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Antagonism between the metabolic responses induced by epinephrine and piroxicam on isolated rat hepatocytes

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most employed therapeutic agents. They have a wide spectrum of biological effects, some of which are independent of cyclooxygenase inhibition, such as the alterations on the components of signal transduction systems. In particular, previous data from our laboratory suggested an antagonism between epinephrine and piroxicam, one of the most prescribed NSAIDs. Thus, this study deals with the epinephrine–piroxicam antagonism recorded for metabolic responses in isolated rat hepatocytes. The obtained results show that epinephrine stimulates lactate and ethanol consumption, stimulates glucose release from lactate only, and has no effect on cellular triacylglycerides content. Otherwise, in a dose-dependent basis, piroxicam stimulates lactate and ethanol consumption accompanied by an increase in triacylglycerides content, without changes in glucose release by hepatocytes. Piroxicam blocks the epinephrine-induced stimulation of glucose release from lactate, and epinephrine blocks the piroxicam-mediated increase in triacylglycerides content from lactate or ethanol. In contrast, the effects of epinephrine and piroxicam, promoting the consumption of lactate and ethanol, are not antagonized or added after the simultaneous administration of both compounds. This last result is probably related to the ability of both compounds to stimulate oxygen consumption. On isolated rat liver mitochondria, μmolar doses of piroxicam partially uncouple oxidative phosphorylation, and paradoxically stimulates an ATP-dependent mitochondrial function as citrullinogenesis. These results show for first time, on isolated rat hepatocytes, an antagonism between the metabolic responses of epinephrine and piroxicam, at the concentration found in plasma after its therapeutical administration.

Keywords: Epinephrine; Piroxicam; Nonsteroidal anti-inflammatory drug (NSAID); Glucose production; Triacylglyceride; Lactate consumption; Ethanol consumption; Mitochondrion; Citrulline synthesis

1. Introduction

The nonsteroidal anti-inflammatory drugs (NSAIDs) comprise one of the most employed therapeutic agents. In 1984, nearly one in seven Americans was treated with a NSAID (Clive and Stoff, 1984), and in 1986, 100 million prescriptions were written for these drugs (Langman, 1988). Their effectiveness to reduce prostaglandin-mediated fever, pain, and swelling, has been attributed to their ability to inhibit the cyclooxygenase, prostaglandin H synthase (Vane, 1971; Ferreira and Vane, 1974; Abramson et al., 1985). However, these drugs have a wide spectrum of biological effects, many of which are determined by other mechanism independent of cyclooxygenase inhibition (Weissmann et al., 1987; Abramson and Weissmann, 1989; Brooks and Day, 1991; Cronstein and Weissmann, 1995; Cashman, 1996). Among these, several reports pointed out modifications on the components of signal transductions systems, a property that may account for diverse effects on membrane-dependent processes. Thus, NSAIDs can modify membrane viscosity, uncoupling receptor signaling events that depend upon the state of membrane fluidity (Abramson et al., 1990); interfere with GTP/GDP exchange at the α-subunit of regulatory G protein (Abramson et al., 1991, 1994); block the pertussis toxin-dependent ADP-ribosylation of the G protein in purified neutrophil

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membranes, and inhibit, in part, the pertussis toxin-sensitive formation of diacylglycerol that follows cell activation (Abramson et al., 1991, 1994; Cronstein and Weissmann, 1995).

In addition, NSAIDs modify the activity of cellular kinases (Earnest et al., 1992; Frantz and O'Neill, 1995; Roy et al., 1995); inhibit Ca^{2+}-uptake in neutrophils (Abramson et al., 1985; Weissmann et al., 1987; Kankaanranta et al., 1996); enhance intracellular levels of cyclic AMP (Abramson et al., 1985; Weissmann et al., 1987); inhibit the activity of phosphodiesterase (Earnest et al., 1992); and inhibit phospholipase C activity (Bomalaski et al., 1986).

Therefore, is not surprising that NSAIDs antagonize some hormone-induced responses. In consequence, NSAIDs inhibit epinephrine-induced stimulation of glycerol production in isolated rat adipocytes (Pimentel-Velazquez et al., 1996), lower the glucagon stimulation of glucose production in rat liver (Wheeler and Epand, 1975), partially attenuate the hypotensive effect of α- and β-adrenoceptor-blocking agents (Durao et al., 1977; Watkins et al., 1980; Ebel et al., 1986; Spence, 1986; Wong et al., 1986; Radack and Deck, 1987; Johnson et al., 1994; De Leeuw, 1996), reverse α-adrenergic inhibition of acute insulin response to glucose (Metz and Robertson, 1980), and reverse the effects of chronic β-adrenoceptor blockade to attenuate adrenergic neurovascular transmission (Daniell et al., 1988). Likewise, hormones also can antagonize NSAIDs-induced responses. Thus, dipyrone- and flunixin-induced analgesia can be reversed by atipamezole (an α_2-adrenoceptor antagonist) (Chambers et al., 1995).

