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Cannabinoid CB₁ receptor-mediated inhibition of noradrenaline release in guinea-pig vessels, but not in rat and mouse aorta

Received: 9 June 2005 / Accepted: 23 August 2005 / Published online: 30 September 2005
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Abstract Cannabinoids exert complex effects on blood pressure related to their interference with cardiovascular centres in the central nervous system and to their direct influence on vascular muscle, vascular endothelium and heart. In view of the relative lack of information on the occurrence of CB₁ receptors on the vascular postganglionic sympathetic nerve fibres, the aim of the present study was to examine whether cannabinoid receptor ligands affect the electrically evoked tritium overflow in superfused vessels (tissue pieces) from the guinea-pig, the rat and the mouse preincubated with ³H-noradrenaline. The cannabinoid receptor agonist WIN 55,212-2 (R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]-pyrrolo[1,2,3-de]1,4-benzoxazinyl] (1-naphthalenyl) methanone) inhibited the evoked tritium overflow in the guinea-pig aorta, but not in that of the rat or mouse. The concentration–response curve of WIN 55,212-2 was shifted to the right by the CB₁ receptor antagonist rimonabant, yielding an apparent pA₂ value of 7.9. The most pronounced (near-maximum) inhibition obtained at the highest WIN 55,212-2 concentration applied (3.2 μM) amounted to 40%. WIN 55,212-2 also inhibited the evoked overflow in guinea-pig pulmonary artery, basilar artery and portal vein, again in a manner sensitive to antagonism by rimonabant. The latter did not affect the evoked overflow by itself in the four vessels, but did increase the electrically evoked tritium overflow from superfused guinea-pig hippocampal slices preincubated with ³H-choline and from superfused guinea-pig retina discs preincubated with ³H-noradrenaline (labelling dopaminergic cells in this tissue). The inhibitory effect of 3.2 μM WIN 55,212-2 on the evoked overflow from the guinea-pig aorta was comparable in size to that obtained with agonists at the histamine H₃, κ opioid (KOP) and ORL₁ (NOP) receptor (1 or 10 μM,

producing the respective near-maximum effects) whereas prostaglandin E₂ 1 μM caused a higher near-maximum inhibition of 70%. Prostaglandin E₂ also induced an inhibition by 65 and 80% in the rat and mouse aorta respectively, indicating that the present conditions are basically suitable for detecting presynaptic receptor-mediated inhibition of noradrenaline release. The results show that the postganglionic sympathetic nerve fibres in the guinea-pig aorta, but not in the rat or mouse aorta, are endowed with presynaptic inhibitory cannabinoid CB₁ receptors; such receptors also occur in guinea-pig pulmonary artery, basilar artery and portal vein. These CB₁ receptors are not subject to an endogenous tone and the extent of inhibition obtainable via these receptors is within the same range as that of several other presynaptic heteroreceptors, but markedly lower than that obtainable via receptors for prostaglandin E₂.

Keywords Rimonabant · WIN 55,212-2 · Cannabinoid CB₁ receptor · Presynaptic receptor · Guinea-pig aorta · Rat aorta · Noradrenaline release · Prostaglandin E₂

Introduction

Cannabinoids can elicit a variety of cardiovascular effects. Acute administration of hashish or marijuana in humans leads to an increase in heart rate whereas the effects on blood pressure are more variable ranging from a slight increase to orthostatic hypotension (the latter occurring at higher doses; for review, see Hollister 1986; Kalant 2004). However, more serious cardiovascular events including cerebral ischaemia and infarction (for review, see Mousouttas 2004) and an increased rate of myocardial infarction shortly after ingestion of cannabis preparations (for review, see Kalant 2004) have been reported as well. There is increasing evidence from animal models that the recently identified endogenous cannabinoid system is implicated in hemorrhagic, endotoxic and cardiogenic shock (for review, see Wagner et al. 1998; Pacher et al. 2005a,b) and in the vasodilated state associated with advanced liver cirrhosis (Batkai et al. 2001; Ros et al. 2002). The endocannabinoid system may also be implicated

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as a compensatory mechanism in various types of arterial hypertension (for review, see Pacher et al. 2005a).

The targets for the cardiovascular effects of the cannabinoids comprise the central nervous system (Niederhoffer and Szabo 2000; Kwolek et al. 2005), the vascular muscle (Gebremedhin et al. 1999), the vascular endothelium (for review, see Pacher et al. 2005b) and the heart (Bonz et al. 2003; Pacher et al. 2004). Many but by far not all effects are mediated via cannabinoid CB₁ receptors. One of the endocannabinoids, anandamide, also activates the TRPV₁ receptor. Anandamide is capable of inducing vasodilation by releasing calcitonin-gene-related peptide (CGRP) from sensory neurones (Zygmunt et al. 1999) and, in addition, leads to a decrease in heart rate and blood pressure by activating the Bezold-Jarisch reflex (Varga et al. 1995; Malinowska et al. 2001). Anandamide also stimulates the so-called abnormal cannabidiol receptor, activation of which is associated with an endothelium-dependent vasodilation, e.g. of the mesenteric arteries (for review, see Pacher et al. 2005b). At this receptor (the exact molecular biology of which has not yet been clarified), cannabidiol, which, like Δ^9 -tetrahydrocannabinol, occurs in high concentrations in cannabis preparations, acts as an antagonist.

Many cannabinoid CB₁ receptors are located presynaptically and their activation causes inhibition of the release of the respective transmitter (Schlicker and Kathmann 2001; Szabo and Schlicker 2005). The possibility that cannabinoids act via CB₁ receptors on vascular postganglionic sympathetic nerve fibres has been considered in only a few studies. Thus, a CB₁ receptor-mediated inhibition of noradrenaline release (or of the endorgan response elicited by noradrenaline) has been shown for the pithed rat and rabbit (Malinowska et al. 1997; Niederhoffer and Szabo 1999; Niederhoffer et al. 2003; Pfitzer et al. 2005) and for isolated mesenteric (Ralevic and Kendall 2002) and renal arteries (Deutsch et al. 1997) from the rat.

