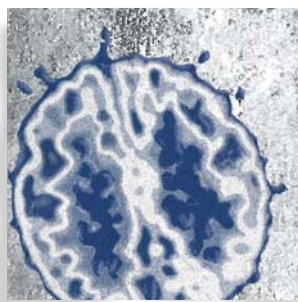


Cellular and molecular mechanisms in the long-term action of antidepressants

Giorgio Racagni, PhD; Maurizio Popoli, PhD



The hypotheses on the pathophysiology of depression /mood disorders and on antidepressant mechanisms have greatly changed in recent years. The classical monoamine hypothesis was revealed to be simplistic, in that it could not explain the temporal delay in the therapeutic action of antidepressants. Converging lines of evidence have shown that adaptive changes in the several mechanisms of neuroplasticity are likely to be the cellular and molecular correlates of therapeutic effect. In this article, several mechanisms of neuroplasticity are analyzed in relation to the mechanism of antidepressants, ranging from changes in gene expression (including neurotrophic mechanisms), to synaptic transmission and plasticity, and neurogenesis. We propose that the current version of the hypothesis of antidepressant mechanism simply be called the "hypothesis of neuroplasticity." In the final section, we also briefly review the main current novel strategies in the pharmacology of depression and the new putative targets for antidepressants, with particular emphasis on non-monoaminergic mechanisms.

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Keywords: mood disorder; depression; antidepressant; neuroplasticity; gene expression; CREB; BDNF; glutamate; NMDA receptor; circadian rhythm

Insufficient knowledge of etiology and pathophysiology of depression

Mood Disorders (MD), particularly major depression, have been estimated to be the fourth major cause of disability worldwide, and may become second only to cardiovascular diseases in the next two decades.¹ A recent consensus document by the European Brain Council estimated the annual cost of MD at 106 billion EUR, with a prevalence of 21 million people across 28 European countries.² Less than 50% of all patients treated with the currently available antidepressants show full remission. However, despite the clear need for better therapies, recent efforts to develop novel antidepressants have been relatively unsuccessful (for discussion see ref 3). A main reason for this is the still-incomplete knowledge of the pathogenetic mechanisms of depression and understanding of antidepressant mechanisms. At the same time, although several animal models have been developed, a model that replicates the etiological factors causing depression in humans, and consequently the symptoms as well, is lacking.⁴ An analysis of the present knowledge of the pathogenetic mechanisms of depression is beyond the scope of this article; therefore this issue will only be briefly addressed when necessary. The

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Translational research

Selected abbreviations and acronyms

AMPA	<i>alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</i>
BDNF	<i>brain-derived neurotrophic factor</i>
CREB	<i>cAMP-response element binding protein</i>
LTP	<i>long-term potentiation</i>
MAOI	<i>monoamine oxidase inhibitor</i>
NMDA	<i>N-methyl-D-aspartic acid</i>
SNRI	<i>serotonin and noradrenaline reuptake inhibitor</i>
SSRI	<i>selective serotonin reuptake inhibitor</i>
TCA	<i>tricyclic antidepressant</i>

main goal of this work is an update of current knowledge of mechanisms of antidepressant drugs, with particular relevance to neuroplasticity. For this purpose, in the following paragraph we report a brief pharmacological classification of currently available antidepressants, based on their primary mechanism of action. Then, we will address the evolutionary process of antidepressants and relevant pharmacological research, from the first version of the monoamine hypothesis to the present hypothesis of neuroplasticity. With regard to the present knowledge of neuroplasticity mechanisms we will analyze how antidepressants impact on distinct levels of these mechanisms, ranging from postreceptor signaling cascades to the regulation of gene expression and synaptic mechanisms. Finally, we will briefly analyze future directions in psychiatric pharmacological research and possible strategies for exploring new targets for antidepressants.

Currently available antidepressants

Brief classification of antidepressants

We arbitrarily classify antidepressants into first- and second-generation drugs (*Figure 1*). First-generation antidepressants (FGAs) include monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), which became available for therapy in the 1960s. MAOIs, such as iproniazide or tranylcypromine, are irreversible inhibitors of the main metabolic enzymes of the monoamine neurotransmitters noradrenaline (NA), serotonin (5-HT), and dopamine (DA), and result in a generalized increase of monoamine levels throughout the central nervous system (CNS).^{5,6} MAOIs are powerful drugs as to their therapeutic efficacy, but their use has been limited by the pronounced and potentially lethal adverse effects, including hypertensive potential. TCAs, intro-

duced shortly after MAOIs, are a variegated class of drugs, named after their chemical structure derived from phenothiazines, including such drugs as imipramine, clomipramine, and amitriptyline. The main pharmacological mechanism of TCAs is the inhibition of membrane transporters for the monoamines, with more or less selectivity, changing from one to the other. TCA treatment results in increased extracellular availability of monoamine neurotransmitters. These are also efficient drugs, and have represented the mainstay of pharmacological therapy of depression for decades, although characterized by a wide profile of adverse effects, mainly owing to variable antagonism for muscarinic, adrenergic, and histaminergic receptors. The mechanism of MAOIs and TCAs represented the main evidence for the monoamine hypothesis of depression and MD, an intrinsically tautological hypothesis which, nevertheless, has driven pharmacological research on depression for over four decades.^{7,8}

Second-generation antidepressants (SGAs) include several different classes of drugs that were developed mainly in the 1980s and 1990s, starting with selective serotonin reuptake inhibitors (SSRIs) and including serotonin and noradrenaline reuptake inhibitors (SNRIs), noradrenaline reuptake inhibitors (NARIs), noradrenergic and specific serotonergic antidepressants (NaSSAs) and 5-HT_{2A}

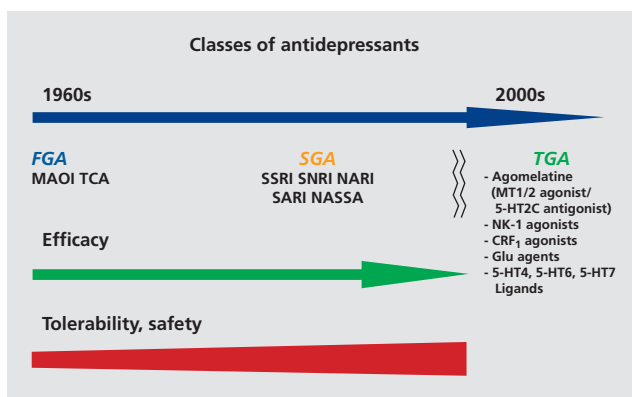


Figure 1. Main classes of antidepressant drugs from the 1960s to present times. FGA, first-generation antidepressant; SGA, second-generation antidepressant; TGA, third-generation antidepressant (only the main classes of antidepressants in development are reported among TGAs, see also *Table III*). MAOI, monoamine oxidase inhibitor; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and noradrenaline reuptake inhibitor; NARI, noradrenaline reuptake inhibitor; SARI, serotonin 2_A antagonist/ reuptake inhibitor; NASSA, noradrenergic and specific serotonergic antidepressant; MT, melatonin; 5-HT, serotonin; NK, neurokinin; CRF, corticotropin-releasing factor; Glu, glutamate

