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The cannabinoid receptor agonist WIN 55,212-2 attenuates the effects induced by quinolinic acid in the rat striatum

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Abstract

The ability of CB_1 receptors to regulate the release of glutamate in the striatum, together with the finding that, in experimental models of Huntington disease (HD), both endocannabinoid levels and CB_1 receptor densities are reduced, has prompted the investigation on the neuroprotective role of the cannabinoids in HD. Quinolinic acid (QA) is an excitotoxin that, when injected in the rat striatum reproduces many features of HD and that acts by stimulating glutamate outflow. The aim of the present study was to test the ability of the cannabinoid receptor agonist WIN 55,212-2 to prevent the effects induced by QA in the rat striatum. In microdialysis experiments, probe perfusion with WIN 55,212-2 significantly and dose-dependently prevented the increase in extracellular glutamate induced by QA. In electrophysiological recordings in corticostriatal slices, the application of WIN 55,212-2 prevented QA-induced reduction of the field potential amplitude. Both effects of WIN 55,212-2 were prevented by the CB_1 receptor antagonist AM 251. In *in vivo* experiments, intrastriatal WIN 55,212-2 significantly attenuated the striatal damage induced by QA, although no significant effects were observed on a behavioural ground.

These data demonstrate that the stimulation of CB_1 receptors might lead to neuroprotective effects against excitotoxic striatal toxicity.

Keywords: Cannabinoids; WIN 55,212-2; Quinolinic acid; Excitotoxicity; Striatum

1. Introduction

In the central nervous system, the physiological actions of cannabinoids are mediated mainly by type 1 cannabinoid (CB_1) receptors (Pertwee, 1997; Freund et al., 2003). CB_1 receptors are densely expressed in the basal ganglia (Herkenham et al., 1990, 1991; Mailleux and Vanderhaeghen, 1992), where they regulate glutamatergic neurotransmission under both physiological and pathological conditions (see Romero et al., 2002, for review). The ability of CB_1 receptors to inhibit the synaptic release of glutamate in the striatum has been consistently reported (Gerdeman and Lovinger, 2001; Huang et al., 2001). A recent report, which demonstrates the localization of striatal CB_1 receptors in the presynaptic active zone (Kofalvi et al., 2005) has provided an anatomical basis for the inhibitory effects of CB_1 receptor agonists on corticostriatal glutamate release.

Since excitotoxic mechanisms are involved in the pathogenesis of several neurodegenerative diseases (Alexi et al., 2000), the ability of CB_1 receptors to downregulate excitotoxic

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glutamate release might be of therapeutic interest. In particular, the finding that in Huntington disease (HD, a devastating and still untreatable neurodegenerative disease) the loss of CB_1 receptor binding in the basal ganglia is one of the earliest neurochemical alterations (Richfield and Herkenham, 1994; Glass et al., 2000), has prompted the investigation of the neuroprotective role of cannabinoids in HD. Interestingly, in transgenic HD mice exposed to an enriched environment, the delayed onset of HD symptoms correlated with a delayed loss of CB_1 receptors (Glass et al., 2004). In models of striatal degeneration induced by mitochondrial toxins in rats, however, the idea that CB_1 receptor agonists could be neuroprotective was not confirmed. Indeed, both Δ^9-tetrahydrocannabinol (Δ^9-THC, the naturally occurring psychoactive constituent of marijuana) and SR141716 (a selective CB_1 receptor antagonist), were found to increase malonate-induced striatal lesions, while a selective CB_1 receptor agonist did not show neuroprotective effects in 3-nitropropionic acid (3-NP)-lesioned rats (Lastres-Becker et al., 2003a, b). Thus, the role of CB_1 receptors in HD and in HD-like striatal degeneration is far from being elucidated, and the ability of CB_1 receptor agonists to exert neuroprotective effects, if any, might depend on the mechanisms responsible for striatal toxicity in a given experimental model.

Quinolinic acid (QA) is an excitotoxin that induces a marked striatal degeneration accompanied by HD-like neurochemical and functional alterations (Beal et al., 1986). In previous studies, we found that QA-induced neurotoxicity depends mainly on the ability of QA to increase extracellular glutamate levels (Popoli et al., 2002, 2003). Such a model may thus be ideal to disclose the potential effects of CB_1 receptor agonists. To the best of our knowledge, however, the possible influence of cannabinoids towards QA-induced effects in the striatum has never been studied.

The aim of the present work was to verify whether CB_1 receptor stimulation prevents QA-induced effects in rats. To this end, the ability of the cannabinoid receptor agonist WIN 55,212-2 to prevent QA-induced increase in extracellular glutamate levels and QA-induced depression of the synaptic response was investigated by means of microdialysis and electrophysiological experiments, respectively. Having found that WIN 55,212-2 significantly prevented QA-induced effects, the potential *in vivo* neuroprotective activity of the cannabinoid receptor agonist was evaluated in QA-lesioned rats.

