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ORIGINAL ARTICLE

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Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB_1 receptors

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Abstract We studied whether serotonin release in the CNS is inhibited via cannabinoid receptors. In mouse brain cortex slices preincubated with [^{3}H]serotonin and superfused with medium containing indalpine and metitepine, tritium overflow was evoked either electrically (3 Hz) or by introduction of Ca^{2+} (1.3 mM) into Ca^{2+}-free K^+-rich (25 mM) medium containing tetrodotoxin. The effects of cannabinoid receptor ligands on the electrically evoked tritium overflow from mouse brain cortex slices preincubated with [^{3}H]choline and on the binding of [^{3}H]WIN 55,212–2 and [^{35}S]GTPγS to mouse brain cortex membranes were examined as well.

In superfused mouse cortex membranes preincubated with [^{3}H]serotonin, the electrically evoked tritium overflow was inhibited by the cannabinoid receptor agonist WIN 55,212–2 (maximum effect of 20%, obtained at 1 μM; pEC_{50}=7.11) and this effect was counteracted by the CB_1 receptor antagonist SR 141716 (apparent pA_2=8.02), which did not affect the evoked tritium overflow by itself. The effect of WIN 55,212–2 was not shared by its enantiomer WIN 55,212–3 but was mimicked by another cannabinoid receptor agonist, CP-55,940. WIN 55,212–2 also inhibited the Ca^{2+}-evoked tritium overflow and this effect was antagonized by SR 141716. Concentrations of histamine, prostaglandin E_2 and neuropeptide Y, causing the maximum effect at their respective receptors, inhibited the electrically evoked tritium overflow by 33, 69 and 73%, respectively. WIN 55,212–2 (1 μM) inhibited the electrically evoked tritium overflow from mouse brain cortex slices preincubated with [^{3}H]choline by 49%. [^{3}H]WIN 55,212–2 binding to mouse cortex membranes was inhibited by CP-55,940, SR 141716 and WIN 55,212–2 (pK_i=9.30, 8.70 and 8.19, respectively) but not by the auxiliary drugs indalpine, metitepine and tetrodotoxin (pK_i<4.5). [^{35}S]GTPγS binding was increased by WIN 55,212–2 (maximum effect of 80%, pEC_{50}=6.94) but not affected by WIN 55,212–3.

In conclusion, serotonin release in the mouse brain cortex is inhibited via CB_1 receptors, which may be located presynaptically and are not activated by endogenous cannabinoids. The extent of inhibition is smaller than that obtained (1) via another three presynaptic receptors on serotoninergic neurones and (2) via CB_1 receptors on cholinergic neurones in the same tissue.

Key words Cannabinoid CB_1 receptor · Serotoninergic neurones · 5-Hydroxytryptamine (5-HT) release · [^{3}H]WIN 55,212–2 binding · [^{35}S]GTPγS binding · Presynaptic receptors · Acetylcholine release · Mouse brain cortex

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Introduction

The psychotropic effects of Δ^9-tetrahydrocannabinol and chemically related compounds are mediated via cannabinoid receptors of the CB_1 subtype. These G protein-coupled receptors, which have been cloned (Matsuda et al. 1990), occur at high densities in the CNS and at much lower densities in the periphery; they are positively coupled to some types of voltage-dependent K^+ channels and to mitogen-activated protein kinase and negatively to adenylyl cyclase and some types of voltage-dependent Ca^{2+} channels (for review see Pertwee 1997; Childers and Breivogel 1998; Ameri 1999). Activation of CB_1 receptors inhibits the release of noradrenaline in the human and guinea-pig brain in vitro (Schlicker et al. 1997; Kathmann et al. 1999) and of acetylcholine in the rat brain in vitro (Gifford and Ashby 1996) and in vivo (Gessa et al. 1997). CB_1 receptor-mediated inhibition of GABA release in the rat brain in vitro is suggested by an electrophysiological study (Szabo et al. 1998) and was measured directly in superfusion experiments (Katona et al. 1999). Furthermore, electrophysiological studies on slices or cultured cells of the rat suggest CB_1 receptor-mediated inhibition of gluta-

mate release (Shen et al. 1996; Lévénès et al. 1998; Ameri et al. 1999). With respect to the modulation of dopamine release by cannabinoids, the situation is more complicated; thus, CB_1 receptor-mediated inhibition was found in the rat brain in vitro (Cadogan et al. 1997; Kathmann et al. 1999) whereas a facilitatory effect of cannabinoids on dopamine release was described in most in vivo studies (for review see Gardner and Vorel 1998).

