Modulation of transmitter release via presynaptic cannabinoid receptors

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Cannabis (marijuana) is not only a frequently abused drug but also has the potential for the development of useful agents for the treatment of emesis, anorexia and multiple sclerosis. In this article, the effects of modulation of transmitter release by cannabinoids in both the CNS and the PNS of various species, including humans, will be discussed. Cannabinoids inhibit neurotransmitter release via specific presynaptic cannabinoid CB_1 receptors. Studies using either the CB_1 receptor antagonist and inverse agonist SR141716 or CB_1-receptor-deficient mice suggest that numerous presynaptic cannabinoid receptors are tonically activated by endogenous cannabinoids and/or are constitutively active. CB_1-receptor-mediated inhibition of transmitter release might explain, for example, reinforcing properties and memory impairment caused by cannabinoids.

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For thousands of years cannabis has been used to treat a variety of diseases, and recently interest has increased again. Established indications for cannabinoids are nausea and vomiting induced by chemotherapy or radiotherapy, and wasting syndrome caused by AIDS. Furthermore, there is evidence that cannabinoids are useful for the treatment of pain, spasticity (occurring, for example, during multiple sclerosis), glaucoma and other disorders[1]. However, the use of cannabinoids is limited by a series of side-effects, including the potential for abuse[2].

Since 1990 our knowledge about cannabinoids has increased enormously as heralded by >2400 articles that were published during this time period (according to a search in the PubMed database, using the keyword 'cannabinoid'). During the past decade, two cannabinoid receptors, termed CB_1 and CB_2, were cloned and their distribution and transduction pathways described. Anandamide and 2-arachidonoyl glycerol were identified as putative endogenous ligands at these receptors, and many research tools were identified, including antibodies, antagonists, antisense oligodeoxynucleotides, and CB_1 and CB_2 receptor knockout mice. These and many other aspects of cannabinoid-related research have been reviewed elsewhere in excellent overviews (e.g. Refs 3–5).

One aspect of cannabinoid receptors has, as yet, attracted only little attention: namely, the fact that many of these receptors are located presynaptically on neurones where their activation causes the inhibition of the release of the respective transmitter. This is one of the many properties that are shared by cannabinoid and opioid receptors; other similarities include coupling to the same type of G protein ($G_{i/o}$) and the same transduction mechanisms (inhibition of adenylyl cyclase, blockade of voltage-dependent Ca^{2+} channels and activation of voltage-dependent K^+ channels) and the antinociceptive and addictive effects exhibited by both cannabinoids and opioids[6]. In the present article (based on *in vitro* and *in situ* studies), we will: (1) provide an overview of the inhibition of transmitter release induced by cannabinoid receptors; (2) discuss the mechanisms of receptor-mediated inhibition of transmitter release; (3) consider the possible occurrence of an endogenous tone at cannabinoid receptors; and (4) discuss three examples of the putative physiological and pathophysiological role of presynaptic cannabinoid receptors.

Evidence for the inhibition of transmitter release by cannabinoid receptors

One of the first examples of a cannabinoid receptor causing inhibition of transmitter release was described by Gill *et al.*[7] in 1970. These authors found that the electrically evoked twitch response of the guinea-pig ileum (which is related to the release of acetylcholine) is inhibited by the cannabis constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) at concentrations of ≥3.2 μM. During the past three decades, cannabinoid receptors causing inhibition of transmitter release have been identified in ~40 experimental models, including four models in human tissues (Table 1) and two models in invertebrate species[8]. In some studies, transmitter release has been determined directly in superfused tissue preparations, whereas in other studies the transmitter release was measured via the end organ response, namely postsynaptic currents (in electrophysiological studies), muscle contraction (in organ bath studies, as in the study by Gill *et al.*[7]) or alteration of blood pressure or heart rate (in pithed animals). Most of the *in vitro* studies were performed on isolated tissues although cultured neurones were used in a minority of studies. Because neurones are not spontaneously active in most of the experimental models, electrical stimulation or (in a few studies) a chemical stimulus was used to evoke transmitter release. The aminoalkylindole WIN552122 (Ref. 4) was used most frequently as a cannabinoid receptor agonist (Table 1) because handling of Δ^9-THC is difficult as a result of its high lipophilicity. WIN552123, the inactive enantiomer of WIN552122, was frequently

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Table 1. Pharmacological properties of cannabinoid receptors causing inhibition of transmitter release[a]

