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Cannabinoid penetration into mouse brain as determined by ex vivo binding

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Abstract

We have used an ex vivo binding assay in the mouse to evaluate the brain penetration of cannabinoid receptor ligands. After intraperitoneal or oral administration, the pharmacological activity linked to the compound was assessed by using by [^{3}H]WIN 55212-2 binding on cerebellar membranes. The brain penetration was high for compounds like methanandamide or Δ^9-tetrahydrocannabinol but poor for synthetic agonists such as (*cis*)-3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-(*trans*)-4-(3-hydroxypropyl)cyclohexanol (CP 55940) or, *R*-(+)-(2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrol[1,2,3-de]-1,4-benzoxazin-6-yl)(1-napthalenyl)methanone monomethanesulfonate (WIN 55212-2). After oral administration the duration of action of Δ^9-tetrahydrocannabinol, methanandamide and WIN 55212-2 is limited and decreased 4 h after administration. The cannabinoid CB_1 receptor antagonist: *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A) exhibited a good brain penetration and a long duration of action.

Keywords: Cannabinoid penetration; Mouse brain

1. Introduction

The natural psychoactive component of the marijuana plant, Δ^9-tetrahydrocannabinol, is known to induce central behavioural effects both in laboratory animals and humans (Martin, 1986; Hollister, 1986). Synthetic agonists such as (*cis*)-3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-(*trans*)-4-(3-hydroxypropyl)cyclohexanol (CP 55940) and, *R*-(+)-(2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrol[1,2,3-de]-1,4-benzoxazin-6-yl)(1-napthalenyl)methanone monomethanesulfonate (WIN 55212-2) induce hypothermia, hypomotility and analgesia in rats and mice. Radioligand binding studies using several compounds including [^{3}H]CP 55940 (Devane et al., 1988) and [^{3}H]WIN 55212-2 (Kuster et al., 1993) have demonstrated that a high affinity receptor for cannabinoids of synthetic or natural origin exists in the brain. Moreover, a cDNA coding for a cannabinoid receptor has been identified and the receptor is present in the central nervous system of rats (Matsuda et al., 1990) and humans (Gérard et al., 1991) (cannabinoid CB_1 receptors). A cDNA for another cannabinoid receptor was isolated from HL60 cells and encodes a protein expressed in immunocompetent cells (cannabinoid CB_2 receptors) (Munro et al., 1993). Both of these proteins are G-protein-coupled receptors, whose activation leads to an inhibition of adenyl cyclase (Howlett and Fleming, 1984). The anatomical distribution of the cannabinoid CB_1 receptor in the rat brain has been characterised by receptor autoradiography (Herkenham et al., 1991) and in situ hybridisation (Matsuda et al., 1993). Cerebellum, hippocampus and basal ganglia exhibit a high density of cannabinoid receptors. Furthermore, an endogenous compound arachidonylethanolamide (anandamide), has recently been isolated and characterised as a ligand of cannabinoid receptors (Devane et al., 1992). In the past, investigations into the central actions of cannabinoids have been hampered by the lack of a potent selective antagonist for the cannabinoid CB_1 receptor., *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride (SR 141716A) was the first compound of this class described (Rinaldi-Carmona et al., 1994). Estimation of brain penetration of cannabinoid receptor agonists and antagonists can be assessed using behavioural paradigms, electrophysiological recordings or

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neurochemical techniques. Brain penetration can also be estimated by measuring the effect of compounds in rat or mouse brain using ex vivo binding methods. This approach has successfully been used for adenosine, angiotensin and cholecystokinin B receptor antagonists (Baumgold et al., 1992; Marshall et al., 1993; Patel et al., 1994; Bertrand et al., 1994). We have thus developed an ex vivo binding assay for estimating the penetration of cannabinoid receptor agonists and antagonists into the mouse brain.

