

Rapid communication

# Cannabinoids inhibit GABAergic synaptic transmission in mice nucleus accumbens

Olivier J. Manzoni\*, Joël Bockaert

CNRS UPR 9023, 141 Rue de la Cardonille, 34094 Montpellier Cedex 05, France

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## Abstract

In mice nucleus accumbens slices, whole-cell patch clamp recording of medium-spiny neurons revealed that cannabimimetics ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone) (WIN-2) and ((-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol) inhibit stimulus-evoked gamma-aminobutyric acid mediated inhibitory post-synaptic currents (IPSC). The actions of WIN-2 were reversed by the selective cannabinoid CB<sub>1</sub> receptor antagonist [*N*-piperidin-1-yl]-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboximide hydrochloride] (SR141716A). WIN-2 modified paired-pulse ratio of evoked IPSCs and decreased miniature IPSC frequency indicating a presynaptic localization of cannabinoid CB<sub>1</sub> receptors. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Nucleus accumbens; GABA ( $\gamma$ -aminobutyric acid); Cannabinoid CB<sub>1</sub> receptor

The nucleus accumbens is involved in the mechanisms at the origin of the rewarding properties of most abused drugs (Pennartz et al., 1994). Morphological studies have described the expression of cannabinoid CB<sub>1</sub> receptors in the nucleus accumbens (Tsou et al., 1998; Ameri, 1999). The physiological roles of cannabinoid CB<sub>1</sub> receptors in the nucleus accumbens are still unknown. We directly tested the presence of cannabinoid CB<sub>1</sub> receptors at the inhibitory synapses in the nucleus accumbens in a brain slice preparation.

The preparation of parasagittal slices of mouse (male C57BL6, 4–6 weeks) nucleus accumbens and the methodology for whole cell patch clamp recordings of evoked and spontaneous IPSC have previously been detailed (Manzoni et al., 1998; Bonci and Williams, 1997). Experiments were done at room temperature and slices were superfused (2 ml/min) with (in mM): 126 NaCl, 2.5 KCl, 1.2 MgCl<sub>2</sub>, 2.4 CaCl<sub>2</sub>, 18 NaHCO<sub>3</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 11 Glucose and 1 kynurenatate to block glutamate receptors, equilibrated with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Whole cell electrodes contained the (mM): CsGluconate 128, NaCl 20, MgCl<sub>2</sub> 1, EGTA 1,

CaCl<sub>2</sub> 0.3, Mg-ATP 2, GTP 0.3, cAMP 0.2 buffered with HEPES 10, pH 7.3. All values are given as mean  $\pm$  S.E.M. Statistical analyses were done with the Mann–Whitney *U*-test or the Kolmogorov–Smirnov test using Statview (Abacus Concepts, USA), *P* < 0.05 was taken as indicating statistical significance. Drugs used: ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone) (WIN-2), ((-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol) (CP 55940) from Tocris Neuramin (England); tetrodotoxin, kynurenatate from Sigma (France); [*N*-piperidin-1-yl]-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboximide hydrochloride] (SR 141716A) was generously provided by Sanofi-Recherche (Montpellier, France).

We found that cannabinoid CB<sub>1</sub> receptor agonists strongly inhibited evoked IPSCs recorded in the core of the nucleus accumbens. Bath-applied WIN-2 (10  $\mu$ M strongly reduced evoked IPSCs (*n* = 4, Fig. 1A). The WIN-2-induced inhibition was prevented by preincubation with the selective cannabinoid CB<sub>1</sub> receptor antagonist (SR141716A) (Fig. 1B) (Rinaldi-Carmona et al., 1994), which by itself had no effect on the IPSCs (not shown). The cannabinoid CB<sub>1</sub> receptor agonist CP 55940 (2  $\mu$ M) which is not structurally related to WIN-2, also reduced evoked IPSCs (Fig. 1C). The variation of the paired-pulse

\* Corresponding author. Tel.: +33-467-14-29-83; fax: +33-467-54-24-32.

E-mail address: manzoni@cciep.montp.inserm.fr (O.J. Manzoni).

