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IDENTIFICATION OF A PRESYNAPTIC CANNABINOID CB_1 RECEPTOR IN THE GUINEA-PIG ATRIUM AND SEQUENCING OF THE GUINEA-PIG CB_1 RECEPTOR

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We studied whether cannabinoid CB_1 receptors occur on the sympathetic neurones innervating the guinea-pig atrium and renal cortex. Atrial and cortical kidney pieces preincubated with [^{3}H]-noradrenaline were superfused and the electrically (3 Hz)-evoked tritium overflow was examined. The evoked overflow in atrium was inhibited by the cannabinoid agonist WIN 55,212-2 maximally by 35%; its concentration-response curve was shifted to the right by the CB_1 antagonist rimonabant (pA_2 8.3), which, by itself, did not affect the evoked overflow. The evoked overflow in the renal cortex was not altered by WIN 55,212-2. The muscarinic agonist oxotremorine and prostaglandin E_2 inhibited the evoked overflow maximally by 55 and 65% in atrium and by 80 and 55% in kidney, respectively. Furthermore, the nucleotide sequence of the guinea-pig CB_1 receptor was determined (GenBank DQ355990). The deduced amino acid sequence has a high homology to the corresponding sequence of man (98.7%) and rat or mouse (99.2%). In conclusion, presynaptic CB_1 receptors leading to inhibition of noradrenaline release occur in guinea-pig atrium but not renal cortex. The deduced amino acid sequence of the guinea-pig CB_1 receptor shows a homology of 99% to the CB_1 receptor sequence of rodents and humans.

Key words: *guinea-pig CB_1 receptor sequence, guinea-pig atrium, guinea-pig kidney, noradrenaline release, presynaptic receptors*

INTRODUCTION

Many effects of cannabinoids are mediated via cannabinoid CB_1 and CB_2 receptors, both of which are G protein-coupled (1). There is increasing evidence

for the existence of additional cannabinoid receptors, including an abnormal-cannabidiol receptor activation of which causes vasodilatation (2); for the latter receptor, which is activated by abnormal-cannabidiol, molecular biological evidence is so far missing. CB_1 receptors are frequently located presynaptically on neurones where they cause inhibition of neurotransmitter release on activation. For identification of presynaptic CB_1 receptors (3) mainly tissues from rat and mouse have been used although in our hands the guinea-pig proved particularly suited for this purpose. For instance, noradrenaline release in the hippocampus and in the aorta is subject to inhibition via CB_1 receptors in the guinea-pig whereas this modulation was not found in the rat and mouse (4, 5). The aim of the present study was to examine whether noradrenaline release in the atrium of the guinea-pig is inhibited by CB_1 receptors. Atrial CB_1 receptors, suggested for the rat (6) although not unanimously (7), could not be shown for the mouse (7 - 9). In addition, we studied whether the abnormal-cannabidiol receptor, which has so far not been shown on a presynaptic site, affects noradrenaline release in the guinea-pig atrium and whether CB_1 receptors inhibit noradrenaline release in another tissue of the guinea-pig, *i.e.* the kidney cortex. The aim of the molecular biological part of our study was to determine the complete nucleotide sequence of the guinea-pig CB_1 receptor since so far only a partial sequence of 330 nucleotides determined by two of us (M.K. and E.S.) was known (GenBank DQ355990).

