

## Comparison of the Effects of Bupivacaine and Ropivacaine on Heart Cell Mitochondrial Bioenergetics

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**Background:** High lipophilic local anesthetics interfere with mitochondrial energy metabolism. These metabolic effects could in part explain some of the toxic effects of local anesthetics, such as bupivacaine-induced myocardial depression. The aim of this study was to compare the bioenergetic effects of the local anesthetics bupivacaine and ropivacaine.

**Methods:** The effects of both local anesthetics on mitochondrial energy metabolism were studied in rat heart isolated mitochondria and in saponin-skinned left ventricle fibers. Oxygen consumption, adenosine triphosphate synthesis, and enzymatic activities of the complexes of the respiratory chain were measured.

**Results:** Bupivacaine and ropivacaine acted, in isolated mitochondria, as uncouplers between oxygen consumption and phosphorylation of adenosine diphosphate. Further, an inhibitory effect of mitochondrial respiration was evidenced with both anesthetics during maximal respiration and was assigned to a direct inhibition of complex I of the respiratory chain. Mitochondrial adenosine triphosphate synthesis was decreased by both mechanisms. However, both in isolated mitochondria and in permeabilized heart fibers, ropivacaine was less potent than bupivacaine. Adenosine triphosphate synthesis was completely suppressed at 3 mM (~0.1%) bupivacaine, whereas 3 mM ropivacaine induced only about a 40% inhibition.

**Conclusions:** Ropivacaine disturbs mitochondrial energy metabolism less than bupivacaine does. The lower lipid solubility of ropivacaine may be responsible for the lesser dose-

dependent effects of this drug on mitochondrial bioenergetics. (Key words: Local anesthetics; myocardium; skinned heart fibers; oxidative phosphorylation.)

MITOCHONDRIA are a potential site of action of general and local anesthetics (LA). Inhaled<sup>1</sup> and intravenous anesthetics such as propofol<sup>2</sup> have been reported to affect different mitochondrial functions (oxidative phosphorylation, calcium accumulation). Several studies have focused on the mitochondrial effects of LA. High lipophilic LA, such as bupivacaine, impair energy metabolism in mitochondria<sup>3-9</sup> or in the cell.<sup>10,11</sup> Such effects could be associated with certain toxic effects of LA. Bupivacaine-induced myocardial depression is still not well known, but some studies suggest that the cardiotoxicity of LA may be the result of the interference with mitochondrial energy transduction.<sup>12,13</sup> Further, the nerve toxicity observed with high lidocaine concentrations could be explained in part by an energy failure of the cell.<sup>14,15</sup>

The mitochondria provide energy in the form of adenosine triphosphate (ATP) for essential cell functions. The mitochondrial respiratory chain is divided into four enzymatic complexes, catalyzing oxidoreduction reactions leading to the oxidation of respiratory substrates (e.g., pyruvate, glutamate, succinate) and to the reduction of oxygen to water (fig. 1). The transfer of electrons along the respiratory chain generates a proton gradient across the inner mitochondrial membrane that is used for ATP synthesis (chemiosmotic hypothesis). Two mechanisms have been described for the interaction of LA with mitochondrial metabolism: (1) an uncoupling effect between oxygen consumption and ATP synthesis by increasing proton membrane permeability, thus dissipating the proton gradient<sup>5-9</sup>; and (2) a direct inhibitory effect of LA (at higher concentrations) on mitochondrial enzyme complexes.<sup>4</sup> All these effects lead to a decrease in ATP synthesis and to a depletion in the ATP content of the cell.<sup>11</sup>

