Naunyn-Schmiedeberg's Arch Pharmacol
DOI 10.1007/s00210-011-0717-8

ORIGINAL ARTICLE

Cannabinoid CB_1 receptor activation, pharmacological blockade, or genetic ablation affects the function of the muscarinic auto- and heteroreceptor

Kirsten Schulte · Nina Steingrüber · Bernd Jergas · Agnes Redmer · Christina Maria Kurz · Rainer Buchalla · Beat Lutz · Andreas Zimmer · Eberhard Schlicker

Received: 9 November 2011 / Accepted: 2 December 2011

Abstract Different types of presynaptic inhibitory $G\alpha_{i/o}$ protein-coupled receptors usually do not act independently of each other but rather pre-activation of receptor X impairs the effect mediated via receptor Y. It is, however, unknown whether this interaction extends to the cannabinoid CB_1 receptor on cholinergic neurones and hence we studied whether its activation, pharmacological blockade, or genetic inactivation affects the function of other presynaptic inhibitory receptors. The electrically evoked acetylcholine or noradrenaline release was determined in superfused rodent tissues preincubated with ^{3}H-choline or ^{3}H-noradrenaline. The muscarinic M_2 receptor, $G\alpha_i$, and $G\alpha_o$ proteins were determined in hippocampal synaptosomes by Western blotting. Hippocampal anandamide and 2-arachidonoyl glycerol levels were determined by LC-MS/MS. The inhibitory effect of the muscarinic receptor agonist oxotremorine on acetylcholine release in hippocampal slices was increased by genetic CB_1 receptor ablation (mouse) and the CB_1 antagonist rimonabant (rat but not mouse) and decreased by a cannabinoid receptor agonist (mouse). In mouse tissues, CB_1 receptor ablation also increased the effect of a δ opioid receptor agonist on acetylcholine release in the hippocampus and the effect of oxotremorine on noradrenaline release in the vas deferens. CB_1 receptor ablation, to a very slight extent, increased $G\alpha_o$ protein levels without affecting either $G\alpha_i$ and M_2 receptor protein or the levels of anandamide and 2-arachidonoyl glycerol in the hippocampus. In conclusion, the CB_1 receptor shows an inhibitory interaction with the muscarinic and δ opioid receptor on cholinergic neurones in the rodent hippocampus and with the muscarinic receptor on noradrenergic neurones in the mouse vas deferens.

Keywords Cannabinoid CB_1 receptor · Muscarinic receptor · Cholinergic neurone · Presynaptic receptor interactions · Endocannabinoids · $G\alpha_i$ and $G\alpha_o$ proteins

Abbreviations

ACh	acetylcholine
5-CT	5-carboxamidotryptamine
$CB_1^{-/-}$ mouse	mouse with CB_1 receptor gene disruption
DAMGO	[D-Ala^2, N-Me-Phe^4, glycerol^5] -enkephalin
DPDPE	[D-Pen^{2,5}]-enkephalin
LC-MS/MS	liquid chromatography associated with tandem mass spectrometry
NA	noradrenaline
S_1, S_2	tritium overflow evoked by the first and second period of electrical stimulation, respectively
SEM	standard error of the mean

Kirsten Schulte and Nina Steingrüber contributed equally to this paper.

K. Schulte · N. Steingrüber · B. Jergas · A. Redmer · C. M. Kurz · E. Schlicker (✉)
Institute of Pharmacology and Toxicology, BMZ, University of Bonn, Sigmund-Freud-Str. 25,
53105 Bonn, Germany
e-mail: e.schlicker@uni-bonn.de

R. Buchalla · B. Lutz
Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University Mainz,
Duesbergweg 6,
55128 Mainz, Germany

A. Zimmer
Institute of Molecular Psychiatry, University of Bonn,
Sigmund-Freud-Str. 25,
53105 Bonn, Germany

Published online: 04 January 2012

t_1, t_2	basal tritium efflux quantified in the 5-min periods starting 15 min after the onset of S_1 and 5 min before the onset of S_2
TEA	tetraethylammonium
Wt	wild-type

Introduction

The cannabinoid receptor of the CB_1 subtype usually serves as a presynaptic receptor. It is located on axon terminals of neurones with a variety of neurotransmitters and leads to the inhibition of their release (Fig. 1; Schlicker and Kathmann 2001; Szabo and Schlicker 2005). A given neurone is equipped with a variety of inhibitory presynaptic receptors many of which, like the CB_1 receptor, are coupled to $G\alpha_{i/o}$ proteins (Fig. 1; Fuder and Muscholl 1995; Boehm and Kubista 2002; Kubista and Boehm 2006). The different types of $G\alpha_{i/o}$-coupled presynaptic receptors do not act independently of each other but rather previous activation of one receptor blunts the effect mediated by another receptor activated subsequently; by contrast, blockade of the first receptor increases the effect mediated by the second one. Such interactions have been frequently shown for presynaptic inhibitory receptors on noradrenergic neurones (for review, see Schlicker and Göthert 1998) but also occur on cholinergic neurones (Hiramatsu et al. 1998; Nishiwaki et al. 1998; Giaroni et al. 1999; Shakirzyanova et al. 2006). However, interactions of presynaptic CB_1 receptors with other presynaptic inhibitory receptors have so far been studied only rarely (Schlicker and Göthert 1998) and interactions of that type on cholinergic neurones have not been studied at all.

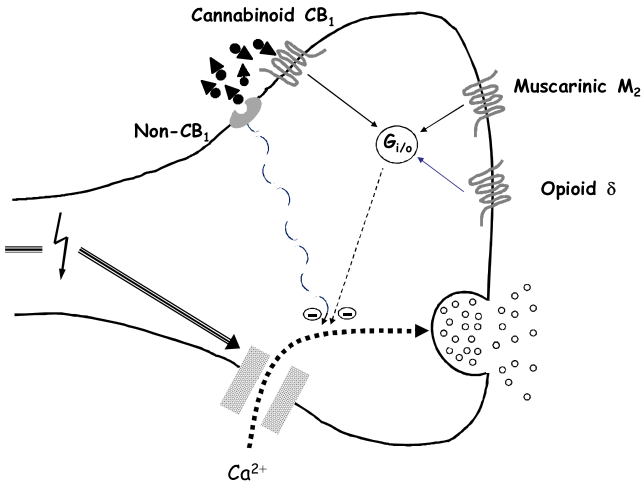

Fig. 1 Rationale of the present study. Shown is the schematic drawing of a nerve ending. An action potential invading the nerve ending leads to the opening of voltage-dependent Ca^{2+} channels and the subsequent fusion of vesicles with the synaptic membrane and exocytotic transmitter release. Transmitter release is inhibited by presynaptic inhibitory $G\alpha_{i/o}$ protein-coupled receptors. Different types of presynaptic receptors do not act independently but influence each other, e.g., in the present study, the M_2 receptor-mediated effect of oxotremorine was increased in hippocampal slices from mice lacking CB_1 receptors. Additional experiments were carried out in order to clarify whether this effect may be related to (a) an increase in M_2 receptor protein, (b) an increase in $G\alpha_{i/o}$ proteins, or (c) an alteration of endocannabinoids (*black structures*) which may act upon transmitter release in a CB_1 receptor-independent manner (note that endocannabinoids have pleiotropic effects as shown here by the *triangle* (CB_1) and the *circle* (non-CB_1)). A second aim of the study was to examine whether, in hippocampal slices from wild-type mice, blockade and activation of the CB_1 receptor increases and reduces the M_2 receptor-mediated effect, respectively. Third, we checked whether the receptor interaction is a more general phenomenon, i.e. also occurs when (a) opioid δ instead of M_2 receptors are considered and (b) a noradrenergically innervated tissue (vas deferens) is examined

Receptor interactions may come into play in patients receiving two drugs acting on different sets of presynaptic receptors. Such an interaction might occur when a patient is prescribed Sativex^R plus a cholinesterase inhibitor simultaneously. Sativex^R is indicated for the treatment of symptoms associated with multiple sclerosis (for review, see Sastre-Garriga et al. 2011) and Δ^9-tetrahydrocannabinol (one of the two components of this brand, the other being cannabidiol) activates presynaptic CB_1 receptors. Cholinesterase inhibitors are indicated for the treatment of paralytic ileus, myasthenia gravis, and Alzheimers's disease (for review, see Taylor 2001) and lead to an increase of acetylcholine levels at presynaptic muscarinic receptors.

