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European Journal of Pharmacology 454 (2002) 145–151

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Effects of pharmacological manipulations of cannabinoid receptors on severity of dystonia in a genetic model of paroxysmal dyskinesia

Angelika Richter[a,*], Wolfgang Löscher[b]

[a] *Institute of Pharmacology and Toxicology, School of Veterinary Medicine, FU Berlin, Koserstr. 20, 14195 Berlin, Germany*
[b] *Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine Hannover, 30559 Hannover, Germany*

Received 2 May 2002; received in revised form 13 September 2002; accepted 20 September 2002

Abstract

Previous studies have shown beneficial effects of the cannabinoid CB_1/CB_2 receptor agonist (*R*)-4,5-dihydro-2-methyl-4-(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6*H*-pyrrolo [3,2,1-ij]quinolin-6-one mesylate (WIN 55,212-2) in dt^{sz} mutant hamsters, a model of idiopathic paroxysmal dystonia (dyskinesia). To examine the pathophysiological significance of the cannabinergic system in the dystonic syndrome, the effect of the cannabinoid CB_1 receptor antagonist *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR 141716A) on severity of dystonia was investigated in dt^{sz} mutants which exhibit episodes of dystonic and choreoathetotic disturbances in response to mild stress. SR 141716A (5 and 10 mg/kg i.p.) failed to exert any effects on the severity of dystonia. While the antidystonic efficacy of WIN 55,212-2 (5 mg/kg i.p.) was confirmed, cannabidiol (which has low affinity to cannabinoid receptors) tended to delay the progression of dystonia only at a high dose (150 mg/kg i.p.). The antidystonic and cataleptic effects of WIN 55,212-2 (5 mg/kg i.p.) were completely antagonized by pretreatment with SR 141716A at doses of 2.5 mg/kg (catalepsy) and 10 mg/kg (antidystonic efficacy). These data indicate that the antidystonic efficacy of WIN 55,212-2 is selectively mediated via CB_1 receptors. The lack of prodystonic effects of SR 141716A together with only moderate antidystonic effects of WIN 55,212-2 suggests that reduced activation of cannabinoid CB_1 receptors by endocannabinoids is not critically involved in the dystonic syndrome. In view of previous pathophysiological findings in mutant hamsters, the antidystonic efficacy of WIN 55,212-2 can be explained by modulation of different neurotransmitter systems within the basal ganglia.

Keywords: Cannabinoid; Basal ganglia; Dystonia; Movement disorder

1. Introduction

Several important developments have been reported since the discovery of a brain cannabinoid receptor (Matsuda et al., 1990). These include the isolation of anadamide as a putative endogenous ligand for this receptor (Devane et al., 1992), the distinction between central (CB_1) and peripheral (CB_2) cannabinoid receptor subtypes (Munro et al., 1993) and the development of the first selective and potent antagonist of the CB_1 receptor, SR 141716A (Rinaldi-Carmona et al., 1994). The high density of cannabinoid CB_1 receptors within the basal ganglia nuclei is consistent with the pronounced effects of cannabinoids on motor function (Herkenham et al., 1991). Numerous recent studies indicated that the well-known cannabinoid-induced catalepsy in rodents is related to inhibition of γ-aminobutyric acid (GABA) release from striatal terminals at the output nuclei of the basal ganglia (Sañudo-Peña et al., 2000) and to interaction with the dopaminergic system (Cadogan et al., 1997; Kathmann et al., 1999).

Since there is evidence that the striatal endocannabinoid system counteracts dopamine-induced facilitation on motor activity by an inhibitory feedback mechanism, cannabinoid CB_1 receptor antagonists could provide improvement in the medical treatment of Parkinson's disease while cannabinoid CB_1 receptor agonists may reduce dyskinesias (Giuffrida et al., 1999; Lastres-Becker et al., 2001). Can-

* Corresponding author. Tel.: +49-30-838-54317; fax: +49-30-838-532112.
E-mail address: angelika.richter@tiho-hannover.de (A. Richter).