Species	Tissue	Transmitter	Method[b]	Inhibition by WIN552122 (%)[c]	Antagonism		Refs
					SR141716[d]	Other strategies	
Human	Hippocampus	NA	Release (slices)	21	+	–	52
		GABA	Release (slices)	49	+	–	53
	Heart	NA	Release (segments)	51 (anandamide)	+	–	54
	Ileum	ACh	Contraction (strips)	85	pA_2=8.2[e]	–	55
Rabbit	Heart	NA	Increase in HR (pithed rabbit)	22	+	–	56
		ACh	Decrease in HR (pithed rabbit)	43	+	–	56
	Resistance vessels	NA	Increase in BP (pithed rabbit)	~90[f]	+	–	57
Guinea-pig	Hippocampus	NA	Release (slices)	50[f]	pA_2=8.2	–	52
	Retina	DA	Release (discs)	73[f]	pA_2=8.3	–	58
	Small intestine	ACh	Contraction (strips or rings)	~85	pK_D=8.0–8.2	Correlation between pK_D and binding affinities	9,28,59
			Release (segments)	35[f]	pK_D =8.0	–	60
		SP	Contraction (rings)	55	pA_2 =8.1	–	28
Rat	Prefrontal cortex	Glutamate	EP (slices)	50	+	–	61
	Hippocampus	ACh	Release (slices)	81	+	Antisense oligodeoxy-nucleotide[g]	10,47
		GABA	EP (slices)	47	+	–	12,22
		GABA	Release (slices)	48	+	–	62
		Glutamate	EP (cultured neurones)	86[f]	+	–	20,21
		Glutamate	EP (slices)	100[f]	+	–	45
	Striatum	DA	Release (slices)	48 (anandamide)	+	–	63
		GABA	EP (slices)	52	+	–	64
		Glutamate	EP (slices)	51[f]	+[e]	–	19,65
	Nucleus accumbens	GABA	EP (slices)	60	+	–	41
	Substantia nigra pars reticulata	GABA	EP (slices)	50	+	–	23
		Glutamate	EP (slices)	54	+	–	66
	Periaqueductal grey	GABA	EP (slices)	63	+	–	67
		Glutamate	EP (slices)	62	+	–	67
	Cerebellum	GABA	EP (slices)	>50	+	–	25
		Glutamate	EP (slices)	88	+	–	25,68
	RVM medulla	GABA	EP (slices)	58	+	–	69
	Heart	NA	Release (tissues)	38 (anandamide)	+	–	70
	Resistance vessels	NA	Increase in BP (pithed rat)	25[f]	+	–	71
	Renal arteries	NA	Release (segments)	~80 (anandamide)	+	–	27
	Vas deferens	NA	Release (tissues)	93	pK_D=7.9	–	30
Mouse	Cerebral cortex	5-HT	Release (slices)	20[f]	pA_2=8.0	–	72
		ACh	Release (slices)	30	+	–	49
	Hippocampus	ACh	Release (slices)	60[f]	pA_2=8.6	Knockout mouse[g]	11,49,73
		GABA	EP (slices)	36	+	Knockout mouse[g]	12, 74
		Glutamate	EP (slices)	54[f]	+	–	18
	Nucleus accumbens	GABA	EP (slices)	70	+	–	42
		Glutamate	EP (slices)	64[f]	+	–	24
	Vas deferens	NA	Contraction (tissues)	100	pK_D=8.6	–	13,15
			Release (tissues)	79	pK_D=9.7–10.5	–	75
	Urinary bladder	ACh, ATP	Contraction (strips)	>80	pK_D=8.1	–	76
	Sympathetic chain	NA	Release (cultured neurones)	69	pK_D=9.3–9.9	–	17

[a]Abbreviations: ACh, acetylcholine; BP, blood pressure; EP, electrophysiological study; HR, heart rate; NA, noradrenaline; RVM, rostral ventromedial; SP, substance P.
[b]Transmitter release was determined directly or via the end organ response [i.e. excitatory (glutamate) or inhibitory (GABA) postsynaptic currents or potentials, contraction, blood pressure or heart rate]. The preparation used is given in parentheses.
[c]The degree of inhibition caused by the maximally effective or the only concentration (or dose) of the cannabinoid receptor agonist WIN552122 used is given as a percentage of the maximum release. Anandamide, an endogenous cannabinoid receptor agonist, was used instead of WIN552122 in the cases indicated.
[d]Inhibition of the effect of WIN552122 or anandamide on neurotransmitter release induced by the cannabinoid receptor antagonist SR141716 is shown with respect to a single agonist concentration (+) or a concentration–response curve (a pA_2 value or similar potency estimate is indicated).
[e]The cannabinoid CB_2 receptor antagonist SR144528 did not reverse the effects of WIN552122.
[f]WIN552123, the enantiomer of WIN552122, was a very weak inhibitor of neurotransmitter release or did not inhibit neurotransmitter release at all.
[g]The effect of WIN552122 was inhibited when rats were pretreated intracerebroventricularly with an antisense oligodeoxynucleotide directed against the CB_1 receptor mRNA but was not affected after pretreatment with a mismatch oligodeoxynucleotide. Furthermore, the effect of WIN552122 was not observed in hippocampal slices from CB_1-receptor-deficient (knockout) mice.

used as a negative control (Table 1). In a few studies, HU210 or CP55940 (more or less chemically related to Δ^9-THC, respectively[4]) or the eicosanoid anandamide (one of the endogenous ligands of cannabinoid receptors[4]) were employed as agonists.

To prove that the effects of the aforementioned agonists indeed involve cannabinoid receptors, four approaches have been chosen. (1) Most frequently, the interaction of the cannabinoid receptor agonist(s) with the CB_1 receptor antagonist SR141716 was examined (Table 1). Whenever used at an adequate concentration,

this highly selective and specific antagonist[4] antagonized the effects of WIN552122 on neurotransmitter release, which suggests that the inhibitory effect of the cannabinoid(s) involves CB_1 receptors. This is particularly likely for those studies in which pA_2 values (or similar estimates of antagonistic potency) were determined; pA_2 values of SR141716 were usually between 8 and 9 (Table 1), which correlates well with its pK_i of ~8.5 at native or recombinant CB_1 receptors[3,4]. (2) In a study on the guinea-pig ileum[9], the potencies of a panel of cannabinoid receptor antagonists were correlated with their pK_i values at CB_1 receptor binding sites, revealing a highly significant correlation and demonstrating that the cannabinoid receptor in this tissue is CB_1 (Table 1). (3) In a study on hippocampal slices, the involvement of CB_1 receptors has been proven by using the CB_1 receptor knockdown approach. Thus, the effect of WIN552122 was blunted when the rats had been pretreated intracerebroventricularly with an antisense oligodeoxynucleotide directed against the CB_1 receptor mRNA but was not affected after pretreatment with a mismatch oligodeoxynucleotide[10] (Table 1). (4) Finally, WIN552122 showed no inhibitory effect on transmitter release in hippocampal slices from CB_1-receptor-deficient mice ('knockout mice')[11,12] (Table 1).

As already mentioned, the identity of the CB_1 receptor in the guinea-pig ileum has been verified by comparing the antagonistic potencies of a series of CB_1 receptor antagonists with their affinities at CB_1 receptor binding sites[9]. However, the potencies of the same antagonists at the cannabinoid receptor leading to inhibition of the electrically induced twitch response in the mouse vas deferens showed a poor correlation with CB_1 receptor binding affinities[9]. This finding might mean that the agonist WIN552122 interacts with two types of receptors in this tissue. CB_1 receptors are undoubtedly present in this preparation[13]. However, selective CB_2 receptor agonists such as JWH015 (Ref. 4) also inhibited the twitch response; these effects were not sensitive to low concentrations of SR141716 (Ref. 14), which suggests that CB_2 receptors might also be present in this preparation[14]. The availability of a selective CB_2 receptor antagonist, SR144528 (Ref. 4), will help to further clarify the occurrence of CB_2 receptors in the mouse vas deferens. SR144528 has already been used in other models; however, at low concentrations, SR144528 failed to antagonize the effects of WIN552122 or CP55940 on neurotransmitter release, which suggests that CB_2 receptors do not play a role in these effects (Table 1).