The aim of the present study was to identify an interaction of the presynaptic inhibitory CB_1 and muscarinic receptors in isolated tissues from mice with CB_1 receptor gene disruption ($CB_1^{-/-}$ mouse generated by Zimmer et al. 1999). Since in hippocampal slices the inhibitory effect of a muscarinic receptor agonist on acetylcholine release (involving M_2 receptors; Zhang et al. 2002) was indeed increased, several series of experiments were done in order to further characterize this effect. First, we studied whether CB_1 receptor disruption influences the amount of the M_2 receptor and of the $G\alpha_i$ and $G\alpha_o$ proteins and the levels of the two major endocannabinoids anandamide and 2-arachidonoyl glycerol in the hippocampus. An increase in the M_2 receptor density would offer a particularly simple explanation for the phenomenon (Fig. 1). An increase in $G\alpha_i$ and $G\alpha_o$ proteins would be a plausible explanation as well since both the CB_1 and the M_2 receptors are coupled to these effectors and the possibility exists that the receptors share the same sets of G proteins (Fig. 1). Another explanation would be that an increase in the levels of the endocannabinoids (which are well-known for their pleiotropic effects) increases the M_2 receptor-mediated effect by a CB_1 receptor-independent mechanism. Second, we examined whether the M_2 receptor-mediated effect in hippocampal slices from wild-type mice is blunted by a cannabinoid receptor agonist and

increased by a CB_1 receptor antagonist, respectively. Third, we studied whether the effect mediated by another presynaptic inhibitory receptor in the same tissue and of the muscarinic receptor in another tissue is affected as well (Fig. 1).

Methods

Animals

Male Wistar rats (150–200 g) and male C57BL/6J mice (2–4 months old; wild-type mice) were purchased from Charles River (Sulzfeld, Germany). Male C57BL/6J mice with disrupted CB_1 receptor gene ($CB_1^{-/-}$ mice; 2–4 months old) were obtained from A. Zimmer (Institute of Molecular Psychiatry, University of Bonn, Germany). Animals were housed in the animal facilities of our department in a temperature- and humidity-controlled environment under a 12-h dark–light cycle with food and water available ad libitum.

Drugs and chemicals used

[Methyl-^{3}H]-choline chloride (specific activity 70.3 Ci/mmol), (R)-(−)-[ring-2,5,6-^{3}H]-noradrenaline (specific activity 53 Ci/mmol; PerkinElmer, Zaventem, Belgium); AF-DX 384 (5,11-dihydro-11-{[(2-[(dipropylamino)methyl]-1-piperidinyl}ethyl)amino]carbonyl}-6H-pyrido(2,3-β1)-(1,4)benzodiazepine-6-one) (Boehringer-Ingelheim, Biberach an der Riss, Germany); desipramine hydrochloride (Novartis, Wehr, Germany); hemicholinium-3, (−)-noradrenaline, oxotremorine sesquifumarate, tetraethylammonium chloride, U-69,593 ((+)-(5α,7α,8β)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide), WIN 55,212-2 (R-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]-pyrrolo[1,2,3-de]1,4-benzoxazinyl](1-naphthalenyl) methanone mesylate) (Sigma Aldrich, München, Germany); 5-carboxamidotryptamine (Tocris, Ballwin, MO, USA); DAMGO ([D-Ala^2, N-Me-Phe^4, glycerol^5]-enkephalin), naltrindole hydrochloride (Bachem, Weil am Rhein, Germany); rauwolscine hydrochloride (Roth, Karlsruhe, Germany); DPDPE ([D-Pen^{2,5}]-enkephalin) (Biotrend, Köln, Germany); rimonabant (Sanofi, Montpellier, France); and sulprostone (Bayer Schering Pharma, Berlin, Germany). Stock solutions of the drugs were prepared with ascorbic acid solution 0.1% ((−)-noradrenaline), dimethyl sulfoxide (rimonabant, WIN 55,212-2), ethanol (sulprostone), or water and diluted with physiological salt solution (see later) to the concentration required. The solvents did not affect basal and evoked tritium outflow by themselves.

For the endocannabinoid determinations, anandamide (AEA), 2-arachidonoyl glycerol (2-AG), and arachidonic acid (AA), and their deuterated analogues AEA-d_4, 2-AG-d_5, and AA-d_8 were obtained from Cayman Chemicals (Ann Arbor, MI, USA). Water (H_2O), acetonitrile, formic acid, ethylacetate, and hexane (all of Fluka LC-MS grade) were obtained from Sigma Aldrich. All stock solutions, intermediate dilutions, and calibration standards were made up with acetonitrile at appropriate concentration levels.

Superfusion studies

Rats, wild-type, and $CB_1^{-/-}$ mice were sacrificed by decapitation and hippocampal, striatal slices (0.3 mm thick, diameter 2 mm), cortex slices (0.3 mm thick, diameter 3 mm), and/or vas deferens pieces (dimensions about 1×1×1 mm) were prepared. Tissues were then incubated (37°C) with physiological salt solution (PSS; Ca^{2+} 1.3 mM) containing ^{3}H-choline 0.1 μM (hippocampal and striatal slices) or ^{3}H-noradrenaline 0.025 μM (vas deferens pieces and cortex slices). Incubation time was 30 min for experiments with brain slices and 60 min for experiments with vas deferens pieces. Subsequently, the preparations were transferred to superfusion chambers and superfused for 110 min at a flow rate of 1 mL/min with PSS (37°C). Ca^{2+} concentration was 1.3 mM for experiments on superfused cortex slices and 3.25 mM for experiments on the other tissues.

The PSS had the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3 or 3.25 (see above), KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, ascorbic acid 0.06, disodium EDTA 0.03, and glucose 10; the solution was aerated with 95% O_2 and 5% CO_2 (pH 7.4). The superfusate was collected in 5-min samples. Tritium overflow was evoked by two 2 min periods of electrical field stimulation after 40 and 90 min of superfusion (S_1 and S_2). Stimulation parameters were 3 Hz, 50 mA, and 2 ms for cortex slices and 3 Hz, 200 mA, and 2 ms for the other tissues. The electrically evoked tritium overflow in hippocampal and striatal slices preincubated with ^{3}H-choline represents quasi-physiological acetylcholine release (Kathmann et al. 1999, 2001a, b) and the evoked tritium overflow in cortex slices and vas deferens pieces represents quasi-physiological noradrenaline release (Schlicker et al. 1992, 2003).

The PSS used for superfusion experiments with ^{3}H-noradrenaline routinely contained an inhibitor of the neuronal noradrenaline transporter, desipramine 1 μM, and an α_2-adrenoceptor antagonist, rauwolscine 1 μM. Hemicholinium 10 μM was present throughout superfusion in all experiments with ^{3}H-choline to block the high-affinity choline uptake. The auxiliary drugs were used to increase the amount of tritium overflow evoked by electrical stimulation and to avoid interference of test drugs with the respective transporters or receptors. One additional reason why rauwolscine was used in the experiments on vas deferens pieces

is that the drug increases the inhibitory effect of oxotremorine on noradrenaline release (Schlicker and Göthert 1998).

The agonists studied were added to the PSS from 62 min of superfusion onwards. When the interaction of an agonist with an antagonist was studied, the latter was present in the PSS throughout superfusion whereas the former was added from 62 min onwards. In those experiments in which the interaction of the cannabinoid receptor agonist WIN 55,212-2 with the muscarinic receptor agonist oxotremorine was examined, WIN 55,212-2 was present in the PSS throughout superfusion whereas oxotremorine was added from 62 min of superfusion onwards.