Ropivacaine, the N-propyl homolog of bupivacaine

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## MITOCHONDRIAL EFFECTS OF LOCAL ANESTHETICS

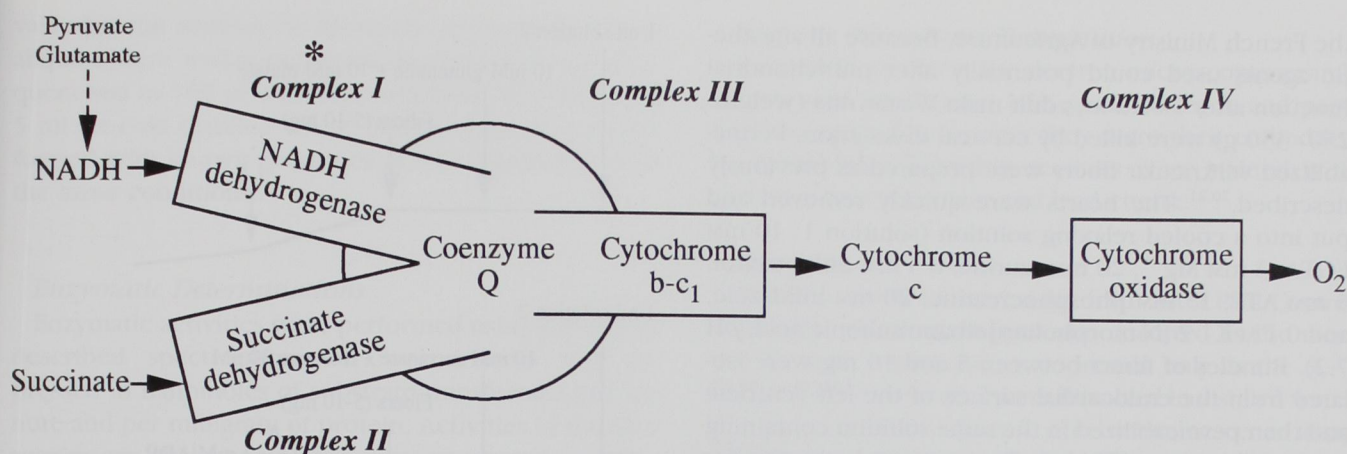


Fig. 1. The mitochondrial respiratory chain. \*Potential site for the action of anesthetics.

(fig. 2), is a new LA now available for clinical use.<sup>16</sup> Unlike bupivacaine, which is prepared and used as a racemic solution, ropivacaine is prepared as a single enantiomer S(-). Its lipid solubility lies between that of lidocaine and bupivacaine.<sup>17</sup> Ropivacaine appears to be less toxic than bupivacaine.<sup>18</sup> However, when the plasma concentration is increased, it produces a toxicity profile similar to that of other LAs, including convulsions and hypotension.<sup>19</sup> The current study was done to define the effects of ropivacaine on mitochondrial bioenergetics and to compare these results with those

observed with bupivacaine. Mitochondrial functions were investigated in isolated rat heart mitochondria and in saponin-permeabilized ventricular fibers.

## Materials and Methods

### Preparation of Saponin-permeabilized Heart Fibers

Care of the animals conformed to the recommendations of the Institutional Animal Care Committee and

|             |  | Molecular Weight | Distribution Coefficient |
|-------------|--|------------------|--------------------------|
| Bupivacaine |  | 288              | 346                      |
| Ropivacaine |  | 274              | 115                      |
| Lidocaine   |  | 234              | 43                       |

Fig. 2. The structure and physicochemical properties of bupivacaine, ropivacaine, and lidocaine. Distribution coefficients were from Strichartz *et al.*<sup>17</sup> \*Asymmetric carbon.



the French Ministry of Agriculture. Because all anesthetic agents used could potentially alter mitochondrial function after isolation, adult male Wistar rats (weight, 250–350 g) were killed by cervical dislocation. Permeabilized ventricular fibers were prepared as previously described.<sup>20,21</sup> The hearts were quickly removed and put into a cooled relaxing solution (solution 1: 10 mM EGTA, 3 mM  $Mg^{2+}$ , 20 mM taurine, 0.5 mM dithiotreitol, 5 mM ATP, 15 mM phosphocreatine, 20 mM imidazole, and 0.1 M  $K^+$  2-[N-morpholino]ethane sulfonic acid, pH 7.2). Bundles of fibers between 5 and 10 mg were isolated from the endocardial surface of the left ventricle and then permeabilized in the same solution containing 50  $\mu$ g/ml saponin. The bundles were washed twice for 10 min each time in solution 2 (10 mM EGTA, 3 mM  $Mg^{2+}$ , 20 mM taurine, 0.5 mM dithiotreitol, 3 mM phosphate, 1 mg/ml fatty-acid-free bovine serum albumin, 20 mM imidazole, and 0.1 M  $K^+$  2-[N-morpholino] ethane sulfonic acid, pH 7.2) to remove saponin. All procedures were done at 4°C with extensive stirring.

The extent of the permeabilization was estimated by determining the activities of the cytosolic lactate dehydrogenase and the mitochondrial citrate synthase in the medium.

#### Mitochondrial Isolation

Rat heart mitochondria were isolated by differential centrifugation as previously described.<sup>8</sup> The mitochondrial pellet was suspended in a medium containing 75 mM sucrose, 225 mM mannitol, 0.1 mM EDTA and 10 mM Tris-HCl, pH 7.2. The protein concentration of the mitochondrial suspension was measured using the Biuret method.

#### Respiration Assay

The oxygen consumption rate was measured polarographically at 30°C using a Clark-type electrode connected to a computer that gave an on-line display of rate values. Solubility of oxygen in the medium was considered to be equal to 450 nmol/l. For permeabilized fibers, respiratory rates were determined in a 1-ml oxygraph cuvette containing one bundle of fibers in solution 2 with 10 mM glutamate plus 10 mM malate, or 10 mM succinate as substrates. The procedure for testing LA on respiration of permeabilized fibers is described in figure 3. Results were expressed in nmol oxygen consumed per minute and per milligram dry weight of fiber.