With respect to the modulation of serotonin release by cannabinoids, only few studies are available so far. Johnson et al. (1976) found that high concentrations of Δ^9-tetrahydrocannabinol facilitated basal and K^+-evoked [^{3}H]serotonin release from rat forebrain synaptosomes to the same extent; these data do not allow to decide whether the physiologically relevant process of exocytotic serotonin release is affected. In experiments on ganglia of the medicinal leech *(Hirudo medicinalis*) and of the mussel (*Mytilus edulis*), the endogenous cannabinoid anandamide failed to affect the K^+-evoked serotonin release (Stefano et al. 1997). It was the aim of the present study to examine whether cannabinoids affect the electrically and K^+-evoked [^{3}H]serotonin release in mouse brain cortex slices. Furthermore, we examined the effects of cannabinoids on the electrically evoked tritium overflow from mouse brain cortex slices preincubated with [^{3}H]choline and on the binding of the cannabinoid radioligand [^{3}H]WIN 55,212–2 and of guanosine 5'-[γ-^{35}S]thiotriphosphate ([^{35}S]GTPγS) in mouse brain cortex membranes.

Methods

Superfusion experiments. Cerebral cortex slices (0.3 mm thick, 3 mm diameter) from male NMRI mice were incubated (37°C) for 60 min with physiological salt solution (PSS) containing [^{3}H]serotonin (0.1 or 0.025 μM), or for 30 min with PSS containing [^{3}H]choline (0.1 μM). Subsequently, the slices were transferred to superfusion chambers (one slice per chamber) and superfused (1 ml/min) with PSS (37°C); the superfusate was collected in 5-min samples. In slices preincubated with [^{3}H]serotonin, tritium overflow was evoked by two 2-min periods of stimulation after 40 and 90 min of superfusion (S_1, S_2); experiments lasted for 110 min. Usually, electrical field stimulation (3 Hz, 100 mA, 2 ms) was applied. In a few experiments, slices were superfused with Ca^{2+}-free K^+-rich (25 mM; the concentration of Na^+ was reduced accordingly) medium containing tetrodotoxin (1 μM) and tritium overflow was evoked by introducing Ca^{2+} into Ca^{2+}-free K^+-rich (25 mM) PSS. In slices preincubated with [^{3}H]choline, tritium overflow was evoked after 40 min by one 2-min period of electrical field stimulation (S_1; 3 Hz, 100 mA, 2 ms); experiments lasted for 60 min.

The PSS was composed as follows (mM): NaCl 118, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.3, glucose 11.1, ascorbic acid 0.06, Na_2EDTA 0.03; it was aerated with 95% O_2 and 5% CO_2. In addition, the PSS routinely contained inhibitors of the respective neuronal transporters, i.e. indalpine (in slices preincubated with [^{3}H]serotonin) and hemicholinium-3 (in slices preincubated with [^{3}H]choline), to increase the amount of tritium overflow. In slices preincubated with [^{3}H]serotonin, metitepine, an antagonist at the presynaptic serotonin autoreceptor, was used for the same reason.

Tritium efflux was calculated as the fraction of the tritium content in the slices at the beginning of the respective collection period (fractional rate of tritium efflux). To quantify effects of drugs on basal efflux, the ratio of the fractional rates in the 5-min period prior to S_2 (t_2) and in the 5-min period 15–20 min after the onset of S_1 (t_1) was determined (t_2/t_1). Stimulation-evoked tritium overflow was calculated by subtraction of basal from total efflux during stimulation and the subsequent 13 min and expressed as percent of the tritium present in the slice at the onset of stimulation (basal tritium efflux was assumed to decline linearly from the 5-min period before to that 15–20 min after onset of stimulation). To quantify drug-induced effects on the stimulated tritium overflow, the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). In some instances, t_1 and S_1 (rather than the respective ratios) were used to quantify effects of drugs on basal and evoked overflow (effect of SR 141716; effects of drugs in slices preincubated with [^{3}H]choline). Apparent pA_2 values for SR 141716 for its antagonism against WIN 55,212–2 were calculated according to formula 4 of Furchgott (1972).

