Simultaneously multiparametric spectroscopic monitoring of tissue viability in the brain and small intestine.

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ABSTRACT

Under body O_2 imbalance, the Autonomic Nervous System is responsible for redistribution of blood flow with preference to the most vital organs (brain, heart), while the less vital organs (intestine, GI tract) are hypoperfused.
The aim of this study was to develop and use an animal model for real time monitoring of tissue viability in the brain, and the small intestine, under various levels of oxygen and blood supply.
Male Wistar rats were anesthetized, the brain cortex and intestinal serosa were exposed and connected by optical fibers to the Multi-Site Multi-Parametric (MSMP) monitoring system. Tissue blood flow (TBF) and mitochondrial NADH redox state were monitored simultaneously in the two organs. The rats were subjected to short anoxia, 20 minutes hypoxia or epinephrine (2& 8µg/kg I.V.).
Under oxygen deficiency, cerebral blood flow (CBF) was elevated, whereas intestinal TBF was reduced. Mitochondrial NADH was significantly elevated in both organs. Systemic injection of Adrenaline showed a dose-depended increase in systemic blood pressure and CBF response whereas, intestinal TBF similarly decreased in both doses. In addition, NADH was elevated (reduced form) in the intestine whereas oxidation was observed in the brain.
In conclusion, our preliminary results may imply the ability of using of the MSMP for monitoring non-vital organs in order to detect early changes in the balance between oxygen supply and demand in the body.

Key words: Mitochondrial NADH, microcirculatory blood flow, brain, small intestine, hypoxia, anoxia, Adrenaline,

1. INTRODUCTION

Many critical conditions, such as shock, sepsis, cardiac arrest, major operations (transplantations, vascular bypasses) and traumatic injuries, are characterized by tissue hypoxia, which often leads to tissue damage [38]. Moderate and acute hypoxia result in metabolic disturbances which may result in cellular energy derangement, often termed "cytopathic hypoxia" [15;16;21;22;33]. Since most of the O_2 absorbed in the body (>95%) is utilized by the mitochondria [44], it is obvious that mitochondrial function plays a crucial role in tissue and organ vitality. Under emergency metabolic states, the autonomic nervous system is responsible for the redistribution of blood in the body in such a manner that vital organs, such as the brain, heart and adrenal glands, is highly perfused and thus protected from hypoxia, while less vital organs are poorly perfused [5;17-19;40]. The decrease in the perfusion of less vital organs may results in mitochondrial dysfunction and cellular energy failure.
Recently, it has been indicated that optimization of oxygen delivery is the best method for prevention, and the only method for treatment, of common intensive care syndromes such as sepsis, Multiple Organ Dysfunction Syndrome (MODS) and acute lung injury [8]. Consequently, a vast amount of resources in critical care research are directed at identifying and analyzing generic markers of incomplete resuscitation, such as splanchnic hypoperfusion, tissue acidosis, and impaired systemic oxygen delivery [34;36;42;43]. Early identification of such factors and prompt corrective interventions may result in a reduced incidence of MODS, reduced length of stay, and an overall improvement of intensive care unit (ICU) outcomes.
Most familiar metabolicparameters monitored in ICU and in the 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 the tissue [6;10;35]. Concerning the location of monitoring, the most common locations are skeletal or abdominal wall muscles, the skin, the conjunctiva and different locations of the GI tract, mainly the sublingual, the stomach and the small intestine [2;9;26].
However, most of the presently available techniques concern overall measurements of oxygen transport and utilization in the body rather then changes at the tissue level, especially in vital organs, thus they are often insensitive, nonspecific, and show abnormality only at a very late stage of disease [12]. In view of that, techniques that directly appraise the tissue energetic state would be an optimal approach for the evaluation of tissue integrity [12]. Mitochondrial NADH redox state is

