

# Anaphylactic Shock

## A Form of Distributive Shock without Inhibition of Oxygen Consumption

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**Background:** The pathophysiology of anaphylactic shock during anesthesia is incompletely characterized. It is described as distributive by analogy with septic shock (anaerobic metabolism, high tissue oxygen pressure [ $P_{tO_2}$ ] values). The  $P_{tO_2}$  profile and its metabolic consequences during anaphylaxis are not known.

**Methods:** Ovalbumin-sensitized anaphylactic shock rats ( $n = 11$ ) were compared to nicardipine-induced hypotension rats ( $n = 12$ ) for systemic hemodynamics,  $P_{tO_2}$ , sympathetic nervous system activation, skeletal muscle blood flow, and interstitial lactate and pyruvate concentrations using combined microdialysis and polarographic Clark-type oxygen probes.

**Results:** In both groups, the time course and the magnitude of arterial hypotension were similar. The ovalbumin group but not the nicardipine group displayed decreased skeletal muscle blood flow (from  $45 \pm 6.2 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$  to  $24.3 \pm 5 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ ;  $P < 0.0001$ ) and  $P_{tO_2}$  values (from  $42 \pm 5$  to  $5 \pm 2$ ;  $P < 0.0001$ ). The ovalbumin group had more intense sympathetic nervous system activation with higher plasma epinephrine and interstitial norepinephrine concentrations. For the ovalbumin group, there was skeletal muscle anaerobic metabolism (lactate concentration increased from  $0.446 \pm 0.105$  to  $1.741 \pm 0.459 \text{ mm}$ ;  $P < 0.05$ ) and substrate depletion (pyruvate concentration decreased from  $0.034 \pm 0.01 \text{ mm}$  to  $0.006 \pm 0.002 \text{ mm}$ ;  $P < 0.05$ ) leading to increased interstitial lactate/pyruvate ratios (from  $17 \pm 6$  to  $311 \pm 115$ ;  $P < 0.05$ ).

**Conclusions:** This profile suggests decreased skeletal muscle blood flow and oxygen delivery. Persistent energy consumption results in decreased  $P_{tO_2}$  and substrate depletion through

anaerobic glycolysis leading to complete failure of cellular energy production. This could explain rapid organ dysfunction and resuscitation difficulties.

ANAPHYLACTIC shock occurring during anesthesia is uncommon (1/10,000–1/20,000 anesthetic procedures)<sup>1</sup> but is a potentially extremely severe condition<sup>2,3</sup> that may lead to death in up to 3–10% of cases.<sup>4,5</sup> The diagnosis of anaphylactic shock can be difficult during anesthesia.<sup>5,6</sup> In addition, adequately conducted resuscitation sometimes does not restore cardiovascular and respiratory homeostasis, even in previously apparently healthy patients. Diagnosis and therapy of anaphylactic shock are challenging because its pathophysiology remains obscure. Anaphylactic shock is commonly classified as a distributive shock.<sup>7</sup> The prototype of distributive shock is septic shock, although the term *distributive* is not defined. All forms of shock are probably distributive in that blood flow of some organs, such as skeletal muscle, is decreased to preserve blood flow to organs, such as the brain and heart. One of the main mechanisms of redistribution is the activation of sympathetic nervous system (SNS), which can counteract local metabolic regulation of blood flow. The canonical description of septic shock includes arterial hypotension that results from a decrease in systemic vascular resistance and anaerobic metabolism. Anaerobic metabolism in septic shock is peculiar in that it is present despite increased skeletal muscle oxygen partial pressure ( $P_{tO_2}$ ). Increased  $P_{tO_2}$  values are related to inhibition of cellular oxygen consumption. This has been attributed to unregulated activation of inflammation, a situation called *cytotoxic hypoxia*.<sup>8</sup>

Anaphylactic shock is also characterized by arterial hypotension due to excessive vasodilatation related to the explosive liberation of preformed and *de novo* synthesized mediators into organs and the bloodstream.<sup>9–11</sup> Despite the wide acceptance of this paradigm, there are several unanswered questions. Is tissue hypoxia present in anaphylactic shock? If present, what are its metabolic consequences? What are the contributions of SNS activation and arterial hypotension to these modifications? To answer these questions, we compared, in Brown Norway anesthetized rats, an ovalbumin-induced model of anaphylactic shock<sup>12</sup> with a model of pharmacologically induced severe arterial hypotension to evaluate

This article is accompanied by an Editorial View. Please see: Hepner DL: From the laboratory to the bedside: Searching for an understanding of anaphylaxis. ANESTHESIOLOGY 2005; 103:1-2.

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Received from the Service d'anesthésie-réanimation, CHU de Nancy Hôpital Central, Nancy, France. Submitted for publication November 5, 2004. Accepted for publication February 7, 2005. Supported by a research grant from Société Française d'Anesthésie-Réanimation, Paris, France.

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‡‡ <http://www.aagbi.org/pdf/Anaphylaxis.pdf>. Accessed June 3, 2005.

metabolic and SNS profiles and regional blood flow in the skeletal muscle.

## Materials and Methods

### Animals

This study, including care of the animals involved, was conducted according to the official recommendations of the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinki Declaration. Therefore, these experiments were conducted in an authorized laboratory under the supervision of an authorized researcher (P. M. M.). Ten-week-old Brown Norway rats weighing 280–310 g (Janvier, Le Genest-St-Isle, France) were chosen as experimental animals.

### Sensitization Protocol

Brown Norway rats were sensitized subcutaneously at day 0, day 4, and day 14 with 1-mg grade VI chicken egg albumin (OVA; Sigma-Aldrich, Saint-Quentin Fallavier, France) mixed with 3.5 mg aluminum hydroxide (OHA<sub>1</sub>; Merck Eurolab, Briare Le Canal, France) diluted in 1 ml saline solution (Chlorure de sodium, Cooper 0.9%; Melun, France).