Tritium efflux was calculated as the fraction of the tritium content in the tissues 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 (for drugs added to the PSS from 62 min of superfusion onward) or the t_1 values obtained in the absence or presence of a given drug were directly compared with each other (for drugs present in the PSS throughout superfusion). Stimulation-evoked tritium overflow was calculated by subtraction of basal from total efflux during stimulation and the subsequent 13 min and expressed as per cent of the tritium present in the tissue at the onset of stimulation (basal 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; for drugs added to the PSS from 62 min of superfusion) or the S_1 values obtained in the absence or presence of a given drug were directly compared with each other (for drugs present throughout superfusion).

Preparation of mouse hippocampal synaptosomes

Hippocampal tissue from 4 wild-type or $CB_1^{-/-}$ mice was homogenized with a Potter–Elvehjem Teflon–glass homogenizer (10 up-and-down strokes at 800 rpm during 1 min) in 2 ml of ice-cold Tris–HCl buffer (Tris 50 mM, pH 7.5; EDTA 5 mM) containing sucrose 10.27% and centrifuged at 1,000×*g* for 10 min (4°C) to remove debris. The synaptosomes were isolated from the supernatant by centrifugation at 12,000×*g* for 25 min (4°C) and were washed once. The resulting pellet was resuspended in 1.6 mL (sucrose-free) ice-cold Tris–HCl buffer and centrifuged again at 12,000×*g* for 15 min (4°C). The synaptosomal pellet was then resuspended in 400 μL (sucrose-free) ice-cold Tris–HCl buffer, snap-frozen and stored at −80°C. The protein content was determined using the Bradford method (Bradford 1976).

Western blot analysis

Ten (for detection of $G\alpha_i$ and $G\alpha_o$ proteins) and 40 μg (for detection of M_2 receptor protein) protein equivalents were mixed with a reducing protein loading buffer and heated at 97°C for 5 min and at 70°C for 10 min, respectively. Samples were separated on 10% sodium dodecyl sulfate (SDS) polyacrylamide gels using the Laemmli buffer system (0.1% SDS, 25 mM Tris, 1.44% glycine) and transferred (Laemmli buffer with 20% methanol) to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Blots were blocked in 5% dried milk protein Tris-buffered saline containing Tween (140 mM NaCl, 10 mM Tris, 0.1% Tween 20). M_2 receptor, $G\alpha_i$, and $G\alpha_o$ proteins were detected by use of goat anti-mouse mAChR M_2 (C-18), rabbit anti-mouse $G\alpha_{i1-3}$ (C-10), and rabbit anti-mouse $G\alpha_o$ (K-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA) polyclonal antibodies. Equal protein loading was checked by mouse monoclonal anti-mouse α-tubulin (DM1A; Dianova, Hamburg, Germany) antibodies. Bands were visualized by peroxidase-conjugated secondary donkey anti-goat (Chemicon, Schwalbach, Germany), goat anti-rabbit (Cell Signaling, Danvers, MA, USA), and goat anti-mouse (Dianova) antibodies employing Amersham enhanced chemiluminescence (ECL) Western Blotting Detection Reagents (GE Healthcare, München, Germany). All bands were quantified by densitometric analysis with QuantityOne software (BioRad, München, Germany).

Endocannabinoid measurements by LC-MS/MS

The isolated hippocampi were stored at −80°C until extraction. Samples were weighed into 2 mL centrifuge tubes, spiked with 50 μL acetonitrile containing the internal standards and homogenized in 500 μL ice-cold 0.1 M formic acid with a 5-mm-steel ball using the TissueLyser II (Qiagen, Hilden, Germany) for 1 cycle of 30 s at 30 Hz. Ethylacetate/hexane (500 μL; 9:1, *v*/*v*) were added to extract the homogenate (for 10 s at 30 Hz), then the tubes were centrifuged for 10 min at 10,000×*g* and 4°C, the upper (organic) phase was removed, evaporated to dryness under a gentle stream of nitrogen at 37°C and re-dissolved in 500 μL acetonitrile. Analyses were performed on a LC-MS/MS system consisting of a 5500 QTrap triple-quadrupole linear ion trap mass spectrometer equipped with a Turbo V Ion Source (AB SCIEX, Darmstadt, Germany), an Agilent 1200 series LC system (degasser, pump and thermostatted column compartment; Agilent, Waldbronn, Germany) and a CTC HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland).

Endocannabinoids were separated with a Phenomenex Luna 2.5 μm C18(2)-HST column, 100×2 mm, combined with a SecurityGuard pre-column (C18, 4×2 mm; Phenomenex, Aschaffenburg, Germany) with solvents A (0.1%

formic acid in 20:80 acetonitrile/water, *v*/*v*) and B (0.1% formic acid in acetonitrile), using the following gradient: 55–90% B (0–2 min), then held at 90% B (2–7.5 min) and re-equilibrated at 55% B (7.5–10 min). The column temperature was 25°C, the flow rate was 0.3 mL/min, and the injection volume was 10 μL. Positive and negative ions were analyzed simultaneously by combining two experiments in "positive-negative-switching" mode. The Turbo V Ion Source was operated with the electrospray ("TurboIon") probe with nitrogen as curtain and nebulizer gas and using the following settings: temperature 550°C, curtain gas 40 psi, GS1 50 psi, GS2 50 psi, and capillary voltage −4,500 V (negative) and +4800 V (positive). The following precursor-to-product ion transitions were used for multiple-reaction monitoring (MRM): Experiment 1 (positive)—AEA *m*/*z* 348.3→62.1, AEA-d_4 *m*/*z* 352.3→66.1, 2-AG *m*/*z* 379.1→287.2, 2-AG-d_5 *m*/*z* 384.2→287.2; Experiment 2 (negative)—AA *m*/*z* 303.1→259.1, AA-d_8 311.0→267.0. Dwell times were 20 ms in Experiment 1 and 50 ms in Experiment 2; pause between MRM transitions was 5 ms and settling time between Experiments 1 and 2 was 50 ms. Data acquisition and analysis were performed using Analyst software (version 1.5.1; AB SCIEX).

Statistics and calculations

Results are given as means±standard error of the mean of *n* experiments. Student's *t* test was used for comparison of mean values; the Bonferroni correction was used when two or more values were compared to the same control. The potency (pEC_{50}) of oxotremorine was determined as the concentration leading to 50 % of its effect at 10 μM since at the latter concentration the maximum inhibition is obtained (Kathmann et al. 2001a). For DPDPE a concentration of 1 μM was considered instead since at that concentration its maximum inhibitory effect occurs (see later, Fig. 3). Apparent pA_2 values were determined according to the formula $pA_2 = \log([A']/[A]-1) - \log[B]$, where [A′] and [A] are the EC_{50} values for the agonist obtained in the presence and absence of the antagonist under study, respectively, and [B] is the concentration of the antagonist (Furchgott 1972).

Results

Superfusion studies

Basal tritium overflow was expressed as t_1 or t_2/t_1. The t_1 value, given as fraction of tissue tritium, was 0.0033±0.0002 and 0.0054±0.0006 min^{-1} in 10 mouse hippocampal and 8 striatal slices preincubated with ^{3}H-choline, respectively, and 0.0093±0.0009 and 0.0049±0.0004 min^{-1} in 10 mouse cortical slices and 10 vas deferens tissue pieces preincubated with ^{3}H-noradrenaline, respectively. The t_1 value in 10 rat hippocampal slices preincubated with ^{3}H-choline was 0.0026±0.0002 min^{-1}. Values were not altered by CB_1 receptor disruption or the drugs under study (not shown). The t_2/t_1 value was close to 0.8 in all experiments of the present study (not shown).