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PII: S0014-2999(02)02477-9

nabinoids have been suggested as potential candidates for the treatment of dystonia, an often intractable syndrome characterized by sustained muscle contractions frequently causing twisting and repetitive movements or abnormal postures (Herkenham et al., 1991; Fahn, 1995). Idiopathic dystonias occur in the absence of lesions within the central nervous system and are thought to be related to dopaminergic dysfunctions (Todd and Perlmutter, 1998). Dystonia is regarded as a basal ganglia disorder, but the pathogenesis of different types of dystonia is not well understood (Fahn et al., 1998). The natural compound of *Cannabis sativa* cannabidiol, which has no substantial effect on cannabinoid CB_1 and CB_2 receptors and undergoes a high degree of nonspecific binding to brain tissue (Pertwee, 1993; Showalter et al., 1996), has been reported to exert beneficial effects in a small number of patients with permanent dystonia (Consroe et al., 1986; Sandyk et al., 1986) and in genetically dystonic rats (Consroe et al., 1988).

Experimental data on the antidystonic efficacy of potent synthetic agonists with high affinity for cannabinoid receptors are obviously still restricted to those in the dt^{sz} mutant hamster (Richter and Löscher, 1994). The dt^{sz} mutant hamster represents one of the few well-characterized animal models of dystonia with high predictive validity (Richter and Löscher, 1998, 2000). This animal model shows the phenomenological characteristics of idiopathic paroxysmal dystonia (dyskinesia), in which episodes of dystonic and choreoathetotic symptoms can be induced by stress (Demirkiran and Jankovic, 1995; Richter and Löscher, 1998). In mutant hamsters, previous studies demonstrated beneficial effects of the potent cannabinoid receptor agonist WIN 55,212-2, an aminoalkylindole with high affinity for CB_1 cannabinoid receptors (Kuster et al., 1993; Showalter et al., 1996), at a relatively high dose of 5 mg/kg (Richter and Löscher, 1994). In contrast to cannabidiol, WIN 55,212-2 shows a low degree of nonspecific binding (Kuster et al., 1993). Since selective cannabinoid receptor antagonists were not available at the time of our initial study in dystonic hamsters (Richter and Löscher, 1994), it remained, however, to be examined whether the antidystonic efficacy of WIN 55,212-2 is actually mediated by the stimulation of cannabinoid CB_1 receptors. The present experiments with the selective cannabinoid CB_1 receptor antagonist SR 141716A were also initiated by recent findings in mutant hamsters, i.e., a reduced basal ganglia output and neurochemical alterations within the striatum (Nobrega et al., 1996; Gernert et al., 2000; Rehders et al., 2000; Richter and Löscher, 2000; Bennay et al., 2001), suggesting that reduced activation of the cannabinoid CB_1 receptor within the basal ganglia by endocannabinoids might contribute to the dystonic syndrome in the hamster model. In view of case reports about beneficial effects of the non-psychoactive compound cannabidiol (see above), examinations of this cannabinoid were included in the present study.

2. Materials and methods

2.1. Animals

The present experiments were carried out in male and female dt^{sz} mutant Syrian golden hamsters which were obtained by selective breeding as described in detail elsewhere (Fredow and Löscher, 1991). The animals were born and kept under the same controlled and constant environmental conditions. All experiments were done in compliance with the German Animal Welfare Act.

2.2. Induction of dystonic attacks and severity-score of dystonia

As reported previously, motor impairments in dt^{sz} hamsters show several features in common with human primary paroxysmal non-kinesigenic dystonia (paroxysmal dystonic choreoathetosis), characterized by long-lasting dystonic attacks (Demirkiran and Jankovic, 1995; Richter and Löscher, 1998). In mutant hamsters, dystonic attacks can be reproducibly induced by a triple stimulation technique (Löscher et al., 1989; Richter and Löscher, 1998), i.e., stressful stimuli consisting of (1) taking the animal from its home cage and placing it on a balance, (2) injection of saline/vehicle (or of drugs), and (3) placement of the animal in a new plastic cage. After this procedure, dt^{sz} hamsters develop a sequence of abnormal movements and postures. Therefore, the severity of dystonia can be rated by following score-system (Löscher et al., 1989; Richter and Löscher, 1998): stage 1, flat body posture; stage 2, facial contortions, rearing with forelimbs crossing, disturbed gait with hyperextended forepaws; stage 3, hyperextended hindlimbs so that the animals appear to walk on tiptoes; stage 4, twisting movements and loss of balance; stage 5, hindlimbs hyperextended caudally; stage 6, immobilisation in a twisted, hunched posture with hind- and forelimbs tonically extended forward. After reaching the individual maximum stage the hamsters recover within 2–5 h. The individual maximum stage of dystonia is usually reached within 3 h after the hamsters were placed in the new cage.

