# Chapter 10
# Chronic Effects of Cannabinoid Drugs on Monoaminergic Systems and the Role of Endocannabinoids and Cannabinoid Receptors in Human Brain Disorders

**Leyre Urigüen, Susana Esteban, Luís F. Callado, María Álvaro-Bartolomé, J. Javier Meana and Jesús A. García-Sevilla**

**Abstracts** The endocannabinoid system and cannabinoid (CB) receptors participate in the regulation of a variety of psychiatric and neurological disorders through a functional coupling with the monoaminergic systems in the brain. Norepinephrine, serotonin (5-HT) and dopamine systems are modulated via inhibitory $CB_1$ receptors by direct or indirect effects. The repeated stimulation of $CB_1$ receptors (and receptor desensitization) can lead to the induction of tolerance on the activity of monoaminergic systems. The chronic administration of CB drugs can also alter the function of presynaptic inhibitory monoamine autoreceptors and heteroreceptors and thus modulate the final effects on these systems. The functional interactions between endocannabinoids, CB receptors, and monoaminergic systems suggest a potential role for CB receptor signaling in the pathophysiology and treatment of various psychiatric and neurological disorders, including drug addiction, which are discussed on evidence from postmortem and living human brain studies.

**Abbreviations**

| | |
|---|---|
| AEA | Anandamide |
| Am | Basolateral amygdala |
| 2-AG | 2-Arachidonoylglycerol |
| CB | Cannabinoid |

J. A. García-Sevilla (✉) · S. Esteban · M. Álvaro-Bartolomé
Laboratori de Neurofarmacologia, Institut Universitari d'Investigació en Ciències de la Salut (IUNICS), Universitat de les Illes Balears (UIB), and Redes Temáticas de Investigación Cooperativa en Salud–Red de Trastornos Adictivos (RETICS-RTA), Palma de Mallorca, Spain
e-mail: jesus.garcia-sevilla@uib.es

L. Urigüen · L. F. Callado · J. J. Meana
Departamento de Farmacología e Instituto Biocruces, Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU), Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Leioa, Bizkaia, Spain

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| | |
|---|---|
| CC | Cerebral cortex |
| CNS | Central nervous system |
| CP55940 | (-)-Cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl) cyclohexanol |
| CP93129 | 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3, 2-b]pyrid-5-one |
| DA | Dopamine |
| DOPA | 3,4-Dihydroxy-phenylalanine |
| DPAT | (±)-8-Hydroxy-2-(di-n-propylamino)-tetralin |
| DR | Dorsal raphe |
| FAAH | Fatty acid amide hydrolase |
| GABA | γ-Aminobutyric acid |
| GLU | Glutamate or glutamic acid |
| GTPγS | Guanosine triphosphate |
| HC | Hippocampus |
| HT | Hypothalamus |
| 5-HT | 5-Hydroxytryptamine or serotonin |
| 5-HTP | 5-Hydroxy-tryptophan |
| HU210 | (6aR)-Trans-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b, d]pyran-9-methanol |
| LC | Locus coeruleus |
| LH | Lateral habenula |
| NAcc | Nucleus accumbens |
| NAE | *N*-Acylethanolamines |
| NE | Norepinephrine |
| OEA | *N*-Oleoylethanolamine |
| PEA | *N*-Palmitoylethanolamine |
| PrH | Prepositus hypoglossal nucleus |
| SD7015 | 1-(2-Iodophenyl)-4-cyano-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxylate |
| SN | Substantia nigra |
| SR141617A | Rimonabant |
| St | Corpus striatum |
| TH | Tyrosine hydroxylase |
| THC | $\Delta^9$-Tetrahydrocannabinol |
| TPH | Tryptophan hydroxylase |
| URB597 | Cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester |
| VTA | Ventral tegmental area |
| WIN55212-2 | R-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]pyrrolol-[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone. |

## 10.1 Introduction

The endocannabinoids (e.g., anandamide (AEA), 2-arachidonoylglycerol (2-AG)) function in the brain as retrograde lipid signaling messengers (Vaughan and Christie 2005; Mechoulam and Parker 2013) which, similarly to cannabinoid (CB) drugs,

mediate their effects through the activation of two inhibitory G protein-coupled receptors termed $CB_1$ and $CB_2$ receptors (Howlett et al. 2002; Pertwee et al. 2010). The predominant $CB_1$ receptor, highly expressed in the central nervous system (CNS), is mainly located on inhibitory γ-aminobutyric acid (GABA) and excitatory (e.g., glutamate) synapses where it regulates the release of the corresponding transmitter (Katona et al. 1999; Schlicker and Kathmann 2001; Hashimotodani et al. 2007). Moreover, numerous nuclei and axon terminals in a variety of brain regions also express $CB_1$ receptors whose function is to inhibit the release of excitatory and inhibitory neurotransmitters (Alger 2002). The brain regions enriched in $CB_1$ receptors include the locus coeruleus/norepinephrine (LC/NE) neurons and axon NE terminals (Oropeza et al. 2007; Carvalho et al. 2010; Scavone et al. 2010) and the dorsal raphe/serotonin (DR/5-HT) neurons and 5-HT terminal fields (Hohmann and Herkenham 2000; Häring et al. 2007). $CB_1$ receptors are also abundant in limbic mood-regulatory dopamine (DA) rich areas (brain reward circuitry) including the ventral tegmental area (VTA), nucleus accumbens (NAcc), and corpus striatum (Herkenham et al. 1991). $CB_1$ receptors, however, are not located on VTA/DA neurons (Matsuda et al. 1993) but rather on presynaptic glutamatergic and GABAergic neurons in the VTA. The anatomical localizations of $CB_1$ receptors indicate that the direct or indirect stimulation/blockade of these inhibitory receptors can result in the fine modulation of the activity of monoaminergic systems in specific brain regions. $CB_1$ receptors display a high level of constitutive activity (Gifford and Ashby 1996), which can exert a tonic control (i.e. ligand-independent activity) on its endocytic cycle (Leterrier et al. 2004) as well as on the function of other receptors (Canals and Milligan 2008). The $CB_1$ receptor basal tone, however, might also be related to the ongoing production of endocannabinoids (AEA and 2-AG) which would stimulate CB receptors given the appearance of constitutive activity (Howlett et al. 2011). In the CNS, the less abundantly expressed and less well understood $CB_2$ receptor is mainly associated with the regulation of neuroinflammatory processes (microglia and immune responses) which can be of importance in the pathogenesis of some psychiatric and neurological diseases (Atwood et al. 2012; Onaivi et al. 2012).

The endocannabinoid system and $CB_1$ receptors participate, in part, in the control of emotional behavior and mood through a functional coupling with monoaminergic systems in the brain (Bambico et al. 2007; Ashton and Moore 2011). These functional interactions have suggested a potential role for $CB_1$ receptor signaling in the neurobiology of various psychiatric disorders (Hill and Gorzalka 2005a, 2005b; Parolaro et al. 2010; Carvalho and Van Bockstaele 2012; Esteban and García-Sevilla 2012). This chapter summarizes and discusses the chronic effects of CB drugs modulating brain monoamine systems (spontaneous neuronal activity, synthesis and release of neurotransmitters) as well as the activity of presynaptic monoaminergic receptors (autoreceptors and heteroreceptors) that regulate the synthesis and release of classic neurotransmitters. The chapter also deals with the possible relevance of the endocannabinoid system and CB receptors in the pathophysiology and treatment of several psychiatric and neurological disorders, including drug addiction, with a special focus on evidence from postmortem and living human brain studies.

