New Strategies in the Development of Antidepressants: Towards the Modulation of Neuroplasticity Pathways

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Abstract: Over the past five decades, the pharmacological treatment of depression has been based on the pathophysiological hypothesis of a deficiency in monoamines, mainly serotonin and noradrenaline. Antidepressants prescribed today, all of them designed to enhance central monoaminergic tone, present several important limitations, including a 2-5 weeks response lag and also a limited clinical efficacy. As it is increasing evident that the abnormalities associated to depression go beyond monoamines, the development of better antidepressants will depend on the identification and understanding of new cellular targets. In this regard, much evidence supports a role for cellular and molecular mechanisms of neuroplasticity, including neurotrophic inputs, in mood disorders, in parallel with the biological features associated to stress conditions. In order to illustrate the possible relevance of neuroplasticity-related pathways for the therapy of depressive states, we here review the biological evidence supporting some therapeutic strategies in a very initial phase of development (modulation of the Wnt/GSK-3β/β-catenin pathway, potentiation of endocannabinoid activity, agonism of 5-HT4 receptors), which involve modulation of downstream mechanisms and neuroplasticity circuits. These strategies also show the existence of mixed mechanisms of action, constituting a nexus between the “classic” aminergic theory and the “new” neuroplasticity hypothesis.

Keywords: Antidepressant, stress, neuroplasticity, β-catenin, endocannabinoid, 5-HT4 receptors.

INTRODUCTION

The hypothesis of a monoaminergic deficiency has determined in the past the basis of therapeutic approaches in the field of depression, as well as of the research in this field [1, 2]. First evidence emerged in the 50’s after observing that the administration of reserpine, an antihypertensive drug, induced depressive states associated with the depletion of the catecolaminergic stores. A few years later, the antidepressant efficacy in humans of iproniazid and imipramine, two compounds developed for non-psychiatric disorders, was accidentally discovered. These findings led to the development of new antidepressant drugs with clinical efficacy: tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs) and, more recently, selective monoamine reuptake inhibitors [3]. Current antidepressants, which elevate central monoamines, are among the most widely prescribed drugs. However, the monoaminergic hypothesis has been questioned repeatedly since it does not explain completely the mechanism of action of antidepressants or the pathophysiology of depression. Several weeks are necessary for their antidepressant efficacy, in spite of the fact that the increase in monoamine concentrations in synaptic cleft occurs in a few hours [4]. On the other hand, it has been demonstrated that some drugs which increase monoamine levels are not effective in depression [5]. Together, these facts suggest that although central monoamines might contribute to the generation of depressive states, the cause of depression is far from being only a simple deficiency of central noradrenaline and/or serotonin activity.

In the late 90’s a new hypothesis was suggested to explain major depression on the basis of the changes in stress-vulnerable hippocampal neurons and the possible role of neurotrophic factors [6]. A morphologic and morphometric analysis of the hippocampus in depressed patients frequently reveals structural changes [7, 8, 9] that go beyond volume loss and include gray matter alterations. To a certain degree, these alterations seem to be reversible in the remission phases of the disease [10]. Furthermore, it is now well documented that chronic antidepressant treatment enhances cell proliferation in adult rodent and nonhuman primate subgranular zone (SGZ) of hippocampus and that the time required for the differentiation and maturation of newborn neurons correlates well with the appearance of clinical response to the antidepressant treatment [11, 12, 13]. Adult hippocampal neurogenesis varies greatly in response to numerous kinds of stimuli [14]. Among these, the biochemical changes associated with stress and depression, such as hypercortisolaemia or alterations in the serotonergic system, not only influence general hippocampal morphology but also affect adult hippocampal neurogenesis [9]. Furthermore, antidepressant treatments also increase BDNF level through an increase in cAMP and further CREB activation that activates a series of genes such as BDNF and other trophic factors [15]. In human, the reduction of the rate of proliferation in hippocampus is accompanied by a reduction in brain derived neurotrophic factor (BDNF) levels in serum [16], lymphocytes and platelets [17] and brain [18], as observed also in animal models [19, 20]. Furthermore, studies in mice show that an increase of BDNF in the dentate gyrus of the hippocampus, but not in the CA1 region, is necessary to obtain behavioral effects after antidepressant treatment [21]. Taken together, all these results have allowed the development of a novel theory on the basis of major depression, supporting a critical role for the cellular and molecular mechanisms of neuroplasticity in the pathophysiology of this disease [22].

The study of the mechanisms underlying depression has been limited by the lack of good animal models to work on. This problem is mainly due to the difficulty in identifying in the animals signs which could correspond to symptoms observed in human...
depression. Another difficulty comes from the lack of a known gene or molecule acting as the main responsible of this illness, as its etiology is still unknown [23]. In humans, besides depressed or irritable mood, depression also includes cognitive symptoms (gait and suicidality), emotional symptoms (anhedonia), homeostatic or ‘neurovegetative’ symptoms (abnormalities in sleep, appetite, weight and energy), and psychomotor agitation or retardation. Only a subset (homeostatic symptoms, anhedonia and psychomotor behavior) can be measured objectively in rodents. Nevertheless and bearing in mind these limitations, some animal models of depression have been generated, including selective breeding (i.e. Flinders sensitive line rats), brain lesions (i.e. bilateral olfactory bulbectomy) and environmental stress (i.e. learned helpless, forced swimming test or chronic mild stress) [24].