Binding experiments. Cerebral cortex from male NMRI mice was homogenized (Potter-Elvehjem) in 25 volumes of ice-cold Tris-HCl buffer (Tris 50 mM, pH 7.5; EDTA 5 mM; sucrose 10.27%) and centrifuged at 1000×g for 10 min (4°C). The supernatant was centrifuged at 35000×g for 10 min and the pellet was resuspended in buffer and frozen at –80°C. The buffer was composed as follows (mM): Tris 50, pH 7.5; EDTA 5 (for binding experiments with [^{3}H]WIN 55,212–2) and Tris 50, pH 7.4; EGTA 0.2; $MgCl_2$ 3; NaCl 100 (for binding experiments with [^{35}S]GTPγS).

Binding experiments with [^{3}H]WIN 55,212–2 were performed in Tris-HCl buffer (Tris 50 mM, pH 7.5; EDTA 5 mM; 1 mg/ml bovine serum albumin) in a final volume of 0.5 ml containing 60–80 μg protein. [^{3}H]WIN 55,212–2 was used at eight concentrations ranging from 0.01 to 10 nM for saturation experiments and at a concentration of 1 nM for competition experiments. The incubation (30°C) was terminated after 90 min by filtration through polyethyleneimine (0.3%)-pretreated Whatman GF/C filters. Non-radioactive WIN 55,212–2 (1 μM) was used to determine non-specific binding (44% of total binding for [^{3}H]WIN 55,212–2, 1 nM). Protein concentration was assayed by the method described by Bradford (1976). Data were analyzed using the programme GraphPadPrism (Prism; GraphPad Software, San Diego, Calif., USA).

Binding experiments with [^{35}S]GTPγS (carried out essentially as described by Selley et al. 1996, with modifications) were performed in Tris-HCl buffer (Tris 50 mM, pH 7.4; EGTA 0.2 mM; $MgCl_2$ 3 mM; NaCl 100 mM; GDP 30 μM; 0.5 mg/ml bovine serum albumin) in a final volume of 0.5 ml containing 3–5 μg protein. [^{35}S]GTPγS was used at a concentration of 0.05 nM. The incubation (30°C) was terminated after 60 min by filtration through Whatman GF/B filters. Non-radioactive GTPγS (10 μM) was used to determine non-specific binding (20% of basal binding).

Statistics. Results are given as means ± SEM of *n* experiments (superfusion experiments) and of *n* experiments in triplicate (binding experiments). Student's *t*-test was used for comparison of mean values; the Bonferroni correction was used when two or more values had to be compared to the same control. The *F*-test was applied in order to evaluate whether the inhibition of [^{3}H]WIN 55,212–2 binding by drugs is better fitted by a one- or two-site model.

Drugs used. (-)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol (CP-55,940; Tocris-Cookson/Biotrend, Cologne, Germany); hemicholinium-3 (ChemCon, Freiburg, Germany); bovine serum albumin, histamine dihydrochloride, prostaglandin E_2 (Sigma, Munich, Germany); indalpine (Rhône-Poulenc, Gennevilliers, France); metitepine maleate (Hoffmann-La Roche, Basel, Switzerland); human neuropeptide Y (gift of Professor A. Buschauer, University of Regensburg, Germany); N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR 141716; Sanofi, Montpellier, France); tetrodotoxin (Roth, Karlsruhe, Germany or ICN, Eschwege, Germany); R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl](1-naphthalenyl) methanone mesylate (WIN 55,212–2), WIN 55,212–3 (S(-)enantiomer of WIN 55,212–2) (RBI/Biotrend, Cologne, Germany); [1,2-^{3}H(N)]-serotonin creatinine sulfate (specific activity 27.5

