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^{3}H-WB4101 AND ^{3}H-DIHYDROALPRENOLOL BINDING PARAMETERS WITH BRAIN NEOCORTEX SYNAPTOSOMES OF RATS TOXIFIED BY CROWN-ETHERS

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The article represents ^{3}H-WB4101 (selective agonist alpha1-adrenoreceptors) and ^{3}H-dihydroalprenolol (selective agonist beta-adrenoreceptors) binding parameters with brain neocortex synoptosomes of rats subjected to intoxication by 12-crown-4, aza-12-crown-4 and thia-12-crown-4 (within 30 days, perorally, in 1/100 LD_{50}).

The K_d indexes of high-affinity pool alpha1-adrenoreceptors composed values from 0.19±0.01 nmol of rats toxified by 12-crown-4 to 2.27±0.33 nmol of rats control group; of low-affinity pool alpha1-adrenoreceptors - from 1.33±0.07 nmol to 4.13±0.33 nmol respectively. The quantity of high-affinity alpha1-adrenoreceptors ranged from 53.7±0.92 fmol/protein mg of rats toxified by aza-12-crown-4 to 168.1±9.48 fmol/protein mg of rats control group. The quantity of low-affinity alpha1-adrenoreceptors varied from 37.1±2.73 fmol/protein mg of rats toxified by 12-crown-4 to 78.4±4.72 fmol/protein mg of rats control group.

The obtained results could be explained by a non-specific action of the xenobiotics upon membrane receptory complexes.

Key words: crown-ethers, adrenergic receptors, synaptosomes, neocortex, ^{3}H-WB4101, ^{3}H-dihydroalprenolol.

Параметри зв'язування ^{3}H-WB4101 та ^{3}H-дигідроалпренололу з синаптосомами неокортекса головного мозку щурів, токсикованих краун-ефірами. Кратенко Р. І. – У статті представлені експериментальні дослідження параметрів зв'язування ^{3}H-WB4101 (селективного агоніста альфа1-адренорецепторів) та ^{3}H-дигідроалпренололу (селективного агоніста бета-адренорецепторів) з синаптосомами неокортекса щурів, токсикованих 12-краун-4, аза-12-краун-4, тіа-12-краун-4 (30 діб, перорально у 1/100 $ДЛ_{50}$).

Величини K_d альфа1-адренорецепторів високоафінного пула складали від 0,19±0,01 нмоль для щурів, токсикованих 12-краун-4, до 2,27±0,33 для тварин контрольної групи; альфа1-адренорецепторів низькоафінного пула – від 1,33±0,07 до 4,13±0,33 нмоль відповідно. Кількість альфа1-адренорецепторів високоафінного пула була від 53,7±0,92 фмоль/ мг білку для щурів, токсикованих аза-12-краун-4, до 168,1±9,48 фмоль/ мг білку для тварин контрольної групи. Кількість альфа1-адренорецепторів низькоафінного пула була від 37,1±2,73 фмоль/ мг білку для щурів, токсикованих 12-краун-4, до 78,4±4,72 фмоль/ мг білку для тварин контрольної групи.

Отримані результати можуть бути пояснені неспецифічним характером дії ксенобіотиків на мембранні рецепторні комплекси.

Ключові слова: краун-ефіри, адренорецептори, синаптосоми, неокортекс, ^{3}H-WB4101, ^{3}H-дигідроалпренолол.

The problem of environment protection has recently been gaining the continuously increasing concern. The enormous quantity of toxic chemicals, alien to the human organism, pollutes Biosphere and reduces population health. Toxic abilities of a goodly number of xenobiotics are very well known, however numerous industrial units of organic synthesis incessantly create many more brand new ones with unpredictable biological effects. It fully applies to compounds of crown-ethers group, the amount of which chemically synthesized and industrially produced has been threateningly rapidly going upwards. Each of them represents itself a macroheterocyclic molecular system with 9-60 atoms in the cycle, a third of which is composed by ether oxygen atoms separated by ethane groups [4]. The main property of macroheterocyclic polyethers is their ability to form stable complexes with salts of alkaline and other metals, involving the cation in their milecular cavity. The property of the ethers to "crown" a cation as well as a crown-like shape of their molecules were considered by C.J. Pedersen to name this class of substances. The exceptional capacities of crown-ethers of forming complexes with metal ions, which possess high electro-conductivity, of dissolving in various lipophilic solvents, of including chiral atoms in their macrocycle determine the wide application of these compounds in electrochemistry, metallurgy, catalysis, organic synthesis, pharmaceutical analysis [4]. However these properties, very useful as they may be in chemistry, could result in damaging biological activity if the xenobiotics enter the organism.

