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The Enzyme Membrain-Associated Complex Activity in Rat Brain under Imidazolin Containing Organic Compounds Action

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Abstract. The aim of the study was to determine the Na⁺, K⁺-ATPase, adenylyl- and guanylyl cyclase synaptosome faction activity in the brain of rats at 30-day toxicity by imidazoline mixtures in 1/10 and 1/100 LD₅₀ doses.

Materials and methods. The paper uses examples of MIM with alkyl radicals C₇₋₉ (MIM7-9) and C₉₋₁₅ (MIM9-15). Experiments carried out on the mature male rats Wistar, weighing 180-220 g. Maintenance and manipulation with animals were carried out according to the principles of bioethics. They were subjected to oral intoxication by probe using by aqueous solutions of compounds once daily per 30 days at a 1/100 LD₅₀ dose. Middle-lethal doses (LD₅₀) were: for MIM7-9 – 1,8 g/kg; MIM9-15 – 5,0 g/kg of body weight. The animals of the control group were injected by the corresponding volume of drinking water. The parameter study performed at 30 days after the start of the experiment. Each group contained 15 animals. Slaughter was performed by decapitation, with pre-anesthesia by sodium thiopental.

The Na⁺+K⁺-ATPase, adenylyl and guanylyl cyclase activity in synaptosomal fraction of rat brain at 30th day of industrial chemical environmental pollutants – imidasoline mixtures influence have been studied in the paper to be necessary for full disclosure of biochemical mechanisms of membranotropic action. Imidasoline-containing mixtures with alkyl radicals C₇₋₉ i C₉₋₁₅ at 1/10 and 1/100 LD₅₀ doses cause the change of membrane-associated enzyme activity to be confirmed by the decrease of Na⁺, K⁺-ATPase, adenylyl cyclase activity on the background of guanylyl cyclase increase. Revealed violations are one of pathogenetic links of biochemical mechanisms of imidasoline mixture membranotropic action to be considered in means of correction developing.

Summarizing the results, it should be made the following conclusions. Against the background of prolonged toxicity by MIM in 1/10 and 1/100 LD₅₀ doses synaptosome fraction of rat brain membrane enzyme activity is changing, as evidenced by decreased activity of Na⁺, K⁺-ATPase, adenylyl cyclase on the background of increasing guanylyl cyclase. Change of membrane-associated enzyme complexes activity in the brain of rats is one of the pathogenetic mechanism of links membrane tropic MIM action to be considered when developing the means of correction.

Keywords: imidasoline mixtures, rats, brain, microsomes, Na⁺+K⁺-ATPase, adenylyl cyclase, guanylyl cyclase.

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Introduction. Actual problem of modern medical biochemistry is a comprehensive studying of the mechanisms of xenobiotic action on humans and means of correction development [7, 5]. Common xenobiotics able adversely affect health, include a imidazoline mixture (MIM). This is associated with large volumes of chemicals, widely used in various sectors of the economy (as the basis for industrial production of detergents, antistatic, anticorrosive agents, adhesive additives, etc.), revenues of the sources of drinking water [1, 4, 14]. The clinical picture of rata cute poisoning by MIM is proved to be the symptoms of the central nervous system disorders in the foreground [14]. Furthermore, electron microscopy studies indicate significant changes architectonics of the nervous tissue, including violation of the integrity of neurons under conditions of prolonged rat intoxication by MIM [14]. Lack of information about the biochemical mechanisms of MIM action on the body encourages them to conduct a comprehensive study, starting primarily with state of the central nervous system. Given the physical and chemical properties of MIM components (the presence of hydrophobic and hydrophilic groups, capacity for chemical reactions to form biologically active compounds, etc.), it is reasonable to research their influence on some characteristics of cell membranes, including membrane-associated enzyme complexes.

The aim of the study was to determine the Na⁺, K⁺-ATPase, adenylyl- and guanylyl cyclase synaptosome faction activity in the brain of rats at 30-day toxicity by imidazoline mixtures in 1/10 and 1/100 LD₅₀ doses.

Materials and methods. The paper uses examples of MIM with alkyl radicals C₇₋₉ (MIM7-9) and C₉₋₁₅ (MIM9-15). Experiments carried out on the mature male rats Wistar, weighing 180-220 g. Maintenance and manipulation with animals were carried out according to the principles of bioethics. They were subjected to oral intoxication by probe using by aqueous solutions of compounds once daily per 30 days at a 1/100 LD₅₀ dose. Middle-lethal doses (LD₅₀) were: for MIM7-9 – 1,8 g/kg;

MIM9-15 – 5,0 g/kg of body weight. The animals of the control group were injected by the corresponding volume of drinking water. The parameter study performed at 30 days after the start of the experiment. Each group contained 15 animals. Slaughter was performed by decapitation, with pre-anesthesia by sodium thiopental.