The aim of the present study was to extend those previous investigations to other blood vessels of other species, in a first step, to the aorta from the rat, guinea-pig and mouse. In addition, we examined whether release-

modulating CB₁ receptors also occur in other vessels of the guinea-pig and, using the CB₁ receptor antagonist rimonabant (former name: SR 141716), whether the vascular CB₁ receptor is subject to an endogenous tone. The occurrence of an endogenous tone has been shown for many CB₁ receptors (for review, see Pertwee 2005), including the CB₁ receptors reducing acetylcholine release from the guinea-pig hippocampus (Schultheiß et al. 2004) and dopamine release from the guinea-pig retina (Schlicker et al. 1996). Furthermore, the extent of inhibition of noradrenaline release via CB₁ receptors and via another four types of presynaptic receptors was compared in the guinea-pig aorta. Finally, radioligand binding studies with ³H-rimonabant were carried out in guinea-pig cortex membranes to exclude that the agonists at those presynaptic receptors might, in addition, have affinity for CB₁ receptors.

Materials and methods

Superfusion studies

Aortae were prepared from male Dunkin–Hartley guinea-pigs, male Wistar rats or male NMRI mice. Pulmonary artery, basilar artery, portal vein, hippocampus and retina were prepared from male Dunkin–Hartley guinea-pigs (for details, see Table 1). Preparations were incubated (37°C) for 60 min with physiological salt solution (PSS; Ca²⁺ 1.3 mM) containing ³H-noradrenaline or ³H-choline (for details, see Table 1). Subsequently, the preparations were transferred to superfusion chambers and superfused (0.5 ml/min) with PSS (37°C) (for Ca²⁺ concentration and auxiliary drugs, see Table 1). The superfusate was collected in 5-min samples; experiments lasted for 60 or 110 min (see Table 1). Tritium overflow was evoked by one or two 2-min period(s) of electrical field stimulation (for stimulation parameters, see Table 1) after 40 and/or 90 min of superfusion (S₁ and S₂). The drugs under study were present in the medium either throughout superfusion or from 62 min of superfusion onward, as indicated in the Results section. The PSS

Table 1 Details of superfusion studies

	Vessels	Hippocampus	Retina
Preparation of tissues	Irregularly shaped pieces ^a	Slices (0.3 mm thick, diameter 2 mm)	Discs (diameter 3 mm)
Preincubation of preparations with medium containing	³ H-noradrenaline 0.025 µM	³ H-choline 0.1 µM	³ H-noradrenaline 0.1 µM
Ca ²⁺ concentration in superfusion medium (mM)	3.25	3.25	1.3
Auxiliary drugs in superfusion medium (µM)	Desipramine 1, rauwolscine 1	Hemicholinium 10, AF-DX 384 1	Nomifensine 10, sulpiride 3.2
Duration of superfusion (min)	110	60	60
Number of stimulation periods	Two, after 40 and 90 min	One, after 40 min	One, after 40 min
Stimulation parameters	3 Hz, 200 mA, 2 ms	3 Hz, 200 mA, 2 ms	1 Hz, 200 mA, 2 ms

^aNo effort was made to ensure that the endothelium remained intact

was composed as follows (mM): NaCl 118, KCl 4.8, CaCl₂ 1.3 or 3.25 (as indicated in Table 1), KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, ascorbic acid 0.06, disodium EDTA 0.03, glucose 10, and the solution was aerated with 95% O₂ and 5% CO₂ (pH 7.4).

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 S₂ (t₂) to those in the 5-min period 15–20 min after the onset of S₁ (t₁) was determined (for drugs added to the PSS from 62 min of superfusion onward) or the t₁ 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 the basal from the total efflux during stimulation and the subsequent 13 min and expressed as a percentage 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 the onset of stimulation). To quantify drug-induced effects on the stimulated tritium overflow, the ratio of the overflow evoked by S₂ over that evoked by S₁ was determined (S₂/S₁; for drugs added to the PSS from 62 min of superfusion) or the S₁ values obtained in the absence or presence of a given drug were directly compared with each other (for drugs present throughout superfusion). The apparent pA₂ value for rimonabant was calculated according to the formula $pA_2 = \log \left(\frac{[A']}{[A]} - 1 \right) - \log [B]$, where [A'] and [A] are the IC_{20%} values for WIN 55,212-2 obtained in the presence and absence of rimonabant and [B] is the concentration of rimonabant.

Binding studies

Cerebral cortex membranes from male Dunkin–Hartley guinea-pigs were homogenised (Potter–Elvehjem) in 25 vol of ice-cold Tris-HCl buffer (Tris 50 mM, pH 7.5; EDTA 5 mM; sucrose 10.27%) and centrifuged at 1,500×g for 10 min (4°C). The supernatant was centrifuged at 39,000×g for 20 min and the pellet was resuspended in buffer (Tris 50 mM, pH 7.5; EDTA 5 mM) and frozen at –80°C.

For binding experiments, membranes were incubated with sucrose-free buffer in a final volume of 0.5 ml containing 40–70 µg protein. ³H-rimonabant was used at eight concentrations ranging from 0.05 to 8 nM for saturation experiments and at 0.5 nM for competition experiments. The incubation (25°C) was terminated after 60 min by filtration through Whatman GF/C filters. CP-55,940 (3 µM) was used to determine non-specific binding. Data were analysed using the programme GraphPadPrism (Prism; GraphPad Software, San Diego, CA, USA).

