Naunyn-Schmiedeberg's Arch Pharmacol (2006) 372: 354–361
DOI 10.1007/s00210-006-0033-x

ORIGINAL ARTICLE

Markus Kathmann · Karsten Flau · Agnes Redmer · Christian Tränkle · Eberhard Schlicker

Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors

Received: 4 October 2005 / Accepted: 4 January 2006 / Published online: 18 February 2006

Abstract The mechanism of action of cannabidiol, one of the major constituents of cannabis, is not well understood but a noncompetitive interaction with mu opioid receptors has been suggested on the basis of saturation binding experiments. The aim of the present study was to examine whether cannabidiol is an allosteric modulator at this receptor, using kinetic binding studies, which are particularly sensitive for the measurement of allosteric interactions at G protein-coupled receptors. In addition, we studied whether such a mechanism also extends to the delta opioid receptor. For comparison, (-)-Δ^9-tetrahydrocannabinol (THC; another major constituent of cannabis) and rimonabant (a cannabinoid CB_1 receptor antagonist) were studied. In mu opioid receptor binding studies on rat cerebral cortex membrane homogenates, the agonist ^{3}H-DAMGO bound to a homogeneous class of binding sites with a K_D of 0.68± 0.02 nM and a B_{max} of 203±7 fmol/mg protein. The dissociation of ^{3}H-DAMGO induced by naloxone 10 μM (half life time of 7±1 min) was accelerated by cannabidiol and THC (at 100 μM, each) by a factor of 12 and 2, respectively. The respective pEC_{50} values for a half-maximum elevation of the dissociation rate constant k_{off} were 4.38 and 4.67; ^{3}H-DAMGO dissociation was not affected by rimonabant 10 μM. In delta opioid receptor binding studies on rat cerebral cortex membrane homogenates, the antagonist ^{3}H-naltrindole bound to a homogeneous class of binding sites with a K_D of 0.24±0.02 nM and a B_{max} of 352± 22 fmol/mg protein. The dissociation of ^{3}H-naltrindole induced by naltrindole 10 μM (half life time of 119±3 min) was accelerated by cannabidiol and THC (at 100 μM, each) by a factor of 2, each. The respective pEC_{50} values were 4.10 and 5.00; ^{3}H-naltrindole dissociation was not affected by rimonabant 10 μM. The present study shows that cannabidiol is an allosteric modulator at mu and delta opioid receptors. This property is shared by THC but not by rimonabant.

Keywords ^{3}H-DAMGO binding · ^{3}H-Naltrindole binding · (-)-Δ^9-Tetrahydrocannabinol · Cannabinoid CB_1 receptor · Rat cerebral cortex · Rimonabant

Abbreviations AEBSF: 4-(2-aminoethyl) benzenesulfonyl fluoride · ^{3}H-DAMGO: ^{3}H-Tyr-D-Ala-Gly-N-methyl-Phe-Gly-ol · ^{3}H-NTI: ^{3}H-naltrindole · CBD: (-)-cannabidiol · THC: (-)-Δ^9-tetrahydrocannabinol

Introduction

(-)-Δ^9-Tetrahydrocannabinol (THC) is the main agent of the pharmacological effects caused by the consumption of cannabis and plays a major role both in its use for recreational and medicinal purposes. However, the non-psychotropic cannabidiol (CBD), several cannabinoid analogues and newly discovered modulators of the endogenous cannabinoid system, so-called endocannabinoids (e.g. anandamide; for review, see Pertwee 1999; Howlett et al. 2002), are also promising candidates for clinical research and therapeutic uses. Cannabinoids exert many effects through activation of G-protein-coupled cannabinoid receptors in the brain and peripheral tissues (for review, see Ameri 1999; Schlicker and Kathmann 2001; Howlett et al. 2002). To date, two types of cannabinoid receptors, CB_1 and CB_2, have been identified, for both of which THC has marked affinity. On the other hand, cannabidiol has a very low affinity for either cannabinoid receptor and little is

M. Kathmann (✉) · K. Flau · A. Redmer · E. Schlicker
Department of Pharmacology and Toxicology,
School of Medicine,
University of Bonn,
Reuterstr. 2b,
53113 Bonn, Germany
e-mail: m.kathmann@uni-bonn.de
Fax: +49-228-735404

C. Tränkle
Department of Pharmacology and Toxicology,
Institute of Pharmacy,
University of Bonn,
Gerhard-Domagk-Str. 3,
53121 Bonn, Germany

known so far about its mechanism of action (for review, see Pertwee 1999; Howlett et al. 2002).