In the mouse vas deferens, a second pitfall should be considered. Thus, the electrically induced twitch response in this tissue is not only inhibited by cannabinoid receptors but also by vanilloid VR1 receptors[15]. Both receptors are activated by anandamide. If the interaction of anandamide with a cannabinoid or vanilloid receptor antagonist is studied, the potency of either antagonist will be underestimated[15].

Cannabinoid receptors are frequently located presynaptically but also occur postsynaptically, for example, in the hippocampus[16]. Thus, it is mandatory to exclude a postsynaptic site of action of drugs if transmitter release is measured indirectly via the end organ response or via postsynaptic currents. For example, in the aforementioned study by Gill *et al.*[7], Δ^9-THC depressed the twitch response of the guinea-pig ileum to transmural stimulation but did not affect the response to exogenously added acetylcholine (which sometimes was even potentiated), suggesting that Δ^9-THC indeed inhibited acetylcholine release. Experiments of this type were carried out in organ bath and electrophysiological studies and in investigations on pithed animals. For electrophysiological studies, additional techniques are available to exclude a postsynaptic action of agonists (Table 2). Only cannabinoid receptors for which a postsynaptic location was excluded by appropriate experiments were considered in Table 1.

However, even if transmitter release is measured directly, the possibility has to be considered that the cannabinoid receptor under study is localized to an interneurone. Again special techniques (Table 2) are useful to exclude this possibility. Furthermore, the presynaptic location of cannabinoid receptors can also be verified morphologically (Table 2).

Mechanism of cannabinoid-receptor-induced inhibition of transmitter release

It has already been mentioned that CB_1 receptors are coupled to $G_{i/o}$ proteins. Consistent with this coupling, pertussis toxin attenuated the CB_1-receptor-mediated inhibition of noradrenaline release in mouse cultured sympathetic neurones[17] and of the glutamate-mediated excitatory postsynaptic currents (EPSCs) in mouse hippocampal[18] and rat striatal[19] slices and in rat cultured hippocampal neurones[20,21].

But what are the subsequent molecular events involved in CB_1-receptor-mediated inhibition of transmitter release? The possible involvement of voltage-dependent Ca^{2+} channels was examined in studies using cadmium, a nonselective blocker of these channels. Cadmium strongly attenuated or abolished the effect of WIN552122 on spontaneous EPSCs in rat striatal slices[19] and on spontaneous GABA-mediated inhibitory postsynaptic currents (IPSCs) in slices of the rat hippocampus[22] and substantia nigra pars reticulata[23].

The question of whether L-, N-, P- and/or Q-type channels are involved was examined in three studies in which glutamate release was determined electrophysiologically. (1) The inhibitory effect of cannabinoids in slices from the mouse nucleus accumbens [parameter measured: field excitatory postsynaptic potentials (fEPSPs)] does not involve any of the four channels[24]. (2) By contrast, the cannabinoids act via N-, but not L-, P- or Q-, type Ca^{2+} channels in rat

Table 2. Presynaptically located cannabinoid receptors[a]

Type of study	Method of identification of presynaptic location	Type of neurone	Tissue	Refs
Electro-physiology	Amplitude of miniature IPSCs and EPSCs not affected:			
	frequency decreased	GABA	Rat hippocampus	31,77
			Rat PAG	67
			Rat cerebellum	25
			Rat RVM medulla	69
		Glutamate	Rat hippocampus	21
			Rat cerebellum	68
			Mouse hippocampus	18
			Mouse NAcc.	24
	frequency unaffected	GABA	Rat hippocampus	12,22
			Rat NAcc.	41
		Glutamate	Rat striatum	19
			Rat cerebellum	25
	Amplitude of Sr^{2+}-induced asynchronous synaptic events not affected	Glutamate	Rat prefrontal cortex	61
			Rat striatum	65
			Rat cerebellum	68
	Increase in paired-pulse ratio	GABA	Rat hippocampus	31,32
			Rat NAcc.	41
			Rat SNr	23
			Rat PAG	67
			Rat RVM medulla	69
			Mouse NAcc.	42
		Glutamate	Rat hippocampus	21,45
			Rat striatum	19,65
			Rat SNr	66
			Rat cerebellum	68
			Rat PAG	67
			Mouse hippocampus	18
			Mouse NAcc.	24
	Increase in the coefficient of variation	Glutamate	Rat hippocampus	20
			Rat striatum	65
			Rat cerebellum	68
	Immunostaining	GABA	Rat hippocampus	12,77
Release	Effect on K^+-evoked transmitter release in slices superfused in the presence of tetrodotoxin	Noradrenaline	Guinea-pig hippocampus	52
		Dopamine	Guinea-pig retina	58
		5-HT	Mouse cerebral cortex	72
		Acetylcholine	Mouse hippocampus	49
	Effect in synaptosomes	Acetylcholine	Rat hippocampus	29
	Immunostaining	GABA	Human hippocampus	53
			Rat hippocampus	62

[a]Abbreviations: EPSCs, excitatory postsynaptic currents; IPSCs, inhibitory postsynaptic currents; NAcc., nucleus accumbens; PAG, periaqueductal grey; RVM, rostral ventromedial; SNr, substantia nigra pars reticulata.

striatal slices (parameter measured: field potential)[19]. (3) Interestingly, the cannabinoid-receptor-mediated effect in rat cultured hippocampal neurones (parameter measured: EPSCs) is related to N- and Q-, but not P-, type Ca^{2+} channels (L-type channels were not examined)[21]. In the latter study, the possibility that the cannabinoid receptor agonist WIN552122 lost its inhibitory effect on the EPSCs only because the Ca^{2+} channel blockers by themselves strongly reduced the size of the EPSCs was also considered. However, this possibility could be excluded because WIN552122 retained its inhibitory effect on the EPSCs when their size was strongly reduced by lowering the Ca^{2+} concentration in the superfusion medium.