For isolated mitochondria, the incubation medium contained 25 mM sucrose, 75 mM mannitol, 100 mM

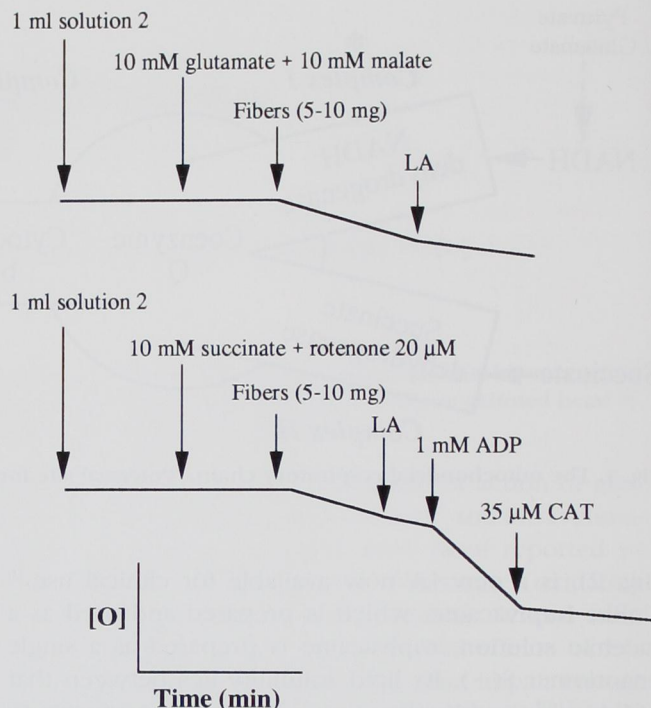


Fig. 3. Oxygen electrode traces showing oxygen uptake in permeabilized heart fibers. Rates were derived from the slope of the curve and expressed as nanomoles of oxygen per minute per milligram of dry weight. Local anesthetics were tested for basal respiration with 10 mM glutamate plus 10 mM malate as substrate and for phosphorylating respiration (*i.e.*, during ATP synthesis). Respiratory control was determined as the ratio of 1 mM adenosine diphosphate-stimulated respiration with 10 mM succinate as the substrate in the presence of rotenone (20  $\mu$ M) to nonphosphorylating respiration in the presence of 35  $\mu$ M carboxyatractyloside (CAT).

KCl, 10 mM Tris-phosphate, 50  $\mu$ M EDTA, 10 mM Tris-HCl, pH 7.2, with 10 mM pyruvate plus 10 mM malate as substrate. Respiration rates were expressed in nmol oxygen per minute and per milligram of mitochondrial protein.

Local anesthetics were added in the oxygraph chamber after equilibrium of the mitochondrial suspension (or permeabilized fibers) with the respiratory substrates. The steady-state action of LA was assumed when the oxygen consumption rate was constant.

#### Measurement of Adenosine Triphosphate Synthesis

Under the same conditions as in the respiration assay, the ATP synthesis rate of isolated mitochondria was determined by bioluminescence measurement (luciferine-luciferase system) of the ATP produced after adding 1 mM adenosine diphosphate.<sup>22</sup> At various time inter-



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vals after the addition of adenosine diphosphate, 20- $\mu$ l aliquots were withdrawn from the oxygraph chamber, quenched in 100  $\mu$ l dimethyl sulfoxide, and diluted in 5 ml ice-cold distilled water. Standardization was performed with known quantities of ATP measured under the same conditions.

#### Enzymatic Determinations

Enzymatic activities were performed using previously described spectrophotometric procedures and expressed in nanomoles of substrate transformed per minute and per milligram of protein. Activities of the four respiratory chain complexes were measured on mitochondria broken down by freeze thawing three times.

**Citrate Synthase.** The enzyme activity of citrate synthase was measured as described by Srere<sup>23</sup> in the presence of 4% Triton (vol/vol) by monitoring at 412 nm and 37°C the formation of thionitrobenzoate dianion from the reaction of coenzyme A and 5,5'-dithiobis(2-nitrobenzoic acid).

**Lactate Dehydrogenase.** The enzyme activity of lactate dehydrogenase was measured by monitoring the disappearance of NADH in the presence of pyruvate.<sup>24</sup>

**Complex I (Reduced Nicotinamide Adenine Dinucleotide Ubiquinone Reductase).** The oxidation of reduced nicotinamide adenine dinucleotide (NADH) by complex I was recorded using the ubiquinone analog decylubiquinone as the electron acceptor.<sup>25</sup> The decrease in absorption resulting from NADH oxidation was measured at 30°C and at 340 nm wavelength. Complex I activity was calculated from the difference in the rate before and after the addition of rotenone (2  $\mu$ M), a specific inhibitor of complex I.

**Complex II (Succinate Dehydrogenase).** Complex II specific activity was measured by monitoring the reduction of 2,6-dichlorophenol indophenol at 600 nm and 30°C, in the presence of phenazine methosulphate.<sup>26</sup>

**Complex III (Ubiquinol Cytochrome c Reductase).** The oxidation of ubiquinol (UQ<sub>1</sub>H<sub>2</sub>) by complex III was determined using cytochrome c(III) as the electron acceptor.<sup>25</sup> The reduction of cytochrome c(III) was recorded at 30°C and at 550 nm.

**Complex IV (Cytochrome c Oxidase).** Complex IV activity was measured by the method described by Wharton and Tzagoloff<sup>27</sup> using cytochrome c(II) as substrate. The oxidation of the cytochrome c was monitored at 550 nm at 30°C.

