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EFFECTS OF THE NONSTEROIDAL ANTI-INFLAMMATORY DRUG MEFENAMIC ACID ON ENERGY METABOLISM IN THE PERFUSED RAT LIVER

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Abstract—The action of mefenamic acid, a nonsteroidal anti-inflammatory drug, on energy metabolism in the isolated perfused rat liver was investigated. Mefenamic acid in the range between 0.1 and 1.0 mM was infused to livers from well-fed rats and from 24-hr fasted rats. The former were perfused with substrate-free Krebs/Henseleit-bicarbonate buffer, allowing the measurement of glycogenolysis and glycolysis from endogenous glycogen. The livers from 24-hr fasted rats, on the other hand, were perfused with Krebs/Henseleit-bicarbonate buffer containing fructose, thus allowing the measurement of fructolysis and glucose synthesis. Oxygen consumption was measured in both cases. When present in the range between 0.1 and 0.5 mM, mefenamic acid increased glycolysis, oxygen uptake, glycogenolysis and fructolysis. Higher concentrations, depending on the perfusion conditions, were inhibitory. Glucose production from exogenous fructose, on the other hand, was inhibited at low mefenamic acid concentrations. In general terms, the effects of mefenamic acid on energy metabolism seemed to be the primary consequence of its uncoupling action on the respiratory chain. This conclusion is supported mainly by the opposite effects on glucose synthesis (inhibition) and oxygen consumption (activation). The intracellular concentration of mefenamic acid is much higher than the extracellular one, a phenomenon which may represent binding to intracellular membrane or proteins.

It has been shown recently by Brass and Garrity [1] that several nonsteroidal anti-inflammatory drugs including meclofenamate, ibuprofen and indomethacin, increase the rate of glycogenolysis in isolated hepatocytes. For another group of nonsteroidal anti-inflammatory drugs, including diflunisal, mefenamate, flufenamate and niflumate, uncoupling of oxidative phosphorylation was demonstrated [2, 3]. Comparison of the structures of the compounds listed above reveals that they have some common features. First, they are all monocarboxylic acids and, second, they all have at least one, but usually two, aromatic rings in their structure. In fact, they may be considered as hydrophobic carboxylic acids, which become more or less water-soluble at alkaline hydrogen potentials (pH). The uncoupling activity, demonstrated for a certain number of these compounds [diflunisal, mefenamate (MEFA), flufenamate and niflumate], strongly suggests that they have a high affinity for biological membranes.

Uncoupling of oxidative phosphorylation influences cell metabolism in several ways. In addition to oxygen consumption activation, several metabolic pathways are affected, because the cell needs to compensate in some way for the diminished intramitochondrial adenosine 5′-triphosphate (ATP) generation. This leads necessarily to an activation of glycolysis and also, if there is enough glycogen within the cell, to an activation of glycogenolysis [4]. Based on this reasoning, we decided to extend the studies of Brass and Garrity [1] to other and yet uninvestigated compounds. Our studies, however, differ from that of Brass and Garrity [1] in two aspects. First, we used the isolated perfused rat liver instead of isolated hepatocytes, because we feel that the intact organ is much closer to the physiological conditions. Second, we measured several related variables and not simply glucose release. Simultaneous measurements of several variables are often useful in the elucidation of the mechanism of action of a given compound.

This article describes experiments performed with mefenamic acid, which uncouples oxidative phosphorylation of isolated mitochondria in the micromolar range [2]. The results will contribute to knowledge concerning the effects of mefenamic acid on the liver.

MATERIALS AND METHODS

Male albino rats (Wistar strain; 200–300 g) received a standard laboratory diet (Purina) and water *ad lib.* prior to the surgical removal of the liver under pentobarbital anesthesia (50 mg/kg). In the experiments in which fructose was infused, the rats were starved for 24 hr prior to the surgical removal of the liver. The perfusion technique described by Scholz *et al.* [5] was used. The perfusion fluid was Krebs/Henseleit-bicarbonate buffer, pH 7.4, saturated with an oxygen/carbon dioxide mixture (95/5%) and containing 25 mg/100 ml bovine serum albumin. Mefenamic acid and fructose were directly dissolved in the perfusion fluid. The fluid was pumped through a temperature-regulated (37°) membrane oxygenator prior to entering the liver via a cannula inserted in the portal vein. Samples of the effluent perfusion fluid were collected in 2- or 4-min intervals and analyzed for D-glucose, L-lactate and pyruvate. D-Glucose was determined by an *o*-tol-

