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Lack of CB_1 receptors increases noradrenaline release in vas deferens without affecting atrial noradrenaline release or cortical acetylcholine release

*,[1]Eberhard Schlicker, [1]Agnes Redmer, [1]André Werner & [1]Markus Kathmann

[1]Institut für Pharmakologie und Toxikologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Reuterstr. 2b, 53113 Bonn, Germany

1 We studied whether cannabinoid CB_1 receptor gene disruption (to yield $CB_1^{-/-}$ mice) affects the electrically evoked tritium overflow from vas deferens and atrial pieces preincubated with [^{3}H]-noradrenaline (NA) ('noradrenaline release') and from cerebral cortex slices preincubated with [^{3}H]-choline ('acetylcholine release').

2 NA release was higher by 37% in vas deferens from $CB_1^{-/-}$ mice than in vas deferens from $CB_1^{+/+}$ mice. The cannabinoid receptor agonist WIN 55,212-2 inhibited, and the CB_1 receptor inverse agonist/antagonist SR 141716, increased NA release in vas deferens from $CB_1^{+/+}$ mice without affecting it in vas deferens from $CB_1^{-/-}$ mice.

3 Atrial NA release did not differ between $CB_1^{+/+}$ and $CB_1^{-/-}$ mice nor did WIN 55,212-2 affect NA release in either strain.

4 Cortical acetylcholine (Ach) release did not differ between $CB_1^{+/+}$ and $CB_1^{-/-}$ mice. WIN 55,212-2 inhibited, but SR 141716 did not affect, Ach release in the cortex from $CB_1^{+/+}$ mice. Both drugs did not alter Ach release in the cortex from $CB_1^{-/-}$ mice.

5 Tritium content did not differ between $CB_1^{+/+}$ and $CB_1^{-/-}$ mice in any preparation.

6 In conclusion, the increase in NA release associated with CB_1 receptor deficiency in the vas deferens, which cannot be ascribed to an alteration of tritium content of the preparations, suggests an endogenous tone at the CB_1 receptors of $CB_1^{+/+}$ mice in this tissue. Furthermore, the effect of WIN 55,212-2 on NA release in the vas deferens and on cortical Ach release involves CB_1 receptors, whereas the involvement of non-CB_1–non-CB_2 receptors can be excluded.
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Abbreviations: Ach, acetylcholine; AM 251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; C57BL/6J, mouse strain; $CB_1^{-/-}$, $CB_1^{+/+}$ mouse, CB_1 receptor-deficient and wild-type mouse; CP-55,940, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; DMSO, dimethylsulfoxide; NA, noradrenaline; NMRI, mouse strain; PSS, physiological salt solution; S_1, S_2, first and second stimulation, respectively; SR 141716, *N*-piperidino-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; t_2, collection period in which basal tritium efflux was determined; WIN 55,212-2, *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl](1-naphthalenyl)methanone

Introduction

Cannabinoid CB_1 receptors serve as presynaptic inhibitory receptors on a variety of central and peripheral neurones (for review, see Schlicker & Kathmann, 2001; Howlett *et al*., 2002). These receptors are activated by endocannabinoids like anandamide, 2-arachidonoylglycerol, noladin ether and virodhamine (Hanuš *et al*., 2001; Porter & Felder, 2001; Porter *et al*., 2002). Many types of presynaptic CB_1 receptors appear to be subject to an endogenous tone as suggested by the fact that CB_1 receptor inverse agonists/antagonists like SR 141716 facilitate the release of the respective neurotransmitter (for review, see Pertwee, 1999; Schlicker & Kathmann, 2001). It is of interest in this context whether lack of CB_1 receptors will lead to the same result.

We have indeed found recently (Kathmann *et al*., 2001b) that in hippocampal slices, in which SR 141716 increases acetylcholine (Ach) release (Kathmann *et al*., 2001a), the release of this transmitter was also increased by CB_1 receptor deficiency (CB_1 receptor knockout mouse ($CB_1^{-/-}$) generated by Zimmer *et al*. (1999) from C57BL/6J mice). This alteration in transmitter release is very specific inasmuch as hippocampal *noradrenaline* (NA) release and *striatal* Ach release (both of which are not subject to modulation *via* CB_1 receptors; Schlicker *et al*., 1997; Kathmann *et al*., 2001a) did not differ between both strains (Kathmann *et al*., 2001b). The aim of the present study was to examine whether parallel effects of SR 141716 and of CB_1 receptor deficiency also occur in other isolated tissues of the mouse. For this purpose, we determined