Statistics

Results are given as means±SEM of *n* experiments (superfusion) and of *n* experiments in duplicate or triplicate (saturation and competition experiments with ³H-rimona-

bant respectively). For comparison of mean values, the *t* test for unpaired data was used (superfusion studies); the Bonferroni correction was used when two or more values were compared with the same control. The *F* test was applied in order to evaluate whether the inhibition of ³H-rimonabant binding by drugs is better fitted by a one- or a two-site model.

Drugs used

[Methyl-³H]-choline chloride (specific activity 86 Ci/mmol), (R)-(-)-[ring-2,5,6-³H]-noradrenaline (spec. act. 53 Ci/mmol; NEN, Zaventem, Belgium); ³H-SR141716A (³H-rimonabant; spec. act. 44 Ci/mmol; Amersham, Braunschweig, Germany); AF-DX 384 (5,11-dihydro-11-{{(2-{2-[(dipropylamino)methyl]-1-piperidinyl}ethyl)amino]carbonyl}-6H-pyrido(2,3-β)(1,4)benzodiazepine-6-one; Boehringer-Ingelheim, Biberach an der Riss, Germany); CP-55,940 ((-)-cis-3-[2[hydroxy-4-(1,1-dimethylheptyl)phenyl]trans-4-(3-hydroxypropyl)-cyclohexanol; Biotrend, Cologne, Germany); desipramine hydrochloride (Novartis, Wehr, Germany); hemicholinium-3, prostaglandin E₂, sulphiride, 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, Munich, Germany); R-α-methylhistamine dihydrogenmaleate (Professor W. Schunack, Institut für Pharmazie, Freie Universität Berlin); nociceptin (Bachem, Bubendorf, Switzerland); nomifensine (Sanofi-Aventis, Frankfurt, Germany); rauwolscine hydrochloride (Roth, Karlsruhe, Germany); rimonabant hydrochloride (SR 141716A; Sanofi-Aventis, Montpellier, France); U-69,593 ((5α,7α,8β)-(+)-N-methyl-N-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl)-benzeneacetamide; MP Biomedicals, Eschwege, Germany). Stock solutions of the drugs were prepared with ethanol (prostaglandin E₂), dimethylsulfoxide (CP-55,940, rimonabant, WIN 55,212-2) or water and diluted with PSS (superfusion experiments) or water (binding experiments) to the concentration required.

Table 2 Electrically evoked tritium overflow (expressed as a percentage of tissue tritium) from superfused vascular tissues from guinea-pig, rat and mouse preincubated with ³H-noradrenaline and the effect of rimonabant

Species	Vessel	S ₁ (% of tissue tritium)		
		No rimonabant	Rimonabant 0.032 µM	Rimonabant 0.32 µM
Guinea-pig	Aorta	5.88±0.73	5.85±0.63	6.44±1.14
	Pulmonary artery	9.59±0.65	N.D.	10.37±1.25
	Basilar artery	10.95±2.59	N.D.	11.82±0.56
	Portal vein	6.07±0.87	N.D.	8.17±1.12
Rat	Aorta	4.42±0.39	N.D.	N.D.
Mouse	Aorta	4.04±0.52	N.D.	N.D.

Tritium overflow was evoked by electrical field stimulation after 40 min of superfusion (S₁). Means±SEM of 3–13 experiments N.D. not determined

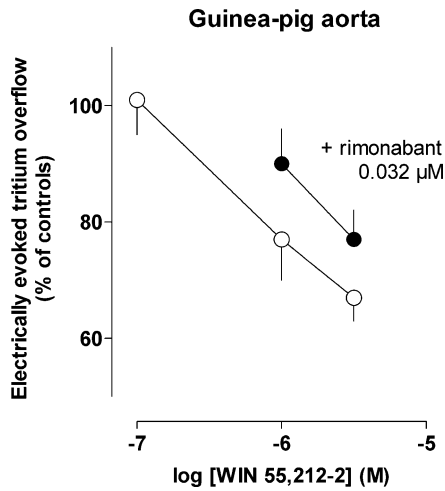
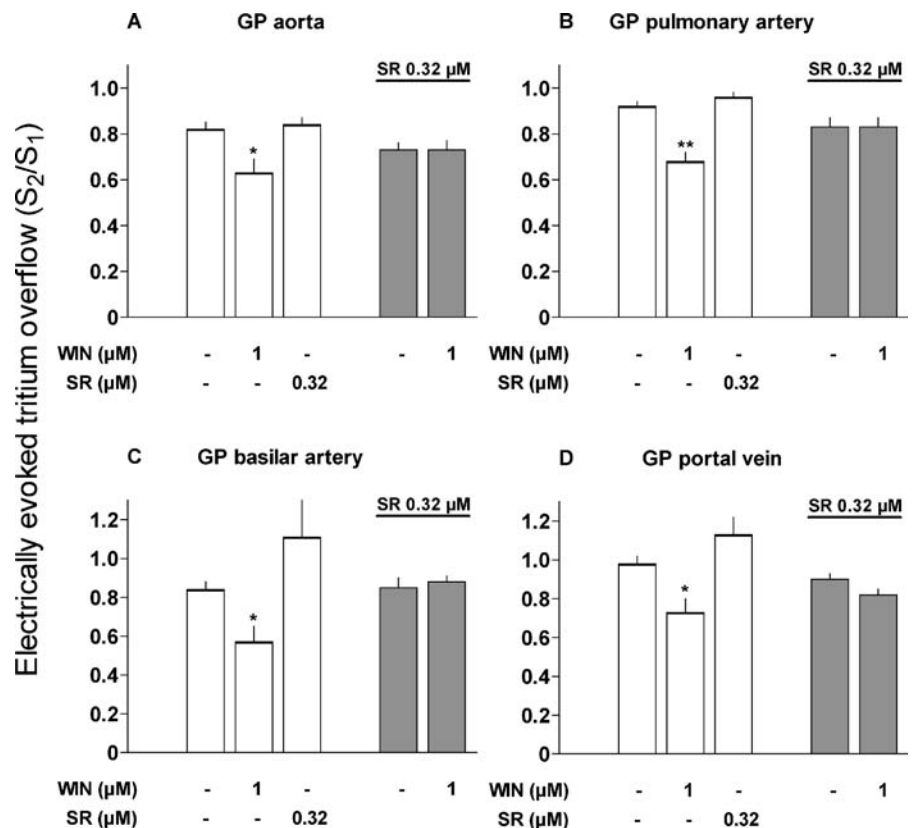


