CELL BIOCHEMISTRY AND FUNCTION
Cell Biochem Funct 2001; **19**: 117–124.
DOI: 10.1002/cbf.904

The uncoupling effect of the nonsteroidal anti-inflammatory drug nimesulide in liver mitochondria from adjuvant-induced arthritic rats

S. M. Caparroz-Assef, C. L. Salgueiro-Pagadigorria, C. A. Bersani-Amado, A. Bracht, A. M. Kelmer-Bracht and E. L. Ishii-Iwamoto*

Laboratory of Biological Oxidations, Department of Biochemistry, University of Maringá, 87020900 Maringá, Brazil

The aim of the present study was to evaluate the changes caused by adjuvant-induced arthritis in liver mitochondria and to investigate the effects of the nonsteroidal anti-inflammatory drug nimesulide. The main alterations observed in liver mitochondria from arthritic rats were: higher rates of state IV and state III respiration with β-hydroxybutyrate as substrate; reduced respiratory control ratio and impaired capacity for swelling dependent on β-hydroxybutyrate oxidation. No alterations were found in the activities of NADH oxidase and ATPase. Nimesulide produced: (1) stimulation of state IV respiration; (2) decrease in the ADP/O ratio and in the respiratory control ratio; (3) stimulation of ATPase activity of intact mitochondria; (4) inhibition of swelling driven by the oxidation of β-hydroxybutyrate; (5) induction of passive swelling due to NH_3/NH_4^+ redistribution. The activity of NADH oxidase was insensitive to nimesulide. Mitochondria from arthritic rats showed higher sensitivity to nimesulide regarding respiratory activity. The results of this work allow us to conclude that adjuvant-induced arthritis leads to quantitative changes in some mitochondrial functions and in the sensitivity to nimesulide. Direct evidence that nimesulide acts as an uncoupler was also presented. Since nimesulide was active in liver mitochondria at therapeutic levels, the impairment of energy metabolism could lead to disturbances in the liver responses to inflammation, a fact that should be considered in therapeutic intervention.

KEY WORDS — nimesulide; liver mitochondria; uncoupler; nosteroidal anti-inflammatory

INTRODUCTION

Mitochondrial function has been shown to be disturbed in several liver pathological conditions such as cirrhosis,[1,2] hepatoma and chronic injuries caused by ethanol[3–5] and thioacetamide.[6] Altered liver mitochondrial functions have also been observed in rats with extrahepatic disease, as inflammation induced by oleyl alcohol or adjuvants.[7,8] Higher sensitivity to uncoupling induced by Ca^{2+} ions, decreased $^{45}Ca^{2+}$ uptake and protein synthesis were demonstrated in liver mitochondria isolated from adjuvant-induced arthritic rats.[8] We have recently reported changes in metabolic processes in the perfused liver and in mitochondria isolated from arthritic rats.[9–11] Depressed gluconeogenesis, increased glucose phosphorylation capacity, higher rates of oxygen uptake and lack of glycogenolytic response to uncouplers were the main effects observed in the perfused rat liver.[9–11] Mitochondria isolated from arthritic rats exhibited higher oxygen uptake dependent on succinate oxidation either in the absence or in the presence of ADP and increased activity of succinate oxidase.[9] We have also demonstrated that nimesulide, 4-nitro-2-phenoxymethane-sulfonanilide, a nonsteroidal anti-inflammatory drug widely employed in inflammatory diseases and pain states,[12] affects several metabolic processes of perfused livers and isolated mitochondria from either normal or arthritic rats.[9] It activates oxygen uptake, glycogenolysis and glycolysis in perfused livers from fed rats and inhibits gluconeogenesis in livers from fasted rats.[9] In isolated

*Correspondence to: Emy L. Ishii-Iwamoto, Department of Biochemistry, University of Maringá, 87.020.900 Maringá, Brazil. Fax:55-44-263-3655. E-mail: eliiwamoto@uem.br

Contract/grant sponsor. Programa de Apoio a Núcleos de Excelência (PRONEX).
Contract/grant sponsor: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Received 26 April 2000
Accepted 28 June 2000

mitochondria nimesulide stimulates oxygen uptake dependent on succinate oxidation in the absence of ADP, inhibits respiration coupled to ADP phosphorylation, decreases the ADP/O ratios and the respiratory control ratios. Although an interference with mitochondrial energy metabolism was suggested as a primary action of nimesulide in the intact liver, no systematic study in liver mitochondria was carried out in order to elucidate the mechanism of action of nimesulide. Thus, as an extension of our previous work, we are now presenting the results of a series of experiments in which several mitochondrial parameters were investigated. These include respiration with NAD^+-dependent substrate, membrane-bound enzymic activities and mitochondrial swelling. The aim was to obtain more experimental evidence for our hypothesis concerning the action of nimesulide in the intact liver, and to verify the extent of the changes induced by the arthritis disease on the mitochondrial activity, including sensitivity to nimesulide.