The electrically evoked tritium overflow was expressed either as S_1 or S_2/S_1. S_1 was used for quantification of the effects of CB_1 receptor disruption, of antagonists and of WIN 55,212-2 (present in the medium throughout superfusion). S_2/S_1 was used to quantify the effects of agonists (present in the medium before and during S_2 only; with the exception of WIN 55,212-2, see above). The alterations of the electrically evoked tritium overflow under the influence of CB_1 receptor disruption and various drugs will be described in the subsequent paragraphs. For the sake of simplicity, the term "^{3}H-acetylcholine release" will be used instead of "electrically evoked tritium overflow from slices preincubated with ^{3}H-choline"; in an analogous manner, "^{3}H-noradrenaline release" will be used instead of "electrically evoked tritium overflow from tissues preincubated with ^{3}H-noradrenaline."

The effect of the muscarinic receptor agonist oxotremorine on ^{3}H-acetylcholine release was compared in hippocampal slices from wild-type and $CB_1^{-/-}$ mice. Oxotremorine inhibited ^{3}H-acetylcholine release (S_2/S_1) in a concentration-dependent manner with pEC_{50} values of 6.2±0.1 and 6.1±0.1, respectively (values not significantly different). The two lower concentrations of oxotremorine did not show differences between the two mouse strains whereas oxotremorine 10 μM had a more marked inhibitory effect in slices from $CB_1^{-/-}$ animals when compared to slices from wild-type mice (Figs. 2 and 4a). The muscarinic M_2/M_4 receptor

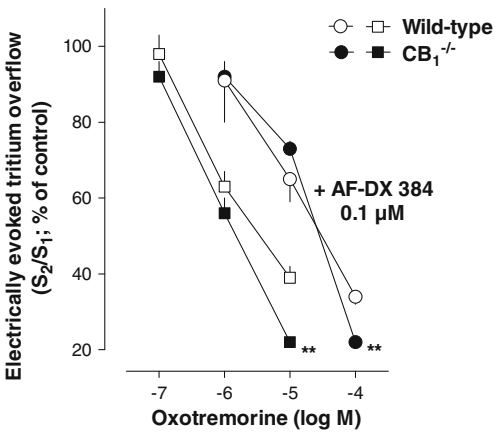

Fig. 2 Influence of CB_1 receptor disruption on the effect of oxotremorine on the electrically evoked tritium overflow from mouse hippocampal slices preincubated with ^{3}H-choline (^{3}H-acetylcholine release). Tritium overflow was evoked after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was formed. Oxotremorine was present in the medium before and during S_2 whereas in part of the experiments AF-DX 384 was present throughout superfusion. Means±SEM of 7–20 experiments. **$P<0.01$, compared with the corresponding value in slices from wild-type mice

antagonist AF-DX 384 0.1 μM shifted to the right the concentration–response curve of oxotremorine in slices from wild-type and $CB_1^{-/-}$ mice, yielding apparent pA_2 values of 8.0±0.2 and 8.3±0.1, respectively (values not significantly different). In this series of experiments, again the two lower concentrations of oxotremorine did not differ whereas 100 μM, its highest concentration, showed a stronger inhibitory effect in slices from $CB_1^{-/-}$ when compared to slices from wild-type mice (Figs. 2 and 4a). CB_1 receptor disruption per se doubled the amount of ^{3}H-acetylcholine release (Table 1). AF-DX 384 0.1 μM by itself increased ^{3}H-acetylcholine release by about 40 % in hippocampal slices of either mouse strain (Table 1).

Next we were interested whether CB_1 receptor disruption also increases the effect of an agonist acting at a $G\alpha_{i/o}$ protein-coupled inhibitory receptor other than the M_2 receptor. Since the knowledge about such receptors on cholinergic hippocampal neurones from mice is very limited, we searched whether high concentrations of six agonists known to inhibit transmitter release in tissues from the mouse (Table 2) do the same with respect to ^{3}H-acetylcholine release in the hippocampus. Only the δ opioid receptor agonist DPDPE inhibited ^{3}H-acetylcholine release (S_2/S_1; Fig. 3, inset). Its maximum inhibitory effect amounted to about 50% (pEC_{50} 7.8) and the δ opioid receptor antagonist naltrindole 0.32 nM shifted to the right the concentration-response curve of DPDPE, yielding an apparent pA_2 value of 10.7 (Fig. 3).

The effect of DPDPE on ^{3}H-acetylcholine release (S_2/S_1) was compared in hippocampal slices from wild-type and $CB_1^{-/-}$ mice. Since the effect of oxotremorine was different for the highest concentrations only (Fig. 2), DPDPE was used at 1 μM, a concentration leading to the maximum inhibitory effect (Fig. 3). Moreover, the extent of inhibition is depicted in columns; Fig. 4b shows that the effect of DPDPE was more marked in hippocampal slices from $CB_1^{-/-}$ than in slices from wild-type mice. This also held true when the inhibitory effect of DPDPE 10 μM was studied in the presence of naltrindole 0.32 nM (Fig. 4b). Naltrindole by itself did not affect the ^{3}H-acetylcholine release (S_1; Table 1).

In addition, the effect of selected ligands (used at maximally active concentrations) on ^{3}H-noradrenaline or ^{3}H-acetylcholine release (S_2/S_1) was examined in tissues apart from the hippocampus. Oxotremorine inhibited ^{3}H-noradrenaline release more markedly in vas deferens tissues from $CB_1^{-/-}$ mice than in tissues from wild-type animals (Fig. 4c). On the other hand, the inhibitory effect of oxotremorine on ^{3}H-acetylcholine release from striatal slices and the inhibitory effect of (unlabelled) noradrenaline on ^{3}H-noradrenaline release in cortical slices did not differ between tissues from either mouse strain (Fig. 4d). CB_1

Table 1 Amount of tritium evoked by electrical stimulation in brain slices and vas deferens tissue pieces preincubated with ^{3}H-choline or ^{3}H-noradrenaline and influence of species, CB_1 receptor disruption, and various drugs

				Electrically evoked tritium overflow (S_1)[a]	
Species	Tissue	Transmitter	Drug(s)	Wild-type	$CB_1^{-/-}$
Rat	Hippocampus	^{3}H-choline		1.87±0.14	ND
			+ Rimonabant 1 μM	2.67±0.17**	ND
Mouse	Hippocampus			2.06±0.23[b]	4.19±0.28*
			+ AF-DX 384 0.1 μM	2.82±0.33	5.93±0.33*, **
				4.23±0.50[b]	6.23±0.74*
			+ Naltrindole 0.00032 μM	3.80±0.60	6.00±0.60*
				4.47±0.53[b]	ND
			+ Rimonabant 1 μM	5.64±0.76	ND
			+ TEA 3,200 μM	6.97±1.23	ND
			+ TEA 3,200 μM + WIN 55,212-2 1 μM	2.79±0.25**	ND
			+ TEA 3,200 μM + rimonabant 1 μM	8.21±1.60	ND
	Striatum			2.67±0.74	3.94±0.67
	Vas deferens	^{3}H-noradrenaline		2.37±0.35	4.99±0.50*
	Cerebral cortex			10.33±0.84	10.14±1.14

ND not determined

Means±SEM of 28 experiments (rat hippocampus) and of 6–13 experiments (mouse tissues). *P<0.05, compared to wild-type; **P<0.01, compared to the corresponding value without the drug under study

[a] The first period of electrical stimulation (S_1) was administered 40 min after the onset of superfusion

[b] The amount of S_1 significantly varied in these three series which were carried out under identical conditions although on different occasions

Table 2 Presynaptic inhibitory receptors other than M_2 and CB_1 receptors identified in various tissues of the mouse

Receptor	Tissue	Neurone	Agonist	Reference
μ opioid	Cortex	Noradrenergic	DAMGO 1 μM	Trendelenburg et al. (2000)
δ opioid	Vas deferens		DPDPE 1 μM	
κ opioid	Vas deferens		U-69,593 1 μM	
α_2-Adrenoceptor	Cortex	Noradrenergic	Noradrenaline 1 μM	Schlicker et al. (1994)
[a]Serotonin 5-HT_{1B}	Cortex	Serotoninergic	5-Carboxamidotryptamine 10 μM	Own unpublished results
Prostaglandin EP_3	Cortex	Noradrenergic	Sulprostone 0.1 μM	Günther et al. (2010)

The table shows the receptors, their locations, and the concentrations of agonists causing the maximum effect at the respective receptors

[a] In the study by Rutz et al. (2006), a presynaptic 5-HT_{1B} receptor could also be identified on *cholinergic* neurones of the mouse cortex

receptor disruption increased ^{3}H-noradrenaline overflow in vas deferens pieces without affecting ^{3}H-acetylcholine release in striatal slices and ^{3}H-noradrenaline release in cortical slices (S_1; Table 1).