2. Methods

2.1. Microdialisys experiments

Under Equithesin (3 ml/kg i.p.) anesthesia, naïve Wistar rats (250–280 g) were implanted with a concentric dialysis probe (mod CMA/12, 3 mm length, Carnegie Medicine, Sweden) into the striatum. Stereotaxic coordinates in mm from bregma, sagittal suture and dura, respectively, were as follows: A = + 1.7, L = + 2.7, V = − 6.2. Twenty-four hours after the implantation the dialysis probe was perfused at a rate of 2 μl/min with a Ringer's solution. After a washout period of at least 90 min, samples were collected every 5 min into a refrigerated fraction collector (mod CMA/170) and then frozen until assay. Since the intracerebral injection of QA induces tremors and convulsions in rodents, these experiments were performed under general anesthesia (Equithesin). Each experimental group was made up of 4 animals. Results were expressed as percentage changes of extracellular glutamate levels induced by probe perfusion with QA (5 mM over 30 min) with respect to basal (pre-drug) values (mean of 4–6 samples collected after the induction of general anesthesia). WIN 55,212-2 (5 and 10 μM) or its vehicle (Ringer solution) were directly administered through the dialysis probe over 20 min before and along with QA perfusion. AM251 (0.5 mg/kg) was administered i.p. 30 min before starting WIN 55,212-2 perfusion. At the end of the experiment, each rat was sacrificed with an overdose of Equithesin, the brain was fixed with 4% paraformaldehyde, and coronal sections (20 μm thick) were cut to verify the probe location. The glutamate content of all samples was measured by reverse-phase high performance liquid chromatography coupled to a fluorometric detector (Perkin Elmer LC240), using a 15-min gradient elution program (methanol from 20 to 80% with 50 mM NaH_2PO_4 and CH_3COONa), and automatic precolumn derivatization with ophthalaldehyde and β-mercaptoethanol. Cysteic acid was used as internal standard. The concentration of the standard was linear ($r^2 = 0.99$) between 0.2 to 25 ng/10 μl. Basal glutamate levels were calculated by comparison of sample peak height with external standard peak height, both corrected for the internal standard peak height and expressed as ng/10 μl without probe recovery correction. Data were processed by two-way ANOVA (factors: treatment and time) followed by post hoc Student's *t*-test.

2.2. Electrophysiological experiments

2.2.1. Extracellular recording from corticostriatal slices

Corticostriatal slices were prepared according to the method previously described by Tebano et al. (2002). Adult male Wistar rats (250–280 g) were decapitated under ether anaesthesia, the brain was quickly removed from the skull and coronal slices (300 μm thick), including the neostriatum and the neocortex, cut with a vibratome. Slices were maintained at room temperature (22–24 °C) in an artificial cerebrospinal fluid (ACSF) containing (mM): 126 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 1.3 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$, 11 glucose, pH 7.3, saturated with 95% O_2 and 5% CO_2. After incubation in ACSF for at least 1 h, a single slice was transferred to a submerged recording chamber and continuously superfused with oxigenated ACSF (33–34 °C, flow rate 2.7–3 ml/min).

Extracellular field potentials (FPs) were recorded in the mediodorsal striatum using a glass microelectrode filled with 2 M NaCl solution (pipette resistance 2–5 MΩ) and evoked by a bipolar twisted NiCr-insulated electrode (50 μm o.d.) placed in the white matter between the cortex and the striatum. The electrodes were positioned in depth where the maximal FP amplitude was observed by recording responses to the single stimuli. Stimulus intensity was then reduced to evoke a FP with an amplitude half of the maximum that could be evoked. Stimulus was delivered every 20 s (frequency 0.05 Hz, duration 100 μs) and three consecutive responses were averaged. Signals were acquired with a DAM-80 AC differential amplifier (WPI Instruments, Waltham, MA) and analysed with the "LTP software" (Anderson and Collingridge, 2001). Three consecutive responses were averaged and 10 min of stable baseline recording preceded drug applications.

In order to evaluate the influence of drugs on neurotransmitter release, experiments were performed using a Paired Pulse Stimulation (PPS) protocol, in which two consecutive pulses are applied with an inter-pulse interval of 50 ms. In control conditions, this protocol elicits a condition of Paired Pulse Facilitation (PPF), in which the amplitude of the FP elicited by the second stimulus (R2) is greater than the amplitude elicited by the first stimulus (R1). The degree of PPF is quantified by the R2/R1 ratio and a modification of this ratio is an indication of a presynaptic action on neurotransmitter release (Manabe et al., 1993; Schulz et al., 1994; Calabresi et al., 1997).

In each experiment, the mean basal FP amplitude (i.e. the mean from the values obtained over the 10-min period immediately preceding drug application) was calculated, and the effects of the drugs were expressed as percentage variation with respect to basal values. QA (1 mM) was applied to slices over 10 min. WIN 55,212-2 was applied over 10 min and then co-applied with QA. AM251 (2 μM) was applied 10 min before and along with WIN 55,212-2. The washout period lasted at least 30 min. Wilcoxon signed rank test and Mann–Whitney *U*-test were used for the statistical analysis of the data.

2.3. In vivo studies in QA-lesioned rats

In these experiments, we wanted to verify whether the ability of WIN 55,212-2 to reduce QA-induced increase in extracellular glutamate was paralleled by a neuroprotective activity *in vivo.*

2.3.1. Surgery and treatments

Adult male Wistar rats (250–280 g) were used. The animals were kept under standardized temperature, humidity and lighting condition, with free access to water and food. Animal care and use followed the directives of the Council of the European Communities (86/609/EEC). All efforts were made to minimize animal pain and discomfort. Animals were anaesthetized with Equithesin (3 ml/kg intraperitoneally, i.p.). QA (210 nmol/0.7 μl) or vehicle (0.7 μl PBS) were bilaterally injected in the striatum (coordinates: A = + 1.7; L = ± 2.7; V = − 4.8 mm from bregma and dura). Groups of 7–10 animals each were treated as follows: sham-lesioned animals (intrastriatal, i.s., injection of vehicle; group "veh-veh"); lesioned animals (i.s. QA; in these animals, 4 μl of WIN55,212-2 vehicle were injected 10 min before QA; group "veh-QA"); animals treated with WIN 55,212-2 (1 μg/4 μl, 10 min before QA plus QA (group "WIN-QA"). The dose of WIN 55,212-2 to be used was calculated from the range of concentrations (5–10 μM) effective in microdialysis studies. Indeed, assuming a delivery of 10–20% of the original concentration through the dialysis probe (Battaglia et al., 2001; Cozzi et al., 2002), and considering that WIN 55,212-2 had been administered at the rate of 2 μl/min over 50 min, we estimated that the amount of WIN 55,212-2 actually delivered to the striatum ranged from 0.5 (10% delivery) to 1 (20% delivery) μg.

