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ORIGINAL ARTICLE

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Cannabinoid CB_1 receptor-mediated inhibition of acetylcholine release in the brain of NMRI, CD-1 and C57BL/6J mice

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Abstract Cannabinoid CB_1 receptors occur as presynaptic receptors producing inhibition of neurotransmitter release. To elucidate their physiological role, experiments on tissues from CB_1 receptor knockout mice would be helpful. We studied whether CB_1 receptor-mediated inhibition of acetylcholine release is detectable in the brain of NMRI mice and of CD-1 and C57BL/6J mice (the latter two strains representing the wild-type strains of the two CB_1 receptor knockout mouse models). Brain slices preincubated with [^{3}H]choline were superfused and tritium overflow was evoked electrically (3 Hz) or by introduction of Ca^{2+} into Ca^{2+}-free K^+-rich medium (35 mM) containing tetrodotoxin.

The *electrically evoked* tritium overflow from NMRI mouse hippocampal slices was inhibited (maximally by 60%) by the cannabinoid receptor agonists CP-55,940 and WIN 55,212-2 but not affected by WIN 55,212-3 (the inactive enantiomer of WIN 55,212-2; pEC_{50}=7.9, 7.4 and <5.5). The concentration-response curve of WIN 55,212-2 was shifted to the right by the CB_1 receptor antagonist SR 141716 (apparent pA_2=8.6). Compared to hippocampal slices from NMRI mice, WIN 55,212-2 1 µM inhibited the electrically evoked overflow (1) from cortical slices from NMRI mice to a lesser extent and from striatal slices not at all, (2) from hippocampal slices from CD-1 and C57BL/6J mice to an identical extent and (3) from hippocampal slices from Sprague-Dawley rats to at least the same extent. SR 141716 0.32 µM abolished the effect of WIN 55,212-2 1 µM in hippocampal slices from NMRI, CD-1 and C57BL/6J mice and in cortical slices from NMRI mice. The electrically evoked tritium overflow from NMRI mouse hippocampal slices was also inhibited by the muscarinic receptor agonist oxotremorine (maximum effect of 85%; pEC_{50}=6.5) and this effect was antagonized by the muscarinic receptor antagonist AF-DX 384 (apparent pA_2=8.3). The *Ca^{2+}-evoked* tritium overflow from NMRI mouse hippocampal slices was inhibited by WIN 55,212-2 in a manner sensitive to SR 141716.

In conclusion, the cholinergic axon terminals of the NMRI mouse hippocampus are endowed with presynaptic CB_1 receptors. Such receptors are also detectable in the hippocampus of CD-1 and C57BL/6J mice. The maximum extent of the CB_1 receptor-mediated inhibition of acetylcholine release is lower than the maximum effect mediated via the autoreceptor.

Keywords Hippocampus · Acetylcholine release · Cannabinoid CB_1 receptors · Muscarinic receptors · Presynaptic receptors · NMRI mouse · CD-1 mouse · C57BL/6J mouse

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Introduction

The psychotropic effects of Δ^9-tetrahydrocannabinol are mediated via cannabinoid CB_1 receptors (Ameri 1999; Pertwee 1999). Part of these receptors is located on nerve endings and activation of these presynaptic CB_1 receptors produces inhibition of the release of the respective neurotransmitter. The physiological role of presynaptic CB_1 receptors can now be determined more precisely since CB_1 receptor-deficient mice have been generated (Ledent et al. 1999; Zimmer et al. 1999). For instance, the question can be addressed whether, in the absence of CB_1 receptors, the release of a given neurotransmitter will change or whether compensatory alterations of other presynaptic receptor systems will appear. Recently, Mascia et al. (1999) found that the morphine-induced increase in dopamine release in the nucleus accumbens in vivo is abolished in CB_1 receptor-deficient mice, as compared to the wild-type strain.

CB_1 receptor-mediated modulation of neurotransmitter release has so far been studied only rarely in the brain of mice. Electrophysiological evidence for the occurrence of CB_1 receptors inhibiting glutamate release in the mouse

brain was presented by Misner and Sullivan (1999), using the hippocampal slice preparation. In a superfusion study on hippocampal slices from various species, the cannabinoid receptor agonist WIN 55,212-2 inhibited noradrenaline release in the human and guinea-pig brain without affecting it in the mouse and rat brain (Schlicker et al. 1997). Nakazi et al. (2000) found that serotonin release in mouse brain cortex slices is inhibited via CB_1 receptors (maximum effect by about 20%); in the same study, however, a single concentration of the cannabinoid receptor agonist WIN 55,212-2 elicited a more marked inhibition of acetylcholine release.