#### Electron Microscopic Examination

Permeabilized fibers for electron microscopic examination were fixed using 2.5% glutaraldehyde and post-fixed in osmium tetroxide; they were then embedded in epoxy resin. Ultrathin sections were stained with saturated uranyl acetate and lead citrate.

#### Chemicals

The HCl salts of LA were a gift from Astra Pain Control (Södertälje, Sweden). Bupivacaine occurred as the racemic mixture; ropivacaine was present only as the S(-) isomer. Local anesthetics were dissolved in distilled water at a concentration of 50 mM. The bioluminescence assay of ATP was performed with the ATP monitoring kit from Bio-Orbit (Turku, Finland). All the other chemicals were from Sigma Chemical Company (St. Louis, MO).

#### Data Analysis

Results were expressed as mean  $\pm$  SD. Data were plotted and analyzed using KaleidaGraph software (Synergy Software, Reading, PA). Concentration-response curves of complex I inhibition were fitted to an equation of the form  $y = \text{Min} + (\text{Max} - \text{Min}) / (1 + (C / \text{IC}_{50}))$ , where C is the drug concentration and  $\text{IC}_{50}$  is the half-inhibitory concentration. Values for  $\text{IC}_{50}$  were derived from this curve fitting and reported as mean  $\pm$  SEM. Statistical analysis was performed using analysis of variance and Student's unpaired *t* test when appropriate. Probability values  $<0.05$  were considered significant.

## Results

#### Effects of Local Anesthetics on Isolated Mitochondria

As previously reported,<sup>6</sup> bupivacaine strongly stimulated mitochondrial respiration, but lidocaine had no effect (fig. 4). Ropivacaine moderately increased respiration, and in the concentration range (0-5 mM), the maximal change in the respiration rate was two times higher with bupivacaine than with ropivacaine. Uncoupled respiration, in the presence of 0.5  $\mu$ M carbonylcyanide *m*-chlorophenylhydrazone, was inhibited by bupivacaine and ropivacaine (fig. 5). This effect could be explained by an impairment in electron transfer at the level of the enzymatic complexes of the respiratory chain. The inhibition was observed at a higher concentration of LA than for uncoupling, and bupivacaine appeared to be a more potent inhibitor than ropivacaine or lidocaine. Finally, both LA effects (uncoupling and



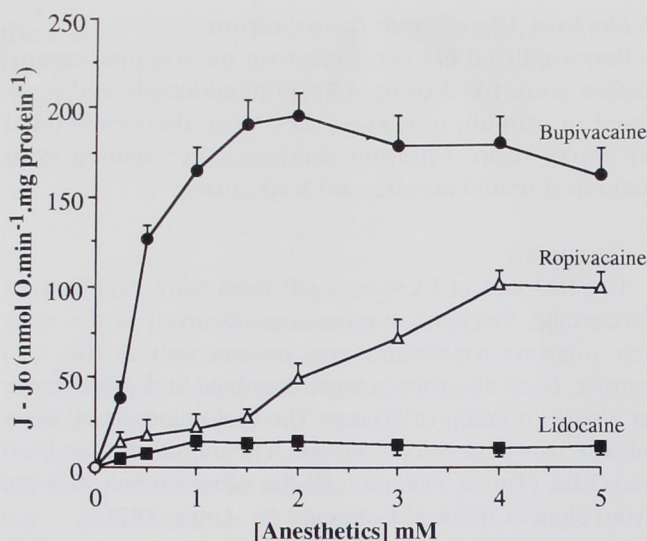


Fig. 4. Dose dependence of respiratory stimulation by local anesthetics. Stimulated oxygen consumption (with 10 mM pyruvate plus 10 mM malate),  $J - J_o$ , is plotted as a function of anesthetic concentration.  $J$  and  $J_o$  are rates of respiration ( $\text{nmol oxygen} \cdot \text{min}^{-1} \cdot \text{mg mitochondrial protein}^{-1}$ ) in the presence and absence of the drug, respectively.  $J_o$  was  $40\text{--}50 \text{ nmol oxygen} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$  with this assay medium. Values presented are from five experiments done with five mitochondrial preparations and are expressed as mean  $\pm$  SD.

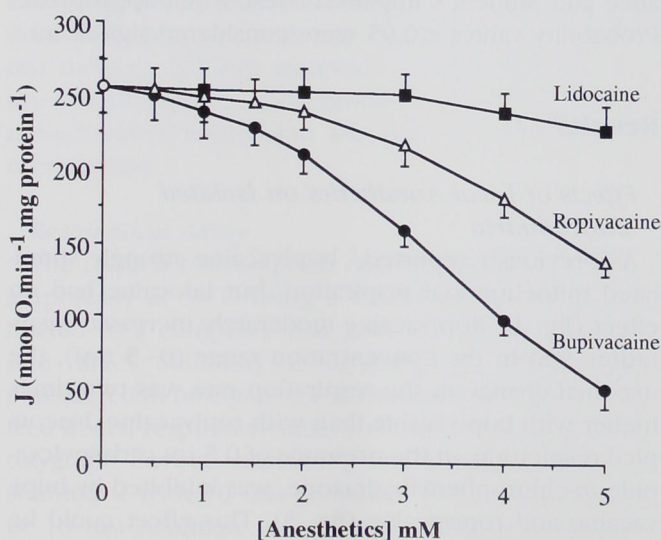


Fig. 5. Inhibition of uncoupled respiration by local anesthetics. The oxygen consumption rate,  $J$  ( $\text{nmol oxygen} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ ), after addition of carbonyl cyanide *m*-chlorophenylhydrazone ( $0.5 \mu\text{M}$ ), is plotted as a function of anesthetic concentration. Values presented are from five experiments done with five mitochondrial preparations and are expressed as mean  $\pm$  SD.