Earlier findings indicate an antianxiety (Guimaraes et al. 1994), neuroprotective and anticonvulsant effect of CBD (Consroe et al. 1981; Martin et al. 1987); CBD also inhibits the release of inflammatory cytokines from blood cells (Srivastava et al. 1998; Malfait et al. 2000). There is evidence for receptor-independent mechanisms of CBD including its antioxidant properties or its direct interaction with cytochrome P450 enzymes (Hampson et al. 1998; Bornheim and Correia 1989). The effect of CBD may also involve stimulation of VR1 receptors or an increase in the level of endogenous anandamide (Bisogno et al. 2001) or may be mediated through an unknown specific receptor. Very recently, Health Canada has approved Sativex (Cannabis sativa L. extract; the ratio of THC to CBD is 2.7 mg : 2.5 mg per spray), a new drug developed by GW Pharmaceuticals; this drug proved successful as adjunctive treatment for the symptomatic relief of neuropathic pain in adults with multiple sclerosis (Rog et al. 2005).

In the present study, the possibility that CBD possesses an allosteric effect on ligand binding to mu and delta opioid receptors (Vaysse et al. 1987) has been further examined, using kinetic binding studies with ^{3}H-DAMGO and ^{3}H-naltrindole, respectively. In such experiments, the dissociation of the radioligand from the orthosteric binding site is induced by a high concentration of an orthosteric ligand and a putative allosteric ligand alters the velocity of dissociation (Christopoulos and Kenakin 2002). For the sake of comparison, THC and the selective CB_1 antagonist rimonabant (previous name SR 141716) were also included in our study.

Materials and methods

Cell membrane preparation

Cerebral cortical tissue from male Wistar rats was homogenized (Potter-Elvehjem) in 25 volumes of ice-cold Tris-HCl-EDTA buffer (TE-buffer: Tris 50 mM; EDTA 5 mM; pH 7.5) containing 10.27% sucrose and centrifuged at 1,000×g for 10 min (4°C). The supernatant was centrifuged at 35,000×g for 10 min (4°C) and the pellet was washed twice with TE-buffer. Finally the pellet was resuspended in TRIS-buffer (Tris 50 mM; pH 7.4) and frozen at −80°C. Protein concentration was assayed by the method described by Bradford (1976).

^{3}H-DAMGO binding assay

To determine mu opioid receptor binding, we used the agonist radioligand ^{3}H-DAMGO (Zhao et al. 2003). In saturation binding experiments, seven concentrations of ^{3}H-DAMGO (0.1 nM to 10 nM) were used in a final volume of 0.5 ml TRIS-buffer containing 10 μM AEBSF (serine proteinase inhibitor; AEBSF was also used in the other types of binding experiments with ^{3}H-DAMGO). Incubation was performed at 25°C and terminated after 60 min by rapid filtration through polyethylenimine (0.3%)-pretreated Whatman GF/C filters. Naloxone (10 μM) was used to determine the non-specific binding (here and also in pseudo-competition and association binding experiments; 16% at 0.5 nM).

Displacement experiments (pseudo-competition experiments) with ^{3}H-DAMGO (0.5 nM) were performed in TRIS-buffer in a final volume of 0.5 ml containing various concentrations of the drugs under study. Incubation was performed at 25°C and terminated after 120 min by rapid filtration through polyethylenimine (0.3%)-pretreated Whatman GF/C filters.

Kinetic experiments were performed in TRIS-buffer containing ^{3}H-DAMGO (0.5 nM). To study ^{3}H-DAMGO association kinetics, assays were prepared in a larger volume of TRIS-buffer allowing withdrawal of several aliquots of 0.5 ml at adequate time intervals over a period of up to 120 min. ^{3}H-DAMGO dissociation kinetics: Radioligand and membranes were incubated for 45 min at 25°C and then naloxone (10 μM) or a combination of naloxone and the drugs under study was added. Assays were carried out in a larger volume of TRIS-buffer allowing withdrawal of several aliquots of 0.5 ml at adequate time intervals over a period of up to 120 min.

^{3}H-NTI binding assay

To determine delta opioid receptor binding, we used the antagonist radioligand ^{3}H-naltrindole (^{3}H-NTI) (Contreras et al. 1993). In saturation binding experiments, eight concentrations of ^{3}H-NTI (0.05 nM to 5 nM) were used in a final volume of 0.5 ml TRIS-buffer. Incubation was performed at 37°C and terminated after 120 min by rapid filtration through polyethylenimine (0.3%)-pretreated Whatman GF/C filters. Naltrindole (10 μM) was used to determine the non-specific binding (here and also in pseudo-competition and association binding studies; 22% at 0.1 nM).

Displacement experiments (pseudo-competition experiments) with ^{3}H-NTI (0.1 nM) were performed in TRIS-buffer in a final volume of 0.5 ml containing various concentrations of the drugs under study. Incubation was performed at 37°C and terminated after 120 min by rapid filtration through polyethylenimine (0.3%)-pretreated Whatman GF/C filters.

Kinetic experiments were performed in TRIS-buffer containing ^{3}H-NTI (0.1 nM). To study ^{3}H-NTI association kinetics, assays were prepared in a larger volume of TRIS-buffer allowing withdrawal of several aliquots of 0.5 ml at adequate time intervals over a period of up to 180 min. ^{3}H-NTI dissociation kinetics: Radioligand and membranes were incubated for 120 min at 37°C and then naltrindole (10 μM) or a combination of naltrindole and the drugs under study was added. Assays were carried out in a larger volume of TRIS-buffer allowing withdrawal of several

aliquots of 0.5 ml at adequate time intervals over a period of up to 300 min.