Optical Tomography and Spectroscopy of Tissue VII
edited by Britton Chance, Robert R. Alfano, Bruce Jason Tromberg, Mamoru Tamura, Eva Marie Sevick-Muraca
Proc. of SPIE Vol. 6434, 64341 N, (2007) .1605-7422/07/$18. doi: 10.1117/12.699445

the most sensitive parameter of oxygen deficiency and is well correlated with tissue pO_2 level under oxygen deficiency conditions [30-32]. Mitochondrial redox state monitoring provides information on the balance between oxygen supply and oxygen demand, and gives indication of the energy formation in the intracellular compartment.
In the present study, we monitored tissue blood flow (TBF) and mitochondrial NADH redox state in the cerebral cortex as well as in the small intestinal serosa. Monitoring of the GI tract was chosen for two reasons. First of all, under critical conditions the small intestine is one of the first organs to be affected [38] making it ideal to serve as a surrogate organ for deterioration of body oxygenation. Additionally, the GI tract is relatively easy to access in experimental as well as clinical situations.
Our hypothesis was that by monitoring the small intestine together with the brain under pathophysiological conditions early detection of the whole body deterioration can be accomplished. In the present study we present some preliminary results of the multi-site multi-organ approach in a rat model under anoxia, hypoxia and adrenaline injection, which are perturbations that closely mimic some of the clinical situations in critical care medicine.

2. METHODOLOGY

The Multi-Site Multi-Parametric (MSMP) monitoring device

Monitoring of the rat brain and small intestine was performed using the Multi-Site Multi-Parametric (MSMP) monitoring system which was developed in our laboratory [23;24]. 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 (Fig 1). The diameter of the probe (including all fibers) is 3mm.
The principle of NADH monitoring from the surface of the tissue (1 mm 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 [28]. The emitted light passes through appropriate filters in order to differentiate between 366nm reflectance and NADH fluorescence (450nm). In addition a specific filter is used in order to prevent red light (laser Doppler flowmeter) from interfering with mitochondrial NADH monitoring. Changes in the reflected light are correlated to changes in tissue blood volume and therefore serve to correct for hemodynamic artifacts in NADH monitoring [27]. The corrected fluorescence (NADH) is obtained by subtracting the reflectance from the fluorescence signal at a 1:1 ratio [27]. Tissue blood flow was monitored using a laser Doppler flowmeter, based on the Doppler shift reflecting the flow of red blood cells in the microcirculation in a depth of 1-2mm [29]. 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 with an IP injection (0.3 ml/100gw) of Equithesin (each ml contains: pentobarbital 9.72 mg, chloral hydrate 42.51 mg, magnesium sulfate 21.25 mg, propylene glycol 44.34 % w/v, alcohol 11.5 % and distilled water). During the entire experimental period, steady anesthesia was maintained by 0.1ml Equithesin injections every 30 min. The rats were placed on a warming tray and body temperature was maintained at 37^o. Polyethylene catheters were introduced into the femoral vein for drug administration, and into the femoral artery for systemic blood pressure monitoring. To prepare the brain for monitoring, the rat was placed in a special mouth holder. A 6 mm diameter hole was drilled in the parietal bone, and the bone was removed (the dura mater remained intact). Two screws were attached to the parietal bone for a better fixation of the MSMP to the brain surface. Then, a special cannula, for the monitoring probe, was placed on the cerebral surface. The cannula together with the two screws were cemented to the skull using dental acrylic cement.
For intestine monitoring, 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 [3].

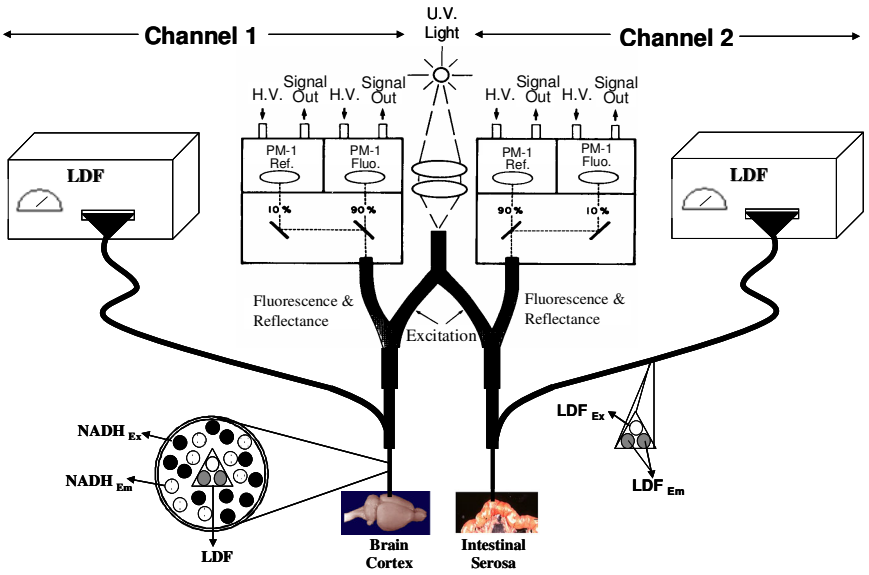

Figure 1: Schematic presentation of the MSMP (Multisite-Multiparametric) monitoring system, and its location on the brain surface and intestinal serosa. Tissue blood flow (TBF) was monitored using the LDF technique – each bundle contains an excitation fiber (LDF_{ex}) and two emission fibers (LDF_{em}). 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 in the tissue.

