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The CB1 receptor agonist WIN 55,212-2 fails to evoke physical dependence in Swiss mice

WIN 55-212,2 agonista receptora CB1 nie wywołuje fizycznego uzależnienia u myszy szczepu Swiss

INTRODUCTION

Marijuana is the most widely used illicit drug among humans, especially young people. Delta-9-tetrahydrocannabinol (THC) is the primary psychoactive component of Cannabis sativa, although the marijuana plant contains many related cannabinoid compounds [15]. Cannabinoids exert their effects through interaction with specific G protein-coupled cannabinoid (CB) receptors. Two types of CB receptors, CB1 and CB2, were characterized [10]. CB1 receptors are found in the central nervous system (CNS), whereas CB2 receptors are present primarily in the immune system [10]. Anandamide, 2-arachidonyl glycerol and noladin ether are the principle endogenous ligands of these receptors [15]. Moreover, several cannabinoid-like agents have been synthesized (CP 55,940, HU 210, WIN 55,212-2 (55,212-2((R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylm ethanonone mesylate)) [15].

CB1 receptors are expressed presynaptically on GABAergic and glutamatergic neurons, where they modulate GABA or glutamate release and indirectly regulate the release of other neurotransmitters, eg. dopamine in the reward system [14,17]. CB1 receptor agonists are in the class of psychoactive agents showing motivational and reinforcing effects [7]. They are implicated in regulation of some neurobiological processes such as food intake, pain perception, motor coordination and cognitive function e.g. learning. Cannabinoid receptor ligands currently used in medicine in North America or Europe are: HU-211 (Dexanabinol, used to treat endotoxic shock, ischemia, and head trauma), dronabinol (Marinol, an appetite stimulant), nabilone (Cesamet, relief pain and nausea), sativex (multiple sclerosis), rimonabant (Acomplia, has been used to reduce appetite – removed from distribution) [2].

WIN 55,212-2 is a potent cannabinoid receptors' agonist that produces effects similar to those of cannabinoid derivatives such as THC. THC undergoes significant binding to cannabinoid receptors at submicromolar concentrations, with similar affinities for CB1 and CB2 receptors. At CB1 receptors,

it behaves as a partial agonist, the size of its maximal effect in several CB1 receptor-containing systems falling well below that of cannabinoid receptor agonists with higher relative intrinsic activity, such as CP55940 and WIN 55,212-2. The relative intrinsic activity of THC at CB2 receptors is even less than its relative intrinsic activity at CB1 receptors. WIN 55,212-2 displays high affinity for both cannabinoid receptors, with moderate selectivity in favor of the CB2 receptor and exhibits high relative intrinsic activity at both CB1 and CB2 receptors [see review 9,10]. WIN 55,212-2 produces cannabis-like effects in humans, however these effects are described as milder and shorter lasting, when compared to THC [13]. It is related to a rapid decrease in the plasma level of WIN 55,212-2 after abrupt cessation of the chronic treatment [13].

It is well known that chronic use or chronic administration of many drugs of abuse results in the development of physical dependence [3,4]. However, cannabinoids' potential to produce dependence is still a controversial issue. In our study, in order to examine the cannabinoid physical dependence phenomenon, we administered CB1 receptor agonist WIN 55,212-2, twice daily for 5 or 7 days. Afterwards, in order to precipitate the withdrawal signs, mice chronically treated with WIN 55,212-2 received an injection of rimonabant (SR141716A), cannabinoid receptors' antagonist.

MATERIAL AND METHODS

A n i m a l s . The experiments were carried out on male Swiss mice weighing 25–32 g (Farm of Laboratory Animals, Warszawa, Poland) at the beginning of the experiments. The animals were kept under standard laboratory conditions (12/12 – h light/dark cycle) with free access to tap water and lab chow (Bacutil, Motycz, Poland), and adapted to the laboratory conditions for at least one week. Each experimental group consisted of 10–15 animals. The experiments were performed between 8.00 a.m. and 8.00 p.m.

All experiments were carried out according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Council Directive of 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the local ethics committee.

Drugs. The compounds tested were: WIN 55,212-2 ((R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinyl methyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanonone mesylate; Tocris Cookson, Bristol, UK) and rimonabant (SR141716A, 5-(p-chlorophenyl) -1-(2,4- dichlorophenyl) -4-methyl-N-piperidinopyrazole-3-carboxamide hydrochloride, gift of Sanofi–Synthelabo, Montpelier, France). The drugs were suspended in one drop of 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) and diluted in saline. Fresh drug solutions were prepared on each day of experimentation. WIN 55,212-2 was administered intraperitoneally (*i.p.*), rimonabant was administered subcutaneously (*s.c.*) in a volume of 10 ml/kg. Control groups received vehicle injections in the same volume and by the same route.