Stress induces a series of responses of the body to maintain the homeostasis in adverse life events. Depression is often characterized by excessive activity of the hypothalamic-pituitary-adrenal (HPA) axis that regulates stress. The dysregulation of the HPA axis, leading to a hypersecretion of corticotrophin releasing factor (CRF) and cortisol is observed in 30 to 50% cases of major depression [25]. Elevated levels of cortisol in some patients with depression can also result from a reduced negative feedback response to cortisol that is reflected by a nonsuppression response in the dexamethasone suppression test. Hypercortisolaemia has been examined as a potential predictor of various outcomes as well as one cause of the brain structural anomalies observed in depressed patients [26].

Some of the animal models used in the study of depression are based on this dysregulation, as it is the case of the chronic mild stress [27], and the corticosterone model [28], in which a depressive-like phenotype and altered hippocampal neurogenesis have been reported. Another valuable model is based on the use of glucocorticoid receptor (GR) heterozygous mice (GR +/-) that exhibits a depression-related phenotype characterized by increased learned helplessness on the behavioral level, neuroendocrine alterations with hypothalamo-pituitary-adrenal (HPA) axis overactivity, and reduced hippocampal BDNF expression [29].

On the other hand, the bilateral ablation of the olfactory bulbs precipitates complex changes of behavioral, neurochemical, and neuroimmunological parameters, similar to those observed in patients with major depression. This animal model has been proposed as a model for chronic psychomotor agitated depression in which there is an impaired serotonin neurotransmission [30, 31]. Bulbectomy is associated with structural changes within the hippocampus that are reversed by chronic antidepressant treatments [32]. This is of particular interest given the hypothesis that stress induced neurodegeneration of hippocampal neurons can be reversed by chronic antidepressant treatment, thereby suggesting that bulbectomy results in an increased vulnerability of the rat to the adverse impact of environmental stress. Moreover, it has also a high predictive validity to chronic, but not acute, antidepressant treatment [30, 31].

FOCUSED ON NEUROPLASTICITY: A ROLE FOR THE WNT/GSK-3β/β-CATENIN PATHWAY IN THE MEDIATION OF ANTIDEPRESSANT RESPONSES

The Wnt/β-catenin pathway has been classically involved in the regulation of cellular proliferation, mainly through the activation of transcription factors at the nuclear level. In this regard, its role in cancer is well known [33]. Activation of the canonical Wnt pathway leads to the inhibition of GSK-3β, allowing β-catenin to be stabilized in the cytosol (see Fig. 1) and translocated to the nucleus, where it activates transcription of target genes [34].

![Diagram](image)

**Fig. (1).** Different pathways that are activated following antidepressant treatments. Activation ofGs or Gi by different receptors (i.e.: serotonin 5-HT₄ or cannabinoid, respectively), produce changes in cAMP levels modulating the activation of cAMP response element-binding protein (CREB) cascade, via cAMP-dependent protein kinase (PKA), PKC and/or Akt proteins, respectively. The activation of CREB leads to an increase of brain-derived neurotrophic factor (BDNF) that binds to tyrosine kinase (trkB) receptors and activates Ras/MAPK pathway, maintaining synaptic function and neural plasticity. BDNF/trkB can also activate CREB through activation of PI3K and Akt, or via MAPK pathway. Regarding Wnt/β-catenin pathway, the canonical pathway acts through activation of frizzled receptors that results in the inhibition of GSK-3β, increasing the amount of cytoplasmic β-catenin that translocates to the nucleus, activating TCF/lefr transcription factors. The Wnt/β-catenin pathway can be also activated through inhibition of GSK-3β by Akt, promoted by G-protein coupled receptors.
In the last years, the role of this pathway in neural development, through the modulation of neural stem cells (NSC), proliferation and differentiation, has been clearly demonstrated [35]. Some of the processes regulated by Wnt/β-catenin activity are: neural differentiation [36], hippocampal formation [37, 38], dendritic morphogenesis [39], axon guidance [40, 41] and synapse formation [42]. Moreover, it also plays a role in spatial learning [43] and memory, including long term potentiation (LTP) phenomena [44]. On the other hand it is of relevance the reported existence of an overexpression of GSK-3β in Alzheimer’s disease, leading to the attempts to develop inhibitors of this kinase as possible therapeutic tools for this disorder [45]. These results reinforce the interest of the Wnt/GSK-3β/β-catenin pathway as a candidate for developing new pharmacological treatments for neurological and psychiatric disorders.

Recent studies have also identified the Wnt/GSK-3β/β-catenin signaling pathway as a key regulator of adult neurogenesis in hippocampus [35, 46] or subventricular zone [47]. NSC in the adult hippocampus express several Wnts as well as their corresponding receptors, and therefore receive Wnt signals produced not only from astrocytes but also from themselves [35, 48]. The autocrine signaling of Wnt, results in the proliferation and multipotency of NSC through the canonical pathway. Because of all that, the possible involvement of Wnt/β-catenin signaling in the responses induced by long-term administration of antidepressants has to be taken into account.