Ci/mmol), [methyl-^{3}H]-choline chloride (specific activity 75 Ci/mmol), [5,7-naphthyl-^{3}H]-WIN 55,212–2 (specific activity 43 Ci/mmol) (NEN, Zaventem, Belgium); guanosine 5'-[γ-^{35}S]thiotriphosphate, triethylammonium salt ([^{35}S]GTPγS; specific activity 1100 Ci/mmol; Amersham, Braunschweig, Germany). Drugs were dissolved in DMSO (CP-55,940, SR 141716, WIN 55,212–2, WIN 55,212–3), citrate buffer (0.1 mM, pH 4.8; tetrodotoxin) or water (other drugs) and diluted with PSS (release experiments), water (binding experiments with [^{3}H]WIN 55,212–2) and buffer (binding experiments with [^{35}S]GTPγS) to obtain the concentration required.

Results

Release experiments

Mouse brain cortex slices were preincubated with [^{3}H]serotonin at a concentration of 0.1 μM (experiments of Fig. 1A and 3) or 0.025 μM (experiments of Fig. 1B and 2) or with [^{3}H]choline (0.1 μM). Slices preincubated with [^{3}H]serotonin were superfused with medium containing indalpine (10μM) plus metitepine (3.2 μM) unless stated otherwise; tritium overflow was evoked electrically (3 Hz) or by introduction of Ca^{2+} (1.3 mM) into Ca^{2+}-free K^+-rich (25 mM) medium after 40 and 90 min of superfusion (S_1, S_2). Slices preincubated with [^{3}H]choline were superfused with medium containing hemicholinium-3 (10 μM); tritium overflow was evoked electrically (3 Hz) after 40 min of superfusion (S_1).

Tritium efflux was collected from 35 min of superfusion onward; the radioactivity in the 5-min sample collected from 35 to 40 min (i.e. immediately before S_1) amounted to about 2200 and 900 dpm in slices preincubated with [^{3}H]serotonin (0.1 and 0.025 μM, respectively), and to about 1000 dpm in slices preincubated with [^{3}H]choline (0.1 μM). Basal tritium efflux was expressed as t_2/t_1 (range of control values was 0.52–0.75) or t_1 (for control values, see Table 1) and was not affected or only slightly (by not more than 17%) increased by the drugs under study (not shown). The evoked overflow was expressed as S_2/S_1 (for control values, see Fig. 1 and legend to Fig. 2) or S_1 (for control values, see Table 1).

In the first series, mouse brain cortex slices were preincubated with [^{3}H]serotonin and tritium overflow was evoked electrically. The electrically evoked overflow (S_2/S_1) was inhibited by WIN 55,212–2 and CP-55,940 (1 μM each) but not influenced by WIN 55,212–3 (1 μM) (Fig. 1A). The effect of WIN 55,212–2 was abolished by SR 141716 (0.32 μM) (Fig. 1A), which, by itself, did not affect the evoked overflow (S_1) (Table 1).