MATERIAL AND METHODS

Materials

Nimesulide was a product of Sigma Chemical Company (St. Louis, MO, USA). All other chemicals (salts and buffers) were from the best available grade (98–99.8% purity) and were purchased from Merck (Darmstadt FRG); Carlo Erba (São Paulo, Brazil) and Reagen (Rio de Janeiro, Brazil). Nimesulide was dissolved in distilled water alkalinized by slow addition of an equivalent amount of KOH. The concentration of the nimesulide stock solution ranged from 6 to 20 mM (pH 8.0).

Animals and induction of arthritis

Male albino rats (Wistar), weighing 180–220 g, were fed *ad libitum* with a standard laboratory diet (Nuvilab®, São Paulo, Brazil). For the induction of adjuvant arthritis, the animals were injected in the left hind paw with 100 µl of Freund's adjuvant (heat inactivated *Mycobacterium tuberculosis*, derived from human strains $H_{37}Rv$), suspended in mineral oil at a concentration of 0.5% (w/v). The animals showing primary and secondary lesions after 14 to 21 days of adjuvant injection were selected for the experiments.[13] The severity of the adjuvant-induced arthritis was evaluated by measuring paw volume changes with a water displacement plethysmometer and by the appearance of nodules in the tail and in one or both ears. Rats of similar ages (nearly 60 days) served as controls. All experiments were conducted according to the rules of the Animal Experiment Ethics Committee of the University of Maringá.

Preparation of mitochondria

Liver mitochondria were isolated by differential centrifugation according to Voss *et al.*[14] For the measurements of oxygen consumption and ATPase activity, a mannitol–sucrose medium was used.[14] For swelling measurements, mitochondria were isolated in a sucrose medium.[15,16] For the assay of NADH oxidase, intact mitochondria were frozen in liquid nitrogen and thawed rapidly at 37°. This procedure was repeated three times.

Determination of oxygen uptake, ADP/O ratio and respiratory control ratio (RC)

Oxygen uptake by intact mitochondria was measured polarographically as described by Voss *et al.*[14] using the incubation medium containing 5 mM disodium phosphate, 10 mM Tris–HCl (pH 7.4), 0.2 mM EDTA, 10 mM potassium chloride, 250 mM mannitol and 50 mg% fatty acid-free bovine serum albumin. Beta-hydroxybutyrate was used as a substrate. The respiratory control ratio (RC) and the ADP/O ratio were calculated according to Chance and Williams.[17]

Mitochondrial swelling

Swelling driven by oxidation of β-hydroxybutyrate was followed spectrophotometrically at 575 nm, as described by Mustafa *et al.*[15] The reaction medium contained: 100 mM sucrose, 10 mM Tris–HCl (pH 7.5) and 0.3 mM EDTA. Sodium acetate (50 mM) was added as a co-permeant anion. The reaction was initiated with the addition of β-hydroxybutyrate (8.0 mM).

The passive swelling in the presence of NH_4Cl was measured as described by Jung and Brierley.[16] Mitochondria were incubated at 25° in a reaction medium containing 150 mM NH_4Cl and 10 mM Tris–HCl (pH 7.4). The reaction was initiated by the addition of nimesulide and the decrease in absorbance was followed spectrophotometrically at 540 nm.

Membrane-bound enzymic activities

The ATPase activity of intact mitochondria was assayed by measuring phosphate release according to Pullman *et al.*[18] The reaction medium contained: 0.2 M sucrose, 12 mM Tris–HCl (pH 7.4) and

50 mM KCl. The reaction was initiated with the addition of 5.0 mM ATP and stopped, after 20 min of incubation, at 37°, by the addition of ice-cold 5% trichloroacetic acid. Phosphate was measured as described by Fiske and Subbarow.[19]

Freeze–thawing-disrupted mitochondria were used as an enzyme source for assaying NADH oxidase. The activity of the enzyme was measured polarographically using a 20 mM Tris–HCl (pH 7.4) medium.[20] The reaction was started by the addition of NADH (1.0 mM).

Determination of protein content

Protein content of the mitochondrial suspensions was measured using the Folin phenol reagent.[21] Bovine serum albumin was used as a standard.

Statistical Analysis

Data in the graphs are presented as means ± standard errors of the mean. The statistical significance of the differences between parameters was evaluated by means of variance analysis and Student's *t*-test. $p \leq 0.05$ was adopted as a criterion of significance. The ED_{50} and ID_{50} were calculated by Lagrange's interpolation formula.