The aim of the present study was to analyze the inhibitory effect of WIN 55,212-2 on acetylcholine release in mouse hippocampal slices (in which this effect was even more pronounced than in cortical slices) and to clarify whether this effect may be located presynaptically. Most experiments were carried out on slices from NMRI mice but the effect of WIN 55,212-2 was also studied in hippocampal slices from the C57BL/6J mouse (the wild-type strain related to the knockout mouse generated by Zimmer et al. 1999) and the CD-1 mouse. The latter strain was used by Ledent et al. (1999) to dilute out the 129/Sv background from the genome of the CB_1 receptor-deficient mouse generated by them (five generations of heterozygous breeding). For the sake of comparison, the effect of WIN 55,212-2 was also studied in hippocampal slices from Sprague-Dawley rats, in which a CB_1 receptor-mediated inhibition of acetylcholine release was described in vitro (Gifford and Ashby 1996; Gifford et al. 1997a, 1997b) and in vivo (Gessa et al. 1997, 1998). Finally, we also studied the autoreceptor-mediated inhibition of acetycholine release in mouse hippocampal slices. It was, thus, possible to compare the inhibitory effects mediated via the CB_1 receptor and the muscarinic autoreceptor. The autoreceptor was chosen for this purpose since, usually, the autoreceptor-mediated inhibition of the release of a transmitter is the most pronounced one.

Materials and methods

Hippocampal, cerebral cortical or striatal slices (0.3 mm thick, 2 mm diameter) were prepared from male NMRI, CD-1 or C57BL/6J mice or from male Sprague-Dawley rats. C57BL/6J mice were purchased from Jackson (Bar Harbor, Me., USA); the other animals were from Charles River (Sulzfeld, Germany). The slices were incubated (37°C) for 30 min with physiological salt solution (PSS) containing [^{3}H]choline 0.1 µM and then superfused with PSS (37°C; 110 min); the superfusate was collected in 5-min samples. 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 (unless stated otherwise), glucose 11.1, ascorbic acid 0.06, Na_2EDTA 0.03; it was aerated with 95% O_2 and 5% CO_2.

Tritium overflow was evoked by two 2-min periods of stimulation after 40 min and 90 min (S_1, S_2). Usually, electrical field stimulation (3 Hz, 200 mA, 2 ms) was applied. In a few experiments, slices were superfused with Ca^{2+}-free K^+-rich medium (35 mM; the concentration of Na^+ was reduced accordingly) and tritium overflow was evoked by introducing Ca^{2+} into this medium. In all superfusion experiments, the PSS contained hemicholinium-3 10 µM (an inhibitor of the neuronal choline transporter) and, only in experiments with Ca^{2+} stimulus, tetrodotoxin 1 µM (a blocker of sodium channels). The antagonists under study (SR 141716, AF-DX 384) were present in the PSS throughout superfusion whereas the agonists (WIN 55,212-2, WIN 55,212-3, CP-55,940, oxotremorine) were added to the PSS from 62 min of superfusion onward. In further experiments, in which tritium overflow was evoked electrically, tetrodotoxin was added or Ca^{2+} ions were omitted from 62 min of superfusion onward.

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 agonists on basal efflux, the ratio of the fractional rates in the 5-min collection periods from 85 min to 90 min (t_2) and from 55 min to 60 min (t_1) was determined. To determine the effects of antagonists on the basal efflux, the t_1-values obtained in the presence and absence of the antagonist under study were compared. 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 collection period before to that 15–20 min after onset of stimulation). To quantify effects of agonists on the stimulated tritium overflow, the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. To determine the effects of antagonists on the evoked overflow, the S_1-values obtained in the presence and absence of the respective antagonist were compared. Apparent pA_2-values were calculated according to formula 4 of Furchgott (1972).

Statistics. Results are given as means ± SEM of n experiments (n refers to the number of animals; t_2/t_1- and S_2/S_1-values are based on one slice per animal whereas t_1- and S_1-values represent the mean of several slices per animal). 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.