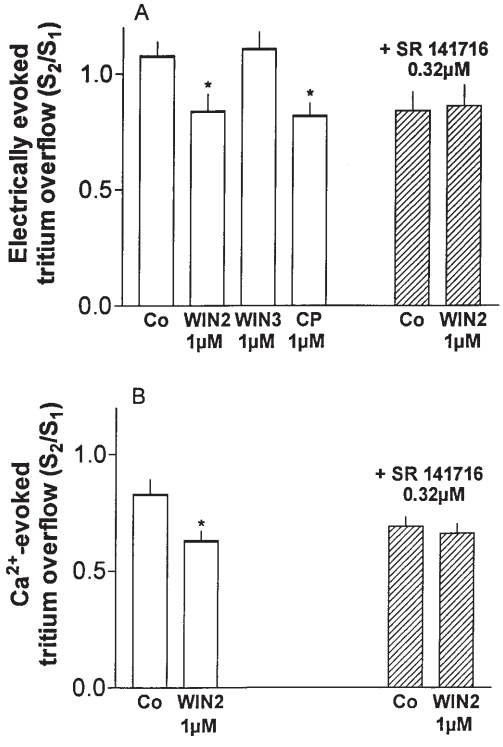

Fig. 1 Effect of WIN 55,212–2 (WIN2), WIN 55,212–3 (WIN3) and CP-55,940 (CP) on the electrically (**A**) and Ca^{2+}-evoked tritium overflow (**B**) from mouse brain cortex slices preincubated with [^{3}H]serotonin, and interaction of WIN 55,212–2 with SR 141716. **A** Slices were preincubated with [^{3}H]serotonin (0.1 μM) and superfused with medium containing indalpine (10 μM), metitepine (3.2 μM) and, when necessary, SR 141716 (0.32 μM) throughout superfusion. The WIN compounds and CP-55,940 were added to the medium from 62 min of superfusion onward. **B** Slices were preincubated with [^{3}H]serotonin (0.025 μM) and superfused with Ca^{2+}-free K^+-rich (25 mM) medium containing indalpine (10 μM), metitepine (3.2 μM) plus tetrodotoxin (1 μM). WIN 55,212–2 and SR 141716 were added to the medium as indicated above. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. *Co* control. Means ± SEM of 4–12 experiments. **P*<0.05

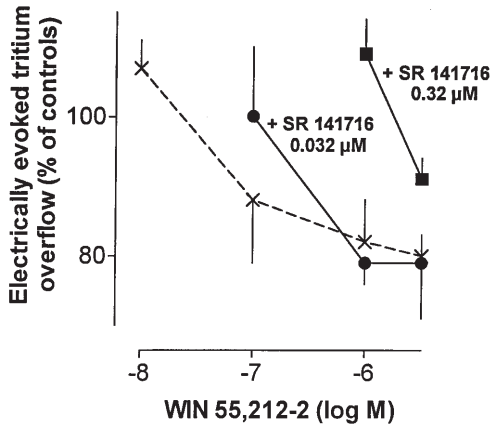

Fig. 2 Effect of WIN 55,212–2 on the electrically evoked tritium overflow from superfused mouse brain cortex slices preincubated with [^{3}H]serotonin (0.025 μM), and interaction of WIN 55,212–2 with SR 141716. The medium contained indalpine (10 μM), metitepine (3.2 μM) and, when necessary, SR 141716, throughout superfusion and WIN 55,212–2 from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). Results are given as percent of the tritium overflow in controls (not shown). The S_2/S_1 (± SEM) values in the three series of controls were 0.74±0.02 (no SR 141716), 0.77±0.04 (SR 141716, 0.032 μM) and 0.72±0.05 (SR 141716, 0.32 μM). Means ± SEM of 5–6 experiments

Table 1 Basal and stimulation-evoked tritium overflow from superfused mouse brain cortex slices preincubated with [^{3}H]serotonin or [^{3}H]choline, and effect of SR 141716 on the evoked tritium overflow. The slices were superfused with medium containing the auxiliary drugs, given below, and, in some instances, SR 141716. Tritium overflow was evoked after 40 min of superfusion (S_1) (and in slices preincubated with [^{3}H]serotonin again after 90 min; not considered in this table), either electrically (3 Hz, 100 mA, 2 ms) or by re-introduction of Ca^{2+} 1.3 mM into Ca^{2+}-free K^+-rich (25 mM) medium. Basal tritium efflux (t_1) was determined in the 5-min sample from 55–60 min. Means ± SEM of 4–10 experiments

Preincubation	Auxiliary drugs present during superfusion (μM)	Mode of stimulation	Basal tritium efflux during t_1: fractional rate (min^{-1})	Tritium overflow evoked by S_1: % of tissue tritium		
			No SR 141716	No SR 141716	SR 141716 (0.032 μM)	SR 141716 (0.32 μM)
[^{3}H]Serotonin (0.1 μM)	Indalpine (10), metitepine (3.2)	Electrical	0.0087±0.0008	9.48±1.18	–	9.05±0.93
[^{3}H]Serotonin (0.025 μM)	Indalpine (10), metitepine (3.2)	Electrical	0.0104±0.0013	8.17±0.81	7.53±0.60	7.85±0.94
[^{3}H]Serotonin (0.025 μM)	Indalpine (10), metitepine (3.2), tetrodotoxin (1)	Ca^{2+}	0.0127±0.0008	6.73±1.09	–	7.56±0.75
[^{3}H]Choline (0.1 μM)	Hemicholinium-3 (10)	Electrical	0.0024±0.0002	1.87±0.31	–	–

