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AM251, cannabinoids receptors ligand, improves recognition memory in rats

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Abstract:
High density of cannabinoid receptors type 1 (CB1) in the brain suggests that endocannabinoid system plays an important role in the functioning of the central nervous system. Natural and synthetic cannabinoids are known to attenuate learning and memory processes. The adverse effects of cannabinoids are reversed by SR141716A, at first reported to be a selective CB1 receptor antagonist, later shown to possess also inverse agonist properties. The present study was performed in an attempt to determine the influence of different doses of AM251, a member of the same cannabinoid group as SR141716A, on recognition memory evaluated in an object recognition test. Because cannabinoids may alter motor function and affect anxiety, the influence of AM251 on psychomotor activity and anxiety was assessed in an "open-field" test and elevated plus maze, respectively. While the lowest dose of AM251 (1.0 mg/kg) significantly improved recognition memory, higher doses (2.5 mg/kg and 5.0 mg/kg) did not have an influence on it. Moreover, AM251 did not affect anxiety but in the highest dose significantly attenuated psychomotor activity in rats. The main finding of the present study indicates that AM251, at the dose of 1.0 mg/kg, improves recognition memory in rats without alteration of their psychomotor activity and anxiety. The pro-cognitive effect exerted by compounds belonging like AM251 to diarylpyrazole group may be beneficial in therapeutic use of these compounds, especially in patients with cognitive dysfunctions.

Key words:
AM251, cannabinoids, recognition memory, psychomotor activity, anxiety, rat

Abbreviations: AM251 – synthetic cannabinoid [N-(piperdin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxyamide], CB – cannabinoid receptor, CNS – central nervous system, Δ^9-THC – Δ^9-tetrahydrocannabinol, SR141716A – synthetic cannabinoid [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride]

Introduction

Cannabis sativa L. remains the most often used plant for its recreational and therapeutic (antiemetic, anticonvulsive, antinociceptive) properties [15]. Cannabinoids, Δ^9-tetrahydrocannabinol and Δ^8-tetrahydrocannabinol (Δ^9-THC, Δ^8-THC), the main biologically active constituents of marijuana and cannabis derivatives, are well known for their psychoactive effects. The clinical observations supported by many experimental data revealed that cannabinoids' intoxication is associated with various side effects, especially dysfunction of cognitive processes [9, 19, 27, 30].

The discovery and subsequent cloning of cannabinoid receptors (CB) followed by the finding of an endogenous, produced upon demand, ligands for these receptors: anandamide (ANA) [13] and 2-arachidonylglycerol (2-AG) [32], began an intensive work on the relevance of endogenous cannabinoid system in physiological and

pathological conditions. Cannabinoids have been used in therapy of glaucoma, motor dysfunction and chronic pain [2, 21]. Moreover, they have been used in therapy of nausea and vomiting during cancer chemotherapy, stimulation of appetite in AIDS wasting syndrome, in therapy of drug dependence, as well as in obesity [1, 24, 37]. Recently, it has been reported that AM251 attenuates the reinstatement of nicotine place preference in rats what may contribute to the development of more effective pharmacoterapies of nicotine dependence [8].

Cannabinoids exert their pharmacological effects through pertussis toxin-sensitive Gi/o protein-coupled membrane receptors CB1 [31] and CB2 [44]. The peripheral receptor CB2, is the most abundant on cells of the immune system and has been also found in the microglial cells of the central nervous system (CNS) [7, 18, 34]. CB1 receptor is located predominantly in the CNS, and belongs to one of the most abundantly expressed neuronal receptors. In the brain, high density of CB1 receptors was found in the structures associated with cognition and movement: hippocampus, amygdala, septum, brain cortex, globus pallidus, substantia nigra, lateral caudate putamen and cerebellum [2, 20, 38, 48]. Moreover, recently two novel orphan G-protein coupled receptors GPR55 and GPR119 have been implicated as targets of cannabinoids' action [6, 17, 23, 42].

