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Differential effects of norepinephrine on brain and other less vital organs detected by a multisite multiparametric monitoring system

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 emergency situations, the protection of the most vital organs in the body, the brain and the heart, may result in a decrease in tissue perfusion, mitochondrial dysfunction and energetic failure, in "less-vital" organs (skin, G-I tract, kidney etc.). One of these pathways includes the secretion of epinephrine and norepinephrine. The aim of the present study was to test the effect of norepinephrine injection on the vitality of four different organs (vital and "less-vital") monitored simultaneously.

Material/Methods: The four organs monitored were, the brain- a vital organ and three "less vital" organs-the kidney, liver and testis. The vitality of the organs was evaluated by monitoring the levels of microcirculatory tissue blood flow (TBF) and the level of mitochondrial NADH measured from the surface of each organ. The hemodynamic state, TBF, was monitored by the laser Doppler flowmetry (LDF) and the metabolic state was evaluated by surface fluorometry-reflectometry.

Results: The results indicated that following norepinephrine bolus injection the increase in mean arterial pressure (MAP) was associated with an increase in cerebral blood flow and a decrease in all three "less vital" organs TBF. As a result, NADH levels in the brain remained stable whereas in all other organs NADH increased due to the hypoperfusion developed and the lack of enough oxygen in these organs.

Conclusions: The significant correlation between the hemodynamic state of the organs and its mitochondrial redox state may serve as an indicator of tissue vitality under "Brain Sparing" conditions.

key words: **mitochondrial NADH • microcirculatory blood flow • norephinephrine • multiorgan monitoring • body vitality**

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BACKGROUND

Pathophysiological events develop in many emergency clinical situations such as hypoxemia, hypotension, sepsis, and cardiac arrest, are mostly involved with the deterioration of body metabolic state, negative oxygen balance and energy failure. As a compensatory mechanism, the sympathetic pathways are activated, via the secretion of epinephrine and norepinephrine (NE), leading to blood flow redistribution in the body in such a manner that blood flow to the most vital organs (brain, heart and adrenal medulla) will increase whereas, blood flow to "less vital" organs (GI tract, skin, kidney) will decrease. This is the reason why NE injection serves as a conventional animal model for the investigation of various critically situations in patients.

It has been stated often that the autonomic nervous system integrates diverse organ systems by redistributing the cardiac output-CO in response to the requirements of the whole animal rather than to the needs of local tissues [1].

The brain, which is considered as the most vital organ, is highly affected by NE. The central adrenergic neurons have several functions in the brain: they enhance cerebral vascular tone by action on α-receptors sites [2,3] and influence metabolic rate of brain tissue by acting on β-receptors sites on the cell membrane [4]. Additionally NE modulates the response of the cerebral cortex to increase in brain metabolic demand. NE may mediate its effect by potentiating Na^+/K^+-ATP'ase or through its effects on vascular reactivity or both [5]. Studies in which a depletion of brain NE levels was induced, showed a disturbance in cerebral microvascular tone and an increase in cerebral blood vessels vulnerability to hypertension [6].

Since catecholamines influence the cerebral blood flow they have also an influence on the energetic state of the brain. As was observed, NE administration caused the oxidation of NADH [7] and a change in the rate of oxidative metabolism activity [8]. The relationship between oxygen demand and supply is not affected by NE depletion when energy demand is low.

The kidney which is a "less-vital" organ (as compared to the brain) poses also its own autoregulation mechanisms, which is independent of circulating humoral or neurogenic factors but governed by intrinsic renal mechanisms [9]. Several studies, using different techniques, have clearly revealed the presence of adrenergic fibers on tubular and vascular elements of the kidney [10,11]. The activation of α_1-adrenergic receptors in the afferent and efferent arterioles, induce arteriolar constriction leading to RBF and GFR decrease although not at the same degree [12–16]. As for the metabolic activity of the renal tissue, here, the aerobic respiratory activity is the main source for energy, producing 95% of the ATP in the kidney [9]. In 1962 Chance et al. [17] found that NADH is the most sensitive component, in the respiratory chain, to tissue oxygen levels, since then several studies used the fluorometric monitoring technique to evaluate renal energetic state [18,19].