RESULTS

Effects of nimesulide on respiration of liver mitochondria from normal and arthritic rats

Figure 1 shows the rates of oxygen uptake in the presence of exogenously added ADP (state III respiration) or after ADP exhaustion (state IV respiration) and the ADP/O ratios and the respiratory control ratios (inserted graphs) as a function of nimesulide concentrations. In these experiments, β-hydroxybutyrate was used as a substrate. In panel A, mitochondria from normal rats and in panel B, mitochondria from arthritic rats were used. In the absence of nimesulide, the rates of both state III and state IV respiration were significantly higher in mitochondria from arthritic rats. No difference was found in the ADP/O ratio, but the respiratory control ratio of arthritic rats was significantly lower (inserted graphs). When nimesulide was added to the incubation medium in the concentration range between 40 to 240 μM, state III and state IV respiration were progressively increased either in mitochondria isolated from normal or arthritic rats. In addition, nimesulide clearly decreased the ADP/O ratios and the respiratory con-

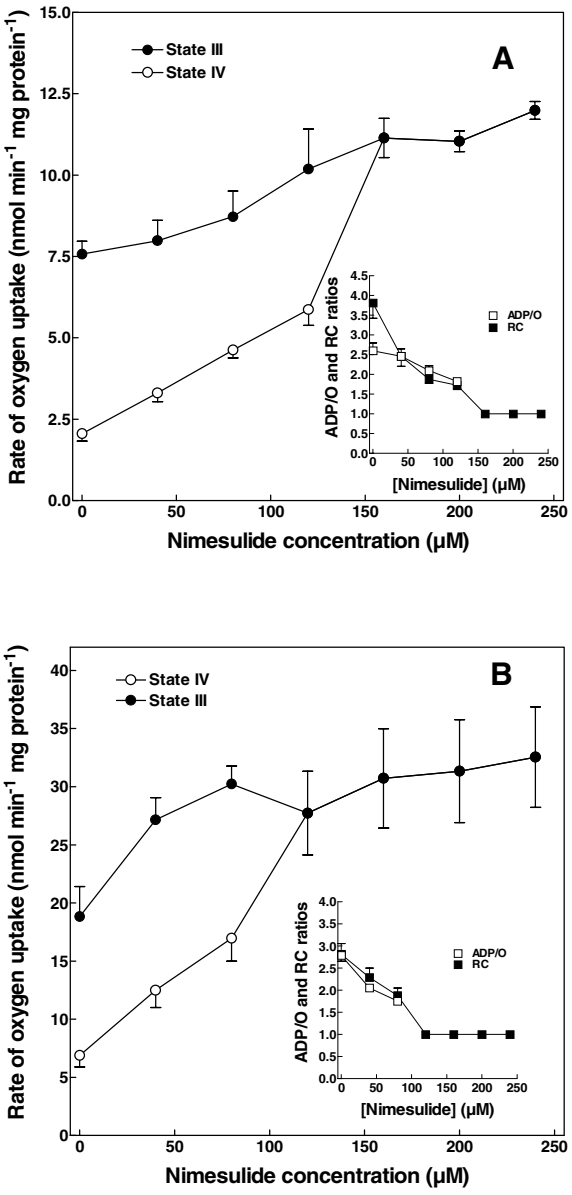

Figure 1. Effects of nimesulide on the respiratory activity of rat liver mitochondria isolated from normal (A) or adjuvant-induced arthritic (B) rats. Mitochondria (0.6–1.15 mg protein) were added to the reaction medium in the closed vessel of the oxygraph (temperature 37°C). The reaction was initiated by the addition of β-hydroxybutyrate (8.0 mM) and the oxygen consumption was followed polarographically over approximately 5 min. After this time 125 nmol of ADP were added. Rates of oxygen consumption were computed from the slopes of the polarographic records. The ADP/O ratios and the respiratory control ratios were evaluated according to Chance and Williams.[17] Each data point is the mean ± SEM of four (normal) or six (arthritic) independent experiments. State III respiration (●–●); state IV respiration (○–○); ADP/O ratios (□–□); respiratory control ratios (■–■)

trol ratios in both experimental series (inserted graphs). Mitochondria isolated from arthritic rats exhibited higher sensitivity to nimesulide as indicated by the values of ED_{50} for the stimulation of state IV respiration. The calculated ED_{50} was 117 ± 8.85 and 62.18 ± 13.69 mM ($p = 0.018$) in mitochondria from normal and arthritic rats, respectively. Moreover, complete abolition of respiratory control was found at 120 μM in the arthritic series whereas the same occurred at 160 μM in the control series.