antagonists/ reuptake inhibitors (SARIs). All the SGAs are based on the monoamine hypothesis, with a primary mechanism consisting of monoamine reuptake inhibition and/or antagonism for selected monoamine receptor(s). SSRIs, including fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, and the recent addition escitalopram, have largely been substituted for TCAs in clinical therapy, owing to a more favorable profile of adverse effects. SNRIs (venlafaxine and duloxetine), NaSSAs (mainly mirtazapine), and NARIs (reboxetine) are also considered as primary choices for treatment of depression. However, although most of the SGAs are superior to TCAs with regard to adverse effects, none of them has offered a substantial improvement in efficacy over TCAs, and SGAs are considered at best comparable to TCAs in this respect (*Figure 1*). For a detailed discussion on the mechanism of action of the different drug classes see ref 8. Finally, in our classification we call third-generation drugs (TGAs) novel compounds that are in most cases characterized by nonmonoaminergic mechanisms (although some of these have been in development for quite a while). TGAs will be analyzed in the last chapter of this article, dealing with new targets for the development of antidepressants.

Monoamine hypothesis of depression: inconsistencies

As addressed above, the monoamine hypothesis of depression and mood disorders was mainly based on the mechanism itself of the first antidepressant drugs, MAOIs and TCAs. Additional evidence was based on the prodepressive effect of the antihypertensive reserpine, which depletes storage vesicles containing noradrenaline and other monoamines. The basic version of the hypothesis stated that depression was due to reduced availability of monoamines, particularly noradrenaline and serotonin, and that antidepressants exerted their therapeutic action by increasing the extracellular availability of monoamines, particularly at synaptic level.⁹ However, the hypothesis was soon criticized because it was evident that increased availability of monoamines, due to inhibition of reuptake or metabolism, developed in a matter of hours, could not be the direct mechanism of the therapeutic effect, which develops only after several weeks. Therefore, in the following decades, with the progress of pharmacological research, updated versions of the hypothesis have followed, as schematized in the following section.

Evolution of antidepressants

The monoamine hypothesis has much evolved from the 1960s to present times, along with the revolutionary changes that have affected the neurosciences (*Table I*). Part of the increased knowledge of intracellular, gene expression, and synaptic mechanisms has been incorporated into the hypothesis, contributing to building up its present version. However, it is the opinion of these authors that pharmacological research on psychiatric disorders has still insufficiently taken advantage of the translational opportunities offered by the present state of neuroscience research, and that this is one of the reasons for the present lack of new drugs in psychiatry (for a discussion of this issue see refs 3,10).

In order to explain the discrepancy between the timing of the primary pharmacological action of antidepressants and therapeutic effect, early changes to the monoamine hypothesis took into account the sensitivity of monoamine receptors. It was shown that a consistent change induced by TCAs was the desensitization of the β -adrenoceptor, and consequently it was suggested that changes in the sensitization state of this and other receptors, rather than increased monoamine availability per se, was a correlate of therapeutic efficacy.^{11,12} In parallel, it was suggested that the sensitivity of monoamine receptors was also involved in the pathophysiology of depression. The most refined example of this stage of the hypothesis was the explanation of the action of SSRIs, largely based on a number of studies by the de Montigny

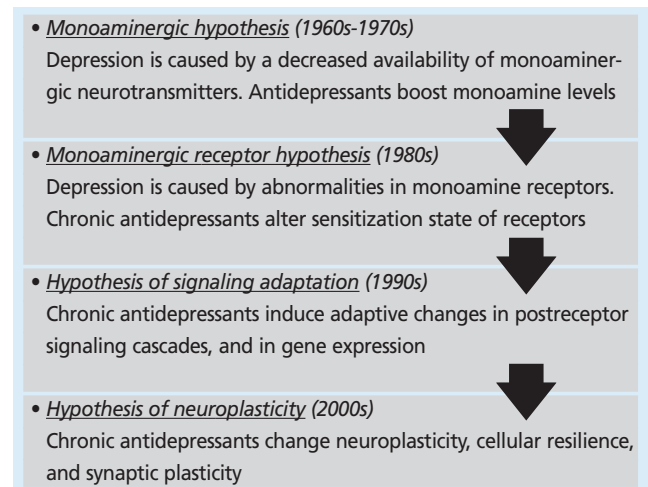


Table I. Evolution of hypotheses on the pathophysiology/pharmacotherapy of mood disorders.

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group, with the opposite changes induced by acute and chronic drug treatment in the sensitization of 5-HT_{1A} receptors and consequently in the firing rate of serotonergic neurons originating in the raphe nuclei.⁷ This evidence-based scheme proposed that desensitization of 5-HT_{1A} receptors and increased firing rate of serotonergic neurons during treatment was a correlate of therapeutic action. However, although satisfactory for SSRIs, this framework could not explain the action of other antidepressants. Additionally, the time required for the receptor sensitivity changes was still not long enough to account for the several weeks required for the onset of action of most antidepressants. At the same time, during the 1980s, the knowledge of postreceptor signaling mechanisms was progressing at a fast pace. Once these mechanisms were understood and described better, it was proposed that slow adaptive changes in postreceptor signaling cascades and downstream mechanisms could be more appropriate mediators of the delayed action of antidepressants,¹³ with changes in gene expression representing plausible downstream effectors of this action (*Table I*). The present and updated version of the hypothesis, which we call the “hypothesis of neuroplasticity,” integrates postreceptor intracellular signaling cascades with the mechanisms of gene expression (including epigenetic mechanisms) and several other processes, including synaptic mechanisms, neurotrophic mechanisms, and neurogenesis. We think this is the best definition at present, because neuroplasticity nicely encompasses all the mechanisms that have been linked to the action of anti-

depressants (including neurotrophic pathways). See *Table II* for a definition of molecular/cellular neuroplasticity. An important corollary of this hypothesis is that neuroplasticity can be advantageous, such as that induced by some antidepressants,¹⁴ but can also be maladaptive, such as that recorded in human brain studies with depressed patients or in animal models of stress and mood disorders.¹⁵⁻¹⁷ An additional interesting concept, which is also receiving experimental validation, is that maladaptive plasticity contextual to the pathological state can at least partly be reversed by antidepressant treatments.¹⁸

Mechanisms of neuroplasticity and the action of antidepressants

What is the meaning of neuroplasticity? Neurobiologists call neuroplasticity the complex of the several processes whereby the brain senses, adapts, and responds to external and internal stimuli of various nature. We address here only molecular and cellular forms of neuroplasticity, which can be both structural and functional in nature; the manifestations of neuroplasticity under both these respects can assume many forms. We have schematically divided these forms into three major categories (listed in *Table II*): (i) modifications of gene expression; (ii) modifications of synaptic transmission; (iii) neurogenesis.