*Author for correspondence; E-mail: e.schlicker@uni-bonn.de
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the influence of CB_1 receptor gene disruption on NA release in the vas deferens and in the atrium. NA release in the vas deferens is inhibited *via* presynaptic CB_1 receptors subject to an endogenous tone (Rinaldi-Carmona *et al*., 1994; Pertwee *et al*., 1996), whereas atrial NA release is not affected by presynaptic CB_1 receptors at all (Trendelenburg *et al*., 2000). Our study was extended to cerebral cortex slices in which Ach release was determined. This experimental model differs from the latter two and from the models considered in our previous study (Kathmann *et al*., 2001b) in that the release of the transmitter is inhibited *via* presynaptic CB_1 receptors, which are, however, not subject to an endogenous tone, that is, Ach release is not facilitated by SR 141716 (Kathmann *et al*., 2001a).

Methods

C57BL/6J mice with disrupted CB_1 receptor gene ($CB_1^{-/-}$ mice) were obtained from A. Zimmer (Klinik für Psychiatrie und Psychotherapie, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany) and C57BL/6J mice of the wild-type strain ($CB_1^{+/+}$ mice) were purchased from Jackson (Bar Harbor, ME, U.S.A.). Animals were bred in the animal facilities of our department where they were housed in a temperature- and humidity-controlled environment under a 12-h dark–light cycle with food and water available *ad libitum*. The following tissues were prepared from 2- to 4-month-old animals: pieces from the vas deferens and the atrium and slices (0.3 mm thick, 3 mm diameter) from the cerebral cortex. Pieces from the atrium and cerebral cortex slices were obtained from animals of either sex. The preparations were incubated (37°C) for 30 min with a physiological salt solution (PSS) containing [^{3}H]-choline 0.1 μM or [^{3}H]-NA 0.025 μM (see below). The PSS was composed as follows (mM): NaCl 118, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_2$ 1.2, $CaCl_2$ 1.3 (if not stated otherwise), glucose 10, ascorbic acid 0.06, Na_2EDTA 0.03; it was aerated with 95% O_2 and 5% CO_2.

The preparations were then superfused at 1 ml min^{-1} (37°C; 110 min) and the superfusate was collected in 5-min samples; the $CaCl_2$ concentration in the superfusion fluid was 3.25 mM. The vas deferens and atrium pieces preincubated with [^{3}H]-NA were superfused with PSS containing desipramine 1 μM and rauwolscine 1 μM. Cortical slices preincubated with [^{3}H]-choline were superfused with PSS containing hemicholinium-3 10 μM. (Desipramine and rauwolscine were used for the blockade of the neuronal NA transporter and the presynaptic α_2-adrenoceptor, respectively, whereas hemicholinium-3 was used to block the high-affinity choline uptake.) Tritium overflow was evoked by two 2-min periods of electrical field stimulation after 40 and 90 min (S_1, S_2); the stimulation parameters were 3 Hz, 200 mA, and 2 ms. In each of the three preparations and regardless of the mouse strain, addition of tetrodotoxin 1 μM or omission of Ca^{2+} ions (from 62 min of superfusion onward) inhibited the electrically evoked tritium overflow by 88% or more (results not shown), suggesting that the electrically evoked tritium overflow represents quasi-physiological release of NA or Ach, as appropriate.

At the end of superfusion, the tritium remaining in a given preparation and the tritium of all superfusion samples collected from this preparation were added up to allow for determination of the tritium content at any time of the superfusion experiment. Tritium efflux was calculated as the fraction of the tritium content in the preparations at the beginning of the respective collection period (fractional rate of tritium efflux). To quantify basal tritium efflux, the fractional rates in the 5-min collection period from 85 to 90 min were determined (t_2). The electrically 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 preparation 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 the onset of stimulation). To quantify the stimulated tritium overflow, the tritium overflow evoked by S_1 or the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined, as explained in the Results section. As a measure for tritium accumulation, the tritium remaining in the preparation at the end of the superfusion was determined.

