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Potential Molecular and Cellular Mechanism of Psychotropic Drugs

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Psychiatric disorders are among the most debilitating of all medical illnesses. Whilst there are drugs that can be used to treat these disorders, they give sub-optimal recovery in many people and a significant number of individuals do not respond to any treatments and remain treatment resistant. Surprisingly, the mechanism by which psychotropic drugs cause their therapeutic benefits remain unknown but likely involves the underlying molecular pathways affected by the drugs. Hence, in this review, we have focused on recent findings on the molecular mechanism affected by antipsychotic, mood stabilizing and antidepressant drugs at the levels of epigenetics, intracellular signalling cascades and microRNAs. We posit that understanding these important interactions will result in a better understanding of how these drugs act which in turn may aid in considering how to develop drugs with better efficacy or increased therapeutic reach.

KEY WORDS: Antipsychotics; Mood stabilizers; Antidepressants; Epigenetics; Intracellular signalling; MicroRNA.

INTRODUCTION

Schizophrenia and mood disorders are chronic and severe psychiatric disorders that affect the lives and functioning of many people worldwide.^{1,2)} There have been some successes in developing pharmacological treatments to treat such disorders that have been generally divided into categories based on their primary clinical response and are thus termed antipsychotics, mood stabilizers and antidepressant drugs. However, these generalised divisions are being challenged by, for example, the use of second generation antipsychotic as effective treatments for bipolar disorder.³⁾ The clinical use of psychotropic drugs in the treatment of psychiatric disease has been occurring for decades, mechanisms of action by which the drugs induce their therapeutic benefits are far from being fully defined. This lack of understanding is inhibiting efforts to identify why current drugs are only partially effective, having high rates of relapse (which may be

due to non-compliance due to side-effects), poor impact on cognitive and functional impairment all of which result in ongoing psychosocial disability and diminished well-being.⁴⁻⁶⁾ Important components of the mechanism of action of available psychotropic drugs are their ability to modulate a number of intra-cellular pathways and in this regard, the understanding of the molecular and cellular pathways that are modulated by current drug is necessary to aid in consideration as to how to develop new drugs with few side-effects, improved efficacy and broader therapeutic reach. Thus, in this review, we discuss the direct and indirect molecular mechanisms of antipsychotics, mood stabilizers and antidepressant drugs on the critical markers of gene expression. These markers include DNA methylation, histone modification (epigenetics), intracellular signalling pathways and post-transcription (microRNA) processes.

MAIN SUBJECTS

Epigenetics

Epidemiological research suggests that the aetiology of psychiatry disorders is underpinned by interaction between genes and the environment.^{7,8)} Epigenetics is the study of potentially heritable changes in levels of gene expression that occur without a change in the DNA sequence and involve mitotically-heritable factors, such as DNA

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methylation and histone modification.⁹⁾ DNA methylation changes the appearance and structure of DNA and interferes with the transcriptional machinery by cytosine methylation, specifically on cytosine-phosphate-guanine (CpG) island.^{10,11)} This is because CpG methylation inhibits transcription factors requiring cytosine/guanine (CG)-rich sites to bind to the DNA or facilitates the assembly of transcription repressor complexes that contain histone deacetylases and methylases that mediate chromatin remodeling-activates.¹²⁾ At the most basic level, a cytosine that is followed by guanine can be methylated on the CpG dinucleotide sequences.¹⁰⁾ The mechanism involves the methylation of cytosines which in turn recruit methyl-DNA binding proteins such as methyl CpG binding protein 2 (MeCP2). Methyl-DNA binding proteins then recruit macromolecular complexes of chromatin proteins which compact chromatin structure, limiting accessibility of transcriptional machinery, and thus suppress gene transcription (Fig. 1).¹⁰⁾

Histones are the primary protein components of eukaryotic chromatin and play a role in gene regulation. There are 5 histone proteins: histone 1 (H1), histone 2A (H2A), histone 2B (H2B), histone 3 (H3), and histone 4 (H4). The histone tail amino acid residues are subject to covalent modifications, such as acetylation, phosphorylation and methylation.¹³⁾ In the biochemical mechanism, the acetylation is catalyzed by histone acetyl transferase (HAT) and deacetylated by histone deacetylase (HDAC). Phosphorylation is catalyzed by protein kinases (PK) and reversed by protein phosphatase (PP). Methylation is catalyzed by histone methyltransferase (HMT) and the reverse reaction is catalyzed by histone demethylases (HDM). Depending on the amino acid residue involved, there can be different effects on gene transcription. Acetylation or phosphorylation are linked to gene activation¹⁴⁾ while methylation is more complicated; having various roles in the regulation of gene expression,¹⁵⁾ most