MATERIALS AND METHODS

Superfusion studies

All procedures conformed to the German animal welfare regulations (Tierschutzgesetz). Male Dunkin-Hartley guinea-pigs (Charles-River, Sulzfeld, Germany; weight range 180-530 g) were housed in the animal facilities of the Institut für Pharmakologie und Toxikologie with free access to water and food pellets. Animals were decapitated and atrial or cortical kidney pieces were prepared with a scalpel (dimensions about 1 x 1 x 1 mm). Tissues were then preincubated with [^{3}H]-noradrenaline 0.025 μM for 30 min and superfused at 37°C for 110 min with physiological salt solution (PSS) at a flow rate of 1 ml/min. The PSS was composed as follows (mM): NaCl 118, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10, ascorbic acid 0.06, Na_2EDTA 0.03; it was aerated with 95% O_2 and 5% CO_2. The concentration of $CaCl_2$ was 1.3 mM when the PSS was used for incubation of the tissue pieces and 3.25 mM (and in one series 1.63 mM) when it was used for superfusion. The PSS used for superfusion routinely contained an inhibitor of the neuronal noradrenaline transporter, desipramine 1 μM, and an α_2-adrenoceptor antagonist, rauwolscine 1 μM (if not stated otherwise). The superfusate was collected in 5-min samples. Tritium overflow was evoked by two 2-min periods of electrical field stimulation after 40 min (S_1) and 90 min (S_2) of superfusion. The stimulation parameters were 3 Hz, 200 mA and 2 ms. Agonists (including the cannabinoid receptor agonist WIN 55,212-2, its enantiomer WIN 55,212-3, CP-55,940, abnormal-cannabidiol, the muscarinic receptor agonist oxotremorine and prostaglandin E_2) were added to the PSS from 62 min of superfusion onward. Antagonists (including the cannabinoid CB_1 receptor antagonist rimonabant and the muscarinic M_2/M_4 receptor antagonist AF-DX 384) were present in the PSS throughout superfusion.

Cloning and sequencing of the cDNA of the guinea-pig CB_1 receptor

Total RNA from the hippocampus of male Dunkin-Hartley guinea-pigs was obtained using TriPure® reagent (Roche, Mannheim, Germany). RNA/DNA-containing samples were subjected to DNAse I (Roche) treatment to exclude any trace of genomic contamination from preparations. RNA was then reverse transcribed into cDNA with AMV (avian myeloblastosis virus) reverse transcriptase (Roche). Polymerase chain reaction (PCR) was performed using the Expand High Fidelity^{PLUS} System (Roche) in a DNA Thermal Cycler (Hybaid, Heidelberg, Germany) for 35 cycles (94°C and 54°C for 1 min each and 72°C for 1.5 min), followed by an additional 3 min extension at 72°C. The primers used were: human CB_1 sense 24-mer primer 5′-ACT GAG GTT ATG AAG TCG ATC CTA-3′ and human CB_1 antisense 24-mer primer 5′-AGG GAG GCA TCA GGC TCA CAG AGC-3′ (position 140-163 and 1559-1582, respectively, from GenBank X54937). The amplified DNA was subcloned into pGEM-4Z (Promega, Mannheim, Germany) and sequenced at GATC Biotech (Konstanz, Germany).

Data analysis and statistics

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 periods from 55 to 60 (t_1) and 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 effect of agonists on tritium overflow, the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1) and was compared to the S_2/S_1 value obtained in tissues not exposed to the respective agonist. To quantify the effect of antagonists on tritium overflow, the S_1 value obtained in the presence of the antagonist was compared to that obtained in its absence. S_2/S_1 values were calculated only from such tissues in which the S_1 value was at least 0.9% of tissue tritium.

To quantify the potencies of WIN 55,212-2, oxotremorine or prostaglandin E_2 for their inhibitory effects on the electrically-evoked tritium overflow (S_2/S_1) the concentrations producing the half-maximum effect (pIC_{50}) were determined graphically (see later, *Table 1*). To determine the antagonistic potencies for rimonabant (against WIN 55,212-2) and for AF-DX 384 (against oxotremorine) apparent pA_2 values were calculated according to the formula $pA_2 = \log ([A']/[A] - 1) - \log [B]$ where [A′] and [A] are the IC_{50} values for WIN 55,212-2 (or oxotremorine) in the presence and absence of the respective antagonist and [B] is the concentration of the respective antagonist.

Results are given as means ± standard error of the mean (SEM) of n experiments. Student's t-test was used for comparison of mean values; the Bonferroni correction was used when two or more values were compared to the same control.

For alignment of amino acid sequences, the GeneDoc software (Boston, MA, USA) was used.