The Na⁺, K⁺ – ATPase activity in synaptosome fraction of rat brain was evaluated by the difference between the content of inorganic phosphorus in the absence and presence in the incubation medium 1 mM uabayne accordance with the methodical recommendations [6]. The samples were put to incubation medium containing 100 mM NaCl, 20 nM KCl, 50 nM tris-HCl (pH 7,6), 3 mM MgCl₂, 0,5 mM CaCl₂, 0,5 mM EDTA, 3 mM ATP. The incubation was carried out at 37°C per 30 minutes; The reaction was stopped by the addition of 15% trichloroacetic acid. Precipitation of denatured proteins was performed at 3500 g per 10 minutes. For synaptosome fraction extraction the tissue was homogenated in 50 mM tris-HCl buffer (pH 7,5) with 5 mM theophylline та 4 mM MgCl₂ at cold in glass hemegenisator (80 up/down); homogenate was centrifugated at 1500 g at 4°C during 5 minutes, supernatant was centrifugated at 18000 g at 0-4°C during 30 minutes, final clot was rehomogenisated in 1,5 ml of the same buffer [2]. Adenylyl cyclase activity (AC) in synaptosome fraction was determined by method [15], guanylyl cyclase (GC) – by method [13]. The enzyme activity was judged about by the accumulation of the products of enzymatic reactions – cAMP and cGMP. The incubation medium for AC activity determination contained 50 mM tris-HCl buffer (pH 7,5), 5 mM creatine phosphokinase, and for GC determination – 50 mM tris-HCl buffer (pH 7,5), 10 mM theophylline, 4 mM MgCl₂, 4mM creatine phosphate, 0,1 mg/ml creatine phosphokinase. Substrates responses were prepared at concentrations: АТФ – 2 mM, ГТФ – 1 mM.

Statistical analysis was performed using the computer application package for the processing of statistical information Statistica 6. 1 (StatSoft, Inc., USA). In the case of the normal distribution of data parametric characteristics – the average indicator (M) and standard deviation (s) were used; in its absence nonparametric – median (Me) and interquartile scope. The critical level of significance during testing statistical hypotheses was accepted p < 0,05.

Results and discussion. The groups of rats undergoes oral administration per 30 days by MIM in 1/10 LD₅₀ dose observed statistically significant (p ≤ 0,005) compared with the control decrease Na⁺, K⁺-ATPase activity of synaptosome fraction of the brain by an average of 37% (**table 1**). Prolonged exposure by MIM7-9 in 1/100 LD₅₀ dose was also accompanied by statistically significant relative to the control group of animals, decrease enzyme activity, but less pronounced (at 16%) than the 1/10 LD₅₀ dose. Comparison between a distribution rate in the group of animals administered by MIM9-15 in a 1/100 LD₅₀ dose, and the control did not show any statistically significant difference (p = 0,065).

Table 1

Na⁺, K⁺-ATPase activity in synaptosome fraction of rat brain at 30th day of imidazolin containing organic mixture influence (μmol P_i/h-mg of protein, n = 15; Me [25%; 75 %] or M ± s)

Mixture	Dose, LD ₅₀	
	1/10	1/100
MIM7-9	8,2 ± 2,21 p < 0,001	12,3 [7,9; 13,7] p = 0,014
MIM9-15	10,4 [7,7; 13,4] p = 0,005	12,1 ± 2,82 p = 0,065
Control	14,6 ± 3,60	

Note: p – level of significance compared with control.

At the 30th day of observation in synaptosome fraction of rat brain statistically significant (p < 0,001) compared with the control reducing the activity of adenylyl cyclase (AC) was also found (**table 2**). 1/10 LD₅₀ dose thus was more toxic than 1/100 LD₅₀. MIM7-9 and MIM9-15 in 1/10 LD₅₀ dose reduced AC activity in relation to the control, respectively 58 and 50%, and in a 1/100 LD₅₀ dose – 41 and 26%. On the background these changes, there was a statistically significant increased activity of guanylate cyclase (GC). The most expressive effect (p < 0,001) at the same time MIM7-9 commit: on 88% (in the case of 1/10 LD₅₀) and 66% (in the case of 1/100 LD₅₀). For MIM9-15 in 1/10 LD₅₀ dose increase (p ≤ 0,002) GC activity was 54%, and in a dose 1/100 LD₅₀ – 32%.