PROCEDURE

T r e a t m e n t s . WIN 55,212-2 physical dependence was induced by repeated *i.p.* injections, two times daily during 5 or 7 days (8.00 a.m. and 8.00 p.m.). The control groups were treated with the

vehicle following the same schedule. On the test day (day 6 or 8), mice received the morning injection and 1.45 h later they were placed in a circular glass observation area for a 15 min period. At the end of this period, the CB1 cannabinoid receptor antagonist rimonabant (10 mg/kg, *s.c.*) was administered to precipitate the withdrawal syndrome. The somatic signs of withdrawal were evaluated for 15 min, 30 min after rimonabant administration. The frequency of following behavioural signs was counted: wet dog shakes, front paws tremor, jumping and sniffing. General body tremor, chewing, ptosis, piloerection, hunched posture and genital licks were scored: one score point for appearance or 0 for non-appearance of each sign given per period of 5 min.

Body weight changes. During chronic WIN 55-212,2 or vehicle treatment and at the test day (before and 30 min after rimonabant injection) animals were weighed precisely.

Statistical analysis. The data were analyzed by the analysis of variance (ANOVA) followed, when appropriate, by post-hoc comparison using Tukey's test. The confidence limit of $P < 0.05$ was considered as statistically significant. For WIN 55,212-2 withdrawal signs, the results were expressed as the scores and the total number of abstinence signs. The data are expressed as means ± SEM.

RESULTS

In the present experiments we revealed that rimonabant (10 mg/kg), administered to animals chronically treated with cannabinoid agonist – WIN 55,212-2 (0.5, 1 or 2 mg/kg), does not induce withdrawal signs (Tables 1 and 2). The subgroup of WIN 55,212-2- treated animals did not display statistically significant increases in appearance of any withdrawal symptoms, neither before nor after injection of rimonabant on the day 6 and 8 as compared to vehicle-treated groups (Tukey test, $p>0.05$).

Table 1. WIN 55,212-2 withdrawal precipitated by administration of rimonabant on the day 6

	before injection of rimonabant									
	wet dog shakes	jumping	paw tremor	sniffing	body tremor	chewing	ptosis	piloerec-tion	hunched posture	licking
vehicle	0.17±0.17	0.0±0.0	0.0±0.0	16.00±3.06	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.00±0.26	0.67±0.21
WIN 55,212-2 0.5 mg/kg	0.0±0.0	0.32±0.26	0.08±0.08	11.83±1.28	0.17±0.11	0.0±0.0	0.0±0.0	0.0±0.0	0.67±0.31	0.25±0.13
WIN 55,212-2 1 mg/kg	0.11±0.11	0.22±0.22	10.11±0.73	10.11±0.73	0.55±0.17	0.0±0.0	0.11±0.11	0.11±0.11	0.67±0.29	0.11±0.11
WIN 55,212-2 2 mg/kg	0.0±0.0	0.0±0.0	0.21±0.13	7.40±0.58	0.0±0.0	0.0±0.0	0.21±1.33	0.21±1.33	1.00±0.44	0.83±0.29

after injection of rimonabant										
	wet dog shakes	jumping	paw tremor	sniffing	body tremor	chewing	ptosis	piloerection	hunched posture	licking
vehicle	0.0±0.0	0.0±0.0	0.0±0.0	14.33±2.72	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.33±0.33	1.17±0.31
WIN 55,212-2 0.5 mg/kg	0.0±0.0	0.08±0.08	0.08±0.08	6.83±0.37	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.25±0.13	0.58±0.23
WIN 55,212-2 1 mg/kg	0.0±0.0	0.0±0.0	0.33±0.17	6.67±0.37	0.44±0.24	0.11±0.11	0.33±0.23	0.0±0.0	0.44±0.34	0.78±0.22
WIN 55,212-2 2 mg/kg	0.0±0.0	0.0±0.0	0.10±0.10	7.53±2.37	6.33±0.33	0.11±0.11	0.53±0.27	0.11±0.11	0.0±0.0	1.00±0.39

