Comparative Pharmacological Analysis of the M-Cholinoreceptors in *Daphnia* and Rats

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The efficacy of M-cholinolytics in the prevention of the toxic effect of some cholinomimetics is comparatively assessed in experiments on *Daphnia magna* and albino rats. It is proposed that the M-cholinoreceptor population is heterogeneous in *Daphnia* as well as in rats.

Key Words: *cholinoreceptors; cholinolytics; pharmacological analysis; Daphnia magna; rats*

Daphnia (class Crustacea) are interesting objects for comparative biological investigations due to the similarity of their physiological and biochemical systems to those of mammals [8]. It is thought that there are structures characteristic for the cholinergic nervous system in *Daphnia* [1,11]. The ability of some cholinolytics to reduce the toxic effects of anticholinesterase compounds in experiments on *Daphnia* may testify to the presence of cholinoreceptors in them [6]. On the whole, however, the *Daphnia* cholinergic system is practically unstudied. A search for test objects is ongoing in pharmacological investigations to obtain alternative techniques for screening of preparations. There are several requirements that are placed upon test objects, the main one of which is the ability to respond in a manner similar to laboratory animals and man. Interest in the comparative study of *Daphnia* derives especially from the fact that *Daphnia magna* is a legally recognized bioobject for the indication of xenobiotics and determination of water quality [7].

The aim of the present investigation was to assess comparatively the efficacy of M-cholinolytics in the prevention of the toxic effect of some cholinomimetics in experiments on *Daphnia magna* and albino rats.

MATERIALS AND METHODS

A comparative study of the activity of central M-cholinolytics atropine, glypine, benactyzine, amedine, benzhexol, and spasmolytin was performed according to their antagonism to the toxic action of the cholinomimetic arecoline and the organophosphorus compounds (OPC) armine or dimethyldichlorvinyl phosphate (DDVP) in experiments with *Daphnia* and albino rats. *Daphnia* breeding was performed as described elsewhere [5] at 17-22°C and two-day *Daphnia magna* were used for tests. Tested hydrobionts were placed in glasses with 25 ml dechlorinated settled tapwater. The measure of cholinolytic activity of the preparations was the EC_{50} index, which is the concentration of cholinolytics preventing the death of 50% of *Daphnia* poisoned by armine and arecoline, which were added to the incubatino medium simultaneously with the cholinolytics. The mean lethal concentrations (LC_{50}) of armine and arecoline were determined as well as LC_{50} of the cholinolytics themselves. When the activity of the cholinolytics was studied, the concentrations of armine (1.5 LC_{50}) and arecoline (2.5 LC_{50}) were chosen so that EC_{50} of atropine was 1 mg/liter for poisoning by both toxins. The cholinoblocking activity of the preparations was evaluated in

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tests on male albino rats weighing 200-250g according to the ED_{50} index, the dose of preparation preventing the death of 50% of animals poisoned by DDVP or arecoline. The doses of the cholinomimetics were chosen so that ED_{50} of atropine was 1 mg/kg for intoxication with arecoline and DDVP. The dose of arecoline inducing tremor in animals was 17 mg/kg. DDVP was used at 10 mg/kg (1.5 LD_{50}). Cholinolytics were injected s.c. 30 min prior to i.p. administration of arecoline and i.m. injeciton of DDVP. Indexes of cholinomimetic toxicity and cholinolytic efficiency in experiments on *Daphnia* and rats were determined after Prozorovskii [4].

RESULTS

The data presented in Table 1 show that all tested M-cholinolytics have a protective effect in *Daphnia* poisoned with arecoline and armine, thus proving the existence of M-cholinoreceptors in the hydrobionts. Attention is drawn to the fact that the cholinoblockers fall into three groups according to their toxicity as follows: low-toxic compounds (atropine, benactyzine), substances with moderate toxicity (benzhexol, amedine, and spasmolytin), and high-toxic compounds (glypine). Moreover, the protective effect of cholinolytics is manifested in nontoxic concentrations. The levels of mean effective concentrations of tested M-cholinolytics (EC_{50}) determined in the test of antagonism with arecoline and armine in daphnia experiments are ranked in the same sequence as the corresponding indexes of drug activity (ED_{50}) in rats poisoned with DDVP and arecoline. This result attests that the cholinergic structures of *Daphnia* are closed to rat muscarinic receptors according to their pharmacological properties. Subsequent analysis of the data confirmed this conclusion.