2.3.2. Behavioural experiments

Two weeks after surgery, rats were subjected to the open-field test to assess motor/exploratory behaviour as well as to the plus-maze test to evaluate anxiety levels. In fact, previous experiments have shown that QA-lesioned rats show alterations in motor/exploratory behaviours when placed in a novel environment, including enhanced rearing frequency and altered responses to novelty. In addition, QA-lesioned rats show behavioural disinhibition when assessed in the plus-maze test, displaying reduced anxiety and risk assessment behaviour (Scattoni et al., 2004).

2.3.2.1. Open-field test. Spontaneous behaviour was videorecorded for 25 min in an open field arena (35 × 35 cm) made of black plexiglas with a light grey bottom subdivided by black lines into 7 × 7 cm squares. A novel object was introduced in the arena after 20 min from the beginning of the observation. Recordings were then scored by an observer blind to the treatments received by each animal, and the following behavioural categories were analysed by "The Observer" (Wageningen, NL) a software package for collection and analysis of observational data: number of crossings (crossing the square limits with both forepaws), frequency of rearing behaviour, and time spent in contact with the novel object.

2.3.2.2. Plus maze test. The elevated plus-maze comprised two open arms and two closed arms that extended from a common central platform. The apparatus was constructed from Plexiglas (black floor, clear walls) and elevated to a height of 60 cm above the floor level. Rats were individually placed on the central platform facing a closed arm and allowed to freely explore the maze for 10 min. Videotapes were scored by a highly trained observer using the above mentioned software. Conventional measures were the frequencies of total, open and closed entries (arm entry = all four paws into an arm), and percentage of time spent in open, closed and central parts of the maze [e.g. (time open/session duration) × 100].

2.3.3. Evaluation of the striatal damage

The striatal damage induced by QA was evaluated as previously described (Popoli et al., 2002, 2004). Briefly, four weeks after the lesion, the animals (QA only, $N = 10$; WIN 55,212-2 + QA, $N = 7$) were decapitated, the brains removed and immediately frozen. For each brain, serial 20-μm coronal sections were cut on a cryostat microtome, stained with Cresyl violet and examined by light microscopy. In QA-lesioned animals, a series of at least five consecutive sections showing a maximal lesion was identified; within this series, the mean amount of ventricular enlargement (ventricular area in μm^2) was measured. Images were captured by a colour digital camera and analysed by the IASS software (Delta Sistemi, Rome, Italy).

3. Results

3.1. WIN 55,212-2 prevents QA-induced increase in extracellular glutamate levels

Mean basal glutamate levels in the striatum were 1.66 ± 0.03 μM. As previously reported, probe perfusion with 5 mM QA significantly increased striatal glutamate outflow (Fig. 1). This effect was reduced and abolished by direct striatal perfusion of 5 and 10 μM WIN 55,212-2, respectively (Fig. 1). Two-way ANOVA showed that both time ($P < 0.0001$) and treatment ($P < 0.0001$) factors, as well as their interaction ($P < 0.001$) were significant. Basal glutamate levels were not affected by WIN 55,212-2. The selective CB_1

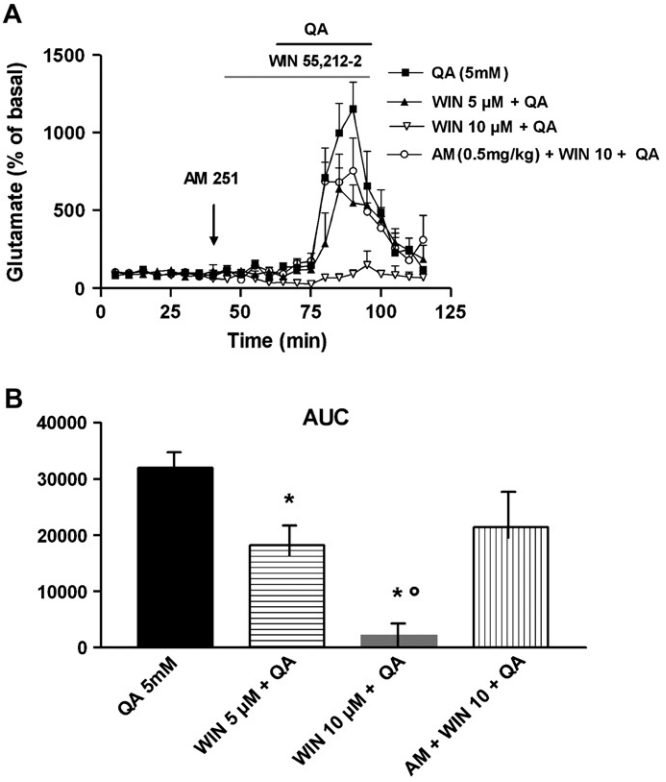

Fig. 1. WIN 55,212-2 prevents quinolinic acid-induced glutamate outflow in the striatum. In rats implanted with a dialysis probe in the striatum, perfusion of 5 mM QA induced a marked increase in the extracellular glutamate levels. Probe perfusion with WIN 55,211-2 (5 and 10 μM) reduced QA-induced glutamate outflow in a concentration dependent way. The effect of WIN 55,211-2 10 μM was completely prevented by pretreatment with the CB_1 receptor antagonist AM 251 (0.3 mg/kg i.p., at the time indicated by the arrow in panel A). The graph in panel A represents the average time-course of changes in extracellular glutamate levels derived from 4 animals/group. Values are expressed as percentage of baseline values (mean ± S.E.M.). The period of probe perfusion is indicated by the horizontal bar. The histograms (B) show the mean (±S.E.M.) AUC values from each group. * = $P < 0.05$ versus QA and ° = versus WIN 5 μM according to two-ways ANOVA and post-hoc Student's test.