Table 2 WIN 55,212-2 withdrawal precipitated by administration of rimonabant on the day 8

before injection of rimonabant										
	wet dog shakes	jumping	paw tremor	sniffing	body tremor	chewing	ptosis	piloerection	hunched posture	licking
vehicle	0.53±0.22	1.50±0.96	0.67±0.42	2.17±1.04	0.0±0.0	0.0±0.0	0.17±0.17	0.0±0.0	0.67±0.33	0.17±0.17
WIN 55,212-2 1 mg/kg	0.11±0.84	0.0±0.0	1.43±0.48	1.57±0.78	0.28±0.18	0.0±0.0	0.0±0.0	0.0±0.0	1.29±0.47	0.28±0.28
WIN 55,212-2 2 mg/kg	3.71±1.25	2.29±1.54	0.71±0.36	6.14±0.46	0.86±0.46	0.14±0.14	0.0±0.0	0.0±0.0	1.00±0.38	0.71±0.42

after injection of rimonabant										
	wet dog shakes	jumping	paw tremor	sniffing	body tremor	chewing	ptosis	piloerection	hunched posture	licking
vehicle	0.53±0.34	0.0±0.0	0.53±0.34	0.0±0.0	0.33±0.33	0.17±0.17	1.17±0.41	0.33±0.21	0.0±0.0	0.17±0.17
WIN 55,212-2 1 mg/kg	1.86±0.63	0.0±0.0	2.00±0.84	1.43±0.68	0.44±0.24	0.43±0.20	0.0±0.0	0.0±0.0	0.57±0.36	0.57±0.21
WIN 55,212-2 2 mg/kg	0.57±0.33	0.71±0.47	1.43±0.75	1.29±0.61	0.57±0.37	0.0±0.0	0.14±0.14	0.0±0.0	1.43±0.53	0.43±0.33

Moreover, our data do not show any changes in body weight of mice after chronic administration of WIN 55,212-2 (Fig. 1). Two-way ANOVA does not yield a significant difference between WIN 55,212-2-treated animals and vehicle-treated ones during neither 6 days of administration [treatment

effect $F_{3,392}$=0.02, p=0.995; day effect $F_{6,394}$=0.02, p=0.999; treatment x day interaction $F_{18,394}$=0.00, p=1.0] nor after 8 days of administration of WIN 55,212-2 [treatment effect $F_{2,405}$=0.01, p=0.99; day effect $F_{8,405=}$0.24, p=0.983; treatment x day interaction $F_{16,405}$=0.23, p=0.999]. Additionally, the body weight of mice does not change neither before nor after injection of rimonabant on the day 6 and 8 of experiments as compared to vehicle-treated groups (Tukey test, p>0.05) (Fig.1).

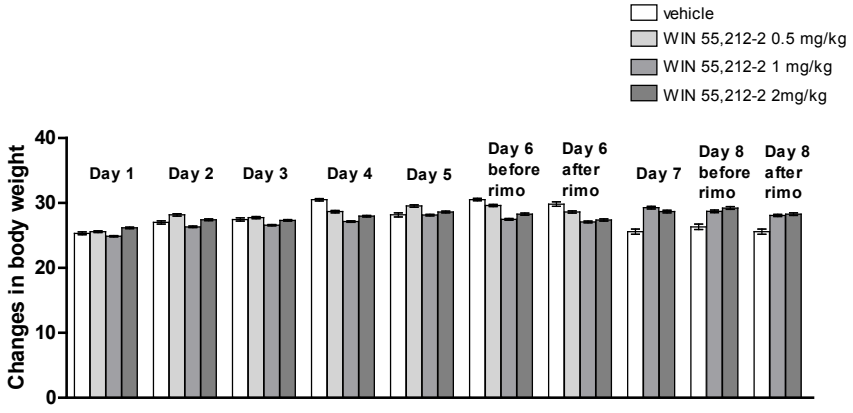

Fig. 1. Changes in body weight (mean±S.E.M., g) of mice during chronic (5 or 7 days, two times/ day) administration of WIN 55,212-2 (0.5, 1 or 2 mg/kg, i.p.) or vehicle and at the test day before and after rimonabant (rimo) administration. *N*= 12-18 mice per group

DISCUSSION

The present study does not show that administration of rimonabant in mice chronically treated with the cannabinoid agonist WIN 55,212-2 (0.5, 1 and 2 mg/kg, 5 or 7 days, twice a day) precipitated several signs that could be interpreted as a withdrawal syndrome. Some studies suggest that the observed signs should include wet dog shakes, jumping, front paw tremor, sniffing, body tremor, chewing, ptosis, piloerection, hunched posture and licking [6]. Moreover, we have not evaluated the changes in body weight of mice neither after chronic treatment of WIN 55,212-2, nor after rimonabant administration.

A large number of psychoactive derivatives have been identified in *Cannabis sativa* preparations and their potential ability to produce physical dependence is still a controversial issue. Physical dependence on THC was demonstrated in humans many years ago [11]. The results of more recent studies on cannabis withdrawal have provided evidence of the existence of physical dependence in heavy/daily cannabis users. The withdrawal syndrome included aggression, irritability, anxiety decrease appetite, restlessness, sleep difficulties, depression mood chills, shakiness, sweating and stomach pain [5,11]. However the withdrawal syndrome in light or nondaily users is still unclear [see review 5].

