
Effects of Cannabinoids on Neurotransmission

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Abstract The CB₁ cannabinoid receptor is widely distributed in the central and peripheral nervous system. Within the neuron, the CB₁ receptor is often localised in axon terminals, and its activation leads to inhibition of transmitter release. The consequence is inhibition of neurotransmission via a presynaptic mechanism. Inhibition of glutamatergic, GABAergic, glycinergic, cholinergic, noradrenergic and serotonergic neurotransmission has been observed in many regions

of the central nervous system. In the peripheral nervous system, CB₁ receptor-mediated inhibition of adrenergic, cholinergic and sensory neuroeffector transmission has been frequently observed. It is characteristic for the ubiquitous operation of CB₁ receptor-mediated presynaptic inhibition that antagonistic components of functional systems (for example, the excitatory and inhibitory inputs of the same neuron) are simultaneously inhibited by cannabinoids. Inhibition of voltage-dependent calcium channels, activation of potassium channels and direct interference with the synaptic vesicle release mechanism are all implicated in the cannabinoid-evoked inhibition of transmitter release. Many presynaptic CB₁ receptors are subject to an endogenous tone, i.e. they are constitutively active and/or are continuously activated by endocannabinoids. Compared with the abundant data on presynaptic inhibition by cannabinoids, there are only a few examples for cannabinoid action on the somadendritic parts of neurons in situ.

Keywords Acetylcholine · Axon terminal · CB₁ cannabinoid receptor · GABA · Glutamate · Neurotransmission · Noradrenaline · Presynaptic inhibition · Transmitter release

1

Introduction

As described in the chapter by Mackie (this volume), the CB₁ cannabinoid receptor is widely distributed in the central and peripheral nervous system. One of the primary consequences of activation of CB₁ receptors is the inhibition or activation of ion channels. For example, voltage-dependent calcium channels are typically inhibited by cannabinoids, whereas several kinds of potassium channels are activated. Theoretically, due to their influence on ion channels, cannabinoids can change the function of neurons in several ways. By acting in the dendrites, they can interfere with the conduction of synaptic currents to the soma of the neuron. By acting in the soma, they can interfere with the generation of action potentials. By acting on ion channels in axon terminals, they can inhibit transmitter release from the terminals; the consequence is inhibition of neurotransmission with a presynaptic mechanism. Inhibition of neurotransmission appears to be, at present, the best-characterised electrophysiological effect of cannabinoids, and this review focuses on this effect. Before analysing the presynaptic effect, we describe cannabinoid effects on ion channels and the anatomical evidence for the presence of cannabinoid receptors in axon terminals. Presynaptic inhibition by endogenous cannabinoids released by postsynaptic neurons—retrograde signaling—is described in the chapter by Vaughan and Christie (this volume).

2

Effects of Cannabinoids on Ion Channels

The somadendritic region of most neurons is accessible for electrophysiological studies. In contrast, direct electrophysiological recording from axon terminals of

mammals is either impossible or extremely difficult. Accordingly, we know relatively well how cannabinoids change the function of ion channels in the somadendritic region. Our knowledge on electrophysiological changes in axon terminals is limited; we can only assume that ion channels are influenced similarly as in the somadendritic region. In this section, effects on the somadendritic region are dealt with.

2.1

Effects of Cannabinoids on Voltage-Gated Ion Channels

2.1.1

Calcium Channels

In the majority of studies, cannabinoids depressed voltage-dependent calcium channels. According to the first observations, activation of CB₁ receptors inhibits N-type voltage-dependent calcium channels in neuronal cell lines (Caulfield and Brown 1992; Mackie and Hille 1992; Mackie et al. 1993). No inhibition occurred in pertussis toxin-treated cells, indicating the involvement of G proteins containing G $\alpha_{i/o}$ subunits. Later, this observation was extended to isolated rat hippocampal neurons and cerebellar granule cells (Twitchell et al. 1997; Nogueron et al. 2001). In isolated rat sympathetic ganglion neurons that previously had been injected with CB₁ receptor cRNA, cannabinoids also inhibited N-type calcium channels (Pan et al. 1996). Q-type calcium channels were also inhibited in CB₁ receptor-transfected AtT20 cells (Mackie et al. 1995). The endogenous cannabinoid (endocannabinoid) anandamide inhibits T-type calcium channels; this effect is, however, not mediated by CB₁ receptors (Chemin et al. 2001).

There are at least two examples for stimulation of calcium channels by cannabinoids: L-type calcium currents in a neuronal cell line (Rubovitch et al. 2002) and in retinal rods of the tiger salamander (Straiker and Sullivan 2003) were enhanced by cannabinoids.

2.1.2

Potassium Channels

Activated CB₁ receptors can also change the function of several types of potassium channels. In oocytes and AtT20 cells artificially expressing the CB₁ receptor, stimulation of inwardly rectifying potassium channels was repeatedly observed (Henry and Chavkin 1995; Mackie et al. 1995; Garcia et al. 1998; McAllister et al. 1999). Potassium A currents in cultured hippocampal neurons are stimulated by cannabinoids (Deadwyler et al. 1995; Mu et al. 2000). The effects of cannabinoids on potassium M currents in hippocampal brain slices have also been studied; M currents were inhibited, which means an enhancement of neuronal excitability (Schweitzer 2000). The potassium K current is inhibited by cannabinoids in cultured hippocampal neurons (Hampson et al. 2000). As in the case of calcium channels, anandamide can elicit a CB₁ receptor-independent effect on potassium

channels, i.e. it inhibits the acid-sensitive background potassium channel TASK-1 (Maingret et al. 2001).

2.1.3

Sodium Channels

In an early study, Turkanis et al. (1991) showed that Δ^9 -tetrahydrocannabinol inhibits voltage-dependent sodium channels; the involved primary receptor was not identified in this study. More recently, it was observed that anandamide and the synthetic CB₁/CB₂ receptor agonist WIN55212-2 inhibited voltage-dependent sodium channels in synaptosomes prepared from mouse brain (Nicholson et al. 2003). Since the effects were not attenuated by the CB₁ receptor antagonist AM251, the involvement of CB₁ receptors can be excluded.

2.2

Effects of Cannabinoids on Ligand-Gated Ion Channels

The function of several types of ligand-gated ion channels is changed by cannabinoids—as a rule, these effects are not mediated by CB₁ receptors. In isolated rat nodose ganglion neurons, cannabinoids inhibited serotonin-3 (5-HT₃) receptor-mediated currents (Fan 1995). This observation was verified and extended in a recent study. In HEK293 cells expressing the human 5-HT_{3A} receptor, several cannabinoids inhibited the 5-HT-evoked current (Barann et al. 2002). CB₁ receptors could not be involved in this effect, since HEK293 cells do not express CB₁ receptors.

The function of AMPA-type glutamate receptors (Akinshola et al. 1999) and nicotinic acetylcholine receptors (Oz et al. 2003), expressed in oocytes, was inhibited by anandamide. These effects are, again, CB₁ and CB₂ receptor-independent.

2.3

What Is the Functional Consequence of the Inhibition of Somadendritic Ion Channels?

The majority of the experiments in which the effect of cannabinoids on somadendritic ion channels was studied were carried out on cell lines, on cells artificially expressing the CB₁ receptor or on isolated neurons. It is not known whether the effects also occur under natural conditions. For example, cannabinoid receptor agonists did not influence voltage-dependent calcium channels in caudate-putamen medium spiny neurons (Szabo et al. 1998), although these neurons are known to synthesise CB₁ receptors. It is conceivable that in neurons under physiological conditions, the density of somadendritic CB₁ receptors is too low for modulation of certain ion channels. Alternatively, the coupling mechanism between receptor and ion channel may not be functional.

Another important question also remains unanswered. We basically do not know how modulation of somadendritic ion channels by cannabinoids affects the excitability or integrative capacity of neurons. There are only a few experiments in which neurons were studied *in situ* (in brain slices), and cannabinoid effects were restricted to the somadendritic region of the neurons (by blockade of the synaptic input of the neurons), and cannabinoids elicited an effect. One such experiment was carried out by Kreitzer et al. (2002): cannabinoids lowered the firing rate of cerebellar interneurons and this was attributed to the activation of barium-sensitive potassium channels. In the experiments of Himmi et al. (1998), cannabinoids changed the firing rate of nucleus tractus solitarius neurons in brain slices; since the synaptic input was not blocked, it is not known whether the change in firing rate was due to an effect on the neurons themselves, or to an effect on their synaptic input.

3

Anatomical Evidence for the Presence of CB₁ Cannabinoid Receptors in Axon Terminals

The wide distribution of the CB₁ receptor in the nervous system is described in the chapter by Mackie (this volume). The prerequisite for presynaptic inhibition of neurotransmission is that the receptor is localised in axon terminals. The following paragraph lists known examples for localisation of CB₁ receptors in axon terminals.

In the cerebellum, CB₁ receptors in terminals of basket cells can be seen at the light microscopic level (Tsou et al. 1998; Diana et al. 2002). Electron microscopical studies have indicated that a great portion of CB₁ receptors in the caudate-putamen (Rodriguez et al. 2001), hippocampus (Katona et al. 1999, 2000; Hájos et al. 2000) and amygdala (Katona et al. 2001) is in axon terminals. Comparison of the site of CB₁ receptor synthesis (which was determined by *in situ* hybridisation) with the distribution of receptor protein (which was determined with receptor autoradiography and immunohistochemistry) indicates localisation of CB₁ receptors in terminals of parallel fibres in the cerebellum and in terminals of striatonigral neurons in the substantia nigra pars reticulata (compare, for example, Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993; Tsou et al. 1998). The changes in the CB₁ receptor distribution pattern during neurodegeneration accompanying Huntington's disease and experimentally elicited neurodegeneration also suggest that CB₁ receptors in the substantia nigra pars reticulata are localised in striatonigral axon terminals (Herkenham et al. 1991; Glass et al. 2000).