Interestingly, an upregulation of this system has been reported following electroconvulsive therapies [46]. Moreover, it is well known that the treatment with lithium modifies GSK-3β activity [49, 50], further supporting its role in antidepressant responses.

We have demonstrated that chronic administration of antidepressants (14 days), such as the serotonin-noradrenaline reuptake inhibitor (SNRI) venlafaxine [51] (Figs. 2,3) or the serotonin reuptake inhibitor (SSRI) fluoxetine (unpublished data) (Fig. 3) resulted in the nuclear accumulation of β-catenin in the subgranular cell layer of the dentate gyrus of the hippocampus [47] (Figs. 2,3). β-catenin expression was increased in both membrane and nuclear fractions of the hippocampus [51] (Fig. 3), after venlafaxine treatment, and immunoelectron microscopy studies further demonstrated an increased presence of β-catenin at the nuclear level. In parallel with that, increased cell proliferation (BrdU incorporation) in subgranular zone (SGZ) was achieved after chronic treatment with a high dose of venlafaxine [51]. Electroconvulsive therapy, known to present antidepressant efficacy also results in an increased expression of β-catenin in cytosolic and nuclear fractions of the hippocampus [46].

Interestingly, we have very recently found that the antidepressant-like behavior observed following the administration of the SSRI fluoxetine in combination with a 5-HT₁A antagonist for 7 days is paralleled by an increase of β-catenin in the hippocampal membrane fraction but not in the nuclear one, in contrast to the results obtained with classical antidepressants. These results are not accompanied by an increase in hippocampal proliferation [52]. Taking into account the dual role of β-catenin in proliferation and in cell adhesion, these results from our group may reflect an increased neuroplasticity instead of a pro-proliferative response [12].

As already mentioned, for a number of years, GSK-3β has been identified as a target for the treatment of Alzheimer’s disease and other cognitive disorders [53]. Interestingly, the reviewed studies strongly suggest that the axis Wnt/GSK-3β/β-catenin can also play a role in affective disorders: it is tempting to speculate that this is due to its involvement in central nervous system proliferation and differentiation. In this regard, GSK-3β has been very recently identified as a key regulator of neural progenitor homeostasis [54]. Du et al. [55] have recently identified a kinesin signaling complex that mediates the regulatory role of GSK-3β in mood behavior.

It is noteworthy that β-catenin, being a part of the N-cadherin/β-catenin complex attached to the cell membrane, plays an important role in cell-cell adhesion [56]. In the membrane, β-catenin is located pre- and postsynaptically associated to synapses [57, 58] and recruits synaptic scaffolding molecules for the development of new synapses [59]. This β-catenin-dependent non-canonical cascade could also contribute to the regulation of mood.

On the other hand, frizzled receptors and GPCRs can interact through several pathways [60, 61]. Some GPCRs act through Gq and/or Gi activating PKB/Akt which inhibits GSK-3β via phosphorylation. These receptors can also activate Gs proteins that would activate PGE2, PI3-K, PKB/Akt, leading to the inhibition of GSK-3β. Another of the pathways activated by GPCRs is through Gq or G12,13, that activates PLCβ and PKCs, inhibiting GSK-3β [60]. Taken together, these data support the possible existence of interactions between the GSK-3β/β-catenin pathway and other...
neurotransmission systems involved in depression, including monoamines.

The pharmacological modulation of the different elements of the β-catenin pathway with antidepressant purposes will be clarified in the near future. Direct modulation at the level of Wnts or β-catenin activity is a possible approach. It is well known that GSK-3β is one of the targets of lithium, a mood stabilizer used in the treatment of manic disorders [62]. Li⁺ inhibits this enzyme at a therapeutically relevant concentration, inducing a number of biochemical features in the central nervous system [62].

Inhibitors of GSK-3β activity have shown antidepressant activity [63, 64]. Interestingly, a number of patents regarding inhibition of GSK-3β as a mechanism of therapy for neuropsychiatric disorders are being launched including treatment of depression among their claims. They include, among many others, imidazole substituted pyrimidines, benzoimidazole derivatives, cycloalkylaminooquinolones and triazolopyrimidine derivatives.

ENDOCANNABINOIDS AND MOOD: IS THERE A LINK?

Cannabinoid receptors, the molecular target of Δ⁹-tetrahydrocannabinol, are physiologically engaged by the endocannabinoids, a family of arachidonic acid derivatives, which include anandamide and 2-arachidonoylglycerol [65]. After release, anandamide is rapidly removed from the extracellular space and subsequently hydrolyzed by the enzyme fatty acid amide hydrolase (FAAH). Cannabinoid CB₁ receptors, which are negatively coupled to adenyl cyclase via Gᵢ/o proteins (Fig. 1) mediate most of the actions of both endogenous and exogenous cannabinoids in the central nervous system [65]. The involvement of these receptors in the regulation of mood has attracted much attention in the recent years, supported by different types of evidence: reduced levels of circulating endocannabinoids together with an up-regulation of CB₁ receptor mRNA and protein expression have been found in animals subjected to different models of depression as well as in prefrontal cortex samples from victims of major depression [66, 67, 68]. Furthermore, CB₁ receptor knockout animals have been reported to present “depressive-like” behavior [69]. From the pharmacological point of view, several studies indicate that the administration of drugs modulating CB₁ receptor activity might result in antidepressant-like responses [70-75]: the report by Gobbi et al. [76] showing the antidepressant-like activity of URB597, an inhibitor of endocannabinoid metabolic degradation, from a group of O-arylcarbamates, casted light on the relevance of this system as a possible target for the treatment of depression (Fig. 4) [76,77]. As it will be discussed below, the overall interpretation of these studies is complex, as some of them show conflicting results.