receptor antagonist AM251 (0.5 mg/kg i.p., 30 min before starting QA perfusion) prevented the inhibitory effects of WIN 55,212-2 10 μM towards QA (Fig. 1). When administered alone, AM251 did not influence basal or QA-stimulated extracellular levels of glutamate ($N = 4$, not shown).

3.2. WIN 55,212-2 prevents QA-induced FP reduction in corticostriatal slices

In electrophysiological experiments in corticostriatal slices, WIN 55,212-2 (2 μM over 20 min) elicited a significant depression of FP amplitude ($-43.05 \pm 3.32\%$; $N = 5$, $P < 0.05$) which reached $25.47 \pm 1.27\%$ of basal after 30 min of washout ($P = 0.001$ *vs* control) (Fig. 2). In agreement with the known ability of WIN 55,212-2 to inhibit presynaptic neurotransmitter release, WIN-induced reduction of FP amplitude was accompanied by an increase in the magnitude of PPF measured through the R2/R1 ratio (1.21 ± 0.5 and 1.74 ± 0.13 in control conditions and after WIN application, respectively; $N = 5$, $P < 0.03$) (Fig. 2C). Both effects of WIN 55,212-2 (FP depression and increase in the R2/R1 ratio) were completely prevented by the selective CB_1 receptor antagonist AM251 2 μM (Figs. 2B, C). When applied alone, AM251 did not influence synaptic transmission (data not shown).

In order to evaluate the influence of CB_1 receptor stimulation on QA-induced effects, a concentration of WIN 55,212-2 (1 μM) which did not significantly influence on its own the FP amplitude ($-14.30 \pm 7.42\%$, $N = 8$, $P > 0.05$) or the R2/R1 ratio (1.30 ± 0.08 *vs.* 1.25 ± 0.47, $N = 8$, NS, Fig. 3C) was used. Consistently with previous observations from our group (Tebano et al., 2002; Popoli et al., 2004), the application of QA (1 mM over 10 min, $N = 12$) to corticostriatal slices induced a strong reduction of FP amplitude ($-91.28 \pm 2.15\%$), followed by a partial recovery after washout ($-54.1 \pm 6.39\%$; $P < 0.001$ *vs* control, Figs. 3A, B). The effects elicited by QA on corticostriatal slices appeared to depend, at least in part, on the stimulation of presynaptic glutamate release. QA, in fact, reduced the R2/R1 ratio under a protocol of PPS (control: 1.254 ± 0.046, QA: 1.117 ± 0.044, $N = 12$ $P < 0.02$ *vs* control, Fig. 3C). Both QA-induced FP disappearance and R2/R1 reduction were significantly attenuated by WIN 55,212-2 1 μM, a concentration which did not influence the FP on its own (FP amplitude: $-51.79\% \pm 12.37$, $N = 8$, $P < 0.02$ *vs* QA alone; R2/R1 ratio: 1.283 ± 0.041, $N = 8$, $P < 0.02$ vs QA alone, Figs. 3B, C). The inhibitory effects of WIN 55,212-2 were completely blocked by AM251 2 μM ($P < 0.005$ *vs* WIN + QA, $N = 5$; Figs. 3A–C). AM251 alone did not influence QA effects ($N = 3$, not shown). The recovery of the FP response after washout was not significantly influenced by WIN 55,212-2 (mean FP amplitude at the recovery: 45.90 ± 6.39 and $61.91 \pm 10.74\%$ after QA alone and WIN + QA, respectively; $P > 0.05$; $N = 12$ Fig. 3B), probably because of the high lipophilicity of the drug which can not be easily washed out from the slices (Brown et al., 2003; Melis et al., 2004). Indeed, in a separate series of experiments we found that, when WIN 55,212-2 was applied over 20 min (i.e. the same period as in the experiments performed with WIN + QA), and then washed out, the FP amplitude decreased up to $67.19 \pm 7.56\%$ of basal ($N = 4$) 30 min after the washout. Thus, it is very likely that, although