Drugs used. [Methyl-^{3}H]choline chloride (specific activity 75 Ci/mmol; NEN, Zaventem, Belgium); AF-DX 384 (5,11-dihydro-11-{[(2-{2-[(dipropylamino)methyl]-1-piperidinyl}ethyl)amino]carbonyl}-6*H*-pyrido(2,3-β)(1,4)benzodiazepine-6-one; Thomae, Biberach an der Riss, Germany); CP-55,940 ((-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; Tocris-Cookson/Biotrend, Cologne, Germany); hemicholinium-3 (ChemCon, Freiburg, Germany); oxotremorine (Sigma, Munich, Germany); SR 141716 (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; Sanofi, Montpellier, France); tetrodotoxin (ICN, Eschwege, Germany or Roth, Karlsruhe, Germany); 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), WIN 55,212-3 (*S*(-)-enantiomer of WIN 55,212-2; RBI/Sigma, Munich, 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 to obtain the concentration required. Diluted DMSO and citrate buffer did not by themselves affect basal or evoked tritium overflow.

Results

Basal tritium efflux

Basal tritium efflux was expressed as t_1 or t_2/t_1. t_1-values, obtained under a variety of experimental conditions, are given in Table 1. Average t_2/t_1-values were between 0.6 and 0.8 in the various experimental groups (not shown). The drugs under study and omission of Ca^{2+} ions from the medium did not affect basal tritium efflux (not shown).

Table 1 Basal and stimulation-evoked tritium overflow from superfused brain slices preincubated with [^{3}H]choline, and effect of SR 141716 on the evoked overflow. Tritium overflow was evoked after 40 min of superfusion (S_1; and again after 90 min; not shown), either electrically or chemically. When tritium overflow was evoked electrically (3 Hz, 200 mA, 2 ms), the medium contained hemicholinium 10 μM and, when necessary, SR 141716; the Ca^{2+} concentration was as indicated below. In a few experiments tritium overflow was evoked by introduction of Ca^{2+} into Ca^{2+}-free K^+-rich (35 mM) medium; in these experiments, the medium contained hemicholinium-3 10 μM, tetrodotoxin 1 μM and, when necessary, SR 141716. Basal tritium efflux (t_1) was determined in the 5-min sample from 55–60 min. Means ± SEM of 4–10 experiments

Mode of stimulation	Ca^{2+} concentration (mM)	Species, strain	Brain region	Basal tritium efflux during t_1 (fractional rate; min^{-1}) No SR 141716	Tritium overflow evoked by S_1 (% of tissue tritium)			
					No SR 141716	SR 141716 (0.032 μM)	SR 141716 (0.32 μM)	SR 141716 (1 μM)
Electrical	1.3	Mouse, NMRI	Hippocampus	0.0024±0.0002	1.25±0.17	1.76±0.36	2.73±0.79*	–
	1.3		Cerebral cortex	0.0022±0.0003	1.73±0.19	–	1.80±0.26	–
	1.3		Striatum	0.0030±0.0004	2.30±0.63	–	–	–
	1.95		Hippocampus	0.0022±0.0002	3.11±0.28	–	–	–
	1.95	Mouse, CD-1		0.0023±0.0002	4.18±0.60	–	5.80±0.78	–
	1.95	Mouse, C57BL/6J		0.0023±0.0003	2.50±0.26	–	4.45±0.70*	–
	2.6	Mouse, NMRI		0.0028±0.0006	2.92±0.43	–	–	–
	2.6	Rat, Sprague-Dawley		0.0028±0.0005	1.91±0.20	–	–	–
Ca^{2+}	1.3	Mouse, NMRI		0.0029±0.0002	7.54±1.02	–	–	6.64±0.78

*P<0.05, compared to „No SR 141716"

Electrically evoked tritium overflow

The evoked tritium overflow was expressed as S_1 or S_2/S_1. S_1-values, obtained under various experimental conditions, are shown in Table 1. S_2/S_1-values in control experiments (no addition of drugs before S_2) were close to unity or slightly higher; some examples are given in the next paragraph and in Figs. 2, 3, 4.

