Qualitative Assessment of Selective Blockade of M_4-Cholinoreceptors in the Whole Organism

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Quantitative assessment of selective blockade of M_4-subtype muscarinic receptors was performed by the number of pilocarpine-induced movements of lower jaw in rats. Three antagonists (atropine, cyclodol, and glipin) were used in the experiments. Glipin produced the most potent blockade of M_4 receptors in the whole organism compared to other test antagonist.

Key Words: *subtypes of muscarinic receptors; selective activity of muscarinic antagonists*

The role of blockade of muscarinic receptors in the regulation of cerebral functions during extrapyramidal disorders caused by neuroleptic haloperidole is well studied. However, of five subtypes of muscarinic receptors only M_1, M_2, and M_3 were studied, because no methods were available for evaluation of other two subtypes [1-3].

The striatum plays a prominent role in the pathogenesis of extrapyramidal disorders. This structure contains a great number of M_4 muscarinic receptors. The functional role of these receptors is little studied.

Our aim was to develop a method of qualitative assessment of selective blockade of M_4 muscarinic receptors in the whole organism.

MATERIALS AND METHODS

The study was carried out on albino outbred rats weighing 160-180 g. The rats were kept on unrestricted food and water diet under standard illumination. Pilocarpine was used as a cholineggic muscarinic agonist, while commercial atropine, glipin, and cyclodol were applied as cholinergic blockers (CB). CB were injected subcutaneously 30 min prior to intraperitoneal injection of pilocarpine. The mean effective doses were calculated by probit analysis using Prozorovskii tables. The results were analyzed statistically using Student's t test at $p<0.05$.

RESULTS

Intraperitoneal injection of 4 mg/kg pilocarpine (PC) to rats affects M_4 receptors, which is manifested in a series of 2-3 and more specific vertical movements of the lower jaw not related to explorative and food-procuring behavior [4].

We evaluated the effect of the test ligand on the number of pilocarpine-induced lower jaw movements (LJM). In parallel, physiological saline was injected to 6 rats and spontaneous LJM not induced by pilocarpine were counted. The mean number of LJM and the double standard deviation 2σ served as the control values.

Experimental rats received subcutaneous injections of the test CB in 4-5 logarithmically increasing doses (two animals per dose, Table 1). After 30 min PC was injected intraperitoneally in a dose of 4 mg/kg. After 10 min, LJM were counted over 5 min. The effect of CB on specific activity was assessed alternatively in positive-negative scale. The effect was considered as positive, if the number of LJM decreased to the control level.

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TABLE 1. Assessment of Blockade of M_4 Cholinergic Receptors with Atropine

Atropine dose, ng/kg	Number of LJM in the presence of atropine	Comparison with threshold*	Effect	Effect in the group
1.58	3	<29	+	2/2
	14	<29	+	
1.26	49	>29	—	1/2
	9	<29	+	
1.0	17	<29	+	1/2
	100	>29	—	
0.8	79	>29	—	0/2
	84	>29	—	

Note. *control number of LJM.

TABLE 2. Blocking Activity (Mean Effective Dose) of Muscarinic Antagonists to M_4 Cholinergic Receptors ($M \pm m$)

Agent	ED_{50} (mg/kg)	Statistical error
Atropine	1.4	0.12
Cyclodol	2.1	0.2
Glipin	0.28	0.031

The data were used to calculate the mean effective dose (ED_{50}) of CB by its capacity to prevent the effect of pilocarpine injected in a dose of 4 mg/kg. It was established that the examined agents differed by their M_4 receptor blocking capacity in the whole organism (Table 2). Their blocking activity decreased in the following order: glipin>atropine>cyclodol.

Thus, capacity of CB to decrease the number of specific LJM induced by pilocarpine in rats allows quantitative evaluation of M_4 cholinergic receptor blockade. In the whole organism, glipin produced the most potent blockade of M_4 receptors compared to atropine and cyclodol.

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