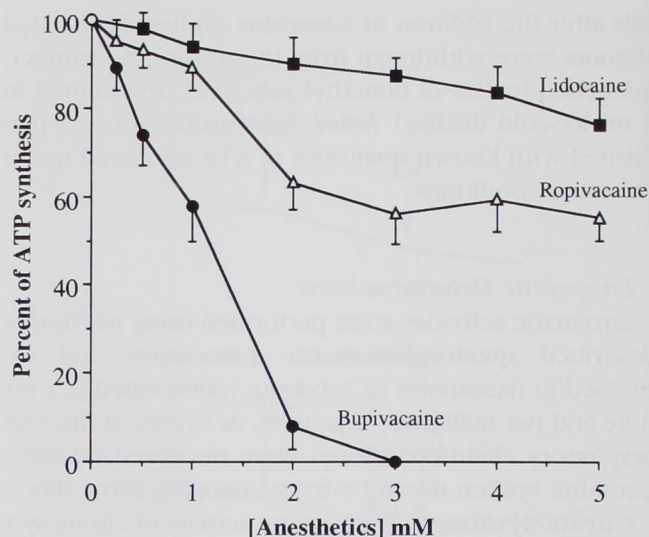


Fig. 6. Effects of local anesthetics on mitochondrial adenosine triphosphate (ATP) synthesis. ATP produced was measured by bioluminescence after addition of 1 mM adenosine diphosphate in the oxygraph cuvette. The ATP synthesis rate in the presence of local anesthetics is given as the percentage of the ATP production in the absence of local anesthetics. Data are presented as mean  $\pm$  SD ( $n = 5$ ).

inhibition of respiration) disturbed the oxidative phosphorylation and led to a decrease in ATP synthesis by mitochondria. Bupivacaine entirely inhibited ATP synthesis at 3 mM ( $\sim 0.1\%$ ), whereas at 5 mM ( $\sim 0.15\%$ ) of ropivacaine, an ATP synthesis was still observed (fig. 6). However, ropivacaine also decreased the ATP-to-oxygen ratio, showing that both LAs had the same type of effects on oxidative phosphorylation.

#### Effects of Local Anesthetics on Enzymatic Complexes of the Respiratory Chain

The effects of bupivacaine and ropivacaine on the respiratory chain were studied in the four separate enzymatic complexes of the inner mitochondrial membrane. Table 1 shows the effects of a high concentration (5 mM) of LAs on these complexes. Complex I (NADH ubiquinone reductase) was strongly inhibited by bupivacaine and ropivacaine ( $P < 0.05$ ). Complex II (succinate dehydrogenase) was significantly inhibited by both LAs ( $P < 0.05$ ), but only up to 20%; complexes III and IV were not affected at all. The inhibitory effect of LA on complex I was concentration-dependent (fig. 7). The  $IC_{50}$  for ropivacaine ( $0.36 \pm 0.03 \text{ mM}$ ) was not significantly different from that of bupivacaine ( $0.38 \pm 0.04 \text{ mM}$ ). These results indicate that both LAs had nearly



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**Table 1. Effects of Local Anesthetics on the Enzymatic Complexes of the Respiratory Chain**

|                  | Complex I | Complex II | Complex III | Complex IV  |
|------------------|-----------|------------|-------------|-------------|
| Control          | 358 ± 15  | 497 ± 26   | 1,346 ± 80  | 4,350 ± 335 |
| Bupivacaine 5 mM | 73 ± 8*   | 406 ± 18*  | 1,297 ± 78  | 4,109 ± 403 |
| Ropivacaine 5 mM | 85 ± 7*   | 424 ± 21*  | 1,410 ± 97  | 3,966 ± 372 |

Values are mean ± SD (n = 3). The activities of complexes are measured as described in "Materials and Methods" and are expressed as nmol substrate transformed · min<sup>-1</sup> · mg mitochondrial protein<sup>-1</sup>.

\*  $P < 0.05$  versus control.

the same effects on the activities of isolated enzymatic complexes after disruption of the mitochondrial membranes.