In a few instances, it was shown that CB₁ receptors are not uniformly distributed in a neuron, but are preferentially localised in the axon terminal. For example, CB₁ receptors were well visible in cerebellar basket cell terminals, but not in the somata of these neurons (Diana et al. 2002). Preferential localisation of CB₁ receptors in axon terminals was also observed in hippocampal neurons (Twitchell et al. 1997; Irving et al. 2000).

4

Effects of Cannabinoids on Neurotransmission in the Central Nervous System

Two methods were used to study the effect of cannabinoids on presynaptic axon terminals. The more frequently used electrophysiological approach measures neurotransmission. In brain slices or neuronal cultures, electrical currents in postsynaptic neurons are recorded with patch-clamp or microelectrode techniques. Presynaptic axon terminals are electrically stimulated and the postsynaptic current resulting from stimulation of ligand-gated ion channels of postsynaptic neurons by the released transmitter is determined. The change in the postsynaptic current amplitude is a measure of the change in synaptic transmission.

In the other method, the release of endogenous or radiolabelled neurotransmitters from presynaptic axon terminals is determined chemically. Although this latter method shows directly what happens at the level of axon terminals, it does not measure “neurotransmission”.

In electrophysiological experiments, cannabinoids inhibited neurotransmission. The inhibition was always due to inhibition of transmitter release from axon terminals and never to interference of cannabinoids with the postsynaptic effects of the neurotransmitters. The experiments in which transmitter release was determined neurochemically also indicated that cannabinoids inhibit transmitter release from axon terminals. In most instances the presynaptic cannabinoid receptors can be classified as CB₁ receptors (but some exceptions are given in Tables 1 and 2). Effects of cannabinoids on the release of individual transmitters are discussed below. Effects of cannabinoids on neurotransmission have also been reviewed by Schlicker and Kathmann (2001).

4.1

Fast Excitatory Neurotransmission

Activation of CB₁ receptors inhibits the release of the excitatory neurotransmitter glutamate in many brain regions and in the spinal cord (Table 1).

Inhibition was seen in nuclei belonging to the extrapyramidal motor control system: caudate-putamen, globus pallidus and substantia nigra pars reticulata (Fig. 1 shows an example of presynaptic inhibition of glutamatergic neurotransmission in the substantia nigra pars reticulata; see Fig. 6 for an overview of cannabinoid effects on neurotransmission in the extrapyramidal motor control system). Inhibition of neurotransmission was also observed in the ventral tegmental area, hippocampus and the nucleus accumbens—these regions are parts of the limbic system. Inhibition of the excitatory synaptic transmission in the hippocampus could contribute to the anticonvulsive effect of cannabinoids. Purkinje cells in the cerebellar cortex receive excitatory inputs from parallel fibres and climbing fibres; both kinds of excitatory inputs are inhibited by activated CB₁ receptors (see Fig. 7 for an overview of cannabinoid effects on neurotransmission in the cerebellar cortex). Moreover, cannabinoids depress the glutamatergic neurotransmission

Table 1. Inhibition of glutamatergic neurotransmission (*continued on next page*)

Neurotransmitter	Species	Region	Method	Mechanism of presynaptic inhibition	Reference(s)
Glutamate	Rat	Cortex (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Auclair et al. 2000
Glutamate ^a	Rat	Cortex (cell culture)	Endogenous glutamate chemically determined	IP ₃ is involved	Ferraro et al. 2001
Glutamate	Rat	Caudate-putamen (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Gerdeman and Lovinger 2001
Glutamate	Rat	Caudate-putamen (brain slice)	Electrophysiology (patch clamp, extracell. recording)	The inhibition is pertussis toxin sensitive Vesicle release machinery not inhibited Ca ²⁺ channels involved	Huang et al. 2001, 2002
Glutamate	Rat	Caudate-putamen (brain slice)	Electrophysiology (extracell. recording), [³ H]glutamate release	Glutamate uptake is inhibited, glutamate causes presynaptic inhibition via mGluR	Brown et al. 2003b
Glutamate	Mouse	Nucleus accumbens (brain slice)	Electrophysiology (patch clamp, extracell. recording)	Vesicle release machinery inhibited Ca ²⁺ channels not involved K ⁺ channels involved cAMP and PKA not involved	Robbe et al. 2001
Glutamate	Mouse	Globus pallidus (brain slice)	Electrophysiology (patch clamp)	No details given	Wallmichrath and Szabo 2003
Glutamate	Rat	Ventral tegmental area	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Mellis et al. 2004
Glutamate	Rat	Substantia nigra pars reticulata (brain slice)	Electrophysiology (patch clamp)	No details given	Szabo et al. 2000
Glutamate	Rat	Hippocampus (brain slice)	Electrophysiology (extracell. recording)	No details given	Ameri et al. 1999; Ameri and Simmet 2000
Glutamate	Rat	Hippocampus (brain slice)	Electrophysiology (extracell. recording)	No details given	Al-Hayani and Davies 2000

Table 1. (continued)

Neurotransmitter	Species	Region	Method	Mechanism of presynaptic inhibition	Reference(s)
Glutamate	Rat	Hippocampus (cell culture)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited Ca ²⁺ channels involved	Sullivan 1999
Glutamate	Rat	Hippocampus (cell culture)	Electrophysiology (patch clamp), microfluorometry (Ca ²⁺ spikes determined)	The inhibition is pertussis toxin sensitive	Shen et al. 1996, Shen and Thayer 1999, Kouznetsova et al. 2002
Glutamate? GABA?	Rat	Hippocampus (cell culture)	Release of the vesicle marker FM1-43	No details given	Kim and Thayer 2000
Glutamate	Rat	Hippocampus (synaptosomes)	Endogenous glutamate chemically determined (4-AP evoked release)	The inhibition is pertussis toxin sensitive Ca ²⁺ channels involved Vesicle release machinery not inhibited PKA not involved	Wang 2003
Glutamate	Mouse, rat	Hippocampus (brain slice)	Electrophysiology (patch clamp)	No CB ₁ receptors are involved	Hájos et al. 2001; Hájos and Freund 2002
Glutamate	Rat, mouse	Hippocampus (synaptosomes)	[³ H]Glutamate release	No CB ₁ receptors are involved	Kófalvi et al. 2003
Glutamate	Mouse	Hippocampus (brain slice)	Electrophysiology (patch clamp)	The inhibition is pertussis toxin sensitive Vesicle release machinery inhibited	Misner and Sullivan 1999
Glutamate	Mouse	Amygdala	Electrophysiology (patch clamp, extracell. recording)	The inhibition is pertussis toxin sensitive Vesicle release machinery inhibited K ⁺ channels involved	Azad et al. 2003
Glutamate	Rat	Cerebellum (brain slice)	Electrophysiology (patch clamp)	Ca ²⁺ channels not involved Vesicle release machinery inhibited K ⁺ channels involved Ca ²⁺ channels only indirectly involved	Levenes et al. 1998, Daniel and Crepel 2001
Glutamate	Rat	Cerebellum (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited	Takahashi and Linden 2000

Table 1. (*continued*)

Neurotransmitter	Species	Region	Method	Mechanism of presynaptic inhibition	Reference(s)
Glutamate	Rat	Cerebellum (brain slice)	Electrophysiology (patch clamp)	No details given	Kreitzer and Regehr 2001
Glutamate	Mouse	Cerebellum (brain slice)	Electrophysiology (patch clamp)	No details given	Maejima et al. 2001
Glutamate	Mouse	Synaptosomes from whole brain	Endogenous glutamate chemically determined (veratridine-evoked release)	No CB ₁ receptors are involved Na ⁺ channels inhibited	Nicholson et al. 2003
Glutamate	Rat	Spinal cord (cord slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Morisset and Urban 2001

4-AP, 4-aminopyridine; cAMP, cyclic adenosine monophosphate; extracell., extracellular; GABA, γ -aminobutyric acid; PKA, protein kinase A.

^aIn this exceptional study, cannabinoids did not decrease glutamate release, but increased it.