Drugs

The following drugs were used: R-(-)-[ring-2, 5, 6-^{3}H]-noradrenaline (specific activity 53.0 Ci/mmol) (NEN, Zaventem, Belgium); abnormal-cannabidiol (Abn-CBD; 4-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol; Biotrend, Köln, 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; Tocris, Bristol, United Kingdom); desipramine hydrochloride (Novartis, Wehr, Germany); oxotremorine sesquifumarate (1-(4-[1-pyrrolidinyl]-2-butynyl)-2-pyrrolidinone; Sigma-Aldrich, Steinheim, Germany); prostaglandin E_2, 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), WIN 55,212-3 (*S*(-)-enantiomer of WIN 55,212-2; Sigma, München, Germany); rauwolscine hydrochloride (Roth, Karlsruhe, Germany); rimonabant hydrochloride (SR 141716; *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; Sanofi-Aventis, Montpellier, France); tetrodotoxin (ICN, Eschwege, Germany or Roth, Karlsruhe, Germany). Drugs were dissolved in dimethylsulfoxide (DMSO) (rimonabant, WIN 55,212-2, WIN 55,212-3, CP 55,940), ethanol (abnormal-cannabidiol, 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.

RESULTS

Superfusion studies

Atrial and renal pieces were usually superfused with medium containing desipramine 1 μM, rauwolscine 1 μM and Ca^{2+} at a concentration of 3.25 mM (if not stated otherwise). Basal tritium efflux (t_2/t_1) was 0.77 ± 0.03 in 12 control experiments in atrial pieces (fractional rate of tritium efflux during t_1 was 0.0022 ± 0.0001 min^{-1}) and 0.59 ± 0.03 in 9 control experiments in renal pieces (fractional rate of tritium efflux during t_1 was 0.0043 ± 0.0003 min^{-1}). Solvents (DMSO or ethanol) and the drugs under study did not influence basal tritium efflux (results not shown). The electrically evoked tritium overflow was also not altered by the solvents (not shown) but affected by the drugs under study as described in the subsequent paragraphs.

In *atrial pieces*, the electrically-evoked tritium overflow (S_2/S_1), which was 0.77 ± 0.03 in 4 controls, was almost abolished by tetrodotoxin 1 μM (0.09 ± 0.03; n = 4, $P < 0.001$) or by omission of Ca^{2+} ions from 62 min of superfusion onward (0.07 ± 0.03; n = 4, $P < 0.001$). The cannabinoid receptor agonist WIN 55,212-2 inhibited the electrically-evoked tritium overflow. *Fig. 1a* shows its concentration-response curve; the maximum effect, obtained at 10 μM, amounted to about 35% and the pIC_{50} value was 6.6 (*Table 1*). The concentration-response curve was shifted to the right by rimonabant 0.032 and 0.1 μM, yielding apparent pA_2 values of 8.2 and 8.4, respectively (*Fig. 1a*, *Table 1*). At either concentration, rimonabant did not affect the evoked overflow (S_1) by itself although there was a tendency towards a facilitatory effect (*Table 2*). Unlike WIN 55,212-2, its enantiomer WIN 55,212-3 and abnormal-cannabidiol did not affect the evoked overflow (S_2/S_1) (*Fig. 1a* and *b*). For the sake of comparison, the inhibitory effect of the muscarinic receptor agonist oxotremorine and of prostaglandin E_2 on the evoked tritium overflow (S_2/S_1) was examined as well (*Fig. 1*). The maximum inhibitory effect was about 55 and 65%, respectively (for pIC_{50} values, see *Table*

