Effect of M_2 Muscarinic Receptor Blockade in Rats with Haloperidol-Produced Catatonic Syndrome

S. G. Dagaev, O. A. Fil'ko, L. G. Kubarskaya, A. V. Khrabrova, L. A. Mukovskii, R. I. Glukhova, V. I. Sanotskii, and V. B. Dolgo-Saburov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 5, pp. 535-537, May, 2004
Original article submitted October 2, 2003

We studied the functional role of individual subtypes of muscarinic cholinoceptors in the pathogenesis of neuroleptic parkinsonism in rats. Blockade of M_4 receptors prevented the development of extrapyramidal disorders, which was abolished by simultaneous blockade of M_2 receptors. The data suggest that various subtypes of muscarinic receptors are involved in the regulation of dopamine concentration.

Key Words: *cholinoceptor blockers; selective ligands; catalepsy*

Impairment of neurotransmission in dopaminergic structures regulated by the cholinergic system underlies the pathogenesis of various neurodegenerative diseases of the central nervous system, including Parkinson's disease and Alzheimer's disease.

On the basis of published data on the interaction of various cholinolytic drugs with individual receptors in the whole organism [1-4,6] a mathematical model was constructed, which takes into consideration the influence of different muscarinic cholinergic receptors on the interaction between the cholinergic and dopaminergic systems. Neuroleptic parkinsonism (catalepsy induced by high concentrations of haloperidol) is a suitable model for this purpose. This model shows that the correction of extrapyramidal disorders (EPD) with cholinoceptor blockers is determined by two major factors: (1) blockade of M_4 muscarinic receptors and (2) minimum effect on M_2 muscarinic receptors. The higher is the affinity of the ligand for M_2 muscarinic receptors, the lower is its ability to correct EPD. Here we studied the role of M_2 muscarinic receptors blockade in the correction of EPN during experimental catalepsy.

Laboratory of Biochemistry, Laboratory of Medicinal Toxicology, Laboratory of Toxicology, Laboratory of Psychopharmacology, Laboratory of Applied Pharmacology, Institute of Toxicology, Russian Ministry of Health, St. Petersburg

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 160-180 g and obtained from the Rappolovo nursery.

Catalepsy was produced by intraperitoneal injection of haloperidol in a dose of 10 mg/kg. The effect was considered to be positive, when the rats retained a vertical rearing posture with leaning on the wall (10 cm) for 2 min. Each animal was tested no more than 3 times.

Cholinolytics (cyclodol, amedin, and pentifin) were injected subcutaneously 30 min before administration of haloperidol in 4-5 logarithmically increasing doses. Control animals received physiological saline. The rats were tested for 15 min at an interval corresponding to the onset of catalepsy in control animals (35-45 min). The results were expressed in an alternative form (catalepsy—no catalepsy). Activity was determined by the mean effective dose (probit analysis) [5]. In experiments with methoctramine and SL-2, the test preparations were injected intraperitoneally in combination with cholinergic receptor blocker.

Pentifin (group of acetylene aminoalcohols) and SL-2 (group of methoctramine) were synthesized at the Institute of Toxicology. Other preparations were obtained from commercial companies.

0007-4888/04/1375-0468

RESULTS

We studied two groups of cholinergic receptor blockers. Group 1 included preparations capable of preventing the development of EPD (pentifin, cyclodol, and amedin). Group 2 included selective ligands of M_2 muscarinic receptors (methoctramine and SL-2).

The mean effective dose of group 1 preparations preventing the development of EPD during haloperidol-produced catalepsy was predetermined. The mean effective dose of preparations *in vitro* blocking various subtypes of muscarinic receptors were also determined in previous pharmacological studies.

We evaluated the doses of preparations selectively blocking M_2 muscarinic receptors *in vivo*. They were determined in the test for prevention of tremor produced by arecoline in a dose of 1.5 U_{50} (4.05 mg/kg intraperitoneally). These doses of methoctramine and SL-2 were 0.066 and 0.086 mg/kg intraperitoneally, respectively. Pretreatment with methoctramine and SL-2 in the specified doses had no effect on M_4 muscarinic receptors. It was determined pharmacologically by changes in the number of specific mandibular movements caused by pilocarpine [1,9,10].

Pentifin, cyclodol, and amedin prevented the development of EPD (Table 1). Pentifin was most potent in this respect. The mean effective anticataleptic dose of pentifin was lower than that of cyclodol and amedin by 20 and 10 times, respectively. Combined treatment with group 2 preparations (methoctramine and SL-2) significantly increased the mean effective dose of cholinergic receptor blocker preventing the development of EPD (by 3-6 times, $p<0.01$). We conclude that during combined administration, a highly selective M_2 receptor blocker counteracted the protective effect of cholinergic receptor blocker belonging to group 1.

Previous studies revealed an interaction between systems containing various subtypes of muscarinic receptors and regulating dopamine content. M_4 muscarinic receptors are localized primarily in the striatum. Histochemical assays showed that these postsynaptic receptors are present on projection neurons of the ventral neostriatum [10,14]. Activation of M_4 muscarinic receptors with cholinergic substances in animals produces specific behavioral reactions analogous to tremor in patients with Parkinson's disease [9,10]. Study of secondary messengers demonstrated competitive interrelations between M_4 muscarinic receptors and D_1 receptors localized on striatal neurons and projecting to the substantia nigra and globus pallidus [11]. These data indicate that M_4 muscarinic receptors play a key role in the regulation of locomotor function, which is controlled by dopamine.

