PHARMACOLOGY AND TOXICOLOGY

Effect of M_4-Cholinoceptor Blockade on Haloperidol-Produced Catatonic Syndrome in Rats

S. G. Dagaev, A. B. Kosmachev, O. A. Fil'ko, L. G. Kubarskaya, A. V. Khrabrova, V. A. Beliaev, N. P. Podosinovikova, N. M. Libman, V. I. Sanotskii, and V. B. Dolgo-Saburov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 2, pp. 174-177, February, 2004
Original article submitted March 25, 2003

We studied the relationship between the efficiency of muscarinic receptor antagonists in preventing haloperidol-induced catatonia and their activity in tests for the interaction of ligands with various subtypes of muscarinic receptors (M_1-M_4) in rats. Mathematical modeling showed that affinity of the ligand for M_4 receptors positively affects its ability to correct extrapyramidal disorders (catatonic syndrome) produced by haloperidol, while affinity for M_2 receptors had a negative effect on this characteristic.

Key Words: *muscarinic receptor antagonists; M-cholinoceptor subtypes; haloperidol; catatonia*

Haloperidol is extensively used in clinical practice as a neuroleptic drug. Long-term treatment with neuroleptics causes extrapyramidal disorders, which makes it necessary to terminate therapy and decrease the dose of preparations.

Among aminoalcohols synthesized at the Institute of Toxicology Pentifin, a central muscarinic receptor antagonist, is most potent in preventing the development of haloperidol-produced catatonic syndrome [8,9].

Here we evaluated a role of M_4 muscarinic cholinergic receptor blockade in the protective effect of muscarinic receptor antagonists.

MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 160-220 g. Functional activity of the cholinergic system was determined by pharmacological tests for the state of M_1, M_2, and M_3 muscarinic receptors [2]. Tremor was produced by pilocarpine and arecoline in doses of 70 and 17 mg/kg, respectively. The dose of pilocarpine causing salivation was 35 mg/kg.

Functional activity of M_4 muscarinic receptors was estimated by the method [11], which is based on detecting specific mandibular movements (MM) produced by pilocarpine (4 mg/kg intraperitoneally). MM were counted for 5 min beginning from the 10th minute after pilocarpine administration. The number of MM for each rat was compared with the threshold value for intact animals receiving physiological saline. It was calculated as the average number of spontaneous movements with a 2σ deviation toward the maximum. The test was considered positive when the number of MM surpassed the threshold value.

The positive effect of haloperidol was considered to be achieved when the rat retained a vertical position by staying on the hindlimbs and placing the forelimbs on a 10-cm wall not less than for 2 min. Testing was performed for 15 min at an interval equal to the latency of haloperidol-produced catalepsy in control

Institute of Toxicology, Russian Ministry of Health, St. Petersburg

0007-4888/04/1372-0152

animals. Each rat was tested no more than 3 times. Haloperidol was injected intraperitoneally in a dose of 10 mg/kg.

The results were expressed in an alternative form (effect-no effect). The effect of muscarinic receptor agonists developed 3-7 min after treatment and persisted for 10 min. Haloperidol caused catalepsy 35-45 min after treatment. Muscarinic receptor blockers were injected subcutaneously in 4-5 increasing doses (logarithmic scale) 30 min before intraperitoneal administration of pilocarpine, arecoline, and haloperidol. Activity of muscarinic receptor blockers was estimated by the mean effective dose (ED_{50}). This dose was determined by probit analysis using tables of V. B. Prozorovskii [7]. Experiments were performed in 3-5 repetitions. The mean and standard error were calculated.

We studied the effects of selective M_2 receptor antagonists on antiparkinsonic activity of Pentifin. Pentifin was injected subcutaneously in increasing doses. M_2 receptor antagonists were administered intraperitoneally in specially selected doses. Doses of metoctramine and SL-2 were 0.066 and 0.086 mg/kg, respectively. Pentifin, SL-2, SL-22, and SL-23 were synthesized at the Institute of Toxicology. Other preparations were obtained from commercial sources.

The mathematical model was constructed in several stages by the method of multiple regression analysis. Equation coefficients and their significance were determined after selection of variables. The significance of this model was estimated by means of dispersion analysis. The prognostic value of an equation was evaluated experimentally [1].