There is considerable evidence that the inhibitory effect of cannabinoid receptor agonists on transmitter release involves sites downstream of the voltage-dependent Ca^{2+} channels (i.e. direct effects on the release machinery). The evidence is again based on electrophysiological experiments in which miniature EPSCs (mEPSCs) or miniature IPSCs (mIPSCs) were studied in the presence of tetrodotoxin (Table 2). The fact that the frequencies of mEPSCs or mIPSCs, which are Ca^{2+} independent, were reduced by a cannabinoid receptor agonist suggest a direct influence on the release machinery. Interestingly, the CB_1 receptors markedly differ in this respect. For example, in rat cerebellar slices, WIN552122 reduced the frequency of the mIPSCs without affecting the frequency of the mEPSCs (Ref. 25).

CB_1 receptors are also coupled to K^+ channels, including voltage-dependent K_M (Ref. 16) and K_A channels[3,5] and voltage-independent GIRK channels[3,5]. The possibility that the CB_1-receptor-mediated inhibition of transmitter release is related to the opening of K^+ channels had also to be considered. In the mouse nucleus accumbens, the inhibitory effect of WIN552122 on the evoked fEPSPs was attenuated by $BaCl_2$ or 4-aminopyridine (blockers of K_A and GIRK channels, respectively) and was abolished by their combined administration[24]. $BaCl_2$ and 4-aminopyridine did not change the effect of adenosine, which acts at another presynaptic inhibitory receptor; furthermore, the effect of WIN552122 remained completely suppressed when the Ca^{2+} concentration in the superfusion medium was reduced to compensate for the marked increase in the amplitude of the fEPSPs caused by the K^+ channel blockers[24]. Both $BaCl_2$ and 4-aminopyridine did, however, not influence the effect of WIN552122 on the evoked IPSCs in rat hippocampal slices[22].

The possibility that inhibition of adenylyl cyclase, the third signal transduction pathway coupled to CB_1 receptors[3,5], is involved in CB_1-receptor-mediated inhibition of transmitter release has been examined in two studies. Addition of forskolin, which leads to an enhanced synthesis of cAMP, did not affect the inhibitory effect of WIN552122 on the fEPSPs in the mouse nucleus accumbens[24]. By contrast, forskolin markedly attenuated the effect of WIN552122 on the (acetylcholine-related) twitch response of the guinea-pig ileum longitudinal muscle[26]. An attenuation was also obtained with 8-bromo-cAMP, a lipophilic cAMP analogue, and isobutylmethylxanthine (IBMX), an inhibitor of cAMP degradation. Forskolin, 8-bromo-cAMP and IBMX did not affect the twitch response by themselves nor did they influence the contraction induced by exogenously added acetylcholine.

Finally, the CB_1-receptor-mediated inhibition of noradrenaline release in renal artery segments of the rat[27] and of dopamine release in ganglia of two invertebrate species[8] has been abolished by N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, which suggests that the CB_1

Table 3. Release-modulating cannabinoid receptors that show endogenous activation and/or constitutive activity

Species	Tissue	Transmitter	Method[a]	Criteria arguing for endogenous activation and/or constitutive activity[b]		Refs
				Facilitated by SR141716 (μM)	Other criteria	
Guinea-pig	Hippocampus	Noradrenaline	Release (slices)	0.32	–	52
	Retina	Dopamine	Release (discs)	0.32	–	58
	Small intestine	Acetylcholine	Contraction (rings or strips)	0.01–1	–	28,59
	Small intestine	Acetylcholine	Release (segments)	1	–	60
	Small intestine	Substance P	Contraction (rings)	0.01–1	–	28
Rat	Prefrontal cortex	Glutamate	Electrophysiology (slices)	1	–	61
	Hippocampus	Acetylcholine	Release (slices)	0.1–1	Facilitated by AM281 (0.1–10 μM)	47,78
	Hippocampus	Acetylcholine	Release (synaptosomes)	0.0003–0.3	–	29
	Hippocampus	GABA	Electrophysiology (slices)	–	Inhibited by AM404 (20 μM)	31
	Hippocampus	Glutamate	Electrophysiology (slices)	1	–	45
	Striatum	Glutamate	Electrophysiology (slices)	5	Facilitated by AM281 (5 μM)	19
	Vas deferens	Noradrenaline	Contraction (tissues)	0.003–3	Facilitated by LY320135 (0.3–30 μM); inhibited by PMSF (200 μM)[c]	30
Mouse	Hippocampus	Acetylcholine	Release (slices)	0.32	Increased acetylcholine release in CB_1 receptor knockout mice	11,49
	Vas deferens	Noradrenaline	Contraction (tissues)	0.032	–	79
	Urinary bladder	Acetylcholine, ATP	Contraction (strips)	0.1	–	76

[a]Transmitter release was determined directly or via end organ response [excitatory (glutamate) or inhibitory (GABA) postsynaptic currents or potentials or contraction].
[b]SR141716, AM281 and LY320135 are cannabinoid CB_1 receptor antagonists and inverse agonists. AM404 is an inhibitor of the reuptake of endocannabinoids.
[c]Abbreviation: PMSF, phenylmethylsulfonyl fluoride (an inhibitor of the degradation of endogenously formed cannabinoids).

receptors in these models might act by releasing nitric oxide. This mechanism does, however, not apply for the CB_1 receptor involved in inhibition of the (acetylcholine- or substance-P-related) twitch response in the guinea-pig ileum (because of a lack of effect of L-NAME)[28].