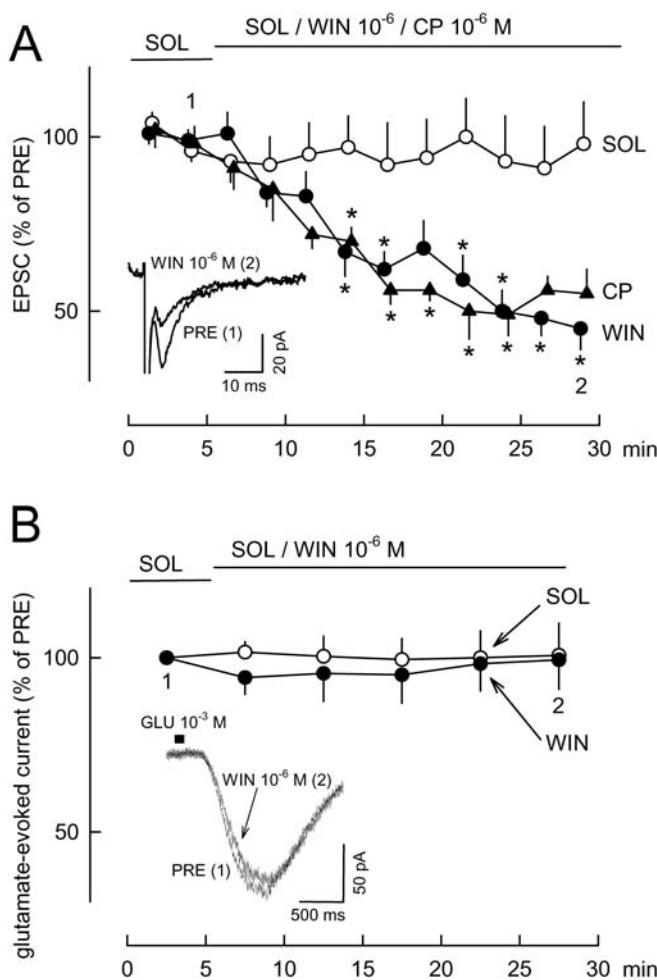


Fig. 1A, B. Cannabinoids inhibit glutamatergic synaptic transmission in the substantia nigra pars reticulata (SNR) of the rat via a presynaptic mechanism. The major glutamatergic afferent input of SNR neurons originates in the subthalamic nucleus. **A** SNR neurons were patch-clamped and their glutamatergic afferent axons electrically stimulated. The stimulation elicited excitatory postsynaptic currents (EPSCs) in SNR neurons. EPSCs remained stable in solvent (SOL)-superfused slices. The synthetic cannabinoid agonists WIN55212-2 (WIN) and CP55940 (CP) inhibited the EPSCs. **B** SNR neurons were patched-clamped and glutamate (GLU) was pressure-ejected from a pipette in their vicinity. Glutamate-evoked currents remained stable in SOL-superfused slices. Superfusion of WIN also did not change the glutamate-evoked currents. This observation indicates that cannabinoids do not interfere with the postsynaptic effect of glutamate; thus, the inhibition of neurotransmission seen in panel **A** is due to presynaptic inhibition of glutamate release from axon terminals. In both panels, a typical original recording obtained in a WIN experiment (*inset*) and the statistical analysis are shown. PRE, initial reference period. See Szabo et al. (2000) for details of the experiments. *, Significant difference from SOL ($p < 0.05$)

between primary sensory fibres and neurons in the dorsal horn of the spinal cord: this effect could be the basis of the spinal analgesia produced by cannabinoids.

According to recent observations, some effects of cannabinoids on glutamatergic transmission in the hippocampus are not mediated by CB₁ receptors (and also not by CB₂ receptors). Synthetic cannabinoids depressed excitatory neurotransmission also in brain slices from CB₁ receptor-knockout mice and in the presence of some CB₁ antagonists (Hájos et al. 2001; Hájos and Freund 2002). Similarly, cannabinoid-evoked glutamate release from hippocampal synaptosomes was resistant to CB₁ antagonists and persisted in CB₁ receptor-knockout mice (Köfalvi et al. 2003; but in a similar preparation, effects were sensitive to a CB₁ antagonist; Wang 2003). Based on such observations, the existence of a new cannabinoid receptor was postulated. It must be noted that the involvement of known non-cannabinoid receptors or ion channels—for which cannabinoids might possess a hitherto unrecognised affinity—was not excluded in these studies.

Prolonged exposure of G protein-coupled receptors to their agonists leads to desensitisation due to diminished coupling of the receptors with G proteins and receptor internalisation. This phenomenon was observed also in the case of CB₁ receptor-mediated inhibition of neurotransmission. Cannabinoid-evoked inhibition of glutamatergic and γ -aminobutyric acid (GABA)ergic neurotransmission in the nucleus accumbens was diminished by treatment of animals for 1 week with natural and synthetic cannabinoids (Hoffman et al. 2003a). Cannabinoid-evoked inhibition of excitatory neurotransmission between cultured hippocampal neurons was also strongly desensitised by a 24-h treatment of the neurons with a cannabinoid (Kouznetsova et al. 2002).

4.2

Fast Inhibitory Neurotransmission

CB₁ receptor-mediated inhibition of GABAergic neurotransmission has been observed in many brain regions, belonging to different functional systems (Table 2).

Thus, cannabinoids depress cerebral cortical GABAergic neurotransmission. Neurotransmission is also depressed in nuclei belonging to the extrapyramidal motor control system: caudate-putamen, globus pallidus and substantia nigra pars reticulata (Fig. 6 also shows cannabinoid effects on inhibitory neurotransmission in the extrapyramidal motor control system). GABAergic synaptic transmission in the cerebellum, a major brain region involved in motor control, is inhibited as well (Fig. 7 also shows cannabinoid effects on inhibitory neurotransmission in the cerebellar cortex). Figure 2 shows inhibition of GABAergic neurotransmission in the cerebellar cortex, and Fig. 3 shows that the inhibition is due to the inhibition of GABA release from presynaptic axon terminals. In several nuclei belonging to the limbic system (e.g. hippocampus and amygdala), activation of CB₁ receptors leads to depression of inhibitory neurotransmission. Inhibition of GABA release in the ventral tegmental area—where the mesolimbic reward pathway originates—could explain the euphoria produced by cannabinoids. The rostral ventromedial medulla oblongata and the periaqueductal grey in the midbrain are involved in nocicep-

Table 2. Inhibition of GABAergic neurotransmission (*continued on next page*)

Neurotransmitter	Species	Region	Method	Mechanism of presynaptic inhibition	Reference(s)
GABA	Mouse	Cortex (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited	Trettel and Levine 2002, 2003
GABA	Rat	Caudate-putamen (brain slice)	Electrophysiology (patch clamp)	No details given	Szabo et al. 1998
GABA	Mouse	Nucleus accumbens (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Manzoni and Bockaert 2001
GABA	Rat	Nucleus accumbens (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery weakly inhibited	Hoffman and Lupica 2001; Hoffman et al. 2003a
GABA	Mouse	Globus pallidus (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited	Engler and Szabo 2003, 2004
GABA	Rat	Substantia nigra pars reticulata (brain slice)	Electrophysiology (patch clamp)	No details given	Chan et al. 1998, Chan and Yung 1998
GABA	Rat	Substantia nigra pars reticulata (brain slice)	Electrophysiology (patch clamp)	No details given	Wallmichrath and Szabo 2002a
GABA	Mouse	Substantia nigra pars reticulata (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited	Wallmichrath and Szabo 2002b
GABA	Rat	Ventral tegmental area (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited	Szabo et al. 2002
GABA (GABA _B -mediated transmission)	Rat	Ventral tegmental area (brain slice)	Electrophysiology (patch clamp)	No details given	Riegel et al. 2003
GABA	Rat	Hippocampus (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited	Hájos et al. 2000
GABA	Rat	Hippocampus (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited K ⁺ channels not involved	Hoffman and Lupica 2000

Table 2. (*continued*)

Neurotransmitter	Species	Region	Method	Mechanism of presynaptic inhibition	Reference(s)
GABA	Rat	Hippocampus (brain slice)	Electrophysiology (patch clamp)	No details given	Hoffman et al. 2003b
GABA	Rat	Hippocampus (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Wilson and Nicoll 2001
GABA	Rat	Hippocampus (cell culture)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Irving et al. 2000
GABA	Rat	Hippocampus (cell culture)	Electrophysiology (patch clamp)	No details given	Ohno-Shosaku et al. 2001
GABA	Rat	Hippocampus (brain slice)	[³ H]GABA release	No details given	Katona et al. 1999
GABA	Mouse	Hippocampus (brain slice)	Electrophysiology (patch clamp)	No details given	Hajos et al. 2001
GABA	Man	Hippocampus (brain slice)	[³ H]GABA release	No details given	Katona et al. 2000
GABA	Man	Hippocampus (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited	Nakatsuka et al. 2003
GABA	Rat, mouse	Amygdala (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery not inhibited	Katona et al. 2001
GABA	Mouse	Amygdala (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Azad et al. 2003
GABA	Rat	Periaqueductal grey (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Vaughan et al. 2000
GABA	Rat	Rostral ventromedial medulla oblongata (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Vaughan et al. 1999
GABA	Rat	Cerebellum (brain slice)	Electrophysiology (patch clamp)	Intracellular Ca ²⁺ concentration decreased	Kreitzer and Regehr 2001

Table 2. (*continued*)

Neurotransmitter	Species	Region	Method	Mechanism of presynaptic inhibition	Reference(s)
GABA	Rat	Cerebellum (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Diana et al. 2002
GABA	Rat	Cerebellum (brain slice)	Electrophysiology (patch clamp)	Intracellular Ca^{2+} concentration decreased	Szabo et al. 2004
GABA	Mouse	Cerebellum (brain slice)	Electrophysiology (patch clamp)	Vesicle release machinery inhibited	Yoshida et al. 2002
GABA	Mouse	Synaptosomes from whole brain	Endogenous GABA chemically determined, Veratridine-induced release	No details given	Nicholson et al. 2003
GABA, glycine	Rat	Superficial medullary dorsal horn	Electrophysiology (patch clamp)	No CB_1 receptors are involved Na^+ channels inhibited	Jennings et al. 2001

GABA, γ -aminobutyric acid.