It was demonstrated that NE increases microvasculature resistance and decreases vascular volume in a dose dependent manner in the liver [20–22]. The first measurements of NADH in the liver using the fluorometric technique were made by Chance who monitored NADH in liver slices from anesthetized rats [23]. Following studies measured NADH fluorescence in rat hepatocyte suspensions and on the surface of perfused rat liver, using a fiber optice probe [24–26]. The changes in fluorescence intensity monitored in the perfused liver were explained by a change in the amount of reduced pyridine nucleotides in the tissue [27].

While in most of the organs blood flow is proportional to the metabolic demands of the tissue, in the testis blood flow is low in relation to its rate of oxygen uptake [28]. Several mechanisms of autoregulation exists in the testis including the neurological mechanism mediated via adrenergic stimulation which effects small blood vessels (adrenergic nerve terminals were observed in the epididymal duct tissue) [29,30].

As previously indicated, catecholamines could induce rapid and dose dependent decrease in testicular blood flow [31,32] via an increase in testis vascular resistance [33,34].

The aim of the present study was to develop and use the multiorgan (including vital and "less-vital" organs) monitoring approach in real time following NE administration in a rat model.

By using NE injection in a rat, together with the Multi-site multiparametric monitoring system (MSMP) we were able to evaluate the hemodynamic and metabolic state of four different organs simultaneously. Our monitoring approach also enabled us to estimate the differences in the responses of the brain, a vital organ and "less-vital" organs such as the kidney, liver and testis. Consequently, the monitoring of "less vital" organs may indirectly provide information concerning the state of vital organs.

MATERIAL AND METHODS

Animal preparation

All experimental protocols were approved by the institutional animal care committee under the instructions of the National Institute of Health.

Wistar male rats (250–300 g) were anesthetized by 0.3 ml/100 gr Equithesin I.P injection (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 water). During the entire experiment, the rats were maintained anesthetized by addition of 0.1 ml Equithesin every half-hour. In addition, a heating pad was placed under the rat to maintain body temperature at 37°C. At the end of the experiments the rats were sacrificed by inhalation of pure N_2.

A midline incision was made in the skin, exposing the skull. Two holes were drilled in the skull. A 3.5 mm hole was drilled in the left parietal bone for the fixation of a cannula in which the monitoring probe was inserted, and a second small hole, in which a screw was inserted for better fixation of the light guide holder to the skull. The cannula was then fixated to the skull using dental acrylic cement. Then the rat was turned over on its back for further operation. A hole in the experimental table, beneath the head, allowed the insertion of the pro-

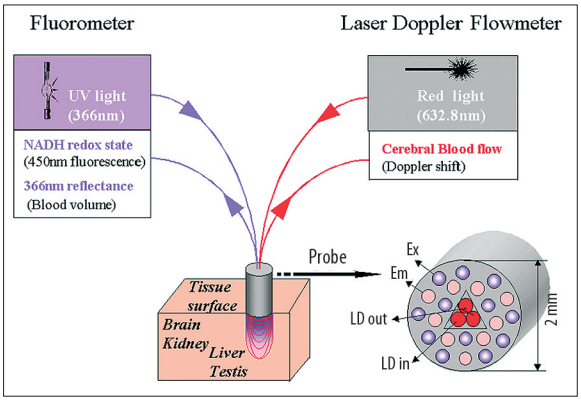

Figure 1. The Multi Site-Multi Parametric Monitoring System (MSMP). A schematic presentation of the one out of four probes and its way of connection to the photomultiplier (for NADH monitoring) and to the laser Doppler flowmeter (for TBF monitoring).