The broad variety of antagonic effects showed by NSAIDs on hormone-induced responses lead to suggest that this antagonism must comprise several not yet analyzed responses. Because it is well established that epinephrine produces an increase in hepatic gluconeogenesis and lactate consumption (Sacca et al., 1983; Pilkis and Granner, 1992; Pilkis et al., 1988), we analyzed the influence of piroxicam, a NSAID, on this ''classical'' metabolic effect induced by epinephrine. Furthermore, as previous data in our laboratory showed a piroxicam dose-dependent increase in triacylglycerides content on isolated rat hepatocytes (Saldaña-Balmori et al., 1996), we also tested the effect of epinephrine on this metabolic response induced by piroxicam. Finally, and in order to obtain a more elaborated picture of piroxicam–epinephrine interactions, we analyze piroxicam effects on some mitochondrial functions and compare them with the well-known effects of epinephrine on mitochondria (Reinhart et al., 1982; Halestrap et al., 1985; Taylor et al., 1986; Quinlan and Halestrap, 1986; Gonzalez-Manchon et al., 1988; Halestrap, 1989; Korzeniewski and Froncisz, 1992; Garcia et al., 1997).

It is noteworthy that in spite of the previous reports showing piroxicam effects on hepatic glucose metabolism (Dorronsoro de Cattoni and Battellino, 1992; Salgueiro-Pagadigorria et al., 1996a), the influence of this therapeutic agent on the hepatic metabolic responses to epinephrine has not been yet analyzed.

Thus, on basis to the extensive use of piroxicam, one of the most prescribed NSAIDs in the United States (American Academy of Family Physicians, 1997), the obtained results, are of interest because this work shows, for first time, an antagonism between piroxicam and some metabolic responses induced by epinephrine on isolated hepatocytes.

2. Materials and methods

Male Wistar rats (200–250 g) were fed ad libitum with a commercial diet (Nutricubos from Mexico) and they were fasted 48 h before treatment, but free access to water was allowed. Animals were anesthetized with ether, and isolated hepatocytes were prepared by the method of Berry and Friend (1969), using slight modifications as described by Guinzberg et al. (1987). Cell viability was assayed by the trypan blue exclusion method; experiments were performed only when more than 90% viability was recorded.

Once isolated, liver cells, equivalent to 10–15 mg protein, were incubated for 60 min at 37°C with continuous shaking in Krebs-Ringer bicarbonate buffer: 120 mM NaCl, 5.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 12 mM $NaHCO_3$, 1.2 mM $CaCl_2$, adjusted to pH 7.4, in an atmosphere of O_2:CO_2 (95%:5%). The incubation media was supplemented with 50 mM sodium lactate pH 7.4, or 50 mM ethanol as substrates, in the absence or in the presence of epinephrine (10^{-6} M) and piroxicam (10^{-9}–10^{-4} M).

After incubation, glucose release (Fales, 1963), lactate consumption (Gutmann and Wahlefeld, 1974), triacylglycerides content (Gottfried and Rosenberg, 1973), ethanol consumption (Bernt and Gutmann, 1974), and protein concentration (Bradford, 1976) were quantified as detailed previously by Saldaña-Balmori et al. (1996). Each assay was routinely performed in duplicate.

All measurements were made from the same hepatocytes batches, however, some data on lactate and ethanol consumption, as well as triacylglycerides content data were presented originally in a previous report from our laboratory (Saldaña-Balmori et al., 1996). These data were completed with new experiments, and reanalyzed in order to integrate them with the glucose release data.

Mitochondria from rat liver were prepared by the method of Schneider and Hogeboom (1950), with the modifications proposed by Siess (1983), as detailed previously by Zentella de Piña et al. (1989), with an isolation medium containing 0.3 M mannitol, 0.5 mM EGTA, 5 mM TES [*N*-tris-(hydroxymethyl)-2-aminoethane sulfonic acid], and 0.1% bovine serum albumin, pH 7.4. Mitochondrial respiration was recorded with a Clark O_2 electrode at 22°C. Mitochondrial citrulline formation was assayed in the medium described by Corvera and García-Sáinz (1982);

after 10-min incubation was stopped by the addition of perchloric acid (3% final concentration). Citrulline was determined by the method of Boyde and Rahmatullah (1980).

Epinephrine, L-lactate, bovine serum albumin (fraction V), collagenase (type IV) were obtained from Sigma (St. Louis, MO, USA). Other reagents were analytical grade either from Merck (México) or Sigma.

Statistical analyses were performed with the software SigmaStat for Windows ver. 1.0 (Jandel Scientific, 1994), employing one-way analysis of variance (ANOVA), followed by an all pairwise multiple comparison procedure using the Dunnett's method. If normality test or equal variance test failed, the nonparametric Kruskal–Wallis one-way ANOVA on ranks was employed.

3. Results

The effect of piroxicam on epinephrine-stimulated gluconeogenesis from lactate is showed in Fig. 1. In this figure can be observed that piroxicam, from 10^{-9} M, blocked the epinephrine-induced activation of glucose synthesis on rat liver hepatocytes ($P < 0.003$), meanwhile piroxicam per se did not modify glucose production in the absence of epinephrine at any of the tested concentrations (1 nM to 0.1 mM). This last result contrasts with those of Salgueiro-Pagadigorria et al. (1996a), who reported a piroxicam dose-dependent inhibition on gluconeogenesis (33%–80%) in isolated perfused rat liver, though this discrepancy can be explained by the greater concentrations of the NSAID assayed in the latter study (0.2–1 mM). Indeed, data in Fig. 1 show a not statistically significant 12% decrease in glucose production with 0.1 mM piroxicam alone, figure is in accordance with the 33% decrease observed by Salgueiro-Pagadigorria et al. (1996a) with 0.2 mM piroxicam.