Thus, studies have found changes in the activity of membrane-associated enzyme complexes in the brain of rats under prolonged exposure by MIM. The main reasons of this can be a direct effect on enzyme complexes or structural changes in membranes (especially due to free radical processes). Previous experiments have demonstrated that MIM 1/10 and 1/100 LD₅₀ doses at 30th day actions cause the ratio of plasma membrane phospholipid fractions changes significantly increasing the percentage of phospholipids lysoform [10] and increase microviscosity of lipid phase [9],

Table 2

Adenylyl and guanylyl cyclase activity in rat brain at 30th day of imidazolin containing organic mixture influence (n = 15; Me [25%; 75 %] or M ± s)

Mixture	Adenylyl cyclase, pmol cAMP/min-mg of protein		Guanylyl cyclase, pmol cAMP/min-mg of protein	
	dose, LD ₅₀			
	1/10	1/100	1/10	1/100
MIM7-9	42,3 ± 5,59 p < 0,001	59,7 [51,8; 69,0] p < 0,001	136,2 [128,6; 160,3] p < 0,001	120,2 [112,3; 135,2] p < 0,001
MIM9-15	50,8 [49,0; 57,7] p < 0,001	75,2 ± 9,41 p < 0,001	111,7 ± 24,62 p < 0,001	95,6 [80,3; 99,5] p = 0,002
Control	101,5 ± 13,69		72,4 [68,4; 79,5]	

Note: p – level of significance compared with control.

which naturally leads, according to many experimental studies [8, 11], to reduce the activity of membrane associated enzymes.

The composition of the membranes of all cells as integral protein that converts the energy of ATP to energy gradient of monovalent ions is an enzyme Na^+ , K^+ -ATPase. It should be noted that this enzyme is involved into numerous cellular functions and processes associated with ion gradients, particularly in providing the electrical excitability of nervous tissue [3]. Therefore, changes in the activity of this enzyme by MIM actions could become an essential reason for their violation. Reducing adenylyl cyclase and increase guanylyl cyclase activity are also a consequence of studied membrane tropic of MIM. As you, The elements of adenylyl cyclase and guanylyl cyclase complexes are known to be integral membrane proteins that's why AC and GC activity are most dependent on the cell membrane [12]. In addition, GC can be activated by free radicals, lipid peroxides, which increased formation is observed in the

organism of experimental animals under prolonged exposure by MIM [4].

Summarizing the results, it should be made the following **conclusions**.

1. Against the background of prolonged toxicity by MIM in 1/10 and 1/100 LD_{50} doses synaptosome fraction of rat brain membrane enzyme activity is changing, as evidenced by decreased activity of Na^+ , K^+ -ATPase, adenylyl cyclase on the background of increasing guanylyl cyclase.

2. Change of membrane-associated enzyme complexes activity in the brain of rats is one of the pathogenetic mechanism of links membrane tropic MIM action to be considered when developing the means of correction.

Perspectives further research. In future the complex research conduction is planned aimed to study the biochemical mechanisms of MIM, including evaluation of antiradical activity and antyperoxid protection.

References

1. Bajpai D. Fatty imidazolines, chemistry, synthesis, properties and their industrial application / D. Bajpai, V. K. Tyagi // Journal of Oleo Science. – 2006. – Vol. 55, № 7. – P. 319-329.
2. Hajos F. An improved method for preparation of synaptosomal fractions in high purity / F. Hajos // Brain Res. – 1975. – Vol. 93. – P. 485-489.
3. Scheiner-Bobis G. The sodium pump. Its molecular properties and mechanisms of ion transport / G. Scheiner-Bobis // Eur. J. Biochem. – 2002. – Vol. 269. – P. 2424-2433.
4. Tyagi R. Imidazoline and its derivatives: an overview / R. Tyagi, V. K. Tyagi, S. K. Pandey // Journal of Oleo Science. – 2007. – Vol. 56, № 5. – P. 211-222.
5. Грузева Т. С. Вплив екологічних чинників на формування глобального тягаря хвороб / Т. С. Грузева // Науковий вісник Національного медичного університету ім. О. О. Богомольця. – 2010. – № 27. – С. 167-168.
6. Казеннов А. М. Исследование активности Na, K-АТФазы в эритроцитах млекопитающих / А. М. Казеннов, М. Н. Маслова, А. Д. Шалабодов // Биохимия. – 1984. – № 7. – С. 1089-1094.
7. Ксенобіотики: накоплення, детоксикація та виведення з живих організмів / [Б. О. Цудзевич, О. Б. Столяр, І. В. Калініна та ін.]. – Тернопіль : Видавництво ТНТУ ім. І. Пулюя, 2012. – 384 с.
8. Курский М. Д. Биомембранология / М. Д. Курский, С. М. Кучеренко. – К. : Вища школа, 1993. – 200 с.
9. Максимова І. Г. Мікрів'язкість мембран клітин крові щурів за дії імідазолінвмісних органічних сумішей / І. Г. Максимова // 36. матер. науково-практ. конф. : Здобутки клінічної та експериментальної медицини. – Тернопіль, 2013. – С. 163-164.
10. Максимова І. Г. Фосфоліпідний склад мембран еритроцитів щурів за умов впливу імідазолінвмісних органічних сумішей / І. Г. Максимова // 36. матер. науково-практ. конф. : Довкілля та здоров'я. – Тернопіль. 2013. – С. 112-114.
11. Огурцов А. Н. Биологические мембраны / А. Н. Огурцов. – Х. : НТУ «ХПИ», 2012. – 368 с.
12. Федоров Н. А. Циклические нуклеотиды и их аналоги в медицине / Н. А. Федоров, М. Г. Радуловацкий, Г. Е. Чехович. – М. : Медицина, 1990. – 176 с.
13. Чирков Ю. Ю. Гуанилатциклаза тромбоцитов крови человека / Ю. Ю. Чирков, И. А. Тыщук, Н. Н. Белушкина // Биохимия. – 1987. – Т. 52, Вып. 6. – С. 956-963.
14. Эколого-гигиеническая характеристика азотсодержащих поверхностно-активных веществ как загрязнителей водоемов / [В. И. Жуков, В. В. Мясоедов, С. А. Стеценко и др.]; под ред. В. И. Жукова. – Х. : Торнадо, 2000. – 180 с.
15. Юдаев Н. А. Активность аденилатциклазы и содержание циклических нуклеотидов в опухолях надпочечников человека при синдроме Иценко-Кушинга / Н. А. Юдаев, С. А. Афиногенова, К. Н. Казеев // Биохимия. – 1981. – Т. 46, Вып. 1. – С. 55-61.

