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# Hormetic Effect of whole Body Hyperthermia (Experimental Study on Rats)

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It is established that hyperthermic exposure is associated with the development of oxidative stress in cells [Finkel, Holbrook, 2000]. The oxidative stress in its essence is related to the process of massive release of free radicals. The possible mechanisms responsible for the development of this process has not been sufficiently examined, although some of them are known. For example, activation of the immune system in response to infection when for elimination of microorganisms, phagocytes begin "shoot" by Hydrogen Peroxide (HP) - a strong oxidant.
It is known that the increase in resistance to oxidative stress is associated with the extension of the vitality of the body [Larsen, 1993]. In particular, it was found that low doses of oxidative stress (induced by, e.g., heat shock) slowing the aging process [Kurapti et al., 2000]. Practically here we have to deal with a phenomenon which is known as "Hormesis". This term originates from the ancient Greek and means "to bring in motion, prodding, acceleration". From a biomedical point of view the term "Hormesis" describes phenomena, when in response to low doses of toxins or any other stressors, the body develops a positive reaction (from a biological standpoint) - an adaptive stress-response, which provide stability of cells to higher (fatal) doses of stressogenic factors stimulating the response (Calabrese et al, 2010).
In recent years the interest to the phenomenon of hormesis has increased enormously, because stress can be physical and chemical, as well as psychological. Even the radiation hormesis, i.e. protective effect of low doses radiactive exposure is under intensive investigation.
The main purpose of this study was to investigate the effects of oxidative stress caused by Whole Body Hyperthermia (WBH) on the behavior of white rats. It should be noted that during the formation of this goal, we did not assume any connection with the phenomenon of hormesis, but the results have forced us to delve into the essence of this particular phenomenon.
For the purpose of comparative evaluation of received results, we found it necessary to use also another method of inducing oxidative stress – chronic administration of HP.

## *Materials and methods*

The experiments were conducted on seven groups of white rats weighing 250-300g (both males and females). State of oxidative stress in animals (before their testing in a multi-way maze) was caused either by WBH or by administration of HP. In particular:

1. The first group of rats instead of regular drinking water for four weeks were allowed to 0.1% solution of HP. We used food-grade HP (Wellness, 35% $H_20_2$); since the beginning of the fifth week the animals began to be trained in the maze for learning the optimal trajectory to get into the nest-box. Prior to the completion of testing (7-8 days), rats, instead of regular drinking water continued to take a 0.1% solution of HP.
2 The second group of rats exposed to WBH in a special hyperthermic chamber. The temperature in the chamber was maintained at the level necessary to achieve a rectal temperature of 40 °C, and this level was maintained for 4 hours. This kind of exposure they received every other day for four weeks. After completion of all hyperthermic exposures, the animals ofthis group were also tested in a multi-way maze.
3 The third group of rats received a combined dose of stress: daily receiving 0.1% HP with drinking water for 4 weeks (similarly to the first group) and every other day the animals were also subjected to WBH, similarly to the second group of animals. Then, the animals of this group were tested in the multi-way maze.

4 A fourth group of rats 15-20 minutes before of each hyperthermic exposure received an intraperitoneal injection (30mg/kg) of a nonselective inhibitor of nitric oxide synthases (NOS) - Nitro-L-Arginine Methyl Ester (L-NAME); After these actions animals of this group were also tested in the maze

5 The animals of the fifth group 15-20 minutes before the beginning of the daily maze sessions, similarly to the fourth group of animals, received an injection of non-selective inhibitor of NOS - L-NAME, but animals of this group were not subjected to WBH.

6 The sixth group consisted of animals, which 15-20 minutes before the start of the daily maze sessions, opposed to the fifth group received an injection of selective inhibitor of inducible nitric oxide synthase (iNOS) Aminoguanidine at a dose of 30 mg/kg.

7 The control group were intact animals, not exposed neither to stressogenic nor pharmacologic factors. A general view of the maze that we used is shown in Figure 1.

