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CROWN-ETHERS – XENOBIOTICS WHICH POSSESS MEMBRANOTROPIC ACTIVITY

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The article represents the negative influence of crown-ethers (12-crown-4, aza-12-crown-4, thia-12-crown-4) on phospholipid composition of erythrocytes and hepatocytes membrane, on the state of brain serotonin receptors and cyclic nucleotides system of white rats. The animals of experimental group were subjected to peroral intoxication by one of the investigated xenobiotics aqueous solutions (1/100 LD_{50}) within 30 days. Crown-ethers increased the percentage of phospholipid lysoforms which may be a consequence of free radical process activation. The xenobiotics also altered the affinity and quantity of serotonin receptors, activity of cyclic nucleotides synthesis and catabolism enzymes, cAMP and cGMP contents. The influence of crown-ethers has a non-specific modulatory character and may be realized via evoked conformational changes of membrane receptory and proteinic complexes, via stimulation of membrane lipid peroxidation process, modification of membrane protein phospholipid microsurroundings, ionic imbalance of cells.

Key words: crown-ethers, membrane, phospholipids, serotonin receptors, adenylate cyclase, guanilate cyclase, phosphodiesterase, cyclic nucleotides.

Краун-ефіри – ксенобіотики, що мають мембранотропну активність. Кратенко Р.І. – Встановлено негативний вплив краун-ефірів (12-краун-4. аза-12-краун-4, тіа-12-краун-4) на фосфоліпідний склад еритроцитів і гепатоцитів, стан серотонінових рецепторів та системи циклічних нуклеотидів головного мозку білих щурів. Тварини експериментальних груп підлягали протягом 30 діб щоденному пероральному одноразовому запалу водними розчинами (1/100 $ДЛ_{50}$) одного з досліджуваних ксенобіотиків. Краун-ефіри підвищували відсоток лізоформ фосфоліпідів, що може бути наслідком активації вільнорадикальних процесів. Ксенобіотики змінювали також спорідненість та кількість серотонінових рецепторів активність ферментів синтезу та катаболізму циклічних нуклеотидів, вміст цАМФ та цГМФ. Вплив краун-ефірів має неспецифічний модуляторний характер і може реалізовуватися через викликані конформаційні зміни мембраних рецепторних і ферментних комплексів, через стимуляцію процесів перекисного окислення ліпідів мембран, модифікацію фосфоліпідного мікрооточення мембранних білків, іонний дисбаланс клітин.

Ключові слова: краун-ефіри, мембрана, фосфоліпіди, серотонінові рецептори, аденілатциклаза, гуанілатциклаза, фосфодіестераза, циклічні нуклеотиди.

Macroheterocyclic crown-ethers belong to widely spread polluters of environmental hydrosphere especially at the places of their industrial production. This is caused by the intensive synthesis of these compounds, and their significant application in chemical and pharmaceutical industry [11]. The insufficient effectiveness of water-cleansing constructions and methods from the given substances results in the high likelihood of crown-ethers invasion in the human

organism in the composition of drinkable water. Previously we showed that in the processes of hydrolytic and thermal aqueous destruction and of biological organism transformation, crown-ethers heterocyclic rings break down to the enormous spectrum of biologically active low-molecular compounds. The majority of these compounds were proved to be far more toxic than their precursors and to cause membranotropic, radiomimetic, gonadotoxic, and other negative effects upon the organism of warm-blooded animals [3; 4]. Besides, crown-ethers themselves, which are quite lipophilic and extremely cumulative substances [4] with complex-forming ionophoric properties, may cause membranotropic action.

Undoubtedly, the constancy of liquid-lipid composition of organism biological membranes is an important criterion in cells homeostasis maintenance. Membrane lipids participate in substances transport, influence the receptors affinity and membrane-bound enzymes activity forming a unified structurally functional ensemble with the receptors and enzymes, and this ensemble is very susceptible to the toxic action of many exogenous biologically active compounds.

Apart from the above mentioned, the receptory apparatus of chemical information discrimination is one of the central links of different xenobiotics influence on organism cells. Biologically active substances receptors are glycoproteinic molecules which are localized on the outer layer of biological membrane or in pre-membrane areas of cells cytoplasm. The main function of membrane receptors is recognition of its own respective ligand out of the plentitude and diversity of information-transport molecules, and triggering the chain of intracellular conversions of the cell response to the signal which has arrived [8].

Intracellular effects of most exo- and endogenic biologically active substances are realized with the participation of second messengers systems, which include cyclic nucleotide system and Ca^{2+}-mobilizing polyphosphoinositole system. Stimulation of cAMP and cGMP formation is proved to be one of the most important mechanisms which mediate physiological and biochemical effects of many biologically active substances, for the main function of these cyclic nucleotides is the transformation of extracellular interactions into intracellular ones [9].