The first experimental series was carried out on hippocampal slices from NMRI mice. The electrically evoked tritium overflow (S_2/S_1), which was 1.30±0.12 in four controls, was inhibited by tetrodotoxin 1 μM by 85% (S_2/S_1 0.20±0.10; P<0.001; n=4) and by omission of Ca^{2+} ions from the medium by 93% (S_2/S_1 0.09±0.04; P<0.001; n=4). Figure 1 shows that the cannabinoid receptor agonists WIN 55,212-2 and CP-55,940 inhibited the electrically evoked tritium overflow in a concentration-dependent manner; the maximum inhibitory effect for both agonists amounted to about 60%. The pEC$_{50}$-values (i.e. concentrations producing an inhibition by 30%) were 7.4 and 7.9, respectively. WIN 55,212-3 (the enantiomer of WIN 55,212-2) at a concentration of 3.2 μM did not affect the evoked overflow. The concentration-response curve of WIN 55,212-2 was shifted to the right by the CB_1 receptor antagonist SR 141716 0.032 μM, yielding an apparent pA_2 of 8.6 (Fig. 1). When present alone, SR 141716 0.032 μM tended to facilitate the electrically evoked tritium overflow (S_1) (Table 1).

In the second series, experiments were performed on slices from three brain regions of NMRI mice. The inhibitory effect of WIN 55,212-2 1 μM on the evoked overflow (S_2/S_1) was most marked in hippocampal slices (inhibition by about 55%) and less marked in cerebral cortical

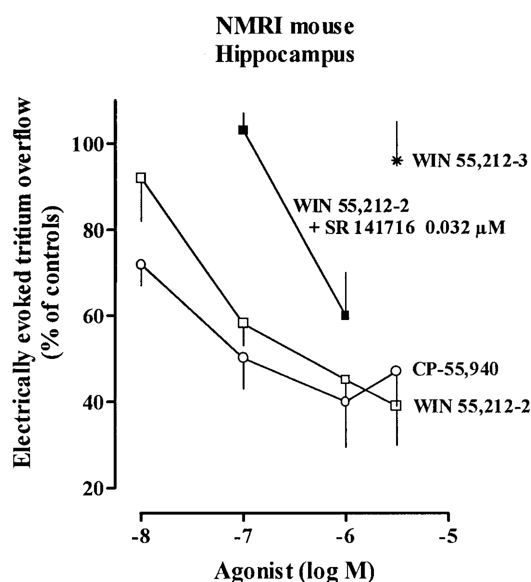

Fig. 1 Effect of WIN 55,212-2, WIN 55,212-3 and CP-55,940 on the electrically evoked tritium overflow from NMRI mouse hippocampal slices, and interaction of WIN 55,212-2 with SR 141716. The slices were superfused with medium (Ca^{2+} concentration 1.3 mM) containing hemicholinium-3 10 μM and, when necessary, SR 141716 throughout superfusion and WIN 55,212-2, WIN 55,212-3 or CP-55,940 from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 min 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. The results are expressed as percent of the S_2/S_1-value in the corresponding controls (not shown). Means ± SEM of 4–5 experiments (n=14 for WIN 55,212-2 1 μM in the absence of SR 141716)

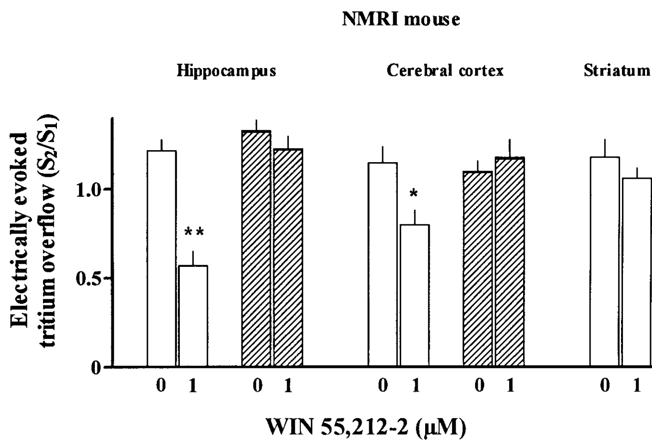

Fig. 2 Effect of WIN 55,212-2 on the electrically evoked tritium overflow from NMRI mouse brain slices (*empty columns*), and interaction of WIN 55,212-2 with SR 141716 0.32 μM (*hatched columns*). The slices were superfused with medium (Ca^{2+} concentration 1.3 mM) containing hemicholinium-3 10 μM 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 min 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. Means ± SEM of 4–6 experiments (n=14 for the first pair of columns from the left). *P<0.02, **P<0.001