High expression of CB1 receptors in the brain suggests that endocannabinoid system plays an important role in the function of CNS. The tonic activity of an endogenous cannabinoid system present in physiological conditions has been proposed to play a modulatory role in processes of learning and memory formation [26, 36]. Cannabinoids' intoxication is known to attenuate learning and memory processes in both humans and animals. There is support for the notion that naturally occurring (Δ^9-THC) and synthetic (CP-55,940 or WIN55,212-2) cannabinoids disrupt working memory in animals evaluated in tests based on conditioning [10, 27, 29]. Moreover, we have previously reported deleterious effect of R-(+)-methanandamide, a stable analogue of endogenous cannabinoid, anandamide, as well as a preferential CB1 receptor agonist CP-55,940 on recognition memory, evaluated in an object recognition test in rats [25].

It has been shown that SR141716A, selective CB1 receptor antagonist, prevented deleterious effects of cannabinoids on memory, without having an influence on cognitive processes when was given alone in non-match-to-position task [30]. Surprisingly, a beneficial effect of SR141716A on learning and memory processes has been also reported. Terranova et al. [47] have shown the ability of SR141716A to facilitate short-term olfactory memory in the social recognition test in rodents. Wolff and Leander [50] as well as Lichtman [26] reported SR141716A improvement of memory in a delayed radial maze task and Takahashi et al. [46] also in the mouse elevated T-maze. The improvement of memory after the blockade of CB1 receptors by SR141716A is in agreement with the enhancement of memory in CB1 receptor knockout mice observed in a two-trial object recognition test [39].

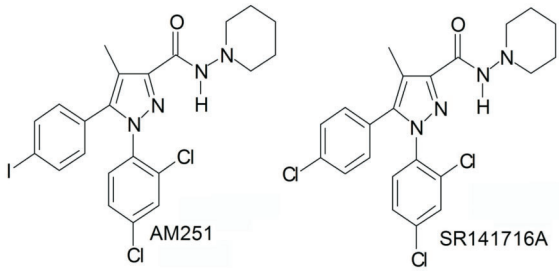

Fig. 1. Structure of AM251 and SR141716A

AM251 and AM281, structurally similar to SR141716A, classified to the group of diarylpyrazoles (Fig. 1.), were introduced as CB1 receptor antagonists [11, 16]. In many experiments selective CB1 receptor antagonist SR141716A reversed the effects of cannabinoids, providing a good evidence for the involvement of CB1-related mechanisms. Investigations performed with radiolabelled AM251 and AM281 confirmed their affinity to CB1 receptor in the brain [11, 16]. Moreover, experiments evaluating their influence on intracellular signal transduction showed that AM251 and AM281, similarly to SR141716A might exert properties of CB1 receptor inverse agonists [11, 33].

The present study was performed in an attempt to determine the influence of AM251, belonging to the same cannabinoids' group of diarylpyrazoles as SR141716A, a CB1 receptor antagonist/inverse agonist [36], on recognition memory. The influence of AM251 was evaluated in an object recognition test, which has no conditioning reinforcement. Moreover, because cannabinoids may alter motor function [2, 10, 22, 40, 43], and may exert neophobia [3], the influence of AM251 on psychomotor activity and on anxiety was evaluated in an "open-field" test and in elevated plus-maze test, respectively.

Materials and Methods

All experiments were approved by the Local Ethics Committee in Białystok and were performed in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Animals

Subjects were experimentally naive white male Wistar rats of laboratory strain, weighing 175–190 g. They were housed in plastic cages (50 × 40 × 20 cm), four animals per cage, in the temperature (22 ± 1°C) and humidity (50–60%) controlled room on a 12-h light-dark cycle beginning at 07:00 h. Food and water were freely available except during tests' period. Experiments were performed in the morning, between 9.00 a.m. and 1.00 p.m.

Drugs

AM251 – [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxyamide] (Tocris), dissolved in DMSO (Sigma), was given intraperitoneally (*ip*) in a single dose of 1.0, 2.5 or 5.0 mg/kg.

In order to evaluate the influence of AM251 on acquisition of information, the compound was given 15 min before learning trial (T1), while in order to evaluate its influence on consolidation of information, AM251 was given immediately after T1 trial in an object recognition test.