Objective. Investigation of membranotropic action which could be a feature of crown-ethers by establishment of negative influence of 12-crown-4, aza-12-crown-4 and thia-12-crown-4 upon erythrocytes and hepatocytes membrane phospholipid composition, as well as serotonin receptors state, and cyclic nucleotides system (cAMP and cGMP concentrations, cyclic nucleotides metabolism enzymes activities) state in white rats brain.

MATERIALS AND METHODS

The investigation involved the usage of 32 male rats of Vistar line (body mass 200-220 g). The animals were divided into three experimental and one control group. The experimental groups of rats were administered with emulsion of the investigated crown-ethers in 1/100 LD_{50} (0.0117; 0.022; 0.0365 g/body mass kg, for 12-crown-4, aza-12-crown-4 and thia-12-crown-4 respectively [3]) daily within 30 days perorally.

The animals of the control group were given water at the same conditions. On the 30th day of the experiment the rats of all groups were anesthetized by sodium thiopental (50 mg/body mass kg [6]) and slaughtered by decapitation with the Guillotine knife. The rat brain neocortex was isolated in the cold and was frozen in liquid nitrogen subsequently for investigation of receptors and membrane-bound enzymes.

Besides, phospholipid composition of hepatocites and erythrocytes was performed using liver and blood of the same animals. For this, erythrocytes were washed out thoroughly by isotonic NaCl solution using triple centrifuging, whereas hepatocytes were obtained by homogenizing rats liver in the Potter's glass homogenizer. Membranes were isolated by general methods using recommendations [1]. Lipid extraction was performed by [2]. Lipid evaporation was carried out in the jet of dry nitrogen. The two-dimensional microthinlayer chromatography was used for the identification of individual phospholipid factions [13] by aid of standard lipid solutions and lipid specific reactions [7]. The quantitative contents of general and individual phospholipids in lipid extracts were evaluated by inorganic phosphorus amount. The latter was determined by molybdenum reagent with the following colorimetry. K_2HPO_4 solution was applied as the standard. Colorimery was performed by using 815nm wave length. The ratio of phospholipid factions was calculated as a phosphorus percentage of each faction phospholipids to the total phosphorus sum of all phospholipids taken as 100 %. For the investigation of erythrocyte phospholipid composition we determined the contents of phosphatidyl choline (PC), sphyngomyeline (SM), phosphatidylserine (PS), lysophosphatidylcholine (LPC) and phosphatidylethanolamine (PEA). In the liver, we additionally investigated the contents of lysophosphatidylethanolamine (LPEA), phosphatidylinositole (PI), phosphatidic acid (PA) and cardiolipin (CL).

The binding parameters of selective ligands with the first and second type serotonin receptors were established by aid of determination of ^{3}H-serotonin (for 5-HT-1 receptors) and ^{3}H-spiperone (for 5-HT-2 receptors) with rat neocortex synaptosomes membranes using the method [15]. The synaptosome faction was obtained with the method [14]. The protein contents were determined by the Lowry method [16]. The protein contents were estimated at 300-500 mkg per sample. The specific ligand binding with receptors was determined as the difference between the general and non-specific bindings. The non-specific binding level amounted at 30 % of the general binding level.

The result calculations were performed by using Sketchard graphs of IBM program "Ligand".

The cyclic nucleotides system state was evaluated by cAMP and cGMP contents determination in rough membrane faction of rat brain neocortex with the usage of cyclic nucleotides "AMERSHAM" standards.

The preparation of rough membrane faction involved homogenization of 200 mg brain tissue in 50 mM tric-HCL-buffer (8 ml), pH 7.5 (5 mM theophyline, 4mM $MgCl_2$) in the cold in a glass homogenizer (80 up/down). Homogenate was centrifuged by 1500 g (0-4^{0}C) within 5 min, supernatant was centrifuged after by

18,000 g (0-4^{0}C) within 30 min. The obtained precipitate was re-homogenized in the same buffer (1.5 ml).

Adenylate cyclase (E.C. 4.6.1.1) activity was determined with the method described by [12] with insignificant modifications; guanylate cyclase (E.C. 4.6.1.2) activity – with the method [10]. The basal level of the enzymes activity was considered. Phosphodiesterase (E.C. 3.1.14.17) activity was determined using the method [17].

RESULTS AND DISCUSSION

The experimental results of phospholipids composition investigation (tab. 1, 2) show crown-ethers as the agents which significantly alter the ratio of rat erythrocytes and hepatocytes membrane phospholipid factions, and the alterations directions were typical for 12-crown-4 and aza-12crown-4.

