

Effects of Bupivacaine on Mitochondrial Energy Metabolism in Heart of Rats following Exposure to Chronic Hypoxia

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Background: Adaptation to chronic exposure to hypoxia alters energy metabolism in the heart, particularly in the left ventricle, which undergoes a loss in oxidative capacity. Highly lipophilic local anesthetics interfere with mitochondrial energy metabolism. The purpose of this study was to compare the effects of bupivacaine on mitochondrial energy metabolism in heart of rats subjected to normoxic or hypoxic environments.

Methods: Male Wistar rats (n = 10) were subjected to hypobaric hypoxia (simulated altitude = 5,000 m, 380 mmHg) for 2 weeks. Control rats (n = 10) were maintained in an ambient normoxic environment. Mitochondrial metabolism (oxygen consumption and adenosine triphosphate synthesis) was assessed using saponin-skinned ventricular fibers. Bupivacaine (0–5 mM) was tested on both left and right ventricles of normoxic or hypoxic heart.

Results: In animals exposed to hypobaric hypoxia for 14 days, cardiac mass significantly increased, and the right-to-left ventricular ratio was approximately twofold (0.48 ± 0.11 vs. 0.22 ± 0.04 , $P < 0.05$). Oxygen consumption and adenosine triphosphate synthesis were significantly lower in the hypoxic left ventricles but not in the right ones. The uncoupling effect of bupivacaine was more pronounced in the left ventricle from hypoxic heart than in the right ventricle; the bupivacaine-induced decrease in the adenosine triphosphate synthesis rate and in the adenosine triphosphate-to-oxygen ratio was significantly greater in the hypoxic left ventricle than in the normoxic one.

Conclusions: Chronic hypoxia impairs cardiac energy metabolism in left ventricles and enhances the depressant effects of bupivacaine on mitochondrial functions.

CHRONIC hypoxia occurs in physiologic (high altitude) or pathologic conditions (chronic pulmonary diseases). Adaptation to chronic hypoxia has been extensively studied in animals and humans.¹ Chronic hypoxia is characterized by a reduction in oxygen supply, and one of the initial adaptive mechanisms is the decrease in energy-requiring reactions, like protein synthesis.² In the heart, mitochondria provide by way of the oxidative phosphorylation more than 95% of the energy supply in the form of adenosine triphosphate (ATP) to support many ATP-dependent processes, like cycling of the contractile proteins or maintaining ion gradients.³

The metabolic adaptation of the cardiac system to chronic hypoxia exposure is still not well understood. Chronic hypoxia leads to pulmonary hypertension. A response to this chronic functional overload is a compensatory increase in right ventricular mass and an elevation of cardiac work, which occurs during conditions of low oxygen availability. By contrast, the left ventricle is not submitted to a pressure overload and should exhibit a different adaptive response. Recently, changes were investigated in the energy metabolism in homogenates of right and left ventricles of rats living in an hypoxic environment.⁴ The major finding was that the oxidative capacity of the left ventricle was diminished by hypoxic adaptation.

Mitochondria are a potential site of action of general and local anesthetics. High lipophilic local anesthetics like bupivacaine impair mitochondrial energy metabolism.^{5,6} Such effects could be associated with certain toxic effects of local anesthetics. Indeed, bupivacaine-induced myocardial depression may in part be explained by an alteration of mitochondrial energy transduction.^{7,8} Bupivacaine induces a decrease in ATP synthesis in the cell through an uncoupling effect between oxygen consumption and ATP synthesis^{9,10} and through inhibition of mitochondrial enzyme complexes.¹¹

The cardiotoxicity of bupivacaine is enhanced in animals by the presence of hypoxia or acidosis.^{12,13} However, the effects of bupivacaine have not been investigated on chronic hypoxic heart. The current study was therefore undertaken to compare the effects of bupivacaine on energy metabolism in left and right ventricles of rats living in normoxic or hypoxic environments. Mitochondrial oxidative phosphorylation was studied in saponin-skinned fibers isolated from left and right ventricles.⁶

Materials and Methods

Chronic Hypoxia

Care of the animals conformed to the recommendations of the Institutional Animal Care Committee and the French Ministry of Agriculture. Male Wistar rats (n = 10), 10–12 weeks old, weighing 250–300 g, were exposed to chronic hypoxia as follows. They were exposed to a simulated altitude of 5,000 m (barometric pressure, 380 mmHg) in a well-ventilated, temperature-controlled hypobaric chamber for 14 days.¹⁴ Free access to a standard rat diet and water was allowed throughout the

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exposure period. The chamber was opened twice a week for a few minutes to clean the cages, to remove the animal that had completed its 2-week stay in the chamber, and to replace it with another for a 2-week stay. By doing this, experiments could be performed on a regular basis, immediately after the chronic hypoxia exposure to limit the time spent in normoxia. Normoxic rats ($n = 10$) were kept in the same room but not in the hypobaric chamber, with the same 12–12 h light–dark cycle. Rat food and tap water were provided *ad libitum*.