### Anesthesia and Surgical Procedure

The surgical procedure was performed during general anesthesia on day 21. Anesthesia was induced with 60 mg/kg intraperitoneal thiopentone sodium (Sanofi Santé animale, Libourne, France) and maintained with intravenous additional doses (2 mg/kg) when required. A fluid-filled polyethylene catheter (ID, 0.58 mm; OD, 0.96 mm; Biotrol Diagnostic, Chennevières Les Louvres, France) was inserted in the right common carotid artery for blood arterial pressure monitoring and blood withdrawal. Another fluid-filled catheter was placed in the left external jugular vein for administration of drugs and fluid maintenance (10 ml · kg<sup>-1</sup> · h<sup>-1</sup>) with lactate-free Ringer's solution (Braun Medical SA, Boulogne, France). The trachea was cannulated, and the lungs were mechanically ventilated with 100% oxygen using a Harvard Rodent respirator model 683 (Harvard Apparatus, Cambridge, MA).

### Hemodynamic Parameter Measurements

Mean arterial pressure (MAP) was recorded using a strain gauge pressure transducer (DA-100; Biopac Systems, Northborough, MA). Through a limited laparotomy, a 20-MHz Hard Epoxy Doppler flow probe (HDP 20, 1. OS; diameter, 1.5 mm; Matec Instrument Companies Inc., Northborough, MA), was positioned around the upper abdominal aorta to measure the upper abdominal aortic blood velocity, taken as an estimate of the cardiac output. The pressure transducer, the Doppler flowmeter, and the electrocardiogram were connected to a desktop computer for continuous data acquisition (Acqknowledge<sup>®</sup> software and MP 100 hardware; Biopac Systems).

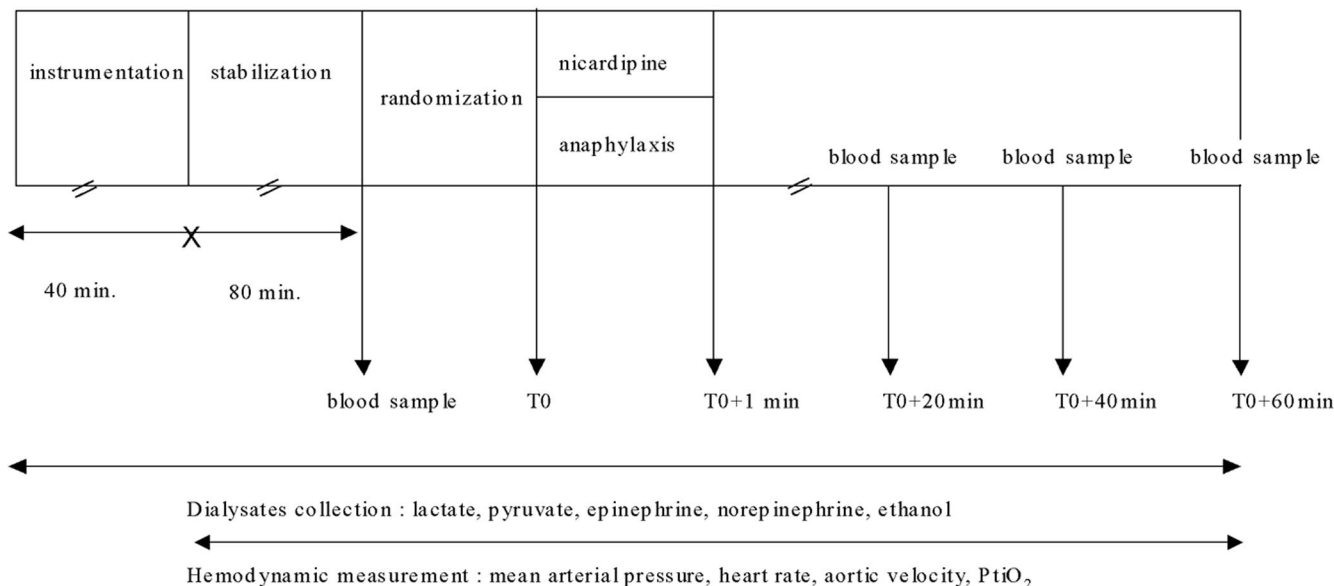
### Collection of Blood Samples, Insertion of Microdialysis Probes, Collection of Microdialysate Samples, and Analytic Procedures

Samples for plasma epinephrine and norepinephrine measurements were drawn into chilled tubes containing 50  $\mu$ l EDTA (5 g/100 ml) solution and sodium metabisulfite (1 g/100 ml). After centrifugation (10 min, 1,200g, 4°C), plasma samples were immediately frozen at -80°C and stored for later analysis.

Linear flexible probes (membrane length, 10 mm; OD, 290  $\mu$ m; ID, 240  $\mu$ m) were custom-made in our laboratory and assembled using a 50-kd molecular weight cut-off polyacrylonitrile membrane (Filtral AN 69 HF membrane; Hospal SA, Lyon, France) glued at both ends to thin (ID, 75  $\mu$ m) inflow and outflow silica tubes (Phymep, Paris, France). Five microdialysis probes were inserted in the two quadriceps muscles using a 27-gauge guiding needle that was passed through the muscle. As soon as the needle traversed the quadriceps, it was removed, and the microdialysis probe was gently pulled back until the dialysis membrane was completely embedded within the muscle. The probes were connected to a microinjection pump (EP 244 Pump; Harvard Pump, Cambridge, MA) and perfused with a lactate-free Ringer's solution at a flow rate of 2  $\mu$ l/min. Probes recovery performances, as determined *in vitro*, were 56  $\pm$  2% for lactates, 58  $\pm$  6% for pyruvates, 40  $\pm$  3% for epinephrine, and 43  $\pm$  4% for norepinephrine.

Interstitial epinephrine and norepinephrine dialysates were collected, using two probes, in plastic Eppendorf microtubes (Eppendorf AG, Hamburg, Germany) kept on ice and in the same collection conditions as plasma samples. The dialysates of two other probes were collected in Eppendorf microtubes to measure interstitial lactate and pyruvate concentrations, respectively.

