<0.01	1.22 ± 0.08	<0.05
S-ketorolac	4	control	111.2 ± 5.6		25.9 ± 1.2		1.47 ± 0.14	
	3	0.10	114.3 ± 6.5	NS	25.2 ± 1.1	NS	1.46 ± 0.06	NS
	3	0.25	117.3 ± 10.7	NS	25.8 ± 1.6	NS	1.48 ± 0.02	NS
	3	0.50	117.0 ± 6.1	NS	27.5 ± 1.9	NS	1.48 ± 0.02	NS
	3	1.00	108.4 ± 15.8	NS	28.1 ± 1.2	NS	1.41 ± 0.12	NS
	3	2.50	80.7 ± 6.2	<0.01	35.5 ± 2.0	<0.01	1.35 ± 0.14	NS

ADP:O ratios were calculated from measured State 3 respiration rates and known amounts of added ADP (588 nmoles). *P* values indicate significant differences from control values.

NS, not significant.

because previous data have indicated that *S*-ibuprofen and *R*- and *S*-flurbiprofen do not form CoA thioesters [8, 9, 11, 12, 23]. However, some studies have concluded that under specific conditions (e.g. at high drug concentrations) *S*-2-APA-CoA thioesters may be formed, particularly with ibuprofen, due to activation of low-affinity ligases [24, 25].

It was evident that there was an optimum added concentration of CoA (10–50 μM) required to achieve a maximum degree of β-oxidation of palmitate in the present mitochondrial preparation. However, as CoA concentration was further increased, the overall oxidation declined. A mechanism by which *R*-ibuprofen might inhibit β-oxidation was by competing for the CoA pool, especially at limiting concentrations of CoA due to the proven ability of this enantiomer to form CoA thioesters. Indeed, with increasing CoA there was a gradual convergence of the data, indicating that this mechanism might account, in part, for the demonstrated inhibition by *R*-ibuprofen. It was somewhat surprising then to observe that both *R*- and *S*-flurbiprofen, which are thought not to form CoA thioesters, displayed a similar convergence with controls as CoA concentrations were increased. A definitive conclusion as to the mechanism of inhibition cannot be made from these data. They are suggestive of competition for the CoA pool and/or a direct effect of the enantiomers on formation of palmitoyl-CoA by long chain CoA synthases, as supported by Knights *et al.* [24]. Therefore, CoA concentration did affect drug inhibition to a degree, although this was not quantitatively determined in the present study.

The effects of the drugs on respiratory parameters indicated a non-CoA-dependent pattern of inhibition which was evident at similar concentrations to those required to elicit an effect on the β-oxidation of palmitate. Similarly, the steepest concentration–effect relationship was consistently evident with *R*-flurbiprofen. Uncoupling of electron transport and phosphorylation of ADP associated with both *R*- and *S*-enantiomers of the drugs used suggests that the parent drugs may enter mitochondria and directly inhibit β-oxidation. A number of lipid-soluble drugs such as amiodarone are known to uncouple oxidative phosphorylation by transporting protons across the inner mitochondrial membrane [26]. The 2-APAs, being weak acids, are structurally suited to do this and are recognised uncouplers [27]. Knights and Drew [28] also reported that ibuprofen

enantiomers decreased the respiratory control ratio. These effects were evident at rather lower concentrations than in the present study, perhaps reflecting the higher incubation temperature (37°) compared to that (30°) used in our studies.

However, effects on respiratory function would not be expected to be stereoselective if the mechanism involved dissipation of the proton gradient by simple diffusion of the enantiomers across the mitochondrial membrane. Indeed, very little stereoselectivity of effect on mitochondrial respiratory parameters was noted for the NSAIDs studied. The data of Mahmud *et al.* [4] indicated a positive relationship between the pK_a of acidic NSAIDs and their ability to act as uncouplers of mitochondrial oxidative phosphorylation. Our results partly confirmed these findings with ketorolac, which has the lowest pK_a (3.49), being the least potent uncoupler. However, we found flurbiprofen (pK_a 4.22) to be a more potent uncoupler than ibuprofen (pK_a 5.20). These complex interactions may reflect the higher pH of our system (pH 8.0 as opposed to 7.4) or the use of individual enantiomers rather than racemic mixtures of the drugs. Furthermore, if the 2-APAs are substrates for carrier-mediated transport, such as has been described for benzoic acid across epithelial cell membranes [29], then relative polarities may not be so important.

Collectively these data support a CoA-dependent interaction, probably associated with depletion of, or competition for, the extramitochondrial pool of CoA, and a CoA-independent mechanism as also concluded by Freneaux *et al.* [2]. Prostaglandin-mediated mechanisms do not account for these non-CoA-dependent effects, at least in hepatic mitochondria, since ketorolac, the most potent inhibitor of prostaglandin synthesis of the three drugs studied, had very little effect on β-oxidation. The relative contribution of each of these drug effects towards inhibition of β-oxidation requires further investigation.

The concentrations required to achieve these effects *in vitro* were much higher than those usually achieved systemically when one considers that free drug concentrations represent less than 1–2% of the total due to plasma protein binding [14, 30, 31]. Consequently, while the present data are of mechanistic interest, it is less likely that these effects will be important *in vivo* for the majority of patients. However, some patients with unexpressed genetic defects of fatty acid β-oxidation [32], those using combinations of drugs, or those with concomitant viral infections [33] do develop hepatic disorders associated with inhibition of fatty acid metabolism by a number of NSAIDs. Data from comparative studies of the rates of β-oxidation and esterification of palmitate suggest that human mitochondria may be more susceptible to these actions than rat mitochondria [34], although data obtained using human lymphocytes also suggested that susceptibility is likely to be preparation-dependent [35]. Effects of 2-APAs mediated by non-prostanoid mechanisms may be exacerbated in tissues other than the liver, such as gastric mucosal cells which are exposed to very high concentrations of the drugs. For example, 2-APA NSAIDs have been shown to increase intestinal permeability [36], and the ulcerative index of *S*-flurbiprofen was reported to be increased in the presence of *R*-flurbiprofen [37].

The present data indicate that the risk of such interactions is probably not greatly lessened by the use only of the *S*-enantiomers of 2-APAs. However, the overall reduction of the burden on cellular respiratory function by removing the *R*-enantiomers from preparations of 2-APAs could have potential advantage, particularly in otherwise metabolically stressed individuals. Finally, it was interesting that there were clear differences between the drugs with respect to these interactions with lipid metabolism. The absence of effect of ketorolac in these studies highlights the biochemical incongruity of drugs of the same pharmacological but different chemical class, the possible clinical relevance of which remains to be demonstrated.

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