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ACTIVITY OF KEY ENZYMES OF ANTIOXIDANT SYSTEM IN RAT BLOOD PLASMA UNDER THE EFFECT OF HISTAMINE AND SODIUM HYPOCHLORITE

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The effects of histamine in 1 $\mu\text{g}/\text{kg}$ and 8 $\mu\text{g}/\text{kg}$ doses that correspond to the doses causing pathological effects at experimental conditions and of sodium hypochlorite in 5 mg/l dose – the lowest concentration of sodium hypochlorite that affects a body by oral administration, on the key enzymes of blood plasma antioxidant system were studied. It was found that histamine used in both concentrations intensified superoxide dismutase activity for 14 days. The simultaneous injections of histamine and sodium hypochlorite caused significant activation of superoxide dismutase in rats. Sodium hypochlorite received by rats with drinking solution caused the same effect. The catalase activity of blood plasma was not significantly affected by histamine, and its activity was significantly increased only under the influence of biogenic amine in 1 $\mu\text{g}/\text{kg}$ dose on the 7th day of the experiment. Sodium hypochlorite caused a decline in catalase activity both in intact animals and in animals that received histamine injections subcutaneously. The injection of histamine in 1 $\mu\text{g}/\text{kg}$ dose caused an increase in glutathione peroxidase activity on the 1st and 7th day of the experiment. Histamine in 8 $\mu\text{g}/\text{kg}$ dose caused the intensification of glutathione peroxidase activity only on the 1st day, followed by the inhibition on the 14th day of the experiment. Sodium hypochlorite received by rats with drinking solution led to general lowering of glutathione peroxidase activity in blood plasma.

Keywords: rats, histamine, sodium hypochlorite, blood plasma, superoxide dismutase, catalase, glutathione peroxidase.

INTRODUCTION

Disturbances of the activity of antioxidant system, and intensification of free radical oxidation of lipids play an important role in the pathogenesis of inflammatory processes (with the leading role of histamine). All cellular structures are sensitive to free radicals, that can cause cell death. It is a well-known fact that the organism can suffer from significant negative effects of histamine, including allergic diseases. Free histamine can cause headaches, catarrh, skin hyperemia, diarrhea, tachycardia or arrhythmia, smooth bronchi muscles spasms, etc. [7, 13]. The histamine is released from tissue basophils,

blood basophils and binds with receptors – H1, H2, H3 and H4. Its interaction with receptors activates adenylyl cyclase and protein kinase A which results in stimulation of biological processes through H2, and inhibition through H3 and H4 receptors. Interaction between histamine and H1 receptor activates phospholipase C and protein kinase C, thus stimulating a biological response [15].

Allergic states are characterized by changes in lipid peroxidation intensity, cytokines synthesis, inhibition of arachidonic acid products metabolism, etc. The dysregulation of metabolic processes in allergic conditions increases expenditure of energy, reduces mitochondria activity and causes ATP deficiency, which in turn leads to changes in basic properties of biomembrane (including ATPase activity).

It is known that histamine activates H2 receptors in the parietal cells of stomach, that is accompanied by an increase in intracellular cAMP and, thus, leads to an increase in H⁺, K⁺-ATPase activity directly through protein kinase A activation. Protein phosphorylation is a necessary precondition for secretory mechanisms.