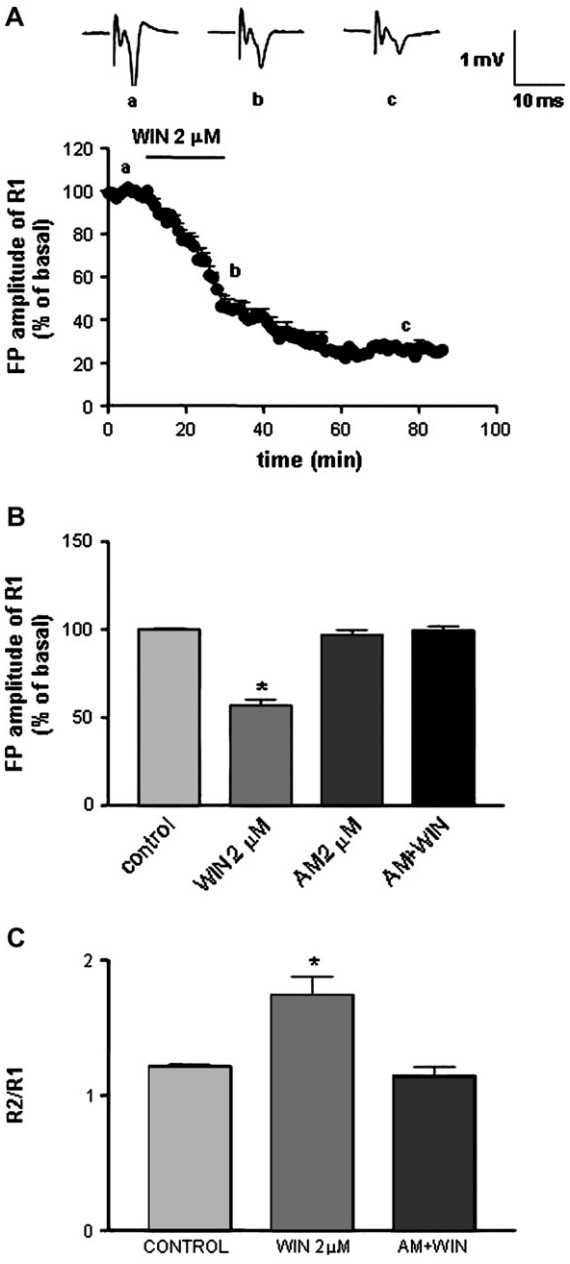

Fig. 2. Effects of WIN 55,212-2 on corticostriatal neurotransmission. Application of 2 μM WIN 55,212-2 induced a strong, long-lasting depression of FP amplitude that did not recover after washing (A). This effect was completely blocked by the selective CB1 receptor antagonist AM251 2 μM (B). The graph in panel A represents the average time-course of changes in FP amplitude derived from 5 experiments. Values are expressed as percentage of baseline values (mean ± S.E.M.). The period of drug application is indicated by the horizontal bar. The histograms (B) show the mean ± S.E.M. of the FP amplitude at the end of the superfusion period. Panel C shows that the WIN-induced reduction of FP amplitude was accompanied by an increase in the magnitude of paired-pulse facilitation ratio (R2/R1) in comparison to control. This effect of WIN 55,212-2 was prevented by 2 μM AM251. The results are expressed as means ± S.E.M. of 5 experiments. * = $P < 0.05$ *versus* baseline (Wilcoxon signed rank test).

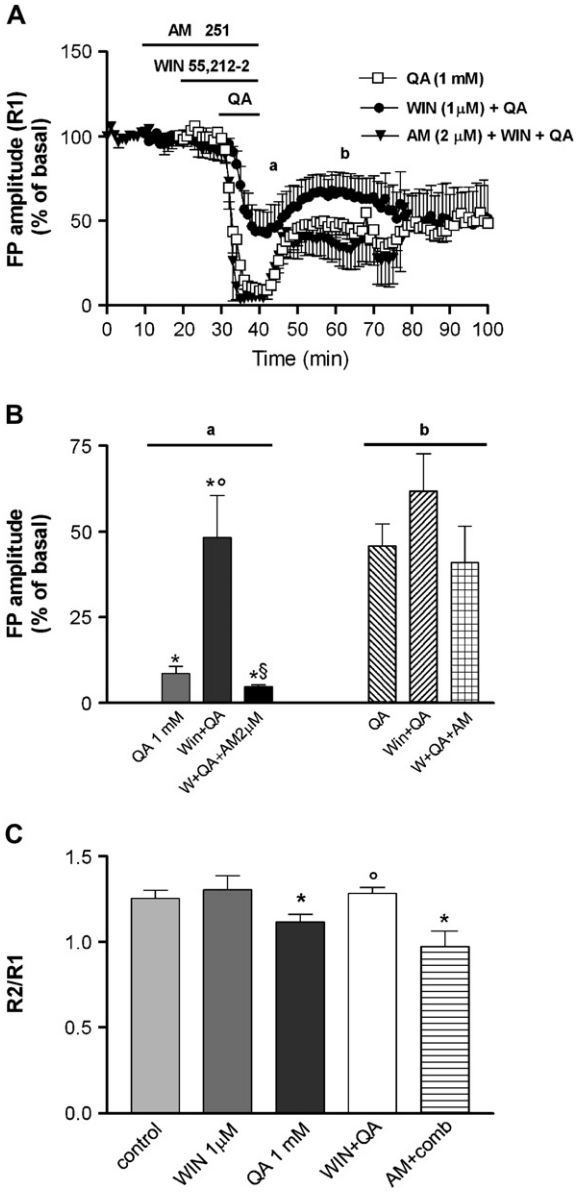

Fig. 3. Influence of WIN 55,212-2 on QA-induced toxicity in corticostriatal slices. Application of 1 mM quinolinic acid (QA) to corticostriatal slices induced a disappearance of the FP which only partially recovered after washout (A and B) Pretreatment with WIN 55,212-2 1 μM attenuated QA-induced FP reduction (Ba) but did not modify the recovery (Bb). The effects of WIN 55,212-2 were completely prevented by 1 μM AM251 (A and B). The graphs in panel A represents the average time-course of changes in FP amplitude; the period of drug application is indicated by the horizontal bars. Values are expressed as percentage of baseline values (mean ± S.E.M.). The histograms in panel B show the mean FP amplitude recorded at the end of drug application (a) and after washout (b). Panel C: under a protocol of PPS, QA reduced the R2/R1 ratio (an index of a facilitation of presynaptic glutamate release). The effect of QA is prevented by 1 μM WIN 55,212-2, while the effect of WIN 55,212-2 is prevented by AM251. The histograms show the mean values of the ratio of the second pulse response to the first pulse response (R2/R1) in control conditions and after the application of QA, WIN 55,212-2 ("WIN"), WIN 55,212-2 + QA ("WIN + QA"), and AM 251 + WIN + QA ("AM + comb"). * = $P < 0.02$ *versus* control (Wilcoxon signed rank test). ° = $P < 0.02$ *versus* QA alone (Mann–Whitney *U*-test). § = $P < 0.005$ versus WIN + QA (Mann–Whitney *U*-test).