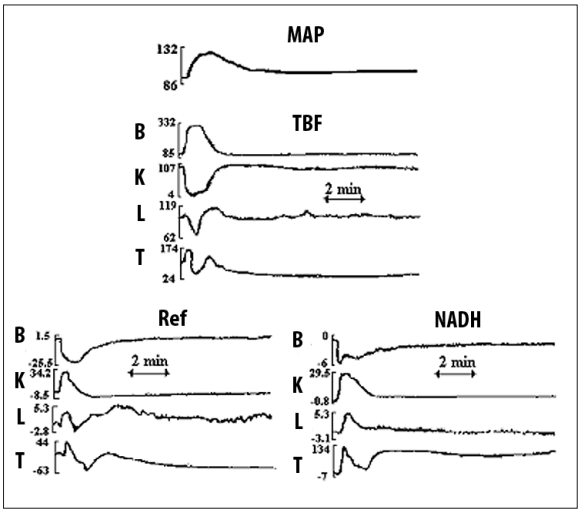

Figure 2. A typical experiment in which 5 µg/100g I.V of Norepinephrine (NE) was injected. The four organs monitored were: Brain (B), Kidney (K), Liver (L), Testis (T). The parameters showen include: Mean Arterial Pressure (MAP), Tissue Blood Flow (TBF), reflectance (Ref) and corrected fluorescence (NADH).

be into the brain cannula. The femoral artery and vein were exposed and cannulated for blood pressure monitoring, via an UTR-disposable pressure transducer (Biometrix, Israel) and NE (A7256 Sigma) injection respectively. For the exposure of the kidney and liver, an abdomen midline section below the rib cage was created. The central lobe of the liver was exposed. Additionally, the left kidney was isolated from the juxtaposed spleen and intestine. Then the right testis was exposed and the probes were placed on each organ. In the liver the probe was placed on the central lobe in its flat area. Another probe was placed on the center of the left kidney where a flat surface exists. All probes, except for the brain, were held in place with micromanipulators during the entire experiment. Parafilm was placed around the tip of these probes and glued to the tissue using cyanoacrylate adhesive [35]. Parafilm was also used for the prevention of dehydration. A black cloth was placed over the parafilm to avoid room light from entering the monitoring sites and causing artifacts.

The Multi-Site Multiparametric monitoring system (MSMP)

Monitoring of the brain, kidney, liver and testis in the rat was performed using the MSMP- Multi-Site Multiparametric monitoring system. Figure 1 presents the principle of one channel of the monitoring system that includes 4 bundles of optical fibers for the NADH redox state measurement, by surface fluorometry, as well as for tissue blood flow (TBF), using Laser Doppler Flowmetry (LDF). Each probe was placed on the surface of the organ monitored. The principle of NADH monitoring from the surface of the brain is that excitation light (366 nm) passes from the fluorometer to the organ via a bundle of optic fibers made of quartz. The emitted light (450 nm), together with the reflected light, at the excitation wavelength (366 nm), was transferred to the fluorometer (containing appropriate filters) via another bundle of fibers [36–38]. Monitoring of the TBF was accomplished using the Perimed® Periflux PF 2B. This device works on the physical principle of the Doppler shift [14]. All the signals monitored during the experiment were transferred at a rate of 100 samples per second to a multi-channel computerized data acquisition and recording system (Labview A/D software, National Instruments Co, USA) for further analysis.

Experimental protocol

One half-hour after surgery, short anoxia (30 sec) was induced by inhalation of pure N_2 for the assessment of organ vitality following with a period of thirty minutes for stabilization. Later on, a volume of 1ml of NE (5 µg/100 g) was injected I.V during one minute and monitoring proceeded for a period of 2.5 hours. In the control group a volume of 1ml saline was I.V. injected.

