EFFECT OF OXYETHILIC XYLIT L-655-2-100 ON THE RECEPTOR APPARATUS AND INTRACELLULAR METABOLISM TRANSMITTER REGULATION SYSTEM

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Abstract. *Effect of oxyethilic xylit L-655-2-100 on of white rats on level of parameters of receptor binding and cyclase transmitter cascade was investigated. It was established that xenobiotic leads to great structural and functional breaches of receptor apparatus state, membrane-structural enzymes activity, and content of intracellular transmitters.*

Key words*: xenobiotic, intracellular metabolism, receptor binding, cyclase transmitter cascade.*

The research of toxic chemical agents effects in the system receptor - mediator and mediator regulation of intracellular metabolism is one of the priorities in modern toxicology. It is known that the structural and functional state of the cell receptor apparatus is largely determined by the of the adenylate cyclase (AC) enzyme activity. Phosphodiesterase (PhDE) as a factor regulating levels of the leading cellular mediators - cyclic adenosine monophosphate (cAMPh) and cyclic guanosine monophosphate (cGMPh) has important role in intracellular

metabolism. Increase of cAMPh content is the earliest sign of stress in the cell. Therefore, excessive activation of cyclic nucleotides often leads to the development of pathological reactions [1 – 3].

The aim of this research was to study the effect of oxyethilic xylit L-655-2-100 as a toxic chemical factor on the receptor apparatus and a system of mediator regulation of the intracellular metabolism in subacute toxicological experiment.

Materials and research methods. The research program included a subacute experiment for 1.5 months for 50 male rats Wistar with an initial body mass 0,18-0,21 kg. During the experiment, the animals of experimental group (n = 25) were subjected to oral exposure of oxyethylic xylit L-655-2-100 at doses of 1/10, 1/100, 1/1000 DL50 (DL50 = 32.9 g / kg of animal mass). This xenobiotic is polyether, it has low toxicity, no cumulative properties, is widely used in various sectors of the national economy for the preparation of polymeric materials, epoxy resins, lacquers, enamels, that does not except its harmful effects on neurotransmitter processes. At the end of the experiment the animals were sacrificed by decapitation under light ether anesthesia, after that we studied parameters of receptor binding (serotonin, dopamine, adrenaline, glucocorticoid receptors) and cyclase mediator cascade (AC - cAMPh, guanylate cyclase (GC) - cGMPh, PhDE, the absorption of ions Ca^{2+}). The control group consisted of 25 rats. The activity of adenylate- and guanilatcyclase systems were determined in suspensions of hepatocytes and brain cells synaptosomes (cortex, stem, cerebellum). Status of cyclase cascade was tested by cAMPh, cGMPh levels, of AC, GC, PhDE activities , and the intensity of Ca^{2+} ions absorption by membrane fractions with help of radioisotope method with using a set of Ria KiT reagents (UK, USA, Slovakia).

Parameters of receptor binding of labeled agonists and antagonists were studied by radioligand binding method. An affinity of ligands to the receptors and the number of binding sites of α_1-, α_2-, β-adrenal; C_1-, C_2-serotonin; D_2-dopamine receptors; the number of glucocorticoid receptors were investigated. The results were analyzed in Scatchard coordinates. Kinetic characteristics were expressed in

the values of the equilibrium dissociation constants (DC) and number of binding sites (B_{max}) [4 – 6]. Statistical analysis was performed by the Student-Fischer indices. Results considered significant at the level of $p < 0.05$. Experimental studies on animals were performed in accordance with "International guidelines for biomedical research using animals" (Strasbourg, 1995) as well as the national "General ethical principles of experiments with animals" (Ukraine, 2001).

Results of researches and their discussion. Analysis of receptor binding parameters indicates an increase in the equilibrium dissociation constant and reducing the number of receptor binding sites of α_1-, α_2- adrenergic receptors in investigated structures of the brain and liver. It demonstrates a decrease of the affinity of ligands to α_1-, α_2- adrenergic receptors under the influence of the given xenobiotic (Table 1).