Statistics

Results are given as mean ± s.e.m. of n experiments. For the t_2 and S_1 values and the tritium content (Figure 1, Table 1 and text), n refers to the number of mice; for the S_2/S_1 values (Figures 2 and 3), n is higher than the number of mice since in some instances two values were obtained from the same animal. The 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

The following drugs were used: [methyl-^{3}H]-choline chloride (specific activity 75–86 Ci mmol^{-1}); R-(-)-[ring-2, 5, 6-^{3}H]-noradrenaline (specific activity 51.8–56.3 Ci mmol^{-1}) (NEN, Zaventem, Belgium); AM 251 (*N*-(piperidin-1-yl)-5-(4-iodo-

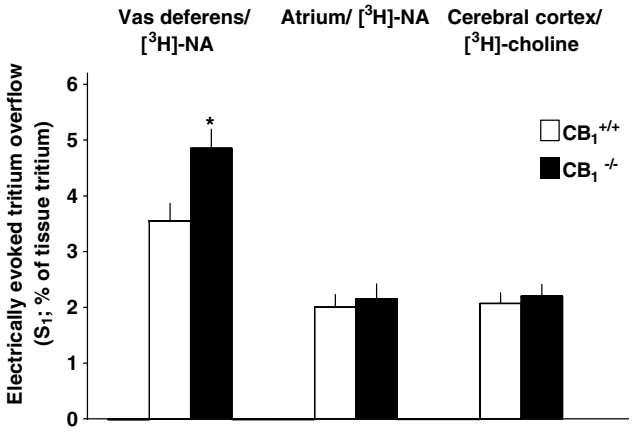

Figure 1 Electrically (3 Hz) evoked tritium overflow from superfused vas deferens, atrium and cerebral cortex preparations from $CB_1^{+/+}$ and $CB_1^{-/-}$ mice. Vas deferens and atrium pieces preincubated with [^{3}H]-NA were superfused with a medium containing desipramine 1 μM and rauwolscine 1 μM. Cortex slices preincubated with [^{3}H]-choline were superfused with a medium containing hemicholinium-3 10 μM. Tritium overflow was evoked after 40 min (S_1) of superfusion (and again after 90 min, not shown). Mean ± s.e.m. of 25 experiments (cerebral cortex) and 12–13 experiments (vas deferens, atrium). *$P<0.05$, compared to the corresponding value from $CB_1^{+/+}$ mice.

Table 1 Tritium content in vas deferens and atrium pieces (preincubated with [^{3}H]-noradrenaline) and in cerebral cortex slices (preincubated with [^{3}H]-choline) from $CB_1^{+/+}$ and $CB_1^{-/-}$ mice

Tissue	*Preincubation with*	n	*Tritium content* (nCi) $CB_1^{+/+}$	$CB_1^{-/-}$
Vas deferens	[^{3}H]-NA	16	6.5±0.8	6.8±0.6
Atrium	[^{3}H]-NA	13	5.8±1.1	5.2±1.1
Cerebral cortex	[^{3}H]-choline	26	46.8±4.1	46.0±3.4

Tissues were superfused for 110 min with a medium containing desipramine 1 μM plus rauwolscine 1 μM (tissues preincubated with [^{3}H]-NA) or hemicholinium-3 10 μM (tissues preincubated with [^{3}H]-choline). The results represent mean±s.e.m. of the tritium content present in the preparations after completion of the experiments.

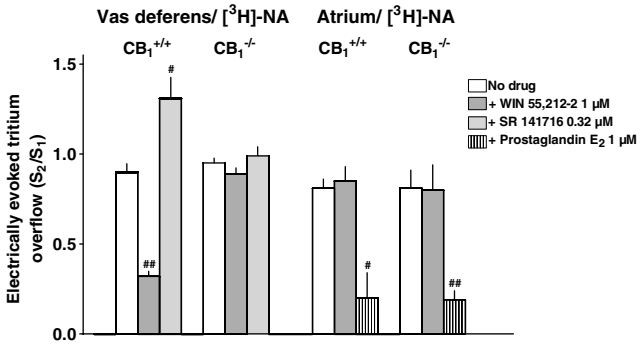

Figure 2 Effect of cannabinoid receptor ligands and of prostaglandin E_2 on the electrically (3 Hz) evoked tritium overflow from superfused vas deferens and atrium pieces from $CB_1^{+/+}$ and $CB_1^{-/-}$ mice. The preparations were preincubated with [^{3}H]-NA. Tritium overflow was evoked twice, after 40 min (S_1) and 90 min (S_2) of superfusion, and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. Desipramine 1 μM and rauwolscine 1 μM were present in the medium throughout superfusion, whereas the cannabinoid receptor agonist WIN 55,212-2, the CB_1 receptor inverse agonist/antagonist SR 141716 or prostaglandin E_2 was added from 28 min before S_2 onward. Mean±s.e.m. of 5–16 experiments. $^{\#}P<0.05$, $^{\#\#}P<0.001$, compared to the corresponding control.

phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide); CP-55,940 ((-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol) (Toc-ris/Biotrend, Köln, Germany); desipramine hydrochloride (Ciba-Geigy, Wehr, Germany); hemicholinium-3 (ChemCon, Freiburg, Germany); prostaglandin E_2 (Sigma, München, Germany); rauwolscine hydrochloride (Roth, Karlsruhe, 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-benzoxazin-yl](1-naphthalenyl)methanone mesylate; RBI/Sigma, München, Germany). Drugs were dissolved in dimethylsulfoxide (DMSO) (AM 251, CP-55,940, SR 141716 and WIN 55,212-2), ethanol (prostaglandin E_2), citrate buffer (0.1 mM, pH 4.8; tetrodotoxin) or water (other drugs) and diluted with PSS to obtain the concentration required.

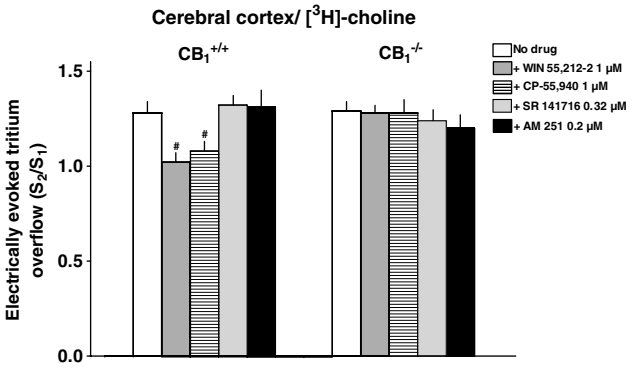

Figure 3 Effect of cannabinoid receptor ligands on the electrically (3 Hz) evoked tritium overflow from superfused cerebral cortex slices from $CB_1^{+/+}$ and $CB_1^{-/-}$ mice. Slices were preincubated with [^{3}H]-choline. Tritium overflow was evoked twice, after 40 min (S_1) and 90 min (S_2) of superfusion, and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. Hemicholinium-3 10 μM was present in the medium throughout superfusion, whereas a cannabinoid receptor agonist (WIN 55,212-2, CP-55,940) or a CB_1 receptor inverse agonist/antagonist (SR 141716, AM 251) was added from 28 min before S_2 onward. Mean±s.e.m. of 5–20 experiments. $^{\#}P<0.05$, compared to the corresponding control.

Diluted DMSO, ethanol and citrate buffer did not affect basal or evoked tritium overflow by themselves.

Results

Basal tritium efflux was expressed as a fractional rate of tritium efflux in the collection period from 85 to 90 min of superfusion (t_2; i.e., in the 5-min period preceding the second stimulation). The t_2 value in vas deferens and atrium pieces and in cortical slices from $CB_1^{+/+}$ mice was 0.0038±0.0004 ($n=15$), 0.0056±0.0003 ($n=13$) and 0.0021±0.0002 min^{-1} ($n=26$), respectively. Basal efflux was not affected by the strain ($CB_1^{+/+}$ vs $CB_1^{-/-}$) and by the drugs under study (AM 251, CP-55,940, prostaglandin E_2, SR 141716, WIN 55,212-2; results not shown).

In the *first* series of experiments, we examined how the lack of the CB_1 receptor affects the electrically evoked tritium overflow in pieces of the vas deferens and the atrium and in cortical slices. Tritium overflow from superfused tissues was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the amount of tritium overflow evoked by S_1 (expressed as percent of tissue tritium) was determined. Tritium overflow in vas deferens pieces (preincubated with [^{3}H]-NA) from $CB_1^{-/-}$ mice exceeded that in vas deferens pieces from wild-type mice by 37% (Figure 1). On the other hand, both strains did not differ with respect to the evoked overflow from atrial pieces preincubated with [^{3}H]-NA and from cortical slices preincubated with [^{3}H]-choline (Figure 1).