Statistics and calculations

Results are given as means±SEM of *n* experiments. Experimental data from the individual binding experiments were analyzed by computer-aided, nonlinear regression analysis using Prism software (Vers. 3.0, Graph Pad, San Diego, Calif., USA). Effects of the allosteric agents on ^{3}H-DAMGO and ^{3}H-NTI equilibrium binding were analyzed by nonlinear regression analysis applying a four parameter logistic equation including a slope factor n_H. The *F*-test was applied in order to evaluate successively whether the inhibition data of ^{3}H-DAMGO or ^{3}H-NTI binding by the test drugs (1) occurred with slopes different from unity and (2) if n_H<1.00, were better fitted by a competition one-site or a two-site model. Dissociation data were fitted applying a monoexponential compared to a double exponential decay function (*F*-test). P<0.05 was taken as criterion for statistical significance.

Drugs used

[Tyrosyl-3,5-^{3}H(N)]-DAMGO (Tyr*-D-Ala-Gly-N-methyl-Phe-Gly-ol; ^{3}H-DAMGO, specific activity 51 Ci/mmol), [3′,7′-^{3}H]-naltrindole (^{3}H-NTI, specific activity 20 Ci/ mmol) (PerkinElmer, Boston, MA, USA); AEBSF (4-(2-aminoethyl)benzenesulfonyl fluoride), (-)-Δ^9-tetrahydrocannabinol (Sigma, München, Germany); (-)-cannabidiol (Sigma, München, Germany or GW Pharmaceuticals, Salisbury, England); DAMGO, naltrindole hydrochloride (Bachem, Weil am Rhein, Germany); rimonabant (N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; SR141716, Sanofi-Aventis, Montpellier, France). Drugs were dissolved in DMSO (cannabidiol, rimonabant, THC) or water (other drugs) and diluted with water to obtain the concentration required.

Results

^{3}H-DAMGO binding

In saturation binding experiments (25°C) on rat brain cortex membranes using seven concentrations of the radioligand ^{3}H-DAMGO and naloxone (10 μM) (to determine non-specific binding), a K_D value of 0.68±0.02 nM with a maximum number of binding sites (B_{max}) of 203±7 fmol/mg protein was determined (Fig. 1a). Scatchard analysis revealed a straight line (Fig. 1a, inset); the coefficient (n_H) obtained by Hill analysis was not different from unity (not shown).

To determine association kinetics, rat brain cortical membranes were exposed to 0.5 nM ^{3}H-DAMGO over a period of up to 120 min, yielding a monoexponential association time course with a half life time $t_{1/2on}$ of 2.69±0.09 min and an apparent rate constant k_{appon} of 0.26±0.01 min^{-1} (Fig. 2a).

To study dissociation kinetics, rat brain cortical membranes were incubated with 0.5 nM ^{3}H-DAMGO for 45 min and then naloxone (10 μM) was added to visualize radioligand dissociation yielding a monoexponential time course with a half life time $t_{1/2off}$ of 7.0±0.9 min and a dissociation rate constant k_{off} of 0.11±0.02 min^{-1} (Fig. 2b). To evaluate the effects of the test compounds, naloxone 10 μM was added together with 30 μM THC, 30 μM cannabidiol or 10 μM rimonabant, respectively (Fig. 3). Both cannabidiol 30 μM and THC 30 μM accelerated ^{3}H-DAMGO dissociation, whereas rimonabant 10 μM had no effect. The accelerating effects of cannabidiol and THC on the dissociation of ^{3}H-DAMGO were further investigated using different concentrations. The dissociation of ^{3}H-DAMGO induced by naloxone 10 μM was accelerated by cannabidiol and THC in a concentration-dependent manner (Fig. 3, inset). The latter drug increased the rate constant of ^{3}H-DAMGO dissociation maximally by a factor of 2; the respective pEC_{50} value was 4.67. In the case of cannabidiol, 100 μM, the highest concentration under study, accelerated ^{3}H-DAMGO control dissociation about 12 fold but did not describe the maximum increase. The pEC_{50} value of 4.38 may, therefore, overestimate the potency of the drug to accelerate dissociation.

In displacement experiments (pseudo-competition experiments), rat brain cortex membranes were incubated for 120 min with medium containing 0.5 nM ^{3}H-DAMGO and various concentrations of the drugs under study. Specific ^{3}H-DAMGO binding was inhibited monophasically by cannabidiol, THC and rimonabant with pIC_{50} values of vav 5.01±0.03 (n_H =−1.46), 4.49±0.04 (n_H =−1.60) and 5.39±0.07 (n_H =−0.73), respectively (Fig. 4a). All slope factors were significantly different from unity (*F*-test, P<0.05). Though inhibition of ^{3}H-DAMGO binding by rimonabant occurred with a shallow slope, a two site competition model did not yield a better curve fit than a one site model (*F*-test, P>0.05). Note that cannabidiol was able to fully suppress ^{3}H-DAMGO binding; for THC and rimonabant, the maximum inhibitory effects could not be determined.