Modifications of gene expression: the role of CREB

As addressed above, throughout the 1980s and 1990s the research on the mechanism of antidepressants has moved from the study of monoamine neurotransmitter levels and sensitization state of membrane receptors to that of postreceptor intracellular signaling pathways. It has been shown that stimulation or inhibition of selected receptors for serotonin and noradrenaline induces adaptive changes in signaling pathways downstream of the receptors, including extensive crosstalk between pathways. In addition, many pathways are also activated by Ca²⁺-channels, glutamate receptors, and receptors for neurotrophins (*Figure 2*). A common downstream function of these intracellular pathways is the regulation of gene expression, through the activation of protein families called transcription factors, that bind to specific domains in the promoter region of genes and regulate mRNA transcription. In this context, the most thoroughly studied factor, both in basic and psychopharmacological

- Modifications of gene expression
 - Activation of signaling cascades
 - Activation of transcription factors
 - Epigenetic changes
 - Activation/repression of different genes
- Modifications of synaptic transmission (synaptic plasticity)
 - Synaptogenesis
 - Alterations of dendritic function
 - Neurite extension
 - Synaptic remodeling
 - Long-term potentiation (LTP)
 - Long-term depression (LTD)
- Neurogenesis

Table II. Major cellular/molecular manifestations of neuroplasticity in the adult brain. Neuroplasticity is the complex of many processes whereby the brain senses, adapts, and responds to external and internal stimuli of various nature.

research, is the protein cAMP-response element binding protein (CREB). CREB function is involved in a wide range of brain mechanisms, including learning and memory, induction of neurotrophic programs, outgrowth of neuronal processes, regulation of circadian rhythms, neurogenesis, pathophysiology of neuropsychiatric and neurodegenerative disorders, and mechanisms of psychotropic drugs.¹⁹⁻²³ CREB is regulated in multiple ways, including acetylation, ubiquitination, glycosylation, and SUMOylation, but the best known form of regulation is represented by phosphorylation at the Ser133 residue by multiple protein kinases.^{18,24-26} There is general agreement

that chronic antidepressant treatments stimulate CREB function, although different results have been reported (see below). It has been shown that, rather than cAMP-dependent pathways, other signaling cascades work as major regulators of CREB function in the brain. In fact, activity-dependent phosphorylation of CREB at Ser133 was shown to be induced in neurons by activation of the Ras-mitogen activated protein (MAP) kinase and the calcium/calmodulin (CaM)-dependent cascades²⁷⁻³⁰ (for discussion see ref 25). Furthermore, it was recently shown that chronic antidepressant treatments significantly activate ERK-MAPK and CaM kinase IV cascades and at

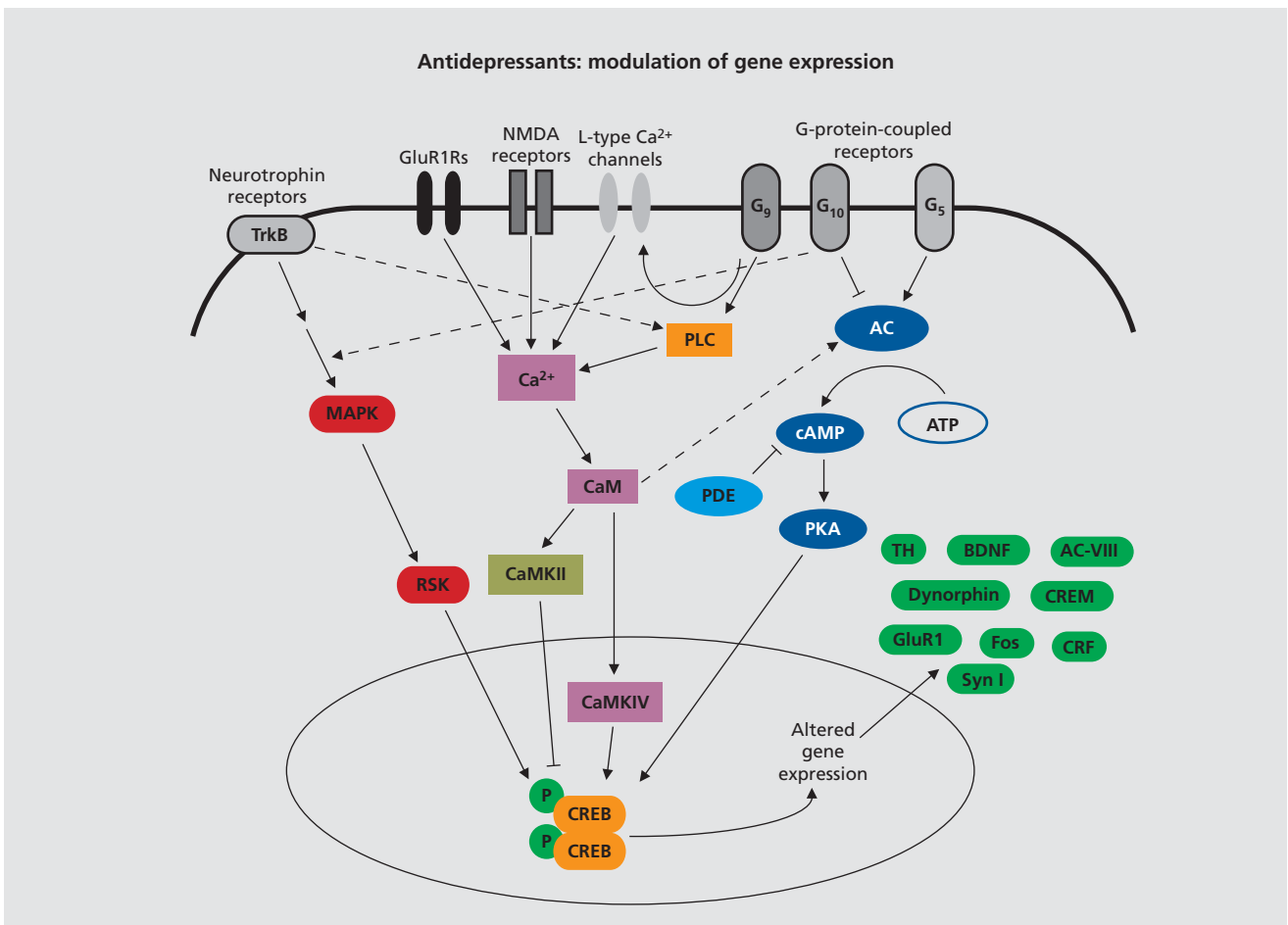


Figure 2. Major signaling cascades involved in the activation of the transcription factor CREB and in the long-term action of antidepressants. A number of genes are depicted, whose transcription is regulated by CREB. Trk B, tyrosine kinase B; MAPK, mitogen-activated protein kinase; RSK, ribosomal S6 kinase; CREB, cAMP response element binding protein; NMDA, N-methyl-D-aspartic acid; Glu, glutamate; PLC, phosphatase C; PDE, phosphodiesterase; AC, adenylate cyclase; cAMP; cyclic adenosine monophosphate; ATP, adenosine triphosphate; PKA, protein kinase A; TH, tyrosine hydroxylase; BDNF, brain-derived neurotrophic factor; AC-VIII, adenylyl cyclase type VIII; CREM, cAMP-responsive element modulator; CRF, corticotropin-releasing factor, Syn I, synaptophysin