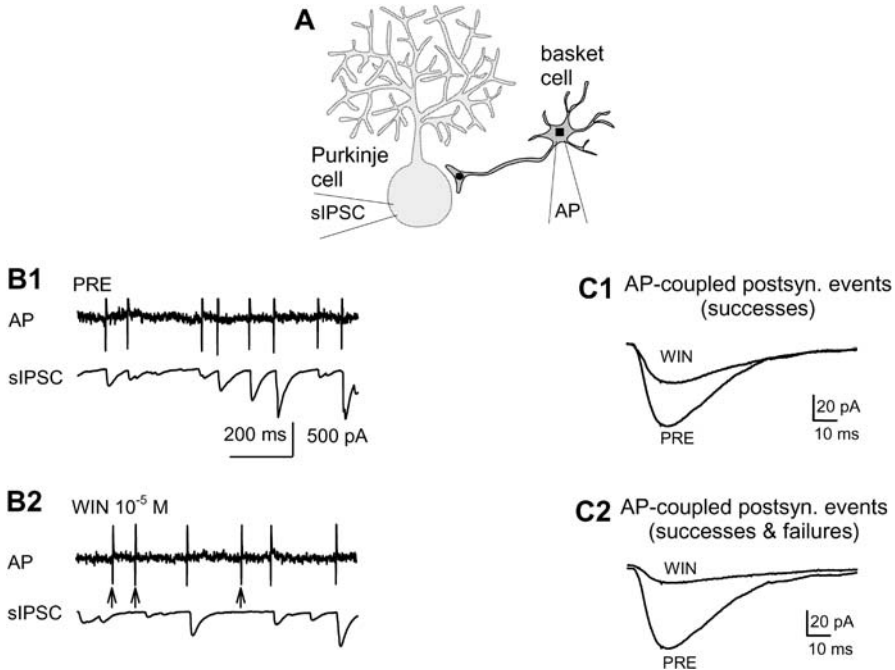


Fig. 2A–C. Cannabinoids inhibit GABAergic synaptic transmission between basket and Purkinje cells in the cerebellar cortex of the rat. **A** The basket cell synthesises CB_1 receptor mRNA (■) and the CB_1 receptor protein (●) is localised in the axon terminal. Action potentials (APs) of a basket cell and spontaneous inhibitory postsynaptic currents (sIPSCs) in a synaptically coupled Purkinje cell were recorded simultaneously. **B1**, **B2** APs and sIPSCs were recorded during the initial reference period (PRE) and during superfusion with WIN55212-2 (WIN). During PRE (**B1**), every presynaptic AP was accompanied by a postsynaptic IPSC: synaptic transmission was always successful. During WIN superfusion (**B2**), synaptic failures appear (marked by arrows). Enhancement of synaptic failure is typical for drugs that decrease probability of transmitter release from the presynaptic axon terminal. **C1** AP-coupled postsynaptic currents were averaged only if transmission was successful. The decrease in amplitude indicates inhibition of neurotransmission by WIN. **C2** All AP-coupled postsynaptic currents were averaged (successes and failures). The WIN-evoked inhibition is greater (than in **C1**), because WIN also increased the number of failures. The figure represents five experiments with a similar outcome. See Szabo et al. (2004) for details of the experiments

tive information processing; in both regions, GABAergic synaptic transmission is inhibited by cannabinoids.

In the above-mentioned experiments, cannabinoids inhibited fast GABAergic transmission by inhibiting GABA release from axon terminals. It is expected that if GABA release is inhibited, then $GABA_B$ receptor-mediated slow inhibitory transmission will be inhibited as well. This was indeed observed in the ventral tegmental area (Riegel et al. 2003).

In addition to GABA, glycine is also involved in fast inhibitory neurotransmission. Activation of CB_1 receptors inhibits both GABAergic and glycinergic synaptic transmission in the medulla oblongata (Jennings et al. 2001; see Table 2).

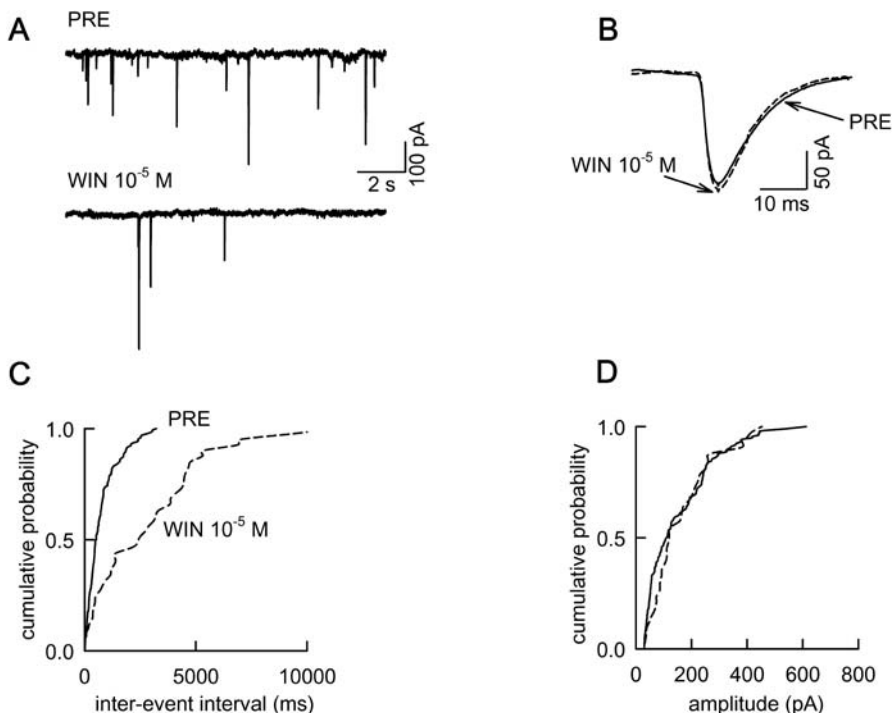


Fig. 3A–D. Cannabinoids inhibit GABAergic synaptic transmission between basket and Purkinje cells in the cerebellar cortex of the rat via a presynaptic mechanism. Miniature inhibitory postsynaptic currents (mIPSCs) were recorded in Purkinje cells in the presence of tetrodotoxin (3×10^{-7} M) during an initial reference period (PRE) and during superfusion with WIN55212-2 (WIN). **A** Original tracings from an experiment with WIN: WIN obviously lowers the frequency of mIPSCs. **B** Averaged mIPSCs from the experiment shown in **A**: WIN does not change the amplitude of mIPSCs. **C**, **D** Cumulative probability distribution plots of inter-event intervals and amplitudes of mIPSCs (same experiment as in **A**): the inhibitory effect of WIN on the frequency of mIPSCs and its lack of effect on the amplitude of mIPSCs is evident. Lack of effect of WIN on the amplitude of mIPSCs indicates that the cannabinoid does not interfere with the effect of GABA on the postsynaptic neuron—this is an indication that WIN inhibited neurotransmission between basket and Purkinje cells (see Fig. 2) via a presynaptic mechanism. Lowering the frequency of mIPSCs by WIN suggests that WIN directly interferes with the vesicle release machinery. The figure represents six experiments with a similar outcome. See Szabo et al. (2004) for details of the experiments

4.3

Neurotransmission via Monoamines and Acetylcholine

A synopsis of the inhibitory effects of cannabinoids on the release of the monoamines noradrenaline, dopamine and serotonin and of acetylcholine in the brain and the retina is given in Table 3. Noradrenaline release is inhibited via CB₁ receptors in the hippocampus of guinea-pig and man but not in the hippocampus of rat and mouse (Table 3, Fig. 4; Van Vliet et al. 2000). Although CB₁ receptors inhibit the release of dopamine from amacrine cells of the retina, contradictory results were obtained with respect to the modulation of dopamine release from

Table 3. Inhibition of the release of monoamines and acetylcholine in the brain and the retina

Neurotransmitter	Species	Region	Method	References
Noradrenaline	Guinea-pig	Cortex, hippocampus, hypothalamus, cerebellum (brain slice)	[³ H]Noradrenaline release	Schlicker et al. 1997
	Man	Hippocampus (brain slice)		
Dopamine	Rat	Caudate-putamen	[³ H]Dopamine release	Cadogan et al. 1997
Dopamine	Rat	Caudate-putamen	NMDA-stimulated [³ H]dopamine release	Kathmann et al. 1999
Dopamine	Guinea-pig	Retina	[³ H]Noradrenaline release	Schlicker et al. 1996
Serotonin	Mouse	Cortex (brain slice)	[³ H]Serotonin release	Nakazi et al. 2000
Acetylcholine	Rat	Hippocampus (brain slice)	[³ H]Acetylcholine release	Gifford and Ashby 1996
		Cortex, hippocampus (synaptosomes)		Gifford et al. 2000
Acetylcholine	Mouse	Cortex, hippocampus (brain slice)	[³ H]Acetylcholine release	Kathmann et al. 2001a
Acetylcholine	Mouse Man	Cortex (brain slice) Cortex (brain slice)	[³ H]Acetylcholine release	Steffens et al. 2003

NMDA, *N*-methyl-D-aspartate

the terminals of the striatonigral axons in the caudate-putamen. Dopamine release was depressed in some studies (Table 3), but not, however, in a study using voltammetry to measure dopamine release (Szabo et al. 1999). Serotonin release was slightly inhibited in the cortex of mice but not affected at all in the cortex of rats (Table 3; Van Vliet et al. 2000). Moreover, cannabinoids inhibit acetylcholine release in the hippocampus and cortex; inhibition also occurs in human cortex (Table 3). However, not all cholinergic neurons are affected by cannabinoids: e.g. acetylcholine release from the cholinergic interneurons of the caudate-putamen is not changed by cannabinoids (Gifford et al. 1997a; Kathmann et al. 2001a).

