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Can the "brain-sparing effect" be detected in a small-animal model?

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Under O_2 imbalance in the body, blood redistribution occurs between more vital organs and less vital organs. This response is defined as the "brain-sparing effect". The study's aim was to develop a new rat model for simultaneous real-time monitoring of tissue viability in a highly vital organ, the brain, and a less vital organ, the small intestine, under various metabolic perturbations and emergency-like situations.

Material/Methods: The cerebral cortex and intestinal serosa were exposed in anesthetized rats and a multi-site multiparametric (MSMP) monitoring system was connected to both. Tissue blood flow (TBF) was monitored using laser Doppler flowmetry and mitochondrial function by NADH fluorometry. The perturbations performed were anoxia (30 sec) and 20 minutes of hypoxia, hypercapnia, or hyperoxia.

Results: Under oxygen deficiency, cerebral blood flow (CBF) increased (315±53% in anoxia and 140±12% in hypoxia), whereas intestinal blood flow decreased (60±11% in anoxia and 56±13% in hypoxia). Mitochondrial NADH significantly increased in both organs (119±2.8% and 151±14% in the brain and intestine, respectively). Under hyperoxia, NADH was oxidized in both organs (up to 9% change). Hypercapnia led to an increase in CBF (143±11%) and oxidation of mitochondrial NADH (by 10%), with no significant changes in the intestine.

Conclusions: The two organs respond significantly differently to lack of O_2 by activating the sympathetic nervous system. Monitoring less vital organs may indicate an early response to emergency situations. Therefore, a less vital organ could be used as a surrogate organ to be monitored in order to spare the brain.

key words: **mitochondrial NADH • tissue viability • microcirculatory blood flow • hypoxia • hypercapnia • hyperoxia**

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BACKGROUND

It is well known that under emergency situations the autonomic nervous system (ANS), mainly its sympathetic branch including the adrenal gland, dominates the compensatory mechanisms of the body related to O_2 deficiency (Figure 1). The rapid compensatory reaction to a decrease in blood volume (hypovolemia), for example, includes redistribution of blood flow (and consequently O_2) to various organs, giving preference to the most vital organs in the body, namely the brain, heart, and adrenal glands [1–5]. The less vital organs and tissues are hypoperfused and O_2 supply decreases [6]. Nevertheless, only very few studies had proved the concept of blood-flow redistribution developed under an emergency situation in animal models [7–9].

Many critical conditions are characterized by tissue hypoxia, which often leads to "cytopathic hypoxia" [10–14]. Since most of the oxygen in the body is utilized by the mitochondria [15] to produce ATP, it is obvious that mitochondrial function plays a crucial role in tissue and organ vitality. Recently it has been indicated that optimization of oxygen delivery is the best method for the prevention and the only method for the treatment of common intensive care syndromes such as sepsis, multiple organ dysfunction syndrome (MODS), and acute lung injury [16]. Consequently, a vast amount of resources in critical care research is directed at identifying and analyzing generic markers of incomplete resuscitation [17–21]. Today, the most familiar parameters being monitored in ICUs and operating rooms include pCO_2, pH, pO_2, Cyt aa_3, and tissue blood flow (TBF), some of which are monitored in the blood and some in various tissues [22–27]. However, most of these techniques are often insensitive, nonspecific, and show abnormality only at a very late stage of disease [17]. In view of this, techniques that directly appraise the tissue energetic state would be an optimal approach for the evaluation of tissue integrity [28].

The mitochondrial NADH redox state is the most sensitive parameter of oxygen deficiency and is well correlated with tissue pO_2 level under oxygen-deficiency conditions [29–31]. Therefore, in the present study we aimed to simultaneously monitor tissue blood flow and mitochondrial NADH fluorescence in the brain, a vital organ, and the small intestine, a less vital organ, under oxygen deficiency (anoxia and hypoxia) and hypercapnia.Our hypothesis was that by monitoring the small intestine together with the brain using this unique monitoring device in different acute stressful conditions, early detection of a systemic impaired metabolic state of the body can be accomplished assuming that this model will help to characterize the "brain-sparing effect" developed under various emergency situations.

MATERIAL AND METHODS

The multi-site multi-parametric (MSMP) monitoring system

Monitoring of the rat brain and small intestine was performed using a multi-site multi-parametric (MSMP) monitoring system developed in our laboratory. Each channel of this monitoring device contains a bundle of optical fibers for NADH redox state monitoring using the fluorometric technique and another bundle of fibers for tissue blood flow (TBF) monitoring using laser Doppler flowmetry (Figure 2). The diameter of the probe (including all fibers) is 3 mm.