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uidine method [6]. L-Lactate and pyruvate were measured enzymatically with L-lactate dehydrogenase [7]. To minimize interference by mefenamic acid at 340 nm, the spectrophotometric measurements were performed at 367 nm. At the latter wavelength, mefenamic acid absorbs much less than at 340 nm, whereas the extinction coefficient of NADH is decreased only 50% [7]. Interference by mefenamic acid was excluded by running a blank for each sample. The latter procedure was necessary, because the mefenamic acid concentration in the venous perfusate varied at different times after the onset of the infusion. The oxygen concentration in the venous perfusate was monitored continuously, employing a teflon-shielded platinum electrode, and the rates of uptake were calculated from the arterio-venous concentration differences and the flow rate. The rates of metabolite release (glucose, L-lactate and pyruvate) were calculated from the venous concentrations and the flow rate. All rates were referred to the wet weight of the liver.

The perfusion apparatus was built in the workshops of the University of Maringá. Bovine serum albumin and all enzymes and coenzymes used in the metabolite assays were products of the Sigma Chemical Co. (St. Louis, MO, U.S.A.). The reagent grade chemicals were from Merck (Darmstadt, FRG), Carlo Erba (São Paulo, Brazil) and Reagen (Rio de Janeiro, Brazil). Mefenamic acid was purchased from Squibb Indústria Química S.A. (São Paulo, Brazil) and purified before use in the experiments.

RESULTS

Effects on glycogen catabolism and oxygen uptake. To study glycogen catabolism, livers from well-fed rats [8] were perfused in an open system and with substrate-free perfusion fluid. Under the latter conditions, the rates of L-lactate, pyruvate and glucose release provide a good approximation of the rate of glycogen catabolism [9]. Figure 1 shows the mean results of seven identical experiments in which mefenamic acid was infused in step-wise increasing concentrations in the range between 0.1 and 1.0 mM. As shown, mefenamic acid up to 0.5 mM increased oxygen uptake. A dose-dependent response was demonstrated by the fact that the concentration changes from 0.1 to 0.2 and 0.5 mM also resulted in increases in oxygen consumption. It should be noted, however, that infusion of 0.5 mM mefenamate increased oxygen consumption to a relatively small extent, when compared to 0.1 and 0.2 mM. It seems that stimulation at 0.5 mM mefenamic acid was already partially compensated by inhibition, which manifested very clearly when the concentration was increased to 1 mM. At the end of the 1 mM infusion period (at 86 min), however, the rate of oxygen consumption [3 μmol · min^{-1} · (g liver)$^{-1}$] was still above the basal value measured before the onset of mefenamic acid infusion [2.6 μmol · min^{-1} · (g liver)$^{-1}$].

The actions on glucose release and L-lactate production were very similar to that on oxygen uptake. Both variables were increased by mefenamic acid up to 0.2 mM. The changes were very pronounced. Glucose release was increased 3.5 times at 0.2 mM mefenamic acid, whereas L-lactate production was 2.5 times higher than the basal value. A tendency toward diminution at higher concentrations, however, was already visible at 0.5 mM mefenamic acid. In the case of glucose release, a small and transient increase occurred upon infusion of 0.5 mM mefenamic acid, but diminution progressively took place and was enhanced further upon 1 mM mefenamic acid infusion. In the case of L-lactate production, the values found at 0.5 mM mefenamic acid were already lower than those found at 0.2 mM, but a substantial decrease occurred at 1 mM. Upon cessation of mefenamic acid infusion, both variables decreased to values that were lower than those found before the onset of the infusion. Pyruvate production, which is also shown in Fig. 1, was not increased by 0.1 or 0.2 mM mefenamic acid. A slight decrease, however, took place at 0.5 and 1.0 mM.