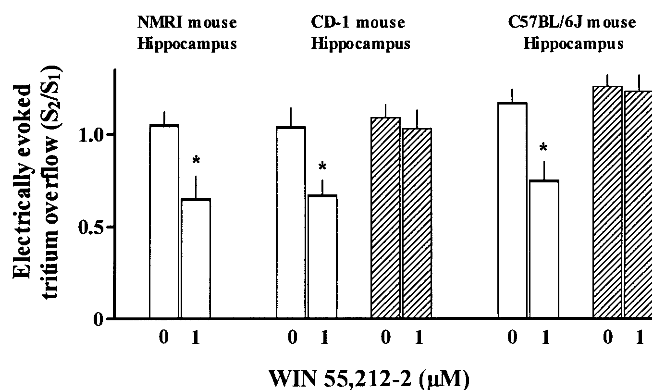

Fig. 3 Effect of WIN 55,212-2 on the electrically evoked tritium overflow from hippocampal slices from NMRI, CD-1 and C57BL/6J mice (*empty columns*), and interaction of WIN 55,212-2 with SR 141716 0.32 μM (*hatched columns*). The Ca^{2+} concentration in the medium was 1.95 mM. For further details, see legend to Fig. 2. Means ± SEM of 6–8 experiments. *P<0.02

slices (inhibition by about 30%); in striatal slices, only a very slight tendency towards an inhibition was found (Fig. 2). SR 141716 0.32 μM abolished the inhibitory effect of WIN 55,212-2 in hippocampal and cortex slices (Fig. 2) and, by itself, facilitated the evoked overflow (S_1) in hippocampal slices without affecting it in cortex slices (Table 1).

The experiments of the third series were carried out on hippocampal slices from NMRI, CD-1 and C57BL/6J mice. Since the S_1-value was very low in preliminary experiments on slices from the latter mouse strain, the Ca^{2+} concentration in the medium was increased from 1.3 mM to 1.95 mM in these experiments. The inhibitory effect of WIN 55,212-2 1 μM on the evoked overflow (S_2/S_1) was about 40% and identical in the three strains (Fig. 3). The inhibitory effect of WIN 55,212-2 in hippocampal slices from CD-1 and C57BL/6J mice was abolished by SR 141716 0.32 μM, which, by itself, facilitated the evoked overflow (S_1) in hippocampal slices from C57BL/6J mice

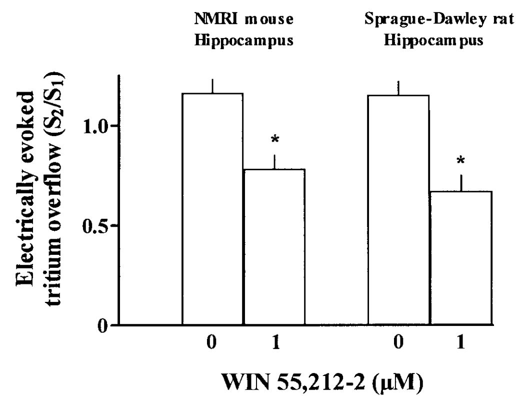

Fig. 4 Effect of WIN 55,212-2 on the electrically evoked tritium overflow from NMRI mouse and Sprague-Dawley rat hippocampal slices. The Ca^{2+} concentration in the medium was 2.6 mM. For further details, see legend to Fig. 2. Means ± SEM of 9 experiments. *P<0.001

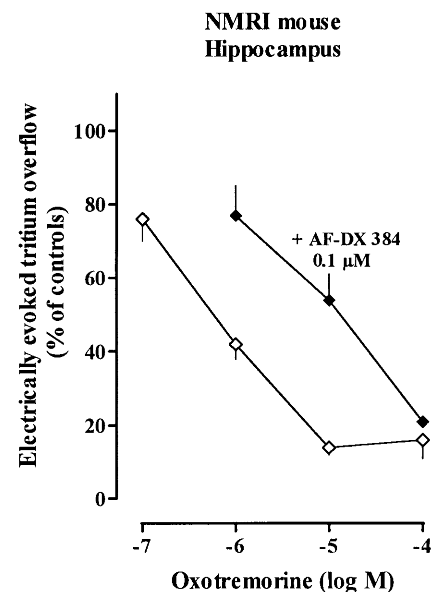

Fig. 5 Effect of oxotremorine on the electrically evoked tritium overflow from NMRI mouse hippocampal slices, and interaction of oxotremorine with AF-DX 384. The slices were superfused with medium (Ca^{2+} concentration 1.3 mM) containing hemicholinium-3 10 μM and, when necessary, AF-DX 384 throughout superfusion and oxotremorine from 62 min of superfusion onward. Tritium overflow was evoked twice, after 40 min 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. The results are expressed as percent of the S_2/S_1-value in the corresponding controls (not shown). Means ± SEM of 4–9 experiments

and tended to increase it in hippocampal slices from CD-1 mice (Table 1).