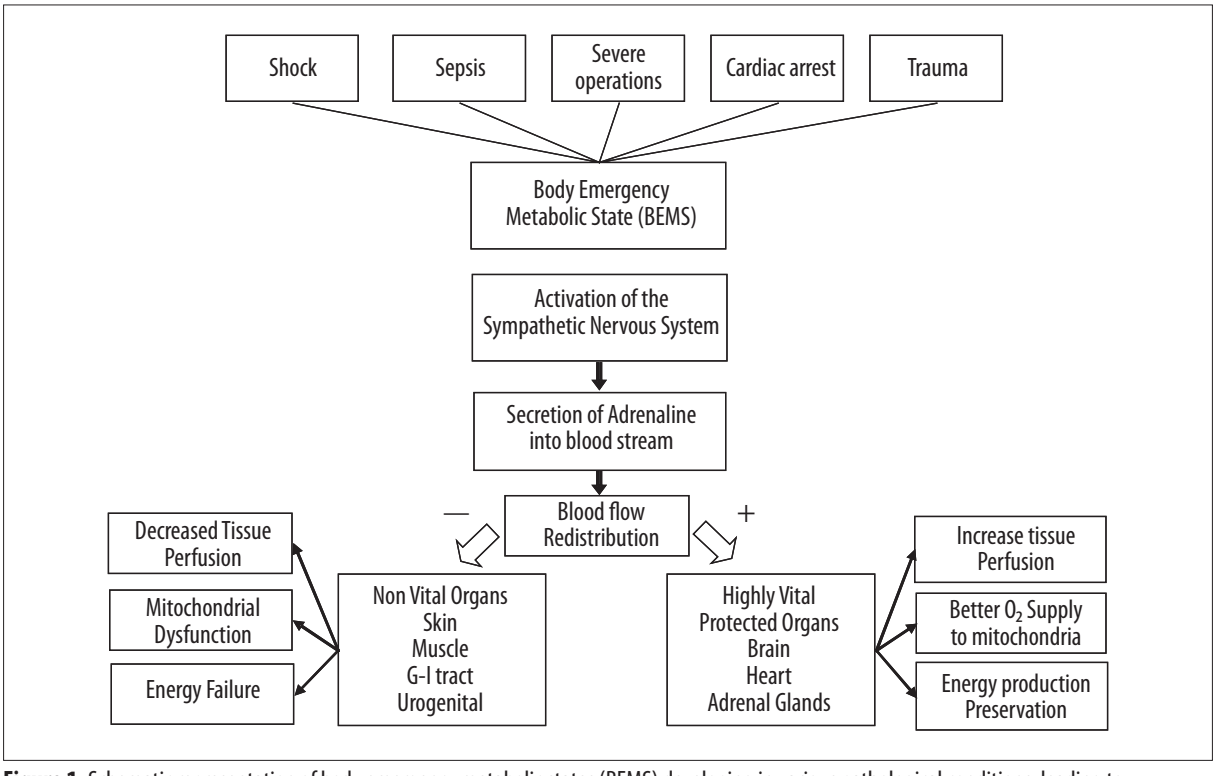

Figure 1. Schematic representation of body emergency metabolic states (BEMS) developing in various pathological conditions, leading to redistribution of the blood flow toward the most vital organs at the expense of less vital organs

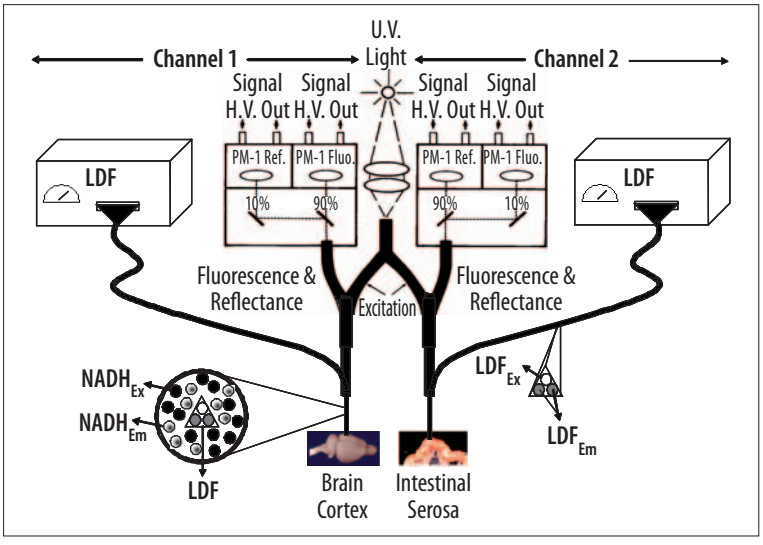

Figure 2. Schematic presentation of the MSMP (multisite-multiparametric) monitoring system and its location on the brain surface and intestinal serosa. Tissue blood flow (TBF) was monitored using the LDF technique: each bundle contains an excitation fiber (LDF_{ex}) and two emission fibers (LDF_{em}). Mitochondrial NADH was monitored by the fluorometric technique using a two-channeled DC fluorometer-reflectometer and a bundle of optical fibers for tissue excitation ($NADH_{ex}$) and emission ($NADH_{em}$). The LDF fibers are located together with the NADH fibers, enabling simultaneous monitoring of TBF and mitochondrial NADH redox state from the same location on the tissue.