Interestingly, a number of studies suggest that brain endocannabinoid system contributes to the chronic effects of antidepressants: long-term administration of antidepressants of different families modulates the levels of anandamide and 2-arachidonoylglycerol, and CB₁ receptor expression in several brain areas [78-80]. Taking into account that a wealth of experimental data supports the existence of cross-talk mechanisms between brain endocannabinoid and 5-HT systems [73, 81, 82, 83, 84, 85], and that the activity of:

![Figure 3](image-url)

**Fig. (3).** β-catenin protein level increase associated to chronic antidepressant treatment in different subcellular fractions. (A) Venlafaxine treatment affects β-catenin protein level in total cell lysate (TCL), membrane-associated and nuclear subcellular fractions of rat hippocampus. (B) Fluoxetine treatment increases β-catenin protein level in total cell lysate (TCL) of rat hippocampus. Densitometric measurement levels were normalized to actin protein amounts and data are expressed as Mean ± S.E.M. *p<0.05; **p<0.01; ***p<0.001, vs vehicle; *p<0.05; **p<0.01, vs venlafaxine 10 mg/kg/day.
of 5-HT neurotransmission is still the main target of most antidepressants available, it is of clear interest the analysis of the possible involvement of 5-HT receptors in the adaptive changes of the endocannabinoid system induced by long-term antidepressant treatment.

In this regard, we have studied the effects of chronic administration of antidepressants on the functionality of the CB1 receptor-dependent signal pathway. Chronic fluoxetine (14 days) induced a significant increase in the maximal ability of the agonist WIN55,212-2 to inhibit adenylyl cyclase (AC) activity in the prefrontal cortex (PFC); this increase was absent in those rats treated with fluoxetine plus the 5-HT1A receptor antagonist WAY100635 [85]. Interestingly, chronic fluoxetine also significantly increased basal cAMP levels in the PFC, this effect not being observed in the animals treated with fluoxetine+WAY100635 or with WAY100635 alone (Fig. 5). Furthermore, immunoprecipitation studies of WIN55,212-2-stimulated [35S]GTPγS labeled Gα subunits revealed that the level of Gα2 and Gα3 subunits activation was significantly increased in the PFC of fluoxetine-treated rats. As reported above for fluoxetine effect on CB1 receptor coupling to AC in this brain area, the 5-HT1A receptor antagonist WAY100635 did not alter the profile of Gα protein activation by WIN55,212-2 when administered alone, but prevented the increased stimulation of Gα2 protein subunits when coadministered with the SSRI [85].

A number of studies have reported modifications of endocannabinoid markers in animal models of depression. In this regard, we have used the olfactory bulbectomized (OB) rat [67] to analyze the density and functionality of CB1 receptor-mediated signaling: a) OB animals exhibited significant increases in both CB1 receptor density ([3H]CP55,490 binding) and functionality (stimulation of [35S]GTPγS binding by the cannabinoid agonist WIN55,212-2) at the prefrontal cortex (PFC) (Fig. 6); and b) the antidepressant-like effect of chronic fluoxetine (10 mg/kg/day, 14 days, s.c) was associated to a full reversal of that enhanced CB1-receptor signaling (Fig 6). Furthermore, chronic fluoxetine increased the ability of the agonist WIN55,212-2 to inhibit adenylyl cyclase (AC) activity (Fig. 7), which could contribute to the behavioral response to the antidepressant in OB rats. The molecular mechanism underlying this relationship between changes in the expression/functionality of CB1 receptor and those on AC activity is unclear. Since fluoxetine per se induces hypersensitization on CB1 receptor-mediated inhibition of AC also in naïve rats [67, 85], it might be speculated that the modifications observed in OB rats along the CB1 receptor-associated signaling cascade at PFC could reflect a compensatory mechanism to maintain endocannabinoid homeostasis.

Although it has been consistently reported that potentiation of endocannabinoid activity by low doses of CB1 receptor agonists or inhibitors of endocannabinoid metabolism results in an antidepressant-like effect in rodents [71, 76, 77] it should be mentioned that
CB₁ receptor antagonists have also been reported to behave as antidepressants in behavioral models [70, 72]. An important consideration is that, although studies specifically addressing the antidepressants effects of CB₁ cannabinoid agonists and antagonists in humans are lacking, the high incidence of depression and anxiety in placebo-controlled clinical trials with the CB₁ receptor antagonist rimonabant for the treatment of obesity, has motivated its withdrawal or lack of approval [86].

