

THE ENDOCANNABINOID SYSTEM: CRITICAL FOR THE NEUROTROPHIC ACTION OF PSYCHOTROPIC DRUGS

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*There is growing evidence that neurotrophins besides their well-established actions in regulating the survival, differentiation, and maintenance of the functions of specific populations of neurons, act as the potential mediators of antidepressant responses. Previous studies on the regulation of nerve growth factor (NGF) levels by psychotropic medications are limited in scope and the underlying mechanism(s) remain elusive. In this review, the latest findings on the effects of pharmacologically heterogeneous groups of psychotropic drugs on NGF contents in the brain regions involved in the modulation of emotions are summarized. Moreover, the therapeutic potentials of the endocannabinoid system which is linked to depression and/or antidepressant effects and appears to interact with neurotrophin signalling, are reviewed. New findings demonstrate that endocannabinoid system is involved in the mechanisms of action of certain psychotropic medications including neurokinin receptor antagonists and that these are mediated via the upregulation of brain regional levels of NGF. This provides a better understanding of the pathophysiological mechanisms underlying neuropsychiatric disorders, leading to novel drug designs. **Biomed Rev 2010; 21: 31-46.***

Key words: endocannabinoids, NGF, psychotropics, brain

INTRODUCTION

Depression is a serious and widespread mental disorder with high relapse rate which is characterized by an array of disturbances in emotional behavior, memory, neurovegetative functions and hedonic processing (1). The neurobiological mechanisms subserving the development, manifestation, and treatment of depression are complex. There is ample evidence that disturbances in monoaminergic signalling and

glucocorticoid activity are involved in the pathophysiology of depression (2-4). However, the monoamine-based antidepressants do not fulfil the expectations in terms of onset of action, efficacy, and tolerability. This urges the need for the development of new antidepressants with novel mechanisms of action. Recent studies suggest a novel - neuroprotective - role for the adipose-derived hormone (adipokine) leptin, which was originally discovered to control energy

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homeostasis and body weight. Indeed, the localization of leptin receptor in limbic structures prompted the researchers to investigate the therapeutic potential of leptin in animal models of depression (5,6). In parallel, there has been growing interest in the assessment of the effects of psychotropic drugs on intraneuronal signal transduction and neurotrophins (7,8). Neurotrophins are family of proteins consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), NT-4/5, and NT-6 which regulate the survival, development, and function of neurons (9,10); both pro-NGF and pro-BDNF should also be appreciated. The cellular actions of neurotrophins are mediated by two types of receptors; a high affinity tyrosine kinase (Trk) receptor, and a low affinity pan-neurotrophin receptor (p75^{NTR}). Each Trk receptor is preferentially activated by one or more neurotrophin; TrkA by NGF, TrkB by BDNF and NT-4/5, and TrkC by NT-3 (Fig. 1), leading to a number of cellular responses (11). Although the long-term trophic effects of neurotrophins depend on gene regulation, the cytoplasmic effectors activated by neurotrophins exert a wide range of more rapid actions including morphogenetic and chemotropic effects on developing neurons (12-14), modulation of neuronal excitability (15), and synaptic transmission (16,17). Neurotrophins are also involved in the specific aspects of neuronal plasticity (18,19), a term used to describe a great variety of changes in neuronal structure and function. Neu-

rotrophins exert neuroprotective effects under pathological conditions which might be important for neurodegenerative and psychiatric diseases (20). Dysfunction in neurotrophin-mediated signalling mechanisms appears to be implicated in the pathogenesis of a number of psychiatric disorders such as psychosis, depression, mania, and obsessive compulsive disorders (21,22). It is worth mentioning that acute or chronic stress decrease cell proliferation and neurogenesis, an effect reversed by antidepressants (23,24). In regards to the mechanism(s) through which antidepressants stimulate neurogenesis, the most convincing hypothesis suggests that antidepressants elicit such an effect by increasing the levels of neurotrophic factors such as BDNF (25). Long-term antidepressant treatment elevates both BDNF protein and mRNA^{BDNF} levels and reverses stress-induced decrease in BDNF (26). Moreover, exogenous administration of BDNF displays antidepressant-like effects in animal models of depression (27). There are further reports suggesting the implication of BDNF in antidepressant action (28-34), however, few studies have measured NGF contents in multiple brain regions following the administration of psychotropic drugs and most of them have been limited in scope, examining a single drug class or brain region (35-37). In addition, the mechanism(s) by which psychotropic medications regulate NGF levels remained elusive. This, has been recently studied in our laboratory which will be discussed in a later part of this paper.