Experimental protocols

After surgery and fixation of the MSMP to the brain and to the intestinal serosa, the following protocols were conducted:
Anoxia: a short anoxia (30 sec) was induced by 100% nitrogen inhalation. This procedure was performed in order to ensure a 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 [29].
Hypoxia: The rat was exposed to a gas mixture of 12% O_2 + 87% N_2 + 1% CO_2 for a period of 20 min, then after the rat was allowed to breathe normal air for 90 minutes. At the end of these periods, the rat was sacrificed by pure N_2 inhalation.
Adrenaline: two boluses of 1ml/kg of Adrenaline in different doses (2 and 8 μg/kg) were I.V. injected at interval of 30 minutes between each other.

3. RESULTS

In order to test the ability of the MSMP device to simultaneously detect changes in the hemodynamic and metabolic states of the brain and the small intestine in a rat model, we used several experimental protocols. The responses of both organs to complete deficit of oxygen (anoxia) and partial deficit of O_2 (hypoxia) are presented in figures 2 and 3.
As seen in Figure 2, N_2 inhalation for 30 sec caused a decrease of 46 mmHg in MAP, followed by an increase of approximately 135% -160% in NADH in the brain and intestinal serosa respectively. With respect to blood flow, a decrease of 50% was observed in the intestine, while in the brain, CBF increased to the level of 133%. Moreover, the kinetics of response in the two organs was different. In the brain CBF increased in a moderate manner whereas in the intestine the decrease in TBF was rapid. During the recovery phase, NADH in the brain returned to its basal level before the intestinal NADH showed full recovery. As for the changes in the reflectance, in both organs they were inversely correlated to changes in tissue blood flow.

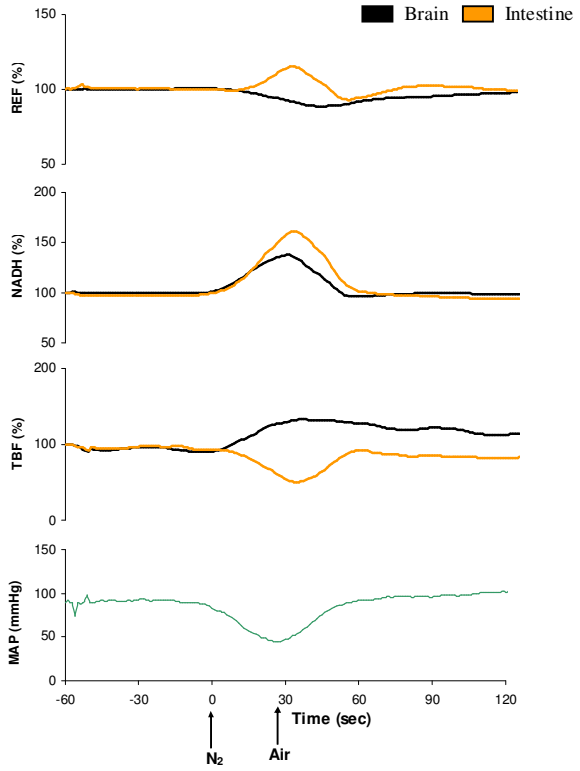

Figure 2: The responses of mean arterial blood pressure (MAP), tissue/cerebral blood flow (TBF/CBF) and mitochondrial NADH in the rat brain and intestinal serosa, to a short session of anoxia (30 sec).