Effects of nimesulide on membrane-bound enzymatic activities

No differences were found in the activities of both NADH oxidase and ATPase in liver mitochondria isolated from normal and arthritic rats. Moreover, nimesulide up to 240 μM did not affect the activity of NADH oxidase (data not shown). However, the ATPase activity of intact mitochondria was stimulated by nimesulide, as revealed by Figure 2. The kinetics of the stimulation were very similar in mitochondria from normal and arthritic rats with a calculated ED_{50} of nearly 30 μM in both experimental series.

The effects of nimesulide on mitochondrial swelling

The effects of nimesulide on mitochondrial swelling dependent on energy generated by the electron transport chain during the oxidation of β-hydroxybutyrate are shown in Figure 3. The maximal changes in absorbance at 575 nm (amplitude of swelling) and the initial rates of decreases in absorbance (inserted graphs) were plotted against the nimesulide concentrations. Measurements of mitochondrial volume changes in the absence of nimesulide showed that mitochondria isolated from arthritic rats swelled slower and reached lower amplitudes than mitochondria from control rats. The addition of nimesulide caused inhibition of both the amplitude and the initial rate of swelling in a dose-dependent manner either in mitochondria isolated from normal or arthritic rats. The curve for the arthritic series was clearly depressed relative to the control curve, reflecting the lower capacity for swelling presented by mitochondria isolated from arthritic rats. Despite this, the kinetics of inhibition was not different in either experimental series. The ID_{50} for the maximal amplitude and initial rate of decreases in absorbance were, respectively, 7.7 and 10 μM in the normal condition, whereas in the

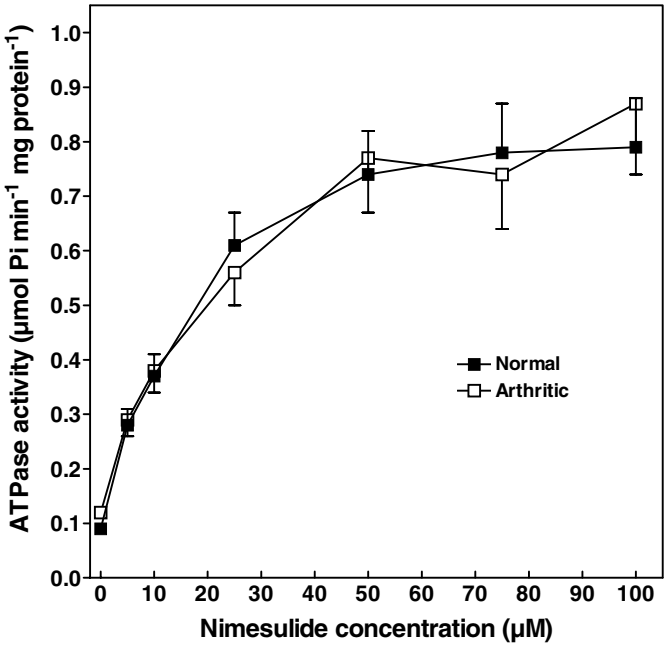

Figure 2. Effects of nimesulide on the ATPase activity. Intact mitochondria (1.6–2.3 mg protein) were incubated in reaction medium at 37°C. The reaction was initiated by the addition of 5.0 mM ATP. After 20 min of incubation the reaction was stopped by the addition of ice-cold 5% trichloroacetic acid and phosphate was assayed as described in Materials and Methods. Each data point is the mean ± SEM of five independent experiments. Mitochondria from normal rats (■–■); mitochondria from arthritic rats (□–□)

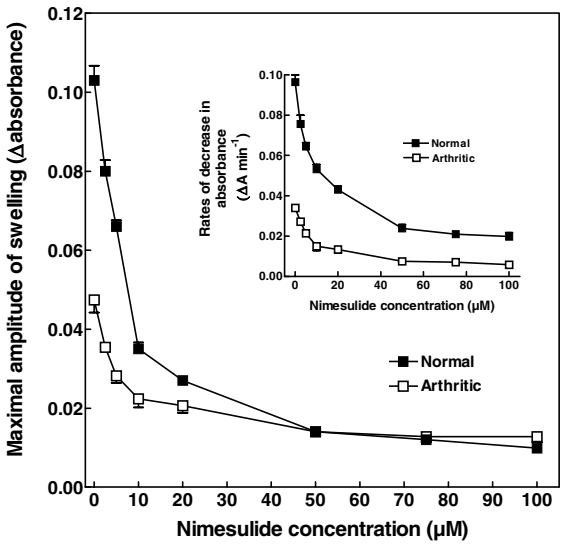

Figure 3. Effects of nimesulide on mitochondrial swelling driven by β-hydroxybutyrate oxidation. Mitochondria (0.79–1.64 mg protein) were incubated in reaction medium at 25°C. The reaction was initiated by additions of sodium acetate (50 mM) and β-hydroxybutyrate (8.0 mM). The decrease in absorbance at 575 nm was recorded. The maximal amplitude of absorbance and the rate of absorbance decrease (inserted graph) were plotted versus the nimesulide concentration. Each data point is the mean ± SEM of five independent experiments. Mitochondria from normal rats (■–■); mitochondria from arthritic rats (□–□)