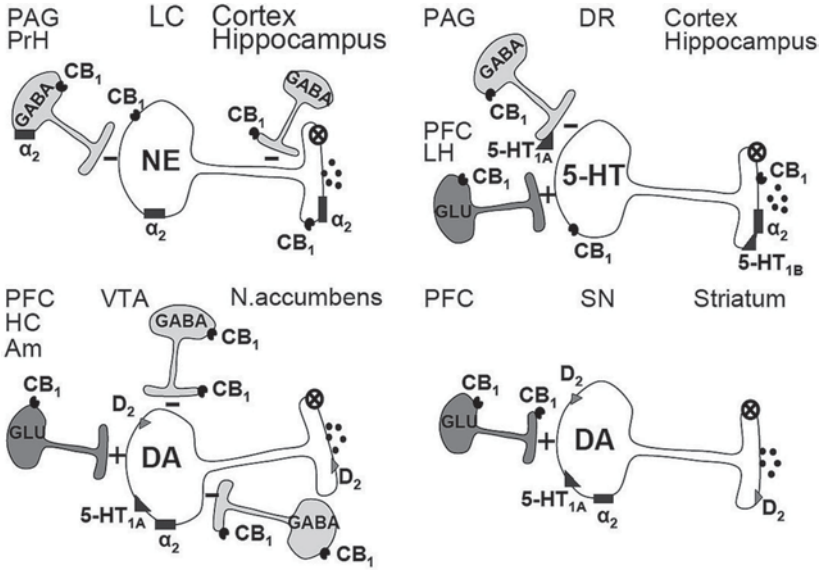


**Fig. 10.1** Neuronal structures and neurotransmitters involved in effects of cannabinoid drugs acting at $CB_1$ receptors on locus coeruleus/norepinephrine (LC/NE) neurons, dorsal raphe/serotonin (DR/5-HT) neurons, ventral tegmental area/dopamine (VTA/DA neurons, and substantia nigra/dopamine (SN/DA) neurons. The most important projections to the LC are GABA local interneurons and GABA afferents from the periaqueductal gray matter (PAG) and the prepositus hypoglossal nucleus (PrH). The relevant neurotransmitter systems that project to the DR are GABA afferents from PAG, and glutamate (GLU) afferents from the medial prefrontal cortex (PFC), and possibly the lateral habenula (LH). The most important projections to the SN are glutamate (GLU) afferents from the medial prefrontal cortex (PFC). The relevant neurotransmitter systems that project to the VTA are glutamatergic (GLU) afferents from the PFC, hippocampus (HC), and basolateral amygdala (Am), as well as GABA inputs from the nucleus accumbens (NAcc) and local GABA interneurons. $\alpha_2$: inhibitory $\alpha_2$-adrenoceptor (somatodendritic and terminal NE autoreceptor and heteroreceptor on 5-HT terminals); $5\text{-HT}_{1A}$: inhibitory somatodendritic autoreceptor; $5\text{-HT}_{1B}$: inhibitory terminal autoreceptor; $D_2$: inhibitory somatodendritic and terminal DA autoreceptor. See the main text for specific comments on the chronic effects and interactions of $CB_1$ drugs regulating monoaminergic systems, including the modulatory role of presynaptic monoaminergic receptors (autoreceptors and heteroreceptors). (Modified from Esteban and García-Sevilla 2012)

## 10.2 Chronic Effects of Cannabinoid Drugs on Brain Monoaminergic Systems. Induction of Tolerance to the Acute Effects of $CB_1$ Agonists

Cannabinoid (CB) drugs modify the functioning of monoaminergic systems via inhibitory $CB_1$ receptors by direct or indirect effects, which depend on receptor localization on monoaminergic neurons themselves and/or inhibitory (GABAergic) and/or excitatory (glutamatergic) regulatory neurons (Fig. 10.1) The acute stimulatory/inhibitory effects of CB drugs on monoaminergic systems have recently been discussed (Esteban and García-Sevilla 2012). In addition, several studies have

investigated the chronic effects of CB drugs on brain monoaminergic systems, and some of them have also assessed the possible induction of tachyphylaxis (neurochemical tolerance) to the acute effects of $CB_1$ receptor agonists (Esteban and García-Sevilla 2012). The long-term regulation of monoaminergic systems by CB drugs can be of importance in the context of the beneficial and deleterious effects of these drugs.

### *10.2.1 Noradrenergic System*

Chronic treatment with URB597 (4 days), a fatty acid amide hydrolase (FAAH) inhibitor, and WIN55,212-2 (8 days), a preferential $CB_1$ receptor agonist, have been shown to markedly increase the spontaneous firing rate of NE neurons and the expression of tyrosine hydroxylase (TH) in the rat LC (Table 10.1). A longer chronic WIN55,212-2 treatment (20 days) in rats was reported not to alter the firing rate of LC neurons (Table 10.1). Notably, repeated treatment with URB597 (resulting in an increased content of AEA) was not associated with the induction of tolerance to its acute enhancing effect on LC/NE neurons (Table 10.1). Chronic WIN55212-2 (5 days) was also shown to increase the synthesis of DOPA/NE in the hippocampus and cerebellum (lack of tolerance) but not in the cerebral cortex (induction of tolerance) of rats (Table 10.1 and Fig. 10.2). Chronic WIN55,212-2 (8 days) also induced an increase in the release of NE in rat brain cortex with a concomitant upregulation of TH in the LC (Table 10.1).

### *10.2.2 Serotonergic System*

Chronic URB597 (4 days) also induced marked increases in the spontaneous firing rate of rat DR/5-HT neurons (Table 10.1; lack of tolerance). The repeated application (three times) of low and high doses of WIN55,121-2 induced biphasic effects on the firing rate (increases and decreases) of rat DR 5-HT neurons (Table 10.1; apparent lack of tolerance). A prolonged WIN55,212-2 treatment in rats (20 days) did not result in alterations of the basal firing rate of DR neurons (Table 10.1), which could indicate the induction of some degree of tolerance to the acute effect of the agonist. Chronic WIN55,121-2 (5 days) in rats did not significantly alter the synthesis of 5-HTP in the cerebral cortex, hippocampus, and cerebellum (Table 10.1; induction of tolerance) (Table 10.1 and Fig. 10.2). In contrast, chronic WIN55,121-2 (5 days), similarly to the acute agonist treatment, also reduced 5-HTP synthesis in rat striatum (Table 10.1; lack of tolerance) (Table 10.1 and Fig. 10.2).

### *10.2.3 Dopaminergic System*

Chronic $\Delta^9$-tetrahydrocannabinol (THC) treatment (14 days) in rats was also reported to enhance the spontaneous firing rate of SN/DA and VTA/DA neu-

**Table 10.1** Effects of chronic treatment with cannabinoid drugs on monoaminergic systems in adult rat brain regions

| Cannabinoid drug (dose and duration of treatment) | Brain region and net effect (% basal change) | Induction of tolerance | Reference |
|---|---|---|---|
| *Norepinephrine system* | | | |
| URB597 (0.1 mg/kg, 4 days) | LC, ↑ firing rate (~50%) | − | Gobbi et al. (2005) |
| WIN55,212-2 (3 mg/kg, 8 days) | LC, ↑ TH expression (125%) | NT | Page et al. (2007) |
| WIN55,212-2 (1 mg/kg, 20 days) | LC, ≈firing rate | NT | Bambico et al. (2010) |
| WIN55,212-2 (4–16 mg/kg, 5 days) | HC/CB ↑ DOPA synthesis (30–41%) | − | Moranta et al. (2009) |
| WIN55,212-2 (4–16 mg/kg, 5 days) | CC, ≈DOPA synthesis | + | Moranta et al. (2009) |
| WIN55,212-2 (3 mg/kg, 8 days) | CC, ↑ NE release (40%) | NT | Page et al. (2007) |
| *Serotonergic system* | | | |
| URB597 (0.1 mg/kg, 4 days) | DR, ↑ firing rate (138%) | − | Gobbi et al. (2005) |
| WIN55,212-2 (0.1–0.2 mg/kg, 3 times) | DR, ↑ firing rate (65–126%) | − | Bambico et al. (2007) |
| WIN55,212-2 (2 mg/kg, 3 times) | DR, ↓ firing rate (64%) | − | Bambico et al. (2007) |
| WIN55,212-2 (0.2–1 mg/kg, 20 days) | DR, ≈firing rate | NT | Bambico et al. (2010) |
| WIN55,212-2 (4–16 mg/kg, 5 days) | CC/HC/CB, ≈5-HTP synthesis | + | Moranta et al. (2009) |
| WIN55,212-2 (4–16 mg/kg, 5 days) | St, ↓ 5-HTP synthesis (29%) | − | Moranta et al. (2009) |
| *Dopaminergic system* | | | |
| THC (5 mg/kg, 14 days) | SN, ↑ firing rate (33%) | + | Wu and French (2000) |
| THC (5 mg/kg, 14 days) | VTA, ↑ firing rate (44%) | − | Wu and French (2000) |
| HU210 (5 μM, 5 applications) | VTA, ↑ firing rate (400%) | − | Cheer et al. (2000) |
| WIN55,212-2 (4–16 mg/kg, 5 days) | St, ↓ DOPA synthesis (25%) | − | Moranta et al. 2009 |