As previously described in detail (e.g., Richter and Löscher, 1998), the dystonic syndrome in dt^{sz} mutants shows an age-dependent time-course. The severity of dystonia reaches a maximum at an age of about 32–42 days (“max-period”, suitable to detect beneficial drug effects). Thereafter, the severity slowly declines. Experiments during the so-called “post-max period” (age: about 45–55 days) allow to determine prodystonic drug effects. Complete remission occurs at an age of about 10 weeks. In the present study, all animals were examined for the presence of dystonia after weaning at the age of 21 days by the triple stimulation procedure three times per week until the animals exhibited constant individual severity scores and latencies to onset of unequivocal dystonic symptoms (stage 2). To obtain reproducible latencies and

avoid onset of dystonia prior or during the triple stimulation technique it was important to keep the time constant and short from taking the animals out of their home cage to placing them in a new cage. Animals that exhibited dystonic symptoms before injections of drug or vehicle were omitted from evaluation.

2.3. Drug treatments

The effects of cannabinoid receptor agonists and antagonists on the severity of dystonia were examined in groups of 5–10 dystonic hamsters. Usually, each group was used for one dose. In cases of repeated testing of drugs (the maximum were two doses), the drug-free interval was 4–5 days. Dystonic attacks were induced by the procedure of triple stimulation, as described above. Since the individual maximum stage of dystonia (score rating system see above) is usually reached within 3 h, the hamsters were observed for 3 h after triple stimulation. For drug testing, control trials were undertaken with the triple stimulation technique, injecting the vehicle (cremophore 10%), and the latencies and severity of the dystonic attacks were noted after placing the animals in the new cage (pre-drug control). Two days later, the drug was administered in the same group of animals and the latency and severity were noted. Furthermore, animals were observed for central adverse effects. In cases of marked reduction of locomotor activity after drug administration, the catalepsy response was determined by quantifying descent latency (in seconds) in a block test in comparison to vehicle control. The hamsters were placed with the forelimbs on a block (6 cm high) and the descent latency, i.e., the time during which the animals maintained this position, was noted. As described for pre-drug-controls, a control trial with vehicle was done 2 days after drug treatment (post-drug control). Hamsters that differed in the maximum severity of dystonia in the pre-drug and post-drug control trials by more than two stages were omitted from the drug evaluation (about 4%). All control and drug trials were done at the same time of the day between 9:00 and 12:00 a.m.

2.4. Drugs

The cannabinoid receptor agonist (*R*)-4,5-dihydro-2-methyl-4-(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6*H*-pyrrolo[3,2,1-ij]quinolin-6-one mesylate (WIN 55,212-2; Biotrend, Cologne, Germany) and the cannabinoid CB_1 receptor antagonist *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR 141716A), kindly provided by Sanofi (Montpellier, France), were dissolved in cremophore EL (10%) prior the experiments. Cannabidiol (Sigma, Deisenhofen, Germany) was suspended in 10% cremophore EL. For all drug administrations, injection volume was 5 ml/kg. For pre- and post-drug control recordings the animals received the same volume of vehicle (i.p.).

2.5. Statistics

The significance of differences (severity of dystonia, latencies to onset of dystonia, adverse effects) between control trials (pre- and post-drug) and drug trial in the same group of animals was calculated by the Wilcoxon signed rank test. The significance of differences between different groups (i.e., effects on dystonia of WIN 55,212-2 administered alone vs. effects after pre-treatment with the antagonist) was evaluated by the Kruskal–Wallis test and, if there was found a significant difference (at least $P < 0.05$), the Mann–Whitney *U*-test was used to analyse which groups differed.