The last microdialysis probe was perfused with a solution containing a known concentration of ethanol (designated as inflow concentration or  $C_{in}$  = 43 mM) to allow muscle blood flow measurements. Briefly, the microdialysis ethanol technique method is based on the principle that ethanol diffuses through the membrane out of the microdialysis probe into the surrounding tissue, in relation to the blood flow. The diffusion of ethanol is enhanced with increased tissue blood flow, resulting in an increased transfer of ethanol from the perfusate to the interstitial fluid, thus resulting in a reduced ethanol concentration in the dialysate outflow ( $C_{out}$ ). To express the muscle blood flow, the outflow-to-inflow concentration ( $C_{out}/C_{in}$ ) ratio of ethanol was calculated from the ethanol concentration in the perfusion medium inflow ( $C_{in}$  = 43 mM) and the collected dialysate outflow ( $C_{out}$  < 43 mM). An increased ratio indicates vasoconstriction, whereas a decreased ratio indicates vasodilatation.<sup>13-15</sup> Previous studies of muscle blood flow in cats where microdialysis probes were perfused at a rate of 2  $\mu$ l/min suggested that a change in



**Fig. 1.** Time course of measurements. Time 0 (T0) corresponds to injection of nicardipine or ovalbumin. PtiO<sub>2</sub> = tissue oxygen pressure.

ethanol Cout/Cin ratio from 0.4 to 0.5 represents a decrease in muscle blood flow of approximately 50%.<sup>15</sup> Skeletal muscle blood flow values were calculated according to the Wallgreen nomogram.<sup>16</sup> The ethanol samples were stored at 4°C for subsequent analysis within 24 h. Ethanol concentrations were determined using the enzymatic method described by Bernt and Gutmann.<sup>17</sup> All other samples were immediately frozen at -80°C and stored for later analysis. Plasma and interstitial epinephrine and norepinephrine concentrations were measured using high-performance liquid chromatography with electrochemical detection as previously described.<sup>18</sup> Lactate and pyruvate dialysate samples were analyzed using enzymatic kinetic methods (Boehringer GmbH Diagnostica, Mannheim, Germany) on a Kone Delta<sup>®</sup> Analyzer (Kone, Fréjus, France).

#### *Tissue Oxygen Partial Pressure Measurement*

A flexible Clark-type polarographic oxygen electrode (diameter, 500 μm; length, 200 mm) computer-supported Licox system (GMS, Mielkendorf, Germany) was introduced in one of the quadriceps muscles. For correction of tissue PtiO<sub>2</sub> measurements, temperature within the muscle was monitored, and PtiO<sub>2</sub> values were adjusted to quadriceps temperature by means of the computer software Licox (GMS). The electrode was calibrated before and after each experiment with room air. The Licox system was connected to a desktop computer for continuous data acquisition (Acqknowledge<sup>®</sup>).

#### *Time Course of Measurements*

At the end of the surgical preparation period, the animals were allowed to stabilize for 80 min to recover from the cellular damage resulting from probe implanta-

tion. This period was called the stabilization period. Plasma epinephrine and norepinephrine measurements were performed at different times during the stabilization period and the study period. All blood samples (500 μl for each measurement of epinephrine and norepinephrine) were compensated volume for volume by 6% hydroxyethyl starch infusion (6% Hesteril<sup>®</sup>; Fresenius Kabi France, Sèvres, France). The total volume of blood withdrawn was 2 ml, corresponding to 10% of the estimated blood volume of Brown Norway rats. At the end of the stabilization period and 5 min before the induction of the shock, baseline values were collected. Dialysates collection was started with 20-min sampling periods. The animals were killed by an overdose of thiopentone sodium after the last blood sample collection (fig. 1).

#### *Induction of Shock*

After the stabilization period, ovalbumin-sensitized animals were randomly allocated into two groups according to a computer-generated randomization list: the anaphylaxis group, in which shock was induced by injecting intravenously in 1 min 1 mg ovalbumin diluted in 1 ml saline solution, and the nicardipine group, in which arterial hypotension corresponding to a 50% decrease in MAP was obtained by injecting intravenously in 1 min 2 μg/100 g (body weight) nicardipine (Novartis Pharma SA, Rueil-Malmaison, France) followed by a continuous intravenous infusion of nicardipine (500 μg · 100 g<sup>-1</sup> · h<sup>-1</sup>). This nicardipine regimen was studied in preliminary experiments (n = 12) and chosen because it resulted in a rapid and persistent decrease of MAP of approximately 50% from baseline values. Time 0 (T0) corresponds to the intravenous injection of ovalbumin or nicardipine,



**Table 1. Time Course of Hemodynamic Data: Mean Arterial Pressure, Heart Rate, and Aortic Velocity in Nicardipine and Anaphylaxis Groups**

T0 ± Minutes	Mean Arterial Pressure, mmHg		Heart Rate, beats/min		Aortic Velocity, cm/s	
	Nicardipine	Anaphylaxis	Nicardipine	Anaphylaxis	Nicardipine	Anaphylaxis
T0 - 5	122 ± 4	123 ± 5	477 ± 13	455 ± 9	24 ± 2	22 ± 2
T0	120 ± 3	127 ± 6	454 ± 13	443 ± 14	27 ± 3	24 ± 3
T0 + 1	87 ± 14*	92 ± 10*	437 ± 10	445 ± 13	31 ± 5	32 ± 4
T0 + 2	66 ± 6*	84 ± 8*	437 ± 12	450 ± 13	29 ± 6	32 ± 3
T0 + 3	67 ± 7*	75 ± 12*	433 ± 11	453 ± 15	31 ± 5	29 ± 2
T0 + 4	69 ± 9*	68 ± 10*	437 ± 10	450 ± 17	30 ± 5	29 ± 4
T0 + 5	62 ± 2*	57 ± 6*	451 ± 14	455 ± 19	27 ± 4	28 ± 5
T0 + 15	59 ± 3*	41 ± 4*	437 ± 13	464 ± 19	27 ± 5	30 ± 4
T0 + 35	53 ± 5*	46 ± 8*	438 ± 13	445 ± 21	23 ± 4	22 ± 4
T0 + 60	53 ± 6*	38 ± 5*	431 ± 18	434 ± 26	23 ± 4	19 ± 4

Time 0 (T0) corresponds to injection of nicardipine or ovalbumin. Values are expressed as mean ± SEM.

\*  $P < 0.0001$  vs. T0.

and the study was performed for an additional 60 min after T0 (T60) (fig. 1).