The principle of NADH monitoring from the surface of the tissue (up to 1 mm in depth) is that excitation light (366 nm) passes from the fluorometer to the tissue via a bundle of optical fibers made of quartz. The emitted light (450 nm fluorescence), along with the reflected light (366 nm reflectance), is transmitted to the fluorometer via another bundle of fibers [32]. The emitted light passes through appropriate filters in order to differentiate between the 366-nm reflectance and the NADH fluorescence (450 nm). In addition, a specific filter is used to prevent red light (laser Doppler flowmeter) from interfering with mitochondrial NADH monitoring. Changes in the 366-nm reflected light are correlated to changes in tissue blood volume and therefore serve as a correct tool for the hemodynamic artifacts in NADH monitoring [33]. The fluorometer is calibrated in such a way that a range of 500 mV is equal to a change of 100% in the monitored signal. The corrected fluorescence (NADH) is obtained by subtracting the reflectance signal from the fluorescence signal at a 1:1 ratio [33].

In general, an inverse linear correlation was clearly demonstrated between the changes in NADH level and tissue metabolic activity. Namely, under conditions in which the oxygen supply to the tissue is prevented (hypoxia, ischemia) and the metabolic activity decreases, the NADH level increases, whereas under conditions in which tissue activity is increased (such as under cerebral cortical spreading depression), NADH is oxidized and its level decreases [34].

Tissue blood flow was monitored using a laser Doppler flowmeter, based on the Doppler shift reflecting the flow of red blood cells in the tissue for a depth of 1–2 mm [35]. All the signals monitored during the experiment were digitized and transmitted to a multi-channeled computerized data acquisition and recording system (Labview A/D software, National Instruments Co, USA) for further analysis.

Animal preparation

All experiments were performed in accordance to the Guidelines of the Animal Care Committee of Bar Ilan University. Wistar male rats (250–300 g) were anesthetized by i.p. injection (0.3 ml/100 g body weight) of Equithesin (each ml containing 9.72 mg pentobarbital, 42.51 mg chloral hydrate, 21.25 mg magnesium sulfate, 44.34% w/v propylene glycol, 11.5% alcohol, and distilled water). During the entire experimental period, anesthesia was maintained by 0.1-ml Equithesin injections every 30 min and the rats were breathing spontaneously. The rats were placed on a warming plate and body temperature was maintained at 37°C. Polyethylene catheters were introduced into the femoral vein for drug administration and into the femoral artery for systemic blood pressure monitoring. To prepare for brain monitoring, the rat was placed in a special head holder. A 6-mm-diameter hole was drilled in the parietal bone and the bone was removed (the dura mater remained intact). A special light guide was then placed above the brain surface. The optical fiber probes of the MSMP device were later placed inside the holder. Two stainless steel screws were inserted into the parietal bone for better fixation of the MSMP holder to the skull surface by dental acrylic cement. Then the rat was placed on its back and a 2-cm incision was performed in the abdomen. A small segment of the small intestine was exposed and the monitoring probe was placed on the intestinal serosa using a micromanipulator and then connected to the intestinal serosa by cyanoacrylate adhesive [36].

Experimental protocols

After surgery and fixation of the MSMP system to the brain and to the intestinal serosa, the following protocols were implemented:

Anoxia

All animals were exposed to a short anoxia (30 sec) induced by 100% nitrogen inhalation. This procedure was performed to ensure the correct placement of the monitoring probe on the monitoring site (the brain and intestinal serosa) as well as to test the organs' response to a complete lack of oxygen [35].

Hypoxia, hypercapnia, and hyperoxia

Three groups of 10 rats each were exposed to the following gas mixtures for a period of 20 min; thereafter the rats