Heart Preparation

Animals were killed by cervical dislocation, and the heart was quickly removed in a normoxic (*i.e.*, equilibrated with air) cooled relaxing solution (solution 1: 10 mM EGTA, 3 mM Mg^{2+} , 20 mM taurine, 0.5 mM dithiothreitol, 5 mM ATP, 15 mM phosphocreatine, 20 mM imidazole, and 0.1 M K^+ -2-[N-morpholino]ethane sulfonic acid, pH 7.2); chemicals were from Sigma Chemical Company (St. Louis, MO). Then, the heart was dissected and weighed. The ratio of right ventricular free wall (RV) weight to the sum of septum plus left ventricular free wall (LV) weight (fresh tissue) was used as an index of right ventricular hypertrophy resulting from chronic hypoxia-induced pulmonary hypertension.

Saponin-skinned Ventricle Fibers

Bundles of fibers between 5 and 10 mg were isolated from the endocardial surface of both left and right ventricles and then permeabilized in solution 1 added with saponin 50 $\mu\text{g/ml}$.¹⁵ Then, 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 dithiothreitol, 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 carried out 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. After 15–20 min of permeabilization, more than 60% of the cytosolic lactate dehydrogenase was found in the external medium, and the mitochondrial citrate synthase activity in the medium remained below 5%.⁶

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/ml. 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 as substrates; 50 μM di(adenosine 5')-pentaphosphate, 20 μM EDTA, and 1 mM iodoacetate

were also added to the cuvette to inhibit extramitochondrial ATP synthesis and ATP hydrolysis.¹⁶ Adenosine diphosphate (ADP)-stimulated respiration, associated with ATP synthesis, was determined in the presence of 1 mM ADP. Basal respiration without ATP synthesis was measured after addition of 70 μM atractyloside and 1 μM oligomycin. Results were expressed in nanomoles of oxygen consumed per minute and per milligram dry weight of fiber.

Bupivacaine HCl (Astra Pain Control, Södertälje, Sweden) was dissolved in dimethyl sulfoxide (DMSO) at 250 mM concentration and was tested in a 0–5 mM concentration range. Control values were obtained in the same conditions in the presence of DMSO. Bupivacaine was added to the oxygraph chamber after equilibration of the mitochondrial suspension with the respiratory substrates. Achievement of the steady state action of local anesthetic was assumed when the oxygen consumption rate was constant.

Measurement of Adenosine Triphosphate Synthesis

Under the same conditions as in the respiration assay, the mitochondrial ATP synthesis rate in skinned fibers was determined by bioluminescence measurement (luciferine-luciferase system) of the ATP produced after addition of 1 mM ADP.¹⁷ The ATP Bioluminescence Assay Kit HS II from Roche Diagnostics GmbH (Mannheim, Germany) was used. At various time intervals after addition of ADP, 10- μl aliquots were withdrawn from the oxygraph chamber, quenched in 100 μl DMSO, and diluted in 5 ml ice-cold distilled water. Standardization was performed with known quantities of ATP measured under the same conditions. The efficiency of oxidative phosphorylation was taken as the ratio of ATP synthesis rate to oxygen consumption rate (ATP/O).¹⁶

Statistical Analysis

Results were expressed as mean \pm SD. Data were plotted and analyzed using SigmaPlot 7.1 and Systat 10.0 (SPSS Inc., Chicago, IL). Comparison of two means was performed using the Student *t* test. Comparison of several means was performed using analysis of variance with *post hoc* Tukey test. All *P* values were two-tailed, and a *P* value of less than 0.05 was required to reject the null hypothesis.