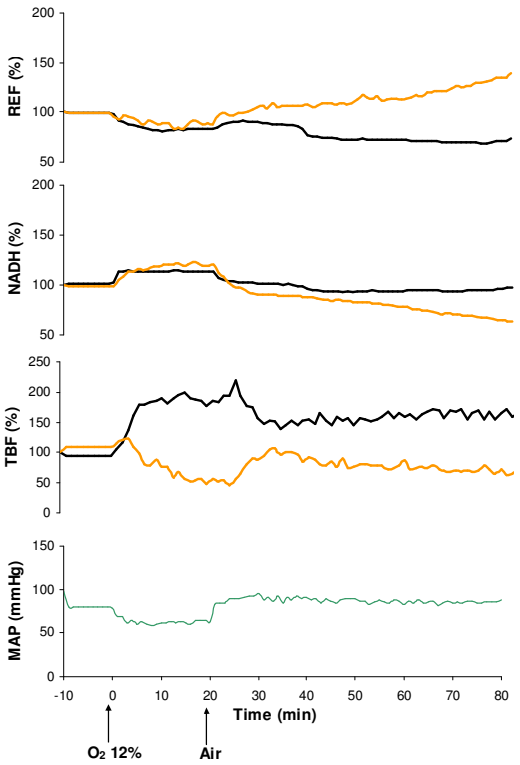

Figure 3: The responses of mean arterial blood pressure (MAP), tissue/cerebral blood flow (TBF/CBF) and mitochondrial NADH in the rat brain and intestinal serosa, to 20 minutes of hypoxia.

Under 20 minutes of hypoxia (Figure 3) MAP decreased by 20 mmHg leading to a decrease of approximately 50% in intestinal blood flow and a 100% increase in CBF. Nevertheless the changes in NADH and in the reflectance were similar in both organs, namely NADH increased while the reflectance decreased by approximately 20%. Following air breathing, reflectance increased in the intestine (30%) and decreased in the brain (40%) and NADH returned to basal levels in the brain whereas in the intestine a massive decrease in NADH was observed. As for TBF in both organs only partial recovery was seen. The brain showed a 50% recovery while in the intestine immediately following air breathing TBF fully recovered then after a decrease of 20% was developed.

Adrenaline injection was used for the evaluation of brain and intestinal responses to activation of the autonomic nervous system. In order to test the effect two doses of 2 μg/kg and 8 μg/kg were used. The responses of both organs to these doses of Adrenaline are presented in figure 4. As seen, the injection of Adrenaline led to an increase in MAP of 18mmHg and 87mmHg in the low and high dosage respectively. Consequently intestinal blood flow decreased to very low levels of 26% and 7% in a dose dependent manner, while CBF increased by 190% and 250% at the low and high dosage respectively. These changes in the perfusion of each organ were associated with changes in the mitochondrial level of NADH in such a way that following injection of 2μg/kg or 8 μg/kg Adrenaline, NADH in the intestine increased to 156% or 186% respectively while the brain showed only a minor decrease of 3% in both doses. Here also reflectance increased in the intestinal serosa in both doses up to 138% and 150% in the low dose and high dose respectively, whereas in the brain a decrease of 12% was observed in both doses.