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the same time induce CREB phosphorylation, while chronic lithium downregulates CREB phosphorylation as well as CaM kinase IV expression and activation in hippocampus.^{31,32}

By contrast, activation of CREB in the nucleus accumbens and other regions by substances of abuse or stress mediates some aspects of drug addiction and depressive/anxiety behaviors.²³ Other transcription factors of primary importance, although less characterized compared with CREB in the mechanism of antidepressants, are the Fos family and NF- κ B.²⁶

It has been suggested that activation of multiple signaling cascades impinging on CREB is required for induction of persistent changes in gene expression.^{33,34} This mechanism could be a way of signaling stimuli of greater significance, deserving to leave a more persistent trace in gene expression and cellular function. We have recently asked whether this notion may apply to the action of antidepressants, by analyzing the time course of activation of multiple signaling cascades and of CREB phosphorylation after antidepressant treatments. Indeed, in our experience CREB activation and expression of a CREB-regulated gene (brain-derived neurotrophic factor, BDNF) seemed to be stronger when multiple signaling cascades were activated early and at the same time during treatments (Musazzi et al, unpublished material). There are more than 100 identified genes regulated by CREB. Among them are such diverse genes as tyrosine hydroxylase (the rate-limiting enzyme in catecholamine biosynthesis), the GluR1 subunit of AMPA receptor for glutamate, the presynaptic protein synapsin I, the neuropeptide corticotropin releasing factor (CRF), BDNF, and many others (*Figure 2*).

Modifications of gene expression: the role of BDNF

BDNF, along with its receptor TrkB, has been widely studied as a gene involved in the regulation of neuroplasticity and cognition, as well as susceptibility to various neuropsychiatric disorders, including Alzheimer's disease, schizophrenia, bipolar disorder, and attention deficit-hyperactivity disorder. Among the CREB-regulated genes, BDNF is by far the one most thoroughly studied with regard to the mechanism of antidepressants and has lately become, together with CREB activation, a sort of readout system in the study of antidepressant mechanisms.^{18,25,35-42} A result of this huge body of research in the last several years is the "neurotrophin hypothesis of

depression," which postulates that a decrease in the levels of BDNF plays a major role in the pathophysiology of depression (and possibly other neuropsychiatric diseases), and that restoration of its levels may represent a critical component in the mechanism of antidepressants.^{38,39,43,44}

Now, while there is no doubt that BDNF may have a primary role, we find it limiting to restrict the present definition of the hypothesis to the neurotrophic effect, because this does not cover all aspects of neuroplasticity. For this reason we prefer to define the present state of the hypothesis on depression and antidepressant mechanisms "hypothesis of neuroplasticity," as addressed above. The neurotrophic hypothesis is based largely on evidence showing that stress and depression-related behavior are associated with reduction of BDNF expression, and that conversely antidepressant treatments increase BDNF expression.³⁸ However, several observations not consistent with this simple framework have been reported: (i) certain stress paradigms have been found to increase BDNF expression or to induce complex patterns of regulation⁴⁵⁻⁴⁸; (ii) many inconsistent data were produced in studies with antidepressant treatments (for a discussion see ref 25); (iii) partial knockout of BDNF in mice did not produce depression-like behavior, but rather reduced response to antidepressants⁴⁹; (iv) BDNF was shown to exert opposing roles in hippocampus/cortical areas vs nucleus accumbens/ventral tegmental area. It was clearly shown that in these latter areas of the brain reward system BDNF (as addressed above for CREB) has a prodepressive action. An elegant study by the Nestler group, using viral-mediated, mesolimbic dopamine pathway-specific knockdown of BDNF, showed that BDNF is required for the development of depressive-like behavior induced by chronic social stress. Effects similar to local knockdown of BDNF were obtained with chronic administration of fluoxetine or imipramine.⁵⁰ Complementary, a recent study showed that knockdown of BDNF in hippocampal dentate gyrus (but not CA1) attenuates the behavioral response to antidepressants, without inducing depressive-like behavior.⁴⁹

Taken together, these studies suggest that: (i) BDNF may have anti- or prodepressive function, depending on the brain areas and circuits; therefore a general increment of its levels or function in the brain could have nonspecific and undesired effects; (ii) the involvement of BDNF in (a) pathophysiology and (b) mechanism of antidepressants, are not necessarily in a simple and direct relationship; behavioral and neurovegetative alterations linked

to the depressive state are likely to require impairment in multiple systems and pathways and the BDNF-TrkB is probably one of the involved pathways, but not the “essential pathway,” as implied by the lack of depressive-like behavior in dentate gyrus BDNF knockdown; (iii) instead BDNF, in the same mice,⁴⁹ seems to be necessary for mediating antidepressant responses. In other words, even though BDNF is not an essential factor in inducing depression, potentiation of its function could be essential for antidepressant mechanism.³⁹

Modifications of gene expression: the regulation of BDNF transcription

The BDNF gene has a complex structure that underscores its potential for regulation. According to the available updated nomenclature, the gene encompasses at least eight noncoding 5' exons that can be spliced to a single 3' exon containing the coding domain for the BDNF protein, generating 11 different transcripts according to the last studies. The previous nomenclature of BDNF transcripts (exons I to V) in the literature cited below has been translated here to the updated nomenclature.⁵¹ The regulation of promoter in exon IV has been extensively characterized.^{21,52} The functional difference among the different BDNF transcripts has not been widely explored thus far but, being among those genes whose transcripts are translocated to different cellular compartments, the delivery of different transcripts may subserve the availability of the message at cell soma, dendrites, axons, according to the needs of plasticity.⁵³ Exon V-containing transcript has been detected in both soma and dendrites, while exon IV-containing transcript expression was found to be limited to the cell body.⁵⁴ A number of studies have analyzed the expression of exons I, II, IV, and V (in the updated nomenclature) in relation to antidepressant treatments, physical exercise, and stress paradigms (reviewed in refs 25, 39). Interestingly, chronic defeat stress, a model of depression, has been shown to downregulate in mouse hippocampus the expression of BDNF IV and V transcripts, by inducing increased repressive histone methylation at respective promoters.⁵⁵ Chronic imipramine treatment reversed this downregulation and increased histone acetylation at these promoters, a modification associated with chromatin decondensation and facilitation of gene transcription, underscoring the role of epigenetic mechanisms in stress response and antidepressant mechanisms.