Substances that are widely used in medicine for the correction of histamine release and metabolism fall into two major groups: histamine receptor blockers (diphenhydramine, Phencarolum, etc.) and histamine containing cell membrane stabilizers (cromolyn sodium, etc.). However, negative side effects (hallucinations, headache, dizziness, convulsions, elevated liver enzymes, etc.) initiated a search for other ways to insure inactivation and reduction of histamine in biological tissues [3]. We hypothesized that sodium hypochlorite (SH) can destroy blood histamine. Sodium hypochlorite solution is an electrochemical model of liver cytochrome P-450 as it effectively acts on various harmful compounds showing disinfecting and detoxifying effects. A significant advantage of sodium hypochlorite as the active oxygen carrier is that it enables to bypass the effect of "protein protection" from harmful metabolites. The SH is always present in the body as one of the main natural factors of infectious agents disintegration in leukocytes [4]. Getting into the bloodstream, SH oxidizes various toxins and metabolites. It is known that histamine is easy to oxidize. There is evidence that SH reduces the histamine content in human blood in case of severe poisoning by psychopharmacological agents [14]. However, there are no reports about the mutual action of histamine and SH at the low concentration on the antioxidant defense system. The fact that SH is used to disinfect water adds to the relevance of this study. Previously, we conducted a research, where we had examined the effect of histamine and SH on various body tissues [1, 2]. The concentration of SH was consistent with therapeutic (20 mg/l). In this series of experiments, we used the lowest concentration of SH (5 mg/l) in order to verify its efficiency under the simultaneous action of histamine in the body.

Thus, a study of this kind is important both in theoretical and practical terms, and will contribute to understanding of the mechanisms of SH and histamine effect in the body. This may open up opportunities for prevention of various types of diseases associated with the release of histamine from tissue basophils and blood basophils.

MATERIALS AND METHODS

For achieving research objectives, experiments were conducted on the nonlinear white male rats for 21 days. The animals were selected on the basis of analogies – 20 animals in each group. The weight of animals was 180–220 g. The first group of animals served as a control group. Animals of the second and third groups received

subcutaneous injections of histamine solutions in 1 and 8 $\mu\text{g}/\text{kg}$ doses, respectively, for 14 days. 0.01 % histamine dihydrochloride solution was used as a stock solution. The chosen doses corresponded to those that cause pathological manifestations in experimental conditions [8]. The animals in the 4th group were administered with a solution of SH at 5 mg/l concentration orally for 14 days. In addition, two more groups were formed in which animals were simultaneously injected with histamine (in both doses mentioned above) and SH (5 mg/l). On the 1st, 7th, 14th and 21st (rehabilitation) days, five animals from each group were decapitated under light ether anesthesia in compliance with the European Convention for the Protection of vertebrate animals used for experimental and scientific purposes (Strasbourg, France 1986) and according to the "General principles of working with animals", adopted by the First National Congress of Bioethics (Kyiv, Ukraine, 2001). Blood Plasma samples were taken. The amount of protein in each sample was determined by Lowry method [11]. Superoxide dismutase activity in the collected samples was determined by V. A. Kostyuk [10], activity of catalase and glutathione peroxidase was determined by M. A. Koroliuk [9] and V. M. Moin [12], respectively. Statistical analysis of the results was conducted using "Excel-2010" program for Windows. A significance in difference between the statistical characteristics of two alternative sets of data was estimated by Student coefficient. Significant difference was considered at $p \geq 0.95$, $p \geq 0.99$, $p \geq 0.999$.

RESULTS AND DISCUSSION

It was found that under the influence of histamine in 1 and 8 $\mu\text{g}/\text{kg}$ doses superoxide dismutase activity (SOD) in blood plasma increases (approximately 26 %) during the entire time of its subcutaneous injection to rats. This indicates an excessive accumulation of $\text{O}_2^{\cdot-}$. It is known that histamine is a potential inflammatory agent produced in the tissue basophils and blood basophils. It degranulates rapidly in response to inflammatory stimuli or certain medical substances [16]. Therefore, an additional injection of histamine into blood vessel causes a release of reactive oxygen species by neutrophilic cells, including $\text{O}_2^{\cdot-}$, which is consistent with the data reported in literature [6]. However, after the rehabilitation period SOD activity returned to control level under the action of histamine in 8 $\mu\text{g}/\text{kg}$ dose and reduced by 31 ± 0.45 % under the influence of histamine in 1 $\mu\text{g}/\text{kg}$ dose (Fig. 1). These results indicate a return of prooxidant-antioxidant status to normal. Oral administration of SH in 5 mg/l dose caused an increase in SOD activity by $27 \pm 0.13 \div 207 \pm 0.01$ % compared to control under a simultaneous influence of histamine in both of the abovementioned doses, as well as compared to the groups of animals that were administered histamine only through subcutaneous injections throughout the experiment. It should be noted that at this concentration SH leads to an increase in the enzyme activity in plasma. A significant intensification of SOD activity was recorded after a rehabilitation period (to 312 ± 0.01 %). The increase in SOD activity may be caused by the influence of SH that damages the structure of mitochondria and endoplasmic reticulum of blood cells (leukocytes, thrombocytes) which can produce (respiratory chain, cytochrome P-450 et al.) $\text{O}_2^{\cdot-}$ as a by-product that is a substrate for SOD and that, in turn, is manifested in the activity of plasma SOD. There are reports that in the experiments performed on neutrophils, fibroblasts and endothelial cells *in vitro*, SH solution (0.025–0.0025 %) causes cytoplasmic vacuolization, swelling of mitochondria and endoplasmic reticulum, dilatation in fibroblasts and endothelial cells and a 90 % inhibition of neutrophil migration, but not cell death [5].