Fig. 1 Effect of WIN 55,212-2 on the electrically (3 Hz) evoked tritium overflow from guinea-pig aorta pieces preincubated with ^3H -noradrenaline, and interaction of WIN 55,212-2 with rimonabant. Following incubation, the tissues were transferred to superfusion chambers and superfused at a flow rate of 0.5 ml/min. The superfusion medium contained WIN 55,212-2 from 62 min of superfusion onward and rimonabant as well as two auxiliary drugs (1 μM desipramine plus 1 μM rauwolscine) throughout superfusion (110 min). Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. Tritium overflow was expressed as a percentage of the S_2/S_1 value in controls (not shown). Means \pm SEM of three to five experiments

Fig. 2 Effect of WIN 55,212-2 and rimonabant on the electrically (3 Hz) evoked tritium overflow from superfused vascular tissue pieces from guinea-pig (GP) preincubated with ^3H -noradrenaline, and interaction of WIN 55,212-2 with rimonabant. *White columns*: the superfusion medium contained WIN 55,212-2 (WIN) or rimonabant (SR) from 62 min of superfusion onward. *Dark columns*: the superfusion medium contained WIN 55,212-2 from 62 min of superfusion onward and rimonabant throughout superfusion (110 min). Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). Means \pm SEM of three to eight experiments. * $P < 0.02$, ** $P < 0.005$, compared with the corresponding control



Results

Transmitter release studies

Basal tritium efflux was not affected by any of the drugs or their organic solvents (DMSO up to 0.03% and ethanol 0.01%) in any of the preparations investigated (results not shown).

To investigate the effects of the drugs on the electrically evoked tritium overflow the drug under study was present in the medium before and during S_2 (the second period of electrical stimulation); its effect on the evoked overflow was calculated as the ratio of the overflow evoked by S_2 over that evoked by S_1 and compared with the corresponding control S_2/S_1 ratio in the absence of the test drug. To study the interaction of WIN 55,212-2 with rimonabant, WIN 55,212-2 was present in the medium before and during S_2 whereas rimonabant was present throughout superfusion. In the experiments designed to examine whether the CB_1 receptor is subject to an endogenous tone (Fig. 4), rimonabant was present throughout superfusion of shorter experiments, which allowed one electrical stimulation only. The control S_1 values are shown in Fig. 4 and Table 2, and the control S_2/S_1 ratios are depicted in Figs. 2 and 3. S_1 and S_2/S_1 values were not affected by DMSO (up to 0.03%) or ethanol (0.01%).

The cannabinoid receptor agonist WIN 55,212-2 inhibited the electrically evoked tritium overflow (S_2/S_1) from guinea-pig aorta pieces preincubated with ^3H -nor-

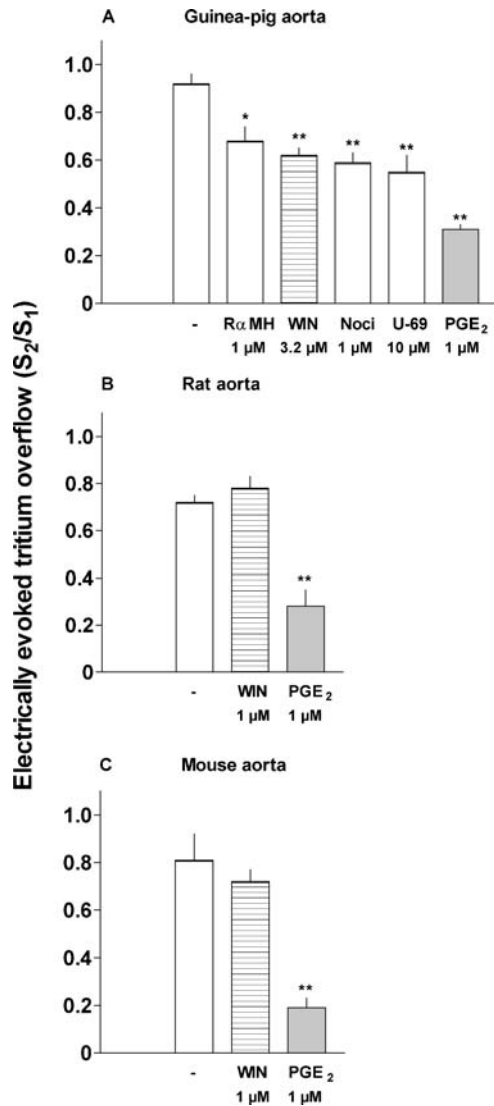


Fig. 3 Effect of WIN 55,212-2 (WIN), prostaglandin E₂ (PGE₂), R- α -methylhistamine (R α MH), nociceptin (Noci) and U-69,593 (U-69) on the electrically (3 Hz) evoked tritium overflow from superfused **a** guinea-pig, **b** rat and **c** mouse aorta pieces preincubated with ³H-noradrenaline. The superfusion medium contained the drug under study from 62 min of superfusion onward (total duration of superfusion 110 min). Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the overflow evoked by S₂ over that evoked by S₁ was determined (S₂/S₁). Means \pm SEM of four to nine experiments. * P <0.05, ** P <0.01

adrenaline in a concentration-dependent manner (Fig. 1). Due to its limited solubility concentrations of WIN 55,212-2 higher than 3.2 μ M could not be examined and its maximum inhibitory effect could not be determined precisely. If one assumes that WIN 55,212-2 inhibits the evoked overflow by a maximum of 40%, the concentration causing the half-maximum effect (i.e. an inhibition by 20%) will be 0.7 μ M. The concentration–response curve of WIN 55,212-2 was shifted to the right by rimonabant 0.032 μ M (Fig. 1), yielding an apparent pA₂ value of 7.9. Rimonabant 0.032 μ M by itself did not affect the evoked overflow (S₁; Table 2).

