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Facilitatory effect of AM281 on recognition memory in rats

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ABSTRACT

Background: Our approach was to determine the influence of a single systemic administration of AM281, synthetic cannabinoid structurally similar to SR141716A, on recognition memory in rats.
Methods: To assess the influence of AM281 on acquisition of information the compound was given intraperitoneally once, at the doses of 0.1, 0.5, 1.0 or 2.0 mg/kg, 15 min before learning trial (T1) and in order to evaluate its influence on consolidation process AM281 was given at indicated doses, immediately after T1 trial in an object recognition test. Since cannabinoids may alter motor function and affect anxiety, the influence of AM281 on psychomotor activity and anxiety was evaluated in an open-field and elevated plus maze test, respectively.
Results: Administration of AM281 at the doses: 0.1, 0.5 and 1.0 mg/kg significantly improved acquisition of information, while 0.1 and 0.5 mg/kg of AM281 significantly facilitated consolidation process. Not only did AM281 not affect locomotor and exploratory activity, but also anxiety.
Conclusion: This is the first evidence that AM281 exerts facilitatory effect on recognition memory in rats. This effect seems to be memory specific because no alterations in animals' psychomotor activity and anxiety were observed.

Introduction

Cannabinoid-based medicines have been increasingly arousing interest concerning their potential utility, especially since some beneficial effects in the treatment of the adverse effects of chemotherapy, appetite stimulation in patients with AIDS, neuropathic pain and sleep disturbances in patients with multiple sclerosis have been observed [1]. Moreover, the promising evidence indicated that manipulation of the endocannabinoid system could be of help in treating several mood and mental disorders such as anxiety and depression [2,3], as well as in preventing memory deficit after morphine withdrawal [4] and the reinstatement of nicotine addiction [5].

A group of synthetic compounds such as SR141617A, AM251, AM281 and LY320135 (Fig. 1) with properties of selective CB1 receptor antagonists has been shown to act also as inverse agonists of this receptor. The phenomenon of cannabinoids' inverse agonism, connected with effects that are opposite in direction from those produced by CB1 receptor agonists, has been observed in experiments performed both *in vitro* and *in vivo* [6].

There is support for the notion that activation of presynaptically localized CB1 receptors inhibits the release of neurotransmitters, *e.g.* glutamate [7] and GABA [8] from rat hippocampal slices, noradrenaline from human and guinea-pig hippocampus [9], and acetylcholine from rat brain slices [10]. The changes in the neurotransmitters release affecting neuronal processes and synaptic plasticity were proved to play a critical role in learning and memory processes [11]. Numerous studies have shown that administration of the main biologically active component of marijuana – Δ9-THC or synthetic CB1 receptor agonists such as WIN55,212-2 or CP55,940, exerts deleterious effect on cognitive processes [12,13].

Since the effects of CB1 receptor agonists on neurotransmitter release have a negative impact on neuronal processes engaged in memory formation, one would expect that compounds possessing an inverse agonistic properties may have a beneficial influence on cognitive processes. Such an effect was shown for SR141716A, which exerted memory-improving effect observed in different experimental tasks in rodents [14–16]. In line with this data is our previous study showing the facilitation of acquisition and

Abbreviations: AM281, cannabinoid [N-(morpholin-4-yl)-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide]; AM251, 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; Δ9-THC, Δ9-tetrahydrocannabinol; RI, recognition index; SR141716A, cannabinoid [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride].

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consolidation of recognition memory in rats observed after single systemic application of AM251 – a congener of SR141716A [17]. However, SR141716A, introduced as a promising anti-obesity drug, was withdrawn from the market due to side effects. It has been recently shown that another CB1 receptor antagonist/inverse agonist–AM281 improves recognition loss induced by naloxone in morphine withdrawal mice [4]. Moreover, Seely et al. [18] have shown that AM281 in contrary to SR141716A and AM251 possessing direct antagonistic property at μ-opioid receptors responsible for significant attenuation of morphine analgesia, exerted only a slight effect.

The present study was performed in an attempt to evaluate whether AM281, similarly to SR141716A and AM251, exerts any beneficial effect on recognition memory in rats, based on discrimination between a familiar and a new object presented at 2-h interval. Since cannabinoids are known to produce motor inhibition [19,20] and affect anxiety [21], all that could influence the animals' behaviour in an object recognition test, their psychomotor activity and fear-related behaviour was evaluated in an open field and elevated plus maze, respectively.