The papers listed in Table 3 and discussed in the preceding paragraph represent *in vitro* studies, and the question arises whether similar results are also obtained *in vivo*. This was examined in a series of studies on rats subjected to *in vivo* microdialysis; the ligands under study were administered intraperitoneally or intravenously. Cannabinoids indeed decrease acetylcholine release in the dorsal hippocampus (Mishima et al. 2002). In the studies by Tzavara et al. (2001, 2003a), in which cannabinoid agonists were not studied themselves, the CB₁ receptor inverse agonist SR 141716, which elicits effects opposite in direction to those of cannabi-

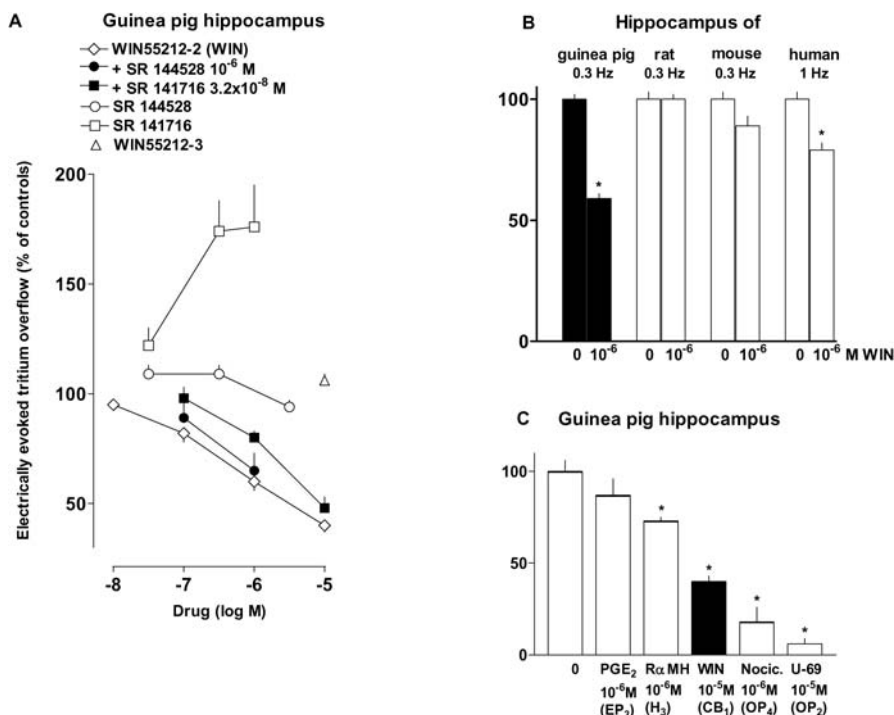


Fig. 4A–C. Cannabinoids inhibit noradrenaline release in the brain. **A** Guinea-pig hippocampal slices were preincubated with [3 H]noradrenaline and superfused. The electrically (0.3 Hz) evoked tritium overflow (which represents quasi-physiological noradrenaline release) was inhibited by WIN55212-2 but not affected by its enantiomer WIN55212-3. The concentration–response curve of WIN55212-2 (WIN) was shifted to the right by a low concentration of the CB₁ receptor antagonist SR 141716 (pA₂ 8.2) but hardly affected by a high concentration of the CB₂ receptor antagonist SR 144528. Given alone, SR 141716 facilitated, whereas SR 144528 did not affect, noradrenaline release. In another series of experiments, not shown here, slices were superfused with K⁺-rich (2.5×10^{-2} M) Ca²⁺-free medium containing tetrodotoxin 10^{-6} M; under this experimental condition WIN inhibited tritium overflow evoked by re-introduction of Ca²⁺ 1.3×10^{-3} M (in a manner sensitive to SR 141716 3.2×10^{-7}), suggesting that the CB₁ receptors are located presynaptically on the noradrenergic axon terminals. **B** WIN inhibited noradrenaline release also in human hippocampus but failed to do so in rat and mouse hippocampus. Although SR 141716 3.2×10^{-7} M counteracted the effect of WIN in human hippocampus, it did not affect noradrenaline release by itself (not shown). (Since noradrenaline release is relatively low in human hippocampus we used a higher stimulation frequency than in hippocampal slices from the three animal species.) **C** In guinea-pig hippocampus, the inhibitory effect of WIN is higher than that of prostaglandin E₂ (PGE₂; acting via prostaglandin EP₃ receptors) and R- α -methylhistamine (R α MH; acting via histamine H₃ receptors), but lower than that of nociceptin (Nocic.; acting via opioid OP₄ receptors) and U-69,593 (U-69; acting via OP₂ receptors). Note that the concentrations of the five agonists cause maximum or near maximum effects at the respective presynaptic inhibitory receptors. *, Significant difference from control ($p < 0.001$). See Schlicker et al. (1997) and Timm et al. (1998) for details of the experiments (some of the data shown here are unpublished)

noid agonists under a variety of conditions (for a more detailed discussion, see Sect. 7), increases noradrenaline release in the prefrontal cortex and anterior hypothalamus, dopamine release in the prefrontal cortex and serotonin release in

the prefrontal cortex and nucleus accumbens. On the other hand, cannabinoids increase rather than decrease striatal dopamine release (Malone and Taylor 1999) and acetylcholine release in the frontal cortex (Verrico et al. 2003). The situation is even more complicated with respect to the effects of cannabinoids on acetylcholine release in the medial prefrontal cortex and hippocampus. Low doses of cannabinoids increase (Acquas et al. 2000, 2001), whereas high doses decrease (Gessa et al. 1998; Carta et al. 1998), the release of this transmitter.

The fact that cannabinoids when given systemically increase rather than decrease transmitter release in various paradigms *in vivo* is in all likelihood not related to the fact that there are also facilitatory cannabinoid receptors. Inhibitory CB₁ receptors occur both on facilitatory and inhibitory neurons of complex neuronal networks and cannabinoids may therefore elicit inhibitory or facilitatory effects on transmitter release, depending on the exact site(s) where they act. Two typical networks in which presynaptic inhibitory CB₁ receptors occur on various sites are depicted in Figs. 6 and 7. The recent study by Tzavara et al. (2003b) shows that the differential effects of cannabinoids on hippocampal acetylcholine release (Gessa et al. 1998; Carta et al. 1998; Acquas et al. 2000, 2001) are due to the fact that the cannabinoids, depending on the dose, act on different pathways, involving dopamine D₁ or D₂ receptors.

5

Effects of Cannabinoids on Neurotransmission in the Peripheral Nervous System

Effects of cannabinoids on the sympathetic nervous system have been studied in isolated tissues and in pithed animals (Table 4). Sympathetic neurons were usually activated by electrical stimulation. Activation of CB₁ receptors led to inhibition of noradrenaline and/or ATP release and, consequently, to inhibition of the effector responses in the heart, in mesenteric and renal blood vessels and in the vas deferens. Figure 5A shows that cannabinoids inhibit sympathetic neuroeffector transmission in the heart. Sympathetically mediated vasoconstriction was inhibited in many tissues of pithed rats and rabbits. Sympathetic tone is depressed during long-term Δ^9 -tetrahydrocannabinol administration in humans; the presynaptic inhibitory effect of cannabinoids on sympathetic axon endings may be the basis of this effect.

Cannabinoids also inhibit transmitter release from cholinergic autonomic neurons (Table 4). As an example, the bradycardia elicited by vagal nerve stimulation is depressed. Figure 5B shows that cannabinoids inhibit parasympathetic neuroeffector transmission in the heart. Electrically evoked contractions of the ileum and urinary bladder can also be inhibited by activation of CB₁ receptors (Table 4).

Finally, cannabinoids inhibit the release of neuropeptides like calcitonin gene-related peptide (CGRP), substance P and somatostatin from sensory neurons (Table 4). Capsaicin or electrical stimulation was used to evoke neuropeptide release. In some of these studies, the endocannabinoid anandamide was used, which has a dual effect on neuropeptide release from sensory neurons. Anandamide possesses an inhibitory effect mediated via CB₁ receptors at low concentrations and

Table 4. Inhibition of neuroeffector transmission in the peripheral nervous system (*continued on next page*)