Table 1. *Effect of L-655-2-100 at dose 1/100 DL50 on receptor binding parameters in experimental animals (M ± m)*

Receptor kinds	Parameters	Organs							
		Brain						Liver	
		Cortex		Stem		cerebellum			
		c	t	c	t	c	t	c	t
α_1-adrenal	DC	4,73± 0,12	17,20± 0,84	3,56± 0,20	15,36± 0,97	3,24± 0,15	12,42 ±0,65	2,38± 0,14	13,56± 0,82
	B_{max}	35,24± 1,30	1,52± 0,18	28,73± 1,15	1,64± 0,21	22,35± 1,26	1,47± 0,18	19,87 ±1,30	1,43± 0,15
α_2-adrenal	DC	6,90± 0,30	18,40± 1,25	7,54± 0,27	16,45± 1,80	2,23± 0,44	19,40 ±1,34	12,74 ±0,56	21,30± 1,66
	B_{max}	3,20± 0,14	2,10± 0,14	3,65± 0,34	2,20± 0,15	3,57± 0,25	2,36± 0,22	4,10± 0,28	2,75± 0,18
β-adrenal	DC	0,26± 0,015	1,23± 0,06	0,17± 0,04	0,97± 0,10	0,28± 0,07	1,65± 0,03	0,46± 0,05	1,95± 0,06
	B_{max}	21,36± 1,30	7,28± 0,32	18,42± 1,22	6,53± 0,27	29,46 ±1,65	9,26± 0,48	38,27 ±1,30	12,32± 0,75
D_2-dopamine	DC	0,58± 0,04	22,3± 0,56	0,40± 0,02	19,72± 0,89	0,35± 0,015	17,50 ±0,94	0,43± 0,02	15,28± 0,62
	B_{max}	86,25± 2,37	7,16± 0,33	74,83± 1,95	6,80± 0,28	67,24 ±1,86	5,65± 0,30	58,64 ±1,37	3,40± 0,26
C_1-serotoni	DC	1,43± 0,08	0,86± 0,16	1,66± 0,07	0,75± 0,08	1,35± 0,05	0,78± 0,05	1,24± 0,015	0,34± 0,012

ne	B_{max}	280,21 ±7,20	345,30± 6,90	230,40± 6,90	275,60 ±6,28	227,34 ±5,80	286,30±7,20	205,20 ±6,50	320,70 ±8,35
C_2-serotonine	DC	0,23± 0,02	0,11± 0,013	0,46± 0,07	0,21± 0,018	0,58± 0,03	0,32± 0,02	0,68± 0,05	0,43± 0,016
	B_{max}	29,74± 0,58	56,30± 1,28	27,32± 0,87	47,26± 2,35	22,46± 0,70	38,40 ±1,65	18,35 ±0,62	31,26± 1,83
Glucocorticoid 2^{nd} type		570,33 ±28,60	1608,72 ±35,60	786,35± 20,42	2875,62±43,80	495,70 ±16,50	624,32±38, 50	480,20 ±13,60	1342,58±12,60

Notes: 1) DC in nmol; B_{max} – fmol/mg protein; 2) $p<0.05$ respectively with control; 3) c - control group, t – test group

Thus, at a dose of 1/100 DL50 in the experimental group compared with intact one for α_2-adrenergic receptors DC value increased in the cortex in 3.63 times, in 4.31 times in the brainstem, in the cerebellum in 3.83 times, in 5.69 times in the liver; B_{max} decreased in the cortex in 23.18 times, in 17.52 times in the brainstem, in the cerebellum in 15.20 times and in 13.89 times in the liver. The trend in changes of receptor binding parameters for α_2- and β-adrenergic receptors was similar. As follows from the dates in Table1 L-655-2-100 at a dose of 1/100 DL50 violated the binding parameters of dopamine receptors also. Parameters of serotonin receptors binding manifested significant differences in influence of L-655-2-100 on the elements of serotoninergic mediator system in all studying structures. A content analysis of glucocorticoid receptors of the 2^{nd}-type revealed a significant increase in their concentration in the experimental group in the cerebral cortex, brainstem, cerebellum, and liver compared to control, respectively, in 2.82, 3.66, 1.26 and 2.79 times.