In an attempt to evaluate, whether during T2 (testing) trial AM251 altered psychomotor activity and level of anxiety in rats, an "open field" test and an elevated plus maze test, respectively, were performed immediately after T2 trial of object recognition test. To limit a number of tests performed on the same group of animals, an "open field" test was performed in groups of rats in which the influence of AM251, given immediately after T1 trial, on consolidation of information (consolidation phase) was evaluated, while an elevated plus maze test was performed when the influence of AM251, given 15 min before T1 trial, on acquisition of information (acquisition phase) was examined. Moreover, in separate groups of rats the influence of AM251 on psychomotor activity of animals was evaluated 15 min after its administration, at the corresponding to T1 trial time of object recognition test. Control animals received a vehicle only at indicated time points.

BEHAVIORAL TESTS

Object recognition test

The apparatus, a gray wooden box (65 × 45 × 45 cm) was placed in a sound-isolated room, where one bulb fastened above the experimental area provided a constant illumination of 40 lux at the level of the test box. Throughout the experiment no cleaning of the box was allowed, in order to saturate it with an olfactory stimuli. The procedure was performed similarly to that described by Ennaceur and Delacour [14]. A day before testing, rats were submitted to a habituation session, whereby they were allowed to explore the apparatus for 5 min. The experimental session, performed on the next day, comprised of two trials. In the first (learning) trial (T1), one object-stimulus, the sample (A), was placed near the rear wall of the box in a location equidistant from the back corners of the box. During the second (testing) trial (T2), a new object (B) was added. Here, each object was placed in a back corner. Presented during T2 object A' was a duplicate of the sample presented in T1 (object A) in order to avoid olfactory traits. To reduce object and place preference effects, from rat to rat, the role (sample or new object) and the position of the two objects during T2 was counterbalanced and randomly permuted. It should be stressed that the objects used during experiments had no natural significance for rats and had never been associated with reinforcement and their weight was such that animals could not displace them. At the beginning of each trial, rats were placed near the centre of the front wall of the box, with their heads oriented in the opposite direction to the object. The duration of T1 and T2 trials were 5 and 3 min, respectively. The recognition, testing trial (T2) started 120 min after T1 trial. The basic measure was the total time spent by rats on objects' exploration during each trial. Exploration of an object was defined as directing the nose at a distance of 2 cm to the object and/or touching it with the nose. Turning around or sitting on the object, as well as resting on the object and sniffing in the air was not considered as exploratory behavior. From this measure, the following variables were defined: A = the time spent on exploration the sample during T1, B = the time spent on exploration new object during T2, (B + A') = the time spent on exploration a duplicate (A') of the familiar object A and a new object (B) during T2. Object recognition was measured by the variable (B – A'). Since (B – A')

may be biased by differences in overall levels of exploration, the variable (B – A)/(B + A') was also computed. Moreover, the recognition index was calculated for each animal and expressed as a ratio: (time B × 100)/(time B + A'). According to Ennaceur and Delacour [14], the recognition index higher than 50%, (when time B is longer than time A') indicates that an animal remembers the familiar object, but the recognition index equal or lower than 50%, (when time B is comparable or shorter than time A') indicates that an animal does not remember the familiar object.

Open-field

Locomotor (crossings of squares) and exploratory activities (rearings and bar approaches) were measured in an "open field" test. The apparatus consisted of a wooden box with a square white floor measuring 100 × 100 cm divided by eight lines into 25 equal squares and surrounded by a 47 cm high wall, as described earlier [5]. Four wooden bars, 20 cm high, were designed as objects of possible interest of the animals and fixed perpendicularly parallel to each other in four line crossings in the central area of the floor. The apparatus was placed in a sound-isolated room and one bulb fastened above the experimental area provided a constant illumination of 75 lux at the level of the test box. The animals were placed in centre of an open-field box and crossings of squares, rearings (rises on the hind legs and looking around), bar approaches (approaches to wooden bars and/or touching them with the nose) were counted for 5 min. The "open field" test was carried out immediately after T2 trial of object recognition test, and in other groups of rats, 15 min after *ip* injection of AM251 or vehicle.