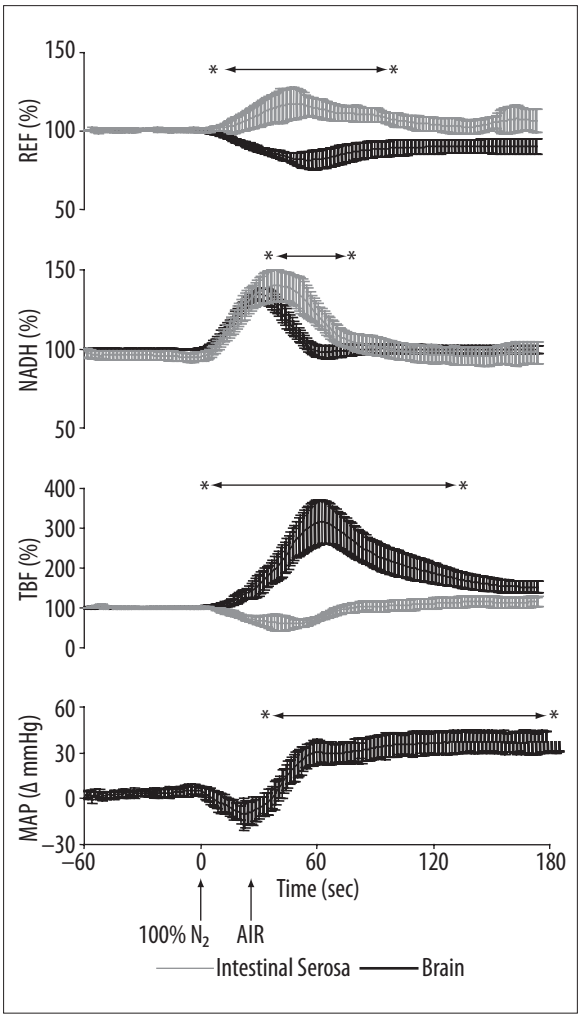

Figure 3. The responses of the brain cortex and intestinal serosa (mean ±*S.E.*) to short anoxia (100% N_2). Ref – reflectance; NADH – mitochondrial NADH redox state; TBF – tissue blood flow; MAP – mean arterial pressure. n=10, (*) $p<0.05$.

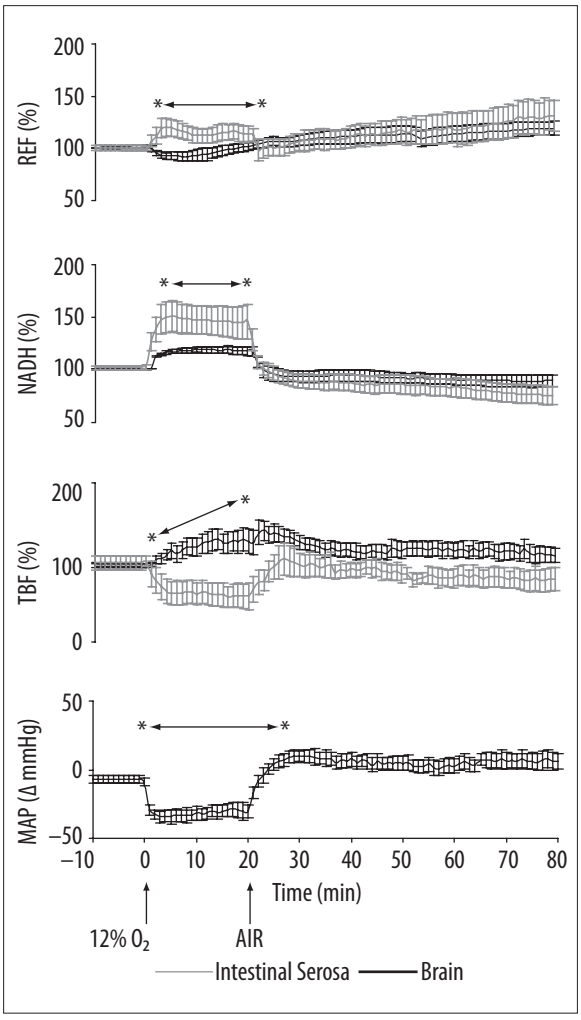

Figure 4. The responses of the brain cortex and intestinal serosa (mean ±*S.E.*) to hypoxia induced by exposure to a low oxygen mixture: 12% O_2 + 87% N_2 + 1% CO_2. Ref – reflectance; NADH – mitochondrial NADH redox state; TBF – tissue blood flow; MAP – mean arterial pressure. n=10, (*) $p<0.05$.

were allowed to breathe normal air for 90 minutes. At the end of these periods, the rats were sacrificed by pure N_2 inhalation.

Hypoxia: 12% O_2 + 87% N_2 + 1% CO_2
Hypercapnia: 21% O_2 + 69% N_2 + 10% CO_2
Hyperoxia: 100% O_2

The main limitation in this study is the inability to withdrawn blood for gas analysis in the same rats that were monitored. This is because the femoral artery (which is the main blood vessel commonly used for blood withdrawal in a rat model) is near the intestine, and any manipulation near the intestinal monitoring site yielded artifacts in the intestinal monitored signals. Furthermore, using another blood vessel for blood withdrawal, such as the carotid artery, will lead to altered cerebral blood supply. Moreover, since the present study is based on the differences in the responses of the brain and intestine to systemic alterations, it is clear that under the various protocols used, both organs are exposed to the same pO_2, pCO_2, and pH levels. Nevertheless, blood pO_2, pCO_2, and pH were evaluated using a group of rats in another study performed previously in our laboratory. In the same species of Wistar rats the same protocol, namely spontaneous breathing of similar mixtures of gases for similar durations, was applied [37]. In addition, we monitored arterial blood pressure in order to achieve important information on the cardiovascular state of the rat.