### Statistical Analysis

Results are expressed as mean ± SEM. Intragroup and intergroup differences were tested by one-way and two-way analysis of variance for repeated measures (Statview; SAS, Cary, NC). When a significant interaction was observed with two-way analysis of variance, the mean values were compared to the baseline values (T0 or T0-5 or T-20 to T0) by the Fisher exact test. The criterion of significance was  $P < 0.05$ .

## Results

Twenty-three ovalbumin-sensitized Brown Norway rats ( $292 \pm 16$  g) were studied and randomly allocated to the anaphylaxis group ( $n = 11$ ) or the nicardipine group ( $n = 12$ ). For all measured variables, baseline values in both groups did not differ during the stabilization period (table 1).

### Hemodynamic Parameter Changes

Hemodynamic data for both groups are presented in table 1. After either nicardipine or ovalbumin injection, the time course and the magnitude of the decrease in MAP were similar throughout the study period. Within

the first 5 min after T0, MAP rapidly decreased to 50% of its baseline value, followed by a further decrease over time. A moderate and transient but not statistically significant increase in aortic blood flow velocity was observed in both groups, followed by a rapid return to baseline values. When the time course of changes in blood flow velocity was analyzed by analysis of variance, despite an increase of aortic velocity values within the 4 and the 15 first minutes after nicardipine or ovalbumin injection, respectively, there were no significant group, time, or time-group interaction effects. No significant differences could be detected in the heart rate response between the two groups. Calculated skeletal muscle blood flows, expressed as  $\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ , are presented in table 2. As compared with baseline values (T-20 to T0 interval), skeletal muscle blood flow was decreased in both groups. At the last time point of the study (T40 to T60 interval), this decrease was significantly greater in the anaphylactic shock group, estimated at approximately 47%, whereas the decrease in the nicardipine group was estimated at approximately 30%. The between-group difference appeared beginning with the T20 to T40 interval and persisted thereafter.

### Sympathetic Nervous System Activation

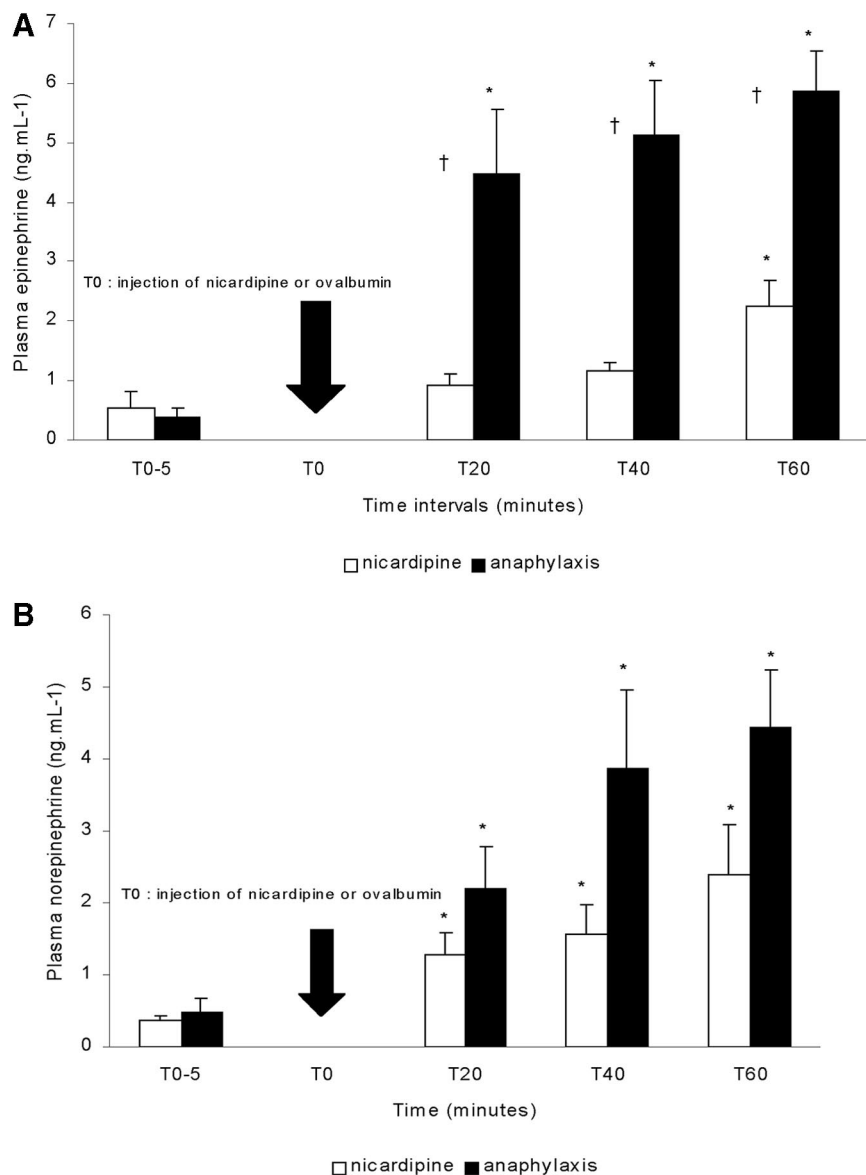
Plasma and interstitial epinephrine and norepinephrine concentrations are presented in figures 2 and 3,

**Table 2. Time Course of Skeletal Muscle Blood Flow in Nicardipine and Anaphylaxis Groups**

Time Interval, min	Cout/Cin Ethanol Ratio		Calculated Skeletal Muscle Blood Flow, $\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$	
	Nicardipine	Anaphylaxis	Nicardipine	Anaphylaxis
T-20 to T0	0.45 ± 0.02	0.47 ± 0.02	53.3 ± 7.7	45 ± 6.2
T0 to T20	0.47 ± 0.03	0.48 ± 0.03	47.6 ± 8.3	40.2 ± 7.7
T20 to T40	0.49 ± 0.03*	0.531 ± 0.03*	42 ± 10*	30.3 ± 8.4*‡
T40 to T60	0.51 ± 0.03*	0.55 ± 0.02*	36.7 ± 8.8*	24.3 ± 5†‡

Results are expressed as mean ± SEM and represent the outflow-to-inflow concentration (Cout/Cin) ethanol ratios and calculated skeletal muscle blood flow.<sup>16</sup> Time 0 (T0) corresponds to intravenous injection of nicardipine or ovalbumin.

