Autoregulation of the brain temperature during whole body hyperthermia

Nodar Mitagravia[1], James Bicher[2], Marina Devdariani[1], Lia Davlianidze[1], Marina Nebieridze[1], Nana Momtselidze[1]

(1) I. Beritashvili Center of Experimental Biomedicine, 14 Gotua St., Tbilisi 0160, Georgia, nodmit@gmail.com

(2) Bicher Cancer Institute, 12099 W. Washington Blvd., #304, Los Angeles, CA 90066, james.bicher@bci-vci.org

Published: http://www.hindawi.com/cpis/medicine/2013/928243/

Autoregulation of the brain temperature during whole body hyperthermia

The aim of this study was revealing the temperature changes in rats brain tissue caused by whole body hyperthermia. Analysis of received results allow to conclude, that the brain has a highly secured system of temperature autoregulation against the exogenous temperature changes. The upper limit of this autoregulation (for rats, at least) is in the range 45 ^{o}C of environment. An important role in the normal functioning of the brain temperature autoregulation system belongs to Nitric Oxide the behavioral disorders, observed in animals after Whole Body Hyperhtermia (sure within the range of brain temperature autoregulation) is hardly associated with the canges in temperature of the Central Nervous System, but rather have to be mediated by impaired blood circulation and oxygen supply to the brain tissues, caused by the rapid deteriorationof the blood rheological properties.

Introduction

In our previous experimetnal studies significant morpho-physiological changes in the rat's brain tissue caused by Local Hyperthermia (43^{0}C, 60 min. exposure) have been revealed [4, 15]. On the **Figure1** we can see the clear-cut edge of damaged tissue in the rats' cerebral cortex. Analysis of the results allowed us to conclude that in the development of these changes essential role belongs to the mechanism associated with intense activation of Nitric Oxide Synthases (NOS), resulting (in the initial phase of Hyperthermic Exposure) in increased oxygenation of exposed brain tissue, and then (in the second phase of exposure), - to changes in blood rheological properties resulting in thrombosis of cerebral vessels [14].

Confirmation of this conclusion is presented on the Figure 2 (A and B). On the Figure2A we can see a sensory motor cortex of rats' brain with a lot of thrombosed cerebral vessels after 60 minutes of hyperthermic exposure in control rats brain and on Figure2B – the similar picture in experimental rats' brain with inhibited production of Nitric Oxide (we can see just a single thrombosed cerebral vessels).

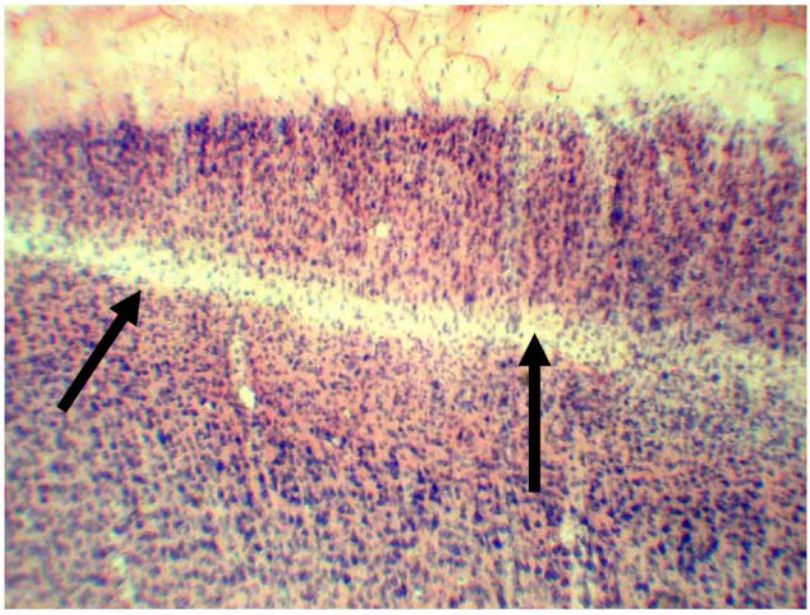

Figure 1. Sensory-motor cortex of rats' brain; 60 minutes hyperthermia (43 ^{o}C); Arrows show the clear-cut edge of damaged tissue. A – malignification: x15