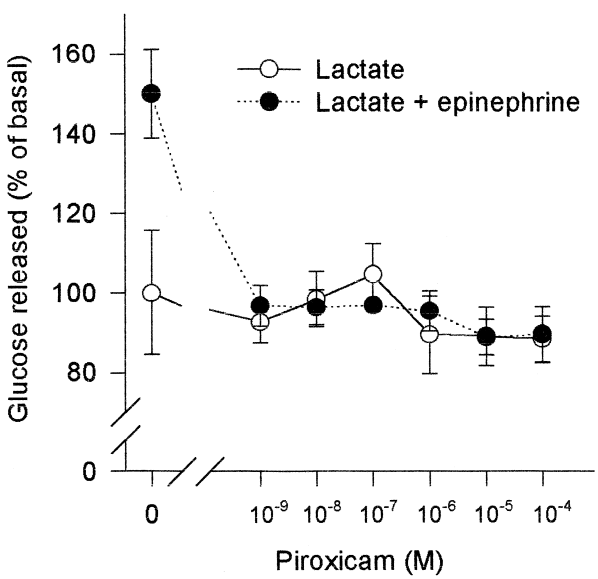

Fig. 1. Effect of different concentrations of piroxicam on glucose synthesis from lactate in rat hepatocytes incubated with 10^{-6} M epinephrine. The isolated cells were incubated as described in Section 2, using 50 mM sodium lactate (pH 7.4) as substrate; (●) in the presence of epinephrine and (○) in the absence of epinephrine. The basal synthesis of glucose, after 1 h of incubation, was 125 ± 20 nmol glucose formed/h per mg of protein. Data expressed as mean ± S.E.M. ($n = 6$–10).

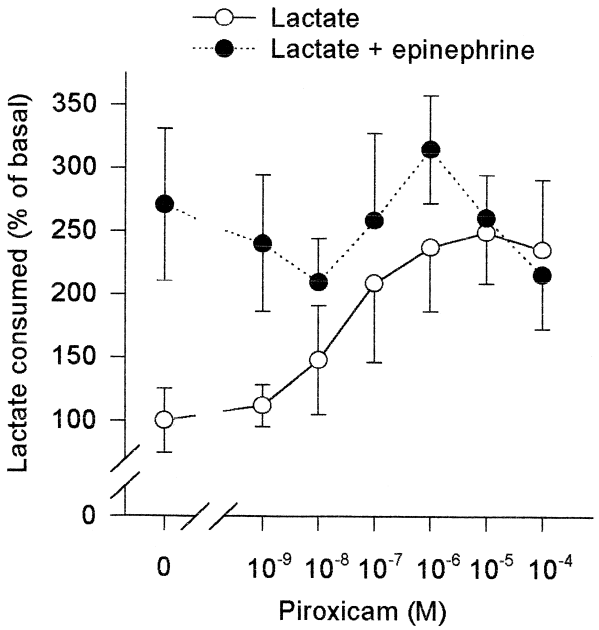

Fig. 2. Effect of different concentrations of piroxicam and 10^{-6} M epinephrine on lactate consumption in isolated rat hepatocytes. The cells were incubated 1 h as described in Section 2 with 50 mM sodium lactate as substrate; (●) in the presence of epinephrine and (○) in the absence of epinephrine. Each point represents the mean ± S.E.M. ($n = 5$–9). The basal lactate consumption was 139 ± 35 nmol/h per mg of protein.

In contrast, the stimulation on lactate consumption induced by epinephrine in isolated rat hepatocytes was not modified by the presence of piroxicam (Fig. 2); however, in this case, piroxicam alone rises the lactate consumption in a dose-dependent basis. This last effect is statistically significant ($P < 0.05$) from 10^{-6} M piroxicam concentration with a maximum response (+150%) at 10^{-5} M. Furthermore, the stimulatory effect of epinephrine and piroxicam on lactate consumption were not additive, suggesting similar sites of action.

The exposure to piroxicam alone increases, on a dose-dependent basis, the content of triacylglycerides in isolated rat hepatocytes incubated for 60 min with 50 mM lactate (Fig. 3). The highest stimulation (51%) was observed in the presence of 10^{-4} M piroxicam, but the effect was statistically significant from 10^{-5} M piroxicam ($P < 0.05$). This finding agrees with previous results from our laboratory, with an in vivo system, where rats treated for 4 to 8 h with piroxicam (10 mg/kg body weight) exhibited a 15% to 25% increase in liver triacylglycerides content, but without statistical significance (Zentella de Piña et al., 1992).

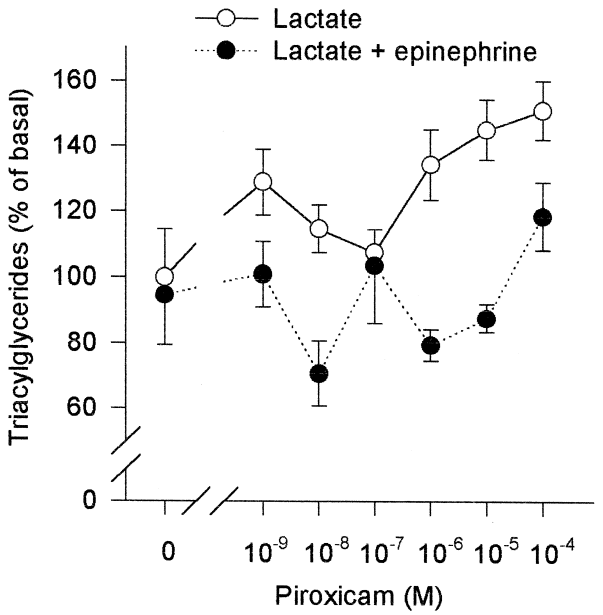

Fig. 3. Effect of different concentrations of piroxicam and 10^{-6} M epinephrine on triacylglycerides content in isolated rat hepatocytes. The cells were incubated 1 h as described in Section 2 using 50 mM sodium lactate as substrate; (●) in the presence of epinephrine and (○) in the absence of epinephrine. Each point represents the mean ± S.E.M. ($n = 5$–7). The basal triacylglycerides content after 1 h of incubation was 29.4 ± 4.3 μg triacylglycerides/mg of protein.