A considerable amount of glycogen was catabolized during the experiments of the type shown in Fig. 1. The livers from *ad lib.* fed rats, such as those used in our experiments, contained 75 mg glycogen/g wet weight before the perfusion experiments.* This corresponds to 470 μmol glucosyl units/g liver. The total amount of glycogen catabolites (glucose, L-lactate and pyruvate) released during the period between 20 and 72 min of perfusion was 230 μmol glucosyl units/g, corresponding to 49% of the quantity originally present as glycogen. If one considers glycogen catabolism during the pre-perfusion period and the amount of pyruvate which was not released, but oxidized within the mitochondria [9], it seems plausible that, at 72 min of perfusion, the time at which the mefenamic acid concentration was shifted from 0.5 to 1.0 mM (Fig. 1), not less than 75% of glycogen had already been catabolized. Diminution of glucose release and L-lactate production by 1 mM mefenamic acid may thus be, in part at least, the consequence of glycogen depletion rather than a true inhibitory effect. Glycogen depletion was also revealed by the fact that cessation of mefenamic acid infusion produced rates of glucose release and L-lactate production that were much smaller than those found before initiation of mefenamic acid infusion (20–30 min). Liver damage caused by the long exposure to high mefenamic acid concentrations, however, may have contributed to the phenomenon.

To get an insight into this question, another series of experiments were performed in which mefenamic acid was infused at a single high concentration (1 mM). Figure 2A shows the mean results (N = 3), which are different from those obtained in the titration experiment of Fig. 1. Glucose release was increased to such high values as 7 μmol · min^{-1} · g^{-1} and remained elevated during mefenamic acid infusion. The L-lactate production was increased less, but it also remained constant. Constancy was also found for oxygen consumption. The total amount of glucosyl units released during 24 min of perfusion, the time at which infusion was discontinued, amounted to 145 μmol/g, representing 31% of the total glycogen originally present. Clearly, in this case, glycogen depletion seems unlikely. This

* Itinose AM, Doi ML and Bracht A, unpublished work.

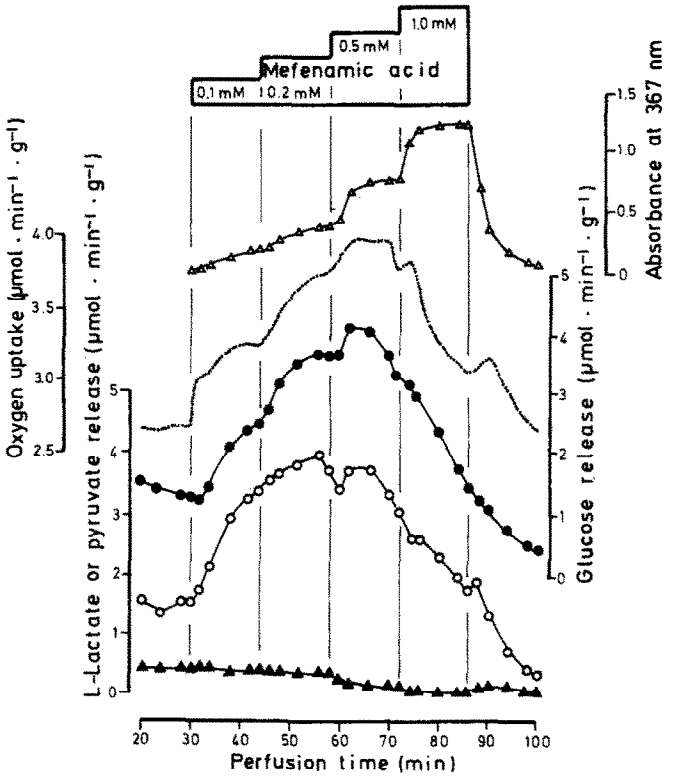

Fig. 1. Effects of mefenamic acid on glucose release from endogenous glycogen, glycolysis (L-lactate and pyruvate production), and oxygen consumption in the isolated perfused rat liver. Livers from fed rats (N = 7) were perfused with Krebs/Henseleit-bicarbonate buffer in a non-recirculating system. Mefenamic acid was infused at 30–86 min of perfusion in step-wise increasing concentrations (0.1, 0.2, 0.5 and 1.0 mM), as indicated by the horizontal bars. The effluent perfusate was sampled in 2-min intervals and analyzed for glucose, L-lactate, pyruvate and mefenamic acid (absorbance at 367 nm). Oxygen consumption was monitored continuously by a platinum electrode. Key: oxygen consumption (trace); glucose release (●—●); L-lactate production (○—○); pyruvate production (▲—▲); and mefenamic acid concentration (△—△).

conclusion is supported by the fact that, even 18 min after the mefenamic acid infusion had been stopped, both glucose release and L-lactate production still had rates somewhat higher than those found initially.