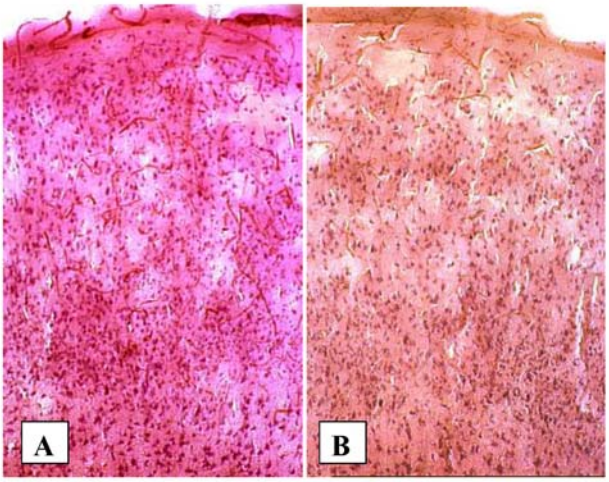

Figure 2. Sensory motor cortex of normal (A) and L-NAME injected (B) rats' brain (x10); 60 minutes hyperthermia (43^{o}C)

In the case of tumor tissue, we believe that the initial thermal hyperemia leads to a deterioration in the process of glycolysis due to increased oxygenation of tissues (Pasteur effect), and subsequent thrombosis leads to the sharp decrease in glucose delivery to tumor cells and to their unconditional death. Based on the foregoing, we attempted to evaluate the possible role of these phenomena in behavioral disturbances in rats observed after Whole Body Hyperthermia [13].
For this purpose, specially made thermocouple we implanted in the subcortical structures of the rats' brain, which allowed to record changes in temperature of brain tissue at different temperatures in HC.
Two series of experiments have beeen carried out - on intact animals, and on animals with previously administered (intraperitoneally) nonselective inhibitor of NOS (Nitro-L-Arginine Methyl Ester - L-NAME). The rectal temperature were also recorded. Raising the temperature in the HC up to 450C and its maintenance on this level during 1 hour, did not lead to temperature increase in the subcortical brain structures of intact animals above the 36-36.50C.. These results led us to temporarily suspend behavioral experiments and to more detailed study of this phenomenon - temperature homeostasis in the brain.

Materials and methods

The used approach was quite simple: specially designed (in Bicher Cancer Institute, Los Angeles, USA) for these experiments thin (300-400 mcm in diameter) teflon-covered thermocouples with a bared active tip (about 1.5-2 mm) were implanted into the brain of experimental animals (rats). Thermocouples were dipped in the subcortical structures (in the area of thalamic nuclei), and their connectors were fixed on the skull.

In one series of experiments on the third day after chronic implantation of a thermocouple, the animals were narcotized (0.15ml/100g, 4% solution of chloral hydrate) and placed in the HC (Figure 3), and by means of insulated cable the thermocouple was connected to the digital meter of temperature (Omega Engineering, Inc., USA). Two series of experiments were carried our: first – on animals wich before the hyperthermic exposure did not receive any pharmacological agents and the second series – on animals which 15 minutes before the beginning of hyperthermic exposure were intraperitoneally injected by nonselective inhibitor of Nitric Oxide Synthase Nitro-L-Arginine Methyl Ester (L-NAME, 50mg/kg).
The temperature in hyperthermic chamber, as well as in animals brain tissue was measured continuously, and the rectal temperature - discretely, in every 15-20 minutes.