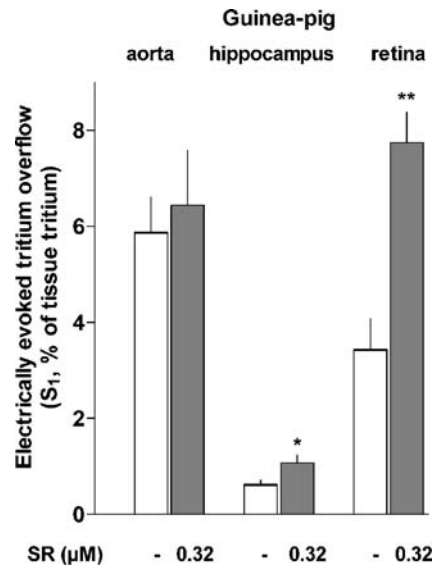


Fig. 4 Effect of rimonabant (SR) on the electrically evoked tritium overflow from superfused guinea-pig aorta pieces (stimulation frequency 3 Hz) and retina discs preincubated with ³H-noradrenaline (1 Hz), and from guinea-pig hippocampal slices preincubated with ³H-choline (3 Hz). The superfusion medium contained rimonabant throughout superfusion (60 min). Tritium overflow was evoked once, after 40 min of superfusion (S₁), and is expressed as a percentage of tissue tritium. Means \pm SEM of three to nine experiments. * P <0.05, ** P <0.01

WIN 55,212-2 (1 μ M) inhibited the electrically evoked tritium overflow (S₂/S₁) not only in the guinea-pig aorta (by 23%), but also in the pulmonary artery, basilar artery and portal vein from this species (by 26, 33 and 26% respectively; Fig. 2). The inhibitory effect was counteracted by rimonabant 0.32 μ M (Fig. 2). Rimonabant by itself did not have a statistically significant effect, but tended to increase the evoked overflow in each of the eight experimental series (Fig. 2 [S₂/S₁]; Table 2 [S₁]).

WIN 55,212-2 (1 μ M) failed to inhibit the electrically evoked tritium overflow (S₂/S₁) in the rat and mouse aorta (Fig. 3B,C). In the aorta from the two rodent species and from the guinea-pig, prostaglandin E₂ (1 μ M) inhibited the evoked overflow by more than 60% (Fig. 3A–C). The evoked overflow from the guinea-pig aorta was also inhibited by the histamine H₃ receptor agonist R- α -methylhistamine, by nociceptin and by the κ opioid receptor agonist U-69,593; the extent of inhibition, representing the near-maximum effects of the respective drugs, was comparable to that obtained with WIN 55,212-2 (3.2 μ M; Fig. 3A).

Finally, the effect of rimonabant on the electrically evoked tritium overflow in the guinea-pig aorta preincubated with ³H-noradrenaline was compared with its effect on the electrically evoked tritium overflow from guinea-pig hippocampal slices preincubated with ³H-choline and guinea-pig retina discs preincubated with ³H-noradrenaline (Fig. 4). In the latter two tissues, rimonabant 0.32 μ M, which was ineffective in the aorta (see also above), facilitated the evoked tritium overflow (S₁) by 70 and 125% respectively (Fig. 4).

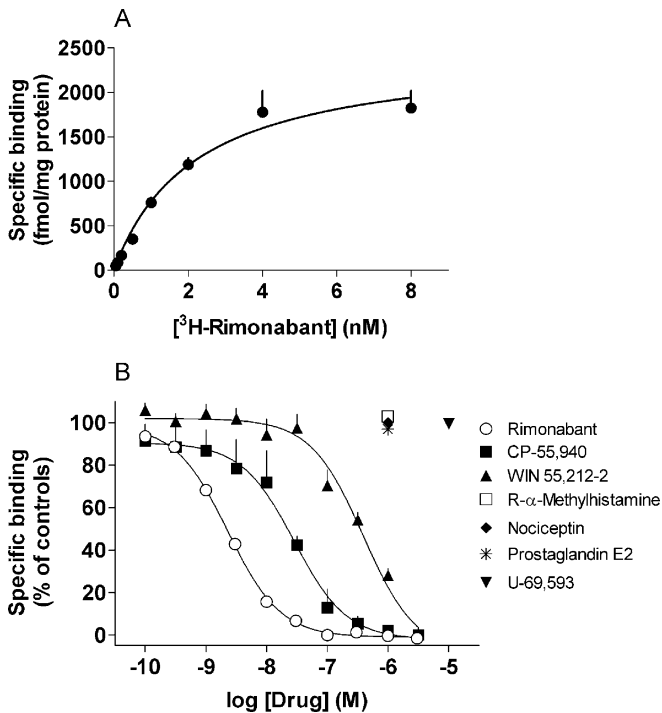


Fig. 5 Specific ^3H -rimonabant binding to guinea-pig cortex membranes. Membranes were incubated (25°C) for 60 min with ^3H -rimonabant. Specific binding was defined as that inhibited by CP-55,940 ($3\ \mu\text{M}$). A Saturation binding experiments. The effect of cannabinoid receptor ligands and of agonists at other presynaptic inhibitory receptors on the binding of ^3H -rimonabant $0.5\ \text{nM}$ is shown in **B**. Means \pm SEM of four experiments in **A** duplicate or **B** triplicate (for some data points, SEM is smaller than the symbols)