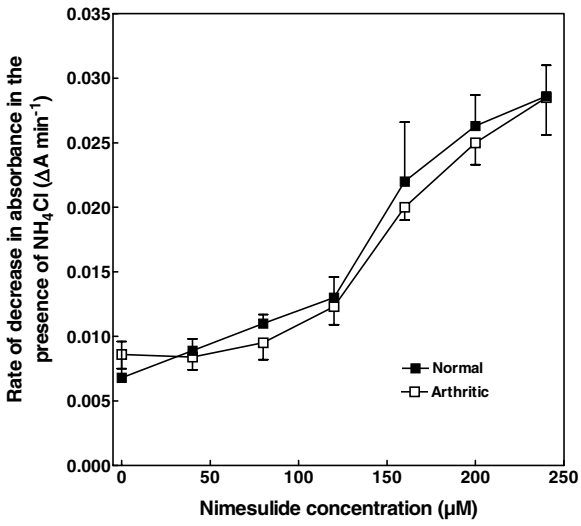

Figure 4. Nimesulide-induced mitochondrial passive swelling in the presence of NH_4Cl. Mitochondria (0.28–0.46 mg protein) were incubated at 25°C in a medium containing 150 mM NH_4Cl and 10 mM Tris–HCl (pH 7.4). The reaction was initiated by the addition of nimesulide. The decrease in absorbance at 540 nm was recorded. The initial rates of absorbance decrease were plotted versus the nimesulide concentration. Each data point is the mean ± SEM of six independent measurements. Key: mitochondria from normal rats (■–■); mitochondria from arthritic rats (□–□)

arthritic condition the corresponding values were 7.3 μM and 4.7 μM.

When isolated non-energized mitochondria are suspended in isosmotic solutions of $NH4^+Cl^-$ a rapid passive swelling occurs in the presence of a protonophore. This allows spectrophotometric measurements of the H^+ transport rate through the inner membrane. Figure 4 shows the initial rates of the changes in absorbance at 540 nm, which occurred upon addition of nimesulide, plotted against the nimesulide concentrations in the mitochondrial suspension. Nimesulide induced passive swelling in a dose-dependent manner in both mitochondria from normal and arthritic rats. The rates did not differ in either experimental series.

DISCUSSION

The results of the present work reveal that mitochondria isolated from adjuvant-induced arthritic rats present some alterations. The most evident were: higher rates of state IV and state III respiration with β-hydroxybutyrate as a substrate; reduced respiratory control ratios and impaired swelling capacity dependent on β-hydroxybutyrate oxidation. A relatively large number of factors are contributing in respiration and energized mitochondrial swelling dependent on β-hydroxybutyrate oxidation. We can dismiss, however, the possibility that the observed differences in the respiratory activity are reflecting changes in the components of complex I, III or IV of the respiratory chain, because no change in the NADH oxidase activity was found. A partial uncoupling of the mitochondria, although suggested by the increase in the state IV respiration and decrease in the respiratory control ratios, is also unlikely, because no alterations in the ADP/O ratios or in the ATPase activity of intact mitochondria were found. Both active mitochondrial swelling measured in the presence of acetate anion and the respiratory activity of intact mitochondria are processes strictly dependent on membrane integrity. It is possible, therefore, that the observed differences are consequences of alterations in the properties of the mitochondrial membrane. Indeed, it has been shown that adjuvant arthritis induces marked changes in phospholipid, arachidonic acid and fatty acid composition of plasmatic[22] microsomal[23] and mitochondrial[24] membranes.