\*  $P < 0.05$ , †  $P < 0.0001$  vs. T-20 to T0. There is a statistically significant difference (‡  $P < 0.05$ ) between the two groups (analysis of variance).



**Fig. 2.** Time course of plasma epinephrine (A) and norepinephrine (B) concentrations (ng/ml) in nicardipine and anaphylaxis groups. Time 0 (T0) corresponds to injection of nicardipine or ovalbumin. (A) Anaphylaxis was characterized by a stronger sympathetic activation with higher plasma epinephrine concentrations occurring early after the injection of ovalbumin and sustained during the entire study (from  $0.38 \pm 0.17$  [T0-5] to  $5.86 \pm 0.67$  ng/ml [T60]; \*  $P < 0.0001$ ). Despite arterial hypotension of similar magnitude, the nicardipine group had a smaller increase in plasma epinephrine concentrations (from  $0.55 \pm 0.27$  [T0-5] to  $2.25 \pm 0.43$  ng/ml [T60]; \*  $P < 0.0001$ ). A between-group difference was observed at T20, T40, and T60 ( $\dagger P < 0.05$ ). (B) Anaphylaxis was characterized by a stronger sympathetic activation with plasma norepinephrine concentrations occurring early after the injection of ovalbumin and sustained during the entire study (from  $0.48 \pm 0.19$  [T0-5] to  $4.44 \pm 0.80$  ng/ml [T60]; \*  $P < 0.0001$ ). Despite arterial hypotension of similar magnitude, the nicardipine group had a smaller increase in plasma norepinephrine concentrations (from  $0.37 \pm 0.06$  [T0-5] to  $2.39 \pm 0.70$  ng/ml [T60]; \*  $P < 0.0001$ ).

respectively. Despite a similar decrease in MAP in the two groups, a stronger sympathetic response was observed in the anaphylactic shock group as attested to by higher plasma epinephrine and norepinephrine concentrations (figs. 2A and B) and a higher increase of interstitial norepinephrine concentration (fig. 3B). In addition, the significantly greater gradient between plasma and interstitial epinephrine concentrations observed in the anaphylactic shock group (figs. 2A and 3A) is consistent with skeletal muscle vasoconstriction (table 2).

#### Tissue Oxygen Partial Pressure Changes

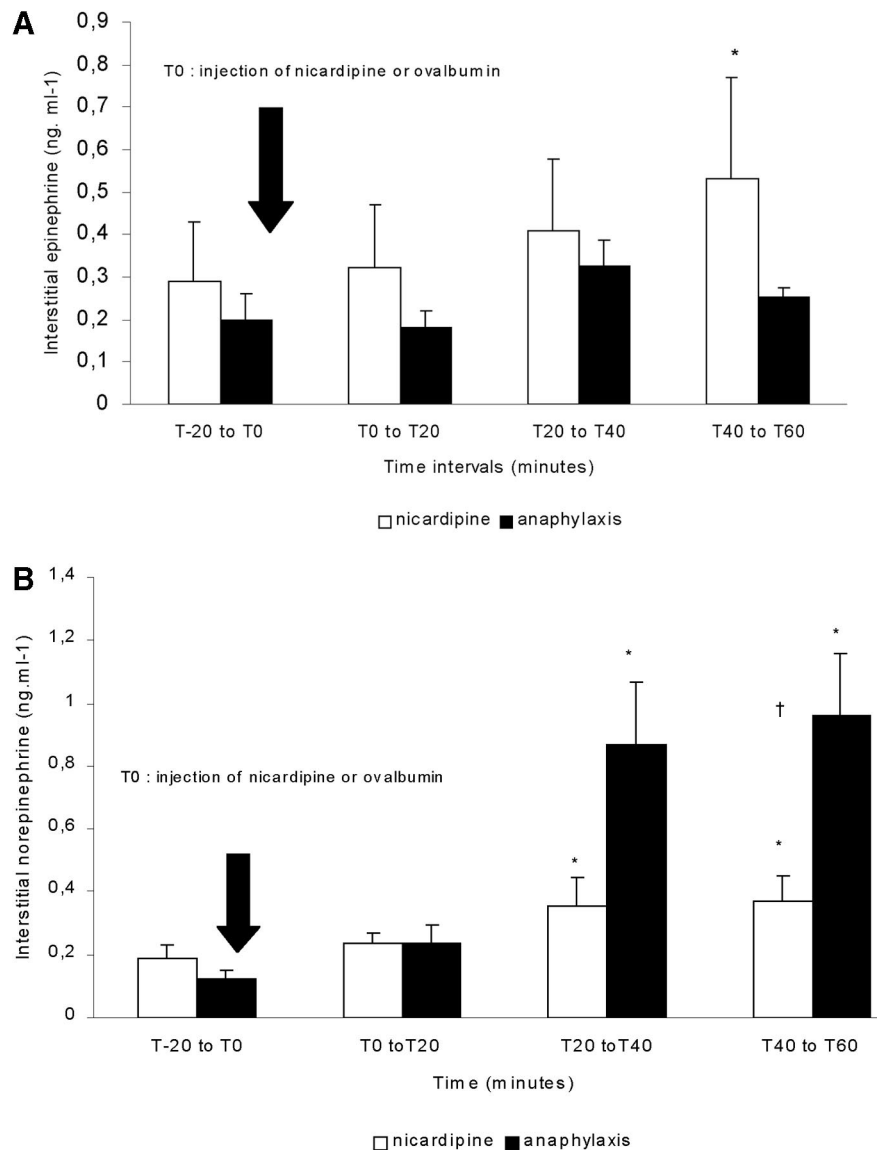
Tissue oxygen partial pressure values for the two groups are presented in figure 4. Anaphylactic shock was characterized by a rapid and sustained 88% decrease of  $P_{tO_2}$  values (from  $42 \pm 5$  mmHg to  $5 \pm 2$  mmHg;  $P < 0.0001$ ), with most of the decrease occurring within the first 5 min after ovalbumin injection (from  $42 \pm 5$  mmHg

to  $18 \pm 5$  mmHg;  $P < 0.0001$ ). In contrast, in the nicardipine group,  $P_{tO_2}$  values were initially maintained. Subsequently in this group, there was a progressive but moderate decrease of  $P_{tO_2}$  values (from  $45 \pm 5$  mmHg to  $34 \pm 8$  mmHg;  $P < 0.05$ ).