Most of the studies that have addressed the effects of chronic antidepressants exposure on the activity of brain endocannabinoid system suggest an enhancement of CB₁ receptor expression in brain areas with a well established role in depression [78-80]. Increased CB₁ receptor density in the rat hippocampus has been reported following long-term administration of the noradrenaline uptake inhibitor desipramine [80], and in the PFC after chronic treatment with the monoamine oxidase inhibitor tranylcypromine or with fluoxetine [80]. Taken together, data available strongly suggest that the forebrain endocannabinoid system may be important for the efficacy of these treatments, reinforcing the interest of simultaneously analyzing the effects of antidepressant administration on the different levels of the CB₁ receptor signal transduction cascade.

In this regard, it is noteworthy that some of the modifications of endocannabinoid neurotransmission found in animal models of depression are normalized by the administration of antidepressants. As here reviewed, the chronic administration of fluoxetine to bulbectomized rats not only exhibits antidepressant-like activity (abolition of motor hyperactivity), but this response is associated to the reversion of the upregulation of CB₁ receptor G coupling functionality present in these animals [67]. The different results described stress the importance of transductional mechanisms: in agreement with the recent theories about depression, the role of endocannabinoid neuromodulation mostly involves downstream molecular, rather than only receptor protein levels.

A relevant issue is the possible relationship between the role of the endocannabinoid system in the pathophysiology of depressive states and the mediation of antidepressant responses, and the classic monoaminergic hypothesis of depression. Our results regarding the blockade of 5-HT₁A receptors with WAY100635 point out to a special involvement of this 5-HT₁ receptor subtype. Interestingly, modifications in the expression of 5-HT₁A and 5-HT₂ receptors, similar to those observed in depression, have been described in cortical areas of bulbectomized animals; furthermore, adequate CB₁ receptor functionality seems to be important for 5-HT₁A receptor-mediated biochemical and behavioral responses, as decreased efficacy of the 8-OH-DPAT to activate Gₛ coupled proteins and impaired anxiolytic and/or hypothermic effects of 5-HT₁A agonists have been detected in CB₁ knockout animals [83, 87]. The present report uncovers the possibility that modulation of CB₁ receptors participates in those effects of chronic fluoxetine that are triggered by 5-HT₁A receptors. In this regard, our results showing a 5-HT₁A receptor-dependent increase in basal AC activity following long-term fluoxetine are in good agreement with previous studies where a role for the activation of Gₛ coupled receptors has been suggested [88, 89]. It is noteworthy that WAY100635 also prevented fluoxetine-induced increase in CB₁ receptor coupling to Gₛ protein, suggesting that up-regulated CB₁ receptor signaling contributes to some extent to 5-HT₁A receptor-mediated modulation of the cAMP cascade after fluoxetine administration. Together with the previous data [73], showing that cannabinoids increase firing activity of
5-HT neurons in the dorsal raphe (mainly regulated by 5-HT₁A receptors), these results illustrate the existence of a link between a “classic” (5-HT) and a “new” target for the development of new compounds for the treatment of depression.

It is noteworthy that endocannabinoid system plays an important role in neuroplasticity, being involved in the regulation of neurogenesis. Recent findings have shown the presence of a functional endocannabinoid system in neural progenitor cells that participate in the regulation of cell proliferation and differentiation [90, 91]. The chronic administration of CB₁ agonist HU-210 promotes embryonic and adult hippocampal neurogenesis and induces anxiety- and antidepressant-like effects [92]. Conversely, the sub-chronic, but not acute, administration of rimonabant causes loss of antidepressant activity and decreases neuronal proliferation in the mouse hippocampus [93]. The fact that cannabinoids, mainly acting through CB₁ receptors, are capable to increase cell proliferation and neurogenesis, consistent with the effects of conventional antidepressants, further supports both the involvement of the endocannabinoid system in mood control and the therapeutic antidepressant potential of cannabinoid agonists.

From the analysis of the different studies regarding the involvement of the endocannabinoid system in depressive disorders, it can be concluded that: 1) a deficiency in endocannabinoid signaling induces symptoms of depression in humans, as well as a “depressive-like” behaviour in rodents; 2) chronic antidepressant treatment modulate CB₁ receptor-dependent signaling; 3) facilitation of endocannabinoid neurotransmission results in the production of biochemical and behavioural responses similar to those reported for “classic” antidepressants. Therefore, the potentiation of endocannabinoid activity could be of interest in depression. Two main pharmacological approaches are possible: the direct activation of CB receptors, mainly of the CB₁ class, and the augmentation of the endogenous endocannabinoid tone through the inhibition of the degradation of endocannabinoids. The first approach can be achieved by administering CB agonists: in this regard, Δ⁹-tetrahydro-cannabinol has been shown to induce antidepressant effects, especially at low doses and under acute administration [67]. However, the possible induction of psychotomimetic effects by CB₁ receptor agonists strongly limits the future of these compounds. The fact that acute cannabidiol, a non psychotomimetic compound, is able to induce antidepressant-like responses in rodents opens an interesting perspective.

The potentiation of anandamide activity though the inhibition of FAAH constitutes a new target for the treatment of depression: the pharmacological profile of URB597 and other pioneering compounds includes antidepressant-like responses in animal models of stress and depression [77], but without other typical cannabinoid responses, such as rewarding effects. In fact, a number of compounds are being recently patented under the hypothesis of antidepressant efficacy as a result of inhibition of FAAH, including, among others, carbamates, imidazole derivatives, substituted heterocyclic urea compounds, heteroaril substituted urea compounds and isoxazolines.