The influence of histamine in 1 $\mu\text{g}/\text{kg}$ dose caused changes in rats blood plasma catalase activity. This influence led to a $90\pm 0.07\%$ increase in CAT activity on the 7th day of biogenic amines injection, indicating the formation of large amounts of H_2O_2 , and its further decrease on the 21st day (rehabilitation) by $81\pm 0.23\%$ (Fig. 2).

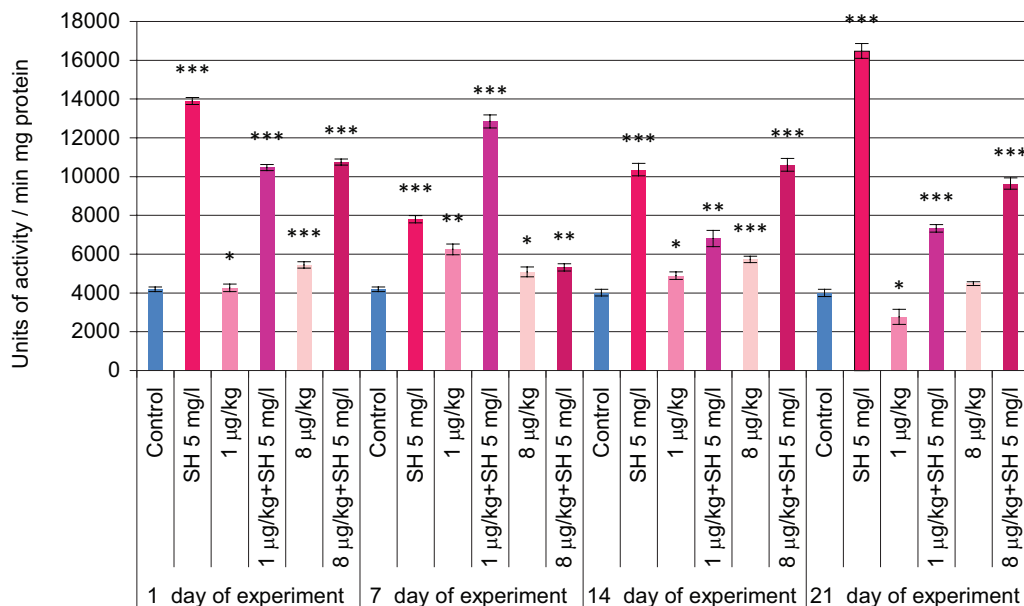


Fig. 1. The activity of superoxide dismutase in rat blood plasma after injection of histamine in 1 and 8 $\mu\text{g}/\text{kg}$ concentrations, influence of SH (5 mg/l), simultaneous influence of SH and histamine on the 1, 7 and 14 days of experiment and after the rehabilitation period (21 day). * – $p \geq 0.95$; ** – $p \geq 0.99$; *** – $p \geq 0.999$