Otherwise, the triacylglycerides content of the isolated rat hepatocytes was not modified by 1-h incubation with 10^{-6} M epinephrine, using 50 mM lactate as substrate (Fig. 3). This result agrees with previous reports indicating that epinephrine does not affect cellular triacylglycerides content in liver (Brindle and Ontko, 1986). However, it can be observed that the piroxicam effect on triacylglycerides content was attenuated by epinephrine, showing again a mutual antagonism in the effect of both compounds.

In isolated rat hepatocytes, piroxicam stimulates lactate consumption (Fig. 2), keeping constant glucose production (Fig. 1), but increasing triacylglycerides content (Fig. 3). Taken together, these three results reveal that in this system a proportion of the lactate consumed after stimulation with the NSAID is used in the triacylglycerides synthesis. In order to obtain an integrated view of the piroxicam effects on isolated rat hepatocytes, we decided to elaborate a three-dimensional plot with lactate consumption, glucose release and triacylglycerides content data (see Fig. 4). Assuming a linear relationship between these three metabolic responses the next equation was proposed:

$$[\text{triacylglycerides content}] = m_1 \times [\text{lactate consumption}] + m_2 \times [\text{glucose release}] + c. \quad (1)$$

The adjusted constant and their standard deviations fitted by least squares are:

$m_1 = +0.093 \pm 0.048$ (percent triacylglycerides content/percent lactate consumption)

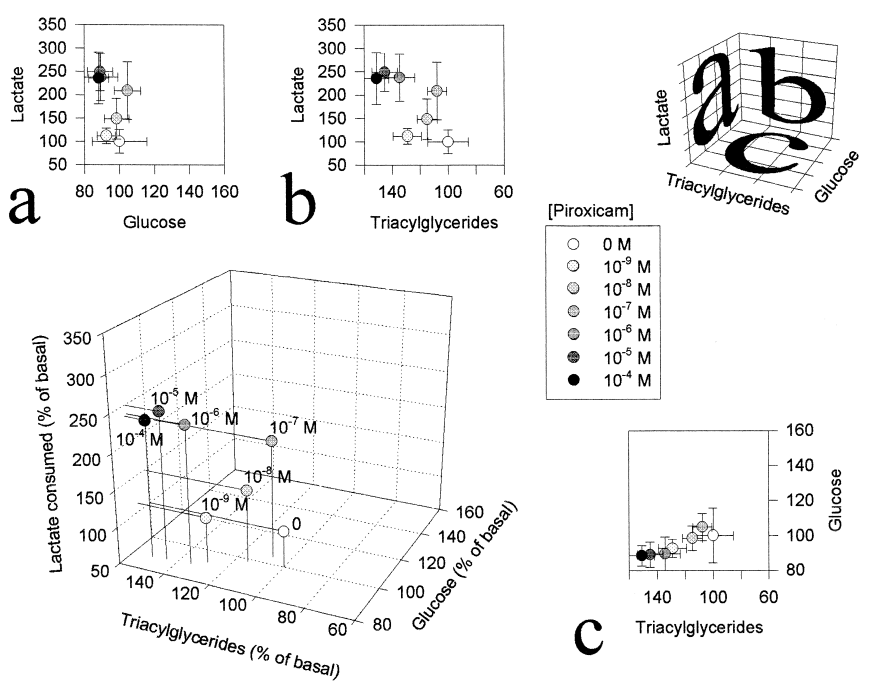

Fig. 4. Three-dimensional plot of (10^{-9}–10^{-4} M) piroxicam effect on glucose production, lactate consumption and triacylglycerides content in isolated rat hepatocytes. The inserted bidimensional plots (a–c) show lateral views of the three-dimensional plot. Each point represents the mean, with the S.E.M. bar showed in the bidimensional plots. A guide to read the bidimensional projections is included at the upper right. Piroxicam concentrations are shown with a gray scale: data without piroxicam are showed in white, and with piroxicam in different gray tones; the coordinates origin in the three-dimensional plot is located at the front in relation to the viewer. Figs. 4 and 5 maintain the same scales for easier comparisons.