3. Results

As shown in Fig. 1, the selective cannabinoid CB_1 receptor antagonist SR 141716A did not exert any effects on the severity of dystonia at the relatively high doses of 5 and 10 mg/kg (Fig. 1). Particularly, the examinations in mutant hamsters which had passed the age of most marked expression of paroxysmal dystonia (post-max period) demonstrate the lack of prodystonic effects. Furthermore, SR 141716A failed to exert any significant effects on the latency to onset of dystonia (Table 1). Obvious central adverse effects were not induced at the tested doses, but the group of mutant hamsters treated with 10 mg/kg during the max-period appeared to be more aggressive against handling.

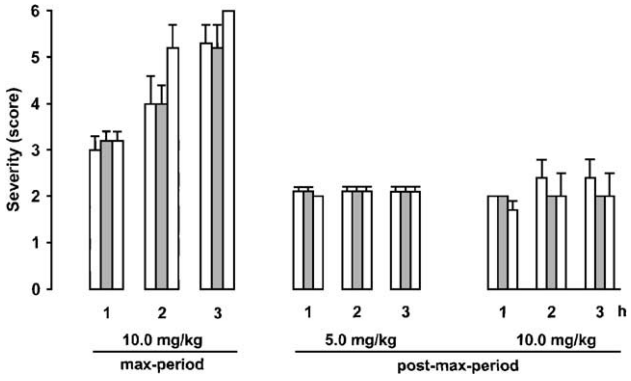

Fig. 1. Effect of the cannabinoid CB_1 receptor antagonist SR 141716A on severity of dystonia after intraperitoneal injections of 5.0 and 10 mg/kg in mutant hamsters at the age of maximum severity of dystonia (max-period; age: 36 days) and in older animals which had passed the age of most marked susceptibility (post-max; age: 49–53 days). Usually, the individual maximum severity of dystonia is reached within 3 h after induction of dystonia by triple stimulation including the i.p. injection of drug (black bars) or vehicle for pre- and post-drug controls (open bars). The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after administration of SR 141716A or vehicle, reflecting the progression of dystonia in dt^{sz} hamsters after treatment with the active compound and control recordings. Control recordings were undertaken 2 days before (pre-drug control) and 2 days after (post-drug control) the drug trial. Data are shown as means + S.E.M. of 6 (10 mg/kg, max-period), 8 (5 mg/kg, post-max) or 9 (10 mg/kg, post-max) dystonic hamsters. Absence of S.E.M. bars indicates that all hamsters had reached the same severity.

Table 1
Effects of the cannabinoid CB_1 receptor antagonist SR141716A and of the cannabinoid receptor agonist cannabidiol on latency to onset of dystonic attacks in genetically dystonic hamsters

Dose (mg/kg)	Latency on (min)			*n*
	Pre-drug	Drug	Post-drug	
SR 141716A				
5.0 (post-max)	13.6 ± 1.9	12.3 ± 2.0	16.3 ± 2.9	8
10.0 (max)	8.7 ± 0.4	8.6 ± 1.6	10.9 ± 2.1	6
10.0 (post-max)	12.6 ± 1.3	13.0 ± 3.5	12.8 ± 0.4	9
Cannabidiol				
50.0 (max)	12.8 ± 1.2	13.0 ± 2.6	10.3 ± 1.5	9
100.0 (max)	6.4 ± 2.5	7.8 ± 1.4	7.0 ± 1.1	5
150.0 (max)	7.9 ± 0.6	10.9 ± 1.7	9.4 ± 1.2	7

The latency to onset of dystonia after injections of WIN 55,212-2 was not evaluated because of preceding injections (of vehicle or SR 141716A). Latency was determined as the time (minutes) to the first unequivocal signs of dystonic attacks (stage 2). Data are shown as means ± S.E.M. of the number of animals indicated (*n*).

In order to examine whether SR 141716A antagonizes the effects of WIN 55,212-2, at first, one group of dt^{sz} hamsters received 5 min after the injection of the vehicle the cannabinoid receptor agonist at a dose of 5 mg/kg, which was previously found to exert antidystonic effects (Richter and Löscher, 1994). Supporting previous data, the cannabinoid receptor agonist WIN 55,212-2 reduced the severity of dystonia during the first, second and third hour after injection (administered 5 min after vehicle injections) in comparison to pre- and post-drug control recordings with two injections of the vehicle at intervals of 5 min (Fig. 2).