Elevated plus-maze

The procedure was performed according to Pellow et al. [35] in a sound-isolated room. The apparatus consisted of four arms: two open, 50 × 10 cm, and two closed arms 50 × 10 × 40 cm, with an open roof. The arms were arranged such that the two open arms were opposite to each other and connected with the central (neutral) area 10 × 10 cm. The apparatus was elevated 80 cm above the floor and one bulb fastened above the experimental area provided a constant illumination of 75 lux at the level of the apparatus. Rats were placed in the neutral area of the maze, facing one of the open arms. The number of entries and the time spent in each type of arm, as well as the time spent in the neutral area was counted for 5 min. The elevated plus-maze test was carried out immediately after T2 trial of object recognition test.

The tests: object recognition, open-field, and elevated plus-maze were recorded on a videotape (mini DV standard) using a digital camcorder. Simultaneously, observers took the measurements during all behavioral experiments manually. After each session the measurements were finally counted and once again behavior of each rat was evaluated corresponding to the videotaped data.

Statistical analysis

The results of experiments were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. F-ratios, degrees of freedom, and p values were reported only for significant differences. In all comparisons between particular groups, a probability of 0.05 or less ($p \leq 0.05$) was considered significant.

Results

Effect of AM251 on recognition memory

Recognition memory measured by variable (B – A') was significantly different between the groups. ANOVA of three injected with different doses of AM251 and respective control groups yielded $F_{3,34} = 8.00$, $p < 0.0005$, $F_{3,32} = 31.85$, $p < 0.0005$, for acquisition and consolidation phase of recognition memory, respectively. *Post-hoc* comparison with Dunnett's test showed significant improvement of recognition memory in 1.0 mg/kg of AM251 treated rats (Tab. 1 and Tab. 2). AM251 given at the dose of 1.0 mg/kg, both 15 min before the learning trial (T1) and immediately afterwards, improved recognition memory in rats measured by the difference in time of exploration of a duplicate (A') of the familiar object A and a new object B in T2 trial (variable B – A') presented in 2 h delay in comparison with the respective control groups of rats.

Moreover, for each animal a recognition index was calculated and expressed as a ratio: (time B × 100)/(time B + time A') (Fig. 2). ANOVA of the recognition index of injected with AM251 and respective

Tab. 1. Effect of AM251 on acquisition of information evaluated in an object recognition test in rats

Variables (s)	Treatment			
	Control	AM251		
		1 mg/kg	2.5 mg/kg	5 mg/kg
(B – A')	–4.300	6.545***	–2.800	–1.571
	(1.897)	(1.591)	(2.031)	(1.428)
A	30.200	21.181	8.800*	12.14
	(6.392)	(4.850)	(2.851)	(5.804)
(B + A')	25.700	19.454	15.400*	9.000*
	(3.464)	(2.832)	(2.604)	(2.507)
(B – A')/(B + A)	–0.133	0.298	–0.099	–0.065
	(0.062)	(0.479)	(0.106)	(0.144)

AM251 was given *ip* once, 15 min before T1 trial of object recognition test. Variables were described in Materials and Methods section. Table presents the means ± SEM (in parentheses) of the values obtained from 7–11 rats. * $p < 0.05$, *** $p < 0.0005$ *vs.* control group of rats (ANOVA and Dunnett's test)

Tab. 2. Effect of AM251 on consolidation of information evaluated in an object recognition test in rats

Variables (s)	Treatment			
	Control	AM251		
		1 mg/kg	2.5 mg/kg	5 mg/kg
(B – A')	–2.900	4.636***	–3.250	–2.142
	(1,120)	(0.766)	(1.359)	(1.724)
A	26.000	26.181	30.750	15.571
	(3.852)	(5.144)	(5.450)	(5.051)
(B + A')	20.700	13.363	14.500	14.142
	(3.279)	(2.744)	(2.897)	(2.947)
(B – A')/(B + A)	–0.150	0.412	–0.284	–0.035
	(0.057)	(0.055)	(0.093)	(0.194)