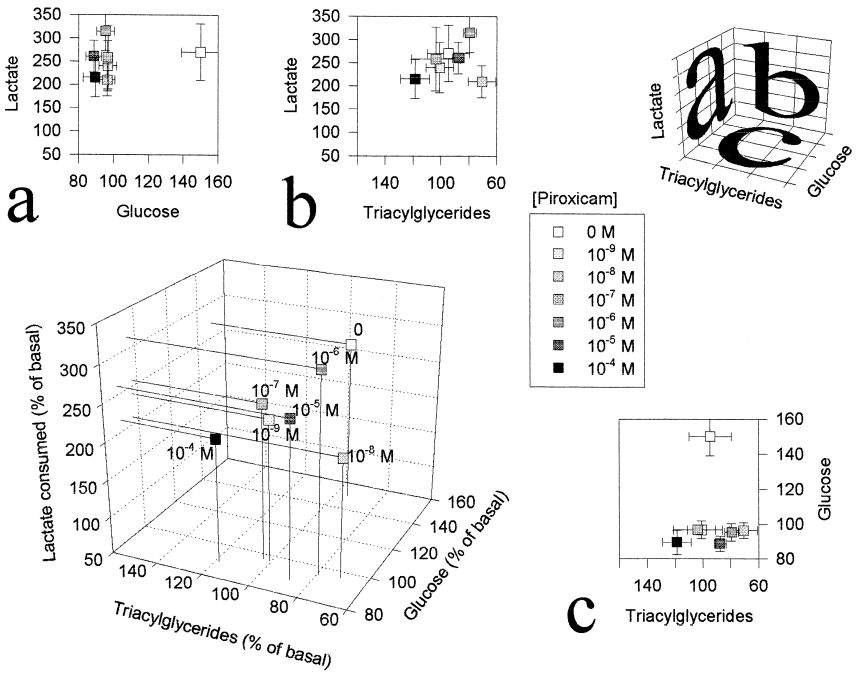

Fig. 5. Three-dimensional plot of 10^{-6} M epinephrine plus (10^{-9}–10^{-4} M) piroxicam effect on glucose release, lactate consumption and triacylglycerides content in isolated rat hepatocytes. The inserted bidimensional plots (a–c) show lateral views of the three-dimensional plot. Data are presented as in Fig. 4, and maintain the same scales for easier comparisons.

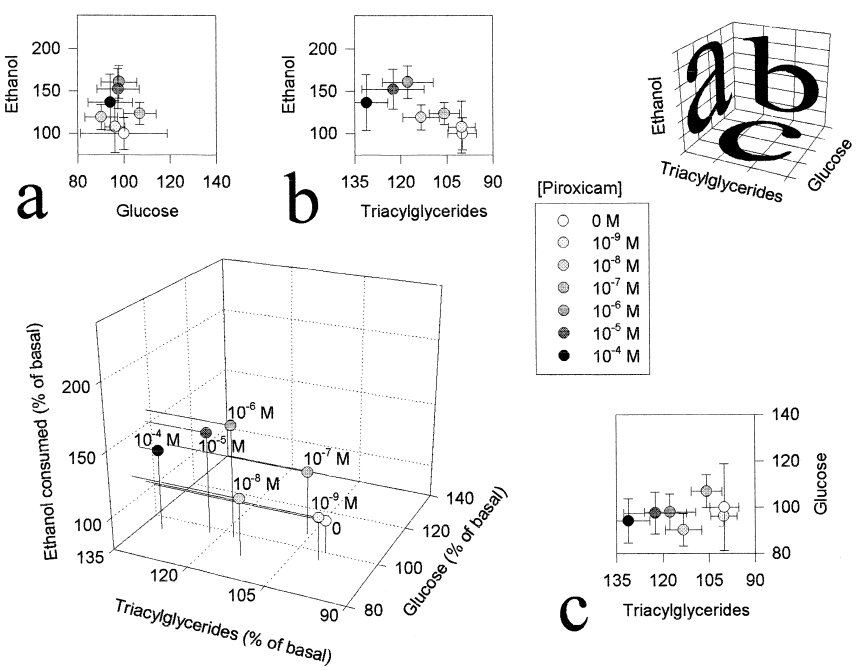

Fig. 6. Three-dimensional plot of (10^{-9}–10^{-4} M) piroxicam effect on glucose release, ethanol consumption and triacylglycerides content in isolated rat hepatocytes. Cells were incubated as described in Section 2 replacing lactate by 50 mM ethanol as substrate. The inserted bidimensional plots (a–c) show lateral views of the three-dimensional plot. Each point represents the mean, with the S.E.M. bar showed in the bidimensional plots. Data are presented as in Fig. 4. The basal activities of untreated hepatocytes after 1-h incubation were as follows: ethanol consumption: 228 ± 43 nmol/h per mg of protein; ($n = 4$–6); triacylglycerides content: 29.4 ± 1.4 μg triacylglycerides/mg of protein ($n = 6$–9); glucose release: 108 ± 20 nmol glucose/h per mg of protein ($n = 5$–9). Figs. 6 and 7 maintain the same scales for easier comparisons.

$m_2 = -2.32 \pm 0.47$ (percent triacylglycerides content/percent glucose release)

$c = 328.5 \pm 49.6$ (percent triacylglycerides content)

and the obtained regression coefficient (r^2) is equal to 0.923 ($P < 0.01$).

The results show that an increase in triacylglycerides content is followed by a parallel activation in lactate consumption and a decrease in glucose release, reinforcing the suggestion that under the experimental conditions described, at least a proportion of consumed lactate is channeled to the synthesis of triacylglycerides.

On the other hand, the uptake of lactate is stimulated by epinephrine and it is used for glucose synthesis (see white square in Fig. 5); when piroxicam is added to this system with epinephrine, the uptake of lactate remains elevated but it is not channelled toward glucose synthesis (see gray squares in Fig. 5), neither toward triacylglycerides synthesis, since the stimulation in triacylglycerides content promoted by piroxicam is blocked by epinephrine; therefore, the stimulation of an alternative metabolic pathway must be considered. This last possibility is supported by the lost of correlation between triacylglycerides content, lactate consumption and glucose release observed with the simultaneous presence of epinephrine and piroxicam. Thus, the regression coefficient for fitting these last data to Eq. (1) is $r^2 = 0.07$, which strengthens the point that under the experimental conditions described, another metabolic fate of lactate should be enhanced, for instance, liver respiration. Furthermore, epinephrine promotes mitochondrial oxygen consumption coupled to ATP synthesis (Taylor et al., 1986; Breton et al., 1987; Korzeniewski and Froncisz, 1992; Garcia et al., 1997).