2. Material and methods

The brain penetration of cannabinoid ligands was evaluated following intraperitoneal (i.p.) or oral (p.o.) administration. Male CD1 mice (20–25 g) (Charles River, France) were treated with the compound (prepared as a suspension or a solution in 1% polysorbate 80) at doses ranging from 0.6 to 80 mg/kg. The animals were killed by decapitation at various times after i.p. and p.o. administration. Their brains were quickly removed and the cerebellum was dispersed (1:8, w/v, to avoid excessive dilution of the administered compound) in HEPES–HCl buffer (20 mM, pH 7.4) containing 10 mM $MgCl_2$ and 0.05% fatty acid free bovine serum albumin with a glass–Teflon pestle homogeniser. Aliquots (25 μl) of the cerebellar homogenate were incubated with 0.1 ml of [^{3}H]WIN 55212-2 (final concentration 1 nM) and 0.175 ml of either WIN 55212-2 (final concentration 10 μM) or buffer at 30°C for 60 min (at which time steady state conditions are reached). The samples were then diluted with 4 ml of ice-cold buffer and immediately filtered under vacuum through Whatman GF/B filters, which were rinsed twice with 4 ml of buffer. The radioactivity was counted for each filter in 10 ml of Ready Solv®. Each determination was performed in triplicate. The inhibition of [^{3}H]WIN 55212-2 binding was measured for each dose of compound, and the half-maximal inhibitory dose (ID_{50}) was determined from binding data by linear regression analysis of the relationship between the logarithm of the administered dose and the percentage inhibition observed. In order to evaluate the apparent cerebellar levels and relative cerebellar penetration of the compound, standard curves were constructed by incubating crude brain homogenate from untreated mice with various concentrations of each inhibitor under the same conditions as above.

3. Results

3.1. Determination of the IC_{50}s in vitro

The inhibition of [^{3}H]WIN 55212-2 binding was determined under our experimental conditions by using crude cerebellar homogenates from mouse brain. In all cases, total [^{3}H]WIN 55212-2 binding was about 1299 ± 28 d.p.m. (14.8 fmol/mg of protein). from which $\sim 185 \pm 5$ d.p.m. (2.1 fmol/mg of protein) represented non-specific binding. The inhibition of [^{3}H]WIN 55212-2 binding measured as in the ex vivo binding assay corresponded to IC_{50} values of 0.15 ± 0.07 for WIN 55212-2; 0.50 ± 0.30 for CP 55940; 3.30 ± 0.80 for SR 141716A; 45.00 ± 22.00 for Δ^9-tetrahydrocannabinol; 46.00 ± 6.00 for methanandamide and 321.00 ± 34.27 nM for cannabinol (Fig. 1A).