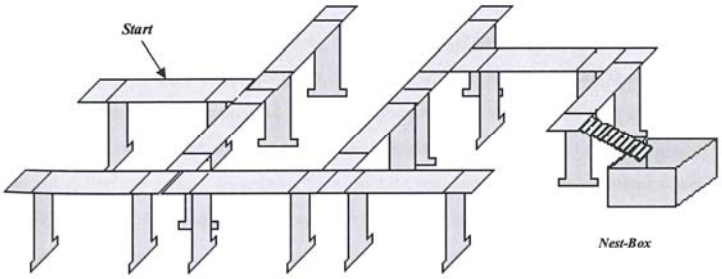


*Figure 1.*

It consists of separate bridges (30 cm high), which makes it possible to easily change the configuration, complicate or simplify the task - getting into the nest-box. At the first start of animals through the maze (the first day of the maze session), experimenter usually helps animal in finding the optimal trajectory from a start to destination point (nest-box), and later they learn independently, by trial and error. The experiments were carried out without food reward, the incentive to move through the maze is the getting rid of not ethological situation - being on the maze platforms. Conditions in the experimental room (lighting, location of objects, etc.) until the completion of the experiments remained strictly unchanged. Assessment of testing is carried out for the two indicators - the number of errors committed (deviations from the optimal trajectory) and the time spent for the passage from start point to the nest-box. Registration of the named indices starts from the second day of training, so as in the first day of the majority of the animals received assistance from the experimenter. At the end of experiments from each experimental group two animals were randomly selected for a blood test (analysis of rheological properties), and two more for morphometric studies of the sensorimotor cortex (the results of these studies will be discussed in a separate article).

## *Results*

From the very beginning of analysis of the received data we have to underline that no statistically significant differences in maze learning from the point of view of reducing the number of errors (deviations from the optimal trajectory) in between of groups has not been observed. On the seventh day of training (five starts per day) almost all of the animals were able to pass a maze without a single error, but if we compare the time spent for maze passage differences have been identified in between of different groups.

Figure 2. Shows the change of time required for passage of the maze by the different groups of animals. In particular, there are the control group and the groups that underwent oxidative stress by

administration of HP or WBH, as well as the group that received combined action of both stress factors.

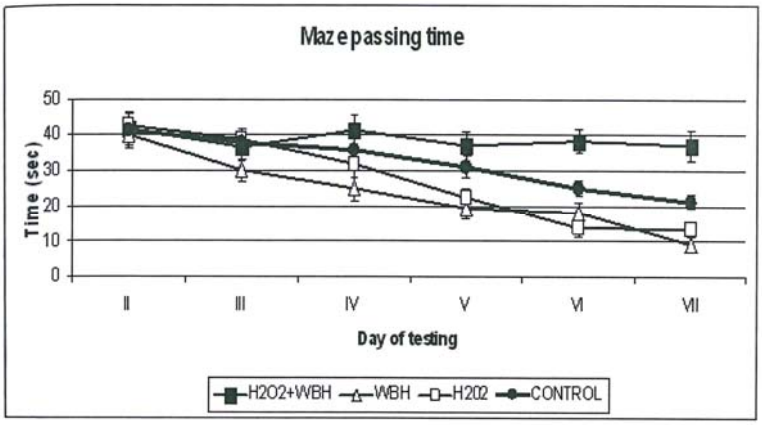


*Figure 2.*

Figure 2. Time required for passage of the maze by the control group and the groups that underwent oxidative stress by administration of $H_20_2$ or by WBH, as well as the group that received combined action of both stress factors.

It was found that animals that were exposed to stressors either chronic administration of HP or WBH, significantly increased their behavioral activity. In comparison with the control animals they behaved on the maze platforms very lively and energetic, and at the end of the seven day training, they were able unmistakably pass all the way almost twice as fast as the control animals (Fig. 2)

In contrast to first two groups, the animals from the third one, that received the combined action of both WBH and HP, dramatically slowed the behavioral activity, animals looked depressed and to achieve the final target at the end of the seventh day of the maze sessions, the needed time two times greater than the control animals and three times more than the group that underwent the action by one of stress-factors.

As indicated in the methodical part of this paper we also had other groups of animals. In one of them (fourth group) the animals every day (in duration of four weeks) were administered by L-NAME, and besides, they every other day underwent the WBH. Testing of animals from this group in maze showed that time for maze passage does not differ from that observed in animals that were exposed only WBH, or those that have taken just HP solution (Figure 3, curve with open triangles).