Рис. 1. Активність супероксиддисмутазу в плазмі крові щурів на 1-шу, 7-му, 14-ту та 21-шу добу дослідю за дії гіпохлориту натрію в дозі 5 мг/л і гістаміну відповідно в дозі 1 і 8 мг/кг маси тіла тварин, а також за одночасного впливу гіпохлориту натрію (20 мг/л) і гістаміну (відповідно 1 і 8 мг/кг маси тіла тварин). * – $p \geq 0,95$; ** – $p \geq 0,99$; *** – $p \geq 0,999$

Simultaneous administration of histamine in 1 $\mu\text{g}/\text{kg}$ dose and SH at 5 mg/l concentration to rats led to a significant decrease in the CAT activity (by $84\pm 0.23\%$ relative to the control and by $92\pm 0.2\%$ relative to a group of animals that received only histamine (1 $\mu\text{g}/\text{kg}$)) on the 7th day of the experiment. Thus, SH leads to a decrease in CAT activity. SH administered orally to intact animals led to a decrease in CAT activity in blood plasma on the 1st and 14th day of the experiment by $85\pm 0.32\%$ and $50\pm 0.14\%$, respectively. So, plasma CAT does not significantly affect histamine influence, whereas SH causes downward CAT activity trend both in intact animals and animals which were injected with histamine subcutaneously (Fig. 2).

On the 1st day of the experiment, we detected an increase in SOD activity. At the same time, glutathione peroxidase (GPO) activity increased too under the influence of histamine in 1 $\mu\text{g}/\text{kg}$ dose and 8 $\mu\text{g}/\text{kg}$ by $60\pm 0.16\%$ and $69\pm 0.03\%$, respectively, whereas CAT activity did not change significantly. These results indicate that under the influence of histamine, a small amount of H_2O_2 is formed in blood the plasma which is eliminated by the GPO (Fig. 3).

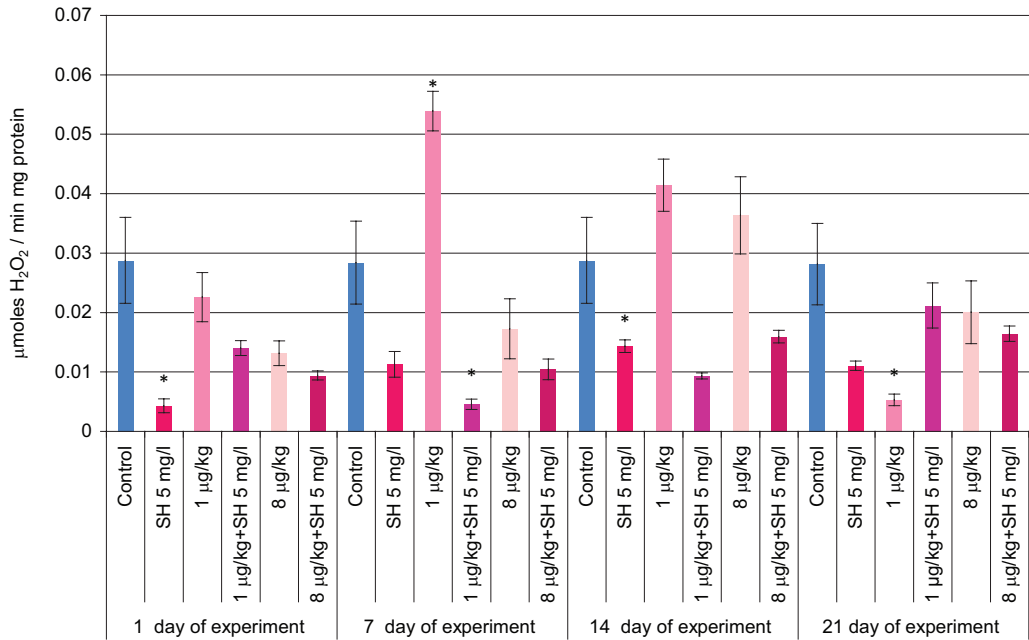


Fig. 2. The activity of catalase in rat blood plasma after injection of histamine in 1 and 8 µg/kg concentrations, influence of SH (5 mg/l), simultaneous influence of SH and histamine on the 1st, 7th and 14th day of the experiment and after the rehabilitation period (21st day). *— $p \geq 0.95$