In the fourth experimental series, hippocampal slices from NMRI mice and Sprague-Dawley rats were examined. Since S_1 was very low in preliminary experiments on slices from rats, the Ca^{2+} concentration was increased to 2.6 mM in this set of experiments. The inhibitory effect of WIN 55,212-2 1 µM on the evoked overflow (S_2/S_1) was 42% in hippocampal slices from rats and tended to be slightly higher when compared to the effect in mice (33% inhibition; Fig. 4). If one compares the effect of WIN 55,212-2 in the first pairs of columns from the left in Figs. 2, 3 and 4 (for which Ca^{2+} concentrations of 1.3, 1.95 and 2.6 mM were used, respectively), an inverse relationship between the Ca^{2+} concentration and the extent of the effect of WIN 55,212-2 is evident.

The fifth series was again carried out on hippocampal slices from NMRI mice (identical experimental conditions as in the first series). Figure 5 shows that the electrically evoked tritium overflow (S_2/S_1) was inhibited by the muscarinic receptor agonist oxotremorine; the maximum inhibition amounted to 85%. The pEC_{50}-value (concentration producing an inhibition by 42.5%) was 6.5. The concentration-response curve of oxotremorine was shifted to the right by the muscarinic receptor antagonist AF-DX 384 0.1 µM, yielding an apparent pA_2-value of 8.3. AF-DX 384 did not affect the evoked overflow (S_1) by itself (not shown).

Ca^{2+}-evoked tritium overflow

In NMRI mouse hippocampal slices, the tritium overflow evoked by introduction of Ca^{2+} into Ca^{2+}-free K^+-rich (35 mM) medium, expressed as S_2/S_1, was inhibited by WIN 55,212-2 1 µM by about 30% (Fig. 6). The effect of WIN 55,212-2 was strongly attenuated by SR 141716 1 µM, which, by itself, did not affect the evoked overflow (S_1; Table 1).

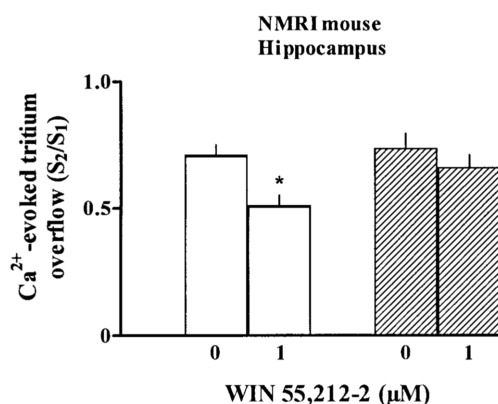

Fig. 6 Effect of WIN 55,212-2 on the Ca^{2+}-evoked tritium overflow from NMRI mouse hippocampal slices (*empty columns*), and interaction of WIN 55,212-2 with SR 141716 1 µM (*hatched columns*). The slices were superfused with Ca^{2+}-free K^+-rich medium (35 mM) containing hemicholinium-3 10 µM, tetrodotoxin 1 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 min and 90 min of superfusion (S_1, S_2), by introduction of Ca^{2+} 1.3 mM into the medium, and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. Means ± SEM of 4–6 experiments. *P<0.02

Discussion

The electrically evoked tritium overflow from hippocampal slices from NMRI mice preincubated with [^{3}H]choline is tetrodotoxin-sensitive and Ca^{2+}-dependent, suggesting that it represents quasi-physiological acetylcholine release. The following findings are in harmony with the view that the inhibitory effect of WIN 55,212-2 on acetylcholine release was mediated via CB_1 receptors. Thus, this effect (1) was not shared by its enantiomer, WIN 55,212-3, (2) was mimicked by CP-55,940, another cannabinoid receptor agonist belonging to an entirely different chemical class, and (3) was antagonized by the CB_1 receptor antagonist SR 141716. WIN 55,212-2 retained its inhibitory effect in slices kept depolarized by high K^+, in which tritium overflow was evoked by introduction of Ca^{2+} ions and impulse propagation along the axons was prevented by tetrodotoxin. This finding suggests (but does not prove with absolute certainty; for review, see Starke 1981) that the CB_1 receptor activated by WIN 55,212-2 is located presynaptically on the cholinergic nerve endings. The presynaptic location of the receptor is also suggested by the inverse relationship between the degree of inhibition by WIN 55,212-2 and the Ca^{2+} concentration in the medium, a typical property of effects mediated via presynaptic receptors (Starke 1977).