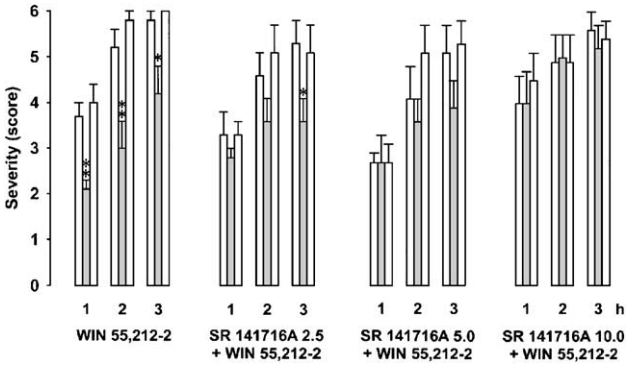

Fig. 2. Effect of the cannabinoid CB_1/CB_2 receptor agonist WIN 55,212-2 (5 mg/kg i.p.) injected 5 min after administration of the vehicle or 5 min after administration of the cannabinoid CB_1 receptor antagonist SR 141716A at a dose of 2.5, 5.0 or 10 mg/kg on severity of dystonia in mutant hamsters at the age of maximum severity of dystonia (max-period; age: 35–40 days). The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after WIN 55,212-2 alone and after pretreatments with SR 141716A. Asterisks indicate significant differences of the severity of dystonia in comparison to the pre- and post-drug control (*$P<0.05$, **$P<0.01$). Data are shown as means + S.E.M. of 10 (WIN 55,212-2 5 min after vehicle injection), 8 (WIN 55,212-2 5 min after injection of 2.5 mg/kg SR 141716A), 7 (WIN 55,212-2 5 min after injection of 5 mg/kg SR 141716A) or 9 (WIN 55,212-2 5 min after injection of 10 mg/kg SR 141716A) mutant hamsters. For further explanation, see Fig. 1 legend.

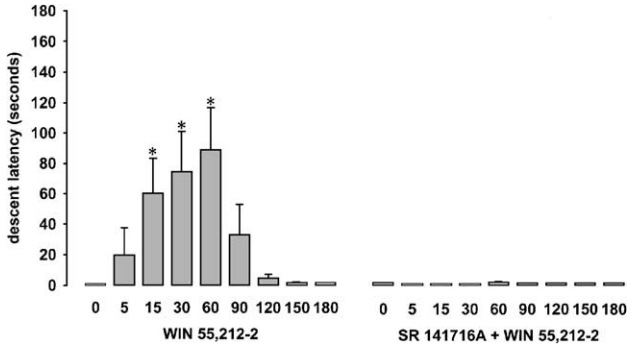

Fig. 3. Catalepsy, measured as descent latency (seconds) in a block test, provoked by the cannabinoid CB_1/CB_2 receptor agonist WIN 55,212-2 at a dose of 5 mg/kg 15 to 60 min after i.p. injection. The catalepsy response was completely prevented by pretreatment with SR 141716A at a dose of 2.5 mg/kg injected i.p. 5 min prior WIN 55,212-2 (5 mg/kg i.p.). Asterisks indicate a significant increase of the descent latency after treatment with WIN 55,212-2 vs. vehicle injections (0 min) (*$P<0.05$). Data are shown as means + S.E.M. of 10 (WIN 55,212-2 5 min after vehicle injection) or 8 (WIN 55,212-2 5 min after injection of 2.5 mg/kg SR 141716A) mutant hamsters. Absence of S.E.M. bars indicates that all hamsters had reached the same descent latency.

Behavioral side effects were loss of spontaneous motor activity and catalepsy, as determined by the descent latency in a block test (Fig. 3). The descent latency was significantly increased 15–60 min after the injection of WIN 55,212-2 in comparison to vehicle control (0 min). Thereafter, the animals showed periods of spontaneous locomotor activity and ataxia.

