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Basic Science

Effects of the Pharmacological Pre-treatment and Global Brain Ischemia on the Body Temperature in Experimental Rats

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Abstract

The aim of this study is to examine the influence of the pharmacologic pre-treatment and global brain ischemia on the one hand, and on the other hand, the influence of anaesthesia on the body temperature in experimental rats. The study involves 48 white female experimental rats (n=48), strain - Wistar, with body weight ranging from 230 g to 390 g, and age range of 3-5 months. The rats were divided into two groups. All of the rats were anesthetised and underwent various pharmacologic pre-treatments (with erythromycin and physiologic solution) and global brain ischemia with a reperfusion period. The obtained results demonstrate increased body temperature during the anaesthesia, a decrease during ischemia in both groups, and also a significant increase of the temperature 18 hours after the induced global brain ischemia. However, no significant temperature differences were registered between the groups. It can be concluded that the pre-treatment with a single dose of the macrolide antibiotic erythromycin does not cause any significant variations of the body temperature in experimental rats, and this under conditions of anaesthesia and global brain ischemia.

Introduction

The phenomenon of pre-treatment with chemical substances with small molecules is used as a procedure for achieving cerebral protection. Nowadays, one of the ways to induce tolerance towards ischemia is the pre-treatment with specific pharmacologic substances with small molecules and it is called "pharmacologic pre-treatment".

The first examinations in which the hypothermia was used for cerebral protection, demonstrated that the deep hypothermia increases the tolerance towards ischemia (1, 2).

Later, it was proven that the changes in the temperature of the brain do influence the post-ischemic survival in experimental animals (1-5). Quite different from hypothermia, hyperthermia worsens the result after the brain ischemia in several experimental animals (6,7).

The aim of this study is to examine the influence of the pharmacologic pre-treatment and global brain ischemia, on the one hand, and that of anaesthesia, on the other hand, on the body temperature of experimental rats.

Material and methods

The study involved 48 white female laboratory rats (n=48), strain - Wistar, with body weight ranging between 230 g and 390 g, and age range between 3 and 5 months. All of the animals were provided human care, in compliance with the Principles of Laboratory Animal Care of the Institute of Biology, Faculty of Natural Sciences and Mathematics.

All of the animals involved in the study were anesthetised, underwent different pharmacologic pre-treatment and global brain ischemia with a period of reperfusion. The body temperature was being monitored during 6 different intervals.

Protocol

Prior to experiment onset, animals with increased body temperature (n=3) were excluded from the experiment.

Depending on the pharmacologic pre-treatment, the experimental animals were divided into two major groups.

The first group (Er) consisted of 35 rats (n=35). Six hours prior the anaesthesia, they were given intramuscular inducing dose of erythromycin (25 mg/kg erythromycin lactobionat). The second, control group (C), consisted of 10 experimental animals (n=10); they were administered appropriate quantity of 0.9% of physiologic solution.

The light regime for the animals was 12 hours, and the analyses were performed during the same time period. After the six-hour-period of hunger and pharmacologic pre-treatment, the rats were anesthetised with intra-muscular combination of midazolam 4 mg/kg and ketamin 60 mg/kg.

The carotid arteries showed up in the anesthetised animals with the longitudinal incision of about 1cm was performed laterally from the middle line on the neck above the hyoid muscle. The fasciation and the muscles were dislocated, which has demonstrated triad of carotide arteries, jugular veins and n.vagus. The carotid arteries were prepaired bilaterally. Then by sole ligatures from surgical stitch of nylon (Prolene 1, Ethicon, manufacturer- Jonson and Jonson Int., W742) were ligated and so remained for 15 minutes. This provides global brain ischemia. Than, the stitch was removed and the blood flow through the carotids was enabled again, i.e. reperfusion was enabled. After the end of the ten-minute reperfusion, the wound was surgically closed, and the animals were put in their cages. The heads and the bodies of the animals were covered with a blanket during the whole experiment.

The body mass and body temperature of the rats were measured prior to experiment onset. The body temperature was measured with a rectal thermometer, 6 times, in particular: prior the experiment onset and anaesthesia (t0), during the anaesthesia (t1), after the surgery and before the ligation of the carotid arteries (t2), during the ischemia (t3), during the reperfusion (t4) and 18 hours after the induced global brain ischemia (t5).