5-HT₄ RECEPTORS: A TARGET FOR FASTER ANTIDEPRESSANT RESPONSES?

5-HT₄, through the activation of different receptor subtypes, plays a pivotal role in the mediation of the effects of most currently used antidepressants. 5-HT₁A and 5-HT₁D receptor subtypes have been classically identified as those mediating the majority of actions of 5-HT in the regulation of mood [1, 2]. In line with that, the existence of changes in the properties of these receptors in depressed patients has been proposed [94, 95]. Furthermore, the effects of chronic treatment with antidepressants on 5-HT₁A receptors, at both pre- and postsynaptic levels have received considerable interest: in the case of raphe presynaptic receptors, a desensitization at both biochemical and functional levels has been consistently found [96, 97, 98, 99, 100].

In the recent years, increasing attention is being paid to 5-HT₄ receptors. These receptors are widely distributed in brain areas including basal ganglia, hippocampus, amygdala or cortex [101, 102, 103]. These receptors belong to the superfamily of G-protein coupled receptors which are positively coupled to adenylate cyclase (AC) [104] leading to the intracellular accumulation of cAMP. This increase of cAMP observed after the application of 5-HT₄ agonists contributes to the neuronal excitability of pyramidal cells of hippocampus by inhibiting potassium channels [105, 106, 107]. It is well known that 5-HT₄ receptors control important functions in peripheral nervous system [108, 109]; however, some of their brain functions are also of possible clinical relevance since this receptor has been implicated in memory, anxiety, anorexia and recently in depression [110, 111, 112, 113].

The presence of the 5-HT₄ receptors in cortical and limbic areas suggests a potential role in learning and memory, as well as in affective behaviour. In fact, an increase of 5-HT₄ receptors in cortical and striatal areas was described in post mortem brain from depressed patients [114]. Over the last few years, evidence indicates that 5-HT₁A agonists might exert antidepressant-like effects with a greater onset than the classical antidepressant drugs. It has been recently shown that acute and subchronic (3 days) administration of 5-HT₄ receptor agonists (prucaloride, 2.5 mg/kg and RS67333, 1.5 mg/kg) induces an increase of dorsal raphe nucleus 5-HT neuronal mean firing [115]. This is expected to result in an increase of 5-HT release in postsynaptic areas. More interestingly, there are many evidences that 5-HT₄ receptor agonists are effective in improving the behavioural performance in some experimental models widely used to assess antidepressant-like effects in a similar way of SSRIs, with a faster onset of action. Lucas et al. reported in 2007 that acute or subacute administration of the partial agonist RS67333, reduced immobility time in FST [113]. Furthermore, in two animal models considered to be predictive of chronic antidepressant response, a partial reversion of the hyperactivity induced by olfactory bulbectomy and chronic mild stress was reported after 3 days treatment with RS67333. Interestingly, studies carried out in the hippocampus showed that 3 days treatment with RS67333 induce a slight increase in neuronal proliferation in the dentate gyrus [113] (Fig. 8) suggesting the involvement of neuroplasticity mechanisms in this pharmacological response. This study has been pioneer in the identification of a new target for the development of new antidepressant drugs. In this context, the antidepressant-like response for another partial 5-HT₄ receptor agonist (SL65.0155) has been also reported in a rodent paradigm of depression (FST), associated to increased levels of several neurotrophic factors including BDNF, pCREB and the antiapoptotic protein Bel-2 [116]. In addition, it has been reported that 3 days of treatment with RS67333 not only increases extracellular 5-HT concentrations, but potentiates the increase in 5-HT elicited by the classical SSRIs [117, 118].

Recent data from our laboratory have confirmed the antidepressant-like effects of RS67333, showing that seven days of treatment are required to obtain a full antidepressant response in the novelty suppressed feeding and sucrose consumption after chronic corticosterone. They also suggest that, after seven days of treatment, increased hippocampal cell proliferation is accompanied by the accumulation of β-catenin in amplifying neural progenitors promoting their differentiation to immature neurons and the increase in BDNF expression [119]. It has also been reported an increase in BDNF expression following acute administration of another partial 5-HT₄ agonist (SL65.0155) [116].

On the other hand, the modulation of 5-HT₄ receptors by antidepressants strongly supports the role of these receptors in the control of mood. In this regard, our group has recently shown for the first time that long term treatment with antidepressants modulate 5-HT₄ receptors at different levels of the 5-HT₄ transductional
pathway [120, 121]. Our results show that while chronic administration (21 days) of fluoxetine [120] or venlafaxine [121] does not modify the expression of 5-HT 4 mRNA, these drugs decrease their density (Table 1) suggesting that changes observed are due to post-transcriptional alterations. The downregulation of 5-HT 4 receptors has also been demonstrated with paroxetine after chronic treatment [122].