As shown in Fig. 2, pretreatment with SR 141716A 5 min prior to the injection of 5 mg/kg WIN 55,212-2 counteracted the antidystonic efficacy of the cannabinoid receptor agonist (Kruskal–Wallis, $P<0.05$). The antidystonic efficacy of WIN 55,212-2 was antagonized during the 1 h of observation by all tested doses of SR 141716A (post-hoc, $P<0.05$). The maximum severity reached at the end of the third hour was only counteracted by pretreatment with the highest dose of 10 mg/kg SR 141716A (post-hoc, $P<0.05$). At lower doses, SR 141716A did not significantly counteract the reduction of the maximum severity, possibly because its duration of action was shorter than that of WIN 55,212-2. The catalepsy caused by WIN 55,212-2 was already prevented after the injection of 2.5 mg/kg SR 141716A (Fig. 3; Kruskal–Wallis, $P<0.0001$; post-hoc: 15–60 min, $P<0.01$). Instead of a hypolocomotion observed after single treatments with WIN 55,212-2, the animals appeared to be more active after combined treatment with the antagonist.

Cannabidiol, the natural cannabinoid with low affinity for cannabinoid receptors, did not exert any significant effects on the severity of dystonia and on latency to onset of dystonia at doses of 50, 100 and 150 mg/kg i.p. (Fig. 4, Table 1). At the highest dose of 150 mg/kg, however, cannabidiol tended to delay the progression of dystonia, as indicated by the lower severity score within the first hour

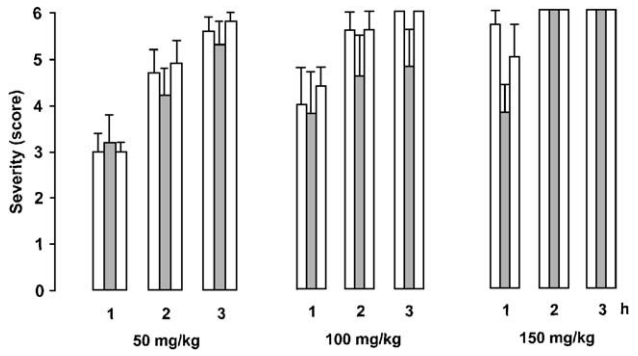

Fig. 4. Effect of the non-psychoactive cannabinoid cannabidiol (50, 100 and 150 mg/kg i.p.) on severity of dystonia in mutant hamsters at the age of maximum severity of dystonia (max-period; age: 35–37 days). The figure shows the average of the maximum individual severity scores of dystonia reached within the first, second and third hour after injections of cannabidiol (black bars) or of vehicle for pre- and post-drug control (open bars). Data are shown as means + S.E.M. of 5 (100 mg/kg), 7 (150 mg/kg) or 9 (50 mg/kg) mutant hamsters. For further explanation, see Fig. 1 legend.

after administration ($P < 0.05$ vs. pre-drug control, but not significant vs. post-drug control). Indeed, the latency to the maximum severity (stage 6) was significantly increased after administration of 150 mg/kg to 74.4 ± 5.5 min ($P < 0.05$) vs. 48.6 ± 4.1 (pre-drug control) and 51.0 ± 8.5 min (post-drug control). Cannabidiol did not cause any observable adverse effects at the tested doses.

4. Discussion

The present data confirm and extend previous finding of antidystonic effects of the cannabinoid receptor agonist WIN 55,212-2 in dt^{sz} mutants (Richter and Löscher, 1994). Since the beneficial effect could be antagonized by pretreatment with the selective cannabinoid CB_1 receptor antagonist SR 141716A, the antidystonic efficacy is mediated by the activation of CB_1 receptors. In contrast to case reports about beneficial effects of cannabidiol at oral doses of 100–200 mg/kg in dystonic patients (Consroe et al., 1986; Sandyk et al., 1986) and a reduction of motor disturbances in *dt* mutant rats at a dose of 80 mg/kg i.p. (Consroe et al., 1988), this natural cannbinoid exerted only moderate effects at a high dose of 150 mg/kg in the hamster model. This finding underlines the importance of CB_1 receptor activation for antidystonic activity, because cannabidiol shows low affinity to cannabinoid receptors and undergoes a high degree of nonspecific binding (Pertwee, 1993; Showalter et al., 1996). In line with recent suggestions that selective cannabinoid CB_1 receptor agonists may be effective against dyskinesias (Giuffrida et al., 1999; Self, 1999), the present data show that the synthetic cannabinoid receptor agonist WIN 55,212-2 is more potent than the non-psychoactive cannabinoid cannabidiol to ameliorate paroxysmal dyskinesia. Central adverse effects may, however, limit the therapeutic potential of potent cannabinoid CB_1 receptor agonists. Furthermore, it should be noted that the antidystonic efficacy of WIN 55,212-2 was not very marked (see below).