Results

Physical Characteristics

Chronic hypoxia induced an hypertrophy of the heart. Cardiac mass increased significantly (1.61 ± 0.20 and 1.13 ± 0.16 g in hypoxic and normoxic animals, respectively, $P < 0.05$) with also a significant increase in the heart weight-to-body weight ratio (0.54 ± 0.06 g/100 g and 0.37 ± 0.03 g/100 g, respectively, $P < 0.05$). The

Table 1. Physical Characteristics of Right and Left Ventricles

	Normoxic Rats (n = 10)	Hypoxic Rats (n = 10)
BW (g)	309 ± 21	290 ± 18
RVW (mg)	0.14 ± 0.03	0.32 ± 0.07*
LVSW (mg)	0.64 ± 0.08	0.67 ± 0.09
RVW/BW (10 ⁻³)	0.44 ± 0.08	1.08 ± 0.23*
LVSW/BW (10 ⁻³)	2.08 ± 0.22	2.29 ± 0.29
RVW/LVSW	0.22 ± 0.04	0.48 ± 0.11*
Right ventricular hypertrophy (%)	100	226 ± 47*
Left ventricular (+septum) hypertrophy (%)	100	104 ± 14

Data are mean ± SD.

**P* < 0.05 versus normoxic animals.

BW = body weight; RVW = right ventricular free wall weight; LVSW = left ventricular free wall plus septum weight.

hypertrophy of hypoxic heart was associated with an increase in the RV mass (table 1). As a consequence of the chronic hypoxia-induced pulmonary hypertension, right-to-left ventricular ratio values markedly increased compared with those in control rats (0.48 ± 0.11 and 0.22 ± 0.04, respectively, *P* < 0.05).

Oxidative Capacity of Chronic Hypoxic Heart

Saponin-skinned fibers of left ventricle from hypoxic animals oxidized substrates at much lower rates than those from rats kept in ambient air. Basal, without-ADP, and ADP-stimulated oxygen consumption rates supported by glutamate were significantly decreased in hypoxic left ventricles. The ATP synthesis was also strongly reduced (approximately -35%), with no changes in the ATP/O ratio (table 2). On the contrary, chronic exposure to hypoxia was not associated with any decrease in oxidative phosphorylation in the right ventricle.

Effects of Bupivacaine on Chronic Hypoxic Heart

In the normoxic heart, bupivacaine induced an uncoupling of oxidative phosphorylation. The uncoupling effect was characterized by an increase in the basal, without-ADP, oxygen consumption and by a concomitant decline in the ATP synthesis. At 5 mM bupivacaine, ATP

synthesis rate was decreased in the left ventricle from 30.3 ± 8.7 nmol ATP · min⁻¹ · mg dry weight⁻¹ to 15.6 ± 4.6 nmol ATP · min⁻¹ · mg dry weight⁻¹ (*P* < 0.05); the ATP/O ratio was also significantly reduced (2.0 ± 0.4 vs. 1.3 ± 0.3, *P* < 0.05). The effects of bupivacaine were similar in the right normoxic ventricle (table 3).

In the chronic hypoxic heart, the effects of bupivacaine (at 1 and 5 mM) on mitochondrial energy metabolism were more pronounced in the left ventricles than in the right ones. The decrease in the ATP synthesis rate and in the ATP/O ratio was significantly greater in the hypoxic left ventricle than in the normoxic one (fig. 1 and table 3). In contrast, there were no significant differences between the effects of bupivacaine in normoxic or hypoxic right ventricles (table 3).

Discussion

During chronic exposure of animals to hypoxia, the heart metabolism, which is based on strict oxidative processes, is subject to various adaptive changes that affect ATP demand and ATP supply through mitochondrial oxidative phosphorylation.² For technical reasons, the metabolism of right and left ventricles has been compared in only a few studies.^{4,18} Mitochondria isolated from just one ventricle can not be obtained in sufficient quantity for performing a complete study of the energy metabolism. In the current investigation, we used saponin-permeabilized ventricle fibers to characterize alterations in mitochondrial energy metabolism in both ventricles of chronic hypoxic heart and to compare the effects of bupivacaine on oxidative phosphorylation in normoxic and hypoxic animals. Our major findings were twofold: (1) the left ventricle, unlike the right, underwent a loss of oxidative capacity in response to chronic hypoxia; and (2) the mitochondrial effects of bupivacaine appeared to be potentiated by chronic hypoxia in the left ventricle.