Table 1. Antidepressants Effect on 5-HT 4 Receptors

<table>
<thead>
<tr>
<th>Region</th>
<th>Vehicle</th>
<th>Fluoxetine</th>
<th>Venlafaxine</th>
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<tbody>
<tr>
<td>mPFCx</td>
<td>7.3 ± 0.7</td>
<td>6.7 ± 0.7</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>Caudate-putamen</td>
<td>19.8 ± 1.5</td>
<td>15.8 ± 0.8*</td>
<td>14.6 ± 1.1*</td>
</tr>
<tr>
<td>GP</td>
<td>15.6 ± 1.5</td>
<td>10.9 ± 0.9*</td>
<td>11.6 ± 1.1</td>
</tr>
<tr>
<td>SN</td>
<td>12.8 ± 1.5</td>
<td>6.2 ± 0.4**</td>
<td>6.5 ± 0.6**</td>
</tr>
<tr>
<td>CA1, hippocampus</td>
<td>13.9 ± 1.4</td>
<td>9.3 ± 0.9*</td>
<td>8.5 ± 0.9*</td>
</tr>
</tbody>
</table>

Effect of chronic antidepressants (fluoxetine 10 mg/kg/day and venlafaxine 40 mg/kg/day, 21 days, p.o.) on specific [1H]GR113808 binding (5-HT 4 receptors) in rat brain. Data are the mean ± S.E.M and specific binding is expressed as Bmax (fmol/mg tissue). *p<0.05; **p<0.01. One-way ANOVA followed by Dunnett test. mPFCx: medial prefrontal cortex; GP: globus pallidus; SN: substantia nigra.

Among the numerous actions of antidepressants, it has been already mentioned that these drugs might induce changes in post-receptor elements involved in the production of cAMP, such as AC activity [123]. In this sense, we have demonstrated that chronic administration of either a SSRI (fluoxetine) or a SNRI (venlafaxine) induce a decrease, in a dose-dependent way, in the accumulation of cAMP after activation of 5-HT 4 receptors with zacopride (Fig. 9) [120, 121]. It is noteworthy that our results indicate that the administration of RS67333 for 7 days also lead to a functional desensitization of striatal 5-HT 4 receptors [119].

Further evidences of the modulation of 5-HT 4 receptors by antidepressants have come from electrophysiological studies: it has been shown that 5-HT 4 receptors modulate excitatory responses of pyramidal cells of hippocampus [105]. 5-HT 4 receptors are coupled to K+ channels through a signaling pathway that involve Gs proteins, adenylate cyclase and PKA [see 107], so the activation of this receptor leads to the blockade of K+ channels resulting in an increase of neuronal excitability. We have reported that chronic treatment with antidepressants (fluoxetine, venlafaxine) leads to a desensibilization of the response induced by activation of 5-HT 4 receptors (Figs. 10 and 11) measured as a decrease of amplitude of population spike induced by zacopride [120, 121], an effect also observed with other antidepressants, such as imipramine [124].

Taken together, these results support the hypothesis that 5-HT 4 receptors are mediating an important part of the involvement of 5-HT neurotransmission in the regulation of affective disorders. In this regard, an increase in the density of these receptors in ventral hippocampus has been reported in bullectomized rats [125], a
mg/kg/day, 21 days, p.o). Treatment with fluoxetine (10 mg/kg/day, 21 days, p.o) or venlafaxine (40 mg/kg/day, 21 days, p.o) may account for the antidepressant effect of RS67333 in the OB model of depression. Moreover, it could be also beneficial to reduce the enhanced 5-HT4 receptor signaling reported in post mortem frontal cortex from depressed patients [114].

If the exciting results showing that partial agonists of these receptors induce antidepressant-like responses in short periods of time (3 or 7 days) are finally confirmed in humans, this will represent a chance for overcoming one of the main limitations of current therapy: the 2-3 weeks latency in the onset of action. It has been very recently reported that SSRI potentiate the rapid antidepressant-like effects of partial 5-HT4 receptor agonists [118].

Furthermore, the results obtained with partial agonists of 5-HT4 receptors clearly demonstrate that both “classic” amimergic and “new” neuroproliferative hypothesis are complementary, as illustrated by the increase of hippocampal cell proliferation, increase in BDNF expression and, interestingly, augmentation of β-catenin expression. These results are not surprising at all taking into account the well documented role of 5-HT in the development of nervous system, mediated among others by the activation of the 5-HT1A receptor [126] and the pivotal role that this amine plays in both depression and the regulation of adult hippocampal neurogenesis [127, 128].

CONCLUDING REMARKS

Over the past five decades, the pharmacological treatment of depression has been based on the monoaminergic hypothesis of the pathophysiology of this disease [129, 130]. Although this hypothesis has been instrumental in the pharmaceutical development of compounds, the full demonstration of a deficit in monoaminergic neurotransmission as the key factor in the generation of the disease is lacking.

Treatment of depressive disorders with the antidepressants clinically available has undoubtedly resulted in a significant improvement in the quality of life and clinical symptoms of depressive patients. However, as mentioned in the Introduction, a number of relevant issues remain unsolved with regard to antidepressant pharmacology: first, although the increase in monoaminergic tone induced by these drugs is immediate, two to five weeks are required before a significant clinical improvement is achieved. Second, these drugs show lack of efficacy in a number of patients; finally, available antidepressants present a number of adverse reactions that frequently limit their use [2]. Because of all that, the development of drugs showing an antidepressant profile, which overcome the above-mentioned limitations, is strongly required. The success in the search of better antidepressants will surely depend on the identification of new cellular targets which would help in the understanding of the biological basis of depressive disorders.