The increased rates of L-lactate release also resulted in increased L-lactate/pyruvate ratios, inasmuch as pyruvate production was decreased in some situations. In the experiments of Fig. 2A, for example, the L-lactate to pyruvate ratio was 4.1 in the absence, but 8.5 in the presence, of mefenamic acid. The L-lactate to pyruvate ratio is usually interpreted as an indicator for the redox state of the cytosolic NAD^+-NADH couple [10]. It seems, thus, that mefenamic acid increased the redox potential of cytosolic NAD^+-NADH.

Effects on fructose metabolism. In addition to glycogen, the liver also metabolizes fructose at comparable or even higher rates [11]. A considerable fraction of fructose is converted to glucose in an energy-dependent process [11]. A small fraction is oxidized in the respiratory chain, but the main products of fructose catabolism (fructolysis) are L-lactate and pyruvate [11]. The investigation of the effects of mefenamic acid on fructose metabolism was accomplished by the experiments shown in Fig. 3 and 4. Fructose concentration was the same in each experiment (5 mM), but the mefenamic acid concentration was varied in the range between 0.1 mM (Fig. 3A) and 1.0 mM (Fig. 4B). Each experiment was repeated three times with livers from 24-hr fasted rats. Under the latter conditions, the glycogen levels are very low and fructose metabolism can be investigated with little interference by glycogen catabolism. As may be seen in Figs. 3 and 4, fructose infusion caused very pronounced increases in glucose, L-lactate and pyruvate production. Oxygen uptake was also increased. Since the glycogen levels are low, the rate of glucose release is a good approximation for the rate of glucose synthesis from fructose [12]. When mefenamic acid was infused, glucose production was inhibited. At 0.1 mM, the rate of glucose production was already inhibited 45%. Higher concentrations increased inhibition progressively. At 1.0 mM, inhibition was virtually complete. It should be stressed that, at concentrations up to 0.5 mM, inhibition of glucose release was concomitant with oxygen uptake activation. Inhibition of oxygen uptake was only

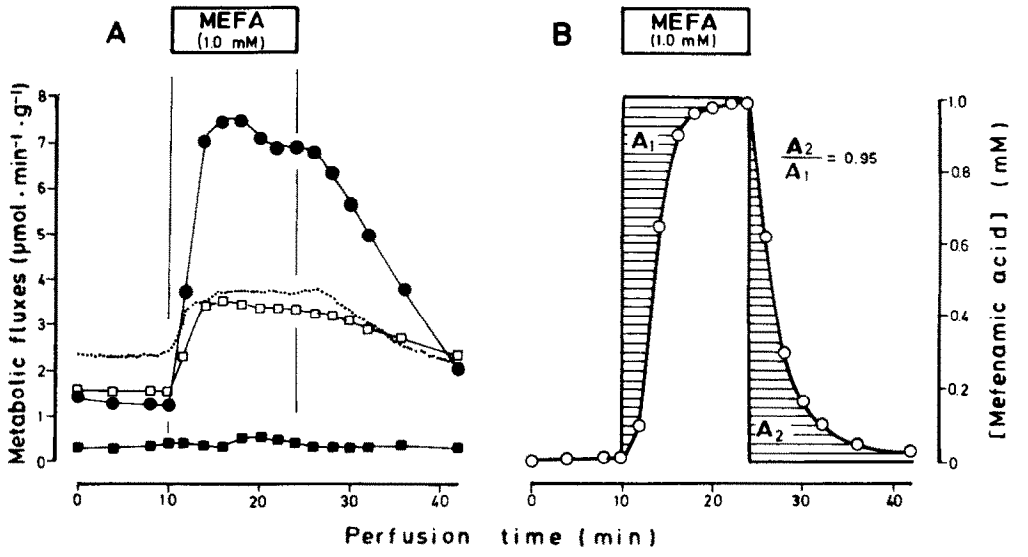

Fig. 2. Effects of 1 mM mefenamic acid (MEFA) on glycolysis, glycogenolysis and oxygen consumption in livers from fed rats. Livers from fed rats (N = 3) were perfused with Krebs/Henseleit-bicarbonate buffer in a non-recirculating system. Mefenamic acid was infused at 10–24 min of perfusion, as indicated by the horizontal bars. The effluent perfusate was sampled in 2-min intervals and analyzed for glucose, L-lactate, pyruvate and mefenamic acid. Oxygen consumption was monitored continuously by a platinum electrode. Key: oxygen consumption (trace); glucose release (●—●); L-lactate production (□—□); pyruvate production (■—■); and mefenamic acid concentration in the effluent perfusate (○—○).