Statistical analysis

Mean ± S.E. values were calculated for each parameter in a group of 9 rats. For NADH calculations, the basal level of NADH was considered to be 0% and a range of 500mV as 100%. As follows; TBF level in death was considered as 0% and the range between basal TBF level and the level monitored in death was considered as 100%. The ANOVA and Duncan's multiple range tests were used to detect significant differences between the four organs. A value of $P<0.05$ was considered to be significant. Significantly different groups were presented with different letters (A,B,C etc.) and groups that were not significantly different one from another were presented with two letters one of each group that they were similar with (AB,BC etc.).

Results

In order to ensure reliable monitoring of four organs simultaneously using the MSMP device, we run control experiments in which monitoring of the brain, kidney, liver and testis was preformed following 1ml saline injection (I.V.). The results showed that monitoring is stable, with no significant changes in any of the parameters (results not shown). In the second step we monitored these four organs under NE injection and the results are as indicated.

In Figure 2 the results of a typical experiment in which NE was injected I.V. (5 µg/100 g) are presented. As seen, follo-

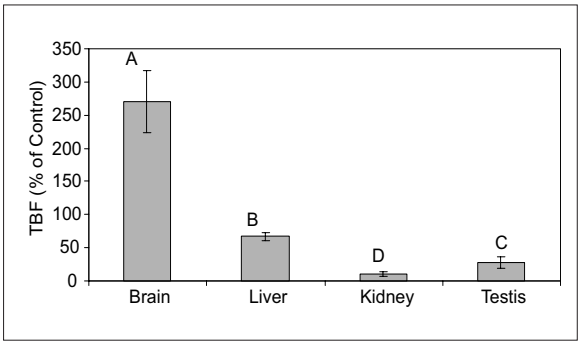

Figure 3. The effect of NE (5 µg/100g I.V.) on Tissue Blood Flow (TBF) monitored in four different organs (brain, kidney, liver and testis, abbreviations are as in Figure 2). Results presented are mean ± S.E (n=9).

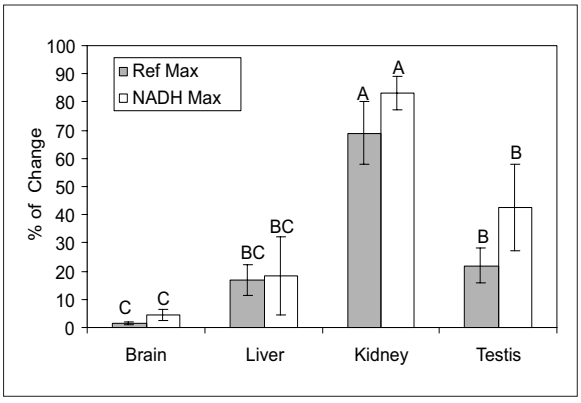

Figure 4. The effect of NE (5 µg/100g I.V.) on the reflectance (Ref) and NADH levels monitored in four different organs (brain, kidney, liver and testis, abbreviations are as in Figure 2). Results presented are mean ± S.E (n=9).

wing NE injection a dramatic increase in mean arterial pressure (MAP) was observed. Consequently an increase in cerebral blood flow (from 85 mV up to 332 mV) was observed while the other organs namely kidney, liver and testis showed a significant decrease in tissue blood flow (a decrease of 103 mV in the kidney, 57 mV in the liver and 150 mV in the testis). Following these changes in tissue blood flow NADH in the brain decreased by 6 mV (became oxidized) while in the rest of the organs an increase in NADH levels was observed (31 mV, 8.4 mV and 141 mV in the kidney, liver and testis, respectively) due to the decrease in O_2 supply.

As for the reflectance (Ref), while the brain showed a decrease in Ref (increase in blood volume) of approximately 25 mV the other organs showed an increase in the Ref signal (decrease in blood volume) of about 30 mV in the kidney, 5 mV in the liver and 44 mV in the testis.