Cannabinoid drugs: URB597, an inhibitor of fatty acid amide hydrolase (FAAH); WIN55,212-2, THC ($\Delta^9$ -tetrahydrocannabinol), and HU210, cannabinoid receptor agonists.

Net effect (% basal change): ↑ increase, ↓ decrease, ≈ no significant change.

Pharmacological tolerance: + induction of tolerance or − lack of tolerance after repeated agonist treatment (chronic effect versus acute effect). *NT* not tested

Brain region: *LC* locus coeruleus, *CC* cerebral cortex, *HC* hippocampus, *CB* cerebellum, *DR* dorsal raphe, *SN* substantia nigra, *St* corpus striatum, *VTA* ventral tegmental area, *TH* tyrosine hydroxylase, *DOPA* 3,4-dihydroxy-phenylalanine, *NE* norepinephrine, *5-HTP* 5-hydroxy-tryptophan

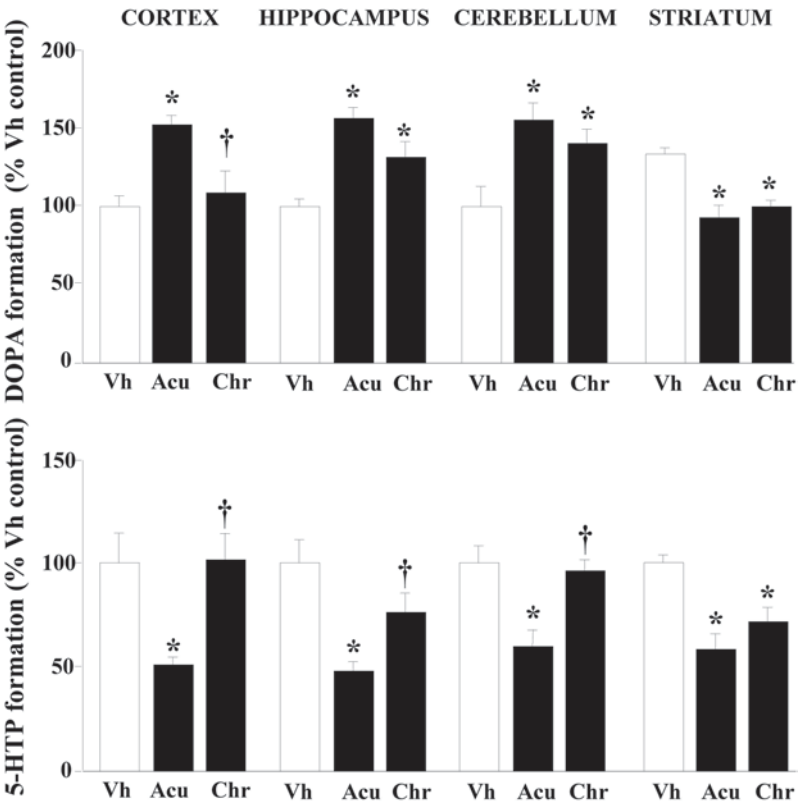


**Fig. 10.2** Acute and chronic effects of the cannabinoid receptor agonist WIN 55,212-2 on DOPA and 5-HTP formation in various rat brain regions, expressed as percentages of vehicle-treated animals (Vh control). Groups of rats were treated (i.p.) with drug Vh ($n=10$), acute WIN (Acu, 8 mg/kg, 1 h, $n=6$) and chronic WIN (Chr, 2–8 mg/kg, twice daily for 5 days, $n=6$). * denotes that $P<0.05$ at least when compared with the corresponding vehicle (Vh)-treated group. † denotes that $P<0.05$ at least when compared with the corresponding acute (Acu)- treated group. (Modified from Moranta et al. 2009)

rons (Table 10.1; induction of tolerance in SN and lack of tolerance in VTA). Similarly, the firing rate of VTA/DA neurons was markedly increased after the repeated in vitro application (five times) of HU210, a selective $CB_1$ receptor agonist (Table 10.2; lack of tolerance). The increase in VTA neuronal activity induced by HU210 was blocked by rimonabant (SR141716A), which by itself was ineffective in altering basal neuronal firing (Cheer et al. 2000). Chronic treatment with WIN55,212-2 (5 days) in rats resulted in a sustained inhibition of DOPA synthesis in striatum (Table 10.1 and Fig. 10.2; lack of tolerance).

These chronic studies in laboratory animals revealed the existence of a complex crosstalk between the endocannabinoid system and monoaminergic neurons in the brain. Notably, chronic CB treatments (FAAH inhibitor and $CB_1$ receptor agonists) are not associated with the induction of tolerance (neurochemical adaptation) to the

acute stimulatory effects of CB drugs on LC/NE, DR/5-HT and VTA/DA neurons (Table 10.1). In contrast, the chronic effects of CB receptor agonists on the synthesis of DOPA and 5-HTP and/or the release of the corresponding neurotransmitter are associated with the induction of tolerance in specific brain regions (Table 10.1 and Fig. 10.2). The process of CB drug tolerance appears to reflect the desensitization of $CB_1$ receptors after repeated drug exposure, the extent of which being dependent on time exposure, agonist efficacy, and the brain region targeted (Sim-Selley 2003). In this context, recent behavioral studies in rhesus monkeys have shown that $CB_1$ receptor tolerance/cross-tolerance (after 14 days THC treatment) is greater for low-efficacy agonists (e.g., THC) compared with high-efficacy agonist (e.g., CP55940), which suggested that differences in $CB_1$ receptor efficacy are relevant in vivo (Hruba et al. 2012). Importantly, the induction of drug tolerance upon $CB_1$ receptor agonist treatment could alter the direct and/or indirect effects of CB drugs modulating the functionality of monoaminergic systems (Fig. 10.1).

## 10.3 Modulation of Presynaptic Monoaminergic Receptors After Chronic Cannabinoid Exposure. Autoreceptors and Heteroreceptors

Presynaptic inhibitory receptors (autoreceptors and heteroreceptors) on monoaminergic neurons are involved in the regulation of neuronal (spontaneous firing rate) activity, synthesis, and release of NE, 5-HT, and DA (Esteban et al. 1996; Ichikawa and Meltzer 2000; Starke 2001; Fink and Göthert 2007). Thus, changes in the function of $\alpha_2$-adrenoceptors and $5\text{-}HT_{1A/1B}$ receptors mediating negative feedback mechanisms in specific neuronal systems (Fig. 10.1) may contribute to the sustained activation of LC/NE, DR/5-HT, SN/DA and VTA/DA neurons induced by chronic CB exposure (Table 10.1). Similarly, the rate-limiting monoamine enzymes TH and tryptophan hydroxylase (TPH) are under the tonic inhibitory control of somatodendritic $\alpha_{2A}$-autoreceptors and $5\text{-}HT_{1A/1B}$-autoreceptors, which regulate the synthesis of the monoamine precursors DOPA and 5-HTP.