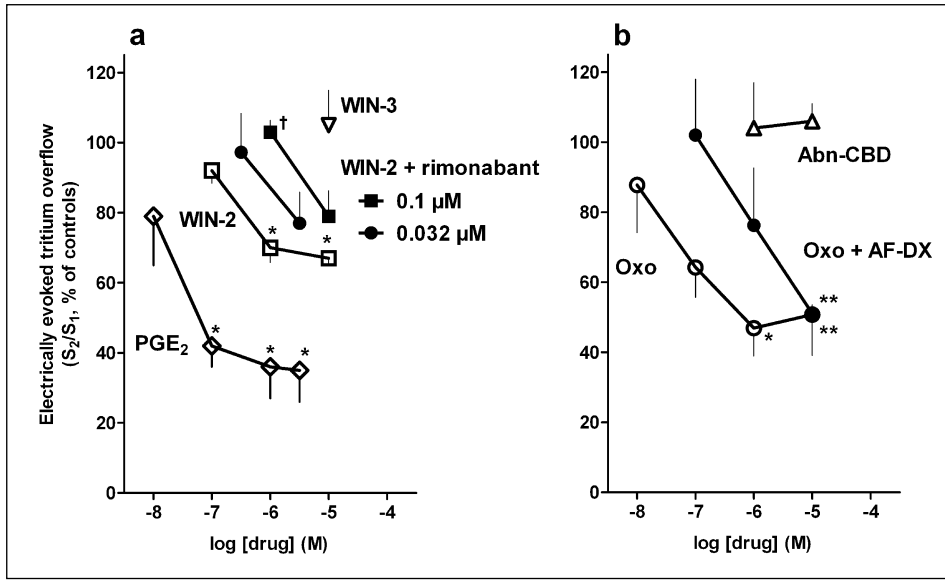

Fig. 1. Effect of WIN 55,212-2 (*WIN-2*), WIN 55,212-3 (*WIN-3*), prostaglandin E_2 (*PGE_2*) (a), abnormal-cannabidiol (*Abn-CBD*) and oxotremorine (*Oxo*) (b) on the electrically-evoked tritium overflow from superfused guinea-pig atrial pieces preincubated with [^{3}H]-noradrenaline, and interaction of WIN 55,212-2 with rimonabant (a) and of oxotremorine with AF-DX 384 0.1 µM (*AF-DX*) (b). Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. Agonists (WIN-2, WIN-3, PGE_2, Abn-CBD or Oxo) were added to the medium from 62 min of superfusion onward whereas antagonists (rimonabant or AF-DX) were present throughout. Results are expressed as percentages of the S_2/S_1 values in the corresponding controls (not shown). The S_2/S_1 value was 0.71 ± 0.02 in controls (no agonist, no antagonist) and was not affected by rimonabant or AF-DX 384. Means ± SEM of 3 - 10 experiments. *P < 0.05, **P < 0.01, compared to the corresponding control (not shown); †P < 0.01, compared to the corresponding value without rimonabant.

1). The concentration-response curve of oxotremorine was shifted to the right by the M_2/M_4 receptor antagonist AF-DX 384 0.1 µM, yielding an apparent pA_2 value of 8.5 (*Fig. 1b*, *Table 1*). AF-DX 384 did not affect the evoked tritium overflow (S_1) by itself (*Table 2*).

The effect of WIN 55,212-2 on the electrically-evoked tritium overflow from atrial pieces was also studied under two different experimental conditions. In the first series, the Ca^{2+} concentration was lowered from 3.25 to 1.63 mM. Under this condition, WIN 55,212-2 1 µM reduced the electrically-evoked tritium overflow (S_2/S_1), which was 0.72 ± 0.05 in 8 controls, by 46% to 0.39 ± 0.06 (n = 8; P < 0.002). In the second series, rauwolscine was omitted from the medium. Under this condition, the S_2/S_1 value in 15 controls was 0.87 ± 0.08; WIN 55,212-2 10 µM tended to decrease this value, which amounted to 0.66 ± 0.09 in 8 experiments. Both lowering of the Ca^{2+} concentration and omission of

Table 1. Maximum inhibitory effects and potencies of agonists and potencies of antagonists in superfused guinea-pig atrial and renal pieces preincubated with [^{3}H]-noradrenaline

	Atrium			**Kidney**		
	Approximate maximum effect (%)	pIC_{50}[1]	pA_2 of rimonabant[2] vs. WIN 55,212-2 and of AF-DX 384[3] vs. oxotremorine	Approximate maximum effect (%)	pIC_{50}[1]	pA_2 of AF-DX 384[3] vs. oxotremorine
WIN 55,212-2	35[4]	6.6	8.3	-	-	-
Oxotremorine	55[5]	7.3	8.5	80[5]	7.2	8.6
Prostaglandin E_2	65	7.7	-	55	7.4	-

[1] – log of the concentration producing the approximate half-maximum effect.
[2] Rimonabant was studied at two concentrations (*Fig. 1a*) and the value given represents the mean of 8.2 (0.032 μM rimonabant) and 8.4 (0.1 μM rimonabant).
[3] Studied at 0.1 μM (*Fig. 1b* and *2b*).
[4] The approximate maximum inhibitory effect in the presence of rimonabant 0.032 and 0.1 μM was assumed to be 35 % (*Fig. 1a*).
[5] The approximate maximum inhibitory effect in the presence of AF-DX 384 0.1 μM is identical to that obtained in its absence, both in the atrium (*Fig. 1b*) and the kidney (*Fig. 2b*).