The statistical processing of the data was carried out by means of the statistical package Statistica 6 for MS Windows XP and MS Excel 2003. The data obtained are shown as a mean value ± standard deviation. Fisher's ANOVA and paired t-test were used for comparison of rectal temperature between groups and between the particular times of measurement in each group; $p<0,050$ was considered as a statistically significant difference.

Results

The experimental design of this study includes a total of 45 female experimental rats. Seven animals died during the reperfusion period, i.e. during the first 18 hours, after the fifteen-minute global brain ischemia. Three of them belonged to the group treated with erythromycin and four were from the control group. The definite group that was examined consisted of 38 rats. The obtained results demonstrate that the body temperature of all the experimental animals, subjects of the examination, was in the frames of the physiologic ranges during the experiment (Fig.1.).

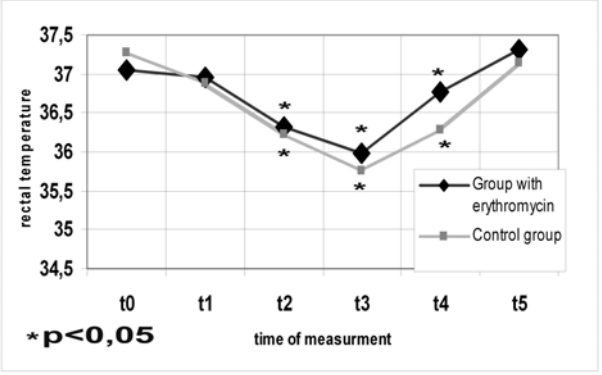

*Figure 1: Average rectal temperature in the 6 times of measurement in both groups of experimental animals, group pretreated with erythromycin and 18 hours of reperfusion and control group with reperfusion. *-p<0,05 point out significant differences compared to time t_0.*

Table1: Significant differences among the means of rectal temperature within the experimental groups (group pretreated with erythromycin and 18 hours period of reperfusion and the control group). n.s point out nonsignificant differences in the means of rectal temperature between particular time of measurement in the group, while p<0,05 denote significant differences. t_0, t_1, t_2, t_3, t_4, t_5, are the different times of measurement.

	Group Erythromycin with reperfusion	Control group
$t_0 : t_1$	n.s	n.s
$t_0 : t_2$	p<0, 05	p<0, 05
$t_0 : t_3$	p<0, 05	p<0, 05
$t_0 : t_4$	p<0, 05	p<0, 05
$t_0 : t_5$	n.s	n.s
$t_2 : t_3$	n.s	p<0, 05
$t_3 : t_4$	p<0, 05	p<0, 05
$t_4 : t_5$	p<0, 05	p<0, 05

Table 1 showed the significant differences among the means of rectal temperature in the group pretreated with erythromycin and 18 hours period of reperfusion and the control group where: t_0 - mark the means of rectal temperature measured prior the experiment onset and anaesthesia, t_1 - denote means of rectal temperature during the anaesthesia, t_2 - define means of rectal temperature after the surgery and before the ligation of the carotid arteries, t_3 indicate the means of rectal temperature measured during the ischemia, t_4 - characterize the means of rectal temperature measured during the reperfusion and t_5 - point out means of rectal temperature 18 hours after the induced global brain ischemia.

The obtained results demonstrate that the induction in the anaesthesia with ketamin, in both groups, group treated with pharmacologic pre-treatment and control group, caused a insignificant increase of the temperature (t_0:t_1). A significant decrease of the temperature happened after the surgery and before the ligation of the carotid arteries (t_2; p<0,05) compare to the period prior the experiment onset and anaesthesia(t_0) in both groups of experimental animals (see Fig.1). Further, ischemia (t_3) significant diminished rectal temperature compare to t_0 ($t_0 : t_3$, p<0,05) in group pre-treated with erythromycin and control group, and significant decrease happened in control group compare to t_2 (t_2:t_3) too (Table 1).

A significant increase of the temperature during the reperfusion (t_4) was registered in both experimental groups. Period of 18 hours after the induced global brain ischemia (t_5) relate to additional increase of the rectal temperature touching (t_4), which finally results with returning of the rectal temperature to the baseline level (t_0 :t_5, n.s).

On basis of the obtained results the following can be concluded:

There are significant differences in each group individually and during the various time periods of the temperature measuring. However, no significant temperature differences were registered between the groups.