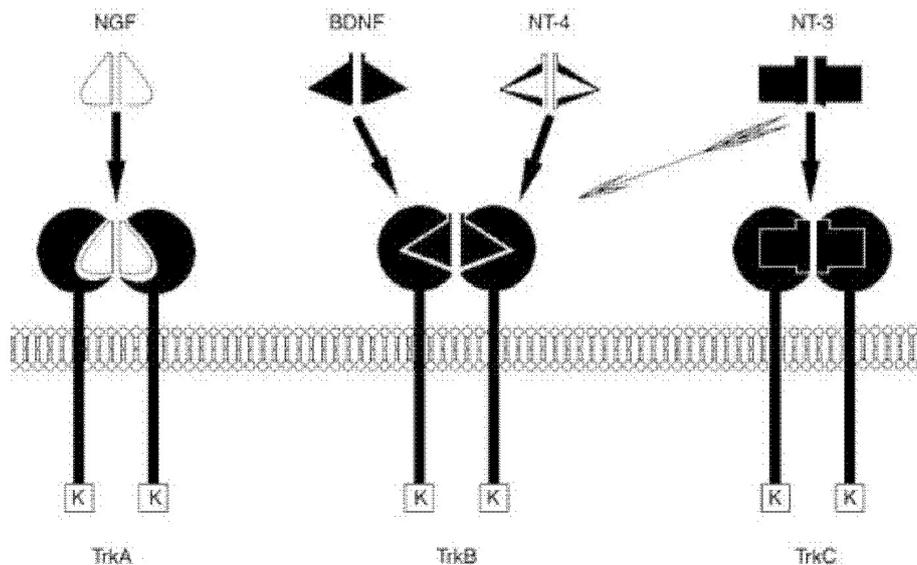


Figure 1. Neurotrophic tyrosine kinase receptors. As shown, tyrosine kinase receptors are a family of receptors with a similar structure. (Adapted from *Nat Rev Mol Cell Bio* 2003; 4: 46-55).

FUNCTIONS AND THERAPEUTIC POTENTIALS OF NGF

The search for target-derived factors that support the survival and growth of motor and sensory neurons led Rita Levi-Montalcini to the discovery of the first neurotrophin, NGF (reviewed in 38-40). Subsequent studies (41-47) showed that NGF (*i*) is synthesized and released by the target tissue of NGF-dependent axons and after binding to its receptors on axon terminals, is internalized and transported in a retrograde manner to the cell body, where it affects neuronal survival and differentiation, (*ii*) circulates throughout the body and is important for maintaining homeostasis, (*iii*) is critical for the memory and attention tasks that rely on the cholinergic septohippocampal pathway, (*iv*) exerts a modulatory role in the neuroendocrine-immune function, as well as inflammatory and neurological disorders, and (*v*) promotes peripheral nerve regeneration in an animal model of sciatic nerve injury. Because of its antioxidant, angiogenic, and insulinotropic properties, NGF may be implicated in the molecular mechanisms of cardiometabolic diseases including coronary atherosclerosis, obesity, type 2 diabetes, and metabolic syndrome (48,49). Moreover, NGF is involved in the suppression of food intake (50), and accelerating wound healing in skin and cornea ulcers (51-53). NGF produced and released by adipocytes and/or mast cells may regulate the sympathetic innervation of adipose tissue. These may link adipose-derived NGF to the pathophysiology of both cardiometabolic and neuropsychiatric diseases (54,55). Interestingly, treatment with nicergoline, an ergot alkaloid derivative with α_1 -adrenoreceptor-blocking and calcium antagonistic properties (56), has been shown to elevate the cerebral blood flow and improve the hemodynamics in aged rats with cerebral ischemia (57). These effects appear to be linked to the nicergoline-induced NGF enhancement in the aged rat brain (58). Therefore, the therapeutic potential of NGF in elderly individuals whose brain neurons are progressively dying with aging, merits further investigation. In recent years, NGF antagonism has been expected to be a highly effective therapeutic approach in acute or chronic pain states, and to be free of the adverse effects of traditional analgesic drugs (59).

NGF AND NEUROPSYCHIATRIC DISEASES

Experimental evidence suggests that antidepressant drugs and electroconvulsive treatment may act by enhancing central nervous system (CNS) levels of neurotrophins. According to *in vivo* experiments, NGF has the capacity to increase circulating concentrations of adrenocorticotropin and cor-

ticosterone (60,61). In addition, *in vitro* experiments have shown that NGF is able to induce the release of hypothalamic vasopressin, a neuropeptide which plays a pivotal role in the formation of social bonding (62,63). Given these considerations, it would be tempting to speculate that NGF is involved in the molecular mechanisms of emotions by acting as a fine modulator of distinct neuroendocrine functions. In this context, the involvement of NGF in neuropsychiatric disorders such as dementia, depression, and schizophrenia has been reported (64-67).