Endogenous tone at cannabinoid receptors

Table 1 shows that cannabinoid receptors activated by exogenously added agonists are detectable in a variety of tissues. A very important question in this respect is whether these cannabinoid receptors are subject to an 'endogenous tone'. Evidence in favour of this has been presented for some, but not all, of the cannabinoid receptor models, using four different approaches (Table 3). (1) CB_1 receptor antagonists and inverse agonists (e.g. SR141716, AM281 and LY320135) influenced transmitter release in a manner opposite to that of cannabinoid receptor agonists (i.e. they increased transmitter release). (2) Similarly, transmitter release was increased in CB_1-receptor-deficient mice compared with wild-type animals. (3) Inhibition of the enzymatic degradation of endogenously formed cannabinoids mimicked the effect of cannabinoid receptor agonists. (4) Inhibition of the reuptake of endogenously formed cannabinoids also mimicked the effect of cannabinoid receptor agonists.

Most of the studies presented in Table 3 do not allow the exact mechanism of the 'endogenous tone' to be identified. Such tone might be caused by endogenously formed cannabinoids such as anandamide or 2-arachidonoyl glycerol accumulating in the biophase of the cannabinoid receptors. A second possibility is that the transduction machinery linked to the cannabinoid receptors is constitutively active (i.e. is working even if the receptors are not activated by a ligand). The results of a study on rat hippocampal synaptosomes[29] are clearly in favour of the latter possibility. In synaptosomes, in which endogenously formed or exogenously added ligands are efficiently removed by the superfusion stream and cannot accumulate in the biophase of receptors, SR141716 nonetheless facilitated acetylcholine release. In other words, this facilitation cannot be explained by the interruption of a tonic inhibition of acetylcholine release caused by endocannabinoids.

Evidence in favour of cannabinoid receptors being tonically activated by endocannabinoids was presented in a study on the rat vas deferens[30], in which the release of noradrenaline (determined as contraction) was inhibited by phenylmethylsulfonyl fluoride (PMSF), an inhibitor of the degradation of endogenously formed cannabinoids[5]. Tonic activation of CB_1 receptors by endocannabinoids is also suggested by a study on rat hippocampal slices[31], in which the amplitude of IPSCs was reduced by AM404, which is an inhibitor of the reuptake of endocannabinoids[5]; the effect of AM404 was counteracted by SR141716.

Depending on the experimental model, the first or the second, or even both, mechanisms might hold true. Independent of the mechanism(s), the occurrence of an endogenous tone is remarkable. Presynaptic receptors are usually classified as auto- and heteroreceptors, that is, receptors serving the modulation of transmitter release by the transmitter itself or by another transmitter and/or mediator, respectively. An endogenous tone is generally shown by autoreceptors but less frequently by heteroreceptors. The fact that some presynaptic cannabinoid heteroreceptors are subject to an endogenous tone has important consequences: (1) the cannabinoid system might play an important

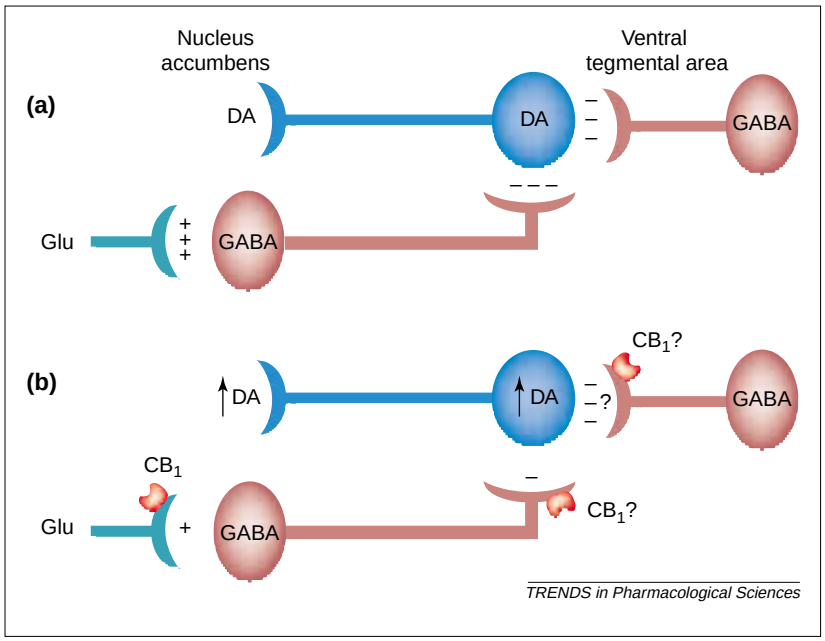

Fig. 1. Effect of Δ^9-tetrahydrocannabinol (Δ^9-THC) on the mesolimbic reward system. (a) The dopaminergic fibre tract (blue) projecting from the ventral tegmental area to the nucleus accumbens shell region is under the tonical inhibitory control of a GABA-containing interneurone (red) in the ventral tegmental area and a long-loop GABA-containing feedback neurone (red) projecting from the nucleus accumbens to the ventral tegmental area. (b) Many drugs of abuse, including Δ^9-THC, increase dopamine release in the nucleus accumbens. The facilitatory effect of Δ^9-THC on dopamine release might theoretically be explained by a direct effect on the dopamine-containing neurone (extremely unlikely) or by activation of presynaptic inhibitory cannabinoid CB_1 receptors on the two GABA-containing neurones (more likely). A more plausible explanation would be that Δ^9-THC activates presynaptic inhibitory CB_1 receptors on the glutamatergic afferents (green) to the long-loop GABA-containing feedback neurone. For further details, see text. Abbreviations: +, excitatory influence; –, inhibitory influence; DA, dopamine; Glu, glutamate.

physiological role, both in the CNS and in the periphery; and (2) not only cannabinoid receptor agonists but, in addition, antagonists/inverse agonists at these receptors might be developed as therapeutic agents.

(Patho)physiological relevance of cannabinoid-receptor-mediated inhibition of transmitter release

The role played by endogenously formed cannabinoids at presynaptic cannabinoid receptors within the neuronal network of the CNS has been further elucidated very recently. It is known that depolarization of both hippocampal pyramidal cells and cerebellar Purkinje cells is associated with an inhibition of the glutamatergic or GABAergic inputs received by these cells[31–33]. This retrograde signalling requires readily releasable and freely diffusible molecules capable of inhibiting transmitter release via a presynaptic site. Using selective antagonists, retrograde signalling from the rat hippocampal pyramidal cell to its GABAergic input[31,32] and from the rat cerebellar Purkinje cell to its glutamatergic input[33] has been shown to involve endocannabinoids. Thus, the retrograde signalling was abolished by SR141716 (Refs 31,32) and/or other CB_1 receptor antagonists[31–33] but was not affected by antagonists of metabotropic glutamate receptors[31–33], $GABA_B$ (Refs 32,33) or adenosine A_1 receptors[33].