Materials and methods

Animals

The experiments were conducted on naïve white male Wistar rats of laboratory strain, weighing 175–190 g. Animals were housed four to plastic cages (50 cm × 40 cm × 20 cm), in the temperature-controlled (22 ± 1 °C) and humidity-controlled (50–60%) room on a 12-h light–dark cycle beginning at 07:00 a.m. Standard laboratory food and tap water were freely available except during tests' period. Experiments were performed between 9:00 am and 1:00 pm., in a sound-isolated room. All experiments were approved by the Local Ethics Commission in Białystok.

Drugs

AM281 [N-(morpholin-4-yl)-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide] (Tocris), dissolved in DMSO solution (Sigma), was given intraperitoneally (*ip*) at a single dose of 0.1, 0.5, 1.0 or 2.0 mg/kg in a volume of 1 ml/kg, according to the experiment setting depicted in Fig. 2. AM281 doses and pre-treatment time was chosen on the basis of our previous study [17] and literature data [14–16,22].

Behavioural tests

Behavioural tests were performed blind to the treatment by two persons–one that injected animals and another one that performed tests without knowledge what they received.

Behavioural tests were recorded on videotapes (mini DV standard) using a digital camcorder. Simultaneously, observer took the measurements during all behavioural experiments manually. After each session the measurements were finally counted and once again the behaviour of each rat was evaluated correspondingly to the videotaped data.

Object recognition test

The procedure was performed according to Ennaceur and Delacour [23], described in details in our previous study [17]. The apparatus, a grey wooden box (65 cm × 45 cm × 45 cm), had a constant illumination of 40 lux. A day before testing, rats were submitted to 5 min long habituation session. The experimental session performed on the next day comprised two trials. In the first, 5-min long learning trial (T1) one object-stimulus, sample A, was presented. During the second, 3-min long testing trial (T2) performed 2 h later (at the time in which injected with saline rats did not remember the familiar object A) a new object (B) was added, and object A was replaced by its duplicate (A′) in order to avoid olfactory traits.

The basic measure was total time spent by the rats on objects' exploration during each trial. From this measure, the following variables were defined: A = exploration time of the sample presented during T1, B = exploration time of new object presented during T2, (B + A′) = exploration time of a duplicate (A′) of the familiar object A and a new object (B) presented 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 (RI) was calculated for each animal and expressed as a ratio: (B × 100)/(B + A′).

Open-field

The apparatus consisted of a wooden box with a square white floor 100 cm × 100 cm divided by eight lines into 25 equal squares and surrounded by a 47 cm high wall, as described earlier [17]. Four wooden bars, 20 cm high, designed as objects of possible interest for the animals, were located in four line crossings in the central area of the floor. The number of crossings, rearings and bar approaches were counted for 5 min. Moreover, the level of anxiety was evaluated by counting the peripheral and central locomotion (crossings of squares adjacent and not adjacent to the walls, respectively), and latency time to leave the central area.

Elevated plus-maze

The procedure was performed according to Pellow et al. [24]. The apparatus consisted of four arms: two open, 50 cm × 10 cm, and two closed arms 50 cm × 10 cm × 40 cm, with an open roof was raised 80 cm above the floor with constant illumination of 75 lux at the level of the apparatus. The arms were arranged such that the two open arms were opposite to each other and connected with the central (neutral) area 10 cm × 10 cm. The rats were placed in the neutral area of the maze, facing one of the open arms. The number of arm entries and the time spent in each

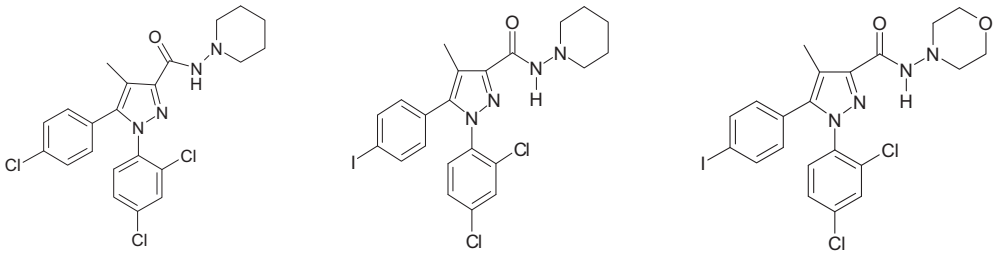

Fig. 1. Structural formulas of synthetic cannabinoids belonging to the group of diarylopyrazoles: SR141716A, AM251 and AM281 [36].