We have recently shown that a wide range of psychotropic drugs including nortriptyline, isocarboxazid, citalopram, risperidone and fluphenazine elevate NGF levels in dose-dependent and brain-region specific fashion (68,69). It is suggested that enhancement of NGF production may be specifically involved in the mechanisms of action of the antibipolar drugs such as lithium (70). However, lack of effects of antipsychotics including haloperidol, clozapine or olanzapine on BDNF or NGF levels in rat hippocampus has also been reported (71). It appears that differences in the experimental protocols, drug type, or dosing regimens might affect the study findings. Second-generation antipsychotics differ significantly with the first-generation antipsychotics in the regulation of brain NGF or BDNF levels, justifying the neurotrophin-mediated neuroprotection induced by the second-generation antipsychotics. Meanwhile, more extended treatments with atypical antipsychotics may be associated with the reduction of neurotrophins (72). These findings may have important clinical implications for the optimal therapeutic management of neuropsychiatric disorders.

Antidepressants, NGF and neurokinin receptors

In recent years, the potential anxiolytic- and antidepressant-like effects of compounds that target the neurokinin (NK) receptors, a class of G protein-coupled receptors which are found in the CNS and peripheral tissues, have attracted a growing interest. In this context, several selective and CNS-penetrating NK receptor antagonists which demonstrate efficacy in the treatment of emesis, anxiety, and depression have been synthesized (73-78). NK₁, NK₂, and NK₃ receptors have been identified in both rodents and humans (79,80). The localization of NK receptors in the cortex, hippocampus, amygdala, and septum may be consistent with the anxiolytic- and antidepressant-like effects of NK antagonists. As compared to the commonly used psychotropic medications, the effects of NK antagonists on neurotrophic factors are poorly characterized. There are a

few studies indicating the differential regulation of the NK₁ receptors and BDNF gene expression during inflammatory pain (81) or the increased levels of BDNF in NK₁ receptor gene knockout mice (82). However, there is no previous report linking the NK antagonists to NGF. We have recently investigated the potential involvement of NGF in the mechanisms of action of NK receptor antagonists. According to our findings, the selective NK receptor antagonists dose-dependently elevate NGF levels in distinct brain regions relevant to the regulation of mood including the frontal cortex (83). As previously reported, the frontal cortex is likely to be involved in depression and antidepressant medications block or reverse stress-induced pathogenic deficits in this brain area (84,85). The NK receptor antagonists can also elevate NGF concentration in the hippocampus which is supposed to be indicative of their neuroprotective effects (83). NGF plays a critical role in the hippocampal plasticity and learning and regulates hippocampal neurogenesis, a process that is mediated by the antidepressant treatment (86). Furthermore, NGF is involved in the cognitive function via the induction of acetylcholine release in the hippocampus (87). It is therefore reasonable to speculate that NK antagonists via enhancement of the hippocampal NGF improve psychopathology and particularly cognitive performance. In a recently conducted study, the NK₁ antagonist, N-acetyl-L-tryptophan, improved the cognitive neurologic outcomes after traumatic brain injury (88). The potential mechanisms through which the NK antagonists exert neuroprotective effects are discussed (89).

We have also shown the differential regulation of NGF levels by NK antagonists in the olfactory bulb and amygdala (83). NGF plays an essential role in the regeneration, maintenance, and development of the olfactory system of mammals (90). In addition, NGF facilitates cholinergic neurotransmission between the nucleus basalis and amygdala which play an important role in the cognitive functions (91). As a whole, it appears that enhancement of the brain regional levels of NGF constitutes an essential part of the biochemical alterations induced by certain psychotropic medications including the NK receptor antagonists.

To gain a mechanistic insight into the process by which psychotropic drugs regulate brain NGF levels, we have investigated the potential involvement of the endocannabinoid system which is linked to depression and/or antidepressant effects (92), and appears to interact with neurotrophin signaling (93-95). This, as well as other therapeutic potentials of the endocannabinoid system will be discussed herein.

THE ENDOCANNABINOID SYSTEM

The endocannabinoid system refers to a group of neuromodulatory lipids and their receptors which are involved in a variety of physiological processes including the regulation of motor activity, nociception, memory, appetite, temperature, brain development, and brain reward processes (96-99). The existence of an endogenous cannabinoid system was demonstrated with the discovery of endogenous brain constituents, anandamide (*N*-arachidonylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG), collectively termed the endocannabinoids, which are synthesized on demand by neuronal cells. Endocannabinoids are not stored in the vesicles like other mediators and are inactivated through re-uptake and enzymatic hydrolysis in both neurons and astrocytes (Fig. 2) (100). They act as retrograde synaptic messengers (Fig. 3) and inhibit the release of different neurotransmitters in the hippocampus, cortex and striatum (101,102). Endocannabinoids are ligands for the cannabinoid receptors including the