Substitution of lactate for a distinct substrate, namely ethanol, might give further insight in the antagonism between epinephrine and the NSAID, as well as in the hepatic metabolic route(s) favored by piroxicam administration. Ethanol was selected because in a previous report from our laboratory it was showed that piroxicam alone stimulates ethanol consumption and modify triacylglycerides content in isolated rat hepatocytes (Saldaña-Balmori et al., 1996). In addition, ethanol elevates fatty acids synthesis (Bremer and Osmundsen, 1984; Brindley, 1988; Yang et al., 1996; Yu and Cronholm, 1997), but inhibits gluconeogenesis (Krebs et al., 1969; Sugano et al., 1980). Figs. 6 and 7 show a three-dimensional plots with ethanol consumption, glucose release and triacylglycerides content data in the presence or absence of piroxicam and epinephrine. In this integrated plot can be observed that piroxicam alone (Fig. 6) activates the ethanol consumption, the maximum effect (+60%) is observed with 10^{-6} M piroxicam ($P < 0.05$). Additionally, piroxicam, in a dose-dependent basis, augments triacylglycerides content in hepatocytes with a maximum effect at 10^{-4} M piroxicam (+31%, $P < 0.002$). The stimulation on ethanol consumption is parallel to the increase of triacylglycerides content in hepatocytes, although the glucose release is not modified by piroxicam. Adjusting this data to Eq. (1), the obtained results are:

$m_1 = +0.477 \pm 0.103$ (percent triacylglycerides content/percent ethanol consumption)

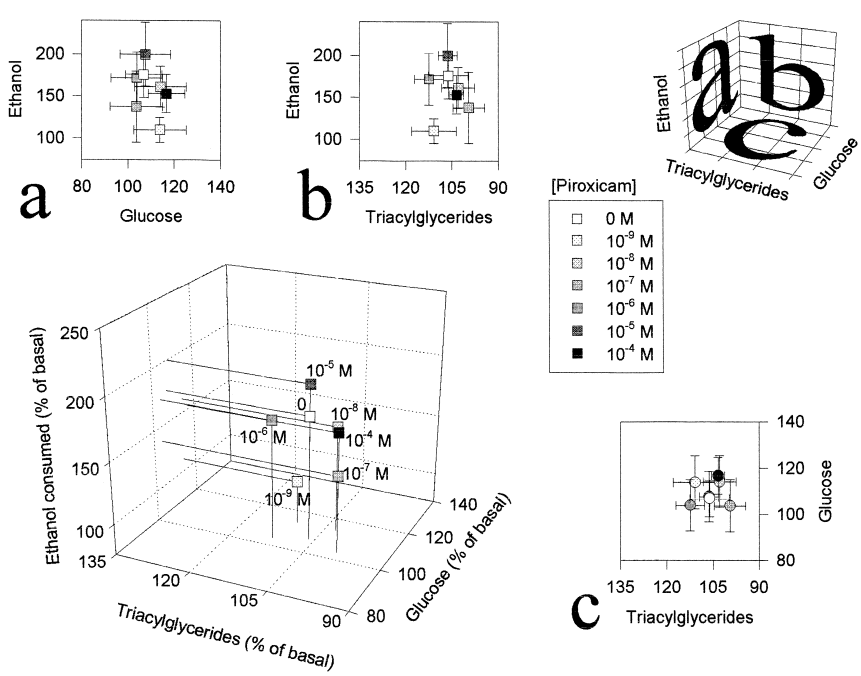

Fig. 7. Three-dimensional plot of 10^{-6} M epinephrine plus (10^{-9}–10^{-4} M) piroxicam effect on glucose release, ethanol consumption and triacylglycerides content in isolated rat hepatocytes. Cells were incubated as described in Section 2 replacing lactate by 50 mM ethanol as substrate. The inserted bidimensional plots (a–c) show lateral views of the three-dimensional plot. Data are presented as in Fig. 4. Figs. 6 and 7 maintain the same scales for easier comparisons.

$m_2 = -0.73 \pm 0.49$ (percent triacylglycerides content/ percent glucose release)

$c = 124.1 \pm 50.7$ (percent triacylglycerides content)

and $r^2 = 0.838$ ($P < 0.02$)

Epinephrine alone stimulates ethanol consumption 75% ($P < 0.05$), without any rise in the content of triacylglycerides or in glucose release. Simultaneous presence of epinephrine and piroxicam keep ethanol consumption elevated but the increase in triacylglycerides content, observed with piroxicam alone, is abolished. Once again the antagonism between both compounds is manifest. The regression coefficient for fitting these last data to Eq. (1) in the presence of epinephrine and piroxicam is $r^2 = 0.01$, this figure is similar to the value obtained with lactate as substrate under similar experimental conditions. In the same way as it was proposed for lactate, an enhanced metabolic pathway leading to ethanol oxidation seems to prevail in hepatocytes incubated with the hormone and the NSAID.