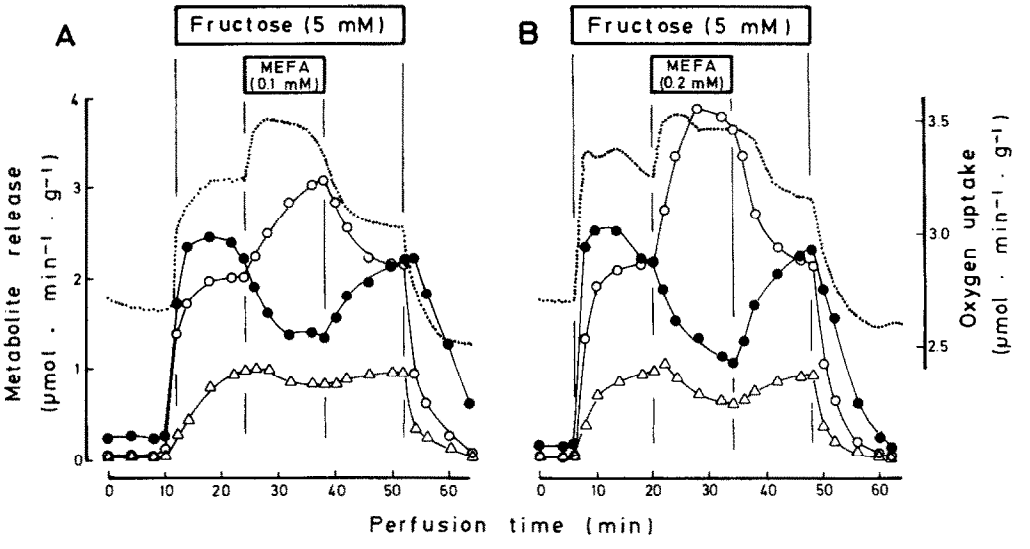

Fig. 3. Effects of 0.1 and 0.2 mM mefenamic acid (MEFA) on fructose metabolism in the isolated perfused rat liver. Livers from 24-hr fasted rats (N = 3 in both cases) were perfused with Krebs/Henseleit-bicarbonate buffer in a non-recirculating system. Fructose (5 mM) and mefenamic acid (A: 0.1 mM; B: 0.2 mM) were infused at the times indicated by the horizontal bars. Oxygen uptake was monitored continuously, and samples were taken for the measurement of L-lactate, pyruvate and glucose production. Key: oxygen uptake (trace); glucose production (●—●); L-lactate production (○—○); and pyruvate production (△—△).

observable at 1.0 mM. The latter phenomenon, however, was preceded by a small and transient activation.

Pyruvate production was inhibited in the whole concentration range. The inhibition was very small at 0.1 mM, but it increased with concentration and at 1.0 mM it was virtually complete. The L-lactate production, in contrast, was increased at low concentrations (0.1 and 0.2 mM). At 0.5 mM mefenamic acid there was a very pronounced transitory increase