Table 2. Effects of Ca^{2+} concentration, rauwolscine, rimonabant and AF-DX 384 on the electrically-evoked tritium overflow in superfused guinea-pig atrial and renal pieces preincubated with [^{3}H]-noradrenaline

		Tritium overflow evoked by S_1 (% of tissue tritium)				
Tissue	Ca^{2+} concentration (mM)	No rauwolscine	Rauwolscine 1 μM plus			
				Rimonabant		AF-DX 384
				0.032	0.1	0.1 μM
Atrium	1.63	-	1.89 ± 0.21*	-	-	-
Atrium	3.25	1.16 ± 0.08**	3.00 ± 0.27	3.27 ± 0.51	3.74 ± 0.35	2.96 ± 0.58
Kidney	3.25	-	3.87 ± 0.33	-	-	3.06 ± 0.34

Means ± SEM of 7 – 19 experiments; the value "Atrium – 3.25 mM Ca^{2+} – Rauwolscine" is based on 41 experiments.
*P < 0.05, **P < 0.001, compared to "Atrium – 3.25 mM Ca^{2+} – Rauwolscine".

rauwolscine markedly decreased the electrically-evoked tritium overflow by themselves (S_1; *Table 2*).

In the experiments on *renal pieces*, the electrically-evoked tritium overflow (S_2/S_1), which was 0.89 ± 0.04 in 3 controls, was again almost abolished by tetrodotoxin 1 μM (0.08 ± 0.03; n = 3, P < 0.001) or by omission of Ca^{2+} ions

from 62 min of superfusion onward (0.07 ± 0.04; n = 3, P < 0.001). High concentrations of WIN 55,212-2 (up to 10 µM) and of another cannabinoid receptor agonist, CP 55,940 (up to 3.2 µM), did not affect the electrically-evoked tritium overflow (S_2/S_1) (*Fig. 2a*). By contrast, oxotremorine and prostaglandin E_2 concentration-dependently inhibited the evoked overflow (*Fig. 2*). The maximum effect was about 80 and 55%, respectively (for pIC_{50} values, see *Table 1*). The concentration-response curve of oxotremorine was shifted to the right by AF-DX 384 0.1 µM, yielding an apparent pA_2 value of 8.6 *(Fig. 2b*, *Table 1*). AF-DX 384 by itself did not affect the evoked tritium overflow (S_1) (*Table 2*).

Cloning and sequencing

Fig. 3 aligns the deduced sequence of the guinea-pig CB_1 receptor to the previously described sequence of the human, mouse and rat CB_1 receptor (for