#### Tissue Lactate, Pyruvate Concentrations, and Lactate/Pyruvate Ratios

Interstitial lactate and pyruvate concentrations as well as lactate/pyruvate ratio values for the two groups are presented in table 3 and figure 5. After allergen challenge, a rapid increase in interstitial lactate concentrations was observed (from  $0.446 \pm 0.105$  to  $1.741 \pm 0.459$  mM;  $P < 0.05$ ) associated with a rapid decrease in interstitial pyruvate concentrations (from  $0.034 \pm 0.01$  mM to  $0.006 \pm 0.002$  mM;  $P < 0.05$ ). A large increase in lactate/pyruvate ratios (from  $17 \pm 6$  to  $311 \pm 115$ ;  $P < 0.05$ ) was observed at the end of the study

**Fig. 3.** Time course of interstitial epinephrine (A) and norepinephrine (B) concentrations (ng/ml) in nicardipine and anaphylaxis groups. Time 0 ( $T_0$ ) corresponds to injection of nicardipine or ovalbumin. (A) The nicardipine group was characterized by a significant increase in interstitial epinephrine concentration at T40 to T60 versus T-20 to T0 ( $*P < 0.0001$ ) as compared with the anaphylaxis group. This resulted in a significantly greater gradient between plasma and interstitial epinephrine concentrations during anaphylaxis as compared with the nicardipine group. (B) Anaphylaxis was characterized by a stronger sympathetic activation with higher interstitial norepinephrine concentrations occurring early after the injection of ovalbumin and sustained during the entire study (from  $0.12 \pm 0.03$  [T-20 to T0] to  $0.96 \pm 0.20$  ng/ml [T40 to T60];  $*P < 0.001$ ). Despite arterial hypotension of similar magnitude, the nicardipine group had a smaller increase in interstitial norepinephrine concentrations (from  $0.19 \pm 0.04$  [T-20 to T0] to  $0.38 \pm 0.08$  ng/ml [T40 to T60];  $*P < 0.001$ ). A between-group difference was observed at T40 to T60 ( $\dagger P < 0.05$ ).



when the pyruvate concentrations were profoundly decreased. In contrast, in the nicardipine group, a moderate increase in lactate interstitial concentrations was observed (from  $0.536 \pm 0.089$  to  $0.843 \pm 0.113$  mM;  $P < 0.05$ ), whereas pyruvate interstitial concentrations were preserved (from  $0.032 \pm 0.006$  to  $0.036 \pm 0.007$  mM;  $P =$  not significant), and therefore, lactate/pyruvate ratios only moderately increased (from  $17 \pm 2$  to  $26 \pm 5$ ;  $P =$  not significant).

## Discussion

The main findings of this study are that (1) anaphylactic shock has a distributive profile characterized by preserved cardiac output and severe skeletal muscle vasoconstriction probably related to the activation of SNS and (2) there is an anaerobic metabolism related to a rapid decrease in skeletal muscle  $P_{tO_2}$ . This is consistent with lack of inhibition of cellular oxygen consumption.

## Methodologic Discussion

The ovalbumin-sensitized Brown Norway rat model has been previously characterized as a suitable model of anaphylactic shock.<sup>12</sup> In Brown Norway rats, hypersensitivity reactions develop that are similar to human anaphylaxis, and circulating antibodies seen after immunization belong to a class of immunoglobulins analogous to human immunoglobulin E.<sup>19</sup> The experimental model and the study design were chosen to reproduce the clinical conditions experienced by patients during anesthesia when anaphylactic shock occurs. This study was performed in ovalbumin-sensitized rats, and extrapolation of our results to humans should be cautious. We deliberately investigated several aspects of the pathophysiology of anaphylactic shock during anesthesia; therefore, this clearly limits extrapolation of these results to anaphylactic shock that occurs in awake animals or humans.

Microdialysis is an *in vivo* technique that was initially

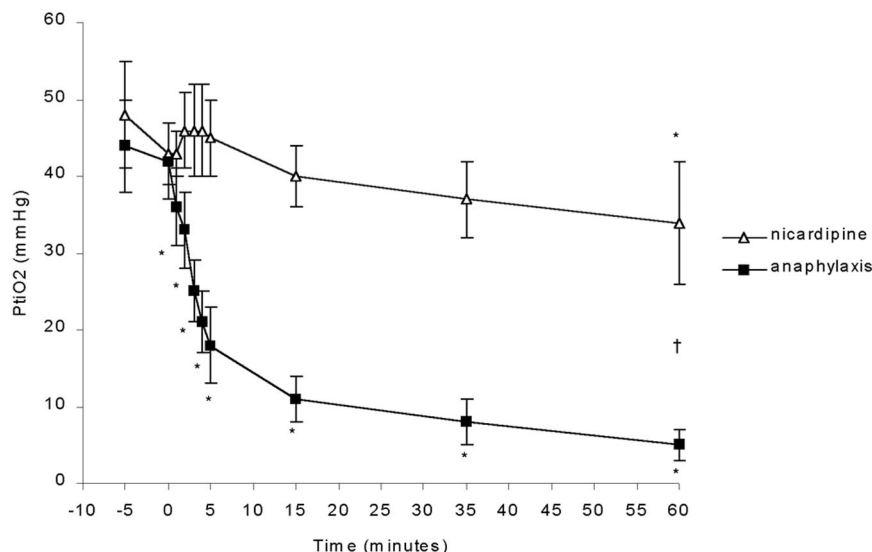


Fig. 4. Time course of tissue oxygen pressure ( $P_{tiO_2}$ ) values in nicardipine and anaphylaxis groups. Time 0 (T0) corresponds to injection of nicardipine or ovalbumin. Anaphylaxis was characterized by an immediate and severe decrease in  $P_{tiO_2}$  values occurring within the first 5 min after ovalbumin injection (from  $42 \pm 5$  mmHg to  $18 \pm 5$  mmHg; \*  $P < 0.0001$ ) and further decrease over time to a nadir value of  $5 \pm 2$  mmHg (\*  $P < 0.0001$ ). In contrast, in the nicardipine group, despite arterial hypotension of similar magnitude,  $P_{tiO_2}$  was initially maintained ( $43 \pm 7$  mmHg) and moderately decreased subsequently to a nadir value of  $34 \pm 8$  mmHg (\*  $P < 0.05$ ). A between-group difference was statistically significant as early as 3 min after T0 ( $\dagger P < 0.05$ ).