Table 1

Influence of crown-ethers upon hepatocytes membrane phospholipid composition, %

	PEA	PC	SM	PS	LPEA	LPC	PI	CL
Control	23.3±2.1	39.4±3.2	16.0±0.7	9.0±0.8	1.3±0.4	1.2±0.5	7.7±0.6	0.8±0.07
12-crown-4	25.4±2.3	50.1±2.5*	12.1±0.9*	8.4.0±0.7	2.4±0.09*	4.9±1.1*	5.4±0.5*	2.5±0.18*
aza-12-crown-4	22.4±1.9	48.5±3.6*	10.2±0.9*	9.3±0.7	2.23±0.07*	2.8±0.3*	7.0±0.6	1.3±0.1*
thia-12-crown-4	23.0±2.1	40.5±3.4	16.8±1.0	8.7±0.8	1.9±0.3	1.7±0.2	7.2±0.5	1.05±0.12

Notes: 1. n=8; 2. *p<0.05

Table 2

Influence of crown-ethers upon erythrocytes membrane phospholipid composition, %

	PEA	PC	SM	PS	LPC
Control	15.5±1.4	46.2±1.7	12.8±0.8	11.3±0.9	3.5±0.6
12-crown-4	14.8±1.9	60.4±3.1*	10.4±1.6	10.3±1.1	5.7±0.4*
aza-12-crown-4	15.4±1.5	61.0±2.3*	11.5±1.1	10.8±1.0	4.9±0.5*
thia-12-crown-4	15.2±1.2	49.2±2.4	12.9±0.9	10.6±0.9	3.9±0.2

Notes: 1. n=8; 2. *p<0.05

Particularly, in hepatocytes, the consequences of the xenobiotics action displayed as the increase in PC and CL contents and decrease in PI (authentically only for 12-crown-4) and SM. The percentage ratio of PS and PEA remained at the control level. The percentage of PEA and PC lysoforms should be noted to get

authentically increased in erythrocytes and hepatocytes of animals having been toxified by 12-crown-4 and aza-12-crown-4. The ratio of erythrocytes and hepatocytes membrane phospholipid factions in the organism of rats toxified by thia-12-crown-4 had only the tendency to alter mostly in the similar way with the two previous experimental groups.

The increase in phospholipids lisoforms contents percentage may be explained by induction of lipid peroxidation. This is verified by the earlier obtained data signifying malonic dialdehyde and dienic conjugates accumulation in liver and blood, blood biochemiluminescence induction, decrease in reduced glutathione contents, and prooxidant protection enzymes activity changes in the organism of rats, intoxified by crown-ethers [4]. The cause of lipid peroxidation enhance is the increased generation of oxygen active forms by microsomal monooxigenase system and formation of xenobiotics biotransformation products (aldehydes, ketones, alcohols), which possess prooxidant effects.

In spite of increased lysoform percentage, the percentage of PEA did not change and the one of PC even grew up, which could have been connected with the increase in metabolism rate of the mentioned phospholipids factions in erythrocytes and hepatocytes of rats of the experimental groups.

As far as CL are the main lipid components of mitochondrial membranes their contents alterations, and, as a consequence, alterations of phospholipid microsurroundings of mitochondrial membrane enzymes may be one of the reasons of bionenergetics processes impairment which is proved by our previously reported experimental results about the decrease in activities of succinate dehydrogenase, monoamine oxidase, and ATPases rat hepatocytes [5].

The decrease in liver PI at the influence of 12-crown-4 may be caused, on one side, by activation of free radical processes, on the other side, by induction of prostaglandins synthesis which is also proved by our experimental results [4].

5-HT-1- and 5-HT-2-receptors selective ligands binding parameters in the brain of rats toxified by the xenobiotics were different from the same indexes of the control animal group. The differences in the indexes of all the three experimental rat groups were similarly directed. The investigated crown-ethers action resulted in the unitypical alterations of selective ligands binding character by both of the receptors types.

The influence of the experimental substances manifested as the increase in receptors affinity and decrease in binding sites quantity. The influencing force of the three individual xenobiotics was not quite different between them. 5-HT-1-receptors affinity increased by 24-36 %, their binding sites quantity decreased by 16-21 %.

K_d differences of 5-HT-2-receptors high affinity pool of experimental animals from the control magnitudes were in the range of 16-32 %, of 5-HT-2-receptors low affinity pool - in 12-25 %. ^{3}H-spiperone binding sites quantity of toxified rats were lower from the control magnitudes by 10-25 % (high affinity pool), and by 17-32 % (low affinity pool).