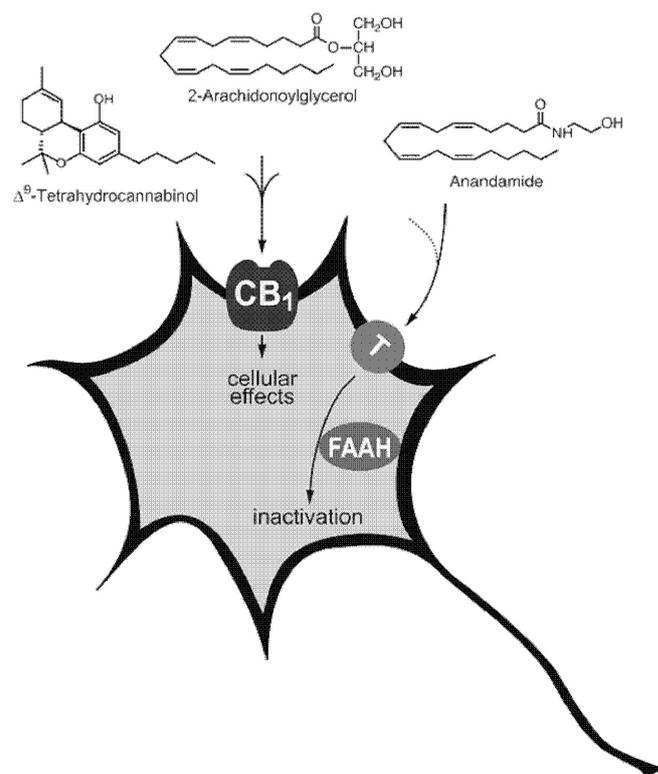


Figure 2. Inactivation of the endocannabinoids. Anandamide and 2-Arachidonoylglycerol are internalized by neurons through a high-affinity transport (T) mechanism. Once inside cells, endocannabinoids are hydrolyzed by fatty acid amide hydrolase (FAAH). (Adapted from *Curr Med Chem* 2002; 9: 663-676).

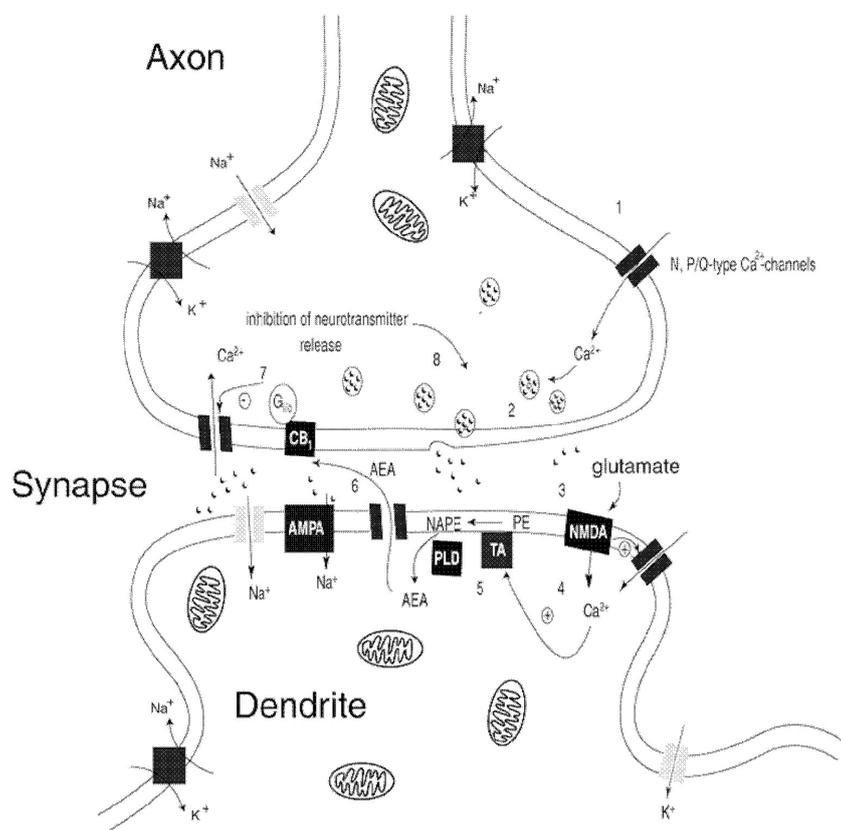


Figure 3. Model of hypothetical retrograde action of anandamide (AEA). (1) Presynaptic depolarization leads to calcium influx which in turn activates glutamate exocytosis. (2) Glutamate diffuses through synaptic cleft and activates postsynaptic glutamate receptors. (3) Activation of NMDA, AMPA and other glutamate subtype receptors leads to postsynaptic depolarization and calcium influx. (4) Elevated post-synaptic calcium levels activate a transacylase (TA) which converts phosphatidylethanolamine (PE) into N-arachidonylphosphatidylethanolamine (NAPE). (5) NAPE is hydrolysed by a phospholipase D (PLD) which yields AEA. AEA is released from the postsynaptic cell and diffuses back to the presynaptic CB₁ receptors. (6) Upon activation of the CB₁ receptors by AEA, Gi/o-proteins are released which inhibit N-, and P/Q-type voltage-sensitive calcium channels. (7) Closing of voltage-sensitive calcium channels results in a reduced release of neurotransmitters (8) (Adapted from *Curr Med Chem* 2002; 9: 663–676).