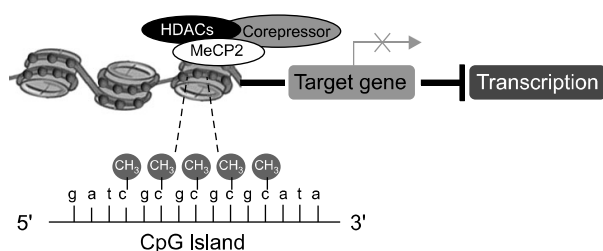


Fig. 1. Schematic of transcriptional suppression by DNA methylation. HDACs, histone deacetylase; MeCP2, methyl CpG binding protein 2; CpG, cytosine-phosphate-guanine; CH₃, a methyl group.

likely because effector proteins, such as transcriptional co-activators, recognize mono-, di-, and tri-methylated epitopes on the chromatin structure with different affinities.¹⁶⁾ Therefore, such particular changes within histone methylation are crucial for determining its effect on gene transcription.

Epigenetic characteristics of antipsychotics

Epigenetics has been shown to be involved in the pharmacological effects of antipsychotics as well as in the pathophysiology of psychiatric illness. The evidence pertaining to the specific effects of antipsychotics on epigenetic mechanism come from both DNA methylation and histone modification studies. At the level of DNA methylation, it has been shown that haloperidol has different effects on DNA methylation in various rat tissues. A study examining the methyl cytosine content of DNA in global methylation from the leukocytes, liver and brain of rats has demonstrated haloperidol treatment induced a decrease in the methyl cytosine content of leukocytes in male rats, but unexpectedly, an increase in females compared to vehicle-treated animals.¹⁷⁾ In addition to leukocytes of rats, haloperidol increased global DNA methylation in the liver in males, but resulted in a significant decrease in the brain in females.¹⁷⁾ These data suggest drugs with a pharmacological profile similar to haloperidol will differentially affect DNA methylation in various tissues and could indicate that sex-specific hormones may play a role in modifying the DNA methylation state by haloperidol. Antipsychotic drugs have also been shown to have an effect on specific epigenetic mechanisms involving the regulation of specific gene regulations. This notion initially came from findings that there are low levels of reelin and glutamic acid decarboxylase 67 (GAD67) are in the brains of people with schizophrenia and bipolar disorder.¹⁸⁻²⁰⁾ These data suggested that the down regulation of these transcripts were due to hyper-methylation of their gene promoters.²¹⁻²³⁾ Subsequently, it has been shown that clozapine and sulpiride, but not haloperidol or olanzapine, induced de-methylation of the hyper-methylated of the reelin and GAD67 promoters in mice pre-treated with L-methionine to hyper-methylate the promoters of these genes in the cortex and striatum of mice brain.²⁴⁾ Together these data would support the argument that one mechanism by which antipsychotic drugs could facilitate improvements in symptomatology is by chromatin remodeling, which at least in the GABAergic system, appears to normalize changes that have occurred in the people with schizophrenia and bipolar disorder. The hypothesis that

chromatin remodelling may be a widespread effect of antipsychotic drugs coming from recent data that haloperidol can induced demethylation and expression of MKP3 gene which in turn inhibited cell proliferation of MIA PaCa-2, human pancreatic cancer cells.²⁵⁾ In addition, a clinical study has revealed that haloperidol treatment is associated with higher levels of global DNA methylation in leukocytes of patients with schizophrenia.²⁶⁾ Therefore, existing data suggests further studies examining the effects of antipsychotic drugs in patterns or levels of DNA methylation may lead to a better understanding of their effects on gene expression and subsequent efficaciousness.