Undoubtedly, the realization of biological action of different compounds, including xenobiotics, on the organism cells primarily involves receptors link of chemical information discrimination. The receptors of biologically active substances are represented by glycoprotein molecules, which are localized on the outer surface of cytoplasmic membrane or/and in cytoplasm itself. The main function of membrane and cytosol receptory molecules is the selection of the correspondent ligand from the diverse variety of signal molecules and triggering the chain of intracellular conversions for the cell response to the signal which has arrived [3]. The data of crown-ethers membranotropic effects obtained earlier allowed us to suggest these xenobiotics to possess influence on the state of membrane-bound molecules, including receptors [8].

Objective. Investigation of ^{3}H-WB4101 (selective agonist of alpha1-adrenergic receptors) and ^{3}H-dihydroalprenolol (selective agonist of beta-adrenergic receptors) binding parameters with brain neocortex synoptosomes of rats subjected to intoxication by 12-crown-4, aza-12-crown-4 and thia-12-crown-4.

MATERIALS AND METHODS

The research used white male rats (body mass 180-210 g) kept at the standard conditions of vivarium. The animals of experimental groups (10 rats in each group) were administered 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 [1]) daily, within 30 days, perorally. The animals of the control group (n=10) were given water under 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 [2]) and slaughtered by decapitation with the Guillotine knife.

Brain neocortex of rats was isolated on ice and frozen in liquid nitrogen. The binding parameters of ^{3}H-WB4101 (selective agonist of alpha1-adrenergic receptors) and ^{3}H-dihydroalprenolol (selective agonist of beta-adrenergic receptors) were determined by radioligand method [5]. For the characteristics of the receptors functional state, dissociation constant (K_d) and maximal quantity of binding sites (B_{max}) were calculated. Synaptosomal faction of rat brain neocortex were obtained by method [6]. During the investigations of alpha1-adrenoreceptors selective ligand binding parameters, it was found that their Sketchard graph had a hyperbolic character which could signify a heterogeneous receptors pool, or an occurrence of negative cooperation in the single receptors pool. Hill's graphic estimation, which was used for the evaluation of the negative cooperation represented a line with an incline equal to 1 to the X-axis. This fact eliminated cooperation effects [7]. Therefore, taking in consideration the existence of several receptors pools, we subsequently used H.E. Rosenthal's method to analyze the material [10]. This method allowed us to reveal two systems of low- and high-affinity binding on the Sketchard graph. The selective ligands used in the research were manufactured by "AMERSHAM", UK. Protein content was determined by Lowry method [9] and composed 300-500 mkg per sample. The specific binding of the ligands with the correspondent receptory pool was displayed as the difference between the general and non-specific bindings values. The level of non-specific binding was not higher than 30 % out of the level of general binding. Experimental results were calculated by traditional methods of parametrical statistics.

RESULTS

The K_d indexes of high-affinity pool alpha1-adrenoreceptors composed values from 0.19±0.01 nmol of rats toxified by 12-crown-4 to 2.27±0.33 nmol of rats control group; of low-affinity pool alpha1-adrenoreceptors - from 1.33±0.07 nmol to 4.13±0.33 nmol respectively. The quantity of high-affinity alpha1-adrenoreceptors ranged from 53.7±0.92 fmol/protein mg of rats toxified by aza-12-crown-4 to 168.1±9.48 fmol/protein mg of rats control group. The quantity of low-affinity alpha1-adrenoreceptors varied from 37.1±2.73 fmol/protein mg of rats toxified by 12-crown-4 to 78.4±4.72 fmol/protein mg of rats control group. The tendency of toxified rats brain neocortex alpha1-adrenoreceptors alterations was untypical. The changes were statistically authentic - we observed the induction of both pools receptors affinity (decrease in K_d values) as well as the reduction of binding sites quantities, compared to the control values. The decrease in the high-affinity pool K_d values composed 92 %, 86 % and 56 %, in the high-affinity pool receptors quantity values - 53 %, 67 % and 37 % for the animals toxified by 12-crown-4, aza-12-crown-4 and thia-12-crown-4 respectively. The decrease in the low-affinity pool K_d values composed 68 %, 55 % and 43 %, in the low-affinity pool receptors quantity values -