CB₁ and CB₂, two G protein-coupled receptors primarily located in the CNS and periphery, respectively (103). While the CB₂ receptors are highly expressed in immune-competent cells, detectable levels of the CB₁ receptors are found not only in central and peripheral neuronal cells, but also in a number of other cell types such as the immune cells, astrocytes, reproductive tissue cells, and endothelial cells in renal and vascular tissues. In the brain, the CB₁ receptors are most abundant in the areas controlling motor, cognitive, emotional, and sensory functions, i.e., the hippocampus, basal ganglia, cerebellum, cortex, thalamus, amygdala, and olfactory bulb (104,105). During the last few years, the endocannabinoid system has emerged as a remarkable topic in the scientific community and many different regulatory actions both in the central and peripheral nervous systems have been attributed to the endocannabinoids. In this context, the synthesis of cannabinoid receptor agonists and antagonists, anandamide uptake blockers, and selective inhibitors of endocannabinoid degradation has triggered an exponential growth of studies (106,107). This has opened new strategies in the treatment of pain, obesity, neurological diseases

such as multiple sclerosis, and the emotional disturbances including depression and anxiety. Signal transduction and biological actions of endocannabinoids are summarized in Table 1.

THERAPEUTIC SIGNIFICANCE OF ENDOCANNABINOIDS

Endocannabinoids and analgesia

Even though non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for their analgesic effects, the underlying mechanism(s) are still puzzling. According to several reports, NSAIDs probably act on targets other than the inhibition of cyclooxygenases to counteract pain (108). The discovery of endocannabinoid binding sites in the substantia gelatinosa, an area involved with the transmission of pain signals, opened new insights into the nociception (109). In this context, antinociceptive effects of the endocannabinoids were reported in several pain models (110–112). Concomitant perfusion of the selective CB₁ receptor antagonist, AM-251, reverses the antinociceptive activity of indomethacin, indicating the involvement of CB₁ receptors in the antinociceptive activity of this NSAID (113).

Table 1. Signal transduction and biological actions of endocannabinoids in the CNS

<p>Biological actions:</p> <p>Cortex, cerebellum and spinal cord Blockade of N-methyl-D-aspartate (NMDA) receptors, control of tremor and spasticity.</p> <p>Basal ganglia, striatum and globus pallidus Control of psychomotor disorders, interference with dopamine transmission, inhibition of GABA-mediated transmission, induction of long-term depression, potentiation of GABA-mediated catalepsy.</p> <p>Thalamus, hypothalamus and hippocampus Control of pain initiation, control of wake–sleep cycles, control of thermogenesis, control of appetite and food intake, impairment of working memory, impairment of memory consolidation, inhibition of long-term potentiation, inhibition of glutamate-mediated transmission.</p> <p>Retina Control of scotopic vision</p>

Endocannabinoids and opioids

The interaction between the endocannabinoids and opioids in treating pain has been extensively reviewed. It is worth mentioning that the endocannabinoids and opiates share a similar pharmacological profile. Both systems induce analgesia, hypothermia, sedation, hypotension, inhibition of intestinal motility and locomotor activity, changes in mood, and depression of the immune function (114-117). Moreover, the mechanism by which the endocannabinoids produce antinociception involves the release of spinal dynorphin (118). It is now well-established that opiates and endocannabinoids exhibit cross-tolerance and/or mutual potentiation for antinociception after chronic treatment (119,120). It is suggested that the widespread reduction of the endocannabinoid 2-AG in the brain of morphine-tolerant rats accounts for the enhanced susceptibility to the neurodegenerative processes and premature aging following chronic exposure to morphine (121). A new insight into the molecular mechanisms of the crosstalk between the opiates and cannabinoids is further provided (122).