Neurotransmitter	Species	Tissue	Method	Reference(s)
Noradrenaline	Mouse	Sympathetic neurons (cell culture)	Electrically evoked [3 H]noradrenaline release	Göbel et al. 2000
Noradrenaline	Rat	Vas deferens	Electrically evoked contraction	Christopoulos et al. 2001
Noradrenaline	Rat	Vas deferens, heart atrium	Electrically evoked [3 H]noradrenaline release	Ishac et al. 1996
Noradrenaline, ATP	Mouse	Vas deferens	Electrically evoked contraction	Pertwee et al. 1992, 2002
ATP	Mouse	Vas deferens	Electrically evoked contraction	Lay et al. 2000
Noradrenaline	Mouse	Vas deferens	Electrically evoked [3 H]noradrenaline release	Trendelenburg et al. 2000; Schlicker et al. 2003
Noradrenaline	Rat	Heart	Electrically evoked cardioaccelerator response	Malinowska et al. 2001
Noradrenaline	Rabbit	Heart	Electrically evoked cardioaccelerator response	Szabo et al. 2001
Acetylcholine			Bradycardia evoked by electrical stimulation of the vagus nerve	
Noradrenaline	Man	Heart atrial appendages	Electrically evoked [3 H]noradrenaline release	Molderings et al. 1999
Noradrenaline	Rat	Sympathetically innervated blood vessels of many organs	Electrically evoked increase in blood pressure in pithed rats	Malinowska et al. 1997
Noradrenaline	Rat	Sympathetically innervated tissues of many organs	Electrically evoked increase in blood pressure and plasma noradrenaline in pithed rats	Niederhoffer et al. 2003
Noradrenaline	Rabbit	Sympathetically innervated tissues of many organs	Electrically evoked increase in blood pressure and plasma noradrenaline in pithed rabbits	Niederhoffer and Szabo 1999
Noradrenaline	Rat	Mesenterial vessels	Electrically evoked noradrenaline release	Ralevic and Kendall 2002
Noradrenaline	Rat	Renal arteries	K $^{+}$ -evoked [3 H]noradrenaline release	Deutsch et al. 1997
Noradrenaline	Guinea-pig	Lung (bronchi)	Electrically evoked [3 H]noradrenaline release	Vizi et al. 2001
Adrenaline	Rabbit	Adrenal medulla	Electrically evoked increase in plasma adrenaline in pithed rabbits	Niederhoffer et al. 2001
			Electrically evoked adrenaline release in isolated adrenal medullary slices	

Table 4. (*continued*)

Neurotransmitter	Species	Tissue	Method	Reference(s)
Acetylcholine, ATP	Mouse	Urinary bladder	Electrically evoked contraction	Pertwee and Fernando 1996
Acetylcholine, NANC-transmitter	Mouse	Colon	Electrically evoked cholinergic and NANC postsynaptic potentials	Storr et al. 2004
Acetylcholine	Guinea-pig	Ileum	Electrically evoked contraction, acetylcholine release, cholinergic postsynaptic potentials	Pertwee et al. 1992, 1996b; Lopez-Redondo et al. 1997; Mang et al. 2001
Acetylcholine, NANC-transmitter	Guinea-pig	Ileum	Electrically evoked contraction	Izzo et al. 1998
Acetylcholine	Man	Ileum	Electrically evoked contraction	Croci et al. 1998
Adenosine	Guinea-pig	Ileum	Electrically evoked adenosine release	Begg et al. 2002
CGRP	Rat	Primary sensory neurons (cell culture)	Basal and capsaicin-evoked CGRP release	Ahluwalia et al. 2003
CGRP, substance P, somatostatin	Rat	Trachea	Capsaicin-evoked CGRP, substance P and somatostatin release	Nemeth et al. 2003
CGRP	Rat	Hind paw skin	Capsaicin-evoked CGRP release	Richardson et al. 1998; Ellington et al. 2002
CGRP	Rat	Spinal cord slices	Electrically evoked CGRP release	Tognetto et al. 2001

CGRP, calcitonin gene-related peptide; NANC, non-adrenergic-non-cholinergic

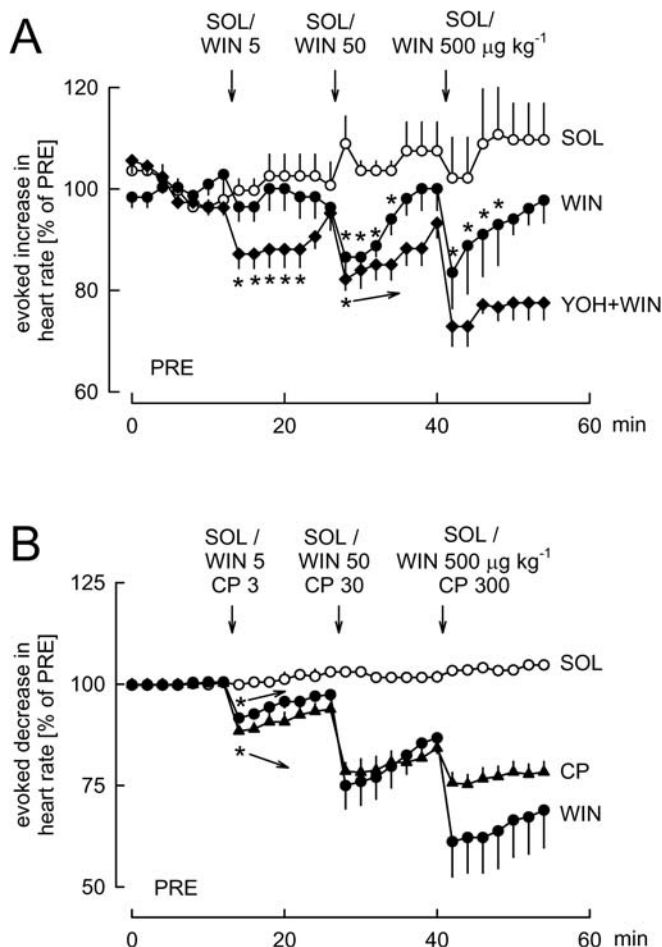


Fig. 5A, B. Cannabinoids inhibit sympathetic and parasympathetic neuroeffector transmission in the heart. **A** Cardiac sympathetic nerves in pithed rabbits were stimulated at a frequency of 1 Hz for 30 s. Solvent (SOL) and WIN55212-2 (WIN) were administered i.v. as indicated by the arrows. One of the WIN groups (YOH+WIN) was pretreated with the α_2 -adrenoceptor antagonist yohimbine (0.5 mg/kg^{-1} ; i.v.) at $t = -14 \text{ min}$. Cardioaccelerator responses are given as percentages of the initial reference value (PRE). WIN inhibited the cardioaccelerator response more strongly in the presence of YOH, probably because YOH prevented concurrent inhibition by endogenous noradrenaline. **B** The right vagus nerve was stimulated at a frequency of 10 Hz for 5 s. SOL, WIN and CP55940 (CP) were administered i.v. as indicated by the arrows. Cardiodecelerator responses are given as percentages of the initial reference value PRE. *, Significant difference from SOL ($p < 0.05$). See Szabo et al. (2001) for details of the experiments

a stimulatory effect mediated via vanilloid receptors (TRPV1, transient receptor potential V1 channel) at high concentrations (Zygmunt et al. 1999; Tognetto et al. 2001; Ahluwalia et al. 2003; Nemeth et al. 2003).

The effect of cannabinoids on peripheral autonomic transmission has been extensively reviewed by Ralevic (2003).

6

Mechanism of the Presynaptic Inhibition

Information regarding the mechanism of presynaptic inhibition is included in Tables 1 and 2. As expected for a $G\alpha_{i/o}$ protein-coupled receptor, the presynaptic inhibition mediated by the CB₁ receptor was sensitive to pertussis toxin in the few cases where this interaction was studied. Moreover, in isolated hippocampal neurons, presynaptic inhibition of excitatory neurotransmission elicited by CB₁ receptor activation could be mediated by several subtypes of $G\alpha_{i/o}$ proteins: $G\alpha_{o1}$, $G\alpha_{i2}$ and $G\alpha_{i3}$ (Straiker et al. 2002). Information on the involvement of second messengers in the presynaptic inhibition by cannabinoids is sparse. For example, the role of $G\beta\gamma$ proteins is not known. Data on the role of the cyclic adenosine monophosphate (cAMP)–protein kinase A messenger system are contradictory (see Tables 1 and 2). After activation of $G\alpha_{i/o}$ protein-coupled receptors, several final mechanisms may lead to inhibition of transmitter release (for review see Thompson et al. 1993; Wu and Saggau 1997; see Fig. 8). Most often, presynaptic inhibition is attributed to inhibition of voltage-dependent calcium channels. In addition, activation of potassium channels and direct interference with the vesicle release machinery can also play a role in presynaptic inhibition. It seems that cannabinoids can use all three mechanisms for producing presynaptic depression (see Tables 1 and 2). Since it is extremely difficult to obtain electrophysiological access to mammalian axon terminals, direct evidence for cannabinoid-evoked modulation of axon terminal ion channels is lacking. Therefore, most of the evidence regarding the mechanism of cannabinoid-evoked presynaptic inhibition is indirect.

6.1

Inhibition of Calcium Channels

As mentioned above, cannabinoids inhibit voltage-dependent calcium channels in somadendritic regions of neurons (see Sect. 2.1.1). It is assumed that such an inhibition also operates in axon terminals and is responsible for presynaptic inhibition. Using microfluorometric methods, it was indeed shown that the action potential-evoked increase in axon terminal calcium concentration is depressed by exogenous and endogenous cannabinoids (Kreitzer and Regehr 2001; Diana et al. 2002; Daniel and Crepel 2001; Brown et al. 2003a; Diana and Marty 2003). Based on the interaction between cannabinoids and calcium channel blockers, Sullivan (1999) concluded that calcium channel inhibition is responsible for the cannabinoid-evoked depression of synaptic transmission.

6.2

Activation of Potassium Channels

As mentioned above, cannabinoids activate several types of potassium channels in the somadendritic region of neurons (see Sect. 2.1.2). Cannabinoid-evoked open-

ing of potassium channels will hyperpolarise axon terminals and shorten action potentials. As a consequence, invasion of axon terminals by action potentials and the activation of calcium channels can be impeded. The duration of calcium influx during the action potential may also decrease. Evidence for the involvement of potassium channels in presynaptic inhibition was obtained by using potassium channel blockers. Thus, potassium channel blockers prevented cannabinoid-evoked presynaptic inhibition (Daniel and Crepel 2001; Robbe et al. 2001; Diana and Marty 2003; Azad et al. 2003) and cannabinoid-evoked inhibition of the action potential-triggered increase in axon terminal calcium concentration (Daniel and Crepel 2001). In contrast, since potassium channel blockers did not affect cannabinoid-evoked presynaptic inhibition, Hoffman and Lupica (2000) excluded a role of potassium channels in presynaptic inhibition.