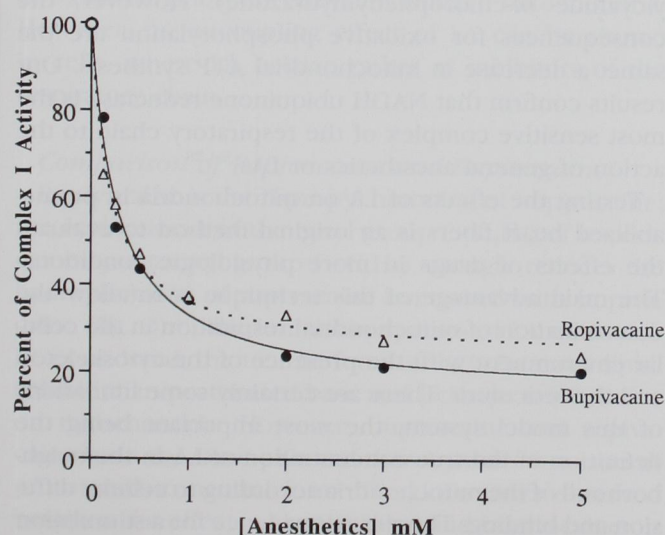
#### *Effects of Local Anesthetics on Mitochondria in Permeabilized Heart Fibers*

Skinned ventricular fibers were obtained by saponin treatment, which allows permeabilization of the sarcolemma and leaves mitochondria and sarcoplasmic reticulum intact. With this technique, it was possible to investigate the mitochondrial metabolism *in situ* inside the skeletal muscle or heart fibers.<sup>20,21</sup> After skinning, external added substrates drove mitochondrial respiration. The optimal incubation time with saponin determined by measuring enzyme release was 20 min. At this time of permeabilization, >60% of the cytosolic

lactate dehydrogenase was found in the external medium, and the mitochondrial citrate synthase activity in the medium remained <5% (data not shown). Further, electron microscopy showed that mitochondrial membranes remained intact after 20 min of permeabilization with saponin (fig. 8).

Whereas a classic uncoupler such as 2,4 dinitrophenol stimulated the respiration rate, bupivacaine and ropivacaine, but not lidocaine, decreased oxygen consumption in permeabilized fibers (table 2). With glutamate plus malate as the substrate, which supplies NADH to complex I, the respiration rate was significantly reduced by both LA, but the effect of bupivacaine at 5 mM (~0.15%) concentration was more potent than that of ropivacaine ( $P < 0.05$ ). On the other hand, LAs slightly decreased oxygen consumption when the respiratory substrate was succinate (*i.e.*, the electron donor to complex II).

As for isolated mitochondria, adenosine diphosphate stimulated oxygen uptake in permeabilized fibers. The respiratory control calculated as the ratio of adenosine diphosphate-stimulated respiration to nonphosphorylating respiration made it possible to evaluate the ability of mitochondria to synthesize ATP. Bupivacaine (1–5 mM) decreased the respiratory control more than ropivacaine ( $P < 0.05$ ). At 5 mM, only bupivacaine completely suppressed the adenosine diphosphate stimulation of oxygen consumption, as in the case of 2,4 dinitrophenol (table 2).



**Fig. 7.** Dose-dependent inhibition of complex I of the respiratory chain by bupivacaine and ropivacaine. Each point represents the average value from three measures. Curves are fitted as described in Materials and Methods section. The half-inhibitory concentrations are equal to  $0.38 \pm 0.04$  mM and  $0.36 \pm 0.03$  mM for bupivacaine and ropivacaine, respectively.

## Discussion

In the current study, we show that bupivacaine disturbs heart cell mitochondrial bioenergetics more significantly than ropivacaine. The dose-dependent effects of LA on oxidative phosphorylation are detailed and, for the first time, the metabolic effects of ropivacaine are compared with those of bupivacaine.