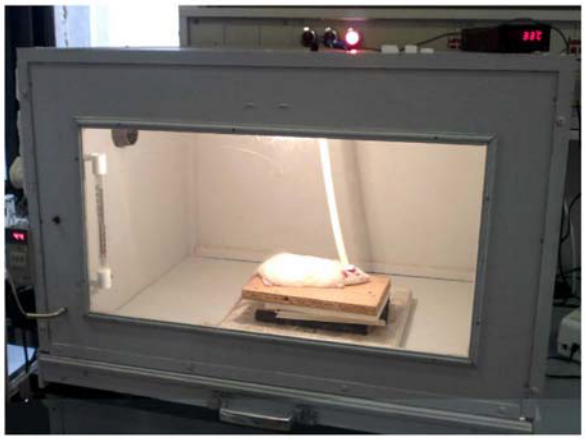

Figure 3. Hyperthermic chamber

The temperature in the hyperthermic chamber was gradually (in duration of 25-30 minutes) increased up to 45^0C and this level was automatically maintained for 60 minutes. Depending on the condition of the animals in some cases we continued the rise of temperature in chamber up to 48-50^0C.

In other groups of animals (without implanted thermocouple) - intacts and with a preliminary administered L-NAME (in the above-mentioned dose) an index of Red Blood Cells (RBC) aggregation - one of the most important rheological parameters of blood was determined at different temperatures in HC.

All received results were evaluated statistically and significance of differences between mean values were assessed by Student's Criterion.

Results

Changes in the brain tissue temperature, when the the temperature in the HC in duration of 30 minutes was increased from 38 to 45^0C, then was maintained on this level (45^0C) for 60 minutes and after that was slowly (in duration of 75 minutes) raised up to 500C, are presented in the Figure4. On this picture we can see the data on changes in temperature in the hyperthermic chamber (dark points) - which is automatically adjusted to a level that is specified by the experimenter, and in the rats' brain tissue (open circles) - continuously measured with a thermocouple implanted in the brain.

As seen on this figure, the temperature in the brain tissue of postoperative animals (before beginning of hyperthermia) is around of 33^0C and begins to rise with the onset of Whole Body Hyperthermia. And when the temperature in the chamber reaches 45^0C the brain temperature stabilizes on the level of 36-36.5^0C inspite of the fact that the 45^0C in the chamber lasts 60 minutes. If, however, we continue rising the temperature in the chamber even just on the one degree of Celsius, the stability of the temperature in the animals brain is disturbed and if we will continue temperature rising, the brain temperature almost linearly will follow to increasing temperature in the chamber. Practically, we were faced with a phenomenon that can be called as the autoregulation of brain temperature, or temperature homeostasis.

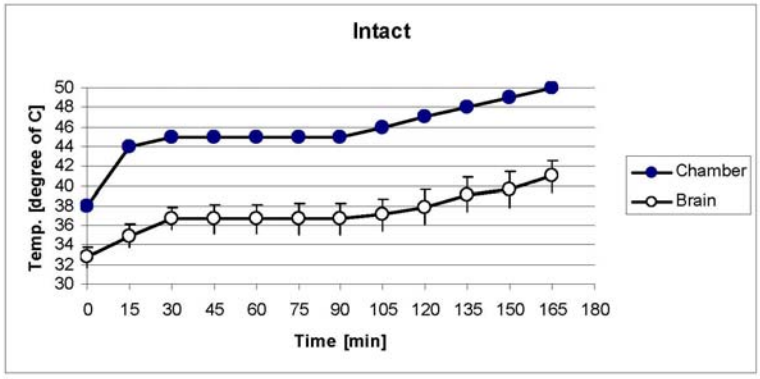

Figure 4. Data on changes in temperature in the hyperthermic chamber (dark points) and in the rats' brain tissue (open circles) – continuously measured with a thermocouple implanted in the brain