The changes in oxidative phosphorylation in the left ventricle were characterized by a similar decline in ATP synthesis and in oxygen consumption. The stability of a

Table 2. Effects of Chronic Hypoxia on Mitochondrial Oxidative Phosphorylation in Right and Left Ventricles

	Oxygen Consumption		ATP synthesis	ATP/O
	-ADP	+ADP		
Normoxic RV	3.0 ± 0.6	13.9 ± 2.7	26.7 ± 7.7	2.1 ± 0.3
Normoxic LV	3.6 ± 0.4	14.7 ± 3.1	30.3 ± 8.7	2.0 ± 0.4
Hypoxic RV	2.9 ± 1.0	11.5 ± 2.8	23.3 ± 8.4	2.1 ± 0.5
Hypoxic LV	2.9 ± 0.3*	10.7 ± 2.5*	19.7 ± 6.6*†	1.9 ± 0.4

Experimental conditions are described in Materials and Methods. Basal, without adenosine diphosphate (ADP), and ADP-stimulated oxygen consumption rates supported by glutamate are expressed in nmol oxygen · min⁻¹ · mg dry weight⁻¹. ATP synthesis rate is expressed in nmol ATP · min⁻¹ · mg dry weight⁻¹. ATP-to-oxygen ratio (ATP/O) is calculated as the ratio of the rate of ATP synthesis to the rate of the concomitant respiration in the presence of ADP. Data are mean ± SD (n = 10).

**P* < 0.05 versus normoxic LV. †*P* < 0.05 versus hypoxic RV.

RV = right ventricular free wall; LV = left ventricular free wall.

Table 3. Comparison of Metabolic Effects of Bupivacaine (5 mM) on Right and Left Ventricles from Hypoxic and Normoxic Rats

	Oxygen Consumption		ATP Synthesis	ATP/O
	-ADP	+ADP		
Normoxic RV	6.1 ± 1.2	10.8 ± 2.7	14.6 ± 5.4	1.4 ± 0.5
Normoxic LV	6.7 ± 0.8	12.3 ± 1.8	15.6 ± 4.6	1.3 ± 0.3
Hypoxic RV	7.6 ± 1.7	11.7 ± 1.7	13.2 ± 4.4	1.2 ± 0.4
Hypoxic LV	6.7 ± 1.5	11.5 ± 2.1	10.6 ± 4.9 [†]	0.9 ± 0.4 ^{*†}

Oxygen consumption and ATP synthesis were measured in the presence of 5 mM bupivacaine. Basal, without adenosine diphosphate (ADP), and ADP-stimulated oxygen consumption rates supported by glutamate are expressed in $\mu\text{mol oxygen} \cdot \text{min}^{-1} \cdot \text{mg dry weight}^{-1}$. ATP synthesis rate is expressed in $\text{nmol ATP} \cdot \text{min}^{-1} \cdot \text{mg dry weight}^{-1}$. ATP-to-oxygen ratio (ATP/O) is calculated as the ratio of the rate of ATP synthesis to the rate of the concomitant respiration in the presence of ADP. Data are mean ± SD (n = 10).

**P* < 0.05 versus normoxic LV. †*P* < 0.05 versus hypoxic RV.

RV = right ventricular free wall; LV = left ventricular free wall.

normal ATP/O ratio indicated that the intrinsic properties of mitochondria were not modified. This has already been observed in heart and liver mitochondria of rats acclimatized to a 4,400-m simulated altitude. No changes in the oxygen or ADP dependence of mitochondrial respiration was shown as a mechanism of adaptation to chronic hypoxia.^{19,20} As in our study, the ATP/O ratio was not different in heart mitochondria from hypoxic or normoxic animals. The decline in the oxidative capacity in the left ventricle of our animals could be interpreted as a reduction of the functional mitochondrial mass, due to a decrease in the number of mitochondria per cardiomyocyte or in the key enzymes of the metabolic pathways.⁴ A rapid decline in protein synthesis has been observed in different hypoxic animal models.²¹ Protein biosynthesis is an ATP-consuming process that is very sensitive to hypoxia. In hypoxia-tolerant animals, the first line of defense against hypoxia is a balanced suppression of ATP demand and ATP supply. The ATP demands of protein synthesis in hypoxic conditions are down-regulated by translational arrest.² Another explanation for the decline in oxidative capacity of the left

ventricle is that it occurs in response to a decrease in global energy demand or substrate availability. Normobaric or hypobaric hypoxia has been found to depress whole body oxygen consumption and protein metabolism. In patients with chronic obstructive pulmonary disease, the decrease in tissue protein content was explained by a low rate of protein synthesis and a high rate of amino acid utilization for gluconeogenesis.^{1,22} On the contrary, increasing energy demand enhances mitochondrial cytochrome content in skeletal muscle and in the heart. This adaptive process could also explain the results observed in the right ventricle. Chronic hypoxia leads to pulmonary hypertension with a chronic overload of the right ventricle. It has been shown that the oxidative capacity of the hypoxic right ventricle is proportional to total protein content and ventricular weight.²³ Therefore, the compensatory increase in muscle mass could maintain the oxidative capacity of the right ventricle at least in this state of adaptation of chronic hypoxia.