Endocannabinoids and appetite

Involvement of the endocannabinoid system in food-seeking

behavior and obesity is well documented. Adipocytes appear to be a source of the endocannabinoids and related compounds. This, may lead to the speculation that adipose tissue is implicated in the peripheral dysregulation of the endocannabinoid system during obesity (123). The endocannabinoid system has been suggested to act as a major mediator between brain, the alimentary system and the adipose tissue (124). Emerging data suggest that tetrahydrocannabinol acts via the CB₁ receptors on hypothalamic nuclei and directly increases the appetite. In parallel, the hypothalamic neurons tonically produce the endocannabinoids which regulate hunger (125). It is worth mentioning that endocannabinoid contents are inversely correlated with the amount of leptin in blood. For example, mice without leptin not only become massively obese, but have higher-than-normal levels of hypothalamic endocannabinoids. Similarly, when these mice are treated with an endocannabinoid antagonist, food intake is reduced (126). Moreover, in CB₁ receptor knock-out mice, the animals tend to be leaner and less hungry than wild-type mice (127). As aforementioned, the endocannabinoid system activate a pathway in the hypothalamus responsible for food-seeking behavior, as well as endocannabinoids might also affect the feeding behavior at the level of taste cells (128,129).

Endocannabinoids and reward system

The presence of the endocannabinoid system in reward circuits and its role in motivational and emotional homeostasis suggests that compounds which modulate cannabinoid signalling might serve as diagnostic or therapeutic tools in drug addiction. In accordance with this rationale, the CB₁ receptor antagonists have been shown to modulate opioid self-administration in rodents (130). Extending this hypothesis, converging research lines have established a role for the endocannabinoids and CB₁ receptors in alcohol dependence (131, 132). According to the findings of an animal study, chronic exposure to nicotine, ethanol, or cocaine induces a brain region-specific changes in the endocannabinoid contents (133), therefore, it is likely that endocannabinoids mediate the anxiety-like symptoms induced by withdrawal from drugs of abuse.

Endocannabinoids and ischaemia-reperfusion injury

Ischaemia-reperfusion is a pivotal mechanism of organ injury during stroke, myocardial infarction, organ transplantation, and vascular surgery. There is a marked increase of endocannabinoid production in various forms of ischaemia-reperfusion which correlate with the degree of tissue injury

and inflammation (134). Direct measurements usually confirm the increase of target tissue endocannabinoid levels following preconditioning. The involvement of the CB₁/CB₂ receptors may be determined by using knockout mice (135).

Endocannabinoids and reproduction

The role of the endocannabinoids in female reproduction is an emerging concept. In embryo implantation timing, the endocannabinoid system plays an important regulatory role (136). Cannabinoid receptors are expressed by the developing embryo and are responsive to the uterine anandamide which modulates the probability of implantation to the uterine wall (137). The likelihood of human miscarriage is increased if uterine anandamide levels are too high or low (138). These findings suggest that proper intake of exogenous cannabinoids can decrease the likelihood for pregnancy for women with high anandamide levels, and alternatively, it

can increase the likelihood for pregnancy in women whose anandamide levels are too low.

Endocannabinoids and cytotoxicity

Since the first reference on antineoplastic action of endocannabinoids on lung adenocarcinoma and leukemia cells, there has been increasing evidence about the cytotoxic, proapoptotic and antineoplastic effects of endocannabinoids *in vivo* and *in vitro* (139,140). Mitogen-activated protein kinase (MAPK)-dependent signaling, ceramide, oxidative stress, and lipid rafts appear to be involved in the triggering of apoptosis by the endocannabinoids (141). These authors represented the cannabinoids as potential anticancer agents either solo or in combination with other cancerostatics.