Statistical analysis

The two-tailed paired Student's *t*-test was used to examine the differences between the responses of the brain and intestinal serosa to various oxygen and CO_2 concentrations at specific time points (in anoxia for each second and in the rest of the groups for each minute). The two-tailed unpaired Student's *t*-test was used to examine the significance of changes in each parameter at specific points after the experimental period compared with its first baseline value. A value of $p<0.05$ was considered to be significant. All values are presented as the average ±*S.E.* In NADH calculations, the basal level before perturbation was considered

Table 1. The major (min/max) changes in the systemic blood pressure and cerebral and intestinal parameters during the induction of anoxia, hypoxia, and hypercapnia.

Perturbation	MAP change	Parameter	Brain	Intestine
Anoxia	−10±8	TBF	315±53	60±11
		NADH	134±3.8	139±10
		REF	81.5±5.7	117±9.7
Hypoxia	−34±4	TBF	140±12	56±13
		NADH	119±2.8	151±14
		REF	92±2	119±8.2
Hypercapnia	+13±14	TBF	143±11	113±12
		NADH	90.3±1.3	93±1.6
		REF	121±2.7	90±7.6

The maximum and minimum changes (mean ±*S.E.*) in tissue blood flow (TBF), mitochondrial NADH redox state (NADH), tissue reflectance (REF), and mean arterial blood pressure (MAP) in the brain and intestinal wall during the induction of three models of acute stress (anoxia, hypoxia, and hypercapnia). The basal level for each parameter was 100% and the changes were monitored within the time the rats were exposed to the specific gas mixtures according to the protocol.

Table 2. Effects of hypoxia (12% O_2, 1% CO_2, 87% N_2) and hypercapnia (10% CO_2, 21% O_2, 69% N_2) on sample blood parameters.

	Control (before hypoxia)	Hypoxia	Control (before hypercapnia)	Hypercapnia
pO_2	108.27±5.59	54.39±3.88***	113.83±7.65	160.49±7.7***
pCO_2	36.42±2.82	30.04±3.18*	37.47±6.83	69.94±5.95**
PH	7.22±0.03	7.27±0.03***	7.26±0.05	7.07±0.05***
% sat.	95.39±0.84	77.42±4.63**	96.43±0.52	97.64±0.13

The levels of pO_2, pCO_2, pH, and the saturation of arterial blood before and following hypoxia or hypercapnia induction. Hypoxia: the rats were exposed to a gas mixture of 12% O_2 + 1% CO_2 + 87% N_2 for 20 minutes and under hypercapnia the rats were exposed to a gas mixture of 10% CO_2 + 21% O_2 + 69% N_2 for 20 minutes. Significance between control levels and the levels measured under treatment are presented as follows (*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$.

100% and NADH reduction is shown by NADH increase above 100% and NADH oxidation is indicated by NADH decrease below 100%.

Tissue blood flow at death was considered 0%, whereas the range of signal from the basal level to the death level was considered as 100%. Mean arterial pressure (MAP) values are presented as the changes in mmHg.

Results

In order to test the ability of the MSMP device to detect changes in the hemodynamic and metabolic states of the brain and the intestine simultaneously in a rat model, we used several experimental protocols. Exposure of the rat to various concentrations of oxygen or CO_2 enabled us to test the differences in the responses of these two organs to oxygen deficiency or surplus.

As it is seen in Figure 3 and Table 1, 100% N_2 inhalation for 30 sec caused a decrease of 10±8 mmHg in MAP, followed by an increase of approximately 140% in NADH in both organs. With respect to blood flow, a decrease of 40±11% ($p<0.01$) was observed in the intestine, while in the brain, CBF increased to the level of 315±53% ($p<0.01$). Moreover, the kinetics of response in the two organs was different. The time needed for CBF to reach maximum hyperemia was 120 sec, whereas in the intestine the maximum decrease in TBF was recorded 40 sec after the beginning of anoxia. During the recovery phase, when the rat breathed normal air, NADH in the brain decreased (oxidized) immediately, whereas in the intestine NADH decreased only 20 sec later. Also, during the recovery phase, CBF continued to increase for 30 sec, even though the rats were breathing air, and the recovery lasted for 90 sec (until CBF reached its basal level). In contrast, TBF in the intestine returned to its baseline level 30 sec after air inhalation. Changes in the reflectance, traced in both organs, were inversely correlated to changes in tissue blood flow.