Binding studies

In *saturation* binding experiments on guinea-pig brain cortex membranes, using ^3H -rimonabant at eight concentrations, a K_D value of $2.12\pm 0.56\ \text{nM}$ with a maximum number of binding sites (B_{max}) of $2,340\pm 420\ \text{fmol/mg}$ protein was determined (Fig. 5A). Scatchard analysis revealed a straight line with a Hill coefficient (n_H) of unity (not shown). Unspecific binding (determined with $3\ \mu\text{M}$ CP-55,940) was 27% for $0.5\ \text{nM}$ ^3H -rimonabant. In *competition* binding experiments, binding of $0.5\ \text{nM}$ ^3H -rimonabant was inhibited monophasically (n_H near unity) by (unlabelled) rimonabant and the cannabinoid receptor agonists CP-55,940 and WIN 55,212-2, yielding $\text{p}K_i$ (\pm SEM) values of 8.72 ± 0.06 , 7.65 ± 0.09 and 6.48 ± 0.11 respectively (Fig. 5B). In the concentrations used for the experiments in Fig. 3, R- α -methylhistamine, nociceptin and prostaglandin E_2 ($1\ \mu\text{M}$ each) and U-69,593 ($10\ \mu\text{M}$) did not affect binding (Fig. 5B).

Discussion

The aim of the present study was to identify release-inhibiting cannabinoid CB_1 receptors in superfused vascular tissue labelled by ^3H -noradrenaline. In some

experiments, we also examined guinea-pig hippocampal slices preincubated with ^3H -choline, which in turn is metabolised to ^3H -acetylcholine. Furthermore, retinal discs were preincubated with ^3H -noradrenaline, which, however, is accumulated in *dopaminergic* amacrine cells (false-labelling; Schlicker et al. 1996). ^3H -noradrenaline rather than ^3H -dopamine was used since the scatter of variation of the results is lower when the former tracer is used (Schlicker et al. 1996). In the vascular and in the two non-vascular tissues electrical stimulation was used to induce quasi-physiological exocytotic release of the respective ^3H -tracer molecule (or of ^3H -acetylcholine in the case of ^3H -choline) and the corresponding endogenous unlabelled neurotransmitter.

Superfusion of vascular pieces, hippocampal slices and retinal discs was routinely performed in the presence of an inhibitor of the high-affinity noradrenaline, choline and dopamine transporter, i.e. desipramine, hemicholinium and nomifensine respectively, to avoid interactions of the drugs under study with the respective transporters and to increase the amount of tritium overflow. In addition, an antagonist of the respective presynaptic inhibitory autoreceptors, i.e. rauwolscine, AF-DX 384 and sulpiride, was present in the medium to avoid interactions of the drugs under study with the respective autoreceptors and again to increase the amount of tritium overflow. There was, however, an additional reason to use the α_2 -adrenoceptor antagonist rauwolscine for the superfusion experiments on vessels. The extent of inhibition of noradrenaline release following activation of presynaptic heteroreceptors (e.g. opioid receptors) is usually increased in noradrenergically innervated tissues, including vessels, when the presynaptic α_2 -autoreceptor is blocked simultaneously (for review, see Schlicker and Göthert 1998). Such an interaction (which might take place at the level of G proteins; Schlicker and Göthert 1998) has also been shown for the presynaptic CB_1 receptor causing inhibition of noradrenaline release in guinea-pig hippocampal slices (Schlicker and Göthert 1998) and may occur in guinea-pig vessels as well, although this has not been studied systematically so far. A direct effect of rauwolscine ($1\ \mu\text{M}$) on CB_1 receptors can be excluded since the drug did not exhibit an affinity for ^3H -rimonabant binding in rat brain cortex membranes (Kathmann et al. 1999).

Noradrenaline release from the guinea-pig aorta was inhibited by the cannabinoid receptor agonist WIN 55,212-2 and its concentration-response curve was shifted to the right by the CB_1 receptor antagonist rimonabant, suggesting that the effect is mediated via the CB_1 receptor. Due to the limited solubility of WIN 55,212-2 full concentration-response curves could not be obtained and the maximum effect could not be determined. The apparent $\text{p}A_2$ of 7.9 is identical to the reference value given in the review by Alexander et al. (2004); it is also close to the values obtained for the presynaptic CB_1 receptors causing inhibition of noradrenaline release in the guinea-pig hippocampus (8.2; Schlicker et al. 1997) and of dopamine release in guinea-pig retina (8.3; Schlicker et al. 1996). The present

study shows that presynaptic inhibitory CB₁ receptors, in addition, occur in guinea-pig pulmonary artery, basilar artery and portal vein.

Presynaptic inhibitory CB₁ receptors could not be identified in the aorta of the rat and the mouse. The possibility that the conditions used for electrical stimulation in the rodent aorta in the present study were not suitable to detect presynaptic modulation can be excluded since prostaglandin E₂ inhibited noradrenaline release by at least 60%. The difference in the aorta of the various species is reminiscent of the lack of CB₁ receptor-mediated modulation of noradrenaline release in the rodent hippocampus, whereas such a mechanism is operative in the guinea-pig hippocampus (Schlicker et al. 1997). On the other hand, the sympathetic nerve fibres innervating the resistance vessels and the mesenteric and renal arteries in the rat are endowed with presynaptic CB₁ receptors (Deutsch et al. 1997; Malinowska et al. 1997; Ralevic and Kendall 2002; Niederhoffer et al. 2003; Pfitzer et al. 2005).