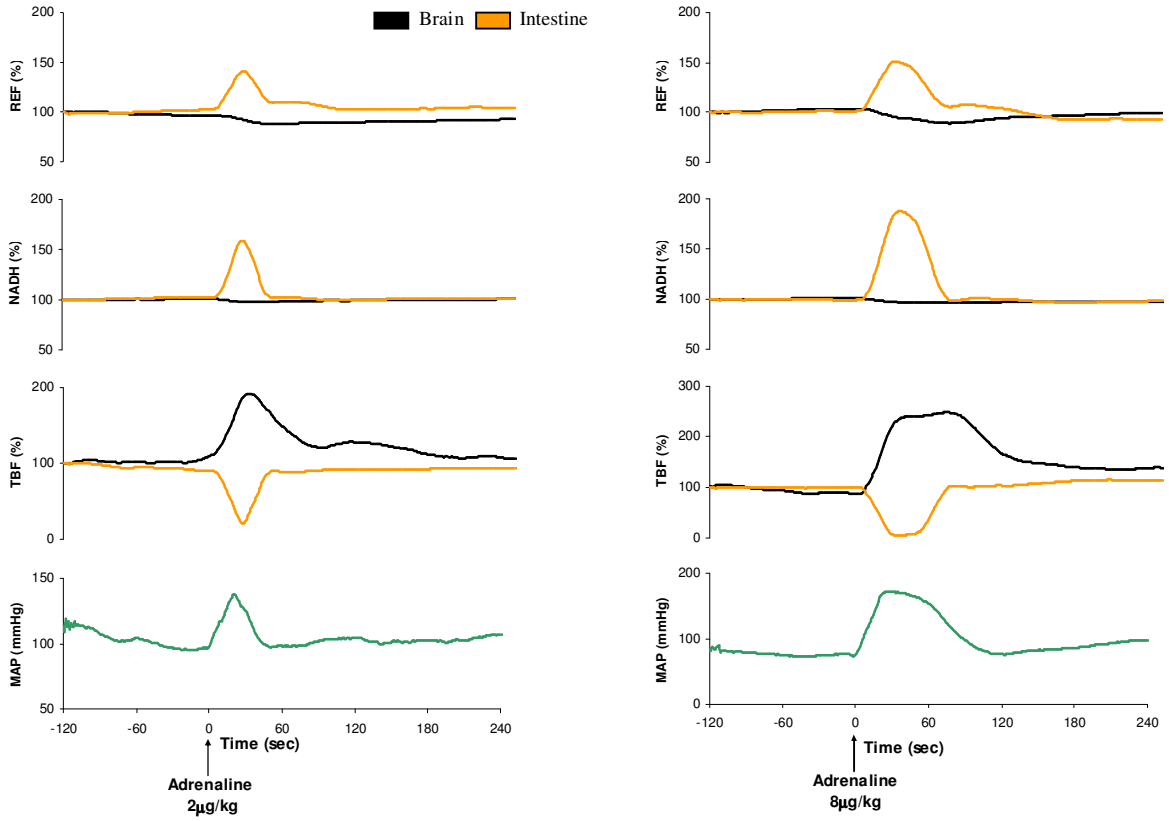

Figure 4: The responses of systemic arterial blood pressure (MAP), tissue/cerebral blood flow (TBF/CBF) and mitochondrial NADH in the rat brain and intestinal serosa, to the injection of 2 doses of adrenaline (A- 2µg/kg, B- 8µg/kg).

In order to evaluate the relation between TBF and mitochondrial NADH level the correlation between those parameters in each treatment was calculated. As presented in figure 5 under deficit of oxygen (anoxia/hypoxia) in the brain positive linear correlation exists between TBF and NADH namely although TBF increased NADH also increased, whereas in the intestine a negative linear correlation exists, as TBF decreased NADH increased. Additionally it seems that for each organ different values of TBF and NADH were observed. When the rats were exposed to air (anoxia/hypoxia off) the responses of the two organs to anoxia versus hypoxia were different. Following recovery from anoxia, the brain showed low correlation between TBF and NADH, in which although TBF was changed (105%-130%), NADH remained steady (at approximately 100%). In the intestine negative linear correlation was seen (r=0.91). After hypoxia the correlation between this two parameters in the brain was also low (r=0.5) whereas in the intestine high linear positive correlation was seen (r=0.8). Here also it can be clearly seen that in this two organs TBF range at two different range of values, namely in the brain most of TBF values were in between 130%-180%, while in the intestine TBF values were in the range of 40%-100%. Nevertheless mitochondrial levels in both are at a similar range (55%-100%). As for the responses to adrenaline, similar patterns of response were seen in both doses. In the brain although TBF was changed (increased or decreased) NADH remained stable whereas in the intestine the changes in TBF were associated with opposite changes in NADH levels, namely a decrease in TBF yielded an increase in NADH levels, whereas an increase in TBF was associated with a decrease in NADH. In was also seen that the high dose of adrenaline caused a larger response of TBF in the brain whereas in the intestine TBF was similar in both doses.