Previous studies in our laboratory showed that by exposing rats (n=6) (breathing spontaneously) to a gas mixture

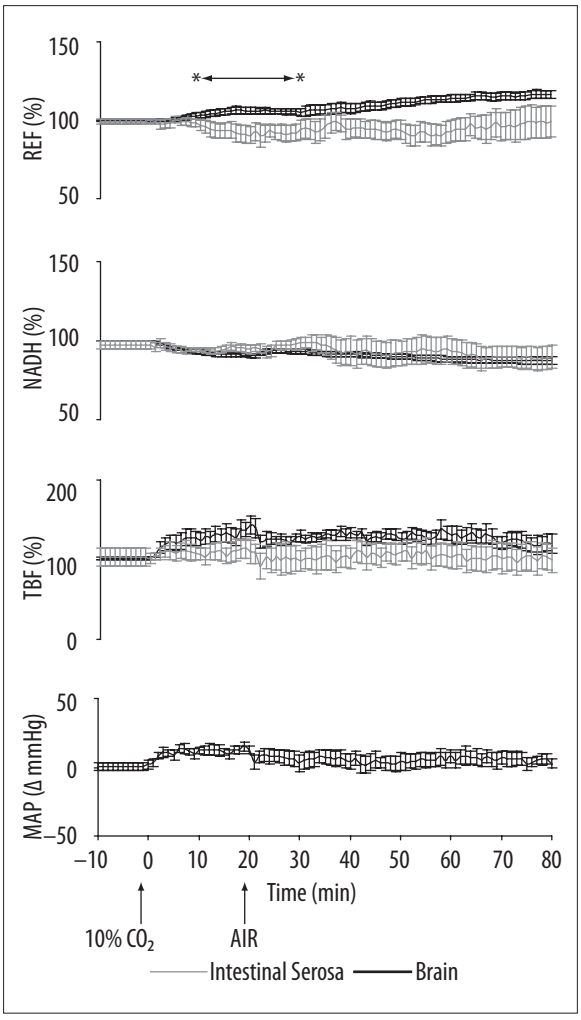

Figure 5. The responses of the brain cortex and intestinal serosa (mean ±*S.E.*) to hypercapnia induced by exposure to a high CO_2 mixture: 21% O_2 + 69% N_2 + 10% CO_2. Ref – reflectance; NADH – mitochondrial NADH redox state; TBF – tissue blood flow; MAP – mean arterial pressure. n=10, (*) $p<0.05$.

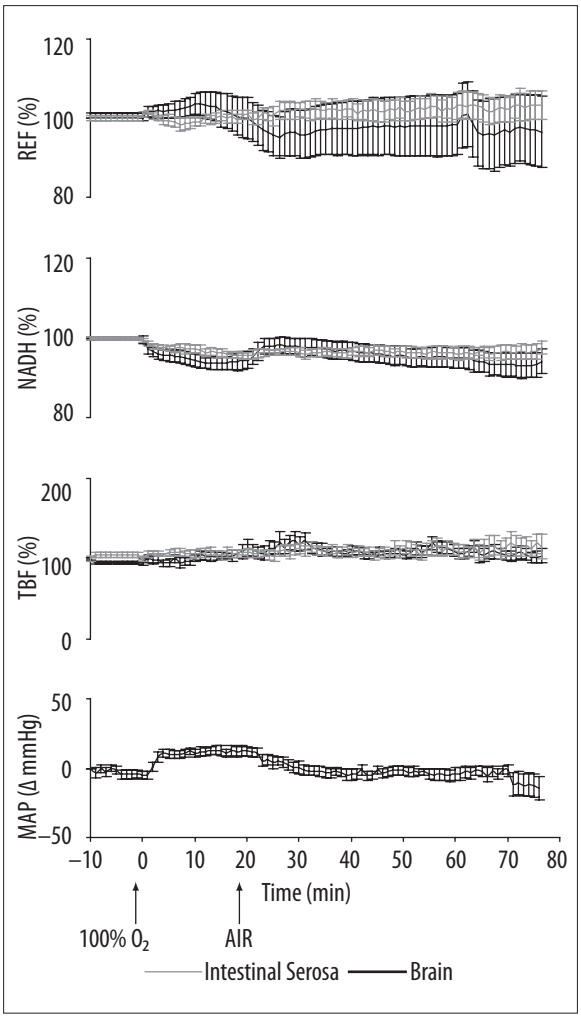

Figure 6. The responses of the brain cortex and intestinal serosa (mean ±*S.E.*) to hyperoxia induced by exposure to 100% oxygen. Ref – reflectance; NADH – mitochondrial NADH redox state; TBF – tissue blood flow; MAP – mean arterial pressure. n=10, (*) $p<0.05$.

of 12% O_2: 1% CO_2: 87% N_2 (hypoxia), pO_2 decreased to 54±4 mmHg, pCO_2 decreased to 30±3 mmHg, and pH increased to 7.27±0.03 (Table 2). The decrease in pCO_2 and the increase in pH level was probably caused by the spontaneous hyperventilation that results from exposure to the low oxygen level in the inspirited air. When testing the effects of hypoxia on the brain and intestinal metabolism, the following results were recorded (Figure 4 and Table 1): MAP decreased by 34±4 mmHg ($p<0.001$) immediately after the rats started breathing the low-oxygen mixture. This change was followed by a decrease in TBF in the intestine to a level of 56±13% ($p<0.05$) and a tendency of CBF increase to a level of 140±12%. Simultaneously, the reflectance in the intestine increased (119±8%, $p<0.05$), while in the brain it decreased (92±2%, $p<0.01$). The changes in the blood supply to the organs produced corresponding changes in the levels of mitochondrial NADH. In the intestine, NADH increased to a level of 151±14% ($p<0.01$) and remained at this level throughout the entire hypoxic period, while in the brain NADH reached a level of 119±2.8% ($p<0.001$). When the rats started breathing air, all the parameters returned to the basal level within 2 min in the intestine, whereas in the brain hyperemia was observed for 11 minutes, followed by full recovery. With reference to NADH and reflectance, full recovery was observed immediately after the rats started breathing air.