AM251 was given *ip* once, immediately after T1 trial of object recognition test. Variables were described in Materials and Methods section. Table presents the means ± SEM (in parentheses) of the values obtained from 7–11 rats. *** $p < 0.0005$ *vs.* control group of rats (ANOVA and Dunnett's test)

control groups of rats yielded $F_{3,34} = 5.78$, $p < 0.005$ and $F_{3,32} = 10.75$, $p < 0.0005$ for acquisition and consolidation of information, respectively. Comparison made with Dunnett's test revealed significant enhancement of recognition index in rats injected with the lowest dose of AM251 (1.0 mg/kg) given both, 15 min before T1 trial and immediately after it, as compared to the respective control groups. In these groups of animals, the recognition index was higher than 50% and reached about 65% in acquisition and 70% in consolidation phase, respectively. The time spent by animals on exploration of a new object B was longer than the time spent on exploration of a duplicate (A') of the familiar object A, what indicates that they remembered the familiar object. In animals injected with higher doses of AM251 recognition index was below 50%, because rats spent comparable or even shorter time on exploration of a new than the familiar object, what indicates that similarly to controls they did not remember the familiar object after 2 h delay.

The experimental data analysis showed that application of AM251 15 min before learning (T1) trial caused shorter time of objects' exploration both in T1 and T2 trials in comparison with the control group

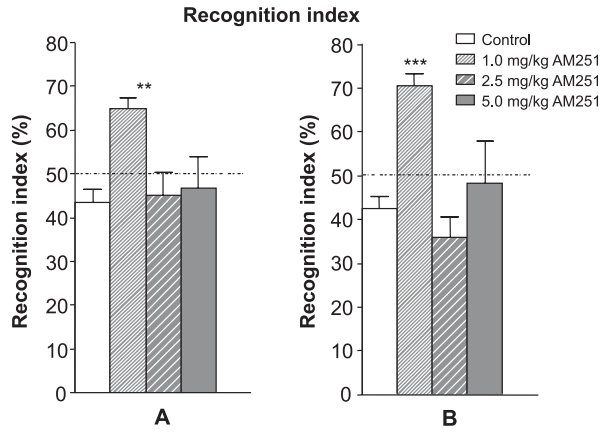

Fig. 2. Effect of AM251 on recognition index in an object recognition test. (**A**) AM251 was given *ip* once, 15 min before T1 trial (when its influence on acquisition of information was evaluated) and (**B**) AM251 was given *ip* once immediately after T1 trial (when its influence on consolidation of information was evaluated). Columns represent the means ± SEM of the values obtained from 7–11 rats. ** $p < 0.005$, *** $p < 0.0005$ *vs.* respective control groups (ANOVA and Dunnett's test)

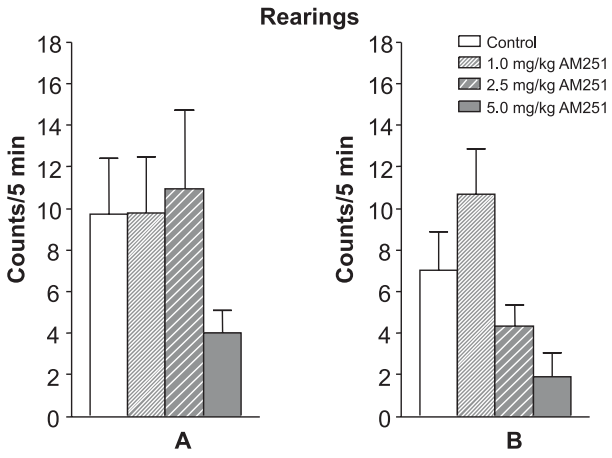

Fig. 4. Effect of AM251 on rearings: (**A**) given *ip* once, 15 min before testing the animals in an "open field" test; (**B**) performed immediately after T2 trial of object recognition test, when its influence on consolidation of recognition memory was evaluated. Columns represent the means ± SEM of the values obtained from 7–11 rats