In an additional series of experiments, the alteration of equilibrium binding by cannabidiol, THC and rimonabant was studied. The experimental design was similar to that used for the experiments in which dissociation kinetics were examined, however, naloxone was omitted. The dissociation curve for naloxone 10 μM (obtained from Fig. 3) is depicted once again in Fig. 5a. Compared to this curve, the curve for cannabidiol and THC, 100 μM each was shifted to the left but showed the same plateau (Fig. 5a). The curve for rimonabant 10 μM was shifted to the right and the maximum level of binding inhibition was lower than that for naltrindole (Fig. 5a).

^{3}H-NTI binding

In saturation binding experiments (37°C) on rat brain cortex membranes using eight concentrations of the

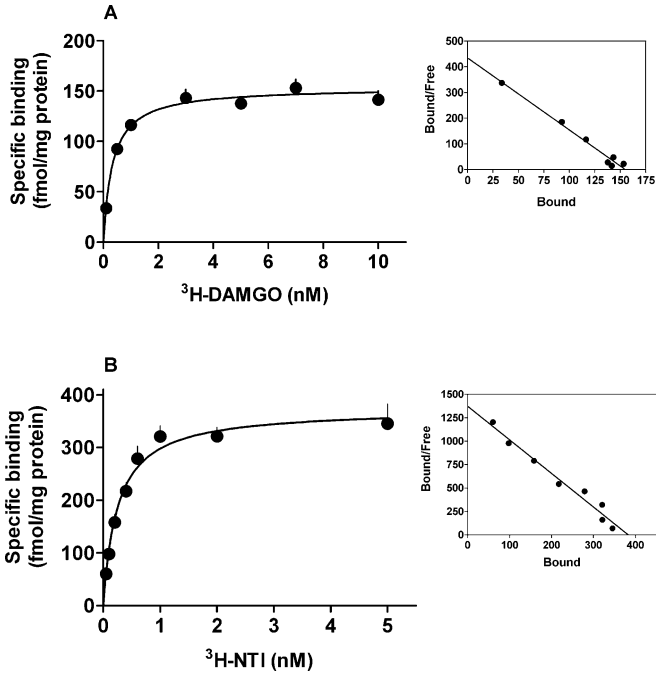

Fig. 1 Saturation of specific ^{3}H-DAMGO (**a**) and specific ^{3}H-NTI (**b**) binding to rat brain cortex membranes. (**a**) Incubation was performed at 25°C and lasted for 60 min and naloxone (10 μM) was used to determine non-specific binding. (**b**) Incubation was performed at 37°C and lasted for 120 min and naltrindole (10 μM) was used to determine non-specific binding. Scatchard analysis of the saturation data is presented in the respective insets. Means from three experiments (in triplicate) are shown (for some data points, SEM is contained within the symbol)

radioligand ^{3}H-NTI and naltrindole (10 μM) (to determine non-specific binding), a K_D value of 0.24±0.02 nM with a maximum number of binding sites (B_{max}) of 352±22 fmol/mg protein was determined (Fig. 1b). Scatchard analysis revealed a straight line (Fig. 1b, inset), Hill analysis yielded a Hill coefficient (n_H) not different from unity (not shown).

To study association kinetics, rat brain cortical membranes were exposed to 0.1 nM ^{3}H-NTI over a period of up to 180 min, yielding a monoexponential association time course with a half life time $t_{1/2on}$ of 27.8±1.2 min and an apparent rate constant k_{appon} of 0.025±0.001 min^{-1} (Fig. 2c).

To determine dissociation kinetics, rat brain cortical membranes were incubated with 0.1 nM ^{3}H-NTI for 120 min and then naltrindole (10 μM) was added to visualize radioligand dissociation yielding a monoexponential time course with a dissociation half life time $t_{1/2off}$ of 118.6±3.1 min and a dissociation rate constant k_{off} of 0.0059±0.0002 min^{-1} (Fig. 2d). In additional experiments, naltrindole (10 μM) was added together with 100 μM THC, 100 μM cannabidiol or 10 μM rimonabant (Fig. 6). Both cannabidiol 100 μM and THC 100 μM accelerated the dissociation of ^{3}H-NTI, whereas rimonabant 10 μM had no effect on the dissociation kinetics. The effects of cannabidiol and THC on the increase in ^{3}H-NTI dissociation kinetics were further investigated using different concentrations. The dissociation of ^{3}H-NTI induced by naltrindole was increased by either drug (Fig. 6, inset). THC increased the k_{off} value maximally by 70% (pEC_{50} value 5.00). The k_{off} value was increased by cannabidiol 100 μM by about the same amount, however, this was not