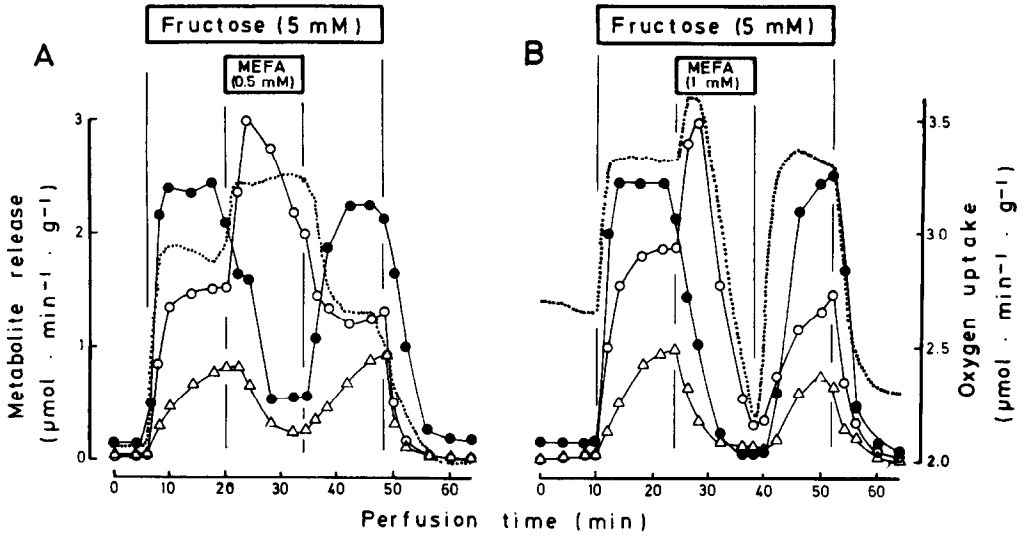

Fig. 4. Effects of 0.5 and 1.0 mM mefenamic acid (MEFA) on fructose metabolism in the isolated perfused rat liver. Livers from 24-hr fasted rats (N = 3 in both cases) were perfused with Krebs/Henseleit-bicarbonate buffer in a non-recirculating system. Fructose (5 mM) and mefenamic acid (A: 0.5 mM; B: 1.0 mM) were infused at the times indicated by the horizontal bars. Oxygen uptake was monitored continuously, and samples were taken for the measurement of L-lactate, pyruvate and glucose production. Key: oxygen uptake (trace); glucose production (●—●); L-lactate production (○—○); and pyruvate production (△—△).

and at 1 mM, after a short overshoot phenomenon, strong inhibition took place. As it happened with glycogen catabolism, the L-lactate to pyruvate ratio increased in the presence of mefenamic acid. Here again, it possibly denotes increased cytosolic NAD^+-NADH potentials [10].

It should be stressed that all effects of mefenamic acid on fructose metabolism were totally reversible. This is particularly important in the case of glucose production, a biosynthetic route strictly dependent on energy and on the structural integrity of the cells. It is, thus, improbable that a short exposure of the liver to even such high concentrations as 1 mM resulted in significant damage to the structure of the cell.

Venous and arterial mefenamic acid concentrations. Since mefenamic acid absorbs light in the ultraviolet range, it can be easily monitored spectrophotometrically. In our experiments this was routinely done at 367 nm ($\varepsilon = 1.5\ mM^{-1} \cdot cm^{-1}$), because the interference of mefenamic acid with the enzymatic measurements of L-lactate and pyruvate had to be excluded. One can expect that mefenamic acid is metabolized in the liver and also that those products which are released into the effluent perfusate may also absorb in the ultraviolet range. Nevertheless, in a situation where the rate of infusion of mefenamic acid is very high compared to the maximal capacity of the hepatic drug-metabolizing systems, interference by metabolic products will be very small. This was the particular situation of the experiment shown in Fig. 2. Mefenamic acid was infused at a concentration of 1 mM and at a rate of $4.5\ \mu mol \cdot min^{-1} \cdot g^{-1}$. Under normal conditions, the maximal rates of drug metabolism in the liver never surpass $0.3\ \mu mol \cdot min^{-1} \cdot g^{-1}$ [13–15], even when two or three different systems are involved as in the case of paracetamol metabolism.* The experimental points shown in Fig. 2B, consequently, may be regarded as corresponding essentially to the mefenamic acid concentration in the venous perfusate at different times after the onset of the infusion. As revealed by Fig. 2B, the venous mefenamic acid concentration increased very slowly until it attained values that were near to the arterial concentration. A venous concentration corresponding to 50% of the arterial one was reached at about 4–5 min after the onset of the infusion. For comparison, in the same experimental system less than 1 min is required for a marker of the extracellular space, such as labeled sucrose, to attain values near the arterial ones [16, 17]. For labeled water, which occupies the whole aqueous space of the liver, it takes less than 2 min in the isolated perfused rat liver [17]. At 2 min, the venous mefenamic acid concentration, however, was only 10% of the arterial one. Similarly, when the infusion was stopped, the concentration of mefenamic acid decreased very slowly. As shown in Fig. 1, the phenomenon observed with a single and high concentration (Fig. 2) also occurred when mefenamic acid was infused in step-wise increasing concentrations. At each step it took at least 10 min until the arterial concentration was reached. At the lower concentrations one can expect that metabolism interferes much more because the metabolic systems are possibly not saturated, allowing a higher fraction to be metabolized.