RESULTS

We studied blockade of M_1, M_2, and M_3 muscarinic receptors [2-6,8-10]. The proposed mathematic model describes antiparkinsonic efficacy of muscarinic receptor blockers depending on their activity in the corresponding pharmacological tests [4]. It was postulated that pharmacological selectivity of cholinolytics determines their ability to prevent the development of extrapyramidal disorders produced by haloperidol. The higher is the ability to block M_1 receptors and the lower is the ability to block M_2 and M_3 receptors, the greater is the efficiency of ligands. Blockade of various subtypes of receptors was described as follows: $Y=-1.0+1.3X_1/X_2+0.9X_1/X_3$, where Y is ED_{50} for muscarinic receptor blockers preventing haloperidol-induced catalepsy; X_1 is ED_{50} for preparations preventing pilocarpine-induced tremor; X_2 is ED_{50} for preparations preventing arecoline-induced tremor; and X_3 is ED_{50} for preparations preventing pilocarpine-induced salivation. The ability of Pentifin to counteract the side effects of haloperidol could not be described by this model. In pharmacological tests Pentifin was not selective to M_1, M_2, and M_3 receptors. We hypothesized that Pentifin has another activity not associated with the muscarinic system. It was found that this preparation possesses no direct dopaminergic activity. We used an additional criterion for the degree of M_4 receptor blockade to study the model, which is based on evaluation of the role of various muscarinic receptors in the development of parkinsonism. This model was calculated by the reciprocal of ED_{50} ($1/ED_{50}$) for each test. It was designated as an efficiency of the cholinergic receptor blocker. The greater is the pharmacological effect in rats of various groups receiving the same dose (per 1 kg body weight), the higher is this index.

The studied cholinolytics prevent the development of catatonic syndrome produced by haloperidol in high doses (Table 1). Norglipin was least potent in preventing extrapyramidal disorders. The efficiency of other cholinolytics varied from 1 (comparable to atropine) to 10. The exception was Pentifin whose blocking efficiency (50) surpassed that of Cyclodol and Amedin by 20 and 5 times, respectively. The effects of blockade of M_1-M_3 receptors were studied previously [3]. We compared antihaloperidol activity and pharmacological reaction characterizing blockade of M_4 muscarinic receptor. Both tests showed that Pentifin, Glipin, and Amedin have the highest activity. Non-coincidence of ranks for their activity necessitates the use of mathematical analyses.

We could not perform regression analysis, since it considers the influence of only one subtype of muscarinic receptors on antihaloperidol activity of the ligand. The equations derived for M_1, M_2, and M_3 receptors had low confidence level. A direct effect of M_4 receptor was described by the following equation: $Y=-1.738+3.967\times X_4$ ($p<0.002$). Further studies of individual preparations showed that this equation has no prognostic value.

We hypothesized that blockade of various subtypes of cholinergic receptors correlates with the ability to prevent locomotor disturbances. The regression model for cholinergic receptor blockers was constructed taking into account the degree of blockade of M_1, M_2, M_3, and M_4 receptors. The derived linear model had high confidence level ($p<0.001$, Fischer's test): $Y=b+a_1\times((X_3)^3/X_1)+a_2\times((X_4)^2/X_2)$, where Y is the efficiency of cholinergic receptor blockers in preventing haloperidol-induced catalepsy. The absolute term was 2.99 ($p<0.05$). Coefficients a_1 and a_2 were 0.16 ($p<0.01$) and 0.03 ($p<0.001$), respectively. These data suggest that M_4 and M_2 receptors constitute the system with antagonistic activity. Blockade of M_4 receptors counteracts the development of catatonic syndrome. However, blockade of M_2 receptor has no correcting effect and, probably, potentiates the development of

TABLE 1. Efficiency of Cholinergic Receptor Blockers ($1/ED_{50}$) in a Dose Equieffective to 1 mg/kg Atropine