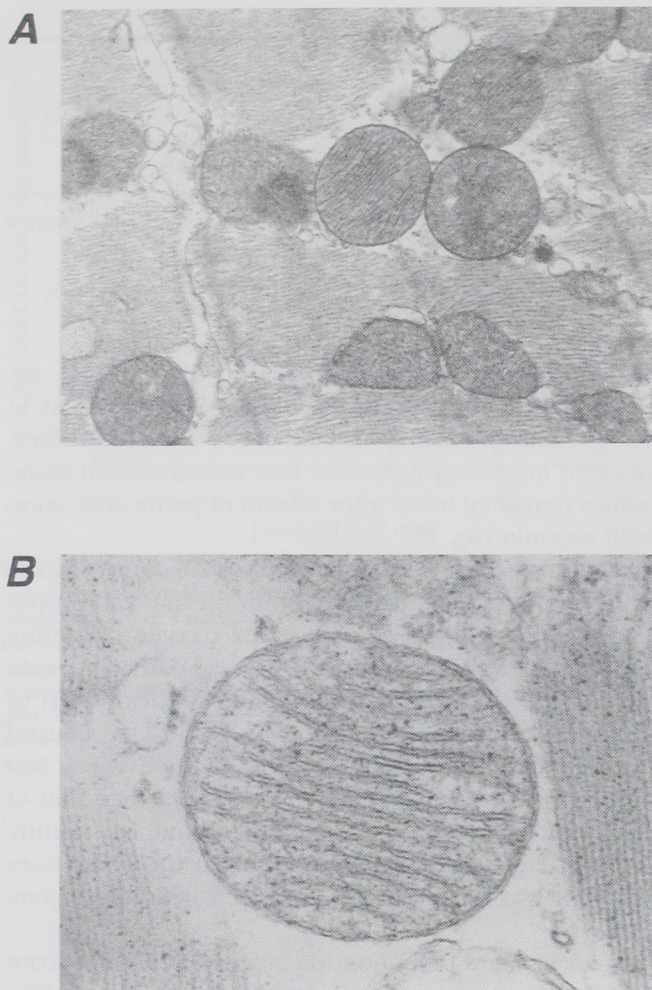


Fig. 8. Ultrastructural study of mitochondria in rat heart fibers after 20 min of permeabilization with saponin (50  $\mu\text{g}/\text{ml}$ ). (A) Magnification,  $\times 15,000$ . (B) Magnification,  $\times 50,000$ .

#### *Effects of Local Anesthetics on Mitochondrial Energy Metabolism*

Mitochondria seem to be one site of action of anesthetics. An earlier review<sup>28</sup> showed that general anesthetics such as barbiturate or halothane not only interfere with cellular respiration but disturb the mechanisms by which mitochondria synthesize ATP (oxidative phosphorylation). More recently, the effects of propofol on respiration in rat liver mitochondria were investigated.<sup>2</sup> Propofol has two main effects: inhibition of the respiratory chain at the level of complex I and uncoupling by modification of the ATPase complex. The potential effects of LA have prompted several studies. Dabadie *et al.*<sup>6</sup> were the first to show that bupivacaine strongly stimulates mitochondrial respiration, and they pro-

posed that bupivacaine acts as a proton carrier uncoupler. The precise mechanism of bupivacaine uncoupling is still being debated,<sup>8</sup> but Sun and Garlid<sup>9</sup> have confirmed that bupivacaine is a true protonophore.

In the current study, we found that bupivacaine and ropivacaine have, as in the case of propofol,<sup>2</sup> two main effects on heart mitochondria: (1) stimulation of oxygen uptake as previously reported, which could be explained by uncoupling of oxidative phosphorylation; and (2) inhibition of the respiratory chain, mainly at the level of complex I (NADH ubiquinone reductase). This apparent contradictory observation of the effects of LA on mitochondrial respiration (*i.e.*, stimulation by uncoupling *vs.* inhibition) has been, in part, previously reported.<sup>9</sup> An explanation could be the following: at state 4 respiration, the rate of respiration is low and membrane potential is high; such conditions could favor the uncoupling effect of LAs. On the other hand, during carbonylcyanide *m*-chlorophenylhydrazone-induced maximal respiration, the high oxygen flux could reveal the inhibitory effect of LAs, regardless of the protonophoretic effect, which could disappear at low membrane potential. Another hypothesis may be that bupivacaine interacts with carbonylcyanide *m*-chlorophenylhydrazone. Until now, no experimental arguments have elucidated this point. In any case, it is difficult to prove a protonophoretic effect of LA in the presence of an already added protonophore (carbonylcyanide *m*-chlorophenylhydrazone). However, the consequences for oxidative phosphorylation are the same: a decrease in mitochondrial ATP synthesis. Our results confirm that NADH ubiquinone reductase is the most sensitive complex of the respiratory chain to the action of general anesthetics or LAs.<sup>3,4,28</sup>

Testing the effects of LA on mitochondria in permeabilized heart fibers is an original method to evaluate the effects of drugs in more physiologic conditions. The main advantage of this technique is to allow the determination of mitochondrial respiration in the cellular environment with the presence of the cytoskeleton and the reticulum. There are certainly some limitations of this model system, the most important being the definition of the true concentration of LA in the neighborhood of the mitochondria according to cellular diffusion and binding. There is no evidence for a stimulation of oxygen consumption by LA, as in the case of a classic uncoupler such as 2,4 dinitrophenol. It appears that LA in skinned fibers acts only as an inhibitor of the respiratory chain. Respiration with glutamate is more decreased than that with succinate. These results may



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**Table 2. Effects of Local Anesthetics on Respiratory Parameters in Permeabilized Heart Fibers**

|                   | Respiration Rate<br>(glutamate + malate) | Respiration Rate<br>(succinate) | Respiratory Control<br>(succinate) |
|-------------------|--|---------------------------------|------------------------------------|
| Control           | 2.33 ± 0.25                              | 4.53 ± 0.52                     | 2.45 ± 0.14                        |
| 2,4 DNP, 7 mM     | 3.96 ± 0.34*                             | 7.72 ± 0.61*                    | 1.00 ± 0.01                        |
| Bupivacaine, 1 mM | 1.92 ± 0.21*                             | 4.08 ± 0.36                     | 1.54 ± 0.08*                       |
| Ropivacaine, 1 mM | 2.10 ± 0.23                              | 4.30 ± 0.31                     | 1.83 ± 0.09*†                      |
| Lidocaine, 1 mM   | 2.31 ± 0.22†                             | 4.48 ± 0.47                     | 2.37 ± 0.13†                       |
| Bupivacaine, 5 mM | 1.04 ± 0.15*                             | 3.62 ± 0.24*                    | 1.02 ± 0.02*                       |
| Ropivacaine, 5 mM | 1.51 ± 0.18*†                            | 3.85 ± 0.27*                    | 1.26 ± 0.07*†                      |
| Lidocaine, 5 mM   | 2.12 ± 0.24†                             | 4.17 ± 0.32†                    | 2.28 ± 0.11†                       |

2,4 DNP = 2,4 dinitrophenol (uncoupler).