Our comparative study performed with nimesulide provides ample evidence that uncoupling of oxidative

phosphorylation is in fact the primary mechanism of action of nimesulide in liver mitochondria from normal and arthritic rats. Nimesulide stimulated oxygen uptake in the absence of ADP (state IV respiration); decreased the ADP/O ratio and respiratory control ratio and stimulated the ATPase activity of intact mitochondria, a combination of events commonly found among uncouplers. Possibly, nimesulide is acting as a protonophore. This is indicated by two additional experimental observations: nimesulide inhibited mitochondrial swelling generated by β-hydroxybutyrate oxidation and induced the passive swelling of the mitochondria in the presence of $NH_4^+Cl^-$ ions. Swelling driven by β-hydroxybutyrate oxidation with acetate as permeant anion depends on active proton ejection,[15] whereas the passive swelling in the presence of $NH_4^+Cl^-$ ions only occurs in the presence of a protonophore. It induces proton exchange across the inner mitochondrial membrane and allows NH_3/NH_4^+ ion accumulation into the mitochondria. All these effects of nimesulide were, thus, those expected for a proton-conducting agent. The finding that nimesulide did not affect the NADH oxidase or succinate oxidase, as previously demonstrated,[9] excludes a direct action on the electron transport chain and provides an additional argument in favour of the hypothesis that uncoupling of oxidative phosphorylation is the predominant if not the only, mode of action of nimesulide in mitochondria.

A comparison of the effects of nimesulide in mitochondria from normal and arthritic rats reveals changes in the mitochondrial responses regarding the respiratory activity and the respiratory control ratio. The stimulation of respiration dependent on β-hydroxybutyrate and the reduction of the respiratory control ratio were induced by nimesulide at a lower concentration range in mitochondria from arthritic rats. This result confirms our previous observation in which succinate was employed as a substrate.[9]

The whole results allow us to conclude that the arthritis disease induces quantitative changes in some properties of mitochondria and in their responses to nimesulide. The question, which now arises, is if the observed differences are reflecting inherent structural or functional dissimilarities or, alternatively, differences generated during the isolation procedures. In our previous work, higher rates of oxygen consumption were observed in perfused livers from fed rats, a result that is in agreement with the higher respiratory activity presented by isolated mitochondria. The finding that the succinate oxidase activity was increased[9] whereas the NADH oxidase activity was not altered suggests that the increased respiration is probably a consequence of activation of specific mitochondrial dehydrogenases, such as succinate dehydrogenase instead of activation of other membrane-bound respiratory chain components. Indeed, changes in the activity of several enzymes have been reported to occur in livers from arthritic rats. Both activation[11] as well as inhibition[7] has been reported. It is possible that the same circulating inflammatory products associated with the inflammatory cachexia in the adjuvant-induced arthritis are also responsible for the observed alterations in mitochondria.[25–29] The increased respiratory activity could reflect an increase in the energy demands of the livers in order to synthesize for example, the acute phase proteins,[30–31] or alternatively, a stimulation of catabolic processes associated with inflammatory cachexia. [25–29]

A possible explanation for the higher susceptibility of mitochondria from arthritic rats to nimesulide, regarding the respiratory activity, may be also related to the reported changes in the membrane properties.[22–24] These changes could favour the interaction of nimesulide with the inner mitochondrial membrane. However, in the intact liver the extent of the changes caused by nimesulide on oxygen uptake was very similar in livers from normal and arthritic rats.[9] We cannot dismiss, therefore, the hypothesis that the observed differences in the mitochondrial properties as well as in the nimesulide effects are partly due to the different behaviour of isolated mitochondria. An alteration in membrane lipid composition and an increase in lipid peroxidation could also increase the fragility of the mitochondrial membranes, rendering the organelles more susceptible to damage or drug actions.

Nimesulide is the first member of the sulfonanilide class for which an uncoupling action has been demonstrated. This property was demonstrated for several other classes of nonsteroidal anti-inflammatory drugs, including several members of carboxylic and enolic acid derivatives.[32–38] This common property of nonsteroidal anti-inflammatory drugs is probably related to their chemical characteristics. They all have two common features, a lipophilic group, frequently aromatic rings and a free acidic group: a carboxylic group, an enolic group or a sulfonanilide group in the case of nimesulide.[12] These two features are also common to protonophore agents such as 2,4-dinitrophenol and FCCP (carbonyl cyanide *p*-trifluoro-metoxiphenil hydrazone).[39–41] The potency of each one of the nosteroidal anti-inflammatories is widely variable. [32–38] Whereas mefenamate, niflumate, piroxicam, flufenamate and diflunisal are active in the concentration range between 1 and 100 μM, salicylic

acid derivatives and sulindac are inactive at concentrations under 5 mM.[32] Nimesulide was active in isolated mitochondria in the concentration range between 2.5 and 250 μM. It can be included, therefore, in the list of the more active group. It should be emphasized that our previous study revealed that nimesulide was active in the perfused rat liver at a similar concentration range.[9] Thus, at therapeutic plasmatic levels of nimesulide[42] (between 20–50 μM) uncoupling of oxidative phosphorylation could become significant with deleterious effects on cell energy metabolism. In the arthritis diseases, as also occurs in other inflammatory processes, the energy requirements of the liver are increased.[25–27] Thus, the impairment of energy metabolism caused by nimesulide could lead to disturbances in the liver responses to inflammation, a fact that should be considered in therapeutic intervention.