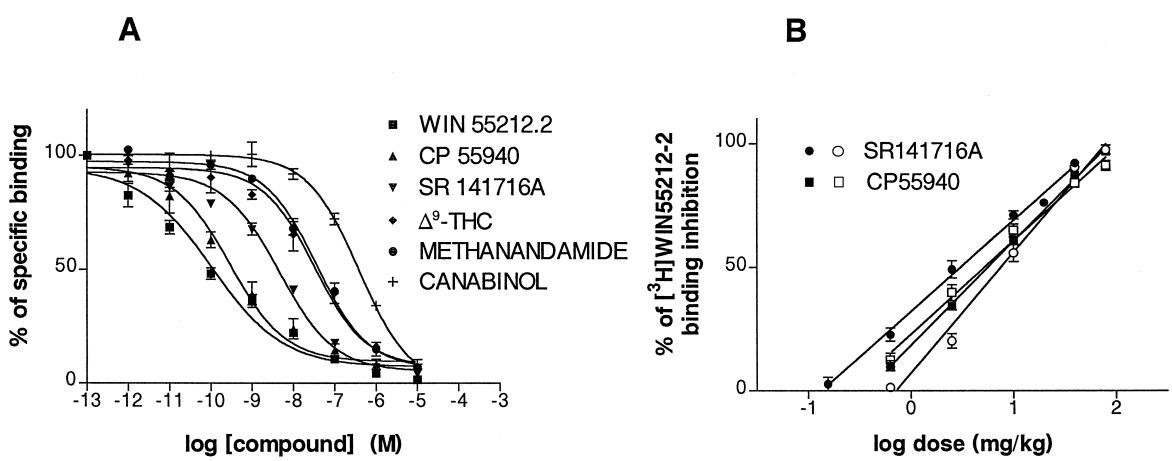

Fig. 1. (A) Inhibition curves determined with cannabinoid receptor ligands using [^{3}H]WIN55212-2 as a ligand and a cerebellar homogenates as described in Section 2. The IC_{50}s obtained are as follows: 0.15 nM for WIN55212-2, 0.50 nM for CP55940, 3.30 nM for SR141716A; 45 nM for Δ^9-THC, 46 nM for methanandamide and 321 nM for cannabinol. Data represent the mean ± S.E.M. from 3 to 6 different experiments each performed in triplicate. (B) For each compound, the inhibition of [^{3}H]WIN55212-2 binding was determined 30 min and 60 min after i.p. (•, ■) and p.o. (◦, □) administration, respectively. Each dose was tested in groups of three mice and the results represent the mean ± S.E.M. of four independent determinations. In each case, the inhibitory effect was well correlated to the logarithm of the dose by linear regression analysis, from which was estimated the half-maximal inhibitory dose (ID_{50}). For SR141716A the ID_{50}s is of 3.0 mg/kg after i.p. administration and 8.3 mg/kg after p.o. administration; for CP55940 the ID_{50}s is of 5.6 mg/kg after i.p. administration and 5.3 mg/kg after p.o. administration.

3.2. Inhibition of [^{3}H]WIN 55212-2 ex vivo binding by cannabinoid receptor agonists and antagonists, kinetic and pharmacological analysis

Five cannabinoid receptor agonists and the cannabinoid receptor antagonist SR 141716A were tested. With the canabinoid receptor agonists a clear hypothermia and a reduction of motility were observed before the sacrifice of the animals. All the drugs, except CP 55940, were more active in inhibiting [^{3}H]WIN 55212-2 binding after i.p. administration than after oral treatment. Nonetheless, for all the compounds, the duration of action following oral treatment was longer than that observed after i.p. treatment. CP 55940 displaces [^{3}H]WIN 55212-2 binding after both i.p. and oral administrations with an ID_{50} around 5 mg/kg, Fig. 1B. Methanandamide exhibited a remarkable inhibitory activity after i.p. ($ID_{50/30\ min} = 3.0$ mg/kg) and oral ($ID_{50/60\ min} = 6.1$ mg/kg) treatments. After intraperitoneal administration the apparent accumulation of cannabinoids in the cerebellum was maximal at 30 min; a rapid decline then occurred and at time 4 h no inhibitory activity was shown with the agonists tested (Table 1). The ID_{50}s obtained with the cannabinoid receptor antagonist SR 141716A were lowest at times 30 and 60 min after i.p. ($ID_{50} = 3.0$ mg/kg) or p.o. ($ID_{50} = 7.0$ mg/kg) administrations (Fig. 1B). After 4 h or 6 h from i.p. or oral treatments, SR 141716A-like pharmacological activity was still observed on [^{3}H]WIN55212-2 binding, suggesting a long duration of action for this compound (Table 1).

The preparation used (cerebellar homogenates) was chosen for its high level of cannabinoid binding sites. For comparison, another ex vivo binding assay was performed by using forebrain homogenates and orally administered CP 55940. An estimated ID_{50} of 5.8 mg/kg was obtained showing that the apparent penetration of CP 55940 into forebrain and cerebellum was similar.

3.3. Inhibition of ex vivo [^{3}H]WIN 55212-2 binding by CP 55940 after transcardiac perfusion

The blood remaining in the brain after the animal was sacrificed could be a source of contamination for binding samples due to the presence of drug in the brain vascular system. Therefore, before removing brains a transcardiac saline perfusion (30 ml) was carried out in order to eliminate this potential problem. The inhibition of ex vivo [^{3}H]WIN 55212-2 binding after oral administration of CP 55940 was evaluated under these conditions. The ID_{50} measured (6.9 mg/kg) did not differ from that obtained without the perfusion step. This result indicates that the level of compound remaining in the blood is likely to be very low.