### *10.3.1 $\alpha_2$-Adrenoceptors*

Chronic treatment of rats with WIN55,212-2 (2-8 mg/kg, 5 days) was associated with the induction of desensitization of somatodendritic and terminal $\alpha_{2A}$-autoreceptors and $\alpha_{2A}$-heteroreceptors regulating the synthesis of DOPA and 5-HTP in brain regions enriched in noradrenergic, serotonergic, or dopaminergic nerve terminals (Moranta et al. 2009). Thus, the ability of the $\alpha_2$-agonist clonidine to decrease the formation of DOPA/NE ($\alpha_2$-autoreceptor), DOPA/DA ($\alpha_2$-heteroreceptor), or 5-HTP/5-HT ($\alpha_2$-heteroreceptor) was markedly reduced or abolished in the cerebral cortex, cerebellum, and striatum of chronic WIN55,212-2 rats (Fig. 10.3). In line

with these findings, chronic WIN55,212-2 in rats (3 mg/kg, 7 days) was reported to reduce $\alpha_2$-adrenoceptor expression in some brain regions (Carvalho et al. 2010). The reduced sensitivity and expression of $\alpha_2$-adrenoceptors (desensitization of autoreceptors and heteroreceptors) modulating brain monoaminergic systems could be the result of an increased NE release induced by $CB_1$ receptor agonists (Oropeza et al. 2005; Page et al. 2007), which in turn would explain the downregulation of postsynaptic β-adrenoceptors induced by chronic THC in the brain (Hillard and Bloom 1982).

### *10.3.2 5-HT$_{1A}$ and 5-HT$_{1B}$ Receptors*

Chronic WIN55,212-2 treatment in rats (2–8 mg/kg, 5 days) was also reported to induce supersensitivity of somatodendritic 5-$HT_{1A}$-autoreceptors regulating the synthesis of 5-HTP in the cerebellum and striatum and of 5-$HT_{1A}$-heteroreceptors modulating DOPA/NE and DOPA/DA in these brain regions (Moranta et al. 2009). Thus, a low dose of the selective 5-$HT_{1A}$ receptor agonist 8-OH-DPAT, which was ineffective in the vehicle-treated rat, reduced 5-HTP formation in the cerebellum and striatum of chronic WIN55,212-2 rats (Fig. 10.3). This increased sensitivity of somatodendritic 5-$HT_{1A}$ auto/heteroreceptors could be the result, in part, of a reduced 5-HT release induced by CB drugs (Nakazi et al. 2000). Chronic WIN55,212-2 treatment in rats (2–8 mg/kg, 5 days) also induced supersensitivity of terminal 5-$HT_{1B}$- auto/heteroreceptors regulating the synthesis of DOPA and 5-HTP. Thus, a low dose of the selective 5-$HT_{1B}$ receptor agonist CP93129 reduced DOPA formation (cerebellum) or potentiated the reduction of 5-HTP formation (cerebellum and striatum) in chronic WIN55,212-2 rats (Fig. 10.3).

The changes in presynaptic monoamine receptor function induced by the sustained stimulation of $CB_1$ receptors (Fig. 10.3) would finally result in less efficient ($\alpha_2$- auto/heteroreceptors) or more efficient (5-$HT_{1A/B}$-auto/heteroreceptors) feedback autoinhibition leading to alterations in the synthesis/release of NE, 5-HT, and/or DA. These adaptations of presynaptic receptor function (autoreceptors and heteroreceptors) in chronically agonist-treated animals could finally modulate the net effects of chronic $CB_1$ receptor stimulation (induction or lack of tolerance) on monoaminergic systems in specific brain regions (Fig. 10.1).

## 10.4 Role of Endocannabinoids and CB Receptors in Human Brain Disorders

Several comprehensive reviews have discussed the potential involvement of the endocannabinoid system and CB receptors in several CNS disorders (most evidence from animal models) with an emphasis on the major psychiatric syndromes major depression and schizophrenia (Bambico et al. 2009; Parolaro et al. 2010; Ashton and

Moore 2011; Gorzalka and Hill 2011; Mechoulam and Parker 2013). Interestingly, the $CB_1$ receptor deficient mouse has been proposed as a useful model of depression (Valverde and Torrens 2012). Animal models of depression (postulated defective endocannabinoid system), however, have shown paradoxical results concerning the regulation of $CB_1$ receptors and the effects of antidepressant drugs (Griebel et al. 2005; Hill and Gorzalka 2005b; Bambico et al. 2007; Mato et al. 2010; Gorzalka and Hill 2011). In the CNS, the less abundant $CB_2$ receptor is mainly associated with the regulation of neuroinflammatory processes which might be of importance in the pathogenesis of neurodegenerative processes such as Alzheimer's disease and Huntington's disease (Fernández-Ruiz et al. 2008). Recently, the $CB_2$ deficient mouse has been proposed as a model of schizophrenia-like behaviors (Ortega-Alvaro et al. 2011). The participation of endocannabinoids and $CB_1$ or $CB_2$ receptors in the pathophysiology and treatment of several psychiatric and neurological disorders is discussed below from data directly obtained in humans.

## *10.4.1 Basal Serum or Cerebrospinal Fluid (CSF) Concentrations of Endocannabinoids. Effects of Psychotropic Medications*

### 10.4.1.1 Major Depression and Schizophrenia

Little is known on the status of endocannabinoids in the pathogenesis and treatment of major depression. In recent studies, the serum concentrations of AEA and 2-AG, but not *N*-palmitoylethanolamine (PEA) or *N*-oleoylethanolamine (OEA), were reported reduced in depressed women relative to matched controls (Hill et al. 2008, 2009). Conversely, in patients with minor depression, serum AEA was increased whereas 2-AG levels showed a similar but statistically insignificant trend (Hill et al. 2008).

In schizophrenia, four studies of the same research group have reported elevated AEA levels in CSF of patients with schizophrenia (Leweke et al. 1999, 2007; Giuffrida et al. 2004; Koethe et al. 2009). Moreover, CSF AEA contents remained high in patients treated with atypical antipsychotics, but they were similar to controls in patients medicated with typical antipsychotics (Giuffrida et al. 2004). No significant differences in serum AEA levels were found among schizophrenia patients and controls (Leweke et al. 2007, Koethe et al. 2009). The neuronal origin of CSF endocannabinoids remains conjectural and it might reflect an elevation in the peripheral content of these lipid signaling messengers. Thus, blood AEA was increased in patients with acute schizophrenia probably as a consequence of the modified immune response observed during the course of the disease (De Marchi et al. 2003). In fact, patients in initial prodromal states of psychosis with lower levels of AEA in CSF showed a higher risk for transiting to psychosis earlier (Koethe et al. 2009).