Under hypercapnia induced by 10% CO_2 in air (in another group of 6 rats), pCO_2 increased to approximately 70±6 mmHg and pO_2 increased to 160.5±7.7 mmHg (Table 2). In the group of rats that were monitored by the MSMP system (Figure 5), hypercapnia induced the following results: There were no significant differences between the brain and intestine, except for the difference in reflectance at the 17th minute, when it increased in the brain to 106±2% and remained constant in the intestine. When testing the effects of hypercapnia on each organ separately, the following results were obtained: In the brain, hypercapnia caused a significant increase in CBF to a level of 143±11% ($p<0.05$) 6 min after hypercapnia induction. The hyperemia continued for approximately 60 min. However, mitochondrial NADH was decreased from the very beginning of hy-

percapnia until the end of the experiment. Considering systemic changes, a significant MAP increase of 13±4 mmHg was seen 3 min after hypercapnia induction, and it continued to increase for 17 min. Hypercapnia effected no significant changes in intestinal TBF. However, a significant decrease in NADH was observed 4 min after the rats were exposed to high CO_2 concentrations. The oxidation of mitochondrial NADH continued for 10 min.

Figure 6 demonstrates the responses of both organs to hyperoxia at 100% O_2. No significant differences between the two organs were observed. Mitochondrial NADH was significantly oxidized in both organs during the entire period of exposure to 100% O_2, reaching levels of 93±1.5% in the brain (p<0.01) and 95±0.6% in the intestine (p<0.001).

Discussion

The aim of the present study was to establish a new small-animal model that would yield conditions similar to some of the emergency situations developed in patients. We used a monitoring system that provide real-time data on microcirculatory blood flow and volume as well as the intramitochondrial redox state of NADH in rats exposed to systemic perturbations. Under critical conditions, blood flow to the gastrointestinal tract is selectively and markedly reduced, and the small intestine is one of the first organs to be affected [38], making it ideal to serve as a surrogate organ to the brain for deterioration of body oxygenation. Additionally, the GI tract is relatively easy to access in experimental as well as clinical situations and provides reliable information.

The three monitored parameters represent the behavior of the microcirculatory hemodynamics and its coupling to intracellular mitochondrial function. In order to evaluate tissue oxygenation under anoxia, hypoxia, hypercapnia, and hyperoxia, we used the intracellular marker located in the mitochondria, which are responsible for generating most of the ATP in the tissue. As shown in Table 2, the perturbations used led to changes in systemic arterial blood parameters. The two monitored organs were exposed to the same internal environment, but their responses were very different. We believe that the use of electrodes for tissue pO_2 measurements is less accurate than the evaluation of mitochondrial NADH redox state since it is very sensitive to tissue blood flow and provides an average level of oxygenation of the microcirculatory blood and extracellular and intracellular compartments. Tissue microcirculatory blood flow together with NADH redox state provides, to the best of our knowledge, the most important information to describe the metabolic activity in the tissue. Since we compared the responses of two different organs in the same body, the absolute level of pO_2 is less important. By measuring the NADH redox state we bypass the need for pO_2 values, which are also very difficult to calibrate *in vivo* due to O_2 consumption by the electrode itself. Moreover, we do not compare our results to previous studies in which similar perturbations were tested, but rather compare the responses of these two organs (brain and intestine) to various conditions in which the rat breathes different gas mixtures.

The systemic effect of hypoxia (for example) is the same in the blood that flows to the brain or the intestine. The difference in the responses of the two organs is the main question, and indeed our multiparametric monitoring approach may provide the answer. When pO_2 is measured, the question that always rises is what the physiological interpretation of the 30 mmHg or 10 mmHg is. In our monitoring approach, the intracellular activity of the mitochondria is assessed and the critical levels for intact activity of the tissue can be evaluated using these data. We know, for example, that an increase in NADH by 50% and more is very significant to the function of the mitochondria. In the past we compared tissue pO_2 with NADH and found that NADH is much more significant [30]. The same was found when we compared HbO_2 oxygenation with NADH. This parameter is also very sensitive to changes in blood flow at the microcirculatory level [29].