ACKNOWLEDGEMENTS

This work was supported by grants from the Programa de Apoio a Núcleos de Excelência (PRONEX) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES

1. Diaz Gil J, Rossi I, Escartin P, Segovia JM, Gonsalvez M. Mitochondrial functions and content of microsomal and mitochondrial cytochromes in human cirrhosis. *Clin Sci Mol Med* 1997; **52**: 599–606.
2. Krähenbühl S, Stucki J, Reichen J. Mitochondrial function in carbon tetrachloride-induced cirrhosis in the rat. *Biochem Pharmacol* 1989; **38**: 1583–1588.
3. Cederbaum AI, Lieber CS, Rubin E. Effects of chronic ethanol treatment on mitochondrial functions damage to coupling site I. *Arch Biochem Biophys* 1974; **165**: 560–569.
4. Schilling RJ, Reitz RC. A mechanism for ethanol induced damage to liver mitochondrial structure and functions. *Biochem Biophys Acta* 1980; **603**: 266–277.
5. Bottenus RE, Spach PI, Filus S, Cunningham CC. Effect of chronic ethanol consumption on energy linked processes associated with oxidative phosphorylation: proton translocation and ATP-Pi exchange. *Biochem Biophys Res Commun* 1982; **105**: 1368–1373.
6. Moller B, Dargel R. Structural and functional impairment of mitochondria from rat livers chronically injured by thioacetamide. *Acta Pharmacol Toxicol* 1984; **55**: 126–132.
7. Barrit GJ, Whitehouse MW. Pathobiodynamics: effect of extrahepatic inflammation on calcium transport and drug metabolism by rat liver mitochondria *in vitro*. *Biochem Med* 1977; **17**: 99–115.
8. Somasundaram S, Sadique J. The role of mitochondrial calcium transport during inflammation and the effect of anti-inflammatory drugs. *Biochem Med Metab Biol* 1986; **36**: 220–230.
9. Caparroz-Assef SM, Bersani-Amado CA, Nascimento EA, Kelmer-Bracht AM, Ishii-Iwamoto EL. Effects of the non-steroidal anti-inflammatory drug nimesulide on energy metabolism in livers from adjuvant-induced arthritic rats. *Res Comm Mol Path Pharmacol* 1998; **99**: 93–116.
10. Fedatto Jr Z, Ishii-Iwamoto EL, Bersani-Amado CA, *et al*. Gluconeogenesis in the liver of arthritic rats. *Cell Biochem Funct* 1999; **17**: 271–279.
11. Fedatto Jr Z, Ishii-Iwamoto EL, Bersani-Amado CA, Maciel ERM, Bracht A, Kelmer-Bracht AM. Glucose phosphorylation capacity and glycolysis in the liver of arthritic rats. *Inflamm Res* 2000; **49**: 128–132.
12. Ward A, Brogden R. Nimesulide: a preliminary review of its pharmacological properties and therapeutic efficacy in inflammation and pain states. *Drugs* 1988; **36**: 732–753.
13. Pearson CM. Development of arthritis, periarthritis and periostitis in rats given adjuvants. *Proc Soc Exp Biol Med*. 1956; **91**: 95–101.
14. Voss DO, Campello AP, Bacila M. The respiratory chain and the oxidative phosphorylation of rat brain mitochondria. *Biochem Res Comm* 1961; **4**: 48–51.
15. Mustafa MG, Utsumi K, Packer L. Damped oscillatory control of mitochondrial respiration and volume. *Biochem Biophys Res Comm* 1966; **24**: 382–385.
16. Jung DW, Brierley GP. Swelling and contraction of potato mitochondria. *Plant Physiol* 1979; **64**: 948–953.
17. Chance B, Williams GR. A simple and rapid assay of oxidative phosphorylation. *Nature* 1955; **175**: 1120–1121.
18. Pullman ME, Penefski HS, Datta A, Racker E. Purification and properties of soluble dinitrophenol-stimulated adenosine triphosphate. *J Biol Chem* 1960; **235**: 3322–3329.
19. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. *J Biol Chem* 1925; **66**: 375–400.
20. Singer TP. Determination of the activity of succinate, NADH, choline and glycerophosphate dehydrogenase. *Meth Biochem Anal* 1974; **22**: 125–175.
21. Lowry OH, Rosebrough NJ, Farr AC, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
22. Ishizuki S, Fujihira E. Peroxidative status of isolated hepatocytes from adjuvant arthritic rats. *Res Commun Chem Pathol Pharmacol* 1984; **44**: 431–443.
23. Buchar E, Janku I. The effect of adjuvant-induced arthritis on rat liver microsomal phospholipid metabolism. *Method Find Exp Clin Pharmacol* 1985; **7**: 469–472.
24. Nemecz G, Bor P, Lepran I, Koltai M, Szeres L. Membrane phospholipid and fatty acid changes in the mitochondrial and sarcolemmal fraction of the heart in adjuvant arthritic rats. *J Mol Cell Cardiol* 1986; **18**: 739–747.
25. Roubenoff R, Roubenoff RA, Kehayias JJ, *et al*. Rheumatoid cachexia: cytokine-driven hypermetabolism accompanying reduced body cell mass on chronic inflammation. *J Clin Invest* 1994; **93**: 2379–2386.
26. Roubenoff R, Freedman LM, Smith DE, Abad LW, Dinarello CA, Kehayias JJ. Adjuvant arthritis as a model of inflammatory cachexia. *Arthritis Rheum* 1997; **40**: 534–539.
27. Tocco-Bradley R, Goerogieff M, Jones CT, *et al*. Changes in energy expenditure and fat metabolism in rats infused with interleukin-1. *Eur J Clin Invest* 1987; **17**: 504–510.
28. Yamaguchi Y, Tanaka Y, Yamada K, Bando Y, Kunitomo M. Abnormal lipid metabolism in adjuvant arthritic rats. *Jpn J Pharmacol* 1989; **50**: 377–386.
29. Besedovsky HO, del Rey A. Interleukin-1 and glucose homeostasis: an example of the biological relevance of immune–neuroendocrine interactions. *Horm Res* **31**: 94–99.