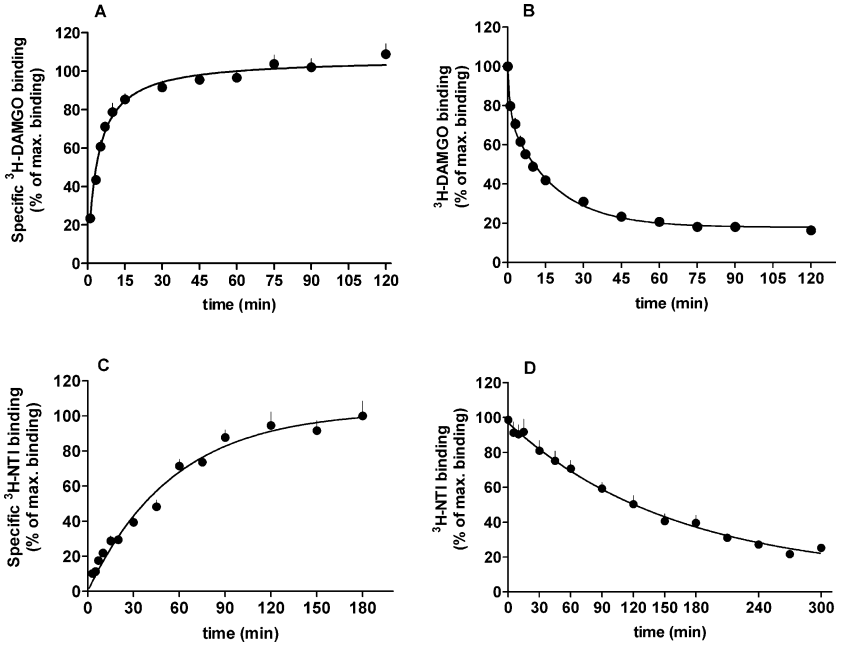

Fig. 2 Association (**a**, **c**) and dissociation (**b**, **d**) kinetics of ^{3}H-DAMGO (**a**, **b**) and ^{3}H-NTI (**c**, **d**) binding to rat brain cortical membranes. (**a**, **b**) For ^{3}H-DAMGO association kinetics, incubation was performed at 25°C for up to 120 min (naloxone 10 μM was used to determine non-specific binding); for ^{3}H-DAMGO dissociation kinetics, incubation was performed for 45 min at 25°C and then naloxone (10 μM) was added to visualize dissociation. (**c**, **d**) For ^{3}H-NTI association kinetics, incubation was performed at 37°C for up to 180 min (naltrindole 10 μM was used to determine non-specific binding); for ^{3}H-NTI dissociation kinetics, incubation was performed for 120 min at 37°C and then naltrindole (10 μM) was added to induce dissociation. Means from four experiments (in triplicate) are shown (for some data points, SEM is contained within the symbol)

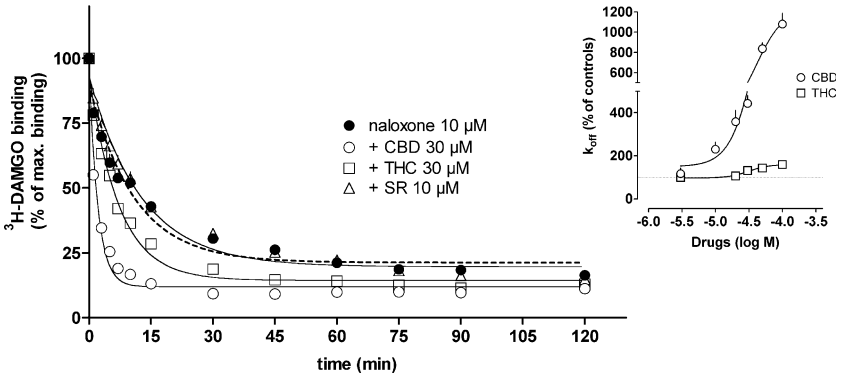

Fig. 3 Dissociation kinetics of ^{3}H-DAMGO binding to rat brain cortical membranes. Incubation was performed for 45 min at 25°C and then naloxone (10 μM) was added alone or in the presence of 30 μM (-)-Δ^9-tetrahydrocannabinol (THC), 30 μM cannabidiol (CBD) or 10 μM rimonabant (SR). *Inset*: Increase in ^{3}H–DAMGO dissociation rate constant K_{off} by various concentrations of THC and CBD. Means±SEM from four experiments in triplicate. *Error bars* are not shown when they are smaller than the symbols

yet the maximum effect. For this reason, the calculated pEC_{50} value (4.10) should represent an overestimation.

In displacement experiments (pseudo-competition experiments), rat brain cortical membranes were incubated for 120 min with medium containing 0.1 nM ^{3}H-NTI and various concentrations of the drugs under study. Specific ^{3}H-NTI binding was inhibited by cannabidiol at a single site with a pIC_{50} of 4.97±0.10 (n_H =-0.83). THC (up to 100 μM) and rimonabant (up to 10 μM) inhibited ^{3}H-NTI binding by no more than 20% (Fig. 4b).