Finally, presynaptic cannabinoid receptors might be involved in two well-known effects of exogenously added cannabinoids: reinforcing properties and cognitive impairment. Δ^9-THC, like other addictive drugs, stimulates the mesolimbic reward system (Fig. 1). Thus, systemic application of Δ^9-THC to animals increases the firing rate of the dopaminergic nerve fibres projecting from the ventral tegmental area to the nucleus accumbens[34] and increases the release of dopamine in the nucleus accumbens[35,36]. Although Δ^9-THC (like amphetamine or cocaine[37]) might increase dopamine release via a direct effect on the dopaminergic axon terminals in the nucleus accumbens (Fig. 1), this is extremely unlikely because both binding[38] and functional studies[39] show that cannabinoid receptors are absent from these neurones. The axon terminals of two GABA-containing neurones in the ventral tegmental area (involved in the effect of opiates on the rewarding system[37]) might be alternative targets for the action of Δ^9-THC (Fig. 1). Both neurones tonically inhibit dopamine-containing neurones and inhibition of GABA release via presynaptic cannabinoid receptors would be expected to increase the activity of the dopaminergic fibre tract. Administration of the cannabinoid receptor agonist HU210 to slices containing the ventral tegmental area indeed increases the firing rate of dopamine-containing neurones[40]. By contrast, *in vivo* administration of Δ^9-THC directly into this brain area does not increase dopamine release in the nucleus accumbens[35].

Therefore, one might consider that the excitatory glutamatergic input to the GABA-containing neurone that projects from the nucleus accumbens to the ventral tegmental area and subserves a long-loop feedback[37] is the target for Δ^9-THC (Fig. 1). When the excitatory input to these tonically active GABA-containing neurones were inhibited via cannabinoid receptors, an increase in the activity of the dopaminergic tract would also be expected. CB_1-receptor-mediated inhibition of glutamate release from neurones in the nucleus accumbens has indeed been shown[24]. There are, however, at least two other mechanisms that might account for the effect of Δ^9-THC on the mesolimbic reward system. Thus, GABA release in the nucleus accumbens is also inhibited via CB_1 receptors and it has been hypothesized that the resulting dis-inhibitory effect is the basis for the increased activity of the dopamine-containing neurones[41,42]. Moreover, the involvement of an endogenous opioid system and of μ_1 opioid receptors located in the ventral tegmental area has been suggested[36].

Acute administration of cannabinoids reversibly impairs cognitive functions both in animals and in humans[43]. In rats, impairment of spatial memory is also observed when the cannabinoid receptor agonist CP55940 is directly administered into the hippocampus[44]. Cannabinoid receptor agonists are also known to impair long-term potentiation (LTP), that is, they interfere with an *in vitro* model of learning

and memory in which high-frequency (e.g. 100 Hz) stimuli are applied to the fibres projecting from the CA3 to the CA1 region of the hippocampus. This type of stimulation depolarizes the postsynaptic membrane to such an extent that the blockade of NMDA receptor channels by Mg^{2+} is overcome and Ca^{2+} influx through these channels can occur. The inhibitory effect of the cannabinoids on LTP might be related to their inhibitory effect on glutamate release[18,20,21,45]; in other words, the amount of glutamate released after administration of cannabinoids is so low that the degree of depolarization caused by glutamate is not sufficient to remove the inhibition of NMDA receptor channels by Mg^{2+}. Consistent with these findings, cannabinoids no longer impair LTP if the postsynaptic membrane is held at a depolarized potential that relieves the blockade of the NMDA channels by Mg^{2+} or if Mg^{2+} is omitted from the recording solution. These data suggest that the inhibitory effect of the cannabinoids on LTP is related to the inhibition of glutamate release via presynaptic CB_1 receptors rather than to an effect on proteins (e.g. protein kinase C).

There is also an alternative explanation for the negative effect of cannabinoids on cognitive skills that implicates the septohippocampal pathway. Anatomical interruption or functional impairment of this fibre tract (e.g. by systemic or topical administration of the muscarinic receptor antagonist scopolamine) has a detrimental effect on certain types of cognitive skills[46]. Cannabinoids inhibit acetylcholine release in the hippocampus of the rat *in vitro*[47] and *in vivo*[48] and of the mouse *in vitro*[49]; by contrast, hippocampal acetylcholine release is increased by SR141716 in the rat[47,48] and mouse[49] and by CB_1 receptor disruption in the mouse[11]. Future studies must examine to what extent acetylcholine, glutamate and perhaps further neurotransmitters are involved in the effects of cannabinoids on cognitive skills. The fact that cognitive skills are subject to an endogenous cannabinoid-mediated tone [as evidenced by an improvement of memory tasks in rodents by SR141716 (Ref. 50) or by CB_1 receptor disruption[51]] is an intriguing hypothesis.