30. Baumgartner WA, Beck FWJ, Lorber A, Pearson CM, Whitehouse MW. Adjuvant disease in rats: biochemical criteria for distinguishing several phases of inflammation and arthritis. *Proc Soc Exper Biol Medic* 1974; **145**: 625–630.
31. Geiger T, Fischer M, Jagher B, Pignat W, Tscherry BJ, Wiesenberg I. Changes in hepatic mRNA levels of acute phase proteins during rat adjuvant arthritis. *Clin Exp Rheumatol* 1992; **10**: 467–473.
32. Miyahara JJ, Karler R. Effect of salicylates on oxidative phosphorylation and respiration of mitochondrial fragments. *Biochem J* 1965; **97**: 194–198.
33. Tomov T. Velichkova-Markova S. Effect of feloran on the oxidative phosphorylation of rat liver mitochondria. *Acta Physiol Pharmacol Bulg* 1983; **9**: 66–72.
34. McDougall P, Markhan A, Cameron I, Sweetman AJ. The mechanism of inhibition of mitochondrial oxidative phosphorylation by the nonsteroidal anti-inflammatory agent diflunisal. *Biochem Pharmacol* 1983; **32**: 2592–2598.
35. Kemmelmeier FS, Bracht A. Effects of nonsteroidal antiinflammatory drug mefenamic acid on energy metabolism in the perfused rat liver. *Biochem Pharmacol* 1989; **38**: 823–830.
36. Salgueiro-Pagadigorria CL, Kelmer-Bracht AM, Bracht A, Ishii-Iwamoto EL. Effects of the nonsteroidal anti-inflammatory drug piroxicam on rat liver mitochondria. *Comp Biochem Physiol* 1196; **113**: 85–91.
37. Petrescu I, Tarba C. Uncoupling effects of diclofenac and aspirin in the perfused liver and isolated hepatic mitochondria of rat. *Biochem Biophys Acta* 1997; **1318**: 385–394.
38. Mahmud T, Rafi SS, Scott DL, Wrigglesworth JM, Bjarnason I. Nonsteroidal antiinflammatory drugs and uncoupling of mitochondrial oxidative phosphorylation. *Arthritis Rheum* 1996; **39**: 1998–2003.
39. Hopfer U, Lehninger AL, Thompson TE. Protonic conductance across phospholipid bilayer membranes induced by uncouplers of oxidative phosphorylation. *Proc Natl Acad Sci USA* 1968; **59**: 484–490.
40. McLaughlin SGA, Dilger J. Transport of protons across membranes by weak acids. *Physiol Ver* 1980; **60**: 825–863.
41. Terada H. The interaction of highly active uncouplers with mitochondria. *Biochem Biophys Acta* 1981; **639**: 225–242.
42. Gandini R, Montalto C. First dose and steady state pharmacokinetics of nimesulide and its 4-hydroxy metabolite in healthy volunteers. *Il Farmaco* 1991; **46**: 1071–1079.