WIN 55,212-2 can prevent QA-induced FP reduction, its intrinsic ability to induce a delayed reduction of FP amplitude may mask the former effect.

3.3. In QA-lesioned rats, WIN 55,212-2 does not effectively prevent behavioural abnormalities while showing a slight neuroprotective effect

3.3.1. Behavioural experiments

Locomotor activity assessed by crossing frequency was not significantly affected by QA lesion, and crossing frequency decreased throughout the four 5-min blocks in agreement with a normal motor habituation profile (data not shown). However, QA lesion affected open rearing, a behavior associated with exploration and visual scanning of a novel environment: rearing was significantly increased in veh-QA in comparison to veh-veh rats on sessions 2, 3 and 4 ($P < 0.05$ after post hoc comparisons performed on the interaction treatment × session). Although rats pretreated with WIN 55,212-2 (1 μg i.s.) did not significantly differ from veh-QA, in these animals a normalization of rearing frequency was observed in session 4 (Fig. 4A).

As previously described (Scattoni et al., 2004), a novel object was placed in the center of the arena at the end of the 20-min-open field test and the animals' behaviour observed for 5 min (session 5). The main treatment effect on time spent in object exploration did not reach statistical significance [$F(2,22) = 2.92$, $P = 0.07$]. However, post-hoc comparisons yielded a significant difference between veh-veh and veh-QA rats ($P < 0.05$): QA lesioned rats spent less time in contact with the novel object than did control rats. Animals pretreated with WIN 55,212-2 showed the same reduction in object exploration as did veh-QA group (Fig. 4B).

QA lesion tended to decrease anxiety-related responses in the plus-maze. As shown in Fig. 4C, time spent in the open arms was significantly increased in veh-QA but not in WIN-QA rats in comparison to veh-veh controls.

3.4. Neuropathological analyses

A marked ventricular enlargement was visible in QA-lesioned animals. In animals pretreated with WIN 55,212-2, the mean ventricular enlargement was slightly though significantly attenuated (Fig. 5).

4. Discussion

The main findings of the present study are as follows: i) WIN 55,212-2 prevented QA-induced glutamate outflow and FP reduction in a concentration-dependent way; ii) the effects of WIN 55,212-2 were fully prevented by AM251, thus indicating a selective involvement of CB_1 receptors; and iii) the ability of WIN 55,212-2 to prevent QA-induced increase in extracellular glutamate is associated with a weak neuroprotective effect in QA-lesioned rats.

The ability of WIN 55,212-2 to prevent QA-stimulated glutamate outflow in striatal microdialysis is reported here for the

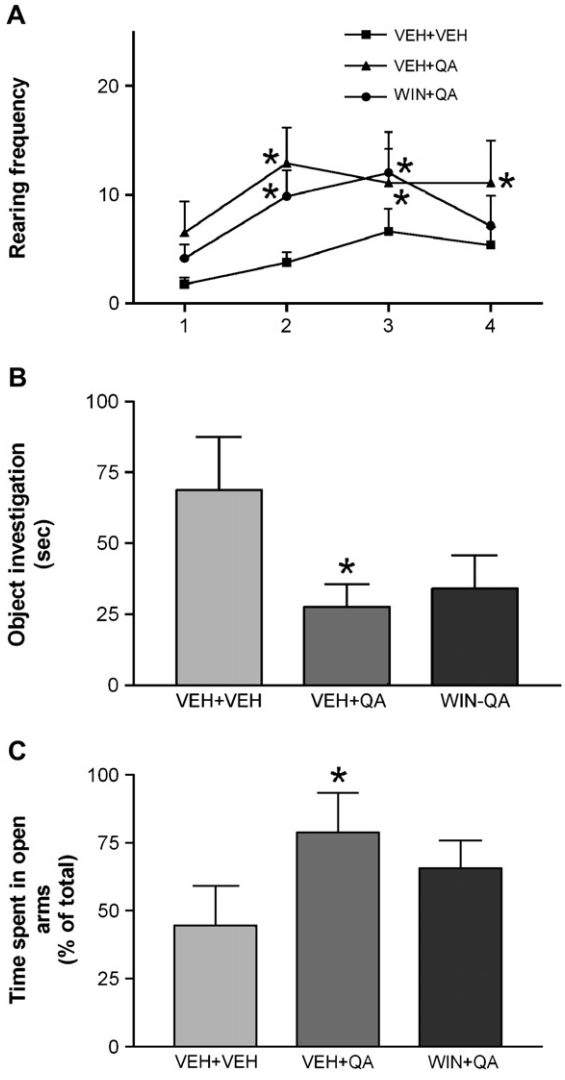

Fig. 4. WIN 55,212-2 does not significantly influence the behavioural abnormalities induced by QA. (A) Rearing frequency throughout the four sessions of a 20 min open-field test, (B) time spent in object investigation during the last session of the open-field, (C) time spent in open arms of a plus-maze by rats receiving bilateral striatal injection of either WIN 55,212-2 (WIN) or vehicle solution (VEH) 10 min before intrastriatal administration of quinolinic acid (QA) or vehicle (VEH). Rats were assessed two weeks after the lesion. n = VEH + VEH = 8; VEH + QA = 10; WIN + QA = 7. Data are means ± SEM. * = $P < 0.05$ versus VEH + VEH controls.