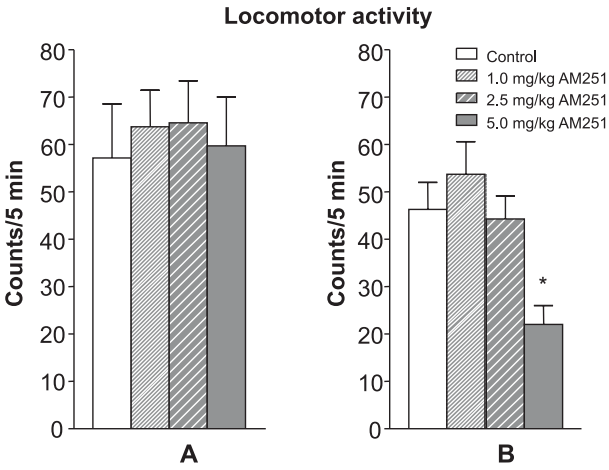

Fig. 3. Effect of AM251 on locomotor activity measured by crossings of squares: (**A**) given *ip* once, 15 min before testing the animals in an "open field" test; (**B**) performed immediately after T2 trial of object recognition test, when its influence on consolidation of recognition memory was evaluated. Columns represent the means ± SEM of the values obtained from 7–11 rats. * $p < 0.05$ vs. control group (ANOVA and Dunnett's test)

(Tab. 1). ANOVA of the time of objects' exploration in AM251 injected animals and control group in T1 and T2 trials yielded $F_{3,34} = 3.59$, $p < 0.05$ and $F_{3,34} = 4.99$, $p < 0.005$, respectively. *Post-hoc* evaluation made with Dunnett's test showed significant decrease of the time of object exploration during T1 trial in animals injected with 2.5 mg/kg and during T2 trial with 2.5 mg/kg and 5.0 mg/kg of AM251 in comparison with the control group. When AM251 was given immediately after T1 trial the time spent on exploration of a duplicate (A') of the familiar object A and a new object B in T2 trial [variable (B+A') in Tab. 2] was comparable between three injected with AM251 groups of rats and insignificantly shorter than in control group.

Effect of AM251 on psychomotor activity evaluated in an "open field" test

Locomotor activity measured by crossings of squares was comparable in control and experimental groups of rats, evaluated in an open field, 15 min after AM251 application (Fig. 3A), while was attenuated in animals injected with the highest dose of AM251 (5.0 mg/kg), when "open field" test was performed immediately after T2 trial of object recognition test (Fig. 3B). ANOVA of three groups injected with AM251 and control group yielded $F_{3,36} = 6.61$, $p < 0.01$. *Post-hoc* analysis with Dunnett's test revealed significant attenuation of locomotor activity in rats treated with 5.0 mg/kg of AM251. Alterations of exploratory activity measured by number of rearings (Fig. 4) and bar approaches (Fig. 5) were also observed. AM251 at the dose of 5.0 mg/kg attenuated the number of rearings in comparison with the respective control groups when "open field" test was performed 15 min after its application (Fig. 4A) and immediately after T2 trial (Fig. 4B) of object recognition test, however, these differences were insignificant.

ANOVA of bar approaches in control group and three groups injected with AM251, in open field performed immediately after T2 trial of object recognition test yielded $F_{3,36} = 6.00$, $p < 0.01$. *Post-hoc* comparison with Dunnett's test indicated significant attenuation of the number of bar approaches in rats treated with the highest dose of AM251 (Fig. 5B).

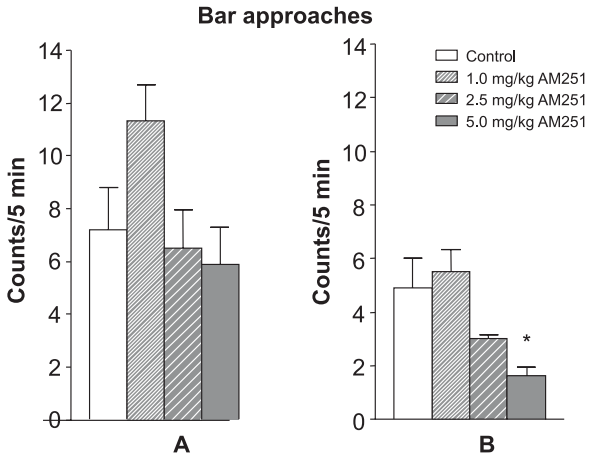

Fig. 5. Effect of AM251 on bar approaches: (**A**) given *ip* once, 15 min before testing the animals in an "open field" test; (**B**) performed immediately after T2 trial of object recognition test, when its influence on consolidation of recognition memory was evaluated. Columns represent the means ± SEM of the values obtained from 7–11 rats. * $p < 0.05$ *vs.* control group (ANOVA and Dunnett's test)