Endocannabinoids and neuroprotection

Regardless of the point of initiation, the neurotoxic events

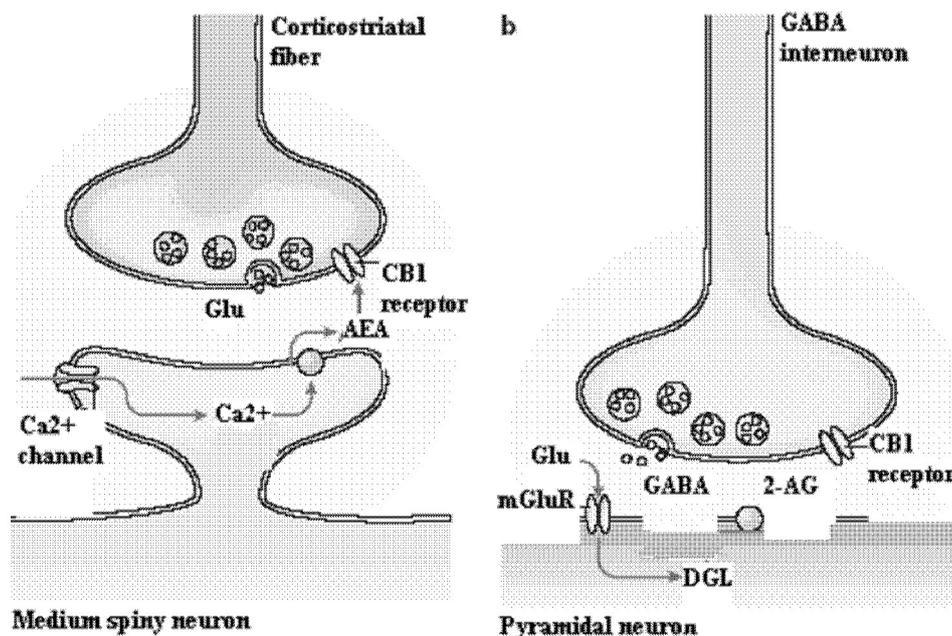


Figure 4. Roles of the endocannabinoids in long-term synaptic plasticity. a) Repetitive activation of corticostriatal fibres causes a persistent reduction of glutamate release, called long-term depression (LTD) which might be mediated by anandamide (AEA). The elevated Ca²⁺ concentrations produced in postsynaptic spines of striatal medium spiny neurons after the stimulation could trigger anandamide formation which in turn might induce LTD by engaging CB₁ cannabinoid receptors on glutamatergic axon terminals. b) High-frequency stimulation of glutamatergic Schaffer collaterals in the hippocampus elicits a prolonged reduction of GABA (γ -aminobutyric acid) release that might be mediated by 2-arachidonoylglycerol (2-AG). This heterosynaptic form of plasticity, called inhibitory-LTD (I-LTD) is induced when glutamate activates metabotropic receptors (mGluR) on pyramidal neurons, eliciting 2-AG formation through the diacylglycerol lipase (DGL) pathway. 2-AG might then travel sideways to engage the CB₁ receptors on contiguous terminals of GABA interneurons, producing I-LTD. (Adapted from *Nat Rev Neurosci* 2003; 4: 873-884).

self-amplify and ultimately lead to cell death (142). A unique opportunity to improve inflammation and neurodegeneration simultaneously might be offered by pharmacological agents that can modulate the activity of cannabinoid receptors (143). In animal model of brain injury, the neuroprotective action of the endocannabinoid 2-AG has been reported (144). In the experimental models of Alzheimer's disease, stimulation of either CB₁ or CB₂ receptor subtypes prevents microglial activation, microglia-mediated neurotoxicity, and neurodegeneration (145). The therapeutic potential of the cannabinoid receptor agonist WIN55212-2 against nigrostriatal degeneration is demonstrated (146). Degeneration of dopaminergic neurons during the experimental Parkinson's disease may be reduced by the agonists of CB₁ or CB₂ receptors (147). A link between the endocannabinoid signalling in the globus pallidus and symptoms of the disease has been reported (148). Moreover, pharmacological agonists of cannabinoid receptors exert beneficial effects in amyotrophic lateral sclerosis (149).

In recent years, a growing interest has been attracted towards the therapeutic potentials of endocannabinoids in multiple sclerosis (MS). According to the historical records from ancient China and Greece, the preparations of *Cannabis Indica* were commonly prescribed to ameliorate MS-like symptoms such as tremors and muscle pain (150). Afterwards, cannabis-based medicine were used for the treatment of MS. Due to the illegality of cannabis and rising number of MS patients undergoing self-medication, there has been much interest in exploiting the endocannabinoid system to provide a legal and effective relief (151). In the experimental models of MS, stimulation of either CB₁ or CB₂ receptors has been beneficial against the inflammatory process (152). In mouse models of MS, a profound reduction and reorganization of the CB₁ receptors has been observed in the cerebellum (153). CB₁ agonists promote both *in vitro* survival of oligodendrocytes and mRNA expression of myelin lipid protein (154). Moreover, the endocannabinoid 2-AG has been shown to stimulate proliferation of a microglial cell line through a CB₂ receptor-dependent mechanism (155). Taken together, these studies point to the exciting possibility that cannabinoid treatment may not only be able to attenuate the symptoms of MS, but also improve oligodendrocyte function. Despite the potential benefits of drugs acting on the cannabinoid receptors, their clinical use is hampered mainly because of their psychotropic effects (156). Therefore, designing cannabinoid derivatives devoid of psychoactive side effects appears to be helpful in the management of the debilitating disease, MS.

In general, cannabinoids exert their neuroprotective responses through activation of the cannabinoid receptors (157,158). However, cannabinoids may also protect neurons independent of the cannabinoid receptors. For example, WIN55, 212-2 may protect cerebral cortical neurons from *in vitro* hypoxia and glucose deprivation in a cannabinoid receptor-independent manner (159). Moreover, exogenous anandamide and 2-AG have been shown to protect cultured cerebral neurons against the toxic levels of the ligands of glutamate, AMPA, and kainate receptors in a cannabinoid receptor-independent fashion (160).