Recently, we have analyzed for the first time the complete pattern of expression of the several BDNF transcripts after treatment with two different antidepressants, fluoxetine and reboxetine, as an attempt to identify molecular signatures of different drugs. In hippocampus, fluoxetine induced BDNF III and IXa and downregulated IV; reboxetine induced VI and IXa and downregulated I and IV. The main difference between the drugs was that fluoxetine selectively induced BDNF III and reboxetine VI. In prefrontal/frontal cortex fluoxetine induced transiently (first 2 weeks) BDNF I and VI, and persistently III and IXa, while it downregulated IV; reboxetine also induced III and IXa. The main difference here was that fluoxetine, in addition to the same two transcripts induced by reboxetine, transiently induced exons I and VI and downregulated IV (Musazzi et al, unpublished data). Further work should investigate whether these differences may represent molecular signatures of distinct drugs.

Synaptic transmission and plasticity: mechanisms of antidepressants

Synaptic plasticity encompasses all forms of neuroplasticity that specifically occur at synapses; both functional and structural forms of plasticity have been described (*Table II*). In many cases this term is referred to activity-dependent modifications of the strength or efficacy of synaptic transmission at glutamate synapses; the most common forms of long-lasting activity-dependent changes in synaptic strength are long-term potentiation (LTP) and long-term depression (LTD).⁵⁶ It has been repeatedly shown that both stress and antidepressant treatments change synaptic plasticity (reviewed in refs 3,18,57,58).

Beyond the monoamine hypothesis: the role of glutamate

Recent neuroimaging and histopathological studies in brain of depressed and bipolar patients revealed the presence of morphometric/functional modifications, including ventricular enlargement, hippocampal and cortical volumetric reduction, and of reduced neurons and glial density.⁵⁹⁻⁶¹ In many of the areas implicated, glutamatergic neurons and synapses predominate, suggesting an involvement of glutamate neurotransmission in the pathophysiology of mood disorders. Indeed, in the last few years numerous lines of evidence have accumulated in favor of a role for glutamate in psychiatric pathophysiology, including the fol-

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lowing: (i) higher levels of glutamate in plasma and brain of patients with mood disorders^{62,63}; (ii) abnormal elevation of glutamate neurotransmission and glutamate levels in cortical/limbic brain areas of depressed patients^{16,64}; (iii) atrophy of apical dendrites in CA3 hippocampal neurons induced by chronic stress, a major factor in pathogenesis of mood disorders¹⁷; (iv) increased amplitude and reduced decay kinetics of NMDA current induced by chronic stress⁶⁵; (v) impaired long-term potentiation (LTP) and facilitated depression (LTD) induced by stress.⁶⁶ Conversely, antidepressant treatments were also shown to affect glutamate neurotransmission: (i) antidepressants downregulate NMDA receptor subunits and dampen NMDA function⁶⁷; (ii) antidepressants may overcome the effects of stress on LTP⁶⁸⁻⁶⁹; (iii) chronic antidepressants reduce depolarization-evoked release of glutamate in hippocampus by modifying presynaptic protein interactions regulating exocytotic release.⁷⁰ Several compounds that modulate glutamate receptors or glutamate neurotransmission at various levels are under development for the treatment of mood disorders (depression, bipolar disorder, anxiety).⁷¹ Some of these putative drugs may work by stabilizing glutamate release when its synaptic level becomes too high, a feature that is now considered as part of the pathophysiology of mood disorders.^{3,15,58,72,73} Recently, it has been suggested that the effect of antidepressants on glutamate transmission may also be mediated by increased AMPA to NMDA throughput in critical neuronal circuits; this action was suggested to be involved in the rapid antidepressant effect of a single ketamine infusion.⁷⁴ However, whereas the studies above, as a whole, strongly suggest that plasticity changes in glutamatergic synapses are involved both in the pathophysiology of stress-related diseases and in the action of therapeutic drugs, little is known as to the cellular and molecular mechanisms involved. In particular, most of the drugs currently used for therapy of affective disorders are based on monoaminergic mechanisms, although for some of them a direct effect on NMDA receptor has been claimed.⁶⁷ Knowledge of the mechanisms whereby drugs interfere with the function of the glutamatergic synapse would be of great help in the design of new drugs and therapies.

Synaptic plasticity: the action of antidepressants on LTP

It has been repeatedly shown that different experimental stress protocols (both acute and chronic) impair hip-

poampal synaptic plasticity, measured as amount of LTP, the main cellular model of synaptic plasticity. There is ample literature on this topic, and the reader is addressed to the numerous reviews available.^{18,58,75} However, the prevalent effect of antidepressants has also been shown to be a reduction of hippocampal LTP, after acute^{68,76-78} or chronic administration.⁷⁹⁻⁸² It has been speculated that antidepressants may induce an LTP-like process which saturates hippocampal synaptic plasticity, so that capacity for further synaptic change is reduced^{83,84}; discussed in ref 58). Interestingly, it has been showed that acute administration of antidepressants (fluoxetine, imipramine, tianeptine) may reestablish LTP after acute stress.^{66,67,85} Recently it was shown that the action of tianeptine (but not of imipramine) could be linked to reversal of stress-induced down-regulation of MEK/ERK-MAPK signaling cascade and activation of Ser831-GluR1 phosphorylation.⁸⁶ However, it is difficult to relate the acute effect on LTP to the therapeutic action of chronic antidepressants; it will be interesting to assess how chronic treatments affect stress-induced impairment of LTP.

Presynaptic mechanisms: the action of antidepressants

Another neuroplasticity-related problem is the effect of stress and antidepressants on the presynaptic release of glutamate. Many studies have shown that different paradigms of stress, or corticosterone administration, induce a rapid and transient increase of extracellular glutamate in prefrontal cortex and hippocampus.⁸⁷⁻⁸⁹ However, in all these studies the outflow of glutamate was measured by *in vivo* microdialysis, a technique that cannot distinguish between exocytotically released glutamate and metabolic glutamate.⁹⁰ For this reason it has been difficult to relate exactly the effect of stress to exocytotic glutamate release.