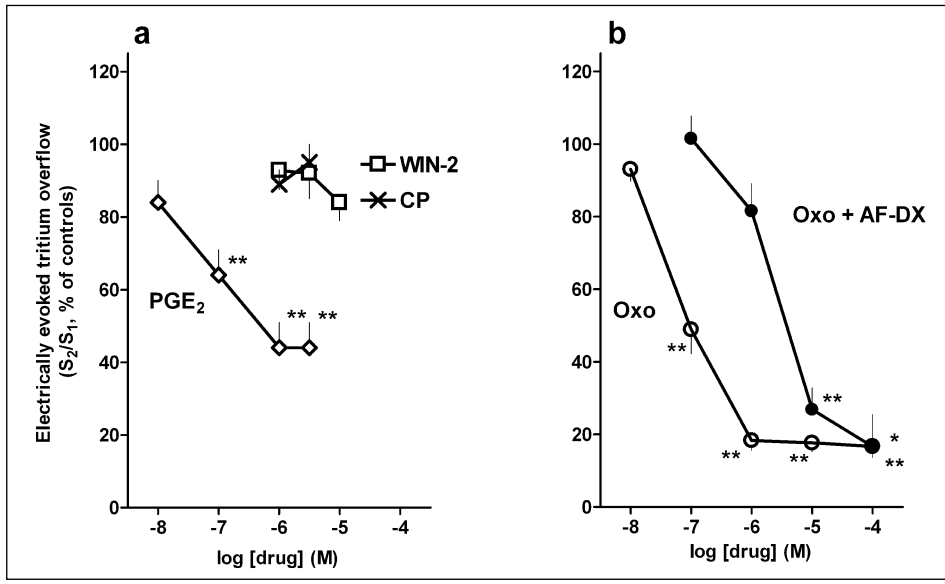

Fig. 2. Effect of WIN 55,212-2 (*WIN-2*), CP 55,940 (*CP*), prostaglandin E_2 (*PGE_2*) (a) and oxotremorine (*Oxo*) (b) on the electrically-evoked tritium overflow from superfused guinea-pig cortical kidney pieces preincubated with [^{3}H]-noradrenaline, and interaction of oxotremorine with AF-DX 384 0.1 µM (*AF-DX*). Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1, S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. WIN 55,212-2, CP 55,940, prostaglandin E_2 or oxotremorine were added to the medium from 62 min of superfusion onward whereas AF-DX 384 was present throughout. Results are expressed as percentages of the S_2/S_1 values in the corresponding controls (not shown). The S_2/S_1 value was 0.94 ± 0.04 in controls (no agonist, no antagonist) and was not affected by AF-DX 384. Means ± SEM of 23 - 31 experiments (WIN 55,212-2 1 µM and its control), of 11 - 16 experiments (CP 55,940 and higher concentrations of WIN 55,212-2) and 3 - 10 experiments (rest). *P < 0.05, **P < 0.01, compared to the corresponding control (not shown).

references, see legend to *Fig. 3*). There is a homology of 99% and an identity of 96 - 97% (*Table 3*). The degree of conservation is even higher when the transmembrane domains (TMDs) are considered; the homology is more than 99% and the identity at least 98.7% (*Table 3*). The 12th amino acid in TMD 1 of the guinea-pig is an Ala as opposed to a Ser in the other three species (*Fig. 3*).

Table 3. Homology and identity between the amino acid sequence of the human, rat and mouse CB_1 receptor and the sequence of the guinea-pig CB_1 receptor (expressed in percent)

		Human	Rat	Mouse
Complete sequence (472 - 473 amino acids)	Homology	98.7	99.2	99.2
	Identity	96.4	97.3	97.0
Sequence in the trans-membrane domains (223 amino acids)	Homology	99.1	99.6	99.6
	Identity	98.7	99.6	99.6