Two procedures are commonly used to investigate the development of physical dependence and the occurrence of withdrawal syndrome in laboratory animals. The first procedure is based on the

chronic administration of drug and after abrupt cessation of treatment the withdrawal symptoms are assessed (spontaneous withdrawal). In the second procedure, withdrawal signs are precipitated by administration of an appropriate receptor antagonist to animals chronic treated with drug of abuse. Needless to say, the experimental parameters and animal model we used do not mimic human practice.

In reference to the laboratory animals, the degree of physical dependence results from the species used, type of ligand, the dosage and the duration of treatment. Several studies failed to report spontaneous withdrawal symptoms following chronic THC administration in experimental animals (rodents, pigeons, dogs) [16]. The main reason may be pharmacokinetic properties of THC (high lipophilicity and long half-life). On the other hand, only few studies have reported the presence of somatic manifestation of spontaneous withdrawal syndrome after chronic administration of synthetic agonists of CB receptors (e.g. CP 55, 940, WIN 55,212-2) [16] but it was rather mild.

There are few studies in which authors have observed withdrawal reactions induced by administration of CB1 receptor antagonists. The characteristic withdrawal signs have been precipitated in rodents chronically treated with THC or WIN 55,212-2 after administration of SR 141716A [1]. However, the schedule of our studies was based on a publication by Castane et al. [6]. The authors of the aforementioned publication evaluated the development of physical dependence after chronic administration of two doses of WIN 55,212-2 (1 and 2 mg/kg, 5 days, twice a day), and observed the withdrawal signs after injection of rimonabant (wet dog shakes, front paw tremor, sniffing, body tremor, piloerection, genital licks, mastication). Nevertheless, Castane's studies were conducted on male CD1 mice, whereas our experiments were carried out on male Swiss mice. The differences between subspecies of these rodents and laboratory conditions may have contributed to the discrepancy between the described studies.

Concerning the neurobiological mechanisms underlying cannabinoid dependence, the data show that the somatic signs are mediated through the CB1 receptor, since in CB1 knockout mice receiving chronic treatment with THC administration, the CB1 receptor antagonist SR141716A did not precipitate any signs of abstinence [12]. Moreover, it has been reported that cannabinoid withdrawal syndrome may be associated with increase of corticotrophin-releasing factor released in the amygdala, compensatory changes in the cyclic AMP in the cerebellum and the decrease in dopamine release in the nucleus accumbens. These changes may be related to the aversive and dysphoric consequences of cannabinoid withdrawal and intensity of the withdrawal syndrome results from the magnitude of tolerance [8].

CONCLUSION

In our studies we haven't observed any withdrawal signs after chronic administration of WIN 55,212-2, so on the basis of the data already published, we can suppose, that in Swiss mice the cannabinoid tolerance develops very slowly, and physical dependence is hard to achieve. In spite of the large amount of information available so far on cannabinoid dependence in animals, this phenomenon is still an unclear issue and requires more study.

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SUMMARY

Potential ability of cannabinoids to produce dependence is still a controversial issue. In our study, in order to examine the cannabinoid physical dependence phenomenon we administered CB1

receptor agonist WIN55,212-2, afterwards, in order to precipitate the withdrawal signs, chronically WIN 55,212-2 treated mice received an injection of rimonabant – an antagonist of CB1 receptors. The present study does not show that administration of rimonabant in mice chronically treated with the WIN 55,212-2 precipitated a signs of withdrawal syndrome.

Keywords: cannabinoids, WIN 55,212-2, rimonabant, dependence, withdrawal, mice

STRESZCZENIE

Prezentowane badania zostały podjęte w celu poszerzenia wiedzy na temat zależności fizycznej rozwijającej się podczas chronicznego podania kannabinoidów, gdyż zjawisko to pozostaje nadal kontrowersyjne. Myszom przewlekle podawano agonistę receptorów kanabinoidowych WIN 55,212-2, zaś w celu indukowania objawów abstynencyjnych podano rimonabant – antagonistę receptorów CB1. W prezentowanych banianach nie zaobserwowano wystąpienia objawów abstynencyjnych, co świadczy o braku rozwoju zależności fizycznej po przewlekłym podaniu WIN 55,212-2.

Słowa kluczowe: kannabinoidy, WIN 55,212-2, rimonabant, zależność fizyczna, objawy odstawienie, myszy