EFFECT OF IMIDAZOBENZODIAZEPINE (Ro 15-1788) ON AGGRESSIVE BEHAVIOR MICE

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In the existing view imidazobenzodiazepine (Ro 15-1788) differs from other blockers of benzodiazepine receptors (CGS 8216, β-carbolines) by being a selective antagonist of benzodiazepine receptors [12]. However, the writers showed previously that Ro 15-1788 has a moderate antiaggressive action and does not abolish aggressive behavior induced by diazepam [4]. Apomorphine in small doses has a similar moderate antiaggressive action, but after repeated injections tolerance to its antiaggressive action (and also to its sedative effect) develops [1]. After prolonged administration of apomorphine the antiaggressive action of Ro 15-1788 also becomes appreciably weaker [4].

The aim of the present investigation was to study any possible similar mechanisms of action of imidazobenzodiazepine (Ro 15-1788) and apomorphine in small doses at the level of dopaminergic and serotoninergic systems.

EXPERIMENTAL METHOD

Experiments were carried out on 110 male Wistar rats weighing 250-300 g and on 275 noninbred male mice weighing 25-30 g. The rats were divided into groups, with 10 animals in each group, and the mice into groups with 15 in each group. The action of a small dose of apomorphine (0.1 mg/kg, subcutaneously) and of Ro 15-1788 (from Hoffmann-La Roche, Switzerland) in a dose of 5.0 mg/kg (intraperitoneally) was compared on the basis of four behavioral tests: aggressiveness induced in rats by electric shocks applied to the feet (footshock), and spontaneous motor activity and haloperidol catalepsy in mice. Footshock aggressiveness was determined in a chamber (30 × 25 × 25 cm) in which a pair of rats received 96 electric shocks with an intensity of 35 V in the course of 3 min. The intensity of aggressive behavior was estimated as the number of fights between the pair of rats. Motor activity was determined with a photoelectric actometer for 30 min. Haloperidol catalepsy was estimated 60 min after intraperitoneal injection of haloperidol in a dose of 0.25 mg/kg (Gedeon Richter, Hungary). The intensity of catalepsy was determined as the length of time the animal remained in an awkward position. Parallel with the behavior tests, the concentrations of serotonin and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in structures of the mouse forebrain were determined [7]. Apomorphine (0.1 mg/kg) and Ro 15-1788 (5.0 mg/kg) were injected immediately before determination of spontaneous motor activity or 30 min before evaluation of the intensity of footshock aggressiveness and haloperidol catalepsy, or before the biochemical investigation. To study the antiaggressive action of apomorphine and Ro 15-1788 the drugs were injected slowly, twice a day for 10 days. The action of a single injection of Ro 15-1788 and of apomorphine also was studied against the background of small doses (0.01-0.03 mg/kg) of pirenperone (from Janssen Pharmaceutica, Belgium), an antagonist of serotonin$_2$-receptors [6], and of haloperidol (0.01-0.05 mg/kg), an antagonist of dopamine receptors. These substances were injected 5 min before apomorphine and Ro 15-1788. The action of apomorphine and Ro 15-1788 also was analyzed in a separate series of experiments after destruction of serotoninergic terminals by parachloramphetamine, injected in a dose of 15 mg/kg twice a day for 7 days before the experiment (from Sigma, USA). Injection of large

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TABLE 1. Effect of Single Injection and Prolonged (twice a day for 10 days) Administration of Apomorphine and Ro 15-1788 on Footshock Aggressiveness in Rats and Serotonin Metabolism in Mouse Forebrain, and Changes in Action of Apomorphine and Ro 15-1788 under the Influence of Small Doses of Pirenperone and Haloperidol (M ± m)