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АКТИВНІСТЬ ФЕРМЕНТНИХ МЕМБРАНОВ'ЯЗАНИХ КОМПЛЕКСІВ У ГОЛОВНОМУ МОЗКУ ЩУРІВ ПРИ ДІЇ ІМІДАЗОЛІНВІСНИХ ОРГАНІЧНИХ СУМІШЕЙ

Максимова І. Г.

Резюме. У роботі вивчено активність Na^+ , K^+ -АТФази, аденілат- і гуанілатциклази у синапсомальній фракції головного мозку щурів на 30-ту добу впливу промислових хімічних забруднювачів довкілля – сумішей імідазоліні, що є необхідним для всебічного розкриття біохімічних механізмів мембранотропної дії. Імідазолінвмісні суміші з алкільними радикалами C_{7-9} і C_{9-15} у дозах 1/10 і 1/100 LD_{50} викликають зміну активності мембранов'язаних ферментів, що підтверджується зниженням активності Na^+ , K^+ -АТФази, аденілатциклази

на тлі підвищення гуанілатциклази. Виявлені порушення є однією з патогенетичних ланок біохімічних механізмів мембранотропної дії сумішей імідазолінів, що необхідно враховувати при розробленні засобів їх корекції.

Ключові слова: суміші імідазолінів, щури, головний мозок, мікросоми, Na^+ , K^+ -АТФаза, аденилатциклаза, гуанілатциклази.

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АКТИВНОСТЬ ФЕРМЕНТНЫХ МЕМБРАНОСВЯЗАННЫХ КОМПЛЕКСОВ В ГОЛОВНОМ МОЗГЕ КРЫС ПРИ ВОЗДЕЙСТВИИ ИМИДАЗОЛИНСОДЕРЖАЩИХ ОРГАНИЧЕСКИХ СМЕСЕЙ

Максимова И. Г.

Резюме. В работе изучена активность Na^+ , K^+ -АТФазы, аденилат- и гуанилатциклазы в синаптосомальной фракции головного мозга крыс на 30-е сутки воздействия промышленных химических загрязнителей окружающей среды – смесей имидазолинов, что необходимо для всестороннего раскрытия биохимических механизмов мембранотропного действия. Имидазолинсодержащие смеси с алкильными радикалами C_{7-9} и C_{9-15} в дозах 1/10 и 1/100 LD_{50} вызывают изменение активности мембраносвязанных ферментов, что подтверждается снижением активности Na^+ , K^+ -АТФазы, аденилатциклазы на фоне повышения гуанилатциклазы. Выявленные нарушения являются одним из патогенетических звеньев биохимических механизмов мембранотропного действия смесей имидазолинов, что необходимо учитывать при разработке способов их коррекции.

Ключевые слова: смеси имидазолинов, крысы, головной мозг, микросомы, Na^+ , K^+ -АТФаза, аденилатциклаза, гуанилатциклаза.

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