Preparation	M_1	M_2	M_3	M_4	Haloperidol
Atropine	1.00	1.00	1.00	1.00	1.00
Amedin	0.90	0.37	0.57	2.50	8.37
Pentifin	0.13	0.07	0.23	10.00	53.48
Cyclodol	0.23	0.06	0.32	0.67	2.73
Glipin	5.39	2.64	5.00	5.00	3.62
Norakin	0.37	0.43	0.16	0.58	3.00
Amizil	0.55	0.49	0.58	0.64	1.28
Tropacin	0.09	0.06	0.06	0.25	1.09
Norglipin	0.15	0.07	0.48	0.17	0.11
SL-22	1.35	0.07	0.96	0.39	8.31
SL-23	17.97	0.38	5.20	5.49	3.78

catalepsy. Similar results were obtained after regression analysis of ED_{50}. The equation appeared as follows: $Y=b+a_1X_1+a_2X_2+a_3X_3+a_4X_4$ ($p<0.001$). The absolute term had a negative value and was insignificant (0.224). Coefficients a_1, a_2, a_3, and a_4 were 0.72 ($p<0.002$), -0.17 ($p<0.01$), -0.49 ($p<0.001$), and 1.14 ($p<0.001$), respectively.

Both models indicate that high affinity of the preparation for M_4 receptors and low affinity for M_2 receptors are of considerable importance. The role of M_1 and M_3 receptors remains unclear.

It was absolutely clear that M_4 muscarinic receptors play a role in the efficiency of preparations. It was necessary to provide the supporting evidence for a negative effect of M_2 muscarinic receptors. The next series was conducted to test this hypothesis. Haloperidol was administered in combination with cholinolytics Pentifin and metoctramine (or SL-2). The dose of the preparation given together with Pentifin was selective to M_2 receptors and did not potentiate excitation of M_4 receptors in pharmacological tests. Metoctramine and SL-2 were injected intraperitoneally in doses of 0.066 and 0.086 mg/kg, respectively. Metoctramine and SL-2 increased anticataleptic ED_{50} of Pentifin by 5 and 7 times, respectively. Our findings suggest that administration of selective M_2 muscarinic receptors antagonist in the specified dose considerably reduced antihaloperidol activity of Pentifin, *i.e.* made it necessary to increase the dose of Pentifin.

The results indicate that affinity of the ligand for M_4 muscarinic receptors improves its ability to correct extrapyramidal disorders (catatonic syndrome) produced by haloperidol. By contrast, affinity for M_2 receptors had a negative effect on this characteristic.

For optimum correction of extrapyramidal disorders, pharmacological selectivity of the ligand should be described by the following ratio: denominator, power function (squared M_4); and numerator, M_2. The efficiency of a selective M_4 receptor ligand Pentifin in preventing catatonic syndrome decreases during combination treatment with M_2 muscarinic receptor antagonists.

This work was partially supported by the Russian Foundation for Basic Research (grants No. 00-04-49432, 03-04-49696).

REFERENCES

1. *Computer Biometrics*, Ed. V. N. Nosov [in Russian], Moscow (1990).
2. A. B. Kosmachev, V. A. Beliaev, O. A. Fil'ko, *et al*., *Eksper. Klin. Farmakol*., **62**, No. 4, 55-58 (1999).
3. A. B. Kosmachev, V. A. Beliaev, A. V. Khrabrova, *et al*., *Ibid*., **61**, No. 5, 3-5 (1998).
4. A. B. Kosmachev, V. A. Beliaev, A. V. Khrabrova, *et al*., *Ibid*., **64**, No. 4, 10-12 (2001).
5. A. B. Kosmachev, A. V. Khrabrova, A. O. Zagrebin, *et al*., *Ibid*., **63**, No. 2, 21-23 (2000).
6. N. P. Podosinovikova, A. B. Kosmachev, V. D. Tonkopii, *et al*., *Ibid*., **65**, No. 1, 73-74 (2002).
7. V. B. Prozorovskii, M. P. Prozorovskaya, and V. M. Demchenko, *Farmakol. Toksikol*., No. 2, 497 (1978).
8. V. D. Tonkopii, A. B. Kosmachev, V. V. Petrov, *et al*., *Khim.-Farm. Zh*., No. 11, 15-16 (2000).
9. V. D. Tonkopii, A. B. Kosmachev, V. V. Petrov, *et al*., *Ibid*., No. 9, 18-19 (2000).
10. A. B. Kosmachev, A. V. Khrabrova, V. A. Beliaev, *et al*., *J. Neurochem*., **71**, Suppl. 1, S21 (1998).
11. J. Mayorga, G. Gianutsos, and J. D. Salamone, *Brain Res*., **829**, 180-184 (1999).