Experimental conditions	Footshock aggressiveness (number of fights during 2 min)		Serotonin, μg/g tissue		5-HIAA	
	I	II	I	II	I	II
Physiological saline	25±3,2	29±3,6	0,50±0,03	0,46±0,02	0,48±0,03	0,45±0,02
Apomorphine (0.1 mg/kg)	11±1,8*	56±4,2*	0,52±0,04	0,47±0,04	0,40±0,03	0,62±0,03*
Ro 15-1788 (5 mg/kg)	15±1,6*	49±3,6*	0,56±0,03	0,32±0,03*	0,55±0,03	0,58±0,04*
Apomorphine (0.1 mg/kg)+Ro 15-1788 (5 mg/kg)	5±0,9*	—	—	—	—	—
Apomorphine (0.1 mg/kg)+haloperidol (0.1 mg/kg)	27±3,7	—	—	—	—	—
Apomorphine (0.1 mg/kg)+pirenperone (0.1 mg/kg)	4±1,2**	—	—	—		—
Ro 15-1788 (5 mg/kg)+haloperidol (0.01 mg/kg)	34±4,2	—	—	—	—	—
Ro 15-1788 (5 mg/kg)+pirenperone (0.01 mg/kg)	3±0,8**	—	—	—	—	—

Legend. I) Single injection, II) prolonged administration. Here and in Table 2: *P < 0.05, **P < 0.02.

TABLE 2. Effect of Apomorphine and Ro 15-1788 on Spontaneous Motor Activity and Haloperidol Catalepsy in Mice and Changes in Action of Apomorphine and Ro 15-1788 on Motor Activity and Serotonin Metabolism in Mice after Injection of Parachloroamphetamine (M ± m)

Experimental conditions	Motor activity (number of impulses in 30 min)	Duration of haloperidol catalepsy, min	Parachloramphetamine (2 × 15 mg/kg)		
			motor activity (number of impulses in 30 min)	serotonin	5-HIAA
				μg/tissue	
Physiological saline	296±28	27±6	323±36	0,28±0,02	0,30±0,03
Apomorphine (0.1 mg/kg)	97±12**	49±4*	53±9***	0,30±0,03	0,54±0,02*
Ro 15-1788 (5 mg/kg)	247±20	9±1*	253±21	0,40±0,04*	0,27±0,02
Apomorphine (0.1 mg/kg)+Ro15-1788 (5 mg/kg)	156±11	—	167±15	—	—
Apomorphine (0.1 mg/kg)+haloperidol (0.05 mg/kg)	187±17	——	—	—	—
Apomorphine (0.1 mg/kg)+pirenperone (0.3 mg/kg)	52±6	—	—	—	—

Legend. ***P < 0.01.

doses of parachloramphetamine causes selective degeneration of serotoninergic terminals in the forebrain and considerable depression of the serotonin concentration [5]. The experimental results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Small doses of apomorphine (0.1 mg/kg) and Ro 15-1788 (5.0 kg/mg) had a moderate antiaggressive action, and if the two were given together, depression of aggressive behavior was potentiated (Table 1). Haloperidol (0.01 mg/kg) abolished the antiaggressive action of both substances, whereas pirenperone (0.1 mg/kg) potentiated the behaviorial effect of a small dose of apomorphine and Ro 15-1788. After prolonged administration, however, the effect of a small dose of both apomorphine and Ro 15-1788 was considerably modified. Both substances had a proaggressive action (Table 1). Injection of a small dose of apomorphine into mice depressed their motor activity significantly (Table 2). Ro 15-1788 caused no significant change in the animals' motor activity compared with the control, but like haloperidol in a small dose (0.05 mg/kg), it depressed the effect of apomorphine. Pirenperone (0.03 mg/kg) and destruction of serotoninergic terminals with parachloramphetamine potentiated the sedative action of apomorphine equally. Apomorphine and Ro 15-1788 had directly opposite actions on the intensity of haloperidol catalepsy (Table 2). Apomorphine potentiated, whereas Ro 15-1788 significantly reduced the behavorial effect of haloperidol (0.25 mg/kg). Acute administration of apomorphine (0.1 mg/kg) did not change serotonin metabolism (Table 1). However, after repeated injections of apomorphine and under the influence of parachloramphetamine a significant rise was observed in the rate of serotonin metabolism (Tables 1 and 2). Unlike apomor-

phine, Ro 15-1788 in acute experiments inhibited serotonin metabolism, but after repeated injections, like apomorphine, it speeded up serotonin turnover.