The benefit obtained by cannabinoid CB_1 receptor stimulation can be explained on the basis of recent pathophysiological findings in mutant hamsters, which are in line with the current concept of basal ganglia dysfunctions in human dystonias (Richter and Löscher, 2000; Hashimoto, 2000). There is evidence that a deficiency of striatal GABAergic interneurons leads by overactivity of monosynaptic GABAergic projection neurons to reduced entopeduncular activity, i.e., to decreased basal ganglia output in dt^{sz} mutants (Gernert et al., 2000; Bennay et al., 2001). Therefore, cannabinoid CB_1 receptor-mediated inhibition of GABA release from striatal terminals at the output nuclei of the basal ganglia (Miller and Walker, 1995; Saňudo-Peňa et al., 1999, 2000) is likely important for the antidystonic activity of WIN 55,212-2. Furthermore, an enhanced striatocortical glutamatergic activity, possibly due to disinhibition via striatal interneurons, and a striatal dopaminergic overactivity seem to contribute to the manifestation of dystonic episodes in mutant hamsters (Nobrega et al., 1996; Rehders et al., 2000; Richter et al., 2002). Interestingly, activation of presynaptic cannabinoid CB_1 receptors can reduce the release of neurotransmitters in different brain regions via inhibition of N-type calcium channels, including the glutamate release from corticostriatal terminals (Gerdemann and Lovinger, 2001; Huang et al., 2001) and the striatal *N*-methyl-D-aspartate (NMDA)-stimulated dopamine release (Kathmann et al., 1999). Otherwise, activation of cannabinoid CB_1 receptors reduces GABAergic inhibitory postsynaptic currents in striatal projection neurons probably by presynaptic inhibition of GABA release from terminals of recurrent axons of these neurons themselves, as shown by in vitro experiments (Szabo et al., 1998). WIN 55,212-2 also increases pallidal activity (Miller and Walker, 1998) and inhibits the glutamate release from subthalamic terminals in the basal ganglia output structures, which seems to be related to an increase of locomotor activity after administration of low doses in rodents (Saňudo-Peňa et al., 2000) and could be relevant for the hyperlocomotion observed in mutant hamsters after pretreatment with SR 141617A. The decrease of the GABA release within the striatum and of the glutamate release in basal ganglia output nuclei might be the reasons for the limited beneficial effects of WIN 55,212-2 in the hamster model. However, further studies in dt^{sz} mutants, e.g., microinjections of cannabinoid receptor agonists and antagonists into basal ganglia nuclei, have to clarify the mechanisms of the antidystonic efficacy of cannabinoid CB_1 receptor activation in paroxysmal dystonia.

The effects of WIN 55,212-2 which are probably important for the antidystonic activity (see above) can be blocked by SR 141716A, but this cannabinoid CB_1 antag-

onist does not produce an effect on its own (Kathmann et al., 1999; Gerdemann and Lovinger, 2001). This can explain the present results, i.e., the antagonism of antidystonic and cataleptic effects of WIN 55,212-2 by pretreatment with SR 141716A, but that this cannabinoid CB_1 receptor antagonist did not aggravate dyskinesia by itself. The latter finding together with recent data (Gernert et al., 2000) argues against a pathophysiological role of the endocannabinoid system in paroxysmal dystonia.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (Lo275/4-3, Ri 845/1-2).We thank Mrs. C. Bartling for her technical assistance.

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