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References

1 Watson, S.J. *et al.* (2000) Marijuana and medicine: assessing the science base. A summary of the 1999 Institute of Medicine report. *Arch. Gen. Psychiatry* 57, 547–552
2 Hall, W. and Solowij, N. (1998) Adverse effects of cannabis. *Lancet* 352, 1611–1616
3 Pertwee, R.G. (1997) Pharmacology of cannabinoid CB_1 and CB_2 receptors. *Pharmacol. Ther.* 74, 129–180
4 Pertwee, R.G. (1999) Pharmacology of cannabinoid receptor ligands. *Curr. Med. Chem.* 6, 635–664
5 Ameri, A. (1999) The effects of cannabinoids on the brain. *Prog. Neurobiol.* 58, 315–348
6 Manzanares, J. *et al.* (1999) Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol. Sci.* 20, 287–294
7 Gill, E.W. *et al.* (1970) Preliminary experiments on the chemistry and pharmacology of cannabis. *Nature* 228, 134–136
8 Stefano, G.B. *et al.* (1997) Morphine- and anandamide-stimulated nitric oxide production inhibits presynaptic dopamine release. *Brain Res.* 763, 63–68
9 Lan, R. *et al.* (1999) Structure–activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. *J. Med. Chem.* 42, 769–776
10 Kathmann, M. *et al.* (1999) CB_1 receptor density and CB_1 receptor-mediated functional effects in rat hippocampus are decreased by an intracerebroventricularly administered antisense oligodeoxynucleotide. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 360, 421–427
11 Kathmann, M. *et al.* (2001) Enhanced acetylcholine release in the hippocampus of cannabinoid CB_1 receptor-deficient mice. *Br. J. Pharmacol.* 132, 1169–1173
12 Hájos, N. *et al.* (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur. J. Neurosci.* 12, 3239–3249
13 Pertwee, R.G. *et al.* (1995) Pharmacological characterization of three novel cannabinoid receptor agonists in the mouse isolated vas deferens. *Eur. J. Pharmacol.* 284, 241–247
14 Griffin, G. *et al.* (1997) Evidence for the presence of CB_2-like cannabinoid receptors on peripheral nerve terminals. *Eur. J. Pharmacol.* 339, 53–61
15 Ross, R.A. *et al.* (2001) Structure–activity relationship for the endogenous cannabinoid, anandamide, and certain of its analogues at vanilloid receptors in transfected cells and vas deferens. *Br. J. Pharmacol.* 132, 631–640
16 Schweitzer, P. (2000) Cannabinoids decrease the K^+ M-current in hippocampal CA1 neurons. *J. Neurosci.* 20, 51–58
17 Göbel, I. *et al.* (2000) Electrically evoked release of [^{3}H]noradrenaline from mouse cultured sympathetic neurons: release-modulating heteroreceptors. *J. Neurochem.* 75, 2087–2094
18 Misner, D.L. and Sullivan, J.M. (1999) Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. *J. Neurosci.* 19, 6795–6805
19 Huang, C.C. *et al.* (2001) Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. *J. Physiol. (London)* 532, 731–748
20 Shen, M. *et al.* (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J. Neurosci.* 16, 4322–4334
21 Sullivan, J.M. (1999) Mechanisms of cannabinoid-receptor-mediated inhibition of synaptic transmission in cultured hippocampal pyramidal cells. *J. Neurophysiol.* 82, 1286–1294
22 Hoffman, A.F. and Lupica, C.R. (2000) Mechanisms of cannabinoid inhibition of $GABA_A$ synaptic transmission in the hippocampus. *J. Neurosci.* 20, 2470–2479
23 Chan, P.K.J. and Yung, W.H. (1998) Occlusion of the presynaptic action of cannabinoids in rat substantia nigra pars reticulata by cadmium. *Neurosci. Lett.* 249, 57–60
24 Robbe, E. *et al.* (2001) Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. *J. Neurosci.* 21, 109–116
25 Takahashi, K.A. and Linden, D.J. (2000) Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. *J. Neurophysiol.* 83, 1167–1180
26 Coutts, A.A. and Pertwee, R.G. (1998) Evidence that cannabinoid-induced inhibition of electrically evoked contractions of the myenteric plexus – longitudinal muscle preparation of guinea-pig small intestine can be modulated by Ca^{2+} and cAMP. *Can. J. Physiol. Pharmacol.* 76, 340–346
27 Deutsch, D.G. *et al.* (1997) Production and physiological actions of anandamide in the vasculature of the rat kidney. *J. Clin. Invest.* 100, 1538–1546
28 Izzo, A.A. *et al.* (1998) Excitatory transmission to the circular muscle of the guinea-pig ileum: evidence for the involvement of cannabinoid CB_1 receptors. *Br. J. Pharmacol.* 124, 1363–1368
29 Gifford, A.N. *et al.* (2000) Cannabinoid receptor-mediated inhibition of acetylcholine release from hippocampal and cortical synaptosomes. *Br. J. Pharmacol.* 131, 645–650
30 Christopoulos, A. *et al.* (2001) Pharmacological analysis of cannabinoid receptor activity in the rat vas deferens. *Br. J. Pharmacol.* 132, 1281–1291
31 Wilson, R.I. and Nicoll, R.A. (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410, 588–592
32 Ohno-Shosaku, T. *et al.* (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29, 729–738
33 Kreitzer, A.C. and Regehr, W.G. (2001) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29, 717–727
34 French, E.D. *et al.* (1997) Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *NeuroReport* 8, 649–652

35 Gardner, E.L. and Vorel, S.R. (1998) Cannabinoid transmission and reward-related events. *Neurobiol. Disease* 5, 502–533

36 Tanda, G. *et al.* (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. *Science* 276, 2048–2050

37 Spanagel, R. and Weiss, F. (1999) The dopamine hypothesis of reward: past and current status. *Trends Neurosci.* 22, 521–527

38 Herkenham, M. *et al.* (1991) Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res.* 547, 267–274

39 Szabo, B. *et al.* (1999) Effects of cannabinoids on dopamine release in the corpus striatum and the nucleus accumbens *in vitro*. *J. Neurochem.* 73, 1084–1089

40 Cheer, J.F. *et al.* (2000) Lack of response suppression follows repeated ventral tegmental cannabinoid administration: an *in vitro* electrophysiological study. *Neuroscience* 99, 661–667

41 Hoffman, A.F. and Lupica, C.R. (2001) Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. *J. Neurophysiol.* 85, 72–83

42 Manzoni, O.J. and Bockaert, J. (2001) Cannabinoids inhibit GABAergic synaptic transmission in mice nucleus accumbens. *Eur. J. Pharmacol.* 412, R3–R5

43 Sullivan, J.M. (2000) Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. *Learn. Mem.* 7, 132–139

44 Lichtman, A.H. *et al.* (1995) Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology* 119, 282–290

45 Ameri, A. *et al.* (1999) Effects of the endogenous cannabinoid, anandamide, on neuronal activity in rat hippocampal slices. *Br. J. Pharmacol.* 126, 1831–1839