The average ± S.E changes in all parameters monitored simultaneously in the four organs are presented in Figures 3–4. As seen in Figure 3, Tissue Blood Flow (TBF) in the brain following NE injection showed a major increase in TBF to the level of 270±47% ($p<0.05$). However, in the other organs; kidney, liver and testis, a decrease in TBF to the levels of 10±3%, 76±6% and 28±9% respectively was seen ($p<0.05$ for all organs). In the testis, however, a rapid phase of hyperemia to the levels of 200±34%, was monitored prior to

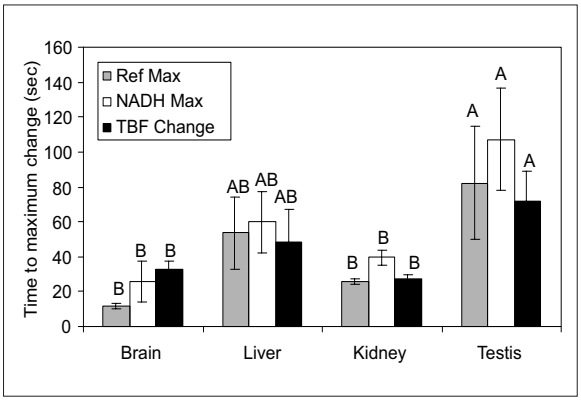

Figure 5. The time needed for the reflectance -Ref, NADH and TBF to reach their maximal change in four different organs (brain, kidney, liver and testis, abbreviations are as in Figure 2). Results presented are mean ± S.E (n=9).

the major decrease in TBF (as demonstrated in Figure 2). This increase in TBF developed 19±3 seconds after NE injection (average results not shown).

The changes in the Reflectance, and NADH levels are presented in Figure 4. As seen the increase in NADH level is the higher in the kidney and testis, 83±6% and 43±15%, respectively. In the brain no significant increase in NADH was measured 4±2% whereas, in the liver the increase in NADH, 18±14%, was significantly different ($p<0.05$) then NADH level monitored in the kidney but not different from NADH level monitored in the testis and the brain. The results of Waller-Duncan T-test of variable showed three significantly different levels of NADH (A,B,C) measured in the kidney, testis and brain respectively. The changes in NADH level in the kidney, liver and testis were inversely correlated with the changes in the TBF namely, the maximal decrease in TBF, which was observed in the kidney, was associated with a maximal increase in NADH levels. As for the testis and liver the increase in NADH level was proportional to the decrease observed in TBF level. However, in the brain the increase of TBF, was associated with stable NADH levels.

Regarding the changes in the reflectance (Ref) levels following NE injection (Figure 4). It was observed that the increases in Ref in the kidney were the higher as opposed to the rest of the organs (69±11%). The maximum Ref levels in the kidney, testis and brain showed three significantly different groups (A,B and C, respectively). While the Ref level in the liver (17±5.5%) was significantly different from the Ref level monitored in the kidney ($p<0.05$) but not different from the level monitored in the testis and brain.

Figure 5 presents the time required for the different parameters to reach their maximum change (mean ± S.E). TBF in the brain reached its maximum level within 33±4 sec. The decrease in TBF was the first to develop in the kidney (27±3 sec) and the liver and testis reached their minimum levels within 48±19 and 72±17 sec respectively (Figure 5).

Following the changes in TBF levels a change in Ref and NADH levels where observed. The time needed for NADH to reach its maximum level was the shorter in the brain and kidney (26±12 and 39±4 sec, respectively). The liver was the next

organ to respond (59±17 sec) and the testis was the last organ in which NADH increased (107±29 sec). As for the kinetics of change in the Ref, the organs responded in the following order: first the brain (12±32 sec) pursuing with the kidney (26±20 sec) and finally the liver and testis (54±2, 82±2 sec).

The results of Waller-Duncan T-test of variable revealed two significantly different groups regarding the kinetics of TBF, NADH and Ref changes following NE administration. The brain and kidney in one group and the testis on the other group while the liver stands between these two significantly different groups.