The identification of the downstream molecular changes induced by antidepressants has allowed the generation of new theories about both the pathophysiology of the depression and the final targets of these compounds, focusing on transduction mechanisms beyond receptors [22]. These findings have also suggested a role for the cellular and molecular mechanisms of neuroplasticity in the mediation of antidepressant responses. The transcription factor CREB is a very good example of that: it has been repeatedly shown that chronic administration of antidepressants might result in the modulation of the levels of expression of this factor, which plays an important role in synaptic plasticity: in agreement with that, a reduction on CREB levels has been found in the brain of depressed patients [131]. In a similar way, a role for the neurotrophin BDNF
in depression appears to be strongly supported: several studies have shown a reduction of BDNF levels in serum of depressed patients, as well as its normalization by chronic antidepressant treatment. In fact, the pharmacological potential of neurotrophic factors as antidepressants is under consideration [132]. Furthermore, in the recent years the relationship between the cellular and molecular modifications induced by chronic stress and those underlying the development of depressive states has been strongly demonstrated.

A huge variety of different agents is currently under development for the treatment of depressive disorders [133]: in agreement with the amimergic hypothesis, many of them are modulators of the different subtypes of 5-HT (5-HT₁, 5-HT₂), noadrenaline and dopamine receptors, also affecting their specific transporters. In addition to that, new targets have been addressed: NK₁ and CRF antagonists, as well as glutamate-acting drugs are being the subject of increasing interest in the recent years. Other families include antiguucocorticoids, estrogenic compounds or melatonin-acting drugs, as it is the case of agomelatine. The analysis of the preclinical and clinical development of those drugs is beyond the scope of this review: our focus has been the role of downstream signaling mechanisms, with special emphasis on neuroplasticity pathways.

As an example, we have here reviewed the evidences supporting the involvement of some neuroplasticity-related (GSK-3β/B-catenin) and neuromodulator (endocannabinoid) pathways in the mediation of antidepressant effects and their relationship with compounds addressed at more “classical” targets (5-HT₁ agonists). New strategies will be developed to address the activation of neuroplastic and neuproliferative circuits. As an example of that, recent studies suggest that a single dose of a NMDA antagonist, ketamine, can produce a rapid antidepressant response in patients resistant to classic antidepressants [134] and this response could be mediated by the mTOR pathway [135]. The activation of mTOR signaling results in rapid increase of synapse-associated proteins and spine number in the prefrontal cortex [135]. These results strongly support mTOR as a new and promising target in the development of antidepressant drugs.

**ABBREVIATIONS**

- GSK-3β = Glycogen synthase kinase 3
- TCAs = Tricyclic antidepressants
- MAOIs = Monoamine oxidase inhibitors
- SGZ = Subgranular zone of the dentate gyrus of the hippocampus
- BDNF = Brain derived neurotrophic factor
- cAMP = Cyclic Adenosine Monophosphate
- CREB = cAMP response element-binding
- HPA = Hypothalamus-pituitary-adrenal axis
- CRF = Corticotrophin releasing factor
- NSC = Neural stem cells
- LTP = Long term potentiation
- SNRI = Serotonin-noradrenaline reuptake inhibitor
- SSRI = Serotonin selective reuptake inhibitor
- 5-HT = Serotonin
- GPCR = G protein-coupled receptors
- PKA = Protein kinases A, B or C
- PGE₂ = Prostaglandin E2
- PI₃-K = Phosphatidylinositol 3-kinases
- PLCβ = Phosphoinositide phospholipase C
- FAAH = Fatty acid amide hydrolase
- CB₁ = Cannabinoid receptor 1
- PFC = Prefrontal cortex
- [³⁵S]GTPγS = Guanosine 5’(γ-thio)triphosphate, [³⁵S]GTPγS
- OB = Olfactory bulbectomized
- AC = Adenyl cyclase
- FST = Forced swimming test
- NK₁ = Neurokinin 1
- NMDA = N-Methyl-D-aspartic acid receptor
- mTOR = Mammalian target of rapamycin
- AC = Adenylyl cyclase
- BDNF = Brain derived neurotrophic factor
- LTP = Long term potentiation
- CREB = cAMP response element-binding
- mTOR = Mammalian target of rapamycin
- NMDA = N-Methyl-D-aspartic acid receptor
- CB₁ = Cannabinoid receptor 1
- 5-HT = Serotonin
- SSRI = Serotonin selective reuptake inhibitor
- SNRI = Serotonin-noradrenaline reuptake inhibitor
- GPCR = G protein-coupled receptors
- PKA = Protein kinases A, B or C
- PGE₂ = Prostaglandin E2
- PI₃-K = Phosphatidylinositol 3-kinases
- PLCβ = Phosphoinositide phospholipase C
- FAAH = Fatty acid amide hydrolase
- CB₁ = Cannabinoid receptor 1
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- NK₁ = Neurokinin 1
- NMDA = N-Methyl-D-aspartic acid receptor
- mTOR = Mammalian target of rapamycin

**CONFLICT OF INTEREST**

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