6.3

Direct Inhibition of the Vesicle Release Machinery

In most nerve terminals, spontaneous and asynchronous quantal transmitter release occurs also in the absence of calcium influx through voltage-dependent calcium channels. Such release events are recorded in electrophysiological experiments either in the presence of tetrodotoxin or calcium channel blockers. The recorded postsynaptic events are called miniature excitatory or inhibitory postsynaptic currents (mEPSCs or mIPSCs). There are many examples for the lowering of the frequency of mEPSCs and mIPSCs by cannabinoids (Tables 1 and 2), including GABAergic synaptic transmission between basket and Purkinje cells in the rat cerebellar cortex (Fig. 3). These observations indicate that cannabinoids are capable of inhibiting neurotransmitter release at a site downward of calcium entry into axon terminals, most probably at the level of the vesicular release machinery. However, it is also clear from Tables 1 and 2 that at many synapses cannabinoids produce presynaptic inhibition without directly interfering with vesicular release.

In conclusion, there are examples for presynaptic inhibition by all three mechanisms: inhibition of voltage-dependent calcium channels, activation of potassium channels and inhibition of the vesicle-release machinery. The inhibitory mechanisms vary in different types of axon terminals. One axon terminal can possess several inhibitory mechanisms (for example, calcium channels and vesicle release can be inhibited simultaneously).

7

Endogenous Tone at Presynaptic Cannabinoid Receptors

There is now increasing evidence that cannabinoid receptors involved in the inhibition of neuroeffector transmission are subject to an endogenous tone (Table 5). A typical example is the presynaptic CB₁ receptors on GABAergic neurons synapsing with the pyramidal neurons in the rat hippocampus (Wilson and Nicoll 2001). Depolarisation of the latter neurons causes an increase in formation of endo-

Table 5. Endogenous tone at CB₁ receptors inhibiting neuroeffector transmission (*continued on next page*)

Neurotransmitter	Species	Tissue	Method	Identification of endogenous tone ¹	Reference(s)
Glutamate	Rat	Cortex	Electrophysiology (patch clamp)	Inverse agonist (SR 141716)	Audair et al. 2000
Glutamate	Rat	Caudate-putamen	Electrophysiology (patch clamp)	Inverse agonist (AM 281)	Huang et al. 2001
Glutamate	Rat	Hippocampus	Electrophysiology (extracellular recording)	Inverse agonist (SR 141716)	Ameri et al. 1999
GABA	Rat	Hippocampus	Electrophysiology (patch clamp)	Inhibitor of endocannabinoid reuptake (AM 404)	Wilson and Nicoll 2001
Noradrenaline	Guinea-pig	Hippocampus	[³ H]Noradrenaline release	Inverse agonists (SR 141716, AM 251, AM 281)	Schlicker et al. 1997, 2002
Noradrenaline	Rat	Vas deferens	Contraction	Inverse agonists (SR 141716, LY 320135), inhibitor of endocannabinoid degradation (phenylmethylsulfonyl fluoride, PMSF)	Christopoulos et al. 2001
Noradrenaline, ATP	Mouse	Vas deferens	Contraction	Inverse agonist (SR 141716)	Pertwee et al. 1996a
Noradrenaline	Mouse	Vas deferens	[³ H]Noradrenaline release	Inverse agonist (SR 141716), knockout mouse generated by Zimmer et al. 1999	Schlicker et al. 2003
Dopamine	Guinea-pig	Retina	[³ H]Dopamine release	Inverse agonist (SR 141716)	Schlicker et al. 1996
Acetylcholine	Man	Cortex	[³ H]Acetylcholine release	Inverse agonist (SR 141716), partial agonist (O-1184), inhibitor of endocannabinoid reuptake (AM 404)	Steffens et al. 2003
Acetylcholine	Rat	Hippocampus	[³ H]Acetylcholine release	Inverse agonists (SR 141716, AM 281)	Gifford and Ashby 1996; Gifford et al. 1997b, 2000
Acetylcholine	Mouse	Hippocampus	[³ H]Acetylcholine release	Inverse agonist (SR 141716), knockout mouse generated by Zimmer et al. 1999	Kathmann et al. 2001a,b
Acetylcholine	Guinea-pig	Hippocampus	[³ H]Acetylcholine release	Inverse agonist (SR 141716)	Schultheiß et al. 2004
Acetylcholine, ATP	Mouse	Urinary bladder	Contraction	Inverse agonist (SR 141716)	Pertwee and Fernando 1996

Table 5. (*continued*)

Neurotransmitter	Species	Tissue	Method	Identification of endogenous tone ¹	Reference(s)
Acetylcholine	Mouse	Colon	Electrophysiology (intracellular recording)	Inverse agonist (SR 141716), knockout mouse generated by Marsicano et al. 2002	Storr et al. 2004
Acetylcholine, substance P	Guinea-pig	Small intestine	Contraction	Inverse agonist (SR 141716)	Pertwee et al. 1996b; Coutts and Pertwee 1997; Izzo et al. 1998
Acetylcholine	Guinea-pig	Ileum	[³ H]Acetylcholine release	Inverse agonist (SR 141716)	Mang et al. 2001
Substance P	Mouse	Spinal cord	Substance P release	Inverse agonist (SR 141716)	Lever and Malcangio 2002

¹Inverse and partial agonists and CB₁ receptor disruption increase, whereas inhibition of endocannabinoid reuptake or degradation decreases, transmitter release.

cannabinoids, which in turn activate the presynaptic inhibitory CB₁ receptors on the GABAergic neurons (Fig. 8; see also the chapter by Vaughan and Christie, this volume). The inhibitory effect is mimicked by a blocker of endocannabinoid reuptake, i.e. AM 404 (in a manner sensitive to the CB₁ receptor inverse agonist SR 141716), suggesting that endocannabinoids are accumulating. This has also been shown in some other paradigms (Table 5) and even in human tissue (Steffens et al. 2003). The same conclusion was reached from experiments in which a blocker of the degradation of the endocannabinoids, i.e. phenylmethylsulfonyl fluoride (PMSF), mimicked the inhibitory effect of the endocannabinoids (Table 5). The third approach was the use of a partial CB₁ receptor agonist, O-1184, which led to an increase in transmitter release, probably by interrupting the inhibition caused by accumulating endocannabinoids (Steffens et al. 2003).

In many studies, SR 141716 or other antagonists/inverse agonists increased transmitter release (Fig. 4; Table 5). Although the reason for their facilitatory effect might be the same as in the case of O-1184, an entirely different explanation has to be considered as well. Thus, presynaptic CB₁ receptors may be constitutively active, i.e. inhibit transmitter release even if they are not activated by endocannabinoids, and in this case inverse agonists would be expected to increase transmitter release as well. Constitutive activity frequently occurs with G protein-coupled receptors expressed in high densities (Seifert and Wenzel-Seifert 2002) and CB₁ receptors are expressed in relatively high densities when compared to other G protein-coupled receptors (Wilson and Nicoll 2002). In at least one of the paradigms shown in Table 5, constitutive activity seems to be the only possible explanation. Thus, SR 141716 increased the Ca²⁺-induced [³H]acetylcholine release in rat hippocampal synaptosomes (Gifford et al. 2000). In synaptosomes as opposed to isolated tissues (used in most of the other studies shown in Table 5), accumulation of endogenously released ligands cannot occur, since the latter are efficiently removed by the superfusion stream (Starke et al. 1989). For further clarification, neutral CB₁ receptor antagonists (which have become available only recently; Hurst et al. 2002; Ruijter et al. 2003) will be useful, since they are expected to facilitate transmitter release if endocannabinoids are accumulating but should be without effect if CB₁ receptors are constitutively active.

The facilitatory effect of inverse agonists on transmitter release was mimicked in some paradigms by the disruption of CB₁ receptors, i.e. transmitter release was higher in tissues from CB₁ receptor-deficient mice when compared to wild-type animals (Table 5). This experimental approach does not allow one to reach a conclusion as to whether the endogenous tone is related to accumulation of endocannabinoids or constitutively active CB₁ receptors; yet it is remarkable that blockade of, or inverse agonism at, CB₁ receptors during the course of the experiment and complete lack of CB₁ receptors have the same consequence.

The fact that presynaptic CB₁ receptors at many sites are activated by endogenous compounds lends further support to the view that the cannabinoid system plays an important regulatory role. It has also great practical relevance since CB₁ receptor antagonists/inverse agonists may be used for therapeutic purposes (for further discussion, see the chapter by Robson, this volume).

8

What Is the Functional Role of Presynaptic Cannabinoid Receptors?

It is evident from Sects. 4 and 5 that presynaptic CB₁ receptors are ubiquitous in the central and peripheral nervous system. Even within one functional system, several components of the neuronal circuitry are equipped with CB₁ receptors. This will be illustrated in two functional systems: the extrapyramidal motor control system (Fig. 6) and the cerebellum (Fig. 7).