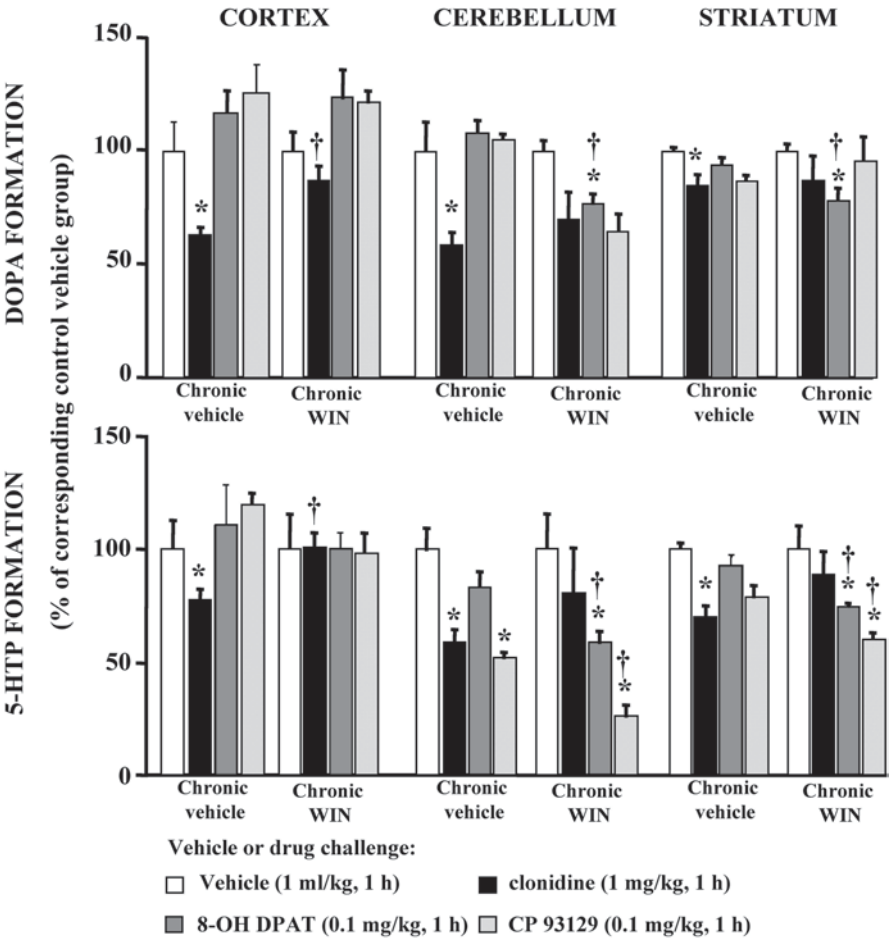


**Fig. 10.3** Acute effects of clonidine ($\alpha_2$-adrenoceptor agonist; 1 mg/kg), 8-OH DPAT (5-$HT_{1A}$ receptor agonist; 0.1 mg/kg), and CP93129 (5-$HTP_{1B}$ receptor agonist; 0.1 mg/kg) on DOPA and 5-HTP formation in various brain regions of chronically vehicle- and WIN55,212-2 (WIN)-treated rats, expressed as percentages of the corresponding control vehicle group. * denotes that $P<0.05$ at least when compared with the corresponding chronic vehicle group. † denotes that $P<0.05$ at least when compared with the corresponding drug challenge in the chronic vehicle group. (Modified from Moranta et al. 2009; data for CP93129, Esteban and García-Sevilla unpublished)

### 10.4.1.2 Stress and Anxiety

Preclinical studies have revealed the involvement of endocannabinoids in the regulation of stress and anxiety through interactions with monoaminergic systems (e.g., see McLaughlin et al. 2012). However, the clinical evidence is scarce (Mechoulam and Parker 2013). In a recent study, social stress exposure evoked a significant increase of blood 2-AG in women immediately following the stress, and both PEA and OEA blood levels declined during the phase of stress recovery (Hill et al. 2009).

Another study has measured circulating endocannabinoids (AEA, 2-AG, and various *N*-acylethanolamides) in healthy subjects after acute stress (Dlugos et al. 2012). The data indicate that stress increased serum AEA and *N*-acylethanolamides, but not 2-AG, immediately after the stress period. Interestingly, anxiety ratings at baseline were negatively correlated with baseline concentrations of AEA in blood (Dlugos et al. 2012).

#### 10.4.1.3 Parkinson's Disease, Alzheimer's Disease, and Huntington's Disease

Two studies have reported an increased content of AEA in CSF of unmedicated patients with Parkinson's disease (Pisani et al. 2005, 2010). Notably, the CSF AEA levels were at least twofold higher in unmedicated patients compared to control subjects. In medicated patients, AEA levels in CSF were indistinguishable from those measured in controls, regardless of the type of treatment with either levodopa or dopamine agonists (Pisani et al. 2005, 2010).

In Alzheimer's disease, the blood concentrations of AEA and 2-AG were found unaltered when compared with those in matched control subjects (Koppel et al. 2009). In the CSF, the content of 2-AG was similar in patients with Alzheimer's disease and controls, and AEA was not detected in any CSF sample (Koppel et al. 2009). This study also reported a lack of correlation between 2-AG in CSF and any measured domain of cognition (Koppel et al. 2009).

In Huntington's disease, a greater content of AEA in lymphocytes, with reduced activity of the enzyme FAAH, have been reported in patients with this neurodegenerative process. Other peripheral markers of the endocannabinoid system were found unaltered (Battista et al. 2007).

### *10.4.2 Basal Content of Endocannabinoids and CB Receptors in the Postmortem and Living Human Brains. Effects of Psychotropic Medications*

#### 10.4.2.1 Major Depression and Schizophrenia

Several studies have assessed the status of $CB_1$ receptors in the pathophysiology of major depression and/or suicide in the human brain. Two independent postmortem studies have reported an increased density of $CB_1$ receptors (agonist radioligand binding sites and receptor protein) and/or a greater $CB_1$ receptor-mediated G-protein activation (agonist stimulated [$^{35}$S]GTPγS binding) in the prefrontal cortex of antidepressant-free depressed suicides (Hungund et al. 2004; Valdizán et al. 2011) (Table 10.2). Interestingly, cortical $CB_1$ receptor-stimulated [$^{35}$S]GTPγS binding was not altered in antidepressant-treated depressed suicides (Valdizán et al. 2011). In line with these findings, the expression of $CB_1$ receptor mRNA has been reported to be greater in the prefrontal cortex of depressed patients when compared with matched

controls (Choi et al. 2012) (Table 10.2). Other postmortem studies, however, did not find significant differences in $CB_1$ receptor immunoreactivity in the prefrontal cortex of subjects with major depression (Eggan et al. 2010). Furthermore, the numerical density of cortical $CB_1$-immunoreactive glial cells was reduced in major depression which could be related to the effects of psychotropic drugs (Koethe et al. 2007) (Table 10.2). The postmortem data (radioligand agonist sites and receptor function) suggest a role for enhanced $CB_1$ receptor signaling in brains of antidepressant-free depressed suicides. These human postmortem findings, however, conflict with the postulated endocannabinoid deficiency in animal models of depression (Gorzalka and Hill 2011; Valverde and Torrens 2012). It should be noted, however, that the consequences of the reported alterations of the endocannabinoid system in depression (human and animal studies) remain to be clarified: e.g., the $CB_1$ receptor has both inhibitory and excitatory effects on synaptic transmission in the prefrontal cortex, indicating complex interactions between endocannabinoids and monoamine systems. Interestingly, an increased content of AEA and 2-AG with upregulation of $CB_1$ receptor density and signaling have been reported in the prefrontal cortex of alcoholic suicides compared with alcoholic nonsuicide subjects (Vinod et al. 2005), which further appears to link sensitization of cortical $CB_1$ receptors to suicide (Table 10.2).

Several studies have assessed the status of endocannabinoids in the pathogenesis and treatment of schizophrenia. Early studies had shown high AEA content in the CSF of schizophrenia subjects (Leweke et al. 1999) and that cannabis abuse could aggravate existing psychosis (Mathers and Ghodse 1992). Recently, 2-AG and AEA contents have been quantified in postmortem brain regions of subjects with schizophrenia (Muguruza et al. 2012). This study has revealed an opposite pattern for the regulation of endocannabinoids in schizophrenia: 2-AG was increased in cerebellum, hippocampus, and prefrontal cortex, whereas AEA and other *N*-acylethanolamines (dihomo-γ-linolenoylethanolamine, PEA, OEA, and docosahexaenoylethanolamine) were decreased in the same brain regions (Muguruza et al. 2012). Interestingly, antipsychotic medications appeared to reduce the content of endocannabinoids in the prefrontal cortex and hippocampus, but not in cerebellum, when antipsychotic-treated and antipsychotic-free subjects were compared (Muguruza et al. 2012).