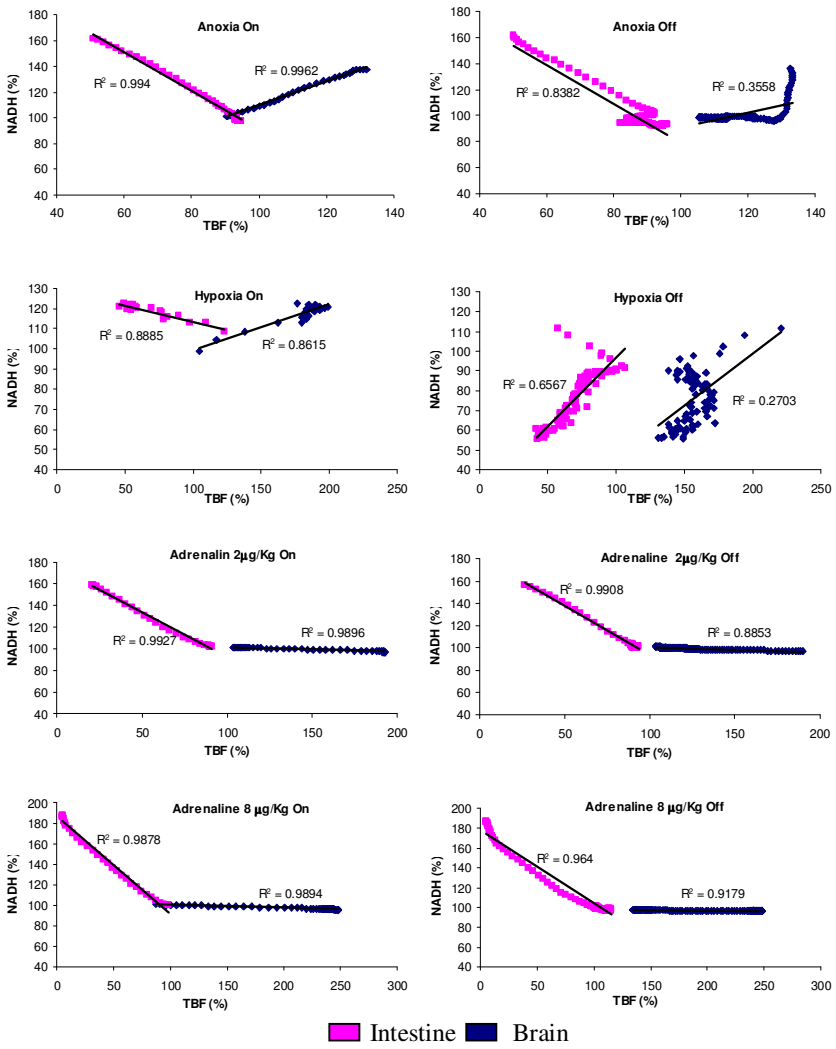

Figure 5: The correlation between tissue blood flow (TBF) and mitochondrial NADH in the cerebral cortex and in the intestinal serosa under anoxia, hypoxia and adrenaline injection. The correlation was tested at two stages: the beginning of the response (On) and the recovery phase (Off).

4. DISCUSSION

The importance of real time monitoring of tissue or organ vitality state is significant under various pathophysiological conditions in intensive care medicine. The vitality of a tissue or an organ is deeply dependent on its level of oxygenation balance [8;12]. Up today the methods used for the evaluation of oxygen balance (supply/consumption) is the monitoring of SvO_2. The SvO_2 is dependent on several variables. Changes in hemoglobin, cardiac output, arterial saturation, or tissue oxygen requirements can lead to changes in the mixed venous saturation [4;7;11;37]. As such, it is not a very specific indicator of a patient's condition. However we believe that the level of tissue oxygenation in organs that are highly susceptible to reduced oxygen delivery, such as the brain, heart and adrenal gland, is one the major factors that influences tissue vitality state [12]. 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 [30-32]. Therefore, in the present study we used the model of simultaneously monitoring the brain and the small intestine, under various critical conditions in a rat model. The conditions being tested included short anoxia, hypoxia (for 20 minutes) and the injection of Adrenaline (2 doses). Our results showed that when oxygen supply to the tissue is impaired (under hypoxia and anoxia), the responses of TBF in the brain were opposite to those in the intestine. Under anoxia and hypoxia, the mechanisms of brain auto-regulation became activated, leading to an increase in CBF, probably 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 [1;45]. On the other hand, in the small intestine, where no auto-regulation mechanisms exist, oxygen deficiency caused a significant decrease in TBF. As for mitochondrial NADH level a significant increase was observed in both organs although TBF responded inversely. In the intestine, under anoxia and hypoxia mitochondrial NADH was reduced due to the decrease in TBF. However, in the cerebral cortex, following anoxia NADH was fully reduced due to the complete luck of oxygen while following hypoxia NADH level remained intact due to the hyperemia which compensated for the reduction in the level of blood oxygenation. These results may point out to the risk involved in the evaluation of organ viability by monitoring only TBF and strength our approach of multiparametric monitoring. The injection of adrenaline was used as a model that mimics the activation of the sympathetic branch of the central nervous system. As expected, following adrenaline injection opposite responses of TBF and mitochondrial NADH level were observed under both doses (2 & 8 μg/kg). MAP increased due to vasoconstriction of peripheral arteries [25;39]. Following this increase in MAP, CBF increased due to the alpha-adrenergic activity [13;20;41], NADH was already well oxidized before the treatment with adrenaline therefore NADH remained stable and showed no further oxidation. However in the intestine TBF decreased [14;46], oxygen supply was interrupted therefore NADH level increased.

ACKNOWLEDGMENTS

This work was supported by the Israel Science Foundation, grant No. 358/04.

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