Figure 6 shows the most important glutamatergic, GABAergic and dopaminergic neuronal connections within the extrapyramidal motor control system. Glutamatergic and GABAergic neurotransmission is inhibited at several sites by cannabinoids. In contrast, dopaminergic transmission may not be influenced. A typical motor effect of high doses of cannabinoids is catalepsy (Compton et al. 1996; Sanudo-Pena et al. 1999). Catalepsy is thought to occur if the GABAergic neurons in the output nucleus of the basal ganglia, the substantia nigra pars reticulata, are firing at a high rate (Kolasiewicz et al. 1988). Among the 11 sites where cannabinoids can act presynaptically, an action at 5 sites would indirectly enhance the firing rate of substantia nigra pars reticulata neurons, and thus would lead to

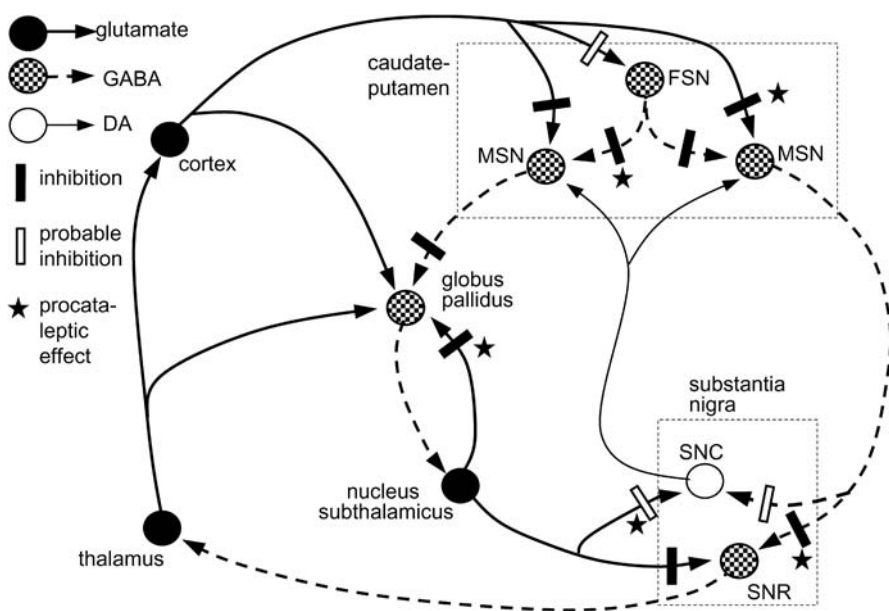


Fig. 6. Effects of cannabinoids on synaptic transmission in nuclei belonging to the extrapyramidal motor control system. DA, dopamine; FSN, fast spiking neuron; MSN, medium spiny neuron; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata. CB₁ receptor-mediated inhibition of neurotransmission was demonstrated at many synapses of this motor control system. In addition to the proved sites of inhibition, inhibition is very probable at additional sites (based on the localisation of the CB₁ receptor). For the sake of simplicity, the pathway including the entopeduncular nucleus (globus pallidus medialis/internus) is not shown

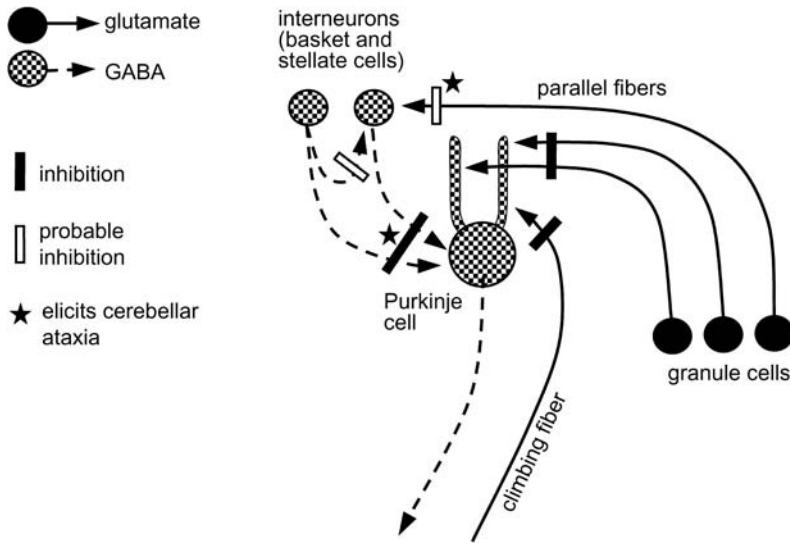


Fig. 7. Effects of cannabinoids on synaptic transmission in the cerebellar cortex. CB₁ receptor-mediated inhibition of neurotransmission was demonstrated at several synapses in the cerebellar cortex. In addition to the proven sites of inhibition, inhibition is very probable at additional sites (based on the localisation of the CB₁ receptor). In addition to synaptic inhibition, activation of CB₁ receptors can also directly decrease the firing rate of interneurons (not shown)

catalepsy. Action at the remaining sites would lead to the opposite effect, i.e. the firing rate of substantia nigra pars reticulata neurons would decrease, which would be an “anticataleptic” effect. In vivo, the balance of all effects obviously favours catalepsy.

Figure 7 shows neuronal connections in the cortex of the cerebellum and the action of cannabinoids on these connections. Activation of CB₁ receptors inhibits glutamatergic as well as GABAergic neurotransmission at altogether five sites. Cannabinoids cause static and gait ataxia, and this is attributed to cerebellar dysfunction (Fränkel 1903; Patel and Hillard 2001). It is thought that the firing rate of Purkinje cells is increased during cerebellar ataxia. Two of the presynaptic cannabinoid effects shown in Fig. 7 would indirectly enhance the firing rate of Purkinje cells; these effects could be the primary events behind cerebellar ataxia. As in the extrapyramidal motor control system, however, inhibitory CB₁ presynaptic receptors are also localised on neurons that play opposite roles in the function of the cortex of the cerebellum.

Further examples for the simultaneous inhibitory effects of cannabinoids on antagonistic components of functional systems can be easily found. For example, cannabinoids inhibit the glutamatergic as well as the GABAergic input of ventral tegmental area dopaminergic neurons (Szabo et al. 2002; Melis et al. 2004) and the sympathetic as well as the parasympathetic input of the heart (Szabo et al. 2001).

What is the physiological role of CB₁ receptors—receptors that are so widely distributed and that simultaneously influence antagonistic components of a given

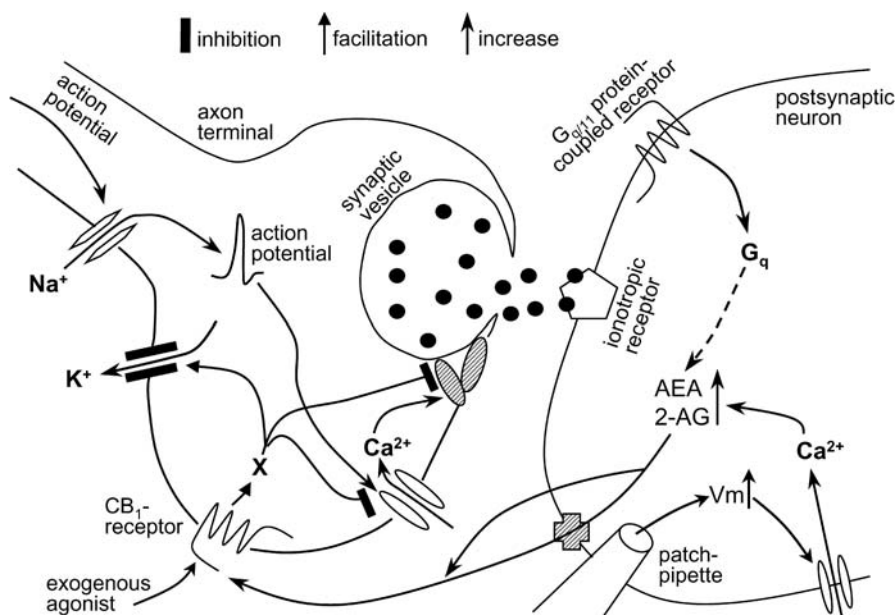


Fig. 8. Effects of cannabinoids on synaptic transmission. Activation of the CB₁ receptor at the presynaptic axon terminal inhibits transmitter release from the synaptic vesicle. Three mechanisms can be involved in presynaptic inhibition (X refers to unknown second messengers): inhibition of voltage-dependent calcium channels, activation of potassium channels and direct interference with the vesicle release machinery. The CB₁ receptor can be activated by exogenous agonists, but also by the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which are released from the postsynaptic neuron by passive and/or facilitated diffusion. The synthesis of endocannabinoids is triggered by a depolarisation-induced (V_m, membrane potential) calcium influx or by activation of G_{q/11} protein-coupled receptors

functional system? One functional role of CB₁ receptors is their participation in retrograde signalling, at least with respect to fast excitatory and inhibitory transmission. Endogenous cannabinoids released from postsynaptic neurons can diffuse to presynaptic axon terminals where they produce presynaptic inhibition (Ohno-Shosaku et al. 2001; Wilson and Nicoll 2001). The trigger for synthesis of endocannabinoids is depolarisation of postsynaptic neurons or activation of G_{q/11} protein-coupled receptors of postsynaptic neurons (see Fig. 8). This phenomenon is called depolarisation-induced suppression of inhibition (DSI; if inhibitory neurotransmission is suppressed by endocannabinoids) or depolarisation-induced suppression of excitation (DSE; if excitatory neurotransmission is suppressed by endocannabinoids). This new research field was reviewed by Wilson and Nicoll (2002) and Freund et al. (2003) and is also reviewed in the chapter by Vaughan and Christie (this volume).

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