The mechanisms through which the exogenous cannabinoids exert neuroprotection in a variety of *in vitro* and *in vivo* models of neuronal injury include: (i) prevention of the excitotoxicity through the CB₁ receptor-mediated inhibition of glutamate transmission. This, is performed by closing the N- and P/Q-type Ca²⁺ channels (161), (ii) reduction of Ca²⁺ influx at both pre- and postsynaptic levels followed by inhibition of subsequent noxious cascades (162), (iii) antioxidant activity mainly owing to the phenol group of various resorcinol-type cannabinoids (163), (iv) suppression of the production of tumor necrosis factor- α (164), (v) activation of the phosphatidylinositol 3-kinase/protein kinase B pathway (165), (vi) induction of phosphorylation of extracellular regulated kinases (166), and (vii) induction of the expression of transcription factors and neurotrophins (167).

Endocannabinoids and mood disorders

As aforementioned, mood disorders represent a chronic debilitating disease group that are highly prevalent worldwide (1). Among all mental disorders, major depression has the highest rate of prevalence and incidence of morbidity. Drugs that directly influence the availability of brain monoamines, are considered as the primary medical treatment of depression (2). Unfortunately, the currently available antidepressant therapies have limited efficacies. Consequently, the research on new drugs for the treatment of mood disorders has become increasingly critical. Compelling evidence suggests that compounds which affect the endocannabinoid signalling not only modulate monoamine-mediated neurotransmission, but also affect the activity of the hypothalamic–pituitary–adrenal (HPA) axis (168). We have shown that activation of the endocannabinoid system is necessary for the suppression of HPA axis activity by tricyclic antidepressant doxepin (169). Recent preclinical evidences indicating the important roles of cannabinoid agonists or endocannabinoid enhancers (e.g. the

fatty acid amide hydrolase (FAAH) inhibitors) in the regulation of mood, have opened a new line of research in antidepressant drug discovery (170). Endocannabinoids are present in moderate to high levels in limbic brain regions including the prefrontal cortex, hippocampus, and amygdala, where the neuronal activity is altered in depression (171). Deficiencies in the endocannabinoid signaling are associated with a behavioral phenotype similar to the symptom profile of severe depression (172). In this context, several pharmacological and somatic treatments against depression have been shown to increase the endocannabinoid neurotransmission which, in some cases, is required for the neurobiological adaptations elicited by these treatments (173). Cannabinoids and the related compounds may also exert a therapeutic potential in anxiety-related disorders (174). Patients treated with cannabinergic compounds for MS or chronic neuropathic pain, might also benefit from the anxiolytic effects of the cannabinoids (175). Studies in animal models have shown that the CB₁ receptor antagonist SR141716A induces anxiety-like responses (176). Meanwhile, psychoactive cannabinoids are able to induce both anxiolytic and anxiety-like reactions which are dose- and context-dependent (177).

The neurobiological mechanism(s) linking the endocannabinoid system with the pathophysiology of mood disorders and antidepressant action have remained somewhat elusive. Similar to the actions of conventional antidepressants, activation of the endocannabinoid signalling may result to the enhancement of serotonergic and noradrenergic transmission, cellular plasticity, and neurotrophin expression within the hippocampus (178, 179). We have shown that chronic exposure to a wide range of psychotropic medications including the NK receptor antagonists induce a significant elevation of both endocannabinoids anandamide and 2-AG in the brain regions which are implicated in the regulation of emotional behaviour and synaptic plasticity (69,83). These findings suggest an existence of intrinsic endocannabinoid activity that contributes to the mechanisms of action of certain psychotropic drugs. In general, brain regional distribution of endocannabinoids following psychotropic treatment suggests that cannabinoid system may be integral to the development and maintenance of effective coping strategies to the emotional responses as well as achievement a better drug efficacy.

In our studies, endocannabinoid contents were increased within the brain regions in which psychotropic drugs were also able to elevate NGF production. Furthermore, CB₁ re-

ceptor neutral antagonist, AM4113, by blocking endogenous cannabinoid activity prevented psychotropic-induced enhancement of NGF (69,83). These findings argue for CB₁-mediated up-regulation of central NGF by psychotropic medications and are in agreement with previous reports suggesting an interaction between the endocannabinoid system and neurotrophins (93-95).

CONCLUSION

During the last few years, the increasing interest in the endocannabinoid system which is involved in the regulation of many cellular and physiological functions, has led to a number of interesting data derived from animal studies. Our recent findings demonstrate that the endocannabinoid system plays a pivotal role in the action of currently used psychotropic medications including the regulation of the HPA axis activity and brain regional levels of NGF. These findings may present an impetus for a better understanding of the complex scenario of the cannabinoid effects in humans as well as the pathophysiological mechanisms underlying neuropsychiatric disorders. Now, the time has arrived for clinical researchers to look at the cannabinoid system as a valuable target for drug discovery, especially in disorders for which no effective therapeutic or prophylactic regimens are presently available. As Eden Phillpotts (1862-1960) wrote, "*The universe is full of magical things patiently waiting for our wits to grow sharper.*"

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