Finally, the effect of oxotremorine 10 μM on ^{3}H-acetylcholine release (S_2/S_1) was studied under the influence of the cannabinoid receptor agonist WIN 55,212-2 and the CB_1 receptor antagonist rimonabant in hippocampal slices from wild-type mice and/or from rats (Fig. 5). Since WIN 55,212-2 at 1 μM markedly decreased ^{3}H-acetylcholine release (S_1) by itself and quantification of results was impossible (results not shown), the appropriate experiments were carried out in the presence of the K^+ channel blocker tetraethylammonium (TEA). In the presence of TEA, WIN 55,212-2 markedly decreased the inhibitory effect of oxotremorine (Fig. 5a). The interaction of oxotremorine with rimonabant 1 μM was examined both in the presence (Fig. 5a) and absence of TEA (Fig. 5b). Under both conditions, the effect of oxotremorine was not affected by rimonabant; the latter, by itself, tended to increase ^{3}H-acetylcholine release by about 20% but the effect did not reach a statistically significant level (Table 1). The interaction of oxotremorine with the same concentration of rimonabant was also studied in hippocampal slices from *rats*; in this series of experiments, rimonabant increased the inhibitory effect of oxotremorine (Fig. 5c). Rimonabant by itself significantly increased ^{3}H-acetylcholine release (S_1) by 43 % (Table 1). The augmentation of the inhibitory effect of oxotremorine by rimonabant was also observed when those experiments were considered only in which ^{3}H-acetylcholine release (S_1) was as low as in all rimonabant-free experiments (Fig. 5c).

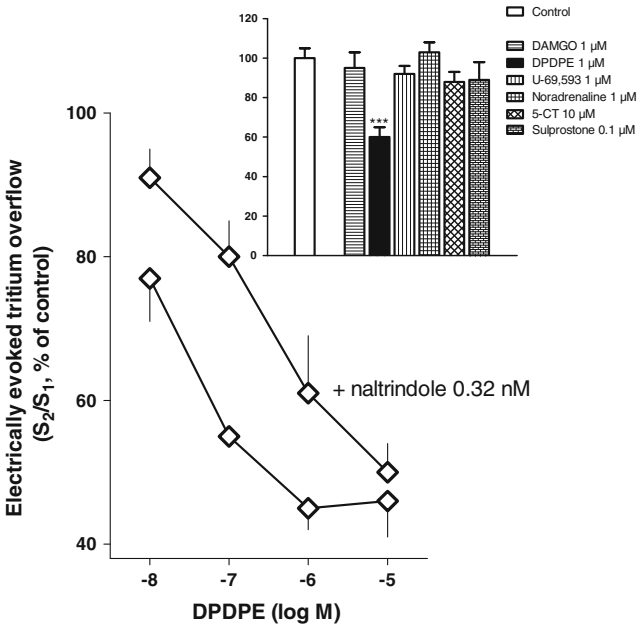

Fig. 3 Effect of DPDPE and of agonists at other presynaptic inhibitory receptors (*inset*) on the electrically evoked tritium overflow from wild-type mouse hippocampal slices preincubated with ^{3}H-choline (^{3}H-acetylcholine release). Tritium overflow was evoked after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was formed. DPDPE or another agonist was present in the medium before and during S_2 whereas in part of the experiments with DPDPE naltrindole was present throughout superfusion. A synopsis between agonists and their respective receptors is given in Table 2. Means±SEM of 4–5 experiments (concentration–response curves) and of 7–9 experiments (*inset*). ***P<0.001, compared with the control

Western blots

The influence of CB_1 receptor disruption on M_2 receptor protein and on $G\alpha_i$ and $G\alpha_o$ proteins in the hippocampus was determined with Western blots. Synaptosomes were used instead of whole tissue in order to have a preparation rich in nerve endings; α-tubulin was used as a reference protein. Figure 6 shows that M_2 receptor protein does not differ between wild-type and $CB_1^{-/-}$ mice. The density of the $G\alpha_o$ protein is significantly higher (by 15%) for $CB_1^{-/-}$ as compared with wild-type mice; for the $G\alpha_i$ proteins, there is only a tendency in this direction.

LC-MS/MS determinations

The levels of the two endocannabinoids anandamide and 2-arachidonoyl glycerol and of arachidonic acid were determined in native hippocampal tissue of wild-type and $CB_1^{-/-}$