The mechanism by which piroxicam stimulates oxidation of lactate or ethanol is probably the same because their consumption increase in a linear way for both substrates, with each added doses of piroxicam (correlation coefficient, $r = 0.918$). Therefore, the effect of the NSAID on mitochondrial oxidation was tested. Fig. 8 shows the effect of piroxicam on oxygen consumption in isolated rat liver mitochondria, in the presence (state 3) or absence (state 4) of added ADP. Piroxicam, assayed at doses comparable to those detected in plasma of patients treated with piroxicam (20 mg daily, (Brogden et al., 1984; Rugstad, 1986)), rises significantly, in a dose-dependent form, the oxygen consumption in resting mitochondria (state 4); whereas in ADP-stimulated mitochondria (state 3), the oxygen consumption remains unchanged. These results confirm an uncoupler activity of piroxicam on oxidative phosphorylation which was previously reported, but using higher concentrations of the anti-inflammatory compound (Mingatto et al., 1996; Salgueiro-Pagadigorria et al., 1996b). Therefore, the mechanism by which piroxicam stimulates oxidation of lactate or ethanol is probably due to a general effect of the NSAID uncoupling oxidative phosphorylation and promoting the oxidation of substrates in mitochondria. This interpretation makes sense with the described linear increase in lactate and ethanol consumption in the presence of different doses of piroxicam.

The antagonism of epinephrine on the stimulative action of piroxicam to elevate the hepatic content of triacylglycerides is probably related to the common ability of both compounds to activate oxygen consumption in mitochondria. In this way, epinephrine in the absence of piroxicam stimulates mitochondria to provide the ATP necessary to sustain gluconeogenesis; but the addition of piroxicam cannot overstimulate mitochondrial activity and therefore the effect of piroxicam increasing the synthesis of triacylglycerides is not observed. Since several other NSAIDs posses the ability to stimulate oxygen consumption in

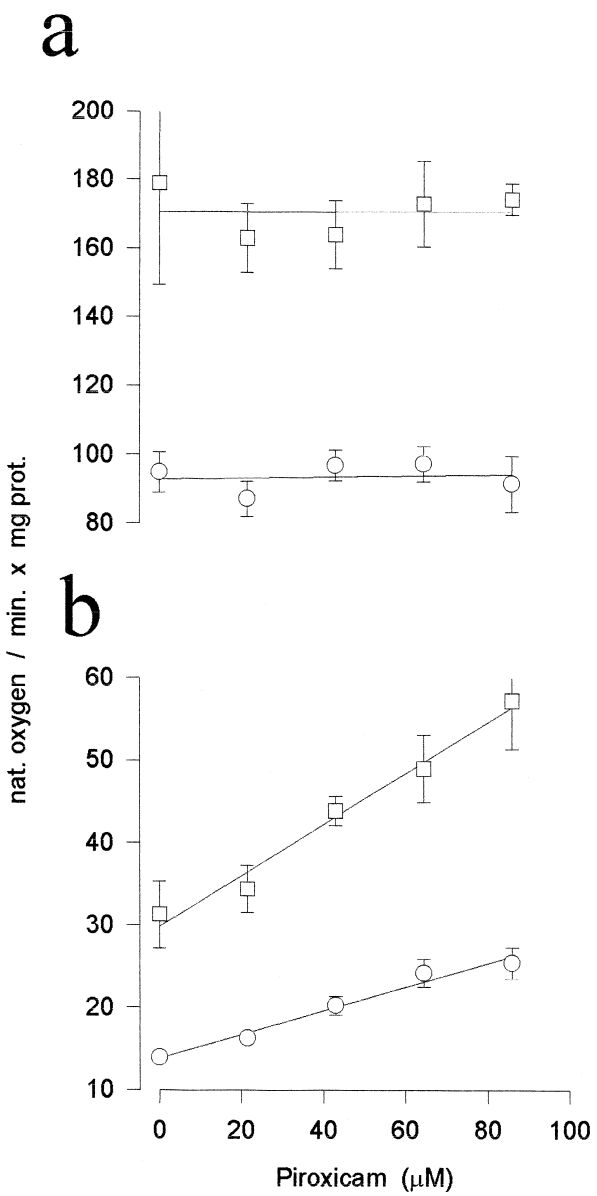

Fig. 8. Effect of piroxicam on oxygen consumption by isolated rat liver mitochondria. Mitochondria were isolated as described in Section 2. Mitochondria (2 mg protein) were added to an incubation mixture containing 260 mM mannitol, 3.3 mM phosphate-tris, 2.6 mM $MgCl_2$, 3.3 mM KCl, 0.44 mM EGTA, and 4.3 mM TES, pH 7.4; in addition, 6.6 mM succinate or 3.3 mM glutamate plus 3.3 mM malate, were added as substrates. Respiration was stimulated with 410 nmol ADP (state 3). (a) ADP-stimulated oxygen consumption (state 3) with succinate (□), or glutamate plus malate (○) as substrates. (b) Resting oxygen consumption (state 4) with succinate (□), or glutamate plus malate (○) as substrates. Each point represents the mean ± S.E.M. ($n = 4$).

mitochondria (Van den Berg and Nauta, 1975; Tokumitsu et al., 1977; McDougall et al., 1983; Baños and Reyes, 1989; Mingatto et al., 1996; Petrescu and Tarba, 1997), this potential mechanism of antagonism could be a more general property of NSAIDs, not restricted to piroxicam alone.