Much attention is given to the role of M_2 muscarinic receptors. They were found on cholinergic interneurons of the striatum. Presynaptic terminals are the major site of localization. Some authors believe that M_2 muscarinic receptors play a role of autoreceptors and are involved in the regulation of acetylcholine release from axons of CC-interneurons [13,15]. Other investigators reported that M_4 muscarinic receptors serve as presynaptic receptors in the striatum [12].

There are published data allocating the major role in the realization and correction of EPD to M_1 and M_2 receptors, to M_1 and M_4 receptors, or to M_1-M_3 receptors EPD [7,8,15].

Blockade of M_4 muscarinic receptors in the striatum prevents the development of EPD and plays a key role in the correction of experimental neuroleptic parkinsonism. Presynaptic M_2 muscarinic receptors in the striatum are the second most important receptors. Selective blockade of striatal M_2 receptors abolishes the effect of ligands blocking M_4 receptors. It can be hypothesized that blockade of M_2 muscarinic receptors is followed by the release of "additional" acetylcholine. Taking into account strong blockade of M_4 muscarinic receptors, it is most likely that M_1 and M_3 muscarinic receptors (not M_4 receptors) are the site for action of "additional" acetylcholine. Previous studies showed that selective activation of M_1 muscarinic receptors reduces the ability of atropine (nonselective muscarinic receptor antagonist) to counteract the cataleptogenic effect of haloperidol. Most probably, CC of this subtype are involved in the acetylcholine-mediated regulation of dopamine concentration [6]. The increase in the mean effective doses of cholinolytics by 3-6 times indicates that they produce the effect even in nonselective doses and can block other subtypes of muscarinic receptors. Moreover, the dose of highly selective pentifin should be increased more significantly compared to that of less selective amedin and cyclodol (by 4-6 and 3-4 times, respectively).

TABLE 1. Mean Effective Doses of CC in Prevention of Haloperidol-Produced EPD in Rats (Catalepsy, $M\pm m$)

Conditions for estimation of ED_{50}	Pentifin	Cyclodol	Amedin
Haloperidol	0.023±0.003	0.34±0.02	0.148±0.018
+methoctramine	0.094±0.001*	1.41±0.02*	0.47±0.08*
+SL-2	0.141±0.015*	1.12±0.06*	0.68±0.05*

Note. *$p<0.01$ compared to haloperidol.

Our results suggest that M_4 muscarinic receptors play a major role in the development of haloperidol-produced EPD. It should be emphasized that M_2 muscarinic receptors are also important in this respect. Combined treatment with two different cholinergic receptor blockers for the correction of EPD during neuroleptic parkinsonism can reduce their therapeutic activity due to blockade of M_2 muscarinic receptors.

This work was supported in part by the Russian Foundation for Basic Research (grant No. 03-04-49696).

REFERENCES

1. S. G. Dagaev, A. B. Kosmachev, N. M. Libman, *et al*., *Byull. Eksp. Biol. Med*., **135**, No. 5, 524-526 (2003).
2. A. B. Kosmachev, V. A. Belyaev, O. A. Fil'ko, *et al*., *Eksper. Klin. Farmakol*., **62**, No. 4, 55-58 (1999).
3. A. B. Kosmachev, V. A. Belyaev, A. V. Khrabrova, *et al*., *Ibid*., **61**, No. 5, 3-5 (1998).
4. A. B. Kosmachev, V. A. Belyaev, A. V. Khrabrova, *et al*., *Ibid*., **67**, No. 4, 10-12 (2001).
5. V. B. Prozorovskii, M. P. Prozorovskaya, and V. M. Demchenko, *Farmakol. Toksikol*., No. 2, 497 (1978).
6. A. V. Khrabrova, A. B. Kosmachev, T. A. Titkova, and A. G. Chigarev, *Eksper. Klin. Farmakol*., **62**, No. 4, 7-8 (1999).
7. J. Gomeza, L. Zhang, E. Kostenis, *et al*., *Proc. Natl. Acad. Sci. USA*, **96**, No. 18, 10,483-10,488 (1999).
8. S. Khan, R. Whelpton, and A. T. Michael-Titus, *Neurosci. Lett*., **293**, No. 3, 179-182 (2000).
9. A. J. Mayorga, M. S. Cousins, J. T. Trevitt, *et al*., *Eur. J. Pharmacol*., **364**, No. 1, 7-11 (1999).
10. A. J. Mayorga, G. Gianutsos, and J. D. Salamone, *Brain Res*., **829**, Nos. 1-2, 180-184 (1999).
11. P. Onali and M. C. Olianas, *Eur. J. Pharmacol*., **448**, Nos. 2-3, 105-111 (2002).
12. J. D. Salamone, M. Correa, B. B. Carlson, *et al*., *Life Sci*., **68**, 2579-2584 (2001).
13. J. F. Smiley, A. I. Levey, and M. M. Mesulam, *Neuroscience*, **90**, No. 3, 803-814 (1999).
14. Z. Yan, J. Flores Hernandez, and D. J. Surmeier, *Ibid*., **103**, No. 4, 1017-1024 (2001).
15. W. Zhang, A. S. Basile, J. Gomeza, *et al*., *Ibid*., **22**, No. 5, 1709-1717 (2002).