A fundamentally different picture is observed if prior to the hyperthermic exposure, the animal is intraperitoneally injected by nonselective Nitric Oxide Synthase inhibitor L-NAME (Nitro-LArginine Methyl Ester) at a dose of 50mg/kg. The results of measurements made on these animals are shown in the Figure 5. Variation of temperature in the hyperthermic chamber presented in this picture (both in duration and in the absolute values of the temperature) is identical to that of the previous picture. However, the dynamics of the temperature variation in the brain is fundamentally changed - a plateau that occurs on the corresponding curve in intact animals, is absent here. The temperature curve, recorded from the brain of animals with inhibited Nitric Oxide Synthase activity, starts smoothly and continuously grow with the onset of hyperthermia, and so lasts until the end of experiment, i.e. until the death of the animal, which usually occurs when the brain temperature reaches the range of 40-41^{0}C.

As for the dynamics of changes in rectal temperature in all experimental conditions we used, it is shown on Figure6. Here we can observe a mixed picture. Analysis showed that the statistical significant difference between the readings at different experimental conditions was observed only in the short time intervals of hyperthermic exposure and do not have a regular character.

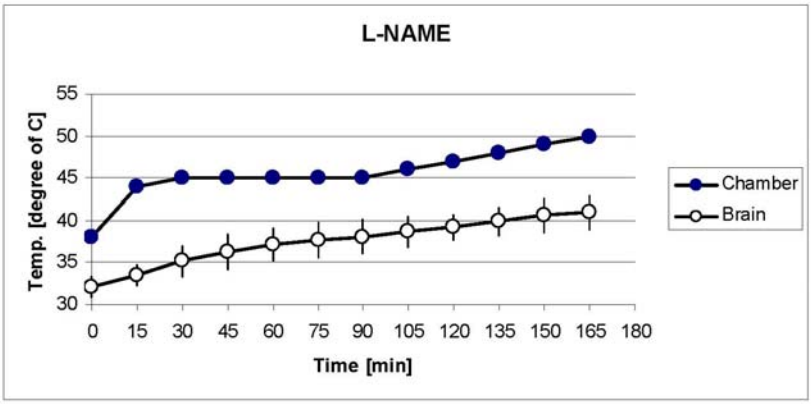

Figure 5. Changes in temperature in the Hyperthermic chamber and in brain tissue of animals that before the beginning of hyperthermic exposure have been intraperitoneally injected L-NAME (50 mg/kg)

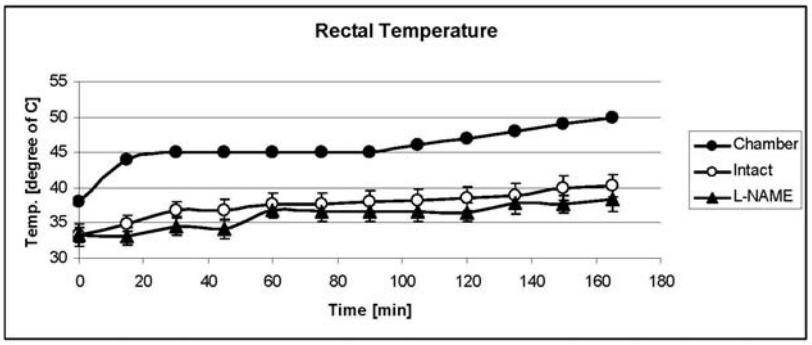

Figure 6. Changes it the temperature (rectal and in chamber) of intact group and in L-NAME (50 mg/kg) injected group of animals

A special series of experiments was conducted to determine the changes in the index of erythrocyte aggregation caused by hyperthermic exposure in intact and L-NAME-administered animals. The results of these measurements are presented in Table 1, where we can see the average values of this index at normal (room) temperature (21-23^{0}C), and 40, 43 and 45^{0}C.