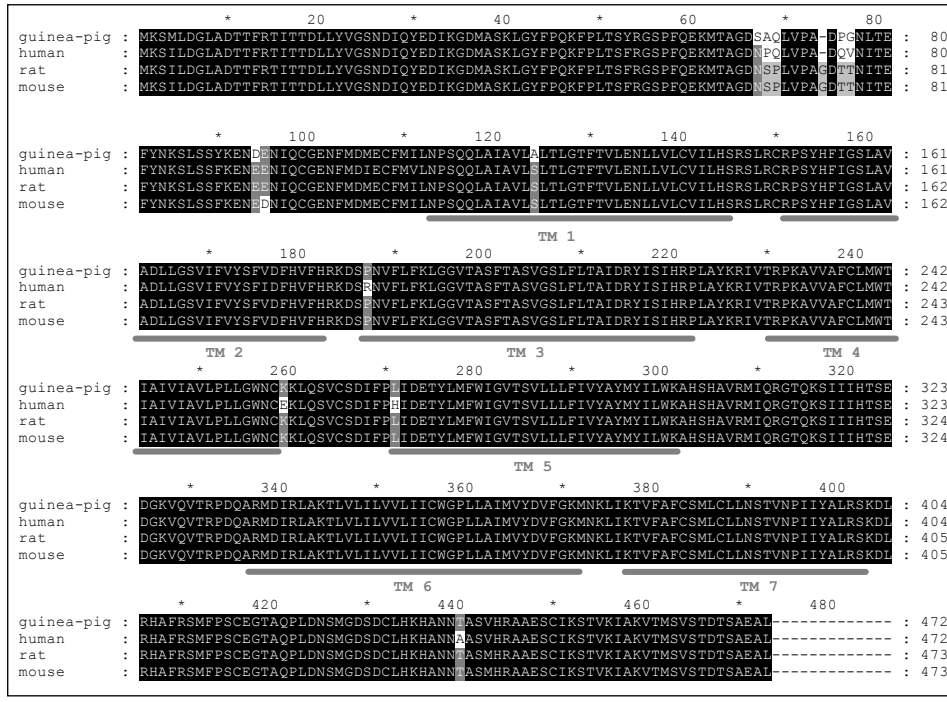

Fig. 3. Alignment of the amino acid sequence of the CB_1 receptor in four mammalian species. The predicted sequences of the guinea-pig (*Cavia porcellus*, this paper), human (*Homo sapiens*; 28), mouse (*Mus musculus*; 29), and rat (*Rattus norvegicus*; 30) are compared. Putative transmembrane (TM) domains are underlined and numbered. Conserved residues are boxed. The *Cavia porcellus* CB_1 sequence has been deposited in the GenBank database under the accession number DQ355990.

DISCUSSION

The present study shows that noradrenaline release in the atrium (but not in the kidney) from the guinea-pig is inhibited by cannabinoid CB_1 receptors and describes the deduced amino acid sequence of this receptor. In the functional experiments of our study the electrically-evoked tritium overflow from superfused tissues preincubated with [^{3}H]-noradrenaline was found to be Ca^{2+}-dependent and tetrodotoxin-sensitive and can therefore be assumed to represent quasi-physiological noradrenaline release. Experiments aimed at the detection of presynaptic receptors although described frequently for the guinea-pig atrium (10 - 12) have, to the best of our knowledge, so far not been reported for the guinea-pig kidney cortex (experiments on renal cortical slices from the rat have, however, been published; 13). Desipramine and rauwolscine were routinely added to the superfusion medium to avoid the possible interference of test drugs with the neuronal noradrenaline transporter and the presynaptic α_2-adrenoceptor, respectively, and in order to increase the amount of noradrenaline release. A third reason for addition of rauwolscine was the fact that the extent of CB_1 receptor-mediated inhibition of noradrenaline release is increased when the presynaptic α_2-autoreceptor (activated by endogenous noradrenaline) is blocked (14).

Noradrenaline release in atrial pieces was inhibited by the cannabinoid receptor agonist WIN 55,212-2. Its concentration-response curve was shifted to the right by the selective CB_1 receptor antagonist rimonabant at an apparent pA_2 of 8.3 (which is in the range of pA_2 values described in many other studies on CB_1 receptors; 1, 4, 5), suggesting that the functional cannabinoid receptor in the guinea-pig atrium belongs to the CB_1 receptor subtype. In harmony with this view WIN 55,212-3 (the enantiomer of WIN 55,212-2) was devoid of an effect at a very high concentration. The possibility that receptors for abnormal-cannabidiol are implicated in the effect of WIN 55,212-2 was a priori not very high since the latter drug does not activate these receptors according to literature data (15). Nonetheless, it was of interest to examine whether abnormal-cannabidiol, which has so far not been examined at presynaptic sites, will affect noradrenaline release; this was, however, not the case.

The effect of WIN 55,212-2 has also been studied under another two experimental conditions, which are closer to the physiological situation, namely at a lower Ca^{2+} concentration (1.63 mM) and in the absence of rauwolscine. At the lower Ca^{2+} concentration, the inhibitory effect of WIN 55,212-2 was more pronounced. The most likely reason for the difference in the degree of inhibition (a very common phenomenon for presynaptic receptors) is that transmitter release is modulated by saturable “release receptors” for Ca^{2+} ions and that the degree of modulation is decreasing as the Ca^{2+} concentration is approaching the saturation level (16). In the second series, carried out at Ca^{2+} 3.25 mM but in the absence of rauwolscine, WIN 55,212-2 tended to decrease noradrenaline release. The remainder of the superfusion experiments was carried out at a Ca^{2+} concentration

of 3.25 mM and in the presence of rauwolscine since otherwise the number of experiments which has to be discarded due to low S_1 values (< 0.9%) is very high.

Under the standard experimental conditions (Ca^{2+} 3.25 mM, rauwolscine present), rimonabant, when given alone, did not affect noradrenaline release, suggesting that the CB_1 receptors under study are not pre-coupled and/or subject to an endogenous tone. On the other hand, pre-coupling and/or an endogenous tone have been identified at many other CB_1 receptors, in most instances based on experiments with rimonabant, which led to effects opposite in direction to those elicited by cannabinoid receptor agonists (3, 15).