Dates of the intracellular mediator cyclase cascade state in the subacute stage in rats under the influence of oxyethilic xylit L-655-2-100 at dose of 1/100 DL50 are given in Table 2.

***Table 2**. Effect of L-655-2-100 at dose 1/100 DL50 on intracellular mediator cyclase cascade state in experimental animals (M ± m)*

Parameter, organ	Control group (n=25)	Test group (n=25)
adenylate cyclase (AC), brainstem, pmol cAMPh/(mg protein·min)	10,60±0,68	0,57±0,02
+ isoproterinol	1,14±0,07	0,360±0,025
+ NaF	1,83±0,09	0,73±0,04
adenylate cyclase (AC), liver, pmol cAMPh/(mg protein·min)	2,35±0,18	0,26±0,03
+ isoproterinol	2,90±0,12	0,320±0,022
+ NaF	3,17±0,15	0,43±0,02
Ca^{2+} absorbtion by brain synaptosomes membranes, imp/(mg protein·min)		
basal	12304,62±48,74	6042,35±36,84
K^{+}-stimulated	18963,76±78,25	10427,32±57,23
Ca^{2+} absorbtion by microsomal membrane of endoplasmic reticulum of hepatocytes, imp/(mg protein·min		
basal	7265,28±46,34	5234,43±38,26
K^{+}-stimulated	9348,77±39,44	6985,37±43,95
adenylate cyclase (AC), cortex, pmol cAMPh/(mg protein·min)	108,25±6,37	62,44±2,78
cAMPh, cortex, fmol/mg tissue	452,68±14,56	243,57±8,46
guanylate cyclase (GC), cortex, pmol cAMPh/(mg protein·min)	0,86±0,04	1,79±0,13
cGMPh, cortex, fmol/mg tissue	46,28±1,18	75,12±2,64
Phosphodiesterase (PhDE), cortex, fmol/(mg protein·min)	3,74±0,26	19,25±0,58
cAMPh, blood serum, pmol/ml	68,34±2,45	116,53±6,70
cGMPh, blood serum, pmol/ml	7,96±0,52	4,66±0,37

Note: $p<0.05$ respectively with control

The observed changes in catalytic activity of adenylate cyclase system corresponded to the kinetics of α_1-, α_2-, β- adrenergic receptors, which is a reflection of the conjugation of these systems work. Studies have shown that xenobiotic reduced adenylate cyclase activity in the brain and liver, respectively in 18.6 times, and in 9.04 times. L-655-2-100 at doses of 1/10 and 1/100 DL50 inhibited the Ca^{2+} absorption by microsomal membrane fractions of endoplasmic reticulum of hepatocytes and brain synaptosomes. Studies have shown that L-655-2-100 in a subchronic exposure altered activity of PhDE, AC, GC, and the content of cAMPh and cGMPh in the cerebral cortex. It should be noted that the xenobiotic inhibited AC activity in 1.74 times, and the production of cAMPh in 1.86 times, and increased activity of PhDE, GC content and cGMPh, respectively, in 5.15, 3.89 and 1.62 times. The blood plasma was observed inverse situation against the cerebral cortex: we had the increase of cAMPh in 1.70 times, and the reduction of cGMPh in the 1.71 times.The dose of 1/1000 DL50 of L-655-2-100 had no effect on the receptor apparatus and intracellular mediator system in experimental animals.

Conclusions. Thus, the results of research found that oxyethilic xylit L-655-2-100 at doses of 1/10 and 1/100 DL50 leads to profound structural and functional disorders of the receptor system, the membrane structured enzymes activity and intracellular neurotransmitters content in the brain structures and liver, which underlies the development of degenerative and destructive processes in cells.

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