In the *second* series, the influence of CB_1 receptor deficiency on the effects of various drugs on the evoked overflow was examined. The drugs were added to the medium before and during S_2 and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. In vas deferens pieces preincubated with [^{3}H]-NA, the evoked overflow (S_2/S_1) was inhibited by the cannabinoid receptor agonist WIN 55,212-2 by 64% and facilitated by the CB_1 receptor inverse agonist/antagonist SR 141716 by 45% in tissues from $CB_1^{+/+}$ mice, but

was not affected in pieces from $CB_1^{-/-}$ mice, (Figure 2). In atrial pieces (preincubated with [^{3}H]-NA) from either strain, WIN 55,212-2 failed to affect the evoked tritium overflow whereas prostaglandin E_2 caused an inhibition by about 75% (Figure 2). With respect to the third model, that is, cortical slices preincubated with [^{3}H]-choline, WIN 55,212 and another cannabinoid receptor agonist, CP-55,940, inhibited the evoked overflow by about 15–20% in slices from $CB_1^{+/+}$ mice, but failed to do so in slices from $CB_1^{-/-}$ mice (Figure 3). SR 141716 and another CB_1 receptor inverse agonist/antagonist, AM 251, did not affect the evoked overflow in slices from either strain (Figure 3).

In the *third* series, we examined whether the lack of CB_1 receptors affects the tritium content determined at the end of superfusion. However, for each of the three experimental models the tritium content did not differ between preparations obtained from either mouse strain (Table 1).

Discussion

The aim of the present study was to examine how CB_1 receptor deficiency affects transmitter release in three isolated tissues differing qualitatively and quantitatively with respect to modulation of transmitter release by presynaptic CB_1 receptors. In the first model, that is, the vas deferens, NA release is inhibited *via* presynaptic CB_1 receptors, as suggested by studies in which transmitter release was determined directly (Trendelenburg *et al*., 2000) or *via* the endorgan response (electrically induced twitch response; Rinaldi-Carmona *et al*., 1994; Pertwee *et al*., 1996). The presynaptic CB_1 receptors in this tissue are subject to an endogenous tone since the CB_1 receptor inverse agonist/antagonist SR 141716 increases NA release (present study) or the electrically induced twitch response (Pertwee *et al*., 1996). The stimulatory effect of SR 141716 may be related to its antagonistic effect against endogenously formed endocannabinoids. Evidence that endocannabinoids can be formed and degraded in vas deferens tissue has been presented recently (Ates *et al*., 2003). Since SR 141716 is an inverse CB_1 receptor agonist, another explanation for the stimulatory effect is that part of the CB_1 receptors is precoupled.

In vas deferens pieces from CB_1 receptor knockout mice, the cannabinoid receptor agonist WIN 55,212-2 and the CB_1 receptor inverse agonist/antagonist SR 141716 failed to affect NA release, confirming that the effects in wild-type animals are indeed related to CB_1 receptors. These results, in addition, exclude the involvement of a non-CB_1–non-CB_2 receptor identified in the hippocampus of CB_1 receptor knockout mice, which is activated by WIN 55,212-2 and blocked by SR 141716 (Hájos *et al*., 2001). The fact that NA release is higher in the vas deferens from knockout mice when compared to wild-type animals (Table 2) is an additional argument that the presynaptic CB_1 receptors are subject to an endogenous tone. The possibility that the difference in NA release between both strains is related to an alteration of the neuronal NA transporter, or the presynaptic α_2-autoreceptor can be excluded since both mechanisms were routinely blocked by desipramine and rauwolscine, respectively. Furthermore, the possibility has to be considered that the increase in NA release in the vas deferens from CB_1 receptor-deficient mice is related to a higher density of the noradrenergic nerve terminals. However, the manner of calculation of the release (S_1) in our experiments as the ratio of the electrically evoked tritium overflow over the tritium content of the preparations *a priori* excludes this possibility. Nonetheless, it was of interest to examine whether the alteration in release might be accompanied by an alteration of the tritium content of the preparations. However, the amount of tritium present in the preparations at the end of superfusion did not differ between wild-type and knockout mice.

The possibility that the increase in NA release in the vas deferens from CB_1 receptor-deficient mice may be a more general phenomenon also has to be considered (e.g., in sympathetically innervated tissues). To exclude this possibility, we used our second model, that is, atrial NA release. Transmitter release in this tissue is not inhibited *via* CB_1 receptors in NMRI (Trendelenburg *et al*., 2000) and C57BL/6J mice (present study). $CB_1^{+/+}$ and $CB_1^{-/-}$ mice did not differ with respect to atrial NA release (Table 2), arguing against the view that CB_1 receptor deficiency generally leads to an increase in transmitter release. The latter conclusion was also reached previously since hippocampal NA release and striatal Ach release, both of which are not modulated by CB_1 receptors, did not differ between $CB_1^{+/+}$ and $CB_1^{-/-}$ mice (Table 2; Kathmann *et al*., 2001b). In order to prove that atrial NA release can be modified *via* presynaptic inhibitory receptors under the experimental conditions of the present study, we used prostaglandin E_2, which caused a strong inhibition in wild-type and knockout mice. A direct inhibitory effect of prostaglandin E_2 on NA release in the mouse atrium has so far not been shown. However, the fact that diclofenac (an