3.4. Relative brain penetration of cannabinoid receptor agonists and antagonists

CP 55940, a highly potent cannabinoid receptor agonist, is described here as crossing the blood brain barrier poorly with a relative brain penetration of 0.00600-0.0065% after i.p. or p.o. administrations, respectively. Similar amounts of i.p. administered WIN 55212-2 penetrated into the brain (0.0060%) (the results obtained after oral administration were less good: 0.0020%). The natural psychoactive terpene Δ^9-tetrahydrocannabinol exhibited good brain penetration after both i.p. ($ID_{50} = 5.8$ mg/kg) or p.o. ($ID_{50} =$ 35.6 mg/kg) administration. The most potent compound, for penetrating into the brain, is the arachidonylethanolamide derivative methanandamide with 0.55 and

Table 1
ID_{50} values for the inhibition of ex vivo [^{3}H]WIN 55212-2 binding by cannabinoid receptor ligands

A	15 min	30 min	60 min	120 min	240 min
CP 55940	7.9 ± 0.9	5.6 ± 0.5	12.5 ± 0.3	39.9 ± 11.7	> 80
WIN 55212-2	11.0 ± 2.0	4.3 ± 0.7	5.7 ± 1.5	27.7 ± 2.9	56.8 ± 12.9
Δ^9-THC	11.3 ± 1.4	5.8 ± 0.6	11.9 ± 0.6	29.5 ± 5.9	80.2 ± 5.8
Methanandamide	4.5 ± 0.1	3.0 ± 0.2	8.4 ± 0.5	19.8 ± 1.2	> 80
Cannabinol	N.D.	23.5 ± 0.6	N.D.	N.D.	N.D.
SR 141716A	3.7 ± 1.0	3.0 ± 0.5	4.3 ± 0.1	13.5 ± 3.6	71.3 ± 8.1
B	30 min	60 min	120 min	240 min	360 min
CP 55940	7.7 ± 0.9	5.3 ± 1.2	8.9 ± 1.2	22.5 ± 1.4	46.6 ± 0.1
WIN 55212-2	25.6 ± 0.5	14.3 ± 1.6	20.0 ± 1.3	57.0 ± 6.1	> 80
Δ^9-THC	35.6 ± 1.8	20.5 ± 2.4	48.9 ± 2.3	84.8 ± 3.3	> 80
Methanandamide	12.0 ± 0.7	6.1 ± 4.5	21.7 ± 3.3	65.0 ± 5.7	> 80
Cannabinol	N.D.	75.6 ± 2.5	N.D.	N.D.	N.D.
SR 141716A	7.0 ± 1.3	8.3 ± 1.3	11.1 ± 0.1	12.8 ± 1.7	35.2 ± 6.3

The inhibition of ex vivo [^{3}H]WIN 55212-2 binding was determined on mouse cerebellar homogenates as described in Section 2 at different periods of time after i.p. (part A) or p.o. (part B) administrations of the compound tested.
The ID_{50} values (mg/kg) correspond to the means (± S.E.M.) of two to four independent experiments, each performed with groups of three mice per dose and with cerebellum samples assayed in triplicate.
N.D.: not determined.
Δ^9-THC: Δ^9-tetrahydrocannabinol.

Table 2
Relative brain penetration of cannabinoid receptor agonists and antagonist

	i.p. (%)	p.o. (%)
CP 55940	0.0062	0.0065
Δ^9-THC	0.45	0.13
Methanandamide	1.11	0.55
WIN 55212-2	0.0060	0.0022
Cannabinol	0.78	0.24
SR 141716A	0.094	0.034

The evaluation of the apparent cerebellar levels and the relative cerebellar penetration of the compound was estimated by constructing standard curves after incubating crude brain homogenate from untreated mice with various concentrations of each inhibitor (see Section 3).
The inhibition of [^{3}H]WIN 55212-2 binding by compounds administered i.p. (after 30 min) or p.o. (after 60 min) was measured under the same conditions.
The half-maximal inhibitory dose (ID_{50}) was determined by a computerised analysis.
Relative penetration of each drug into the cerebellum was calculated by dividing the theoretical amount present in the cerebellum (as estimated from standard curve for IC_{50} determination) by the total amount of drug administered (corresponding to ID_{50})$\times$100.
Δ^9-THC: Δ^9-tetrahydrocannabinol.