On the other hand, several reports have linked schizophrenia with a differential expression of $CB_1$ receptors in the postmortem human brain. A significant upregulation of $CB_1$ receptors (autoradiographic density) has been reported in the different brain regions (including the cingulate cortex and dorsolateral prefrontal cortex) of subjects with schizophrenia, irrespective of the treatment given to the patients (Dean et al. 2001; Zavitsanou et al. 2004; Newell et al. 2006; Dalton et al. 2011; Jenko et al. 2012) (Table 10.2). In line with these findings, a neuroimaging (positron emission tomography (PET)) study has reported a generalized increase in $CB_1$ receptor density in most brain regions of schizophrenia subjects compared to controls, although the increase was significant in the pons only (Wong et al. 2010) (Table 10.2). Interestingly, $CB_1$ receptor binding in the frontal lobe and middle and posterior cingulate regions significantly correlated with the ratio of the brief psychiatry rating score psychosis to withdrawal score (Wong et al. 2010).

**Table 10.2** Basal regulation of brain CB receptors in various psychiatric and neurological disorders

| Brain disorder | Brain region and net effect (% basal change) | Reference |
|---|---|---|
| *Major depression (postmortem)* | | |
| $CB_1$ functional binding | CC, ↑ (45 %) | Hungund et al. (2004) |
| | CC, ↑ (30 %) | Valdizán et al. (2011) |
| $CB_1$ radioligand binding | CC, ↑ (24 %) | Hungund et al. (2004) |
| $CB_1$ immunoreactivity | CC, ≈ | Eggan et al. (2010) |
| | CC, ↓ | Koethe et al. (2007) |
| $CB_1$ mRNA | CC, ↑ | Choi et al. (2012) |
| *Schizophrenia (PET)* | | |
| $CB_1$ availability | BS/pons ↑ | Wong et al. (2010) |
| *Schizophrenia (postmortem)* | | |
| $CB_1$ immunodensity | CC, ≈ (drug-free subjects) | Urigüen et al. (2009) |
| | CC, ↓ (29 %) (treated subjects) | Urigüen et al. (2009) |
| $CB_1$ radioligand binding | CC, ↑ (23 %) | Dean et al. (2001) |
| | CC, ↑ (64 %) | Zavitsanou et al. (2004) |
| | CC, ↑ (25 %) | Newell et al. (2006) |
| | CC, ↑ (22 %) | Dalton et al. (2011) |
| | CC, ↑ (20 %) | Jenko et al. (2012) |
| | STG, ≈ | Deng et al. (2007) |
| $CB_1$ immunoreactivity | CC, ↓ (12–14 %) | Eggan et al. (2008) |
| | CC, ↓ (19 %) | Eggan et al. (2010) |
| | CC, ≈ | Koethe et al. (2007) |
| $CB_1$ mRNA | CC, ↓ (15 %) | Eggan et al. (2008) |
| *Parkinson (PET)* | | |
| $CB_1$ availability | SN, ↓ | Van Laere et al. (2012) |
| *Parkinson (postmortem)* | | |
| $CB_1$ functional binding | CN, ↑ (65 %); P, ↑ (144 %); GP, ↑ (672 %); SN ↑ (53 %) | Lastres-Becker et al. (2001) |
| $CB_1$ radioligand binding | P, CN, ≈ | Farkas et al. (2012a) |
| $CB_1$ mRNA | CN, P, GP, ↓ | Hurley et al. (2003) |
| *Alzheimer (postmortem)* | | |
| $CB_1$ radioligand binding | HP, CN, SN, GP, ↓ | Westlake et al. (1994) |
| | FB, BG, ≈ | Lee et al. (2010) |
| | CC, ↑ | Farkas et al. (2012b) |
| $CB_1$ density | HP, CC, ≈ | Benito et al. (2003) |
| | CC, ↓ | Ramirez et al. (2005) |
| | FB, BG, ≈ | Lee et al. (2010) |
| $CB_1$ functional binding | CC, ↓ | Ramirez et al. (2005) |
| $CB_1$ mRNA | HP, CN, SN, GP, ≈ | Westlake et al. (1994) |
| *Huntington (PET)* | | |
| $CB_1$ availability | CRB, CB, BS, ↓ | Van Laere et al. (2010) |

**Table 10.2** (continued)

| Brain disorder | Brain region and net effect (% basal change) | Reference |
|---|---|---|
| *Huntington (postmortem)* | | |
| $CB_1$ radioligand binding | SN, ↓ | Glass et al. (1993) |
| | St, GP, ↓ | Richfield and Herkenham (1994) |
| | CN, P, GP, ↓ | Glass et al. (2000) |
| $CB_1$ immunoreactivity | GP, ↓ | Allen et al. (2009) |
| *Alcohol dependence (postmortem)* | | |
| $CB_1$ functional binding | CC, ↑ (34%) | Vinod et al. (2005) |
| $CB_1$ radioligand binding | CC, ↑ (39%) | Vinod et al. (2005) |
| $CB_1$ immunoreactivity | CC, ↑ (67%) | Vinod et al. (2005) |
| | Vt, ↓ (26–52%) | Vinod et al. (2010) |
| *Cannabis dependence (postmortem)* | | |
| $CB_1$ radioligand binding | CC, HP, St, SN, ↓ (25–40%) | Villares (2007) |
| $CB_1$ mRNA | CC, St, SN, ↓ | Villares (2007) |
| $CB_1$ radioligand binding | CN, P, ↑ (25%) | Dean et al. (2001) |
| *Cocaine addiction (postmortem)* | | |
| $CB_1$ immunodensity | CC, ↓ (40%) | Álvaro-Bartolomé and García-Sevilla (2013) |
| $CB_2$ immunodensity | CC, ≈ | |
| *Opiate addiction (postmortem)* | | |
| $CB_1$ immunodensity | CC, ≈ | Álvaro-Bartolomé and García-Sevilla (2013) |

Net effect (% basal change): ↑ increase, ↓ decrease, ≈ no significant change
*PET* positron emission tomography
Brain region: *CC* cerebral cortex, *CB* cerebellum, *CRB* cerebrum, *HP* hippocampus, *SN* substantia nigra, *St* corpus striatum, *Vt* ventral striatum, *CN* caudate nucleus, *P* putamen, *GP* globus pallidus, *FB* forebrain, *BG* basal ganglia, *BS* brain stem/pons, *STG* superior temporal gyrus

In other postmortem studies, however, $CB_1$ receptor immunodensity was found decreased (with or without changes in $CB_1$ receptor mRNA) in the prefrontal cortex of antipsychotic-treated subjects with schizophrenia but not in drug-free schizophrenia subjects (Eggan et al. 2008, 2010; Urigüen et al. 2009) (Table 10.2). Other studies did not find alterations in $CB_1$ receptor density or $CB_1$ receptor mRNA in the cingulate cortex and superior temporal gyrus of schizophrenia subjects (Deng et al. 2007; Koethe et al. 2007) (Table 10.2). The reported discrepancies between postmortem studies might be related to confounding factors such as the subtype of schizophrenia or the presence of antipsychotic medications. Thus, a recent study has reported increased $CB_1$ receptor binding in the dorsolateral prefrontal cortex of paranoid schizophrenia subjects when compared with control and nonparanoid schizophrenia subjects (Dalton et al. 2011) (Table 10.2). Mostly, these postmortem studies suggest that the modulation of $CB_1$ receptor density in the prefrontal cortex seems to be a consequence of antipsychotic treatment and it