Temperature in Chamber (^{0}C)	Intact rats	L-NAME injected rats
21-23	1.3±0.1	1.75±0.15
40	30.1±2.1	9.48±0.2
43	33.2±1.8	10.4±0.18
45	40.1±3.5	15.8±0.2

Table 1. The average statistical values and standard errors of RBC aggregation index in normal temperature and in conditions of WBH

Discussion

The bacis mechanisms underlying the regulation of body temperature in humans and animals has always been under the attention of scientists and it should be noted that in this area has been achieved significant progress, both in condition of norm and pathology [6, 9, 11, 22]. However, substantially different picture appears if we consider the issue of temperature regulation in the brain not only in case of external, but also in internal impacts (associated with changes in temperature caused by different levels of brain functional activity) [20].

There is an evidence that the direction of temperature changes in the brain of the cat during visual stimulation is dependent on the frequency of flashing light - at low frequencies, the temperature rised, and decreased at high frequencies [12]. It was also established that under normal physiological conditions, the temperature in the deep brain structures is higher than the temperature of arterial blood, and closer to the brain surface, due to exchange with the environment - the situation is diametrically opposite [17, 20, 24]. It is believed that quantitatively this phenomenon is regulated by the temperature shielding effect of blood flow, which protects the subcortical structures of the brain from penetration of "extracranial cold" [21], although would be more appropriate, instead of "extracranial cold" use the expression "ambient temperature changes."

In accordance with our above described results, the rat's brain has a sufficiently effective system of thermoregulation in case of sharp rise in environmental temperature and the upper limit of this autoregulation is 450C. Further increase of temperature in the hyperthermic chamber (above 450C) leads to disruption of this autoregulation - the temperature in the brain increases in a linear function of chambers' temperature and when it reaches 40-410C – animal dies.

We completely agree with Zhu et al. [25]. who in their study concluded that the depth of the thermal shielding of the brain is critically dependent on cerebral blood flow. This is clearly evidenced by our data

showing that the preliminary (15 minutes prior to hyperthermic exposure) administration of a nonselective inhibitor of Nitric Oxide Synthase (L-NAME, 50mg/kg), completely disrupts the autoregulation of temperature in the of brain tissue and from the very beginning of hyperthermic exposure its dynamics linearly follows the changes of temperature in the chamber.

It is known that a sharp decrease in the synthesis of Nitric Oxide (if not its complete blockade) leads to the same sharp decrease in blood flow and increase in systemic arterial pressure (approximately on 60%) [5]. We know as well that in norm the endothelial Nitric Oxide Synthase controls basal vascular tone, and that the Nitric Oxide is involved in neurogenic vasodilation in response to a rise in body temperature [23]. In addition, it was found that LNAME inhibits the norepinephrine-induced increase of blood flow in brown adipose tissue – in the main thermogenic organ [16]. All the above confirms that Nitric Oxide is an important component in the system of thermoregulation not only for the brain but also for the whole body.

The intensity of the circulation, and hence the degree of maintenance of temperature homeostasis is largely dependent on the rheological properties of blood [8], one of the most important indicators of which is an index of erythrocyte aggregation However, this parameter itself is extremely temperature dependent, which is mainly due to the influence of temperature on the viscosity of the plasma and inter-erythrocyte interaction, promoting their aggregation [18].
Increased blood viscosity, adversely affecting its fluidity, further facilitates the aggregation and such an avalanche-like development of processes leads to formation of vascular thrombosis. This is one of the main and intended purpose of the local hyperthermia in case of tumor tissue, but it is a matter of special consideration and analysis.

Based on the fact that our study was conducted on rats, it is necessary to recall that between species of vertebrates the index of RBC aggregation varies greatly [7] and the lowest is in the rats that have very poorly defined tendency to aggregation of RBC [3]. There is strong reason to believe that Nitric Oxide plays an important role in the regulation of blood rheology, particularly in the phenomenon of RBC aggregation, and that disruption of this regulation is one of the factors causing the development of L-NAME-induced hypertension [3]. It is believed that Nitric Oxide-induced improvement in the deformability of RBC and decrease in their aggregability are the results of Nitric Oxide direct action on RBC, which is considered as a sufficient reason for use of Nitric Oxide donors to improve blood fluidity [19]. But it should be emphasized that this applies only to Nitric Oxide produced by activation of the constitutional forms of NOS, primarily the endothelial one (eNOS).