Рис. 2. Активність каталази у плазмі крові щурів на 1-шу, 7-му, 14-ту та 21-шу добу дослідження за дії гіпохлориту натрію в дозі 5 мг/л і гістаміну відповідно в дозі 1 і 8 мкг/кг маси тіла тварин, а також за одночасного впливу гіпохлориту натрію (20 мг/л) і гістаміну (відповідно 1 і 8 мкг/кг маси тіла тварин). * — $p \geq 0,95$

An increase in the GPO activity in blood plasma of rats was detected on the 7th day of the experiment under the influence of histamine in 1 µg/kg dose, along with an increased CAT activity that indicates the increased content of hydroperoxides which are eliminated by the GPO. In the higher dose histamine caused a decrease of GPO activity to $34 \pm 0.1\%$ (from its initial activity) on the 14th day of the experiment. However, after the rehabilitation period, the GPO activity in blood plasma decreased under the influence of histamine at a lower dose (Fig. 3).

It should be noted, that SH at 5 mg/l concentration caused a significant decrease in GPO activity under the influence of histamine in both studied doses during the experiment. Enzymatic activity was reduced relative to the control group and to the groups of animals that were administered with only subcutaneously histamine. While GPO activity decreased, in contrast SOD activity, increased relative to such in control groups of rats, and groups of animals that received injections of histamine (Fig. 3). So, SH binds to hydroperoxides it eliminates a substrate for GPO. A decrease in the GPO activity is also possible due to inhibition of the enzyme itself, since SH can negatively affect protein structure [5]. This supposition is proved by the GPO activity (indicators) in blood plasma of rats that were administered with SH orally at 5 mg/l concentration, whereby the activity of this enzyme decreased relative to that in control groups during the experiment.

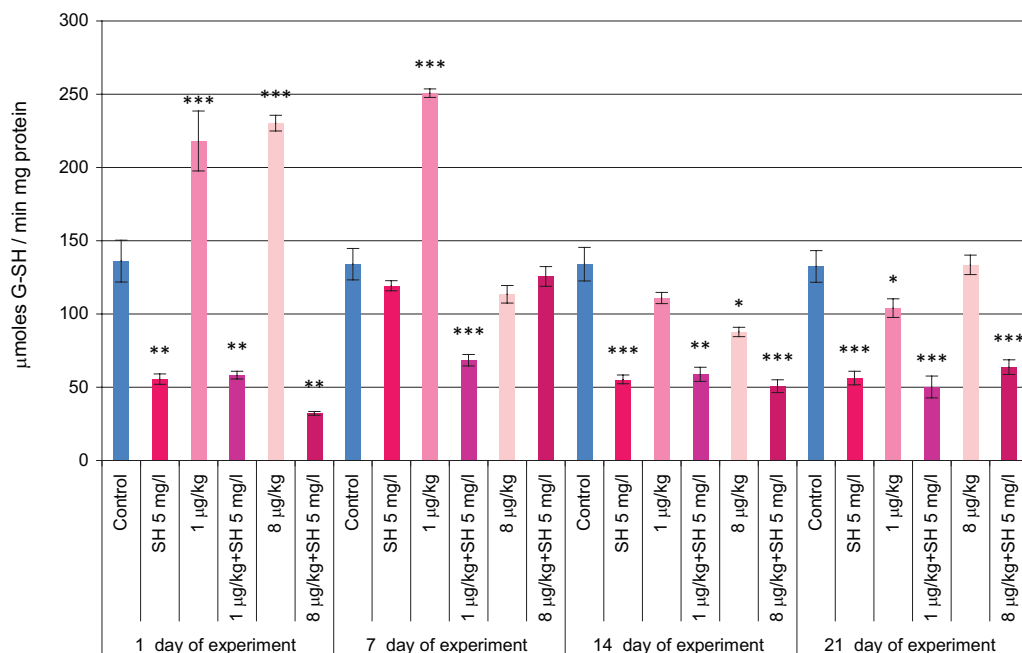


Fig. 3. Glutathione peroxidase activity in rat blood plasma after injection of histamine in 1 and 8 µg/kg concentrations, influence of SH (5 mg/l), simultaneous influence of SH and histamine on the 1st, 7th and 14th day of the experiment and after the rehabilitation period (21st day). * – $p \geq 0.95$; ** – $p \geq 0.99$; *** – $p \geq 0.999$