Table 2 Effects of cannabinoid receptor ligands and of cannabinoid CB_1 receptor deficiency on transmitter release in superfused tissues from C57BL/6J mice

Transmitter	*Tissue*	*Effects of cannabinoid receptor ligands on transmitter release in wild-type mice ($CB_1^{+/+}$)*		*Effect of CB_1 receptor deficiency on transmitter release ($CB_1^{-/-}$ vs $CB_1^{+/+}$)*	*Source*
		WIN 55,212-2 1 μM	*SR 141716 0.32 μM*		
NA	Vas deferens	↓	↑	↑	Present study
NA	Atrium	0	ND	0	Present study
NA	Hippocampus	0	0	0	Kathmann *et al*. (2001b)
Ach	Hippocampus	↓	↑	↑	Kathmann *et al*. (2001b)
Ach	Cerebral cortex	(↓)	0	0	Present study
Ach	Striatum	0	0	0	Kathmann *et al*. (2001b)

Ach, acetylcholine; NA, noradrenaline; ND, not determined. The symbols ↓, (↓) and ↑ indicate marked decrease, slight decrease and marked increase.

inhibitor of prostaglandin synthesis) increases the facilitatory effect of bradykinin on atrial NA release is an indirect evidence that prostaglandins are capable of inhibiting NA release in the atrium of this species (Chulak *et al*., 1998).

The vas deferens (NA release) and the hippocampus (Ach release) resemble each other in that in both tissues transmitter release is increased by SR 141716 and by CB_1 receptor gene disruption (Table 2). In this context, one might wonder how transmitter release will be affected by CB_1 receptor deficiency in a tissue in which transmitter release is inhibited *via* CB_1 receptors, but *not* facilitated by SR 141716. Our third model, that is, Ach release in the cerebral cortex, is such a tissue. It has previously been found that WIN 55,212-2 inhibits, whereas SR 141716 does not affect, Ach release in the cerebral cortex from NMRI mice (Kathmann *et al*., 2001a; Steffens *et al*., 2002) and the same results were obtained in the present study for C57BL/6J mice. To be very sure, we also examined another cannabinoid receptor agonist, CP-55,940, and another CB_1 receptor inverse agonist/antagonist, AM 251, which, as expected, inhibited and failed to affect Ach release in the cortex from wild-type mice, respectively. The fact that cortical Ach release did not differ between wild-type and knockout mice (Table 2) suggests that the lack of CB_1 receptors leads to an increase in transmitter release only in those tissues in which these receptors are subject to an endogenous tone.

Since the presynaptic CB_1 receptors causing inhibition of NA release in the mouse vas deferens are subject to an endogenous tone, the question might arise as to the physiological and/or pathophysiological role played by this receptor. The effects of cannabinoid receptor agonists on sexual functions are not uniform; both aphrodisiac effects and sexual dysfunction have been described in humans and animals (for a review, see Dewey, 1986; Stella, 2001). In a recent study on the sexual behavior of male rats, the cannabinoid receptor agonist HU-210 inhibited the latencies to mounting, intromission and ejaculation (Ferrari *et al*., 2000). These effects may be related to activation of cannabinoid receptors in the brain, but the possibility should be taken into consideration that the inhibitory effect on ejaculation is partially due to an impairment of NA release from the sympathetic neurones innervating the vas deferens (which is also endowed with presynaptic CB_1 receptors in the rat; Christopoulos *et al*., 2001).

In conclusion, the fact that NA release is higher in vas deferens from CB_1 receptor-deficient mice than in vas deferens from wild-type animals is an additional piece of evidence that these presynaptic CB_1 receptors are subject to an endogenous tone. This view is supported by our finding that in cortical slices, in which the presynaptic inhibitory CB_1 receptors according to findings with SR 141716 are not subject to an endogenous tone, Ach release is not facilitated by CB_1 receptor deficiency. The study also shows that the effects of WIN 55,212-2 and/or of SR 141716 on transmitter release in either tissue are related to CB_1 receptors and, in particular, excludes the involvement of non-CB_1–non-CB_2 receptors.

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