applied to investigate the kinetics of various neurotransmitters and metabolites within the extracellular space in several regions of the brain.<sup>20</sup> We previously characterized the combined use of microdialysis and polarographic Clark-type oxygen electrodes<sup>21</sup> for measurement of both oxygen availability and cellular metabolism within the same tissue microenvironment. Specifically, we showed that the combination of the two probes did not modify the accuracy of measurements of the individual probes.<sup>21</sup> The combined use of the two probes allowed us to investigate the consequences of anaphylactic shock on skeletal muscle blood flow distribution (ethanol microdialysis technique), skeletal muscle  $P_{tiO_2}$ , and metabolic aerobic/anaerobic profile.

#### *Anaphylactic Shock Is Characterized by Arterial Hypotension*

Mean arterial pressure values decreased rapidly after allergen challenge (within the first 5 min) to very low values comparable to those measured during anaphylactic shock in anesthetized dogs.<sup>22,23</sup> This was associated with unchanged cardiac output as assessed by preserved aortic velocity values (table 1), therefore indicating a decrease in systemic vascular resistance according to the law of Poiseuille, which describes the pressure–flow–resistance relation.<sup>24</sup> No change in heart rate was ob-

served in our study. Taken together, these results are consistent with preserved cardiac function, at least within the first minutes after allergen challenge. This interpretation is reinforced by the results of another study suggesting that cardiac dysfunction was not a major mechanism of hypotension during anaphylactic shock.<sup>22</sup> Nevertheless, the current study cannot exclude alteration of myocardial contractility, as suggested by other studies.<sup>25–27</sup> It is probable that any alteration of myocardial contractility, if present, was compensated by decreased left ventricular afterload that initially maintained the cardiac output.

#### *Anaphylactic Shock Is Characterized by Redistribution of Blood Flow*

Although there was a decrease in systemic vascular resistance, as described above, it is probable that there was marked heterogeneity within different regional circulations. We investigated skeletal muscle perfusion because this organ can sustain intense vasoconstriction to redistribute cardiac output to organs such as the brain and heart. Skeletal muscle blood flow was investigated directly by the ethanol microdialysis technique and indirectly by the measure of  $P_{tiO_2}$  used to evaluate the balance between oxygen availability and oxygen consumption. The information provided by these two methods is consistent

Table 3. Time Course of Interstitial Lactate and Pyruvate Concentrations in Nicardipine and Anaphylaxis Groups

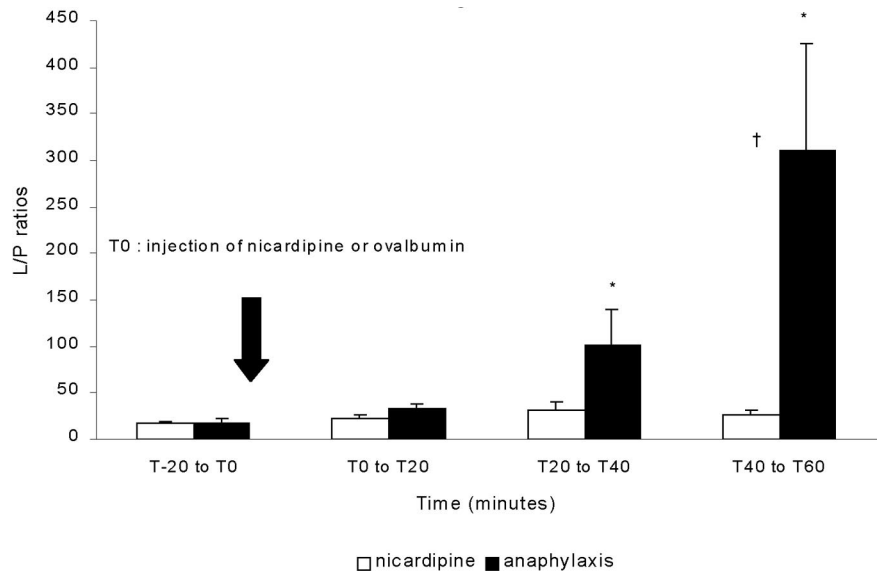
Time Interval, min	Lactate, mm		Pyruvate, mm	
	Nicardipine	Anaphylaxis	Nicardipine	Anaphylaxis
T–20 to T0	$0.536 \pm 0.089$	$0.446 \pm 0.105$	$0.032 \pm 0.006$	$0.034 \pm 0.010$
T0 to T20	$0.676 \pm 0.123$	$0.786 \pm 0.272$	$0.031 \pm 0.006$	$0.022 \pm 0.040$
T20 to T40	$0.866 \pm 0.166^*$	$1.274 \pm 0.314^*$	$0.034 \pm 0.009$	$0.014 \pm 0.030^*$
T40 to T60	$0.843 \pm 0.113^*$	$1.741 \pm 0.459^*$	$0.036 \pm 0.007$	$0.006 \pm 0.002^{\dagger}$

Time0 (T0) corresponds to injection of nicardipine or ovalbumin. Values are expressed as mean  $\pm$  SEM.

\*  $P < 0.05$  vs. T–20 to T0,  $\dagger P < 0.05$  for the between-groups comparison by two way-analysis of variance.