Two different effects of local anesthetics have been found in mitochondria: an uncoupling of oxidative phosphorylation and an inhibition of enzymatic complexes. The uncoupling effect of local anesthetics corresponds to the dissipation of the transmembrane proton gradient, which leads to the decrease in the ATP-to-oxygen ratio (*i.e.*, the efficiency of ATP synthesis). The mechanism of bupivacaine uncoupling has been extensively investigated.^{6,9,10} Tertiary-amine local anesthetics such as bupivacaine act mainly by cycling protons through the membrane. This effect largely depends on the lipid solubility of these molecules.¹⁷ In the cell, the consequences of these mitochondrial effects are a decrease in ATP synthesis rate and a depletion in the cellular ATP pool.⁵ Myocardial depression induced by high concentrations of bupivacaine could in part be explained by this impairment of cell energy metabolism.⁶⁻⁸

Several studies in animals have shown that the cardiotoxicity of bupivacaine is enhanced by the presence of hypoxia.^{12,13} However, the toxic effects of bupivacaine have still not been investigated during chronic hypoxia. The current study shows that the impairment of mito-

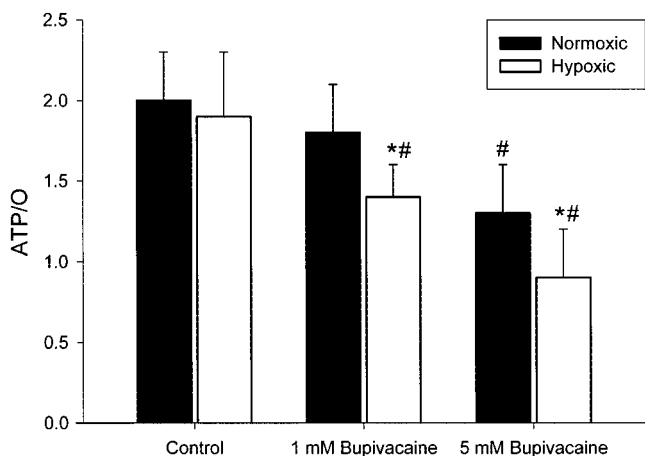


Fig. 1. Effect of bupivacaine (1 and 5 mM) on the ratio of adenosine triphosphate (ATP) synthesis rate to oxygen consumption rate (ATP/O) in the left ventricle from normoxic and hypoxic rats. Experimental conditions are similar to those in table 3. Data are mean ± SD (n = 10). **P* < 0.05 versus normoxic ventricle; #*P* < 0.05 versus control.

chondrial energy metabolism in the presence of bupivacaine is reinforced in the hypoxic left ventricle. The loss of oxidative capacity in the hypoxic left ventricle, which is associated with a reduction of the functional mitochondrial mass, could explain these results. On the right side, where no change was observed in the oxidative phosphorylation during chronic hypoxia, no difference was found between the effects of the local anesthetic in hypoxic or normoxic right ventricles.

Similar changes in mitochondrial energy metabolism may be observed in patients with chronic obstructive pulmonary diseases.^{1,22} Lipophilic local anesthetics could be more toxic in these patients. However, the clinical relevance of this study is questionable. The bupivacaine concentrations showing an effect on mitochondrial energy metabolism are 50–100 times higher than the toxic plasma concentrations. Nevertheless, lipophilic local anesthetics accumulate in tissues and the real concentrations at the cellular level remain unknown.

In conclusion, after a 2-week exposure to chronic hypoxia, the energy metabolism in the left ventricle was impaired with a loss in oxidative capacity. On the contrary, the adaptive mechanisms in the right ventricle allowed mitochondrial metabolism to be maintained. The depressant effects of bupivacaine on mitochondrial functions appeared reinforced in the hypoxic left ventricle.

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