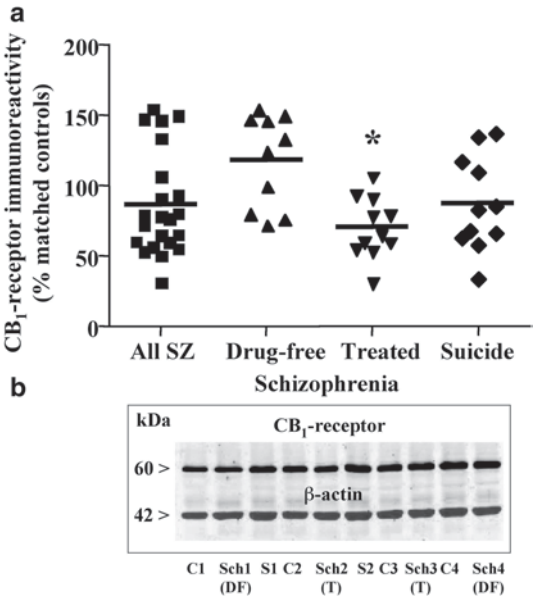


**Fig. 10.4** **a** Immunodensity of cannabinoid $CB_1$ receptor in the prefrontal cortex of drug-free ($n=10$) and antipsychotic-treated suicide schizophrenia subjects ($n=11$) and non-schizophrenia suicide subjects ($n=11$), expressed as a percentage of immunoreactivity in the corresponding matched controls (*$P<0.05$, comparison of antipsychotic-treated and drug-free schizophrenia suicide subjects). **b** Representative immunoblots of $CB_1$ receptor and β-actin for control subjects (C), drug-free schizophrenia (Sch, DF) and antipsychotic-treated schizophrenia (Sch, T) subjects, and non-schizophrenia suicide subjects (S). The molecular masses (kDa) of target proteins were estimated from referenced standards. (Modified from Urigüen et al. 2009)

represents an adaptative mechanism (Fig. 10.4). Since reductions in markers of GABA neurotransmission have been identified in the prefrontal cortex of subjects with schizophrenia (Lewis et al. 2005), a lower $CB_1$ receptor density induced by antipsychotic drugs could reduce the endocannabinoid-mediated suppression of GABA release, thus contributing to the normalization of cognitive functions. Consistent with this hypothesis, selective $CB_1$ receptor antagonists would be beneficial for the treatment of schizophrenia symptoms (Miyamoto et al. 2005). Although rimonabant, the first marketed $CB_1$ receptor antagonist, was suspended because of the induction of depression and suicide risk in some patients with abdominal obesity and coronary artery disease (Nissen et al. 2008), the identification of high-risk patients for these side effects could be important for the safe use of $CB_1$ receptor antagonists in various pathologies (Lazary et al. 2011).

Although these findings in the postmortem and living human brains are important, further studies are still needed to substantiate the status of endocannabinoids and $CB_1$ receptors in the pathogenesis and treatment of major depression and schizophrenia.

#### 10.4.2.2 Parkinson's Disease, Alzheimer's Disease, and Huntington's Disease

In Parkinson's disease the postmortem findings related to $CB_1$ receptors in the basal ganglia (radioligand binding sites and agonist stimulated [$^{35}$S]GTPγS binding) are contradictory (Table 10.2). An early study reported an enhanced stimulation of [$^{35}$S]GTPγS binding by WIN55,212-2 in the caudate nucleus, putamen, lateral globus pallidus, and substantia nigra of subjects with Parkinson's disease (Lastres-Becker et al. 2001). This study also reported an increase in $CB_1$ receptor binding sites in the same caudate nucleus and putamen samples (Lastres-Becker et al. 2001) (Table 10.2). In contrast, a recent autoradiographic study with the $CB_1$ receptor inverse agonist [$^{125}$I]SD7015 demonstrated unchanged $CB_1$ receptor density in the putamen and nucleus caudatus of subjects with Parkinson's disease (Farkas et al. 2012a) (Table 10.2). Other postmortem studies showed reductions in the expression of $CB_1$ receptor messenger RNA (mRNA) in the caudate nucleus, anterior dorsal putamen, and external segment of the globus pallidus (Hurley et al. 2003) (Table 10.2). A recent PET study has reported a reduced $CB_1$ receptor availability in the SN with an increased receptor availability in nigrostriatal, mesolimbic, and mesocortical dopaminergic projection areas (Van Laere et al. 2012) (Table 10.2).

In Alzheimer's disease, compared to normal brains, an early postmortem investigation reported reductions in the density of $CB_1$ receptors in several brain regions (Westlake et al. 1994). In this study, the specific binding of the agonist [$^3$H]CP55940 was strongly reduced in the hippocampus and caudate nucleus and to a lesser extent in the SN and globus pallidus (Table 10.2). In contrast, the expression of $CB_1$ receptor mRNA did not differ between Alzheimer's and control brains (Westlake et al. 1994) (Table 10.2). In line with these findings, G-protein coupling and $CB_1$ receptor protein expression were also shown markedly decreased in the frontal cortex of subjects with Alzheimer's disease (Ramírez et al. 2005) (Table 10.2). In these Alzheimer's brains, moreover, protein nitration was increased, and, more specifically, $CB_1$ and $CB_2$ receptor proteins showed enhanced nitration (Ramírez et al. 2005). In contrast, a recent autoradiographic study with [$^{125}$I]SD7015 has shown upregulation of $CB_1$ receptors in the prefrontal cortex of subjects with Alzheimer's disease (Farkas et al. 2012b) (Table 10.2). Another immunohistochemical study has reported that $CB_1$ receptor density was not modified in hippocampus and entorhinal cortex sections from brains of Alzheimer's disease patients (Benito et al. 2003) (Table 10.2). This latter study also showed that FAAH protein and activity as well as $CB_2$ receptor protein in Alzheimer's disease were selectively overexpressed in glial cells (Benito et al. 2003). Another study has also reported no differences in the immunoreactivity of cannabinoid $CB_1$ receptors in various areas of the forebrain and basal ganglia of subjects with Alzheimer's disease, a negative finding corroborated with saturation binding assays using the antagonist [$^3$H]SR141716A (rimonabant) (Lee et al. 2010) (Table 10.2).

In Huntington's disease, postmortem quantitative autoradiographic studies with [$^3$H]CP55940 revealed a massive loss of $CB_1$ receptors in the SN (pars reticulata) of subjects with this neurodegenerative process (Glass et al. 1993). In an independent autoradiographic investigation, the density of $CB_1$ receptors in striatum and palli-

dum was also markedly decreased in Huntington's disease (Richfield and Herkenham 1994). Similarly, $CB_1$ receptor immunoreactivity was markedly reduced in the globus pallidus of subjects with Huntington's disease (Allen et al. 2009) (Table 10.2). These postmortem findings indicating a loss of $CB_1$ receptors in specific brain regions agree well with the known massive death of GABAergic neurons (enriched in $CB_1$ receptors) in the neostriatum of subjects with Huntington's disease (DiFiglia 1990). An interesting investigation assessed the distribution and density changes of $CB_1$ receptors in the basal ganglia in early, intermediate, and advanced neuropathological grades of Huntington's disease (Glass et al. 2000) (Table 10.2). The results showed that the very early stages of the disease were characterized by a major loss of $CB_1$ receptors in the caudate nucleus, putamen, and globus pallidus externus; the intermediate neuropathological grades were associated with further decreases of $CB_1$ receptors, and advanced neuropathological grades revealed an almost total loss of $CB_1$ receptors (Glass et al. 2000). In line with these findings, a PET study has also reported decreases of $CB_1$ receptor availability in various brain regions (gray matter of cerebrum, cerebellum, and brainstem) in symptomatic patients with Huntington's disease, including the early stages of the disease (Van Laere et al. 2010) (Table 10.2).