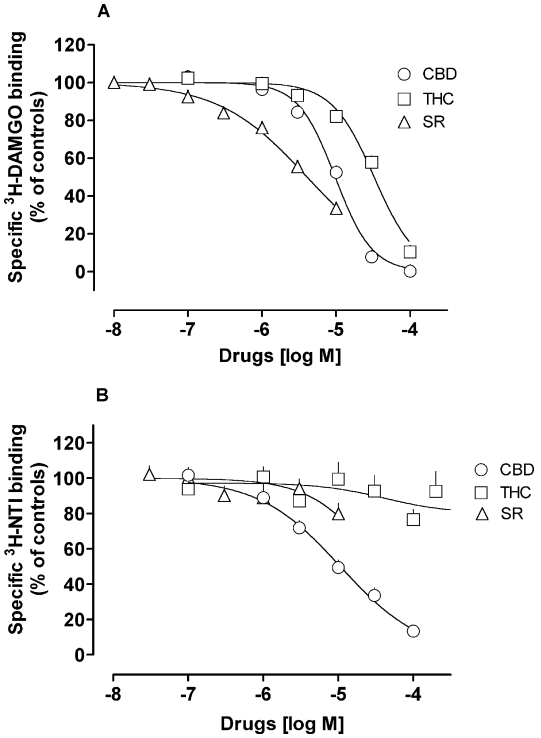

Fig. 4 Effects of (-)-Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and rimonabant (SR) on ^{3}H-DAMGO (**a**) or ^{3}H-NTI (**b**) equilibrium binding to rat cerebral cortical membranes. Unspecific binding was determined using naloxone 10 μM (**a**) and naltrindole 10 μM (**b**), respectively. Means±SEM from 4 experiments in triplicate. *Error bars* are not shown when they are smaller than the symbols

In the final series of experiments, the alteration of equilibrium binding by cannabidiol and THC was studied. The experimental design was similar to that used for the experiments in which dissociation kinetics were examined, however, (unlabelled) naltrindole was omitted. The dissociation curve for naltrindole 10 μM (obtained from Fig. 6) is depicted once again in Fig. 5b. Compared to this curve, the

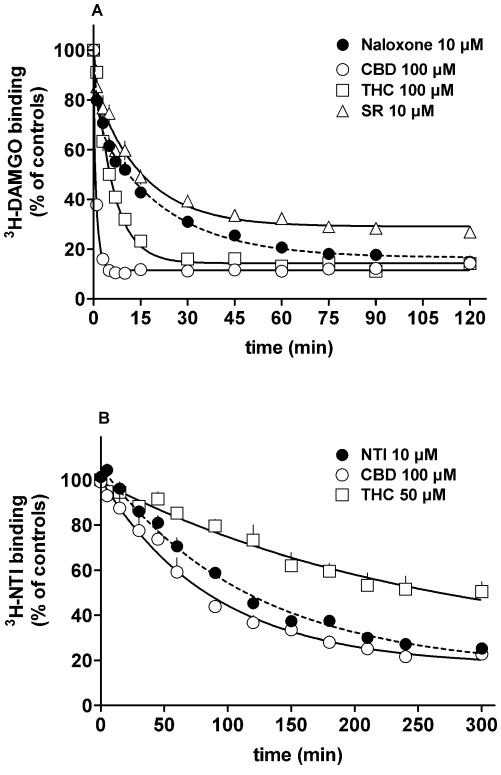

Fig. 5 Time-dependent alteration of equilibrium binding of ^{3}H-DAMGO (**a**) and ^{3}H-NTI (**b**) to rat brain cortical membranes by rimonabant (SR), (-)-Δ^9-tetrahydrocannabinol (THC) and/or cannabidiol (CBD). Incubation was performed for 45 min at 25°C (**a**) and for 120 min at 37°C (**b**) and then SR, THC or CBD was added. For the sake of comparison, the dissociation curves for naloxone 10 μM (**a**; obtained from Fig. 3) and for naltrindole 10 μM (**b**; obtained from Fig. 6) are shown here again. Means±SEM from four experiments in triplicate. *Error bars* are not shown when they are smaller than the symbols