46 Dutar, P. *et al.* (1995) The septohippocampal pathway: structure and function of a central cholinergic system. *Physiol. Rev.* 75, 393–427

47 Gifford, A.N. and Ashby, C.R. (1996) Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55,212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. *J. Pharmacol. Exp. Ther.* 277, 1431–1436

48 Gessa, G.L. *et al.* (1997) Inhibition of hippocampal acetylcholine release by cannabinoids: reversal by SR 141716A. *Eur. J. Pharmacol.* 327, R1–R2

49 Kathmann, M. *et al.* (2001) Cannabinoid CB_1 receptor-mediated inhibition of acetylcholine release in the brain of NMRI, CD-1 and C57BL/6J mice. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 363, 50–56

50 Terranova, J.P. *et al.* (1996) Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. *Psychopharmacology* 126, 165–172

51 Reibaud, M. *et al.* (1999) Enhancement of memory in cannabinoid CB_1 receptor knock-out mice. *Eur. J. Pharmacol.* 379, R1–R2

52 Schlicker, E. *et al.* (1997) Cannabinoid CB_1 receptor-mediated inhibition of noradrenaline release in the human and guinea-pig hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 356, 583–589

53 Katona, I. *et al.* (2000) GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience* 100, 797–804

54 Molderings, G.J. *et al.* (1999) Presynaptic cannabinoid and imidazoline receptors in the human heart and their potential relationship. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 360, 157–164

55 Croci, T. *et al.* (1998) *In vitro* functional evidence of neuronal cannabinoid CB_1 receptors in human ileum. *Br. J. Pharmacol.* 125, 1393–1395

56 Szabo, B. *et al.* (2001) Effects of cannabinoids on sympathetic and parasympathetic neuroeffector transmission in the rabbit heart. *J. Pharmacol. Exp. Ther.* 297, 819–826

57 Niederhoffer, N. and Szabo, B. (1999) Effect of the cannabinoid receptor agonist WIN 55,212-2 on sympathetic cardiovascular regulation. *Br. J. Pharmacol.* 126, 457–466

58 Schlicker, E. *et al.* (1996) Cannabinoid receptor-mediated inhibition of dopamine release in the retina. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 354, 791–795

59 Pertwee, R.G. *et al.* (1996) Further evidence for the presence of cannabinoid CB_1 receptors in guinea-pig small intestine. *Br. J. Pharmacol.* 118, 2199–2205

60 Coutts, A.A. and Pertwee, R.G. (1997) Inhibition by cannabinoid receptor agonists of acetylcholine release from the guinea-pig myenteric plexus. *Br. J. Pharmacol.* 121, 1557–1566

61 Auclair, N. *et al.* (2000) Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. *J. Neurophysiol.* 83, 3287–3293

62 Katona, I. *et al.* (1999) Presynaptically located CB_1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* 19, 4544–4558

63 Cadogan, A.K. *et al.* (1997) Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. *J. Neurochem.* 69, 1131–1137

64 Szabo, B. *et al.* (1998) Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. *Neuroscience* 85, 395–403

65 Gerdeman, G. and Lovinger, D.M. (2001) CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J. Neurophysiol.* 85, 468–471

66 Szabo, B. *et al.* (2000) Cannabinoids inhibit excitatory neurotransmission in the substantia nigra pars reticulata. *Neuroscience* 97, 89–97

67 Vaughan, C.W. *et al.* (2000) Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons *in vitro*. *Mol. Pharmacol.* 57, 288–295

68 Lévénès, C. *et al.* (1998) Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellum. *J. Physiol.* 510, 867–879

69 Vaughan, C.W. *et al.* (1999) Cannabinoid receptor activation inhibits GABAergic neurotransmission in rostral ventromedial medulla neurons *in vitro*. *Br. J. Pharmacol.* 127, 935–940

70 Ishac, E.J.N. *et al.* (1996) Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB_1 receptors on peripheral sympathetic nerves. *Br. J. Pharmacol.* 118, 2023–2028

71 Malinowska, B. *et al.* (1997) Cannabinoid CB_1 receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 356, 197–202

72 Nakazi, M. *et al.* (2000) Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB_1 receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 361, 19–24

73 Zimmer, A. *et al.* (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 5780–5785

74 Ledent, C. *et al.* (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. *Science* 283, 401–404

75 Trendelenburg, A.U. *et al.* (2000) Modulation of ^{3}H-noradrenaline release by presynaptic opioid, cannabinoid and bradykinin receptors and β-adrenoceptors in mouse tissues. *Br. J. Pharmacol.* 130, 321–330

76 Pertwee, R.G. and Fernando, S.R. (1996) Evidence for the presence of cannabinoid CB_1 receptors in mouse urinary bladder. *Br. J. Pharmacol.* 118, 2053–2058

77 Irving, A.J. *et al.* (2000) Functional expression of cell surface cannabinoid CB_1 receptors on presynaptic inhibitory terminals in cultured rat hippocampal neurons. *Neuroscience* 98, 253–262

78 Gifford, A.N. *et al.* (1997) Effect of the cannabinoid receptor SPECT agent, AM 281, on hippocampal acetylcholine release from rat brain slices. *Neurosci. Lett.* 238, 84–86

79 Pertwee, R.G. *et al.* (1996) Agonist–antagonist characterization of 6′-cyanohex-2′-yne-Δ^8-tetrahydrocannabinol in two isolated tissue preparations. *Eur. J. Pharmacol.* 315, 195–201

Chemical names

AM281: 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide

AM404: (all *Z*)-*N*-(4-hydroxyphenyl)-5,8,11,14-eicosatetraenamide

CP55940: (–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol

HU210: (–)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl

JWH015: (2-methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone

LY320135: [6-methoxy-2-(4-methoxyphenyl)benzo[*b*]thien-3-yl][4-cyanophenyl]methanone

SR141716: *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride

SR144528: *N*-[(1*S*)-endo-1,3,3,-trimethylbicyclo[2.2.1]heptan-2yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide

WIN552122: *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-yl](1-naphthalen-yl)methanone mesylate

WIN552123: *S*(–)-enantiomer of WIN552122