In the second series, slices were preincubated with [^{3}H]serotonin and tritium overflow was evoked electrically. Fig. 2 shows the concentration-response curve of WIN 55,212–2 for its inhibitory effect on the evoked overflow (S_2/S_1). The maximum inhibitory effect of WIN 55,212–2 of about 20% was obtained at 1 μM; the negative logarithm of the concentration causing an inhibition of 10% (pEC_{50}) amounted to 7.11. The concentration-response curve of WIN 55,212–2 was shifted to the right by SR 141716 (0.032 and 0.32 μM) (Fig. 2); the apparent pA_2 values for the rightward shifts were 7.93 and 8.11, respectively (mean of 8.02). Both concentrations of SR 141716 did not affect the evoked overflow (S_1) by themselves (Table 1).

In the third series, slices were preincubated with [^{3}H]serotonin and superfused with Ca^{2+}-free K^+-rich (25 mM) medium containing tetrodotoxin (1 μM); tritium overflow was evoked by introduction of Ca^{2+} ions. WIN 55,212–2 (1 μM) inhibited the Ca^{2+}-evoked tritium overflow (S_2/S_1) by 20%; the inhibitory effect was abolished by SR 141716 (0.32 μM) (Fig. 1B), which, by itself did not affect the evoked overflow (S_1) (Table 1).

In the fourth series, slices were preincubated with [^{3}H]serotonin and tritium overflow was evoked electrically. Fig. 3A shows that, compared to WIN 55,212–2 (1 μM) (which inhibited the evoked overflow S_2/S_1 by 22%), histamine (10 μM), prostaglandin E_2 (1 μM) and neuropeptide Y (0.32 μM) caused an inhibition by 33, 69 and 73%, respectively.

In the fifth series, slices were preincubated with [^{3}H]choline and tritium overflow was evoked electrically. WIN 55,212–2 (1 μM) inhibited the electrically evoked tritium overflow (S_1) by 49% (Fig. 3B).

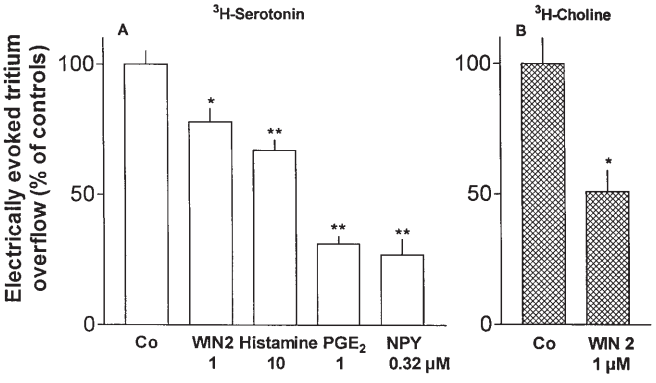

Fig. 3 Effect of WIN 55,212–2 (WIN2), histamine, prostaglandin E_2 (PGE_2) and neuropeptide Y (NPY) on the electrically evoked tritium overflow from mouse brain cortex slices preincubated with [^{3}H]serotonin (0.1 μM) (**A**) or [^{3}H]choline (0.1 μM) (**B**). **A** Slices were superfused with medium containing indalpine 10 μM plus metitepine 3.2 μM throughout superfusion and the drug under study from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). Results are expressed as percent of the S_2/S_1 value in the control (Co) (for the absolute value of the control, see Fig. 1A). **B** Slices were superfused with medium containing hemicholinium-3 10 μM and, when necessary, WIN 55,212–2. Tritium overflow was evoked after 40 min of superfusion (S_1) and expressed as percent of tissue tritium. Results are expressed as percent of the S_1 value in the control (for the absolute value of the control, see Table 1). Means ± SEM of 9–15, and in the case of neuropeptide Y, of five experiments. *P<0.05, **P<0.0025