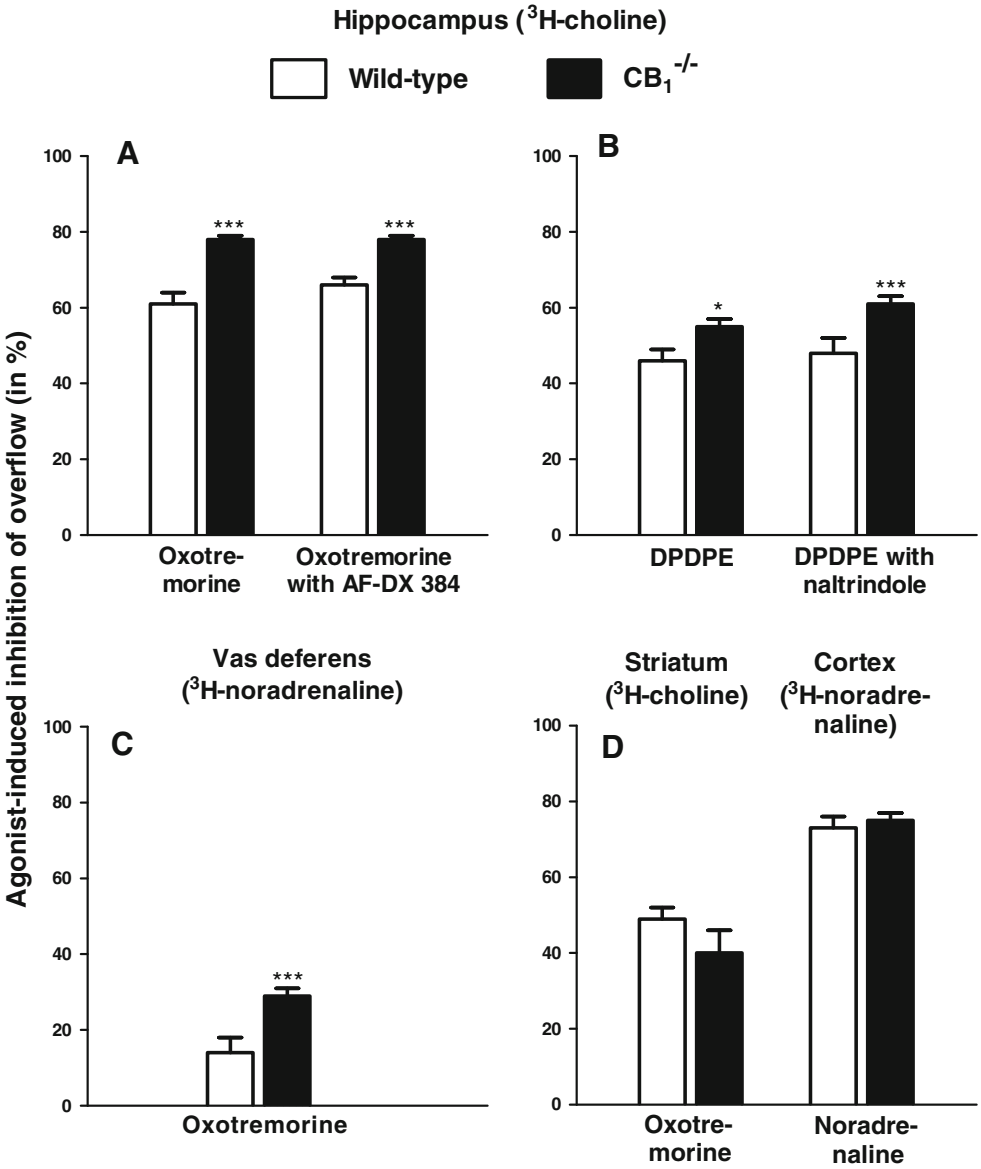

Fig. 4 Effect of CB_1 receptor disruption on the extent of inhibition of ^{3}H-transmitter release obtained with three agonists. Brain slices and vas deferens pieces were prepared from the mouse and then preincubated with ^{3}H-choline or ^{3}H-noradrenaline (as indicated in the four panels). Tritium overflow was evoked electrically to induce ^{3}H-acetylcholine and ^{3}H-noradrenaline release, respectively. Tritium overflow was evoked 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 formed. The agonist under study was present in the medium before and during S_2 whereas the $M_{2/4}$ receptor antagonist AF-DX 384 0.1 μM (**a**) or the δ opioid receptor antagonist naltrindole 0.32 nM (**b**) was present throughout superfusion. The muscarinic receptor agonist oxotremorine was used at 10 μM (or at 100 μM in the presence of AF-DX 384); the δ opioid receptor agonist DPDPE at 1 μM (or at 10 μM in the presence of naltrindole); noradrenaline at 0.32 μM. The effect of each agonist (oxotremorine, DPDPE or noradrenaline) is depicted as $100 \times (1-S_2/S_1$ of agonist divided by S_2/S_1 of control). Means ±SEM of 7–20 experiments. $^*P<0.05$, $^{***}P<0.001$, compared to wild-type

mice. The levels of the three lipids did not differ between both genotypes (Table 3).

Discussion

The aim of the present study was to examine whether alteration of the cannabinoid CB_1 receptor (genetic disruption and activation or blockade of the intact receptor) alters the function of other presynaptic inhibitory receptors. This was studied for the presynaptic muscarinic autoreceptor, leading to inhibition of acetylcholine release in the hippocampus. Our results show that CB_1 receptor disruption increased the maximum inhibitory effect of oxotremorine, considered in the absence or presence of AF-DX 384. This phenomenon was further examined in three additional series of experiments.

First, experiments were carried out in order to clarify the mechanism leading to the increased function of the hippocampal muscarinic autoreceptor in $CB_1^{-/-}$ mice. Thus, the increased maximum effect may be related to an increase in the protein of the muscarinic autoreceptor. The latter is mainly a M_2 receptor in the mouse hippocampus (Fig. 1; Zhang et al. 2002). For this reason, M_2 receptor content was quantified by Western blots; synaptosomes, i.e., isolated nerve endings (Raiteri and Raiteri 2000), were used as preparation rather than whole tissue. However, there was no difference in the M_2 receptor protein between both genotypes.

Next, the possibility had to be considered that an alteration in the transduction machinery behind the level of the muscarinic autoreceptor can account for the observed phenomenon. It is an attractive hypothesis that different types of presynaptic inhibitory receptors on a given axon terminal share a common set of G proteins (Fig. 1; for review, see

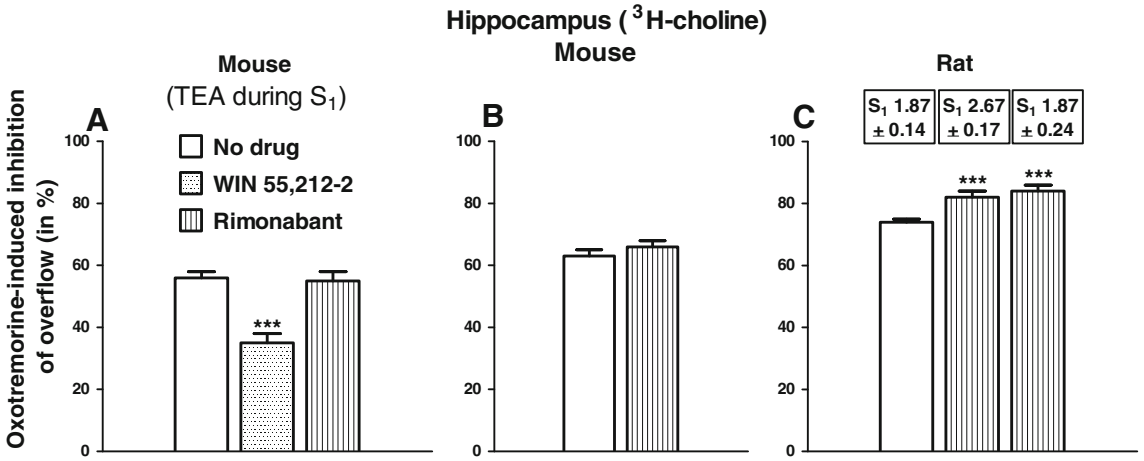

Fig. 5 Effect of CB_1 receptor activation or blockade on the extent of inhibition of ^{3}H-acetylcholine release obtained with oxotremorine. Hippocampal slices from wild-type mice or from rats were preincubated with ^{3}H-choline. Tritium overflow was evoked electrically after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was formed. Oxotremorine 10 μM was present in the medium before and during S_2 whereas the cannabinoid receptor agonist WIN 55,212-2 1 μM or the CB_1 receptor antagonist rimonabant 1 μM was present throughout superfusion. The effect of oxotremorine is depicted as 100 × (1−S_2/S_1 of oxotremorine divided by S_2/S_1 of control). In the experiments of **a**, tetraethylammonium (TEA) 3.2 mM was present during S_1. For the experiments of **c** (*second column*), all values were used; whereas for the values of **c** (*third column*), only the 10 values with the lowest S_1 were considered. Means±SEM of 6–13 (**a**), 22–26 (**b**), 19–22 (**c**, *first* and *second column*) and 10 experiments (**c**, *third column*). ***$P<$ 0.001, compared to the respective group without CB_1 receptor activation or blockade

Schlicker and Göthert 1998). In detail, the effect mediated by one type of inhibitory receptor is blunted when another inhibitory receptor coupled to the same G proteins has been activated beforehand. Conversely, the total lack of a receptor acting on G proteins may have the consequence that the G proteins are exclusively available to other presynaptic inhibitory receptors. We have examined the possible alteration of G proteins by Western blots using two antibodies, one against the three types of $G\alpha_i$ proteins and one against $G\alpha_o$ protein; experiments were again carried out in synaptosomes. Our results show, to the best of our knowledge for the first time, that CB_1 receptor disruption increases the amount of $G\alpha_{i/o}$ proteins. One may wonder whether this effect, which is admittedly very small and became significant for the $G\alpha_o$ protein only, can explain the increased M_2 receptor function observed in our study. On the other hand, one has to consider the possibility that a re-distribution of G proteins within the membrane may occur although the total amount does not change (Zhang and Rasenick 2010). In the latter study, a re-distribution of G proteins between lipid raft and non-raft fractions has been shown following chronic treatment of C6 cells with the selective serotonin reuptake inhibitor escitalopram.