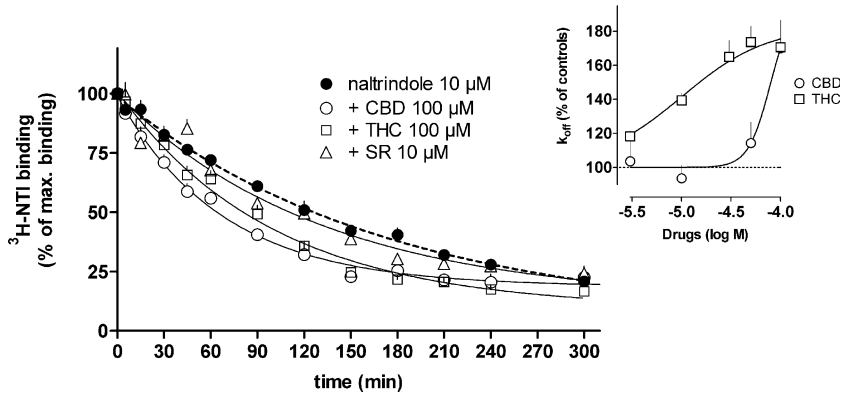

Fig. 6 Dissociation kinetics of ^{3}H-NTI binding to rat brain cortical membranes. Incubation was performed for 120 min at 37°C and then naltrindole (10 µM) was added alone or in the presence of 100 µM (-)-Δ^9-tetrahydrocannabinol (THC), 100 µM cannabidiol (CBD) or 10 µM rimonabant (SR). *Inset*: Increase in ^{3}H-NTI dissociation rate constant k_{off} by various concentrations of THC and CBD. Means± SEM from four experiments in triplicate. *Error bars* are not shown when they are smaller than the symbols

curve for cannabidiol 100 µM was slightly shifted to the left but showed the same plateau (Fig. 5b). The curve for THC 50 µM was shifted to the right and the maximum level of binding inhibition was lower than that for naltrindole (Fig. 5b).

Discussion

The aim of the present study was to further examine the postulated allosteric effect of CBD at mu opioid receptors (Vaysse et al. 1987) and to check whether this drug possesses an allosteric effect also at delta opioid receptors. THC and rimonabant (previous name SR 141716) were also included in this study. The latter drug was chosen since it is chemically unrelated to CBD and is well-characterized as a selective CB_1 receptor antagonist or, more precisely, inverse agonist (Pertwee 2005). THC was used since it showed a noncompetitive interaction with mu and delta (but not with kappa) opioid receptors in saturation experiments (Vaysse et al. 1987). In detail, the latter authors found that THC did not affect the affinity but decreased the density of mu opioid receptors (labelled by ^{3}H-dihydromorphine) and delta opioid receptors (labelled by ^{3}H-D-Pen^2, D-pen^5-enkephalin (^{3}H-DPDPE)). We wanted to further elucidate possible allosteric effects in kinetic binding experiments. Unlike Vaysse et al. (1987), who used the whole rat brain, we employed membranes from the cerebral cortex since both mu and delta opioid receptors exhibit a higher density in the cortex when compared to many other brain regions (Dhawan et al. 1996). For labelling of mu opioid receptors we used the agonist radioligand ^{3}H-DAMGO, which possesses a threefold higher affinity at this receptor than ^{3}H-dihydromorphine (Ulibarri et al. 1987). Although we would have preferred to use ^{3}H-DPDPE for labelling of delta opioid receptors, its extremely high unspecific binding (found in initial experiments and also described by Akiyama et al. 1985) led us to use the antagonist radioligand ^{3}H-naltrindole (^{3}H-NTI) instead.

Saturation binding studies revealed that both radioligands bound to a single class of receptors with K_D and B_{max} values comparable to results obtained from other groups (Zhao et al. 2003; Contreras et al. 1993). Much emphasis was put in the present study on kinetic binding experiments. Association studies were carried out, which, however (although of interest for the design of other types of binding studies), are less suited for the identification of allosteric ligands since a binding of the drug under consideration to the orthosteric binding site cannot be excluded. In contrast, in dissociation studies, the orthosteric binding site is occupied by a very high concentration of a competitive antagonist and the drug under study has to bind to another (i.e., allosteric) binding site (Christopoulos and Kenakin 2002).

Dissociation of ^{3}H-DAMGO from the mu opioid receptor, visualized by naloxone 10 µM, was not affected by rimonabant but accelerated by cannabidiol and THC. Our data demonstrate that the latter two drugs are allosteric modulators of ligand binding kinetics at mu opioid receptors, thus extending the findings obtained by Vaysse et al. (1987) using a different experimental approach. THC increased the dissociation of ^{3}H-DAMGO by a factor of 2; cannabidiol increased the dissociation markedly at least by a factor of 12 (the highest concentration feasible for investigation in this study). The allosteric effect of CBD might explain that this drug at 10 µM slightly shifted to the right the concentration-response curve of DAMGO for its inhibitory effect on the electrically induced twitch response in the mouse vas deferens (Pertwee et al. 2002).

In addition, cannabidiol and THC accelerated dissociation of ^{3}H-NTI from delta opioid receptors, visualized by NTI 10 µM, whereas rimonabant failed to do so. These results show that cannabidiol and THC are allosteric modulators also of ligand binding to the delta opioid receptor. Thus, a noncompetitive interaction with the delta opioid receptor as postulated earlier for THC by Vaysse et al. (1987) was demonstrated here for the first time both for THC and cannabidiol. Both drugs increased the dissociation about twofold although for cannabidiol again the maximum could not be determined precisely in the concentration range feasible under study. An acceleration of radioligand dissociation is also known from e.g. amiloride

in α_{1A}-adrenoceptors (Leppik et al. 1998) and in α_{2A}-adrenoceptors (Leppik et al. 2000).