Rimonabant did not affect noradrenaline release in the four vessels of the guinea-pig under study (although there was a tendency towards an increase). In this respect, the vessels differ from guinea-pig hippocampus and retina in which the CB₁ receptors involved in the inhibition of acetylcholine and dopamine release respectively are subject to an endogenous tone, i.e. rimonabant facilitates transmitter release (Schlicker et al. 1996; Schultheiß et al. 2004). To confirm the organ specificity of a tonical activation of CB₁ receptors, we repeated the experiments on hippocampal slices and retina discs under the conditions of the present study, i.e. using a shorter exposure to rimonabant (40 vs. 60 min) and superfusion chambers with a lower volume. Again, rimonabant markedly increased acetylcholine and dopamine release respectively, demonstrating that a facilitation of transmitter release is possible under the experimental conditions of the present study. The possibility has to be considered anyway that the four guinea-pig vessels are subject to an endogenous tone in vivo (i.e. when they are in their natural surroundings, possibly providing endocannabinoids). On the other hand, rimonabant also failed to increase noradrenaline release in the resistance vessels of the pithed rat (Malinowska et al. 1997; Pfitzer et al. 2005), i.e. in an experimental model more closely resembling the in vivo situation compared with the in vitro experiments of the present study.

The maximum inhibitory effect of WIN 55,212-2 on noradrenaline release in the guinea-pig aorta was 40% or slightly higher. Since an inhibition of 40% appears to be relatively small, the effects of agonists at other presynaptic inhibitory heteroreceptors in the guinea-pig aorta, namely the histamine H₃, ORL₁, κ opioid and prostaglandin EP₃ receptors (at concentrations causing the maximum or near-maximum effect at the respective receptors; Exner and Schlicker 1995; Schlicker et al. 1998; Timm et al. 1998; Bauer et al. 1999), were examined in this study as well. The inhibitory effects were 26% via H₃, 33% via CB₁, 36% via ORL₁, 40% via κ and 66% via EP₃ receptors. The effects of

the same agonists (at identical concentrations) on noradrenaline release from guinea-pig hippocampal slices have been studied by us previously (Szabo and Schlicker 2005 and unpublished), yielding an extent of inhibition of 27% via H₃, 49% via CB₁, 82% via ORL₁, 94% via κ and 21% via EP₃ receptors. The comparisons show that in both experimental models the extent of CB₁ receptor-mediated inhibition of noradrenaline release is moderate, but within the range mediated by other presynaptic inhibitory heteroreceptors. The inhibitory effect of R-α-methylhistamine, nociceptin, U-69,593 and prostaglandin E₂ cannot be attributed to a putative agonistic property at CB₁ receptors in addition to their respective specific properties since the four agonists at the concentrations under study did not have an affinity for CB₁ receptor binding sites determined with ³H-rimonabant in guinea-pig cortex membranes. The lack of affinity of prostaglandin E₂, which like some of the endocannabinoids is derived from arachidonic acid, had also been shown by Howlett et al. (1992) on rat brain membranes, using the radioligand ³H-CP-55,940.

In conclusion, the postganglionic sympathetic nerve fibres innervating guinea-pig aorta, pulmonary artery, basilar artery and portal vein are endowed with presynaptic inhibitory cannabinoid CB₁ receptors, whereas such receptors could not be identified in rat and mouse aorta. The CB₁ receptors in the guinea-pig vessels are not subject to an endogenous tone and the maximum extent of CB₁ receptor-mediated inhibition is within the same range as that of several other presynaptic inhibitory heteroreceptors, except for EP₃ receptors, which mediate a more pronounced inhibition.

Acknowledgements The financial support by the Deutsche Forschungsgemeinschaft (Graduiertenkolleg 246 and grant "Schl 266/5-5") and by the faculty programme "BONFOR" is gratefully acknowledged. We would also like to thank Mrs. D. Petri and Mrs. P. Zeidler for their technical assistance and Professor Schunack and the companies Boehringer-Ingelheim, Novartis and Sanofi-Aventis for gifts of drugs.

References

- Alexander SPH, Mathie A, Peters JA (2004) Guide to receptors and channels. *Br J Pharmacol* 141:S1–S126
- Batkai S, Jarai Z, Wagner JA, Goparaju SK, Varga K, Liu J, Wang L, Mirshahi F, Khanolkar AD, Makriyannis A, Urbaschek R, Garcia N Jr, Sanyal AJ, Kunos G (2001) Endocannabinoids acting at vascular CB₁ receptors mediate the vasodilated state in advanced liver cirrhosis. *Nat Med* 7:827–832
- Bauer U, Nakazi M, Kathmann M, Göthert M, Schlicker E (1999) The stereoselective κ opioid receptor antagonist Mr 2266 does not exhibit stereoselectivity as an antagonist at the orphan opioid (ORL₁) receptor. *Naunyn-Schmiedeberg Arch Pharmacol* 359: 17–20
- Bonz A, Laser M, Kullmer S, Kniesch S, Babin-Ebell J, Popp V, Ertl G, Wagner JA (2003) Cannabinoids acting on CB₁ receptors decrease contractile performance in human atrial muscle. *J Cardiovasc Pharmacol* 41:657–664