first time. This finding is in line with previous reports showing that CB_1 receptor stimulation reduced the release of glutamate from corticostriatal synapses (Gerdeman and Lovinger, 2001; Huang et al., 2001). At least in the range of concentrations used here, WIN 55,212-2 did not affect basal glutamate levels, its effects becoming evident only in conditions of stimulated outflow. This is in agreement with the findings that Δ^9-THC reduced K^+-stimulated, but not basal, glutamate release in striatal slices (Brown et al., 2003). Moreover, at concentrations comparable to those used here (≈1−2 μM), WIN 55,212-2 did not affect resting glutamate outflow but reduced, through a CB_1-mediated mechanism, evoked glutamate release in rat striatal synaptosomes (Kofalvi et al., 2005). It should be noted, however, that the inhibitory effects of CB_1 receptors on glutamate outflow may not be a general mechanism in the brain, since systemic administration of low doses of WIN 55,212-2 has been reported to increase basal extracellular levels of glutamate in the rat cortex (Ferraro et al., 2001). To add further complexity to the matter, at concentrations higher than those used by us, the CB_1 receptor antagonist AM251 was found to reduce glutamate release as well (Kofalvi et al., 2005). In spite of these contrasting observations, however, our results confirm the widely accepted notion that cannabinoids exert an inhibitory control on corticostriatal glutamate release. Interestingly, cannabinoids can also modulate glutamate outflow through non-CB_1 mechanisms, since they still decreased glutamate release in the hippocampus of CB_1-deficient mice (Hajos et al., 2001; Kofalvi et al., 2003). In our experiments, however, the ability of AM251 to abolish the effects of WIN 55,212-2 clearly suggests the involvement of CB_1 receptors in the modulation of striatal glutamate release. It should be noted, nonetheless, that the block by AM251 might be not a definite index of a CB_1-mediated effect. Indeed, a recent report showed that in CB_1 −/− mice, AM251 decreased the inhibitory effect of WIN 55,212-2 on glutamate release (Kofalvi et al., 2005), thus indicating the possible existence of a non-CB_1, AM251-sensitive cannabinoid receptor. Although the above mentioned findings require caution, it is important to point out that in the striatum of CB_1 −/− mice concentrations of WIN 55,212-2 higher than those effective in the rat striatum or in the mice hippocampus (20 μM) were required to inhibit glutamate release (Kofalvi et al., 2005). Moreover, although WIN 55,212-2 could still stimulate GTP-γS binding in some brain areas from CB_1-deficient mice, this did not apply to the striatum (Breivogel et al., 2001; Monory et al., 2002). Thus, although some important species- and region-related differences seem to exist in the pharmacology of cannabinoid receptors, it is very likely that effects induced in the rat striatum by 1−2 μM WIN 55,212-2 and blocked by AM251 can be considered as CB_1-mediated.

In corticostriatal slices, WIN 55,212-2 reduced the FP amplitude. This effect, which was completely prevented by the CB_1 receptor antagonist AM251, was likely mediated by an inhibition of presynaptic glutamate release, as suggested by the increase in the magnitude of PPF. These findings are fully consistent with previous striatal electrophysiology studies on the effects of Δ^9-THC, WIN 55,212-2 or the CB_1 receptor agonist HU-210 (Gerdeman and Lovinger, 2001; Huang et al., 2001; Brown et al., 2003). In agreement with previous studies from our group (Popoli et al., 2004; Tebano et al., 2002), the strong reduction of the FP amplitude induced by 1 mM QA can be considered as a toxic effect, since the electrical response only partially recovered after washout. Such an effect, although qualitatively similar to that induced by WIN 55,212-2, can not be ascribed to an inhibition of presynaptic glutamate release. Indeed, as suggested by the reduction of the R2/R1 ratio under a protocol of PPS, QA rather increases the release of glutamate. Thus, while the reduction of FP amplitude induced by WIN 55,212-2 may be ascribed to a depression of corticostriatal glutamatergic transmission, a different