Tab. 3. Effect of AM251 on anxiety evaluated in an elevated plus-maze test in rats

Variables	Treatment			
	Control	AM251		
		1 mg/kg	2.5 mg/kg	5 mg/kg
Closed arms time (s)	279.900 (4.530)	279.500 (2.212)	275.200 (7.693)	276.500 (5.245)
Closed arms entries	1.800 (0.290)	1.400 (0.163)	1.410 (0.162)	1.900 (0.433)
Opened arms time (s)	6.700 (3.930)	7.200 (2.327)	8.200 (3.990)	10.300 (5.050)
Opened arms entries	0.800 (0.290)	0.900 (0.276)	0.800 (0.326)	1.000 (0.333)
Neutral area time (s)	13.400 (2.684)	12.300 (3.151)	16.600 (4.232)	12.600 (3.063)

Table presents the means ± SEM (in parentheses) of the values obtained from 7–11 rats

Effect of AM251 on anxiety evaluated in elevated plus-maze test

AM251 given in three doses (1.0, 2.5 and 5.0 mg/kg) did not have any influence on anxiety evaluated in elevated plus-maze test performed immediately after T2 trial of object recognition test (Tab. 3). Animals injected with AM251 spent a comparable amount of time in closed and opened arms, as well as in the neutral area in comparison with control animals. Similarly, the number of entries to closed and opened arms was not different in comparison with control group.

Discussion

The main finding of the present study is the facilitation of recognition memory observed after AM251 application, a congener of SR141716A, CB1 receptor antagonist/inverse agonist. AM251 at the dose of 1.0 mg/kg, but not in the higher doses (2.5 and 5.0 mg/kg) significantly improved acquisition and consolidation of information, evaluated in an object recognition test in rats. During T2 trial the animals injected with 1.0 mg/kg of AM251 significantly longer explored a new object than a duplicate of familiar one presented in T1 trial, while animals injected with higher doses of AM251, similarly to control rats, spent a comparable amount of time on exploration of a new and the familiar object. The improvement of discrimination between a duplicate (A') of the familiar object A and a new object B was present also when AM251 was given after the learning trial (T1) what is, according to Dawson and McGaugh [12], an indication that this effect was memory specific, since the compound was administered after the learning experience, unspecific effect resulting from altered perception, motivation and emotion can be ruled out.

Moreover, since the influence of AM251 on acquisition and consolidation of information, given 15 min before and immediately after T1 (learning) trial, respectively, was similar that might indicate that shorter time of objects' exploration in T1 and T2 trials, observed in groups injected 15 min before T1 trial with 2.5 and 5.0 mg/kg of AM251 in comparison with the control groups, probably did not have an influence on the ability of objects' discrimination.

Because processes of memory formation may be affected by non-mnemonic factors, the influence of

AM251 on psychomotor activity and the level of anxiety was also evaluated. AM251 given 15 min before the evaluation of psychomotor activity of rats in an open field, performed in the time related to T1 trial of object recognition test, did not alter the behavior of experimental animals in comparison to the controls. When an "open field" test was performed immediately after T2 trial, a significant attenuation of crossings of squares and bar approaches was observed after the highest (5.0 mg/kg) dose of tested compound. However, when animals were injected with AM251 immediately after T1 trial, the total time spent on exploration of both objects during T2 trial was comparable in animals injected with all tested doses, what indicates that attenuation of psychomotor activity exerted by the highest dose of this compound evaluated in an "open field" test conducted after T2 trial did not account for the effect of AM251 on recognition memory.

To answer the question whether the exploration of the familiar and a new object could be interfered by the level of anxiety and neophobia, immediately after T2 trial of object recognition, the level of anxiety was examined in an elevated plus maze test. AM251 in all tested doses did not alter anxiety in AM251 treated rats in comparison to the control group what indicates that injected with AM251 animals during discrimination between the familiar and a new object in T2 trial did not afraid of the new one.