#### 10.4.2.3 Alcohol Dependence

A hyperactivity of the endocannabinoid signaling system has been reported in the prefrontal cortex of suicidal alcoholic subjects compared to alcoholic subjects dying of causes other than suicide. These suicidal alcoholic subjects showed a greater $CB_1$ receptor density and functionality through G protein signaling, as well as higher contents of AEA and 2-AG in brain (Vinod et al. 2005) (Table 10.2). The same group reported decreased $CB_1$ receptor binding and functionality in the ventral striatum of nonsuicidal alcoholic subjects compared to controls (Vinod et al. 2010) (Table 10.2). However, these parameters were elevated in the suicidal alcoholics when compared to nonsuicidal alcoholic subjects (Vinod et al. 2010). On the other hand, it has been reported that the C allele of the single nucleotide polymorphism (SNP) rs2023239 of the gene that codes for the $CB_1$ receptor is associated with greater $CB_1$ receptor binding in postmortem prefrontal cortex, greater alcohol cue-elicited brain activation in the midbrain and prefrontal cortex, greater subjective reward when consuming alcohol, and more positive outcomes after treatment with a medication that targets the mesocorticolimbic neurocircuitry (Hutchison et al. 2008). In regard to the differences between Cloninger type 1 and 2 alcoholics, reduced AEA contents were observed in the NAcc and frontal cortex in type 1 alcoholics (Lehtonen et al. 2010). These findings suggest that endocannabinoids, and mainly AEA, are increased in specific brain regions of impulsive type 2 alcoholics. In contrast, brain AEA content was decreased in anxiety-prone type 1 alcoholics (Lehtonen et al. 2010).

#### 10.4.2.4 Drug Addiction: Cannabis, Cocaine, and Opiates

Chronic CB drug exposure in laboratory animals leads to drug tolerance and dependence, demonstrating that these drugs of abuse possess addictive properties (Hutcheson et al. 1998; Aceto et al. 2001; Lichtman and Martin 2005). The endocannabinoids can also participate, as a modulatory system, in the mechanisms of other drugs of abuse including cocaine and opiates (Maldonado et al. 2006). For example, a complex crosstalk between CB and opioid receptors has been unraveled (e.g., see Fattore et al. 2011; Scavone et al. 2013). A postmortem study has shown that the chronic abuse of marijuana (heavy user subjects) was associated with reduced $CB_1$ receptor density ([$^3$H]SR141716A antagonist binding) in various regions (NAcc, caudate nucleus, putamen, hippocampus, mesencephalon, and others) of the human brain (Villares 2007) (Table 10.2). Furthermore, the number of $CB_1$ receptor mRNA–positive neurons was also reduced in various brain regions of heavy cannabis users compared with control brains (Villares 2007) (Table 10.2). In marked contrast, significant increases in the density of $CB_1$ receptors, using the agonist radioligand [$^3$H]CP55940, have been reported in the caudate-putamen areas from subjects who had been taking cannabis within 5 days of death, which was independent of a diagnosis of schizophrenia (Dean et al. 2001) (Table 10.2). These striking differences may reflect the use of different radioligand (agonist or antagonist receptor sites), the outcomes of long-term cannabis use, the different routes of cannabis intake, or brain regional differences in the effects of THC in humans.

In laboratory animals, chronic treatment with cocaine was shown to decrease the expression of $CB_1$ receptor mRNA without altering the number of receptor agonist binding sites ($^3$H-CP55940) in rat brain cortex (González et al. 2002). Other studies have shown that chronic cocaine increased AEA content (partly due to FAAH inhibition) and potentiated the effect of the $CB_1$ receptor agonist HU210 in the rat corpus striatum (Centonze et al. 2004). In human cocaine addiction, however, the immunodensity of $CB_1$ receptor protein was markedly decreased in the prefrontal cortex of pure cocaine abusers, whereas receptor protein content was not significantly altered in mixed cocaine/opiate addicts and pure opiate (heroin/methadone) addicts (Table 10.2, Fig. 10.5a). In contrast, cortical $CB_2$ receptor protein in cocaine addicts was similar to that quantified in control subjects (Álvaro-Bartolomé and García-Sevilla, 2013). In mice, acute cocaine exposure increased the activation of mTOR (mammalian target of rapamycin) in brain cortex (Fig. 10.5b). Interestingly, chronic treatment with cocaine was associated with the induction of tolerance to the acute activation of cortical mTOR (Fig. 10.5b). Similarly, the basal activation of mTOR in the prefrontal cortex of long-term cocaine addicts was not significantly altered when compared with that quantified in matched controls (Fig. 10.5b). These postmortem findings strongly suggest that cocaine addiction in humans induces downregulation of $CB_1$ receptors and dampens the associated mTOR signaling in the prefrontal cortex.

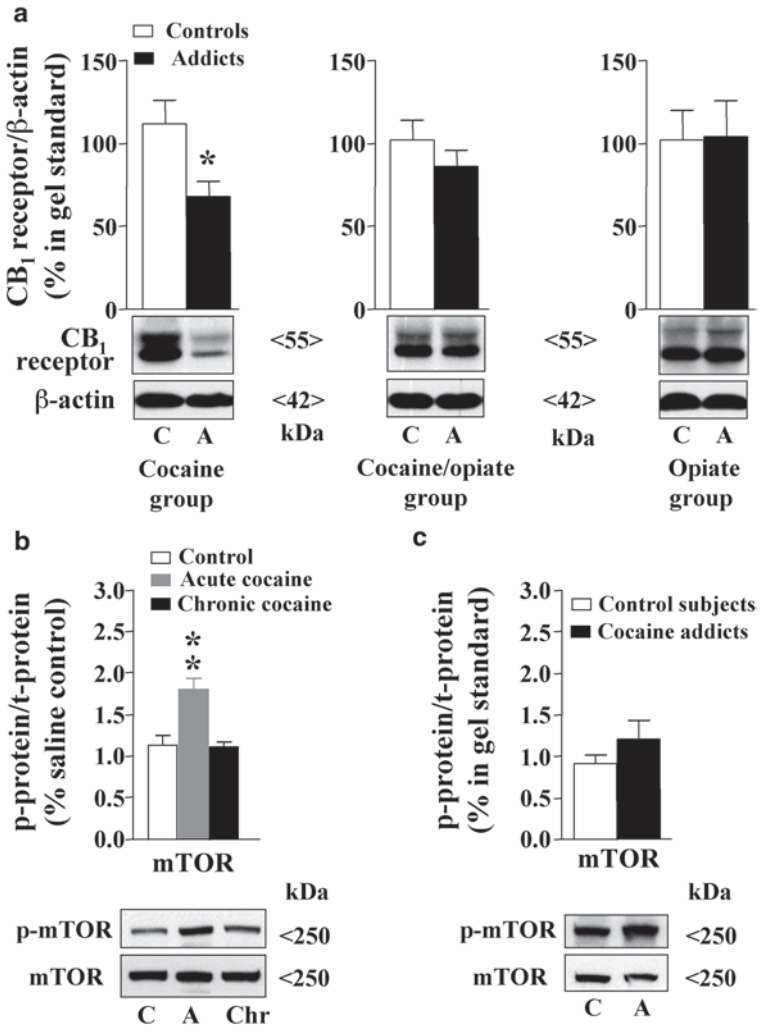


**Fig. 10.5** **a** Immunodensity of cannabinoid $CB_1$ receptor in the prefrontal cortex of control subjects (C, $n=8$–11), pure cocaine addicts (A, $n=9$), mixed cocaine/opiate addicts (A, $n=11$), and pure opiate addicts (C, $n=8$), expressed as mean ± standard error of mean percentages of an in-gel standard (100 %, pool of control samples) (*$P<0.001$ when compared with the corresponding control group, C). **b** Effects of acute (20 mg/kg, i.p. 2 h) and chronic (40 mg/kg, i.p., 7 days) treatments with cocaine on the activation of mammalian target of rapamycin (ratio of phosphorylated mTOR to total mTOR) in mouse brain cortex, expressed as percentages of saline-treated animals (control). **c** Activation of mTOR (ratio of phosphorylated mTOR to total mTOR) in the prefrontal cortex of control subjects ($n=9$) and long-term cocaine addicts ($n=9$), expressed as percentages of an in-gel standard (100 %, pool of control samples). The molecular masses (kDa) of target proteins were estimated from referenced standards. (Modified from Álvaro-Bartolomé and García-Sevilla 2013)

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