We have recently approached the problem by measuring the depolarization-evoked release of glutamate from freshly purified synaptic terminals (synaptosomes) in superfusion.⁷⁰ First, we reported that chronic (not acute) treatment with antidepressants endowed with different primary mechanisms markedly and significantly reduces depolarization-evoked release of glutamate, but not release of GABA, from hippocampal synaptosomes. Interestingly, treatment with the drugs above did not change the release of glutamate (and GABA) induced by ionomycin, a calcium ionophore that, contrary to K⁺

depolarization, does not selectively affect the readily releasable pool of vesicles (RRP). Therefore, our results suggest that antidepressant treatments particularly affect the release of glutamate from the RRP, thereby altering a physiologically relevant pool of neurotransmitter.^{3,70} Looking for molecular underpinnings of this effect, we found changes in selected protein-protein interactions regulating the formation of the core presynaptic 7S SNARE protein complex, that mediates the fusion of synaptic vesicles, and a reduction of SNARE complexes in synaptic membranes (that contain the RRP). These results suggested that one of the modes of action of antidepressants is a stabilization of glutamate release, that could improve the signal to noise ratio in glutamate neurotransmission, when it becomes compromised by an excessive release due to the action of stress-related mechanisms (iii). As a result, glutamatergic neurotransmission will be selectively inhibited (release of GABA was not affected by antidepressants); release of glutamate evoked by neuronal activation will be decreased in the face of unchanged constraint exerted by GABA. This would induce a marked alteration in the balance between excitatory and inhibitory neurotransmission, contributing to dampening excessive neuronal activation following stressful stimuli.⁹¹ Our observation that these effects are measurable only after repeated drug administration is also in line with the well-known property of these drugs of being therapeutically efficient only after chronic treatment.⁸ We suggest that the remarkable effect of traditional antidepressants on depolarization-evoked glutamate release in basal conditions could be linked to the restorative action of these drugs on synaptic plasticity in hippocampus (HC) and hippocampal/prefrontal cortex circuits.^{68,69}

Stress-induced glutamate release: a protective action of antidepressants?

In order to test whether this mechanism is involved in the response to stressful events, we subjected the animals to a standard footshock (FS) stress protocol, similar to that used to induce learned helplessness, a widely used animal model of depression,⁹² and immediately after the stress session measured depolarization-evoked release of glutamate from synaptosomes of prefrontal/frontal cortex (P/FC), obtained from both vehicle and 2-week antidepressant-treated rats.⁹³ We found that FS stress induces a marked and significant (30% to 50%) increase of glutamate

release from P/FC synaptosomes, and that acute FS stress induces accumulation of 7S SNARE complexes in the synaptic membranes (containing the RRP of glutamate vesicles), a finding in line with increased efficiency of glutamate release. Previous chronic treatment with different antidepressants (fluoxetine, desipramine) completely abolished the effect of stress on glutamate release (Musazzi et al, unpublished data). The molecular underpinnings of this drug effect are currently being investigated. Therefore, based on these combined data, we speculate that modulation of stress-induced release of glutamate may be a component in the therapeutic mechanism of antidepressants in both depression and anxiety.

Postsynaptic glutamate receptors: action of antidepressants

Converging evidence suggests that the functional interplay between NMDA and AMPA glutamate receptors in cortical and limbic areas is involved in both the pathophysiology of mood disorders and in antidepressant mechanisms.⁷²⁻⁷⁴

The two types of ionotropic glutamate receptors are often colocalized on the same individual dendritic spines. It has been clearly demonstrated that the induction of LTP in the hippocampal CA1 region requires activation of NMDA receptors, which leads to calcium influx and activation of downstream signaling. This in turn favors the recruiting of AMPA receptors to the postsynaptic membrane, a change that is thought to mediate the expression of LTP.⁵⁶ Several preclinical studies have shown that chronic treatment with different antidepressants induces a reduction in the function or expression of the NMDA receptor. Since the early reports on the antidepressant action of amantadine, various antidepressants, including imipramine and citalopram, have been shown to bind to and inactivate the glycine-binding site of NMDA receptors.⁹⁴ Likewise, functional antagonists of the NMDA receptor were shown to induce behavioral changes similar to antidepressants in preclinical screening tests. Traditional antidepressants have been shown to produce time- and dose-dependent changes in the radioligand binding properties of rat brain NMDA receptors, but it is not clear if this is due to downregulation of receptors, because changes in mRNA expression of NMDA subunits have been only shown in mice.⁹⁵ We have recently investigated this issue and found that chronic fluoxetine and reboxetine induce in rat hip-

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pocampus downregulation of NR1 (the main subunit of NMDA receptor) only locally at synapses, with no changes in total expression.⁹⁶ The same result was found with escitalopram in a genetic animal model of depression.⁸² Therefore, it seems that antidepressant-induced changes in NMDA receptors are more likely to be found at synaptic level.

On the other hand, several lines of evidence support the view that increasing the function of AMPA receptors may result in antidepressant action. First, it has been shown that AMPA receptor activation increases the expression of BDNF (which is a mediator of antidepressant action, see above)⁹⁷ as well as stimulating neurogenesis. Chronic antidepressant treatments have been shown to upregulate the membrane insertion of GluR1, GluR2/3 and synaptic expression of GluR1.^{98,99} As a consequence, AMPA receptor potentiators (sometimes called AMPAkinases) have been developed as potential antidepressants (see the final section).

Interestingly, recent clinical studies found a rapid and sustained antidepressant effect (up to 1 week) of a single infusion of the noncompetitive NMDA antagonist ketamine.^{100,101} In a preclinical study Maeng et al¹⁰² showed that in rats the antidepressant effect (measured in the forced swim test) may last for 2 weeks. They also showed that AMPA receptor throughput is required for the antidepressant effect of ketamine, and suggested that enhancement of AMPA to NMDA throughput in critical circuits is the mechanism of rapid antidepressant effect.¹⁰² The use of ketamine for a rapid antidepressant effect has been proposed as a strategy for treatment-resistant depression.⁷⁴ Intriguingly, acute administration of ketamine increases glutamate release, probably by disinhibiting NMDA receptor-containing GABAergic neurons and in turn enhancing the firing rate of glutamatergic neurons. But, as addressed above, the prevalent effect of traditional antidepressants in limbic and cortical areas seems to be a reduction in glutamate release (particularly if measured as a response to stress; Musazzi et al, unpublished data); how could this riddle be solved? Early observations and our preliminary results may suggest that also traditional antidepressants acutely increase the presynaptic release of glutamate and that reduction of glutamate release is an adaptive change which takes time to develop.^{70,103} Therefore, in this hypothesis, at the beginning of treatment traditional antidepressants might transiently increase presynaptic glutamate release in critical circuits (a feature perhaps linked to worsening of symp-

tomatology, eg, anxiety); in the course of treatment, release of glutamate in limbic/cortical areas, such as hippocampus and prefrontal cortex, will be reduced along with a synaptic reduction of NMDA receptor levels. This combined effect would produce a dampening of glutamate transmission and an enhancement of AMPA- vs NMDA-mediated transmission. More work is required to understand if this is the mode of action of traditional antidepressants, and if quicker redistribution of AMPA- vs NMDA-mediated transmission may speed up the onset of antidepressant action.