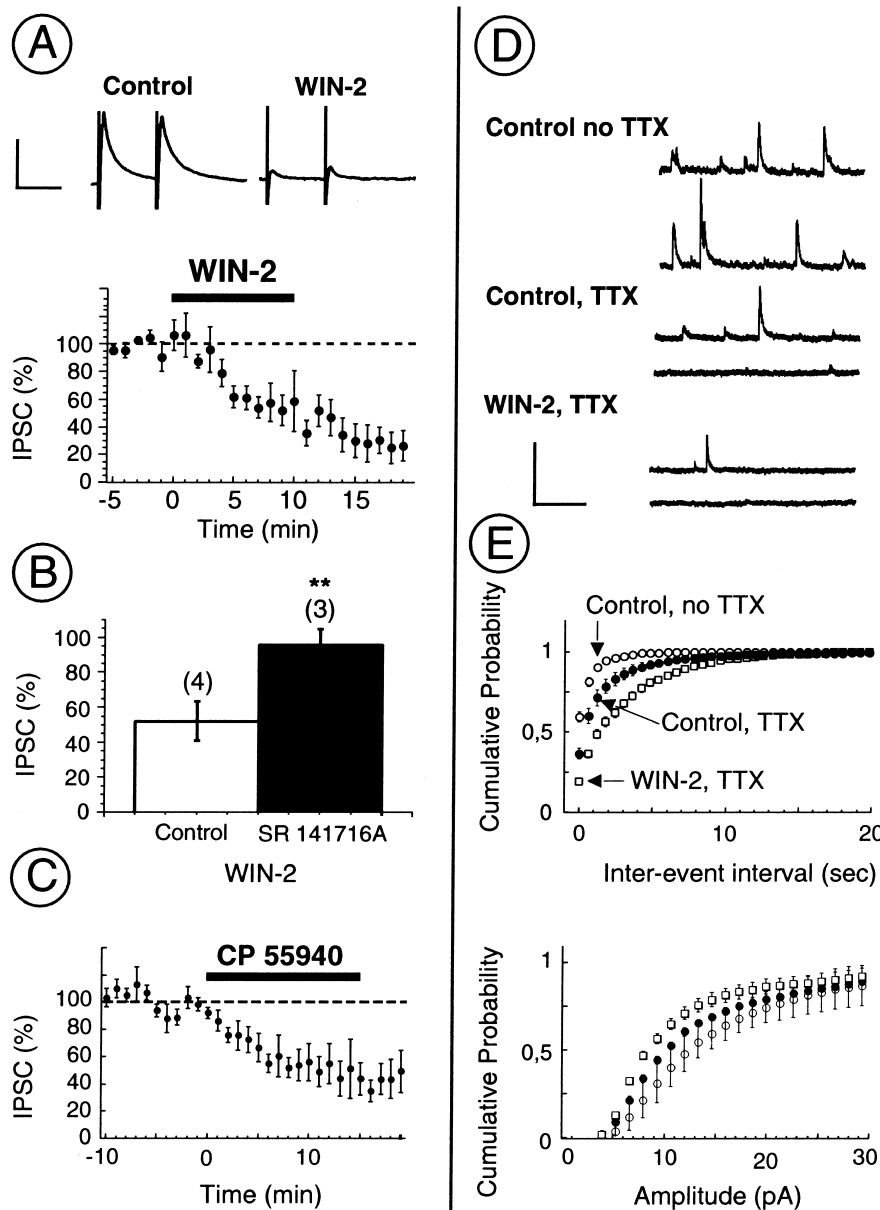


Fig. 1. Cannabinoid CB<sub>1</sub> receptor-mediated inhibition of evoked and spontaneous inhibitory GABAergic synaptic transmission in mice nucleus accumbens. (A) 10  $\mu$ M of the cannabimimetic WIN 55,212,2 reduced evoked IPSCs on average to  $30.3 \pm 9.8\%$  of its basal value ( $n = 4$ ). Scale bar: horizontal 100 ms, vertical 100 pA. Upper graph: traces represent averages of 3–5 consecutive IPSC from a typical experiment included in the averaged data. Lower graph: summary of the time-course of the depressing action of bath-applied WIN-2 in the four cells recorded. Each point is expressed as the percentage of inhibition of basal evoked transmission and the error bar represents the S.E.M. (B) Preincubation of the slices with 10  $\mu$ M of the selective cannabinoid CB<sub>1</sub> receptor antagonist SR 141716A (10  $\mu$ M), was without effect on basal synaptic transmission (not shown) but completely prevented the WIN-2 induced inhibition ( $n = 3$ ,  $P < 0.01$  Mann-Whitney  $U$ -test). (C) The cannabinoid agonist CP 55940 (2  $\mu$ M,  $n = 4$ ), which is not structurally related to WIN-2, also clearly inhibited evoked IPSCs. (D) Raw traces illustrating miniature IPSCs and the effect of tetrodotoxin and WIN-2 plus tetrodotoxin. Scale bar: horizontal 500 ms, vertical 100 pA. (E) Upper graph: the inter-event interval distribution curve was significantly shifted to the right when action potentials dependent gamma-aminobutyric acid (GABA) release was blocked by tetrodotoxin (Kolmogorov–Smirnov test,  $P < 0.0001$ ,  $n = 4$ ). The distribution curve was further displaced in presence of WIN-2 (10  $\mu$ M, Kolmogorov–Smirnov test,  $P < 0.0001$ ,  $n = 4$ ). Lower graph: the effect of tetrodotoxin and WIN-2 plus tetrodotoxin on the amplitude distribution curve. The amplitude distribution curve was shifted to the left when action potentials dependent GABA release was blocked by tetrodotoxin (Kolmogorov–Smirnov test,  $P < 0.0061$ ). The distribution curve was further displaced in the presence of WIN-2 (10  $\mu$ M, Kolmogorov–Smirnov test,  $P < 0.0001$ ).