A negative balance between O_2 supply and O_2 demand has a crucial role in many pathophysiological states. However, under critical conditions, the autonomic nervous system redistributes the supply of blood and more oxygen is provided to the most vital organs compared with less vital ones. This protective mechanism was demonstrated in the present study using real-time monitoring of tissue energy metabolism. Our results showed that when the oxygen supply to the tissue is impaired (under hypoxia), the responses of TBF and mitochondrial NADH in the brain were different from those in the intestine. Under oxygen deficiency, the mechanisms of brain auto-regulation became activated, leading to an increase in CBF due to vasodilatation of small arterioles. This phenomenon is well known in mammals, e.g. rats, where the increase in CBF under hypoxia is mediated by the rostral ventrolateral reticular nucleus of the brainstem [39,40]. On the other hand, in the small intestine, where no auto-regulation mechanisms exist, hypoxia caused a significant decrease in TBF, which correlated to the decrease in mean arterial pressure. Consequently, oxygen supply was interrupted and mitochondrial dysfunction developed, leading to a significant increase in NADH in the intestine. Although NADH also increased in the brain during hypoxia, this increase was significantly lower than the increase observed in the intestine, indicating the ability of auto-regulatory mechanisms to improve tissue oxygenation rather than their ability to prevent energy failure. It is important to emphasize that although the brain and intestine differ in their optical characteristics, a comparison of the NADH levels in these two organs is possible since the responses to a complete lack of oxygen, as observed under anoxia or following death (results not shown), are identical in both organs. NADH monitoring using the fluorometric technique in these two organs is not affected by the differences in their optical nature due to the calibration technique which brings the signal to the same baseline. Furthermore, the fact that NADH increased significantly in both organs although TBF responded inversely may point out to the risk involved in the evaluation of organ viability by monitoring only TBF.

Contrasting responses of TBF in these two organs were also observed under anoxia, where CBF in the brain increased while TBF in the intestine decreased. Nevertheless, NADH increased to the same level in both organs due to the complete depletion of oxygen from the blood. The advantage of the state of the brain following anoxia is observed during the recovery phase, in which mitochondrial NADH was fully oxidized earlier than the NADH in the intestine.

Under hypercapnia, CBF increased due to the elevation in pCO_2. NADH became oxidized before the increase in blood supply, probably due to the increase in respiratory rate (which was observed in real time) leading to an increase in oxygen supply to both tissues. This was also observed in the hypercapnia group presented in a previous study [37], where hypercapnia of 20 minutes increased pO_2 by 47 mmHg, as well as in another previous study [41]. Consequently, NADH was oxidized in the intestine as well, although only for a short duration, since no increase in TBF occurred. In contrast, the blood supply increased in the brain in addition to the increase in the respiratory rate; therefore, NADH oxidation lasted longer.

Under hyperoxia, the only change observed was the oxidation of NADH in both organs, probably due to the increase in blood oxygenation. The response of CBF to high oxygen tension, as indicated in previous studies, seems to be heterogeneous for several reasons: the duration of hyperoxia, artificial ventilation versus spontaneous breathing, CBF monitoring techniques, and the anesthetic used. Transient hyperoxia (2 min) caused an increase in CBF due to hypoventilation, which increased $PaCO_2$ levels [42]. In contrast, chronic hyperoxia is suggested to lead to vasoconstriction of small arterioles in the brain and thus to a decrease in CBF. Other studies, however, showed that hyperoxia does not alter CBF [43]. With regard to monitoring techniques, using laser Doppler flowmetry showed no significant changes in CBF during hyperoxia [44], although other blood-flow monitoring techniques, such as microspheres and H_2 clearance, showed a decrease in CBF under exposure to high pO_2 levels [45].

Since many critical conditions treated in intensive care units and operating rooms involve imbalance between oxygen supply and oxygen demand, which may lead to a general breakdown of homeostasis, it is crucially important to develop a method for the early and reliable detection of these conditions. Such early detection is necessary to mitigate adverse consequences, including ATP catabolism, the production of reactive oxygen metabolites, and the activation of the inflammatory processes, which can lead to progressive cellular dysfunction and cell death [46]. Moreover, during the last decades it has become clear that traditional global methods for the estimation of tissue hypoxia, such as lactate levels and mixed venous pO_2 measurements, are nonspecific and insensitive in detecting regional abnormalities [28]. We believe that the use of the MSMP system in animal models that mimic clinical situations will enable the detection of partial or complete tissue hypoxia at an early phase, before irreversible damage occurs.

CONCLUSIONS

This study expands on and supports our previous work in which we simultaneously monitored tissue blood flow and mitochondrial NADH redox state in four organs, i.e. the brain, kidney, liver, and the testis, in rat models of anoxia and cardiac arrest [47] as well as after norepinephrine injection [48]. Moreover, since intestinal blood flow as well as mitochondrial NADH levels showed a significant response to oxygen deficit, the monitoring of the intestinal wall, or any other less vital organ, could be used for the early detection of whole-body deterioration.

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