Рис. 3. Активність глутатіонпероксидази у плазмі крові щурів на 1-шу, 7-му, 14-ту та 21-шу добу дослідю за дії гіпохлориту натрію в дозі 5 мг/л і гістаміну відповідно в дозі 1 і 8 мкг/кг маси тіла тварин, а також за одночасного впливу гіпохлориту натрію (20 мг/л) і гістаміну (відповідно 1 і 8 мкг/кг маси тіла тварин). * – $p \geq 0,95$; ** – $p \geq 0,99$; *** – $p \geq 0,999$

CONCLUSION

1. Subcutaneous injection to rats of histamine in 1 and 8 µg/kg doses caused an increase in the activity of superoxide dismutase in blood plasma. The influence of sodium hypochlorite and a simultaneous influence of histamine and sodium hypochlorite led to more intense increase in superoxide dismutase activity. The enzymatic activity did not return to normal values even after a rehabilitation period.
2. Histamine significantly affected catalase activity in blood plasma. In case of oral administration of sodium hypochlorite to rats, a trend in a decrease in catalase activity in both intact animals and animals that received subcutaneous injections of histamine was detected.
3. The injections of histamine to rats in 1 µg/kg dose caused an increased glutathione peroxidase activity on 1st and 7th day of the experiment, while histamine in 8 µg/kg dose led to the elevation of glutathione peroxidase activity only on the 1st day, and its inhibition – by the 14th day of the experiment. The action of sodium hypochlorite and a simultaneous influence of histamine and sodium hypochlorite in blood plasma led a significant decrease in glutathione peroxidase activity.

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АКТИВНІСТЬ КЛЮЧОВИХ ЕНЗИМІВ АНТИОКСИДАНТНОЇ СИСТЕМИ ПЛАЗМИ КРОВІ ЩУРІВ ЗА ДІЇ ГІСТАМІНУ ТА ГІПОХЛОРИТУ НАТРІЮ

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У статті досліджено вплив гістаміну у дозі 1 мкг/кг та 8 мкг/кг (дози відповідають тим, які зумовлюють патологічні прояви в експериментальних умовах) і гіпохлориту

натрію у концентрації 5 мг/л (максимально низька концентрація гіпохлориту натрію, яка може впливати на організм при пероральному введенні) на ключові ензими антиоксидантної системи плазми крові щурів. Встановлено, що гістамін у обох досліджуваних концентраціях веде до інтенсифікації супероксиддисмутазної активності впродовж 14-ти діб. Одночасне введення в організм щурів гістаміну і гіпохлориту натрію спричиняє значну активацію супероксиддисмутази. Такий самий ефект чинить і випоювання тваринам тільки гіпохлориту натрію. Каталаза плазми крові незначно реагує на вплив гістаміну, активність її достовірно зростає лише за дії біогенного аміну в дозі 1 мкг/кг на 7-му добу досліді. Гіпохлорит натрію спричиняє зниження каталазної активності, як у інтактних тварин, так і у тварин, яким підшкірно вводили гістамін. Ін'єкції гістаміну в дозі 1 мкг/кг щурам спричиняють зростання активності глутатіонпероксидази на 1-шу і 7-му добу досліді, тоді як гістамін у дозі 8 мкг/кг зумовлює інтенсифікацію роботи глутатіонпероксидази лише на 1-шу добу, а пригнічення – на 14-ту добу досліді. Випоювання щурам гіпохлориту натрію у плазмі крові ведуть до переважаючого пригнічення активності глутатіонпероксидази.

Ключові слова: гістамін, гіпохлорит натрію, плазма крові, супероксиддисмутаза, каталаза, глутатіонпероксидаза.

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