Next, the question was addressed how THC, cannabidiol and rimonabant behave in pseudo-competition experiments. ^{3}H-DAMGO binding was monophasically inhibited by cannabidiol and THC. The fact that the Hill coefficients were markedly higher than unity may be interpreted as a positive homotropic cooperativity between the respective inhibitor molecules. This could imply that two molecules of cannabidiol or THC bind to an allosteric site thereby inhibiting the binding of ^{3}H-DAMGO to the orthosteric (= agonist) binding site. Such a behaviour is also known from the action of tacrine on the binding of orthosteric ligands in muscarinic receptors (e.g., Potter et al. 1989; Tränkle et al. 2003). Unexpectedly, ^{3}H-DAMGO binding was also inhibited by rimonabant and the possibility has to be considered that this drug is an agonist at the mu opioid receptor. A flat inhibition curve with a Hill coefficient lower than unity, as found in our study, frequently occurs when an agonist binds to a G protein-coupled receptor labelled by an agonist radioligand (for review, see Kenakin 1993). The low affinity of rimonabant (pIC_{50} of 5.39) would not contradict the findings by Rinaldi-Carmona et al. (1994), who found that the IC_{50} value of rimonabant at opiate receptors is lower than 1 μM.

The effects of rimonabant, THC and cannabidiol were also studied in binding experiments in which the membranes were exposed to ^{3}H-DAMGO until equilibrium was reached before one of the three drugs was added. These experiments, in which the alteration of equilibrium binding is studied kinetically, differ from the dissociation binding experiments inasmuch as naloxone, which serves to visualize dissociation (= prevention of radioligand re-association) of binding, is *not* used. Such experiments allow to examine kinetically the inhibition of equilibrium binding following the administration of a test drug. The fact that rimonabant inhibited ^{3}H-DAMGO binding and that the half life times of the curves for rimonabant and naloxone are virtually identical is compatible with the view that rimonabant indeed competitively interacts with ^{3}H-DAMGO (Fig. 5a). On the other hand, the dissociation curves for THC and cannabidiol were much steeper than that for naloxone, again suggesting that the former two drugs accelerate the dissociation of ^{3}H-DAMGO (Fig. 5a).

With respect to the effects of drugs on ^{3}H-NTI binding in pseudo-competition experiments, the marked difference between the curves of THC and cannabidiol is remarkable. The fact that THC inhibited ^{3}H-DPDPE binding in the study of Vaysse et al. (1987), but almost failed to inhibit ^{3}H-NTI binding in the present one, is, however, not so surprising since the identification of allosteric phenomena critically depends on the choice of the orthosteric radioligand (Christopoulos and Kenakin 2002). To estimate whether THC may also have an influence on the association of ^{3}H-NTI, experiments were performed in which the alteration of equilibrium binding is studied kinetically. In this additional set of binding experiments (Fig. 5b), cannabidiol (100 μM) and THC (50 μM) were used at concentrations that accelerate the dissociation of ^{3}H-NTI binding induced by naltrindole to the same extent (Fig. 6), equivalent to an increase in k_{off} of about 70% (Fig. 6, inset). The experiments of Fig. 5b show that the time course for cannabidiol 100 μM is similar to the curve for naltrindole 10 μM whereas the time course for THC 50 μM is slower (and shows a lower maximum level of inhibition). The data are compatible with the conclusion that THC not only increases dissociation but, in addition, increases association and thereby weakens its inhibitory effect on equilibrium binding.

Finally, the question has to be addressed whether the allosteric effect of cannabidiol at the mu and delta opioid receptor may explain its in vivo activity. This is unlikely since a single dose oral preparation containing 100 mg of cannabidiol yielded a plasma concentration of about 36 ng/ml or 100 nM in man (Dr. S. Wright, GW Pharmaceuticals, Salisbury, England, personal communication), which is lower by a factor of about 100 than the EC_{50} values determined in the present study. In addition, it is also very unlikely that the effects of THC at mu and delta receptors and the effect of rimonabant at mu receptors contribute to the overall effects of both drugs in vivo. Thus, the K_i values of THC and rimonabant at the cannabinoid CB_1 receptors, which represent the molecular target of the effects of both drugs, is about 50 nM (Pertwee 1999) and 2 nM (Rinaldi-Carmona et al. 1994), respectively. The allosteric effects of THC at mu and delta opioid receptors occur at a 200-fold and the effect of rimonabant at mu opioid receptors at a 1,400-fold higher concentration.

In conclusion, our study shows that cannabidiol is an allosteric modulator of ligand binding to mu and delta opioid receptors. This property is shared by THC but not by rimonabant. All effects occur at very high concentrations and cannot be expected to contribute to the in vivo action of the three drugs.

Acknowledgements This study was supported by grants from the Deutsche Forschungsgemeinschaft (Schl 266/5-5 and Graduiertenkolleg 246 TP 01). We are also indebted to Mrs. P. Zeidler for her skilled technical assistance and to GW Pharmaceuticals and Sanofi-Aventis for gifts of drugs.

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