Finally, metabotropic glutamate receptors (mGlu) are also crucial for the regulation of glutamatergic neurotransmission as well as for other neurotransmitters that are involved in mood disorders and depression. Limited clinical proof of concept for mGlu receptor ligands in the treatment of affective disorders has been achieved. In particular, group II receptor agonists (mGlu2/3) and group I receptor antagonists (mGlu5) have shown activity in animal and/or human conditions of fear, anxiety, or stress.¹⁰⁴

Neurogenesis

The latest addition to the different forms of neuroplasticity was neurogenesis, the generation of new neurons in the adult brain, whose discovery has broken the long-standing dogma that the whole neuronal population in the brain is made up of postmitotic cells.^{105,106} Neurogenesis in adult mammalian brain has been so far described in three areas: the subventricular zone, hippocampal dentate gyrus, and olfactory bulb, although there are reports that it may also occur in cerebral cortex and hypothalamus. It has been estimated that in rodent brain approximately 250 000 new neurons, and about 6% of the granule cell layer, are formed each month.¹⁰⁷ However, in primates this number seems to be much smaller, and it is still debated whether this lower rate of neurogenesis is clinically significant in pathology and in the action of psychotropics. A number of magnetic resonance imaging studies have clearly shown that hippocampal volume may be reduced in depressed patients and that this correlates with recurrence and length of depressive episodes.¹⁰⁸ Although it has been suggested that reduced neurogenesis might be a contributing factor, there is at present no clear evidence supporting this hypothesis. On the contrary the available evidence suggests reduction of neuropil and loss of glial cells as main

factors in the shrinking of hippocampus in depression.¹⁰⁹ However, compelling evidence from preclinical studies showed that different paradigms of stress reduce hippocampal neurogenesis, while antidepressant treatments and interventions that have antidepressant properties, such as physical exercise or environmental enrichment, increase neurogenesis (reviewed in ref 108). To date, the most convincing evidence for a role of neurogenesis in the mechanism of antidepressants was offered by a study in which the knockout of 5-HT_{1A} receptor, or restricted irradiation of the subgranular zone, suppressed neurogenesis and at the same time the behavioral effects of fluoxetine and imipramine in mice.¹¹⁰ Two later studies supported the same conclusion.

Studies in which adult hippocampal neurogenesis was blocked did not show increased anxiety-related behavior or increased susceptibility to the effects of chronic stress, as assayed in preclinical scenes (reviewed in ref 111). For this reason, based on the available evidence, it is likely that neurogenesis in the hippocampus is probably not a major contributor to the etiology of depression, although it may be required for the behavioral effects of antidepressants. Future imaging studies allowing to visualize hippocampal neurogenesis are warranted to understand the role of adult neurogenesis.

Future directions: new targets for antidepressants

As summarized in *Figure 1*, all the available antidepressants are based on acute mechanisms affecting monoaminergic transmission. Although, as addressed above, there is ample evidence that different and converging downstream mechanisms are responsible for therapeutic effect of these drugs, no drug based on non-monoaminergic mechanism has made it to the market so far. The reasons for this are multiple and have been analyzed in recent reviews.^{3,10,112} We believe that four factors have been particularly important for the lack of success in the development of new drugs for psychiatric disorders: (i) lack of adequate diagnostic classification; (ii) lack of adequate animal models; (iii) lack of adequate translational work; (iv) problems in target validation.

First, the present diagnostic and classification system in psychiatry is based on arrays of symptoms, rather than on neurobiology, epidemiology, genetics, or response to treatments. A primary goal in this area is the development of a diagnostic system based on these different

aspects, rather than on the phenomenology of the disease. This is especially timely if one takes into account the recent progress in the knowledge of genetic factors, psychosocial stressors, and most important gene-environment interactions in predisposing for pathology.¹¹³ Second, we still lack adequate animal models of depression and/or anxiety. Most available models are either based on the exposure of “normal” animals to different paradigms of acute or chronic stress, or they are straightforward knockouts for some of the genes that have been involved in depression. Obviously, depressed patients are not gene knockouts; they carry different combinations of gene mutations that (most probably through multiple gene interactions) may combine with adverse life events predisposing for disease. Therefore, what is needed is the development of animal models carrying known human mutations or noncharacterized genetic vulnerability (but with good face, construct, and predictive validity), subjected to validated stress paradigms.^{82,113,114} What seems crucial is to reproduce to some extent the gene-environment interaction that is believed to be central to human depression. Third, there is a lack of sufficient translational efforts applying recent neuroscience research findings and technology to pharmacology and biological psychiatry. In spite of the great development of research on postreceptor signaling cascades, gene expression, epigenetic mechanisms, synaptic plasticity, identification of biomarkers for vulnerability and drug response/resistance by global genomics/proteomics, a large part of current pharmaceutical research is still focused on the stereotype “receptor-ligand” interaction. As a consequence, several recent “novel” drugs in psychiatry are still compounds acting on neurotransmitter receptors or transporters. Although the trend has been changing lately, still a good part of the new basic knowledge needs to be applied to or interfaced with target discovery/validation and clinical research. Fourth, target validation is still one of the main problems in psychiatric pharmacology, because in most cases ultimate validation is missing or may be obtained only when the drug is already on the market. To be added to the previous, a fifth, not strictly scientific, reason was the lack of stimulus from major pharmaceutical companies to take the risks involved in developing new nonmonoaminergic drugs for depression. Differently from other drug fields (eg, cancer, cardiovascular diseases) much of the effort in recent times was directed toward replication and implementation of already known mechanisms (eg, “me-too” drugs).

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However, with all the limitations exposed above, a good number of new compounds are in development. We have listed here only new drug classes that have been in development for some time (some of them for quite a long time) and possibly recent new drugs will be missing here. Most of these compounds are based on peptidergic, glutamatergic or circadian rhythm-related mechanisms, but a few still relate to a monoaminergic mechanism (*Table III*).

NK-1 receptor and CRF-1 receptor antagonists have had a somewhat troubled history. Both drug classes have in turn raised much hope and most companies have had (some still have) these compounds in their pipeline. In the case of NK-1 antagonists, one of them (MK-869) did not separate from placebo in phase II clinical studies and the development was discontinued. However, the hypothesis of using NK-1 antagonists for add-on strategy with SSRIs or SNRIs is still pursued. Antagonists of the CRF1 receptor have also been in development for quite some time. After preclinical development, one of these compounds (R121919) showed antidepressant efficacy in an open-label clinical trial, but later was dropped owing to hepatotoxicity. Other compounds in this class are still in development.