57 %, 48 % and 35 % for the animals toxified by 12-crown-4, aza-12-crown-4 and thia-12-crown-4 respectively.

The K_d indexes of beta-adrenoreceptors composed values from 0.23±0.01 nmol of rats toxified by 12-crown-4 to 0.39±0.02 nmol of rats control group. The quantity of beta-adrenoreceptors ranged from 11.2±0.08 fmol/protein mg of rats control group to 18.4±1.08 fmol/protein mg of rats toxified by aza-12-crown-4. The tendency of brain neocortex beta-adrenoreceptors alterations of all the three experimental animals groups was similar. We observed the induction of binding sites quantities, compared to the control values, i.e. +58 %, + 68 % and + 32 % for the animals toxified by 12-crown-4, aza-12-crown-4 and thia-12-crown-4 respectively, compared to the control values. The affinity of beta-adrenoreceptors towards its selective ligand induced as well: +93 %, + 87 % and + 62 % for the animals toxified by 12-crown-4, aza-12-crown-4 and thia-12-crown-4 respectively, compared to the control values.

CONCLUSION

Disregulational effects of crown-ethers upon the functional state of membrane receptors of biologically active substances, particularly CNS catecholamines, must be a substantial link in biological action mechanism of these xenobiotics. The experimental crown-ethers do not have any structural similarities with the endogenous bio-regulatory molecules, i.e. with hormones or neurotransmitters of adrenoreceptors, that is why the ethers are not likely to cause selective influence on the receptors. The obtained results could be explained with a non-specific action of the xenobiotics upon membrane receptory complexes. Being highly lipophilic compounds, crown-ethers may dissolve in phospholipid bilayer of plasmatic membranes, altering chemical composition and structure of receptors phospholipid microsurrounding causing the changes in the quantity of the active forms and in the affinity of receptors themselves.

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Параметри связывания ^{3}H-WB4101 та ^{3}H-дигидроалпренолола с синаптосомами неокортекса головного мозга крыс, токсикованных краун-эфирами. Кратенко Р. И. – Статья представляет экспериментальные исследования параметров связывания ^{3}H-WB4101 (селективного агониста альфа1-адренорецепторов) та ^{3}H-дигидроалпренолола (селективного агониста бета-адренорецепторов) с синаптосомами неокортекса крыс, токсикованных 12-краун-4, аза-12-краун-4, тиа-12-краун-4 (30 суток, перрорально, в 1/100 $ДЛ_{50}$).

Величины K_d альфа1-адренорецепторов високоаффинного пула составляли от 0,19±0,01 нмоль для крыс, токсикованных 12-краун-4, до 2,27±0,33 для животных контрольной группы; альфа1-адренорецепторов низкоаффинного пула – от 1,33±0,07 до 4,13±0,33 нмоль соответственно. Количество альфа1-адренорецепторов високоаффинного пула была от 53,7±0,92 фмоль/ мг белка для крыс, токсикованных аза-12-краун-4, до 168,1±9,48 фмоль/ мг белка для животных контрольной группы. Количество альфа1-адренорецепторов низкоаффинного пула была от 37,1±2,73 фмоль/ мг белка для крыс, токсикованных 12-краун-4, до 78,4±4,72 фмоль/ мг белка для животных контрольной группы.

Полученные результаты могут быть объяснены неспецифическим характером действия ксенобиотиков на мембранные рецепторные комплексы.

Ключові слова: краун-эфиры, адренорецепторы, синаптосомы, неокортекс, ^{3}H-WB4101, ^{3}H-дигидроалпренолол.

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