Binding experiments

Saturation binding experiments in mouse brain cortex membranes with [^{3}H]WIN 55,212–2 (not shown) yielded a dissociation constant (K_d) of 2.53±0.21 nM and a maximum number of binding sites (B_{max}) of 444±52 fmol/mg protein (n=4). Scatchard analysis (not shown) revealed a straight line with a Hill coefficient (n_H) of unity. In competition experiments, binding of [^{3}H]WIN 55,212–2 (1 nM) was inhibited monophasically by CP-55,940, SR 141716, WIN 55,212–2 and WIN 55,212–3 (Fig. 4; n_H values near unity), yielding pK_i values of 9.30±0.02, 8.70±0.06, 8.19±0.11 and <6, respectively. The following

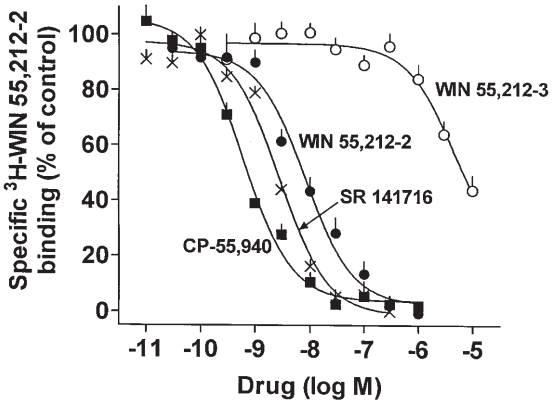

Fig. 4 Effect of cannabinoid receptor ligands on specific [^{3}H]WIN 55,212–2 binding to mouse brain cortex membranes. Membranes were incubated (30°C) for 90 min with [^{3}H]WIN 55,212–2 (1 nM) and 10 concentrations of the drugs under study. Means ± SEM of 3–4 experiments in triplicate (for some data points, SEM is contained within the symbol)

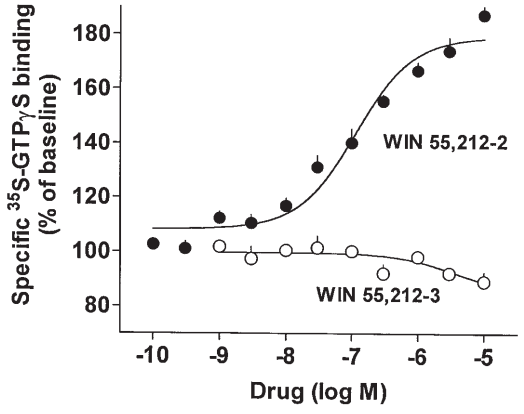

Fig. 5 Effect of WIN 55,212–2 and WIN 55,212–3 on specific [^{35}S]GTPγS binding to mouse brain cortex membranes. Membranes were incubated (30°C) for 60 min with [^{35}S]GTPγS and 9–11 concentrations of WIN 55,212–2 or WIN 55,212–3. Means ± SEM of 3–4 experiments in triplicate (for some data points, SEM is contained within the symbol)

drugs did not affect [^{3}H]WIN 55,212–2 binding: hemicholinium-3, histamine, indalpine, metitepine, neuropeptide Y, prostaglandin E_2 and tetrodotoxin (pK_i<4.5 or, in the case of neuropeptide Y, <5.5; *n*=4, results not shown).

Basal [^{35}S]GTPγS binding in mouse brain cortex membranes was 153±49 fmol/mg protein and was increased by WIN 55,212–2 (maximum effect of 79.4±2.4%, pEC_{50}= 6.94±0.08) but not affected by WIN 55,212–3 (Fig. 5).