In 1987 by Maeda et al [10] showed that at increase in temperature (in a range of 5 to 430C) velocity of fibrinogen-induced RBC aggregation increases. Our data presented in Table 1 show that increase of temperature in hyperthermic chamber leads to multiple, statistically significant increase in the rats' RBC aggregation index, which in contrast to other vertebrates, as already noted, in the norm probably is very low [2]. On the background of NOS inhibition by L-NAME we recorded (see Table 1) statistically significant ($P < 0.05$) decrease in RBC aggregation index at all temperature regimes of hyperthermia. Is this in contradiction with the above-mentioned effect of Nitric Oxide on the aggregation of RBC? We believe that there is not contradiction, because it is well known that hyperthermia stimulates the excessive production of Nitric Oxide, as it causes a significant activation not only constitutional isoforms of NOS, but also the inducible one (iNOS) [1] which is accompanied with sharp intensification of free-radical processes and formation of peroxynitrite.
Along with this, dramatically increases the xanthin oxidase-induced generation of reactive oxygen species [3]. These effects primarily influence the process of RBC aggregation. Free radicals' attack leads to damage not only the membrane of red blood cells, but their cytoplasmic structures also, which leads to an increase in the index of aggregation, to the durability of aggregates and, consequently, to a considerable increase in the shear rate required for their disaggregation [3]. Increased blood viscosity, adversely affecting its fluidity, further facilitates the aggregation and such an avalanche-like development of processes leads to formation of vascular thrombosis. This is one of the main and intended purpose of the local hyperthermia in case of tumor tissue, but it is a matter of special consideration and analysis.

Based on the foregoing it is clear that non-selective inhibition of all isoforms of NOS by means of L-NAME on the background of hyperthermia results (in our experiments) to the reduction of red blood cell aggregation indwx.

Conclusion

The analysis of all the above data led us to the following conclusions:

1. The brain has a highly secured system of temperature autoregulation against the exogenous temperature changes.
2. The upper limit of autoregulation (for rats, at least) is in the range 45^0C of environment.
3. An important role in the normal functioning of the brain temperature autoregulation system belongs to Nitric Oxide.
4. Behavioral disorders, observed in animals after Whole Body Hyperthermia (sure within the range of brain temperature autoregulation) is hardly associated with the changes in temperature of the Central Nervous System, but rather have to be mediated by impaired blood circulation and oxygen supply to the brain tissues, caused by the rapid deterioration of the blood rheological properties.