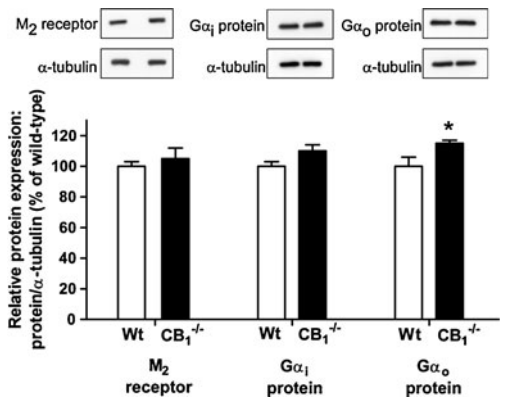

Fig. 6 Western blots for M_2 receptor, $G\alpha_i$, and $G\alpha_o$ protein in hippocampal synaptosomes from wild-type (Wt) and $CB_1^{-/-}$ mice. The change in each protein was estimated by using its corresponding α-tubulin as endogenous control. Ratios from $CB_1^{-/-}$ mice are given as per cent of the corresponding ratios from wild-type animals. Representative Western blots and the means±SEM of 6–7 experiments from 3 (M_2) or 6 (G proteins) membrane preparations are shown. *$P<0.05$, compared to wild-type mice

In this context, the possibility has to be considered that the muscarinic autoreceptors in the wild-type mice possess spare receptors. So, muscarinic and CB_1 receptors may share the same G proteins and absent CB_1 receptors might loan G proteins for previous muscarinic spare receptors, possibly

Table 3 Levels of endocannabinoids and of arachidonic acid in the hippocampus of wild-type and $CB_1^{-/-}$ mice (means±SEM of four experiments each)

	Anandamide pmol/g	2-Arachidonoyl glycerol nmol/g	Arachidonic acid nmol/g
Wild-type mouse	18.3±2.1	10.2±0.5	453±32
$CB_1^{-/-}$ mouse	21.2±1.9	11.4±0.8	509±30

diminishing a previous muscarinic receptor reserve but increasing the functional response upon receptor activation due to a higher availability of G proteins. In order to prove the role of spare receptors a comparison of the concentration-response curves of oxotremorine in transmitter release and radioligand binding studies were necessary. The findings from the present study, namely that the potency of oxotremorine did not differ in slices from $CB_1^{-/-}$ and wild-type mice, speak against muscarinic spare receptors. It is also of interest that the potency of the muscarinic $M_{2/4}$ receptor antagonist AF-DX 384 at the muscarinic autoreceptor did not differ between either mouse strain.

A further explanation for the increased maximum effect of oxotremorine in $CB_1^{-/-}$ mice might be that CB_1 receptor disruption leads to an alteration of the levels of the endogenously formed cannabinoids in the hippocampus. The endocannabinoids and in particular anandamide are well-known for their pleiotropic effects (Fig. 1; for review, see Pertwee et al. 2010). However, the fact that the hippocampal levels of anandamide and of 2-arachidonoyl glycerol were not different speaks against the hypothesis that a non-CB_1 receptor-mediated effect can account for the increased effect via the M_2 autoreceptor. Arachidonic acid was not affected either and this argues against the possibility that pathways fuelled by this fatty acid (e.g., formation of prostanoids and leukotrienes) can explain the differences between the two mouse strains with respect to the M_2 receptor.

Second, we examined whether an alteration of the M_2 autoreceptor function can also be shown if the CB_1 receptor is intact and activated or blocked by an appropriate agonist and antagonist, respectively. When the CB_1 receptor was activated by WIN 55,212-2 in hippocampal slices from wild-type mice, the inhibitory effect via the M_2 autoreceptor was indeed decreased. Since WIN 55,212-2 by itself has a marked inhibitory effect on acetylcholine release and under this condition quantification of the results is impossible, these experiments were carried out in the presence of the K^+ channel blocker TEA during the first period of electrical stimulation (S_1). The latter drug causes an increase in the intraneuronal Ca^{2+} concentration and this in turn leads to a more marked transmitter release (Schulte et al. 2010).

When the CB_1 receptor was blocked by rimonabant, the M_2 autoreceptor-mediated effect was not affected, regardless of whether the experiments were carried out with or without TEA. Under both experimental conditions, rimonabant by itself tended to increase acetylcholine release by about 20 %. When experiments of the same type were performed on hippocampal slices from *rats*, the effect via the muscarinic autoreceptor was, however, increased. In this experimental series, rimonabant by itself increased acetylcholine release by 40 % and it is very plausible that the stronger effect can be explained by the species difference. The increase in acetylcholine release may either reflect the interruption of a tonical inhibitory effect exerted by endocannabinoids, an inverse agonistic effect by rimonabant or both. Regardless of the mechanism(s), it is plausible that the interaction with the muscarinic autoreceptor comes into play only if the effect of rimonabant exceeds a minimum level.

It has already been mentioned that rimonabant and WIN 55,212-2 as well as CB_1 receptor ablation have effects of their own on acetylcholine release and one may argue that the extent of acetylcholine release is the simple explanation for the receptor interactions suggested here. This possibility, however, can be discarded. Thus, in hippocampal slices from rats the augmentation of the autoreceptor-mediated inhibition of acetylcholine release by rimonabant occurred also when experiments with a low level of acetylcholine release were selected (compare the third with the second column in Fig. 5c). Moreover, the extent of inhibition of acetylcholine release by oxotremorine in hippocampal slices from wild-type mice was nearly identical in the experiments of Figs. 4a and 5b although the level of acetylcholine release in the two series (which were performed under identical conditions but on different occasions) differred by a factor of more than 2 (Table 1).

Third, the question arose whether an increase in effect can also be shown for another presynaptic inhibitory receptor at the hippocampal cholinergic neurone of $CB_1^{-/-}$ mice (Fig. 1). There is very limited information in the literature with respect to the occurrence of presynaptic inhibitory receptors on hippocampal cholinergic neurones of the mouse other than CB_1 and M_2 receptors and for this reason

Table 4 Receptors considered in the present study and their interactions with the presynaptic cannabinoid CB_1 receptor

Receptor	Location	Transmitter	Type of receptor	Interaction with CB_1 receptor	Fig.
Muscarinic	Hippocampus	ACh	Autoreceptor	Yes	2, 4a, 5a–c
Muscarinic	Striatum	ACh	Autoreceptor	No	4d
Muscarinic	Vas deferens	NA	Heteroreceptor	Yes	4c
δ opioid	Hippocampus	ACh	Heteroreceptor	Yes	4b
α_2-Adrenoceptor	Cerebral cortex	NA	Autoreceptor	No	4d

ACh acetylcholine, *NA* noradrenaline

we searched for such receptors. Among six possible candidates (Fig. 3 and Table 2), only δ opioid receptor activation leads to inhibition of acetylcholine release; the evidence is based on experiments with a selective δ opioid receptor agonist and antagonist. Since our results with the M_2 receptor had revealed that a difference between wild-type and $CB_1^{-/-}$ mice occurs only for the maximum inhibitory effect, the subsequent experiments with the δ opioid receptor agonist DPDPE were restricted to its maximally active concentration.

CB_1 receptor ablation did increase the maximum effect of DPDPE and this phenomenon occurred again in the presence and absence of an appropriate antagonist (Table 4). Moreover, augmentation is not restricted to cholinergic and central neurones but is also found in a sympathetically innervated tissue; thus, the maximum inhibitory effect of the muscarinic receptor leading to noradrenaline release in the vas deferens (Schlicker et al. 2003; Trendelenburg et al. 2005) was increased (Table 4). The cholinergic neurones of the hippocampus and the noradrenergic neurones innervating the vas deferens have in common that they are equipped with presynaptic inhibitory CB_1 receptors (Kathmann et al. 2001b; Schlicker et al. 2003). Here, the question arises whether presynaptic inhibitory neurones in general are affected or whether the augmentation is restricted to such locations at which presynaptic CB_1 receptors occur. For this reason, two neurones of the mouse not subject to modulation by presynaptic CB_1 receptors were considered, namely the striatal cholinergic neurone (Kathmann et al. 2001b) and the cortical noradrenergic neurone (Trendelenburg et al. 2000; Kathmann et al. 2001b). The muscarinic receptor-mediated inhibition of acetylcholine release in the striatum (by oxotremorine) and the α_2-autoreceptor-mediated inhibition of noradrenaline release in the cortex (by noradrenaline itself) was not affected by CB_1 receptor ablation (Table 4). Taken together, the data are compatible with the view that the augmenting effect of CB_1 receptor ablation is specific for neurones endowed with CB_1 receptors.