Discussion

The binding experiments of our study, carried out with [^{3}H]WIN 55,212–2, show a rank order of inhibition curves typical of CB_1 receptors (for review see Pertwee 1997) and a high density of these receptors in the mouse brain cortex. The coupling of the CB_1 receptors in mouse brain cortex membranes to G proteins is suggested by the activation of [^{35}S]GTPγS binding by WIN 55,212–2 and the lack of effect of its inactive enantiomer WIN 55,212–3. The superfusion experiments show that serotonin release is inhibited by cannabinoid receptors. Thus, two cannabinoid receptor agonists (WIN 55,212–2, CP-55,940) of different chemical structure inhibited serotonin release whereas WIN 55,212–3 failed to do so. Final proof that CB_1 receptors are involved comes from the experiments in which a rightward shift of the concentration-response curve of WIN 55,212–2 by the CB_1 receptor antagonist SR 141716 was shown, yielding an apparent pA_2 value not markedly different from that obtained for other functional CB_1 receptor models (e.g. Coutts and Pertwee 1997; Croci et al. 1998; Schlicker et al. 1996; Schlicker et al. 1997).

The inhibitory effect of WIN 55,212–2 and the antagonism of SR 141716 were also found in experiments in which the nerve endings were kept depolarized by a high K^+ concentration in the PSS and tritium overflow was evoked by introduction of Ca^{2+} ions into Ca^{2+}-free PSS, containing tetrodotoxin for blockade of impulse propagation along the axons. These results may suggest that the CB_1 receptors are located presynaptically on the serotoninergic nerve endings themselves. The CB_1 receptors do not appear to be activated by endogenously formed cannabinoids since SR 141716 by itself did not affect tritium overflow evoked by either type of stimulation.

The maximum extent of CB_1 receptor-mediated inhibition of serotonin release (by 20%) was relatively small. The possibility that the auxiliary drugs indalpine, metitepine and tetrodotoxin masked the effects of cannabinoid receptor agonists due to an accidental high affinity for CB_1 receptors could be excluded by the radioligand binding experiments. Decreasing the concentration of [^{3}H]serotonin during preincubation from 0.1 to 0.025 μM did not increase the extent of the CB_1 receptor-mediated effect, as opposed to a previous study on rat brain cortex slices in which the same procedure markedly enhanced the histamine H_3 receptor-mediated inhibition of serotonin release (Schlicker et al. 1991a).

The possibility that the mouse brain cortex is not particularly suited for the identification of inhibitory presynaptic receptors on serotoninergic neurones had also to be considered. Therefore, we examined the effects of histamine, prostaglandin E_2 and neuropeptide Y, which inhibited serotonin release in rat brain cortex slices in previous studies of our laboratory (Schlicker et al. 1987; Schlicker et al. 1988; Schlicker et al. 1991b). The three ligands, at concentrations eliciting maximum or near-maximum effects at H_3, EP_3 and Y_2 receptors, respectively, inhibited serotonin release in mouse brain cortex very markedly, i.e. by 33, 69 and 73%.

One has also to check whether the mouse brain cortex might be poorly suited for the identification of release-modulating CB_1 receptors. We examined whether WIN 55,212–2 (which had been shown to inhibit acetylcholine release in slices of the rat striatum and nucleus accumbens; Gifford and Ashby 1996) affects acetylcholine release in mouse brain cortex slices. WIN 55,212–2 inhib-

ited acetylcholine release by about 50%. The latter result and previous studies performed with the same superfusion equipment (with CB_1 receptor-mediated inhibitory effects of up to 80%; Schlicker et al. 1996; Schlicker et al. 1997) argue against the possibility that the weak inhibition of serotonin release is related to excessive binding of lipophilic drugs like WIN 55,212–2, CP-55,940 and SR 141716 to the superfusion chambers and the tubing of the superfusion apparatus. Although there are still other explanations for the weakness of effect which have to be considered (e.g. desensitization or internalization of the CB_1 receptors involved; attenuation of the CB_1 receptor-mediated inhibitory effect by a simultaneously occurring facilitatory effect), nonetheless, the explanation appears to be very plausible that the effect per se is relatively small.

In conclusion, serotonin release in the mouse brain cortex is inhibited via CB_1 receptors; the extent of the effect is small. The CB_1 receptors may be located presynaptically on the serotoninergic neurones themselves and are not activated by endogenously formed cannabinoids. The study also shows for the first time that, in mouse brain cortex slices, serotonin release is inhibited by histamine, prostaglandin E_2 and neuropeptide Y and that acetylcholine release is inhibited by WIN 55,212–2. A more detailed pharmacological analysis of these four effects should be the subject of future studies.

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