* Itinose AM, Doi ML and Bracht A, unpublished work.

The approximate amount of mefenamic acid that was retained by the liver during infusion may be calculated from the area between the experimental curve and the arterial levels (area A_1 in Fig. 2B). Conversely, the approximate amount which left the liver after cessation of the infusion is given by the area under the corresponding experimental curve (A_2 in Fig. 2B). The amount retained by the liver, calculated in this way, was equal to 15.8 μmol/g liver. The quantity which came out again after 42 min of perfusion was only slightly smaller and was equal to 14.9 μmol/g. This fact shows that a minor portion of mefenamic acid present within the cells was excreted into bile. The same phenomenon, with similar values, was also described for niflumic acid, a compound which is similar in structure to mefenamic acid [18].

DISCUSSION

The main conclusion allowed by the results obtained in the present work is that the uncoupling action of mefenamic acid, demonstrated in experiments with isolated mitochondria [2], also manifests in the intact liver cells. The following observations support the conclusion: (a) mefenamic acid increases oxygen consumption [4, 16]; (b) it also increases glycolysis and glycogenolysis [4, 8, 9]; (c) glucose production from fructose, a biosynthetic route which is energy dependent, is inhibited [4]; and (d) L-lactate and pyruvate production from fructose (fructolysis) is increased.

Individually, none of the observations listed above can be taken as sufficient evidence for the uncoupling action. The combination of effects, however, provides more security in the interpretation. The most important combination of events is the simultaneous stimulation of oxygen uptake and inhibition of glucose production. Glucose production inhibition always occurs when energy metabolism is affected negatively [4, 12]. This means that oxygen uptake activation did not increase the ATP supply, but rather decreased it. Such a deleterious increase in oxygen consumption in the intact liver was also found for the classic uncoupling agent carbonylcyanide *p*-trifluoromethoxyphenylhydrazone [29]. The increased rates of glycolysis and fructolysis, on the other hand, are also an indication that mitochondrial ATP supply was diminished, making it necessary to increase cytosolic ATP-generating processes. The latter phenomenon is characteristic of compounds which negatively affect mitochondrial functions [8, 20]. The stimulatory action on glycogenolysis, finally, is also a common feature of compounds which directly or indirectly affect oxidative phosphorylation [8, 19–21], although the exact mechanism of this effect still remains a matter of controversy [21].

Brass and Garrity, in their study about the effects of nonsteroidal anti-inflammatory drugs on hepatic glycogenolysis [1], demonstrated that the effect is independent of cyclooxygenase inhibition. As a possible mechanism of action, they suggested that stimulation of glycogenolysis could be related to calcium redistribution within the cell [1]. In fact, inhibition of calcium uptake by anti-inflammatory drugs in isolated hepatic mitochondria has been observed [22], a phenomenon which probably leads to increased cytosolic calcium concentrations. Moreover, it is also clear that increased calcium concentrations stimulate glycogenolysis [23, 24]. The question which now arises is if our proposition, i.e. glycogenolysis activation by mefenamic acid as a primary consequence of uncoupling of oxidative phosphorylation, is compatible with the hypothesis evoked by Brass and Garrity [1]. Actually, calcium fluxes and redistribution within the cell are elicited by several agents and situations especially when energetic parameters are affected [24–26]. One can expect, thus, that all anti-inflammatory drugs which act as uncouplers will also affect calcium distribution within the cell. Consequently, the conclusion also imposes that our interpretation and that of Brass and Garrity [1] have an important common point and are not irreconcilable.