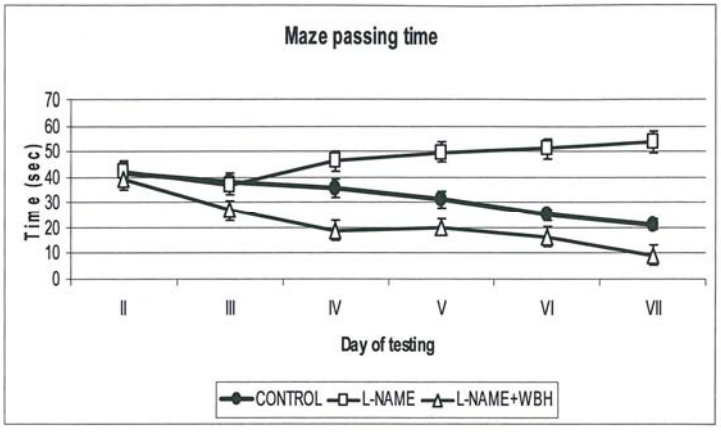


*Figure 3. Time required for passage of the maze by the control group, the groups that underwent L-NAME administration and the group that received combined action of L-NAME and WBH*

The animals of fifth group prior to maze sessions were administered by NOS nonselective inhibitor - Nitro-L-Arginine Methyl Ester - L-NAME (30 mg/kg), but unlike the previous group animals did not get the hyperthermic exposure. As can be seen from Figure 3 the motor activity of the animals in this group declined sharply and at the end of training sessions (7th day) the animals were able to reach the nest-box on average for 55 seconds, which is more than twice greater than the time spent on solving the same problem by animals of control group.

To assess the role of Nitric Oxide, produced by activation of its inducible synthase (iNOS) special (sixth) group of animals instead of L-NAME were administered by selective inhibitor of inducible NOS - Aminoguanidine (30 mg/kg). In this group of animals a statistically significant difference in time needed to pass the maze in comparison with the control group were not revealed (see Figure 4).

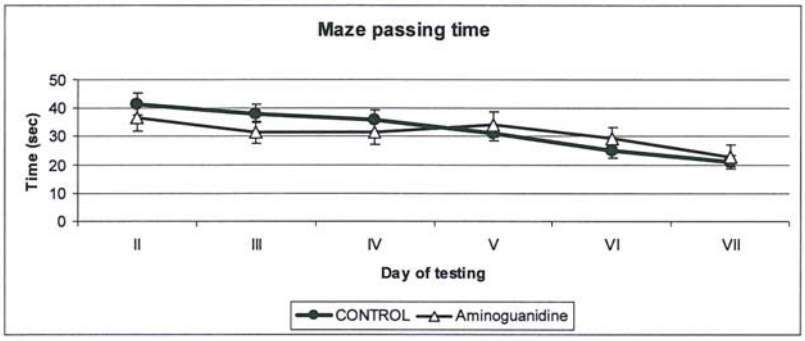


*Figure 4. Time required for passage of the maze by the control group, the group that underwent Aminoguanidine administration*

Despite the described differences in time of the maze passage, as we have already mentioned above, statistically significant difference in the number of errors committed in the process of learning between used groups of animals have not been identified. The learning process in accordance with this criterion in all groups of animals was almost the same.

## *Discussion*

The body's resistance to stresses is one of the most important indicators of its viability and it is clear that the study of the mechanisms that shape this resistance have a fundamentally important character (Michalski, Novoseltsev, 2005).

Oxidative stress, as noted, is involved in the development of many pathological processes (Nunomura et al., 2005; Ramalingam, Kim, 2012; Singh et al., 1995; Valko et al, 2007), but it appears that it may play a significant role in processes of physiological adaptation and regulation of intracellular signal transduction. It is believed that the most appropriate definition of oxidative stress is a "state in which oxidation exceeds the antioxydant systems in the body secondary to a loss of balance between them" (Yoshikawa, Naito, 2002).
Markers that may help in the recognition of oxidative stress in in-vivo conditions are gaining in importance, because the determination of the state of oxidative stress is essential not just for the study of many diseases, but also to improve the efficiency of their treatment (Yoshikawa, Naito, 2002).

The above mentioned proves that for the study of oxidative stress problems it is essential to have an adequate experimental models. To these kind of models can be confidently attributed the

chronic administration of HP (0.1% in drinking water) or the use of whole-body hyperthermia (heat shock), which were used in our work.
There are suggestions that chronic stress can contribute to disorders of learning and memory, and that it is an important contributor in the development of Alzheimer's disease (Nunomura et al., 2005; Jeong et al., 2006). According to the theory of D. Harman (1956, 1972), oxidative stress plays a significant role in the processes of aging. Further development of this theory oddly enough, got in the works, which argue that free radicals may contribute significantly to metabolic health and life expectancy (Ristow, Schneisser, 2011; Guliano, Watson, 2012). This effect is known as mitochondrial hormesis (mitohormesis).