- Deutsch DG, Goligorsky MS, Schmid PC, Krebsbach RJ, Schmid HH, Das SK, Dey SK, Arreaza G, Thorup C, Stefano G, Moore LC (1997) Production and physiological actions of anandamide in the vasculature of the rat kidney. *J Clin Invest* 100:1538–1546
- Exner HJ, Schlicker E (1995) Prostanoid receptors of the EP₃ subtype mediate the inhibitory effect of prostaglandin E₂ on noradrenaline release in the mouse brain cortex. *Naunyn-Schmiedeberg Arch Pharmacol* 351:46–52
- Gebremedhin D, Lange AR, Campbell WB, Hillard CJ, Harder DR (1999) Cannabinoid CB₁ receptor of cat cerebral arterial muscle functions to inhibit L-type Ca²⁺ channel current. *Am J Physiol* 276:H2085–H2093
- Hollister LE (1986) Health aspects of cannabis. *Pharmacol Rev* 38:1–20
- Howlett AC, Evans DM, Houston DB (1992) The cannabinoid receptor. In: Murphy L, Bartke A (eds) *Marijuana/cannabinoids: neurobiology and neurophysiology*. CRC, Boca Raton, pp 35–72
- Kalant H (2004) Adverse effects of cannabis on health: an update of the literature since 1996. *Prog Neuropsychopharmacol Biol Psychiatry* 28:849–863
- Kathmann M, Bauer U, Schlicker E, Göthert M (1999) Cannabinoid CB₁ receptor-mediated inhibition of NMDA- and kainate-stimulated noradrenaline and dopamine release in the brain. *Naunyn-Schmiedeberg Arch Pharmacol* 359:466–470
- Kwolek G, Zakrzaska A, Schlicker E, Göthert M, Godlewski G, Malinowska B (2005) Central and peripheral components of the pressor effect of anandamide in urethane-anaesthetized rats. *Br J Pharmacol* 145:567–575
- Malinowska B, Godlewski G, Bucher B, Schlicker E (1997) Cannabinoid CB₁ receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. *Naunyn-Schmiedeberg Arch Pharmacol* 356:197–202
- Malinowska B, Kwolek G, Göthert M (2001) Anandamide and methanandamide induce both vanilloid VR1- and cannabinoid CB₁ receptor-mediated changes in heart rate and blood pressure in anaesthetized rats. *Naunyn-Schmiedeberg Arch Pharmacol* 364:562–569
- Mousouttas M (2004) Cannabis use and cerebrovascular disease. *Neurologist* 10:47–53
- Niederhoffer N, Szabo B (1999) Effect of the cannabinoid receptor agonist WIN55212-2 on sympathetic cardiovascular regulation. *Br J Pharmacol* 126:457–466
- Niederhoffer N, Szabo B (2000) Cannabinoids cause central sympathoexcitation and bradycardia in rabbits. *J Pharmacol Exp Ther* 294:707–713
- Niederhoffer N, Schmid K, Szabo B (2003) The peripheral sympathetic nervous system is the major target of cannabinoids in eliciting cardiovascular depression. *Naunyn-Schmiedeberg Arch Pharmacol* 367:434–443
- Pacher P, Batkai S, Kunos G (2004) Haemodynamic profile and responsiveness to anandamide of TRPV1 receptor knock-out mice. *J Physiol (Lond)* 558:647–657
- Pacher P, Batkai S, Kunos G (2005a) Blood pressure regulation by endocannabinoids and their receptors. *Neuropharmacology* 48:1130–1138
- Pacher P, Batkai S, Kunos G (2005b) Cardiovascular pharmacology of cannabinoids. In: Pertwee RG (ed) *Cannabinoids (handbook of experimental pharmacology, vol 168)*. Springer, Berlin Heidelberg New York, pp 599–625
- Pertwee RG (2005) Inverse agonism and neutral antagonism at cannabinoid CB₁ receptors. *Life Sci* 76:1307–1324
- Pfitzer T, Niederhoffer N, Szabo B (2005) Search for an endogenous cannabinoid-mediated effect in the sympathetic nervous system. *Naunyn-Schmiedeberg Arch Pharmacol* 371:9–17
- Ralevic V, Kendall DA (2002) Cannabinoids inhibit pre- and postjunctionally sympathetic neurotransmission in rat mesenteric arteries. *Eur J Pharmacol* 444:171–181
- Ros J, Claria J, To-Figuera J, Planaguma A, Cejudo-Martin P, Fernandez-Varo G, Martin-Ruiz R, Arroyo V, Rivera F, Rodes J, Jimenez W (2002) Endogenous cannabinoids: a new system involved in the homeostasis of arterial pressure in experimental cirrhosis in the rat. *Gastroenterology* 122:85–93
- 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, Timm J, Göthert M (1996) Cannabinoid receptor-mediated inhibition of dopamine release in the retina. *Naunyn-Schmiedeberg Arch Pharmacol* 354:791–795
- Schlicker E, Timm J, Zentner J, Göthert M (1997) Cannabinoid CB₁ receptor-mediated inhibition of noradrenaline release in the human and guinea-pig hippocampus. *Naunyn-Schmiedeberg Arch Pharmacol* 356:583–589
- Schlicker E, Werthwein S, Kathmann M, Bauer U (1998) Nociceptin inhibits noradrenaline release in the mouse brain cortex via presynaptic ORL₁ receptors. *Naunyn-Schmiedeberg Arch Pharmacol* 358:418–422
- Schultheiß T, Flau K, Redmer A, Kathmann M, Reggio P, Seltzman HH, Schlicker E (2004) The facilitatory effect of SR141716 on transmitter release in guinea-pig hippocampus is due to its inverse agonist activity at cannabinoid CB₁ receptors. *Naunyn-Schmiedeberg Arch Pharmacol* 369(Suppl 1):R84
- Szabo B, Schlicker E (2005) Effects of cannabinoids on neurotransmission. In: Pertwee RG (ed) *Cannabinoids (handbook of experimental pharmacology, vol 168)*. Springer, Berlin Heidelberg New York, pp 327–365
- Timm J, Marr I, Werthwein S, Elz S, Schunack W, Schlicker E (1998) H₂ receptor-mediated facilitation and H₃ receptor-mediated inhibition of noradrenaline release in the guinea-pig brain. *Naunyn-Schmiedeberg Arch Pharmacol* 357:232–239
- Varga K, Lake K, Martin BL, Kunos G (1995) Novel antagonist implicates the CB₁ cannabinoid receptor in the hypotensive action of anandamide. *Eur J Pharmacol* 278:279–283
- Wagner JA, Varga K, Kunos G (1998) Cardiovascular actions of cannabinoids and their generation during shock. *J Mol Med* 76:824–836
- Zygmunt PM, Petersson J, Andersson DA, Chuang HH, Sorgard M, Di Marzo V, Julius D, Högestätt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400:452–457