Discussion

The temperature of the cerebral tissue differs from the body temperature (8,9) i.e. the temperature of the cerebral tissue is a bit higher than the body temperature. Ward et all. demonstrated that there is a difference in the temperature of the neocortex and the other parts of the brain as a result of the brain metabolism and the differences between the brain and body temperature (10).

The change of the brain temperature about 2-3^{0}C does significantly increase the brain damage after the ischemia, and, it influences the cerebral metabolic response to the ischemia (11). The body hypothermia during the global brain ischemia induces release of neurotransmitters and free fatty acids that modify the cell response towards ischemia (12).

Under normal conditions, the brain temperature depends on the brain blood flow (13), the brain metabolism (14,15) and the temperature exchange with the environment (16-20).

The results from this study illustrated that there was no a significant decrease of the body temperature during the experiment, and the influence from the hypothermia as one of factors important for the cell response to ischemia, is minimised.

The temperature of the rats from both of the groups in the start of the experiment is with almost same values, which indicates the homogeneity of both of the groups.

The use of the anaesthetic ketamin in a combination with midazolam caused a statistically insignificant decrease of the body temperature in both of the groups. The decrease of the body temperature under influence of the anaesthetics is a well known phenomenon that is due to the redistribution of the central body temperature towards the surface.

This mechanism enables increase of the surface body temperature, and decrease of the central temperature.

The production of the heath during the anaesthesia is equal to the basal metabolic temperature (22). The exchange of the heath between the skin and the environment takes place spontaneously. The thermoregulation under conditions of anaesthesia is absent, but it is activated again when the body temperature decreases under the critical values of 34-35^{0}C (23, 24). All the medications that are used in the anaesthetic procedures, including ketamin, influence the normal autonomous control of the thermoregulation. The central body temperature decreases from 0.5 to 1.5^{0}C during the first hour after the induction in the anaesthesia (25).

This experiment involved measuring of the central body temperature, by means of rectal thermometry, and after the induction (t1-t2) a decrease of the body temperature of 0.7±0.5^0 C was registered.

Ketamin that was used as an anaesthetic has a special roll among the other anaesthetics. It increases the artery blood pressure, and during the period of absence of the mechanisms of the cerebral-vascular auto-regulation, it causes cerebral vasodilatation, it passively increases the cerebral blood volume and the intracranial pressure (26, 27).

Although the surgical intervention in this experiment involves a small incision, it results in a further significant decrease of the body temperature, in both of the groups ($p<0.05$). It is due to the loss of the body temperature through the convection and radiation, especially because of the big blood vessels in this region, which were exposed to the influence of the outer temperature.

The ligation of both of the carotid arteries caused an interruption of the flow of artery blood in the brain, after that global brain ischemia took place and it additionally interrupted the regulation of the body temperature in the experimental animals of both of the groups. During the period o the fifteen-minute global brain ischemia, in the examined group of animals (Er), the following was registered: a decrease of the body temperature of 0.9 ± 0.2^{0}C ($p<0.05$) from the basal value; and a significant decrease of 1.1 ± 0.5^{0}C ($p<0.05$) in the control group.

This decrease of the body temperature of the experimental animals in both of the groups, subjects of the examination, is in the frames of the normal physiologic ranges.

There was an increase of the body temperature which after 18 hours reached the basal values in both of the examined groups, and it was a result from the reactive hyperthermia followed by reperfusion (t3-t5). Hyperthermia may persist for at least 3 days in rats, depending of the territory damage by hypoxia (28).

The obtained results do clearly illustrate that there is a quick normalisation of the temperature values in the group treated with erythromycin, also there are prolonged decreased values of the body temperature in the control group.

On basis of this information and of the previously obtained significant values for the change of the body temperature, it can be speculated that the pre-treatment with erythromycin influences the fast recovery of the brain tissue after suffering the global brain hypoxia.

The conclusion is that the pre-treatment with a single dose of the macrolide antibiotic erythromycin does not cause any significant changes in the body temperature of the experimental rats, under influence of anaesthesia and global brain ischemia. The decrease of the temperature of the brain during the global ischemia happens as a result of the complete interruption of the blood flow. The decrease of the temperature in physiologic limits during the period covered by anaesthesia and the period of the global brain ischemia happens because of the influence of the anaesthetics, the surgery and the temperature exchange with the environment through conduction, convection and radiation.

As a final conclusion, it may be speculated that the pre-treatment with erythromycin has a positive influence on the brain tissue that has suffered a global hypoxia.

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