The aging process is associated with a stochastic accumulation of damage at the molecular level and progression of inability to restore them. The use of the phenomenon of hormesis in the study of this process is based on the principle of stimulation of recovery processes by use of repeated exposure to mid-level stress. One of the first versions of this methodical approach was the use of repeated thermal shocks on the culture of human cells. The results of these studies have shown that the use of the principle of hormesis in gerontological research has a very promising future (Rattan, 2005). By activating the stress responses at the cellular level Mattson (Mattson, 2005) concluded that the organisms that in the process of evolution used the toxic agents from the environment to their advantage, often used them as signaling molecules that trigger adaptive stress responses. Examples are nitric oxide (NO) and carbon monoxide (CO), amino acids (e.g. glutamate) and ions of Ca and K.

Analyzing the mechanisms of HP-induced oxidative stress in in-vitro models, Coyle (2004) came to the conclusion that in its development involved the nitric oxide synthase (NOS) and NADPH-oxidase, which, in fact, serve as a source for increased level of reactive oxygen species. The presence of cytoprotective properties of the responses to stressors caused a widespread interest in the creation of pharmacological agents capable of inducing stress reaction, but their level should not go beyond the hormetic reactions.

The fundamental basis for understanding the phenomenon of hormesis curve is the "dose-response", which shows the process of stimulation at lower doses and inhibition - at high.
Low or high doses of stress factors cause, respectively, eustress or strong distress, resulting in activation of moderate or damaging allostatic buffering capacity of the organism. This is true no matter what the nature of the stressor is - the physical, chemical or mental (Cornelius et al., 2013). These authors believe that a well-known concept of preconditioning is a classic manifestation of the phenomenon of hormesis.

On the background of all foregoing, analysis of our results allows to conclude that in our experiments, we observed behavioral manifestations of the phenomenon of hormesis. A very significant increase in behavioral activity aimed at getting rid of from non ethological conditions in response to oxidative stress caused by the introduction of HP (the first group) or hyperthermic exposure (second group), in our opinion indicates that in both cases the dose of induced stress was within the range needed for stimulation of hormetic mechanisms.

Combined exposure of both stressogenic factors (WBH and HP) have apparently brought to the level that is out of functioning of hormetic mechanisms. Roughly similar results were obtained earlier in drosophilas, flying speed of which after application of oxidative stress by HP, was, according to the authors, "dramatically increased» (Grover et al., 2009). And two years earlier than Grover et al, again in Drosophila has been shown that hyperthermic preconditioning (36°C for one hour) improves the locomotor activity (Xiao et al., 2007). In addition, it was found that low dose stress increased also life expectancy of Drosophila (Butov et al., 2001). It is believed that this effect is due to activation of heat shock proteins chaperone functions, resulting in not only in reparation of damage inflicted by stress, but also that having place before applying the exposure of

stressor (Butov et al., 2001). Operation of this mechanism is generally associated with the production of nitric oxide (Romano et al., 2011).

In our experiments, inhibition of production of nitric oxide in the group of animals that hyperthermic exposure was carried out on the background of the non-selective inhibitor of NOS-L-NAME, as we can see on Figure 3, practically there is not any changes in hormetic effect of hyperthermic stress. The same figure clearly demonstrates that in normal animals (without stress exposure), blocking the production of nitric oxide, as compared with the control group, and even more with the group in which against the background of the NOS non selective inhibition was subjected to hyperthermic stress, significantly decreased locomotor activity of animals.

In according to data obtained from the next group of animals, we can conclude that the sharp decrease in locomotor activity on the background of L-NAME, was mediated by inhibition of endothelial isoform of NOS. Figure 4. clearly shows that the selective inhibition of the inducible isoform of NOS by Aminoguanidine, almost did not have any effect on the behavior of rats in a maze – a statistically significant difference from control animals were not detected. It is possible that in the case of non-selective inhibition of NOS, hyperthermic exposure activates another source of oxygen radicals, namely NADPH oxidase (Coyle, 2004) which provides the formation of oxidative stress and induction of hormetic effect.

Anyway, we think that the results of our experiments suggest that for the formation of oxidative stress and accordingly hormetic effect, the presence of nitric oxide is not a necessary factor, at least in case of oxidative stress caused by WBH.

In this presentation, we did not consider such important issues as the activation of the transcription factor Nrf2, as well as the system of heat shock proteins, which without any doubts are involved in the formation of hormetic effect caused by WBH.