However, because 15 min after application of AM251 locomotor and exploratory activities of rats were not altered in comparison to the control group, and level of anxiety was not evaluated, the involvement of neophobia in shorter exploration of object A during T1 trial (when AM251 was given 15 min before it) observed in groups injected with higher doses of AM251 (2.5 and 5.0 mg/kg) could not be excluded.

The results concerning the influence of CB1 receptor blockade on anxiety are not consistent and are dependent on the species and strains of tested animals [3, 19, 20, 50]. In contrary to the observation that AM251 did not alter the level of anxiety reported in the present study, it has been shown that SR141716A given at the dose of 3.0 mg/kg induced an increase of anxiety-like responses, similarly to the cannabinoid receptors agonist CP-55,940 [3].

The memory-improving effect of AM251 observed in our study is in agreement with the results obtained in experiments performed with antagonist/inverse agonist of CB1 receptors, SR141716A. Wolff and Leander [50] have shown that SR141716A at the dose of 1.0 mg/kg, but not in the higher (3.0 mg/kg) or in lower (0.3 mg/kg) doses, improved memory consolidation in rats in a delayed non-match to sample task, conducted in an eight-arm radial maze. Similarly, Lichtman [26] described an improvement of memory acquisition induced by administration of SR141716A, but likewise in the former study failed to show the enhancement of memory consolidation after post-training SR141716A administration at the dose of 3.0 mg/kg in the radial maze in mice. Pro-cognitive effect of SR141716A was also shown in experiments performed in the elevated T-maze on mice [46]. Administration of 1.0 mg/kg of SR141716A before training, and immediately after it produced an improvement of memory acquisition and consolidation, respectively, evaluated 24 h later, while similarly as in previous studies [50], neither lower (0.5 mg/kg), nor higher (2.0 mg/kg) doses were able to improve the acquisition phase of memory formation. However, Terranova et al. [47] described the improvement of short-term memory in a social recognition task after subcutaneous post-trial application of SR141716A in a wider range of doses (0.1–3.0 mg/kg), both in adult and aged rats.

Presented in our and also in other studies results are in agreement with investigations performed in CB1 receptor knockout mice, in which the enhancement of recognition memory was observed in young [39] and old mice [28]. The improvement of cognitive processes in the CB1 receptor knockout mice was in accordance with exhibited by these mice enhancement of long-term potentiation (LTP) of excitatory synaptic transmission, the most accepted candidate for the neural mechanism underlying learning and memory processes [4].

To explain the putative mechanism of pro-cognitive effect of AM251 some possibilities should be considered.

There is support for the notion, that endogenous cannabinoids act as retrograde neurotransmitters activating CB1 receptors located on presynaptic terminals and leading to suppression of neurotransmitter release [49]. Recent findings suggest that endocannabinoids inhibit acetylcholine release in the neocortex through the activation of CB1 receptors [45]. According to Steffens et al. [45], a decrease of cholinergic neurotransmission may contribute to the memory deficits induced by the cannabinoid agonists, while the blockade of CB1 receptors, by their antagonists such as SR141716A or AM251, may lead to the elevation of

the basal level of acetylcholine in brain areas crucial for the cognitive processes.

Second possibility is connected with agonistic properties of AM251 to recently discovered new orphan receptor GPR55, which activation is followed by cellular calcium mobilization [41, 42]. Therefore, activation of this receptor should be taken into consideration in mechanism of AM251 mediated pro-cognitive effects.

Moreover, because AM251 similarly as SR141716A belongs to diarylpyrazole group and in the same dose as the later compound improves memory, therefore proposed for pro-cognitive activity of SR141716A inverse agonism [26] (an opposite effects to that exerted by agonists) should also be considered in memory-improving effect of AM251.

In conclusion, the main finding of the present study indicates that AM251, at the dose of 1.0 mg/kg, improves recognition memory in rats without alteration of their psychomotor activity and anxiety. However, the explanation of the exact mechanism of pro-cognitive activity of AM251 requires further studies. The memory-improving effect exerted by compounds belonging like AM251 to diarylpyrazole group is promising in therapeutic use of these compounds, especially in patients with cognitive dysfunctions.

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