The comparison of drug indexes of cholinolytic activity obtained in experiments on *Daphnia* as well as on rats revealed that the sequence of variations of the given indexes of some cholinolytics depends on the cholinomimetic used. For example, the mean effective concentrations or doses for atropine, glypine, and benactyzine differ insignificantly in arecoline and armine (or DDVP) poisoning. However, the activity of amedine, benzhexol, and spasmolytin is reliably higher in OPC tests than in experiments with arecoline. The noted characteristics of the action of some groups of cholinoblockers are found both in *Daphnia* and in the rat tests.

The differences in the activity of M-cholinolytics which are manifested under conditions of antagonism with some cholinomimetics may be interpreted from the standpoint of the heterogeneity of M-cholinoreceptors. The population of M-cholinoreceptors in the animal organism (especially in rats) has now been shown not to be homogeneous - there are separate subtypes of receptors differing from each other in a number of properties [2,3]. A characteristic property of M-cholinoreceptor subtypes is a dissimilar affinity for some cholinergic ligands, which are classified in this case as selective.

Two groups of muscarinic antagonists isolated in pharmacological experiments demonstrate definite peculiarities in interaction with various subtypes of M-cholinoreceptors *in vitro*. It is known that cholinolytics belonging to the first group, atropine, glypine, and benactyzine, are nonselective (total) muscarinic antagonists. Amedine, benzhexol, and spasmolytin belong to the selective M_1-cholinolytics [9,10].

It may be summarized that the activity of nonselective muscarinic antagonists in the prevention of the toxic effect of various cholinomimetics practically does not differ in experiments on *Daphnia* and rats, whereas the selective M_1-cholinolytic activity in OPC tests is considerably higher than in experiments with arecoline.

TABLE 1. Efficacy of Muscarinic Antagonists in Poisoning of *Daphnia* and Rats with Cholinomimetics ($M \pm m$, $n = 12$)

Cholinolytic	*Daphnia magna*				Rat		
	EC_{50}, mg/liter		EC_{50}, arecoline/EC_{50}, armine	LC_{50}, mg/liter	ED_{50}, mg/kg		EC_{50}, arecoline/EC_{50}, DDVP
	arecoline	armine			arecoline	DDVP	
Atropine	1.34±0.11	1.15±0.21	1.2	107.3±13.0	1.32±0.10	0.87±0.18	1.6
Glypine	0.37±0.11	0.40±0.11	0.9	8.5±1.4	0.50±0.007	0.19±0.03	2.6
Benactyzine	2.80±1.17	2.83±1.02	1.0	124±14.6	2.67±0.15	1.20±0.10	2.6
Amedine	3.69±0.53	0.26±0.07	14.2	21.1±4.7	3.58±1.03	0.32±0.03	11.2
Benzhexol	5.82±0.40	0.32±0.09	18.2	18.2±6.5	22.43±3.38	3.08±0.18	7.3
Spasmolytin	9.22±3.86	1.16±0.48	8.0	21.6±4.9	1080.0±95.3	52.50±9.50	20.6

Note. The efficacy and toxicity of cholinolytics was determined 24 h after incubation with *Daphnia*.

The differential activity of selective muscarinic antagonists in experiments with DDVP and armine on the one hand and with arecoline on the other suggests that the mechanism of toxic action of the mentioned cholinomimetics is mediated via their interaction with various subtypes of M-cholinoreceptors. Thus, the higher activity of M_1-antagonists in OPC tests testifies that the toxic effect of these cholinomimetics results mainly from the excitation of the M_1-cholinoreceptors, whereas in the pathogenesis of arecoline intoxication its interaction with the M_2-cholinoreceptors is more significant. This has been confirmed in other investigations as well [3,9].

Considering the similarity of the results obtained in experiments on rats and *Daphnia* as well as the documented existence of M-cholinoreceptor subtypes in rats, it may be assumed that the population of muscarinic receptors in *Daphnia* is heterogeneous as well.

The closeness of the pharmacological properties of the cholinoreceptors in rats and *Daphnia* makes it possible to recommend the latter as alternative test objects for a study of the specific properties of various cholinotropic ligands.

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