ratio of inhibitory transmission, a phenomenon sensitive to presynaptic manipulations was measured (Manabe et al., 1993). At the peak of the depression induced by 10  $\mu$ M WIN-2, the IPSC was reduced to  $28.2 \pm 13.2\%$  of its

control value while the paired-pulse ratio was up to  $272.2 \pm 88.5\%$  of its control value ( $n = 4$ ), indicating a presynaptic site of action. To confirm a presynaptic localization of cannabinoid CB<sub>1</sub> receptors, the effects of the cannabi-

noid CB<sub>1</sub> receptor agonist on spontaneous miniature IPSCs recorded in the presence of the voltage-dependent Na<sup>+</sup> channel blocker tetrodotoxine (300 nM) were measured. Bath perfusion of WIN-2 caused a marked depression of the miniature IPSC frequency (Fig. 1D). WIN-2 also caused a small but significant modification of the cumulative distribution plot of miniature IPSCs amplitude (Fig. 1D, last graph).

The main finding of this work is that cannabimimetics induce synaptic inhibition in the nucleus accumbens, in agreement with a number of convergent studies showing that cannabinoid CB<sub>1</sub> receptors modulate excitatory and inhibitory transmission in a variety of models (Ameri, 1999). Moreover, the paired-pulse ratio was increased and the miniature IPSC frequency was decreased suggesting that a presynaptic mechanism participates in the inhibitory effects of the cannabinoid CB<sub>1</sub> receptor agonists. The small reduction in the amplitude of spontaneous miniature IPSCs suggests the involvement of postsynaptic cannabinoid CB<sub>1</sub> receptors. In accord with this idea, immunohistochemical analysis has shown the expression of cannabinoid CB<sub>1</sub> receptors in nucleus accumbens cells that “were in the size range and shape typical of medium-sized spiny neurons” (Tsou et al., 1998).

Since the vast majority of cells in the nucleus accumbens are Gamma-aminobutyric acid containing (GABAergic) medium-spiny interconnected by a dense network of recurrent collaterals (Pennartz et al., 1994) and innervated by inhibitory GABAergic interneurons (Koos and Tepper, 1999), the present finding of cannabinoid CB<sub>1</sub> receptors reducing inhibitory synaptic GABAergic currents, suggests a disinhibitory role of cannabinoids in the nucleus accumbens.

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