Another aspect which deserves some comment is the concentration range of the effects described in this work. Mefenamic acid was active in the range between 10^{-4} and 10^{-3} M in the perfusion fluid. From the work of McDougall *et al.* [2], we know that 50% of maximal uncoupling activity in isolated rat liver mitochondria occurs at concentrations between 2 and 4×10^{-5} M. Apparently, in relation to isolated mitochondria, the action in the intact liver was shifted toward higher concentrations, but the phenomenon cannot be interpreted in such simple terms. The reason for this is that mefenamic acid as well as other anions with a hydrophobic side chain has quite a complex behavior. Our experiments have shown that, when 1 mM mefenamic acid was infused, the liver took up approximately 15 μmol/g liver of the drug until the arterial and venous concentrations were equalized. Approximately the same amount was released by the liver after cessation of mefenamic acid infusion. An isolated perfused rat liver weighing 8 g has an intracellular water volume of approximately 5.5 ml, as shown by indicator dilution studies [11, 17]. From the amount of drug taken up and the intracellular aqueous volume one may calculate that the intracellular mefenamic acid concentration, expressed in terms of the intracellular water content, was as high as 21.8 mM, more than 20 times the concentration in the extracellular space (1 mM).

It should be stressed at this point that, by expressing intracellular concentrations in terms of the intracellular water content, one can get a better idea about the relative amount of drug inside the cells, but probably it does not represent the real situation. The assumption that the total amount of drug within the cells is effectively restricted to the aqueous phase brings some practical as well as theoretical difficulties. In the first place one is faced with a concentration gradient between two aqueous solutions (perfusate and intracellular water), which requires a great amount of energy to be built up and maintained. Indispensable would also be an active transport system. There is indeed evidence that some kind of active transport for anionic drugs may be present in the liver [27]. It is doubtful, however, if the transport systems yet described are as efficient as necessary in the present case. For example, in the experiments shown in Fig. 2, one can calculate a

unidirectional influx rate around 4 μmol·min^{-1}·g^{-1}. Such high rates of activity of a supposedly energy-dependent transport system are not compatible with the rates of ATP synthesis within the cell under the conditions of the experiments [9, 28]. Moreover, one can hardly harmonize a rapid energy-dependent transport system with the known uncoupling effect of mefenamic acid inasmuch as the amount of drug in direct contact with the mitochondria would be very high. Uncouplers, when present at very high concentrations, produce irreversible and deleterious alterations in the mitochondrial membranes. Irreversible changes, however, were not found in our experiments. It therefore seems unlikely that the highly concentrative uptake of mefenamic acid is the consequence solely of active transport, with mefenamic acid present in the aqueous phase.

An additional explanation for the concentration gradient would be that a considerable fraction of mefenamic acid is not in the aqueous phase, but rather bound to intracellular proteins or dissolved in the lipid phase. This possibility has considerable theoretical and experimental support. The existence of proteins whose specific function is to bind anions with hydrophobic side chains is a well established fact. One of the best known proteins of this kind is the so-called Z protein [29]. More important than protein binding, however, is possibly the dissolution of the drug within the lipid phase of the membranes, which constitutes a considerable fraction of the cell volume. It is well established that uncouplers are generally substances with high affinity for the lipid phase of the membranes [1, 3, 4, 26]. Correlations between lipophilicity of analogous compounds and their ability to uncouple oxidative phosphorylation have indeed been found [30]. When such substances are allowed to distribute between aqueous and hydrophobic phases, the concentration in the latter one, after equilibrium has been reached, generally surpasses by far the concentration in the aqueous phase.

In view of the latter and more likely explanation, it may be concluded that more important than the concentration in the perfusate is actually the concentration within the mitochondrial membranes. In this sense, the data of McDougall *et al.* [2] are of little help, because their experiments were conducted with more or less the same concentration of mitochondrial protein, and no relation between drug action and protein concentration, which is proportional to the amount of mitochondria present in the incubation system, was established [1]. Consequently, the situation in the liver cells and that of an incubation system containing isolated mitochondria can hardly be compared, and the concentrations for half-maximal activity found by McDougall *et al.* [2] in the latter system are not applicable to the intact organ. On the other hand, perfusate concentrations up to 0.2 mM may be considered physiologic in the sense that they may be attained in animal experiments with doses up to 50 mg/kg. In humans, concentrations which are not very far from 0.1 mM have indeed been reported for several anti-inflammatory drugs [31]. Uncoupling of oxidative phosphorylation may, thus, be contributing to the toxicity of mefenamic acid.

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