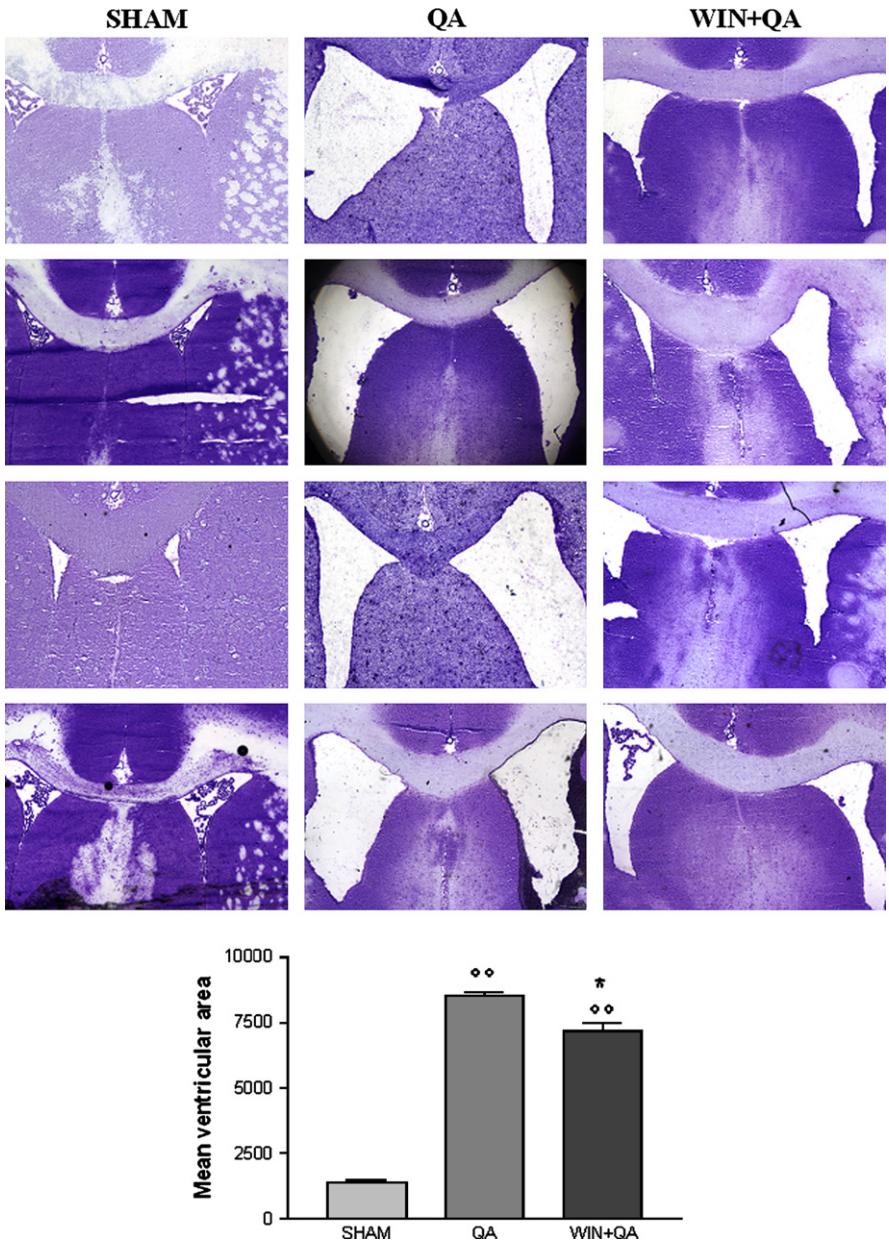

Fig. 5. WIN 55,212-2 significantly reduced the ventricle enlargement in rats lesioned with QA. The representative images reported in the figure show that in rats lesioned with QA, a marked enlargement of lateral ventricles is observed and that the pretreatment with WIN-55,212-2 (1 μg i.s.) slightly though consistently reduced the ventricular enlargement. The histogram shows the mean ventricular area (N = 10 and 7 in QA and WIN + QA groups, respectively). * = $P < 0.05$ versus QA.

mechanism has to be invoked for QA. In this case, the enhanced glutamate levels (most probably achieved by the activation of NMDA receptors, which in turn stimulates presynaptic glutamate release) may reduce the FP amplitude as a consequence of neuronal depolarization. Moreover, and perhaps even more importantly, QA may act by stimulating postsynaptic NMDA receptors, thus leading to neuronal depolarization and consequently loss of the drive for postsynaptic potential. Whatever the mechanism, we found, in nice agreement with the microdialysis results, that WIN 55,212-2 prevented QA-induced effects and that the CB1 receptors are involved in the action of WIN 55,212-2.

The finding that WIN 55,212-2 prevented the effects of QA on glutamate outflow (microdialysis) as well as on the R2/R1 ratio (electrophysiology) confirms the ability of cannabinoids to downregulate excitotoxic glutamate release. Given the key role of excitotoxicity in the pathogenesis of striatal neurodegenerative disorders, the above finding prompted us to verify the possible neuroprotective effects of the CB_1 receptor agonist in a model of striatal excitotoxicity. Specifically, we took advantage of the model of QA-induced striatal damage, in which a chronic and progressive phenotype is thought to be triggered by early excitotoxic events (Popoli et al., 2002, 2004; Scattoni et al., 2004). Although such experiments were performed in a limited number of animals, at the dose used here WIN 55,212-2 significantly, though modestly, reduced the amount of QA-induced ventricular enlargement (an established index of the lesion, see Popoli et al., 2004).

Such a protective activity was however not paralleled by a clear functional effect. Indeed, no significant reduction of QA-induced behavioural effects was observed in rats pretreated with WIN 55,212-2. It would then seem that the anatomical protection provided by WIN 55,212-2 is too modest to have a functional impact. In agreement with this conclusion, in previous experiments aimed at evaluating the neuroprotective activity of other treatments, we found that the ability to prevent QA-induced behavioural abnormalities was paralleled by a reduction of 50% or more of the striatal damage (Popoli et al., 2002, 2004). The apparent discrepancy found between the microdialysis experiments (in which WIN 55-212,2 clearly prevented QA effects) and the studies in lesioned animals (in which the effects of WIN 55-212,2 were modest to absent) might depend on the fact that the dose of QA used to induce a clear striatal degeneration was necessarily higher than that used to acutely stimulate glutamate outflow. In any case, and although further studies with different doses of CB_1 receptor ligands are needed to draw a firm conclusion on the true neuroprotective potential of WIN 55,212-2, the present results do suggest that CB_1 receptor activation may provide protection against striatal excitotoxicity. Whether such results may imply a role of CB_1 receptors in HD remains to be determined in appropriate experimental models. In particular, considering that cannabinoids have shown neuroprotective effects in cells expressing mutated huntingtin (Aiken et al., 2004), and that altered CB_1 gene transcription has been proposed as one of the toxic functions of mutant huntingtin (McCaw et al., 2004), the effects of CB_1 receptor agonists should be evaluated in transgenic HD mice.

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