Discussion

In cases like hypoxemia, hypotension, sepsis, dysoxia and cardiac arrest, the metabolic state of the body will be deteriorated and energy failure will develop. Consequently, a central protection mechanism will be activated towards the redistribution of blood flow to the most vital organs like brain, heart and adrenal gland, while the "less vital" and peripheral organs and tissues like the skin, muscles and G-I tract will undergo vasoconstriction and a decrease in blood flow and oxygen supply will occur [39,40].

The aim of the present study was to use the simultaneously multiple organ monitoring system in real time in vital and "less-vital" organs following NE injection. It is assumed that knowing the kinetics of changes in the hemodynamic as well as the metabolic state of various organs can be used as a detecting tool for oxygen deficiency, in different pathophysiological situations. It is for the first time that microcirculatory blood flow and mitochondrial function (NADH redox state) were monitored simultaneously in vital (the brain) and various "less vital" organs. The changes in TBF were evaluated using the LDF. Cortical studies, in the kidney using the LDF demonstrated that it is a reliable technique and could be used as an indicator for the directional changes in cortical blood flow, as well as whole kidney blood flow, compared to the electromagnetic flow probe and the radioactive microspheres method [41–43]. It was also demonstrated that the LDF provide a good measure of whole organ blood flow in the perfused rat liver [21,35,44], as well as in the rat testis [34], recording rapid changes in blood flow [45].

In the present study an increase of 40 mmHg in arterial blood pressure developed immediately post NE injection. The increase in blood pressure was followed by a significant increase in cerebral blood flow and a decrease in TBF of the three "less vital" organs monitored. As previously indicated epinephrine improves blood flow to the brain probably because of its alpha-adrenergic activity [3,13,46]. Several investigators showed that the destruction of the central noradrenergic system produces an increase in resting CBF and CO_2 reactivity. However, others have found no effects of this procedure on these parameters [47].

In the present study, NE injection caused also the reduction of blood flow in the liver, probably via the activation of β-adrenergic receptors, leading to hepatic microvasculature vasoconstriction [20,48]. The decrease in renal blood flow was also documented [41,49–52]. It is important to clarify that all of the changes in TBF and NADH parameters presented in the present article were a direct result of NE injection since the results of the control group (saline injection) showed no effect on TBF and NADH in the brain as well as the other organs.

The results of the present study show high correlation between tissue blood flow (O_2 supply) monitored by the LDF technique and the energetic state of the liver, kidney and testis (mitochondrial NADH redox state). However, in the brain although TBF increased, NADH remained stable. This is probably since the normoxic brain is well oxidized in normal perfusion levels, thus an increase in TBF has no further beneficial effect on mitochondrial NADH levels. Our study showed high correlation between TBF and the reflectance (Ref) parameter. This is explained due to the fact that Ref is mostly influenced by the absorption properties of the tissue, which is mainly effected by tissue blood volume (TBV). Hence, a reduction in TBF, caused a decrease in TBV, leading to increase in the Ref. Although these four organs have different optical character (the brain and testis are white tissues and the liver and kidney are red tissues) the comparison between TBF and NADH between the organs was possible since both of these monitoring techniques gives relative results meaning each organ was calibrated against its own values in time.

The brain, a vital organ, showed an increase in TBF with no damage to its mitochondrial function and energetic state, while in the kidney, liver and testis, which are defined as "less vital" organs (relatively to the brain), TBF decreased and NADH increased. Our results showed that the brain and kidney are the first organs to respond to NE injection probably due to their own autoregulation mechanisms. The testis showed the latest response in TBF as well as in NADH and Ref parameters. These results may imply that the testis is less important in stress conditions. However, in the liver TBF and NADH parameters were changed in a time course, which was found to be, in between the rate of changes in the brain and kidney as opposed to the testis, probably due to its role in glucose metabolism, which increases at stress situations.

Conclusions

In conclusion, our approach of multiple organ multiparametric monitoring (MSMP) could be a practical tool to evaluate organs vitality under various pathophysiological situations. Moreover, the use of the MSMP in "less vital" organs may serve as a tool for the evaluation of the severity of oxygen deficiency in the whole body.

Acknowledgments

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