**Fig. 5.** Time course of lactate/pyruvate (L/P) ratios in nicardipine and anaphylaxis groups. Time 0 corresponds to injection of nicardipine or ovalbumin. After injection of ovalbumin, a strong but delayed increase in L/P ratios (from  $17 \pm 6$  [T-20 to T0] to  $311 \pm 115$  [T40 to T60]; \*  $P < 0.05$ ) was observed. In the nicardipine group, L/P ratios remained constant. A between-group difference was observed at T40 to T60 ( $\dagger P < 0.05$ ).



with marked vasoconstriction in skeletal muscle, which was of higher amplitude in anaphylactic shock than in the nicardipine group. A decrease of similar amplitude in skeletal muscle blood flow was reported during the hemorrhagic shock model.<sup>28,29</sup>

#### *Anaphylactic Shock Is Associated with Strong Activation of the SNS*

Our results suggest that during anaphylactic shock, activation of SNS in compartments, such as the skeletal muscles (which correspond to 30–40% of the total body mass), might be one of the main mechanisms of blood flow redistribution to metabolically active tissues or organs. In this experimental model of anaphylactic shock, the amplitude of SNS activation was high. The increase in plasma epinephrine and norepinephrine concentrations occurred in both ovalbumin- and nicardipine-induced hypotensive states but was of higher amplitude during anaphylactic shock, where a 15-fold epinephrine increase and a 10-fold norepinephrine increase over baseline values were measured 1 h after allergen challenge. This profile of higher epinephrine than norepinephrine plasma concentrations in anaphylactic shock is different from that observed in the early phase of septic<sup>30</sup> or hemorrhagic shock.<sup>31</sup>

In addition, the reduced skeletal muscle blood flow, probably responsible for the increased plasma/interstitial gradient of epinephrine in the anaphylactic shock group, further confirms the intense vasoconstriction observed in the muscle compartment. Skeletal muscle vasoconstriction could also have modulated the higher norepinephrine (directly released by the sympathetic nerve endings within tissues) interstitial concentrations by reducing its vascular clearance. In addition, skeletal muscle hypoxia may have reduced norepinephrine reuptake, which is an adenosine 5'-triphosphate-dependent mechanism.<sup>32</sup> Moreover, some studies have shown

a significant contribution of  $\alpha$ -adrenergic vasoconstrictor tone to the critical oxygen extraction in the hind limb and the whole body during progressive hypoxia and underscore the significance of an active sympathetic response for efficient tissue oxygen utilization, especially in states in which oxygen delivery to an isolated region is reduced.<sup>33,34</sup> Taken together, our results suggest that in the anaphylactic shock group, the intense vasoconstriction with decreased  $Pt_{iO_2}$  observed in skeletal muscle is probably not only the consequence of low MAP values (see nicardipine group), but we speculate that inflammation, SNS activation, or other unknown mechanisms are probably the main culprits.

#### *Anaphylactic Shock Is Characterized by Skeletal Muscle Anaerobic Metabolism and Low $Pt_{iO_2}$ Values*

One of the main findings of this study is that, in anaphylactic shock, anaerobic metabolism in skeletal muscle is associated with profoundly and rapidly decreased  $Pt_{iO_2}$  values. The decreased  $Pt_{iO_2}$  values observed in the current study are probably the consequence of (1) skeletal muscle vasoconstriction; (2) lack of inhibition of oxygen consumption based on the initial sharp decrease in  $Pt_{iO_2}$  values; or (3) a factor that probably worsened tissue hypoxia, *i.e.*, the SNS-driven increase of oxygen consumption.<sup>35</sup> The  $Pt_{iO_2}$  profile in anaphylactic shock is unique because of its rapid decrease. This  $Pt_{iO_2}$  decrease is similar in amplitude (but not in speed of onset) to that observed in hemorrhagic shock<sup>28,36,37</sup> but differs from that reported in septic shock where animal and human studies have shown that septic shock could be characterized by high<sup>38,39</sup> or low  $Pt_{iO_2}$  values.<sup>40,41</sup> The severe decrease in  $Pt_{iO_2}$  observed during anaphylactic shock was associated with anaerobic metabolism without inhibition of the respiratory chain. Measurement of the different metabolites was



performed in skeletal muscle and thus reflects local production and clearance. If oxygen availability is limited, adenosine 5'-triphosphate production is slowed, and the inhibitory effect of adenosine 5'-triphosphate on phosphofructokinase is relieved so that glycolysis (anaerobic pathway) is stimulated, thus resulting in increased pyruvate production. Pyruvate accumulates and is converted to lactate as long as glucose substrate is available. Excess lactate formed during glycolysis leaves the cells and accumulates in the interstitial fluid, explaining its rapid increase during anaphylactic shock. The persistence of shock leads to glucose depletion and/or the inhibition of phosphofructokinase due to increased proton concentration. This leads to the dramatic decrease in pyruvate concentration and therefore to the impressive increase in lactate/pyruvate ratio. In contrast, in the nicardipine group, the moderate decrease in  $P_{tO_2}$  was associated with preserved aerobic metabolism.

In summary, our results suggest that anaphylactic shock has skeletal muscle  $P_{tO_2}$  and metabolic profiles that clearly are not similar to those reported in septic or hemorrhagic shock.<sup>28,36-41</sup> The sharp and rapid decrease in  $P_{tO_2}$  values is not explained by the level of arterial hypotension alone. The stronger local activation of SNS in anaphylactic shock as compared with the nicardipine group probably contributes to the lower  $P_{tO_2}$  values. Nevertheless, we do not believe that the differences in the amplitude of local SNS activation explain the large difference in  $P_{tO_2}$  values between the anaphylactic shock and nicardipine groups. We speculate that another factor contributing to the rapid decrease of  $P_{tO_2}$  values in anaphylactic shock is the specific inflammatory profile of anaphylaxis. The sharp decrease of  $P_{tO_2}$  values in skeletal muscle, in the absence of inhibition of cellular metabolism, results initially in anaerobic glycolysis, followed over time by depletion of substrates, which finally results in decreased pyruvate production. This results in a sharp increase in lactate/pyruvate ratios, which represents a complete failure of energy production within skeletal myocytes.<sup>35</sup> If this phenomenon occurs in other organs, tissues, and cells, it could result in rapid alteration of cell and organ functions and could explain the failure of well-conducted resuscitation to restore cardiovascular homeostasis even in previously apparently healthy individuals. These results could explain why rapid diagnosis and therapy of anaphylactic shock are mandatory<sup>2</sup> to prevent cellular and organ energy depletion and altered function.

The authors thank Pierre Mairose and Maryline Schwartz (Technicians, Laboratoire d'explorations fonctionnelles métaboliques et endocriniennes Centre Hospitalier Universitaire Brabois, Vandoeuvre les Nancy, France) and Gaille Belle (INSERM 684, Vandoeuvre-les-Nancy, France) for their technical assistance.

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