The results are evidence that apomorphine and Ro 15-1788 may have the same or different effects on different functional systems of the brain. According to existing data apomorphine is a direct agonist of dopamine receptors [8] and does not interact with benzodiazepine receptors [11]. However, according to our data, apomorphine has a direct effect on serotonion$_2$-receptors in small doses, moreover, apomorphine is an antagonist of these receptors. This view is confirmed by a number of facts. After long-term administration, apomorphine potentiates serotonin turnover and increases the sensitivity of serotonin$_2$-receptors [3], pirenperone, an antagonist of serotonin$_2$-receptors, potentiates the effects of apomorphine, and after destruction of serotoninergic terminals by parachloramphetamine, apomorphine has a stronger inhibitory effect on the animals' behavior, and after prolonged administration the animal becomes tolerant to the behavioral (sedative, antiaggressive) effects of apomorphine and, finally, apomorphine displaces labeled ketanserine (an antagonist of serotonin$_2$-receptors) from binding sites in the prefrontal cortex [10]. According to the results of the writers' previous investigations Ro 15-1788 increased the concentration of the dopamine metabolite 3,4-dihydroxyphenylacetic acid equally in the striatum and limbic structures [2]. The different effects of Ro 15-1788 on different functional systems of the brain are thus not connected with its interaction with the dopaminergic system. In all probability this action of Ro 15-1788 is due to its interaction with serotoninergic mechanisms. The writers showed that Ro 15-1788 is an agonist of benzodiazepine receptors concerned with regulation of activity of the serotoninergic system [4]. However, the present investigation demonstrated that these benzodiazepine receptors differ in their effects on serotoninergic mechanisms in different functional systems of the brain. These benzodiazepine receptors have an inhibitory influence on serotonin mechanisms regulating emotional behavior, and located mainly in limbic structures. This hypothesis is supported by the potentiation of the antiaggressive action of apomorphine against the background of Ro 15-1788 and potentiation of the antiaggressive effect of Ro 15-1788 under the influence of small doses of pirenperone, the antagonist of serotonin$_2$-receptors. After prolonged administration tolerance is developed to the antiaggressive action of Ro 15-1788, and as in the case of apomorphine, this is accompanied by a significant increase in the rate of serotonin metabolism. Ro 15-1788 had an opposite effect on serotonin mechanisms concerned with regulation of motor reactions and located mainly in extrapyramidal structures. Unlike pirenperone, and like a small dose of haloperidol, Ro 15-1788 counteracts the sedative effect of apomorphine. In acute experiments Ro 15-1788 inhibits the serotonin turnover and, after long-term administration, reduces the sensitivity of serotonion$_2$-receptors [4]. Probably the antagonism of the cataleptogenic action of haloperidol under the influence of Ro 15-1788 is due to intensification of serotonin processes in the striatum. Another explanation of this different action of Ro 15-1788 on animal behavior may be the fact that extrapyramidal and limbic structures receive their serontoninergic innervation from unequal populations of mesenteric raphe nuclei [9].

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LITERATURE CITED

1. L. H. Allikmets and É. É. Vasar, Zh. Vyssh. Nerv. Deyat., No. 1, 130 (1982).
2. É. É. Vasar, "Pharmacologic analysis of neuromediator mechanisms of apomorphine and clonidine aggressiveness," Author's Abstract of Candidate's Dissertation, Tartu (1983).
3. É. É. Vasar and L. H. Allikmets, Byull. Éksp. Biol. Med., No. 6, 70 (1983).
4. É. É. Vasar, L. K. Rägo, and L. H. Allikmets, Zh. Vyssh. Nerv. Deyat., No. 5, 864 (1983).
5. T. Archer, S.-O. Ogren, and S. B. Ross, Neurosci. Lett., 34, 75 (1982).
6. S. C. Colpaert, C. J. E. Niemegeers, and P. A. J. Janssen, J. Pharmacol. Exp. Ther., 221, 206 (1982).
7. C. J. Earley and B. E. Leonard, J. Pharmacol. Methods, 1, 67 (1978).
8. A. M. Earnst, Psychopharmacology, 10, 316 (1967).
9. K. Fuxe, and G. Jonsson, Adv. Biochem. Psychopharmacol., 10, 1 (1974).
10. J. E. Leysen, C. J. E. Niemegeers, J. M. Van Nueten, et al., Mol. Pharmacol., 21, 301 (1982).
11. C. R. Mackerer, R. L. Kochman, B. A. Biderschenk, et al., J. Pharmacol. Exp. Ther., 206, 405 (1978).
12. H. Möhler and J. G. Richards, Nature, 294, 763 (1981).