References

1. Arnaud C., Godin-Ribuot D., Bottary S., Peinnequin A., Joyeux M., Demenge P., Ribuot C. iNOS is a mediator of the heat stress-induced preconditioning against myocardial infarction in vivo in the rat. Cardiovascular research, 2003, 58, 118-125, 118-125.
2. Baskurt Oguz K., Robert A. Farley, and Herbert J. Meiselman. Erythrocyte aggregation tendency and cellular properties in horse, human, and rat: a comparative study. *Am J Physiol Heart Circ Physiol*, **273** (1997) H2604 - H2612.
3. Baskurt OK, Meiselman HJ, Kayar E. Measurement of red blood cell aggregation in a "plate-plate" shearing system by analysis of light transmission. Clin. Hemoreol. Microcirc., 1998, 19, 307-314.
4. Bicher HI, Mitagvaria NP, Nebieridze MI. The role of local blood flow intensity, blood rheological properties and free radicals in development of local hyperthermia-induced morphological changes in cerebral tissue of the rat. Bicher Cancer Institute. 2010, Los Angeles, CA, USA. http://www.bichercancerinstitute.com/Papers/
5. Bor-Kucukatay M, O Yalcin, O Gokalp, D Kipmen-Korgun, A Yesilkaya, A Baykal, M Ispir, UK Senturk, I Kaputlu, and OK Baskurt Red blood cell rheological alterations in hypertension induced by chronic inhibition of nitric oxide synthesis in rats. *Clin Hemorheol Microcirc*, **22** (2000) 267-275.
6. Holdcroft A. Body temperature control : in anaesthesia, surgery and intensive care. 1980, London : Bailliere Tindall, (1980) 179 p.
7. Kumaravel M and M Singh. Aggregation and deformability of erythrocytes in leprosy. *Indian J Exp Biol,* **33** (1995) 408-415.
8. Lim HJ, YJ Lee, JH Nam, S Chung, and S Shin Temperature-dependent threshold shear stress of red blood cell aggregation. *J Biomech*, **43** (2010) 546-550.
9. Mackowiak PA, ed. Fever : Basic Mechanisms and Management, (Lappincot, Philadelphia), 1997, pp 506.
10. Maeda N, M Seike, and T Shiga Effect of temperature on the velocity of erythrocyte aggregation. *Biochim Biophys Acta*, **904** (1987) 319-329.
11. Maier CM, Steinberg GK. Hypothermia and Cerebral Ischemia : Mechanisms and Clinical Application, 2004, (Humana, Totowa) pp, 188.
12. McElligott JG and R Melzack Localized thermal changes evoked in the brain by visual and auditory stimulation. *Exp Neurol*, **17 (**1967) 293-312.
13. Mete F, Kilic E, Somay A, Yilmaz B. Effects of heat stress on endocrine functions & behaviour in the pre-pubertal rat. Indian J Med Res. 2012;135:233-239.
14. Mitagvaria NP, Bicher JI, Lazrishvili IL, Devdariani MI, Nebieridze M., Gobechia LSh, Sikharulidze N. proceedins of XXX Meeting of ICHS, Tbilisi, 2011, 4.
15. Mitagvaria NP, Bicher JI. Cerebral Blood Flow Regulation, 2009, Nova Science Publishers, New York.
16. Nagashima T, H Ohinata, and A Kuroshima Involvement of nitric oxide in noradrenaline-induced increase in blood flow through brown adipose tissue. *Life Sci*, **54** (1994) 17-25.
17. Nelson DA and SA Nunneley Brain temperature and limits on transcranial cooling in humans: quantitative modeling results. *Eur J Appl Physiol Occup Physiol*, **78 (**1998) 353-359.
18. Neumann FJ, H Schmid-Schonbein, and H Ohlenbusch Temperature-dependence of red cell aggregation. *Pflugers Arch*, **408 (**1987) 524-530
19. Starzyk D, R Korbut, and RJ Gryglewski Effects of nitric oxide and prostacyclin on deformability and aggregability of red blood cells of rats ex vivo and in vitro. *J Physiol Pharmacol*, **50 (**1999) 629-37.
20. Sukstanskii AL and DA Yablonskiy. An analytical model of temperature regulation in human head. *J Therm Biol*, **29** (2004) 583-587
21. Sukstanskii Alexander L. and Dmitriy A. Yablonskiy Theoretical model of temperature regulation in the brain during changes in functional activity. *PNAS*, **103** (2006) 12144 -12149.
22. Swan H. Thermoregulation and Bioenergetics, 1974, Elsevier, N.Y.
23. Taylor W. F. and V. S. Bishop A role for nitric oxide in active thermoregulatory vasodilation. *Am J Physiol Heart Circ Physiol*, **264** (1993) H1355 - H1359
24. Van Leeuwen GM, JW Hand, JJ Lagendijk, DV Azzopardi, and AD Edwards. Numerical modeling of temperature distributions within the neonatal head. *Pediatr Res*, **48** (2000); 351-356
25. Zhu Mingming, Joseph J. H. Ackerman, Alexander L. Sukstanskii, and Dmitriy A. Yablonskiy. How the body controls brain temperature: the temperature shielding effect of cerebral blood flow. J Appl Physiol, **101** (2006) 1481 - 1488.