Experimental conditions are described in "Material and Methods" and in figure 3. Respiration rate is expressed in  $\text{nmol O} \cdot \text{min}^{-1} \cdot \text{mg dry weight}^{-1}$ . Respiratory control was determined as the ratio of ADP-stimulated respiration to nonphosphorylating respiration. Values are mean ± SD from at least five experiments.

\*  $P < 0.05$  versus control.

†  $P < 0.05$  versus bupivacaine, at same concentrations.

be explained by the specific inhibition of complex I. However, the drastic decrease in respiratory control with succinate indicates that LA could disturb oxidative phosphorylation by other mechanisms, such as changes in ATP synthase complex or in mitochondrial membrane integrity. These observations must be compared with results of our previous work<sup>11</sup> that showed that bupivacaine decreases oxygen consumption in cells and the ATP content in cytosol and mitochondria. In summary, lipophilic LAs certainly appear to be uncouplers of oxidative phosphorylation in isolated mitochondria. However, no uncoupling effect of LA can be shown in cells, where LA behave rather as inhibitors of the respiratory chain.

#### Comparison of Ropivacaine and Bupivacaine

Ropivacaine is the N-propyl homolog of bupivacaine; the replacement of the butyl group ( $-\text{C}_4\text{H}_9$ ) of bupivacaine by a propyl group ( $-\text{C}_3\text{H}_7$ ) strongly reduces the hydrophobicity of ropivacaine compared with bupivacaine (fig. 2).<sup>17</sup> The ionization species of LA must also be considered; the neutral form of LA is far more lipid-soluble than protonated base and perhaps more active on mitochondria. However, bupivacaine and ropivacaine have the same pKa and thereby the same ratio of protonated to neutral form. The differences observed between the effects of ropivacaine and bupivacaine on mitochondria could be a result of the lower partition coefficient of ropivacaine. It has been shown that only high lipophilic LAs have significant effects on isolated mitochondria. As reported in liver mitochondria,<sup>6</sup> we have shown in the present study that lidocaine, which

has the lower partition coefficient, does not significantly change mitochondrial respiration and ATP synthesis. Local anesthetic partition coefficients correlate with anesthetic action potency *in vitro* and *in vivo*.<sup>17,29</sup> We hypothesize that partition coefficients also correlate with the metabolic effects of LA on mitochondria isolated from rat heart.

Further, ropivacaine is prepared as a single enantiomer S(−), whereas bupivacaine is used as a racemic solution. There is no evidence for a stereoselectivity of the effects of LA on mitochondria, and bupivacaine and ropivacaine have the same potency of inhibition on isolated complex I. Differences between both LA on the inhibition of mitochondrial respiration in permeabilized fibers could be explained by the higher ability than ropivacaine of the hydrophobic anesthetic bupivacaine to reach mitochondrial complexes through biological membranes. However, comparison of (+) and (−) enantiomers of bupivacaine, which is being investigated in our laboratory, will be necessary to confirm this hypothesis.

#### Clinical Relevance of this Study

Is mitochondrial function affected by the action of LA *in vivo*? The LA-induced conduction block is classically explained by the inhibition of neuronal sodium channels.<sup>30</sup> However, the mechanisms of the toxic effects of LA, especially neuronal or cardiac, are still poorly understood. Myocardial depression induced by high concentrations of bupivacaine could be explained in part by an impairment of cell energy metabolism.<sup>12,13</sup> Acute cardiotoxicity from accidental intravenous injec-



tion of bupivacaine occurs in a few minutes, a time that could appear too short for ATP levels buffered by phosphocreatine reserves to decrease to a subcontractile level. Nevertheless, when mitochondrial NADH oxidation is partially inhibited by an inhibitor of complex I, such as LA, ATP is initially maintained, but phosphocreatine is strongly decreased, thus suggesting that mitochondrial ATP synthesis cannot meet the demand.<sup>31</sup> In such conditions, contractile failure has been observed.<sup>32</sup> Further, irreversible conduction block in isolated nerve by high concentrations of LA, which is time- and concentration-dependent, is compatible with a loss of cell energy supply.<sup>14,15</sup> The LA concentrations showing an effect on energy metabolism are 50–100 times higher than the toxic plasmic concentrations. However, lipophilic LAs accumulate in tissues, and the real concentrations at the cellular level remain unknown. Further, regional anesthesia implies high local concentrations of LA (in the millimolar range)<sup>33</sup> for which disturbances of mitochondrial bioenergetics may occur.

In conclusion, this study shows that the effects of ropivacaine on mitochondrial bioenergetics are less potent than those of bupivacaine, perhaps a result of a difference in lipid solubility.

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