1.11% of orally and intraperitoneally administered methanandamide reaching the mouse brain (Table 2).

4. Discussion

The use of an ex vivo binding assay for examining cannabinoid receptor agonists and antagonist penetration into the brain could be of value for the development of new compounds of therapeutic interest in psychiatry and neurology. Cannabinoid CB_1 receptors are mainly expressed in the brain (Herkenham et al., 1991; Matsuda et al., 1993) with low expression in the periphery. For example, cannabinoid CB_1 receptor mRNA have been found in testis (Gérard et al., 1991) and blood cells (Galiègue et al., 1995). The role of peripheral CB_1 cannabinoid receptors is not clearly understood. Little is known about the effect of cannabinoids on testicular physiology. The inhibition of the peripheral cannabinoid type 1 receptor by SR 141716A has been used to counteract haemorrhagic shock-induced hypotension (Wagner et al., 1997) and this effect is probably mediated by blood monocytes. In peripheral tissues cannabinoid CB_2 receptors are more abundant than cannabinoid CB_1 receptors. Thus, the actions of cannabinoid receptor agonists, after oral or intraperitoneal injection, occurs mostly, first, on cannabinoid CB_2 receptors. Indeed, all the classical cannabinoid receptor agonists recognise cannabinoid CB_1 as well CB_2 receptors, and some of them such as WIN 55212-2 have a slight preference for cannabinoid CB_2 receptors. The latter are mainly present in immunocompetent cells from the spleen and the blood. It is now clear that the immunomodulatory properties of cannabinoids are mediated mainly by cannabinoid CB_2 receptors. Before reaching the brain, a cannabinoid receptor agonist or antagonist will probably interact with peripheral receptors. The importance of this phenomenon on cannabinoid physiological actions is presently unknown but has to be kept in mind. Interestingly enough, SR 14176A is devoid of affinity for cannabinoid CB_2 (Rinaldi-Carmona et al., 1994) receptors and consequently will interact only with peripheral and central cannabinoid CB_1 receptors. The high lipophylicity of natural cannabinoids and derivatives like CP 55940 explains why these compounds were expected to enter the brain easily. We show here, by using an ex vivo [^{3}H]WIN 55212-2 binding assay, that after oral or intraperitoneal administration cannabinoid receptor agonists (Δ^9-tetrahydrocannabinol, CP 55940, WIN 55212-2 and methanandamide) and the cannabinoid receptor antagonist SR 141716A do penetrate into the brain. For all the compounds tested the maximal inhibitory effect is reached 30 and 60 min after i.p. or p.o. treatments, respectively. Four hours after i.p. administration all the compounds were eliminated from the brain while 6 h after oral administration CP 55940- and SR 141716A-like inhibitory activities were still observed. WIN 55212-2 and methanandamide were not active 6 h after oral administration, and Δ^9-tetrahydrocannabinol apparently exhibited the fastest elimination kinetics, being absent from the brain at 4 h after oral administration. These observations may be explained by different pharmacokinetic or metabolic profiles, for the compounds tested.

This study discloses a rapid and reliable measure for brain penetration of cannabinoid receptor ligands using an ex vivo binding technique. Natural psychoactive terpenes and lipidic compounds such as methanandamide are the best brain penetrating molecules when compared to the synthetic cannabinoid receptor agonists and antagonists. As expected, the central activity of these cannabinoids may thus be explained by both the potency of the compounds for binding to the receptor and the ability of the molecule to reach the brain.

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