The effect of WIN 55,212-2 in the hippocampus of NMRI mice tended to be lower than its effect in the hippocampus from Sprague-Dawley rats, in which CB_1 receptor-mediated inhibition of acetylcholine release has been previously shown in vitro and in vivo (see Introduction for references). Like in the rat (Gifford and Ashby 1996; Gifford et al. 1997a, 1997b), the CB_1 receptor-mediated effect was greater in the hippocampus than in the cerebral cortex whereas no effect was obtained in the striatum. This pattern well agrees with the distribution of mRNA for CB_1 receptors in the rat brain. Thus, CB_1 receptor mRNA was detected in the septal nuclei (Mailleux and Vanderhaeghen 1992) in which the perikarya of the cholinergic neurones projecting to the hippocampus and cerebral cortex are located; on the other hand, cholinergic interneurones of the striatum were CB_1 receptor mRNA-negative (Hohmann and Herkenham 2000).

The effect of WIN 55,212-2 was identical in hippocampal slices from NMRI, CD-1 and C57BL/6J mice. This finding is not trivial since the mouse strains differed markedly with respect to the magnitude of acetylcholine release and the effect of SR 141716 when given alone. The facilitatory effect of SR 141716 might be related to the fact that endocannabinoids accumulate in the biophase of the CB_1 receptors and that this tonical inhibition is interrupted by the antagonist. An alternative explanation is that the CB_1

receptors are precoupled to their effectors and that, even in the absence of endocannabinoids, SR 141716 facilitates acetylcholine release due to an inverse agonistic effect (which has been shown at recombinant CB_1 receptors; MacLennan et al. 1998; Pan et al. 1998). Note that the present experimental approach does not allow to decide which of the two possibilities holds true. The explanation for the less marked facilitatory effect of SR 141716 in hippocampal slices from CD-1 mice, as compared to hippocampal slices from C57BL/6J mice, may be the marked difference in the control level of acetylcholine release. The same explanation may hold true also for the fact that, in hippocampal slices from NMRI mice, SR 141716 facilitated the electrically evoked, without affecting the K^+-induced, acetylcholine release.

With respect to the (patho)physiological role of the CB_1 receptors inhibiting acetylcholine release in the hippocampus, one has to consider that septohippocampal cholinergic neurones are important in learning and memory (Fischer et al. 1992; Zola-Morgan and Squire 1993). Thus, one may hypothesize that these CB_1 receptors might contribute to the inhibitory effects of cannabinoid receptor agonists (for review, see Ameri 1999) and the facilitatory effect of SR 141716 (Terranova et al. 1996) on learning and memory.

Our study also shows that acetylcholine release in hippocampal slices from NMRI mice can be inhibited via muscarinic autoreceptors. It was beyond the scope of the present paper to determine the muscarinic receptor subtype involved; however, the apparent pA_2-value of 8.3 for AF-DX 384 (an antagonist possessing a slight selectivity for M_2 and M_4 over M_1, M_3 and M_5 receptors; Alexander and Peters 1999) would be in harmony with an M_2 or M_4 receptor. (The very same conclusion has been reached recently by Iannazzo and Majewski (2000) for the muscarinic autoreceptor in cerebral cortex slices from Swiss mice.) In our study, the maximum inhibition mediated via the autoreceptor (85%) exceeded that mediated via the CB_1 heteroreceptor (60%) by about 40%.

In conclusion, acetylcholine release in the hippocampus of the NMRI mouse is inhibited via CB_1 receptors, which are most probably located presynaptically on the cholinergic nerve endings themselves, and via muscarinic autoreceptors. CB_1 receptor-mediated inhibition of acetylcholine release is also detectable in the cerebral cortex but not in the striatum of NMRI mice. The fact that CB_1 receptor-mediated inhibition of acetylcholine release also occurs in the hippocampus of CD-1 and C57BL/6J mice will offer the opportunity to use the present model in the CB_1 receptor-deficient mice generated by Ledent et al. (1999) and Zimmer et al. (1999).

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