Because piroxicam resembles an uncoupler of oxidative phosphorylation in mitochondria, its effect at the μmolar

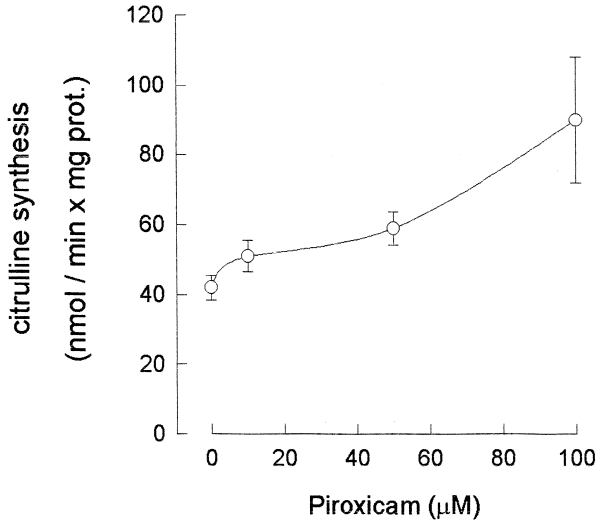

Fig. 9. Effect of piroxicam on citrulline synthesis in isolated rat liver mitochondria. Mitochondria (4.5 mg protein) were isolated as described in Section 2 and incubated with: 5 mM KH_2PO_4, 15 mM KCl, 3 mM $MgCl_2$, 16 mM $KHCO_3$, 1 mM EDTA, 10 mM succinate, 10 mM L-ornithine, 10 mM NH_4Cl, 4 μg rotenone, and 75 mM Tris in 2 ml final volume. Each point represents the mean ± S.E.M. ($n = 4$).

range, impairing the ATP-dependent function in mitochondria was explored. In this way, the effect of piroxicam on citrullinogenesis was tested. This activity was selected because comprises an indispensable step in the urea cycle and is one of the most important ATP-dependent function in liver mitochondria. Indeed, carbamyl phosphate synthetase I, responsible of citrullinogenesis, is the most abundant enzyme in liver mitochondria (Raijman and Jones, 1976). Fig. 9 shows the effect of piroxicam on citrullinogenesis in isolated rat liver mitochondria. Piroxicam, in the μmolar range, increases citrulline synthesis on isolated rat liver mitochondria. This last effect shows that the mild uncoupler effect of piroxicam described above do not impede this important mitochondrial ATP-dependent function in the liver. Furthermore, the increase in triacylglycerides content induced by piroxicam is observed also at this μmolar range, suggesting that this energy-dependent stimulation is related to the effects of piroxicam on mitochondria.

4. Discussion

In more integrated experimental models, other NSAIDs showed also an antagonism with the activation of metabolic pathways by catecholamines. Thus, indomethacin and salicylate decrease epinephrine- and glucagon-induced glycogenolysis in vivo (Wheeler and Epand, 1975; Ganguli et al., 1979; Miller et al., 1983, 1985), and naproxen inhibits glycogenolysis stimulated by norepinephrine in perfused rat liver (Nascimento et al., 1995). However, an explanation of this antagonism becomes particularly difficult in the in vivo experiments since NSAIDs modify the plasma levels of hormones, such as insulin, glucagon and epinephrine (Bybee et al., 1978; Metz and Robertson, 1980). Hence, the use of isolated hepatocytes seems to be the suitable model to explore, in deep, the antagonism of epinephrine and piroxicam. Such antagonism probably is unrelated to the ability of NSAIDs to inhibit prostaglandin synthesis because prostaglandins produce a similar effect: inhibit hepatic glucagon- and epinephrine-stimulated glycogenolysis in isolated rat hepatocytes (Wheeler and Epand, 1975; Brass and Garrity, 1985; Brass et al., 1987; Brass and Garrity, 1990; Okumura and Saito, 1990; Okumura et al., 1988a,b).

It is important to considerate that patients treated with piroxicam (20 mg daily) usually show steady-state plasma concentrations in the range from 13 to 40 μM (Brogden et al., 1984; Rugstad, 1986). Therefore, the piroxicam effects described in this paper are medically relevant since they are statistically significant at the therapeutical concentration range for piroxicam or even below this range.

Interestingly, other effects of different NSAIDs at nanomolar concentrations, quite below their therapeutical range, have been reported previously: the median inhibitory concentration (IC_{50}) values for cyclooxygenase-2 activity in intact cells are 3 nM for diclofenac (Pilbeam et al., 1997) and 24 nM for piroxicam (Frölich, 1997); the IC_{50} values for cyclooxygenase-1 are 1 nM for flunixin and flurbiprofen (Riendeau et al., 1997); the IC_{50} values for the potentiation of morphine inhibition of γ-aminobutyric acid-dependent synaptic transmission in rat neurons are 6 nM for indomethacin, and 57 nM for piroxicam (Vaughan, 1998). Inclusive, Raisz et al. (1989) pointed out that piroxicam at very low concentrations (10^{-10} M) paradoxically elevates prostaglandin production in cultured rat calvaria. In addition, the reported inhibitory action of piroxicam on glucose release induced by epinephrine is statistically significant from 10^{-9} M (Fig. 1).

In conclusion, the antagonism of piroxicam on epinephrine-induced glucose production is observed at nmolar range, whereas piroxicam effects on mitochondrial functions are significative only at μmolar range. Thereof, the nmolar action of the NSAID might interfere with the transduction system switched on by the hormone, and this nanomolar action seems to be independent of the μmolar effects of piroxicam on mitochondrial functions.

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