In conclusion, the present study shows for the first time that the CB_1 receptor exhibits an interaction with other presynaptic receptors on cholinergic neurones (Table 4). Activation of the CB_1 receptor blunts, whereas its genetic deficiency or pharmacological blockade (at least under certain circumstances) increases the function of other presynaptic inhibitory receptors. The mechanism is unclear although a decreased and increased availability of $G\alpha_{i/o}$ proteins, respectively, would be a plausible explanation. The receptor interactions disclosed in the present study would also offer an explanation for potential pharmacodynamic drug interactions between cannabinoid receptor agonists on the one hand (e.g., Δ^9-tetrahydrocannabinol in Sativex^R) and cholinesterase inhibitors on the other.

Acknowledgments The financial support by the Deutsche Forschungsgemeinschaft to B.L., A.Z., and E.S. within the "Forschergruppe 926" is gratefully acknowledged. We would also like to thank Mrs. D. Petri and Mrs. C. Schwitter for their skilled technical assistance and the companies Bayer Schering Pharma, Boehringer Ingelheim, Novartis, and Sanofi-Aventis for gifts of drugs.

References

Boehm S, Kubista H (2002) Fine tuning of sympathetic transmitter release via ionotropic and metabotropic presynaptic receptors. Pharmacol Rev 54:43–99

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Fuder H, Muscholl E (1995) Heteroreceptor-mediated modulation of noradrenaline and acetylcholine release from peripheral nerves. Rev Physiol Biochem Pharmacol 126:265–412

Furchgott RF (1972) The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. Handb Exp Pharmacol 33:283–335

Giaroni C, Somaini L, Marino F, Cosentino M, Senaldi A, De Ponti F, Lecchini S, Frigo G (1999) Modulation of enteric cholinergic neurons by hetero- and autoreceptors: cooperation among inhibitory inputs. Life Sci 65:813–821

Günther J, Schulte K, Wenzel D, Malinowska B, Schlicker E (2010) Prostaglandins of the E series inhibit monoamine release via EP_3 receptors: proof with the competitive EP_3 receptor antagonist L-826,266. Naunyn-Schmiedeberg's Arch Pharmacol 381:21–31

Hiramatsu M, Murasawa H, Mori H, Kameyama T (1998) Reversion of muscarinic autoreceptor agonist-induced acetylcholine decrease and learning impairment by dynorphin A (1-13), an endogenous κ-opioid receptor agonist. Brit J Pharmacol 123:920–926

Kathmann M, Bauer U, Schlicker E (1999) CB_1 receptor-mediated functional effects in rat hippocampus are decreased by an i.c.v. administered antisense oligodeoxynucleotide. Naunyn-Schmiedeberg's Arch Pharmacol 360:421–427

Kathmann M, Weber B, Schlicker E (2001a) Cannabinoid CB_1 receptor-mediated inhibition of acetylcholine release in the brain of NMRI, CD-1 and C57/BL/6J mice. Naunyn-Schmiedeberg´s Arch Pharmacol 363:50–56

Kathmann M, Weber B, Zimmer A, Schlicker E (2001b) Enhanced acetylcholine release in the hippocampus of cannabinoid CB_1 receptor-deficient mice. Brit J Pharmacol 132:1169–1173

Kubista H, Boehm S (2006) Molecular mechanisms underlying the modulation of exocytotic noradrenaline release via presynaptic receptors. Pharmacol Ther 112:213–242

Nishiwaki H, Saitoh N, Nishio H, Takeuchi T, Hata F (1998) Relationship between inhibitory effect of endogenous opioid via mu-receptors and muscarinic autoinhibition in acetylcholine release from myenteric plexus of guinea pig ileum. Jpn J Pharmacol 77:279–286

Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. Pharmacol Rev 62:588–631

Raiteri L, Raiteri M (2000) Synaptosomes still viable after 25 years of superfusion. Neurochem Res 25:1265–1274

Rutz S, Riegert C, Rothmaier AK, Buhot MC, Cassel JC, Jackisch R (2006) Presynaptic serotonergic modulation of 5-HT and acetylcholine release in the hippocampus and the cortex of 5-HT_{1B}-receptor knockout mice. Brain Res Bull 70:81–93

Sastre-Garriga J, Vila C, Clissold S, Montalban X (2011) THC and CBD oromucosal spray (Sativex^{R}) in the management of spasticity associated with multiple sclerosis. Expert Rev Neurother 11:627–637

Schlicker E, Göthert M (1998) Interactions between the presynaptic α_2-autoreceptor and presynaptic inhibitory heteroreceptors on noradrenergic neurones. Brain Res Bull 47:129–132

Schlicker E, Kathmann M (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol Sci 22:565–572

Schlicker E, Behling A, Lümmen G, Göthert M (1992) Histamine H_{3A} receptor-mediated inhibition of noradrenaline release in the mouse brain cortex. Naunyn-Schmiedeberg's Arch Pharmacol 345:489–493

Schlicker E, Kathmann M, Exner HJ, Detzner M, Göthert M (1994) The 5-HT_3 receptor agonist 1-(m-chlorophenyl)-biguanide facilitates noradrenaline release by blockade of α_2-adrenoceptors in the mouse brain cortex. Naunyn-Schmiedeberg's Arch Pharmacol 349:20–24

Schlicker E, Redmer A, Werner A, Kathmann M (2003) Lack of CB_1 receptors increases noradrenaline release in vas deferens without affecting atrial noradrenaline release or cortical acetylcholine release. Brit J Pharmacol 140:323–328

Schulte K, Kumar M, Zajac JM, Schlicker E (2010) Noradrenaline release in rodent tissues is inhibited by interleukin-1β but is not affected by urotensin II, MCH, NPW and NPFF. Pharmacol Rep 63:102–111

Shakirzyanova AV, Buhkaraeva EA, Nikolsky EE, Giniatullin RA (2006) Negative cross-talk between presynaptic adenosine and acetylcholine receptors. Eur J Neurosci 24:105–115

Szabo B, Schlicker E (2005) Effects of cannabinoids on neurotransmission. Handb Exp Pharmacol 168:327–365

Taylor P (2001) Anticholinesterase agents. In: Hardman JG, Limbird LE, Goodman Gilman A (eds) Goodman & Gilman's the pharmacological basis of therapeutics, 10th edn, McGraw-Hill, New York, pp 175–191

Trendelenburg AU, Cox SL, Schelb V, Klebroff W, Khairallah L, Starke K (2000) Modulation of ^{3}H-noradrenaline release by presynaptic opioid, cannabinoid and bradykinin receptors and ß-adrenoceptors in mouse tissues. Brit J Pharmacol 130:321–330

Trendelenburg AU, Meyer A, Wess J, Starke K (2005) Distinct mixtures of muscarinic receptor subtypes mediate inhibition of noradrenaline release in different mouse peripheral tissues, as studied with receptor knockout mice. Brit J Pharmacol 145:1153–1159

Zhang L, Rasenick MM (2010) Chronic treatment with escitalopram but not R-citalopram translocates $G\alpha_s$ from lipid raft domains and potentiates adenylyl cyclase: a 5-hydroxytryptamine transporter-independent action of this antidepressant compound. J Pharmacol Exp Ther 332:977–984

Zhang W, Basile AS, Gomeza J, Volpicelli LA, Levey AI, Wess J (2002) Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. J Neurosci 22:1709–1717

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TJ (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci 96:5780–5785