Taking into account that presynaptic CB_1 receptors, involved in the inhibition of noradrenaline release, are present in the human (17) and guinea-pig atrium, as opposed to the rodent atrium (7 - 9), one might assume that the guinea-pig is a better predictor for human presynaptic CB_1 receptors than rodents. A similar phenomenon was reported for the modulation of noradrenaline release in the hippocampus, which is inhibited via presynaptic CB_1 receptors in human and guinea-pig, unlike in rat and mouse brain (4). A plausible reason for the similarity between human and guinea-pig might be that the CB_1 receptor densities on the noradrenergic neurones are higher for the latter two species than for rodents but systematic inter-species comparisons are so far lacking.

For the sake of comparison, the inhibitory effects of oxotremorine and prostaglandin E_2 on atrial noradrenaline release have been examined as well. The maximum inhibitory effect obtainable with oxotremorine (via muscarinic receptors; 55% inhibition) and prostaglandin E_2 (via EP receptors; 65% inhibition) is by far higher than that obtainable via CB_1 receptors (35% inhibition). The occurrence of presynaptic inhibitory muscarinic and EP receptors in the guinea-pig atrium had been reported in the past (10 - 12). In two of the latter studies (11 – 12), noradrenaline release was not determined directly but rather via the endorgan response and this may be the reason why the conclusion was reached that the presynaptic muscarinic receptor should be classified as an M_3 receptor (12) whereas according to our experiments the classification as an M_2 or M_4 receptor would be more plausible. Our conclusion is based on the fact that the apparent pA_2 value of AF-DX 384 of 8.5 is in the range of pA_2 values of this antagonist for M_2 and/or M_4 receptors but markedly higher than its pA_2 values for M_1, M_3 and M_5 receptors (1).

Unlike in the atrium, WIN 55,212-2 did not affect noradrenaline release in the guinea-pig kidney cortex; CP 55,940, another potent cannabinoid receptor agonist, was ineffective as well. In harmony with the present findings, WIN 55,212-2 also failed to inhibit noradrenaline release in mouse renal pieces examined under very similar experimental conditions (18). On the other hand, evidence for the occurrence of presynaptic inhibitory CB_1 receptors in segments of rat renal arteries has been presented (19). The possibility that noradrenaline release in our experimental model is unresponsive to modulation via presynaptic inhibitory receptors in general can be discarded since oxotremorine and

prostaglandin E_2 caused a robust inhibition (maximum effects of about 80 and 55%, respectively). The data obtained with AF-DX 384 again suggest that the muscarinic receptor belongs to the M_2 or M_4 subtype.

The atrial CB_1 receptor described in the present study adds to the long list of presynaptic inhibitory CB_1 receptors identified in tissues from the guinea-pig, including retina (20), vessels (5), small intestine (21 - 24), several brain regions (4) and lung (25). Nonetheless, the full amino acid sequence of the guinea-pig CB_1 receptor is unknown. Therefore, we cloned and sequenced the guinea-pig CB_1 receptor cDNA. The degree of homology and even identity of the deduced amino acid sequence to the sequence of human, rat or mouse is very high (> 96%). The percentages are still higher for the amino acids within the TMDs (> 98%). Interesting enough, however, the 12th amino acid residue of the first transmembrane domain, which is a Ser in the CB_1 receptor of human, rodents and even mountain paca (*Agouti taczanowskii*), a species closely related to the guinea-pig (26), is an Ala in the case of the guinea-pig CB_1 receptor. According to the literature, there are so far no data whether this amino acid residue is involved in the binding of cannabinoid receptor ligands (rimonabant, WIN 55,212-2 and anandamide) in other species (27).

In conclusion, the guinea-pig CB_1 receptor, the deduced amino acid sequence of which shows a homology of 99% to the CB_1 receptor of humans and rodents, causes inhibition of noradrenaline release in the atrium. In the latter tissue, noradrenaline release is inhibited to a more marked extent via presynaptic muscarinic and prostaglandin EP receptors but not affected by an agonist of the abnormal-cannabidiol receptor. In the renal cortex of the guinea-pig, noradrenaline release is not subject to inhibition via presynaptic CB_1 but via presynaptic muscarinic and prostaglandin EP receptors.

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