The crown-ethers action resulted in reduction of adenylate cyclase activity in rats'neocortex. 12-crown-4 displayed 43 % fall of this enzyme activity whereas aza-12-crown-4 and thia-12-crown-4 showed only 28 and 18 % falls respectively. All results are authenticable compared to the control magnitude. The reduction in the adenylate cyclase activity correlated with decrease in cAMP contents: - 47 %, - 25 % and - 17 % for 12-crown-4, aza-12-crown-4, and thia-12-crown-4 respectively.

The opposite character of the xenobiotic influence was found for the system "guanylate cyclase - cGMP". 12-crown-4, aza-12-crown-4, and thia-12-crown-4 action led to the induction of guanylate cyclase activity by 122, 81, and 54 %, and to increase in cGMP contents by 105, 77, and 48 %, respectively, and authentically, compared to the control magnitude.

The activity of phosphodiesterase - a catabolic enzyme of cyclic nucleotides metabolism manifested its induction in the organism of rats toxified with 12-crown-4, aza-12-crown-4, and thia-12-crown-4 by 108, 77 and 15 % respectively compared to the control magnitude (authenticable only for the first two substances).

Thus, the obtained results display the pronounced influence of crown-ethers upon the system of cyclic nucleotides in experimental rats'neocortex. This influence shows the alterations of cyclic nucleotides catabolic and anabolic enzymes activity as well as the changes in cAMP and c GMP contents.

The experimental crown-ethers do not have a similarity in chemical structure with endogenic bioregulation molecules - hormones and neurotransmitters, which realize their specific influence upon cells, particularly, via cyclic nucleotides, as second messengers. Thereby, we cannot predict a selective influence of crown-ethers upon receptory and post-receptory links of intercellular information realization whatsoever. The obtained data may be explained by non-specific modulatory character of these xenobiotics influence on membrane receptory, enzymic, channel-forming protein complexes. This specific action could be a consequence of crown-ethers ability to evoke conformational rearrangements of the mentioned protein complexes, to stimulate membrane phospholipid peroxidation, to alter lipid microsurroundings of membrane receptors and enzymes.

Besides, the investigated crown-ethers may lead to cellular ionic imbalance via their negative action on channel-forming proteins, as well as via the ability of these substances to form complexes with biogenic elements. The ionic imbalance could be one of the reasons of observed alterations in the cyclic nucleotides system of rats toxified by crown-ethers.

The alterations, which are developed by influence of crown-ethers are one of the reasons, and a reflection of metabolic processes impairment inherent to organism cells at conditions of xenobiotic toxic action.

CONCLUSIONS

1. Crown-ethers significantly influence phospholipid composition of erythrocytes and hepatocytes. The increase in phospholipids lysoforms percentage contents is a consequence of free-radical lipid oxidation process.

2. Macroheterocyclic crown-ethers result in alterations in systems of rat neocortex serotonin receptors and cyclic nucleotides. These alterations concern affinity and quantity of the receptors, as well as activity of cyclic nucleotides catabolic and anabolic enzymes, cAMP and cGmp contents.

3. The influence of crown-ethers bears a non-specific modulatory character and is realized via evoked conformational changes membrane receptory and enzymic complexes, via stimulation of membrane phospholipid peroxidation, modification of membrane proteins phospholipid microsurroundings, ionic imbalance of cells.

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Краун-эфиры – ксенобиотики, которые обладают мембранотропной активностью. Кратенко Р.И. – Установлено негативное влияние краун-эфиров (12-краун-4. аза-12-краун-4, тиа-12-краун-4) на фосфолипидный состав эритроцитов и гепатоцитов, состояние серотониновых рецепторов и системы циклических нуклеотидов головного мозга белых крыс. Животные экспериментальных групп подвергались на протяжении 30 суток каждодневной пероральной одноразовой затравке водными растворами (1/100 $ДЛ_{50}$) одного из исследуемых ксенобиотиков. Краун-эфиры повышали процент лизоформ фосфолипидов, что может быть последствием активации свободнорадикальных процессов. Ксенобиотики также изменяли сродство и количество серотониновых рецепторов, активность ферментов синтеза и катаболизма циклических нуклеотидов, содержание цАМФ та цГМФ. Влияние краун-эфиров имеет неспецифический модуляторный характер и может реализовываться через вызванные конформационные изменения мембранных рецепторных и ферментных комплексов, через стимуляцию процессов перекисного окисления липидов мембран, модификацию фосфолипидного микроокружения мембранных белков, ионный дисбаланс клеток.

Ключевые слова: краун-эфиры, мембрана, фосфолипиды, серотониновые рецепторы, аденилатциклаза, гуанилатциклаза, фосфодиэстераза, циклические нуклеотиды.