Compounds acting on glutamate transmission represent a large and variegated class of potential antidepressants.⁷¹ As addressed above, the interest in glutamate as a potential target in depression and mood disorder is not new; however, recently this interest was revived by several key findings, such as the many morphological and functional changes found with depression in areas where glutamate transmission predominates, the documented effects of stress on glutamatergic neurons and circuits, the striking sustained antidepressant effect of a single infusion of ket-

amine (see above). The psychotomimetic properties of ketamine are a limit to its clinical use, but similar compounds less endowed with these properties would be interesting drugs that could greatly fasten the onset of action. Weaker NMDA antagonists, such as memantine, or compounds acting on modulatory sites of the NMDA receptor could be viable alternatives to reduce NMDA-mediated throughput. Another possibility is interfering with glutamate release, which could be upregulated after acute or chronic stress; drugs that have a reduction of glutamate release as component of their mechanism of action are already on the market (riluzole, lamotrigine). A feasible strategy of limiting glutamate release could be the use of ligands for metabotropic receptors, that could be safer than compounds directly affecting the machinery of release. AMPAkinases, drugs potentiating the function of AMPA receptors have also been in development for some time. Tianeptine, an antidepressant that has been for some years in the market, has shown unique properties in the regulation of neuroplasticity, and this effect seems to be mediated by its modulation of the glutamatergic system.¹¹⁶⁻¹¹⁹

A novel approach to depression, regulation of circadian rhythms, has been the basis for the development of an antidepressant with an entirely new mechanism of action. Changes in the sleep-wake cycle and in the periodicity of circadian rhythm profoundly influence the state of mood. Sleep disturbances and depression/mood disorders are interlinked.¹²⁰ Among the typical and recurring features of depressed individuals is insomnia with early-morning awakenings; indeed, disturbed sleep is one of the diagnostic criteria in DSM-IV. Likewise, it has been shown that manipulations of circadian rhythms, such as total or REM sleep deprivation or phase advance in the sleep-wake cycle, may have therapeutic action in the treatment of depression.¹²¹ It is not clear whether sleep disturbances are part of the clinical picture of depression or represent a causative factor; some studies have shown that changes in sleep architecture persist into the remission phase, while improvement in clinical state is frequently preceded by sleep changes.^{120,121} The first (and so far only) antidepressant in this class is agomelatine, an agonist of MT₁/MT₂ melatonergic receptors and antagonist of serotonin 5-HT_{2C} receptor. Agomelatine was shown to induce resynchronization of circadian rhythms and to be efficient in preclinical studies with different animal models of depression. The antidepressant efficacy of the drug in humans was positively tested in several clinical trials,¹²²

New nonmonoamine-based antidepressants.

- NK-1 receptor antagonists
- CRF1 antagonists
- Glutamatergic agents (NMDA blockers, AMPAkinases, mGlu modulators, riluzole, lamotrigine)
- Melatonergic (MT_{1/2}) agonist / 5-HT_{2C} antagonist (agomelatine)

New monoamine-based antidepressants

- 5-HT₄ and 5-HT₆ agonists
- 5-HT₇ antagonists

Table III. New antidepressants in development or marketed. NK, neurexin; NMDA, N-methyl-D-aspartic acid; CRF, corticotrophin-releasing factor; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; mGlu glutamate; 5-HT, serotonin

and its regulation of the sleep-wake cycle has been proven.^{123,124} A recent study of long-term (10 months) treatment showed efficacy of agomelatine against placebo, while the percentage of patients reporting adverse effects was similar in the two groups.¹²⁵ Furthermore, it presents clinical benefits such as respect of sexual function, absence of discontinuation symptoms, and no effect on body weight.^{126,127} Agomelatine could be the first antidepressant with a really new mechanism of action to hit the market which will also achieve a better quality of remission by directly acting on the residual symptoms.

Finally, among the novel compounds in development there are also a few monoamine-based putative antide-

pressants, namely agonists or antagonists of the most recently characterized subtypes of serotonin receptors, 5-HT₄, 5-HT₆, and 5-HT₇ (Table II).

Conclusion

Overall, the monoamine hypothesis of depression and antidepressant mechanisms has become, over nearly half a century, a hypothesis of neuroplasticity. We are able today to identify new targets for antidepressants with nonmonoaminergic mechanisms. As a result, there are a good number of such compounds in development, which, in the treatment of mood disorders, gives hope for novel, more effective, and safer antidepressants. □

Mecanismos celulares y moleculares en la acción a largo plazo de los antidepresivos

Las hipótesis acerca de la fisiopatología de los trastornos del ánimo y la depresión, y de los mecanismos antidepresivos han cambiado mucho en los últimos años. La clásica hipótesis monoaminérgica mostró ser simplista, en cuanto a que no podía explicar el retardo temporal en la acción terapéutica de los antidepresivos. Líneas convergentes de evidencia han mostrado que los cambios de adaptación en varios mecanismos de neuroplasticidad son probablemente los correlatos celulares y moleculares del efecto terapéutico. En este artículo se analizan algunos mecanismos de neuroplasticidad en relación con el mecanismo de los antidepresivos, que van desde cambios en la expresión génica (incluyendo mecanismos neurotróficos) hasta la transmisión sináptica y la plasticidad, y la neurogénesis. Se propone que la versión actual de la hipótesis del mecanismo de los antidepresivos sea llamada simplemente la "hipótesis de la neuroplasticidad". En la sección final se revisan brevemente las principales estrategias actuales en la farmacología de la depresión y los nuevos blancos para los antidepresivos, con especial énfasis en los mecanismos no monoaminérgicos.

Mécanismes cellulaires et moléculaires dans l'action à long terme des antidépresseurs

Les hypothèses sur la physiopathologie des troubles dépressifs/de l'humeur et sur les mécanismes antidépresseurs ont bien changé ces dernières années. L'hypothèse classique monoaminérgique s'est révélée simpliste, car elle ne peut expliquer le délai d'action des antidépresseurs. Des preuves convergentes indiquent que plusieurs mécanismes adaptatifs de la neuroplasticité semblent être des corrélats cellulaires et moléculaires de l'effet thérapeutique. Dans cet article, plusieurs mécanismes de neuroplasticité sont analysés en relation avec le mécanisme des antidépresseurs, s'échelonnant entre les modifications de l'expression du gène (y compris les mécanismes neurotrophiques), jusqu'à la neurogenèse, et la plasticité et la transmission synaptiques. Nous proposons que la version actuelle de l'hypothèse du mécanisme antidépresseur s'appelle simplement « l'hypothèse de la neuroplasticité ». Au dernier chapitre, nous revoyons aussi rapidement les principales stratégies actuelles dans la pharmacologie de la dépression et les nouvelles cibles généralement admises des antidépresseurs, en insistant particulièrement sur les mécanismes non monoaminérgiques.

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