Antipsychotic drugs have also shown to regulate histone modification by modifying histone structure which is achieved by changing protein methylation, phosphorylation and acetylation.¹³⁾ For example, it has been reported that haloperidol impact on phosphorylation and acetylation of histone H3. A study demonstrated that haloperidol induces both phosphorylation and acetylation of histone H3, defined phospho-serine 10 in conjunction with acetyl-lysine 14 (H3pS10-acK14), genome wide, in the mouse striatum by blocking dopamine D2-like receptor signalling.²⁷⁾ By contrast, another study has suggested haloperidol increases levels of Ser10 phosphorylated histone H3 in the striato-pallidal medium spiny neurons (MSNs) of the dorsal striatum without causing a change of acetylation at lysine14 histone H3.²⁸⁾ More recently, it has been reported that chronic clozapine and risperidone, but not haloperidol, treatment strongly decreased histone H3 acetylation at the metabotropic glutamate receptor 2 (mGlu2) promoter in mouse frontal cortex.²⁹⁾ In this study, HDAC2 mRNA levels were reported to significantly increase by clozapine treatment but there was no change in the methylation pattern of CpG sites at the mGlu2 promoter; data suggest DNA methylation does not seem to contribute to the regulation of the mGlu2 gene. Potentially consistent with these finding in mice, are the findings of decreased histone H3 acetylation at the mGlu2 promoter and increased expression of HDAC2 in the frontal cortex of people with schizophrenia who had been treated with atypical antipsychotics. Together, these findings suggest that atypical antipsychotic drugs can down-regulate mGlu2 expression through an epigenetic mechanism that involves decreased histone acetylation at its promoter and interaction of HDAC2 to the mGlu2 promoter.²⁹⁾

In conclusion, available data suggest antipsychotic drug treatment appears to have complex and region-dependent effects on epigenetic mechanisms. This is sup-

ported by the finding that olanzapine, clozapine, clomipramine resulted in significant increases in acetylation of histone H3 in accumbens, in levels of HDAC2 in striatum and in HDAC3 in both striatum and cingulate cortex from mice brain whereas olanzapine alone induces a significant increase in HDAC5 in amygdala.³⁰⁾ Understanding these complex mechanisms will be necessary to fully comprehend the effects of antipsychotic drug treatment on DNA methylation and histone modification.

Epigenetic characteristics of mood stabilizers

Valproic acid (VPA) is a drug that can act as a mood stabiliser and has one mechanism of action by which it acts as a HDAC inhibitor to cause hyper-acetylation of histones and enhanced gene expression.³¹⁾ As an example, VPA can inhibit methionine-induced reelin promoter hyper-methylation leading to a down-regulation of reelin expression as well as inducing an increase in GAD67 and enhanced total acetylated histone H3 levels.³²⁾ VPA also causes hyper-acetylation of histone H3 in the α -synuclein promoter region in neuroblastoma SH-SY5Y cells.³³⁾ Furthermore, VPA was shown to induce hyper-acetylation of both histones H3 and H4 in genome-wide DNA methylation in primary rat astrocytes, but did not affect the levels of DNA methyltransferases1 (DNMT1), which catalyses the methylation of cytosines in CpG dinucleotides.¹⁰⁾ It showed no interaction of the histone modification and DNA methylation.³⁴⁾ Importantly, VPA and lithium are both widely used mood stabilizers and therefore it is significant that both drugs have been shown to reduced DNA methylation of brain derived neurotrophic factor (BDNF) promoter in peripheral blood mononuclear cells (PBMCs) of patients with bipolar disorder.³⁵⁾ This finding is balance by another study that found that the levels of DNA methylation at BDNF promoter were not significantly affected by mood stabilizers, including VPA, lithium and carbamazepine,³⁶⁾ whereas these mood stabilizers were associated with hypo-methylation in SLC6A4 CpG3 and 4 sites. Significantly these sites have been reported to be hyper-methylated in patients with bipolar disorder.³⁷⁾ Another study to examine regional specific changes in mice demonstrated VPA, lamotrigine and lithium increased levels of acetylated histone H3 in nucleus accumbens while carbamazepine, lamotrigine and levetiracetam induced a significant increase in HDAC3.³⁰⁾ In addition, the treatment with VPA, lamotrigine and levetiracetam results in significant increase in HDAC3 in cingulate cortex and HDAC5 in amygdala in mice brain,³⁰⁾ providing the information re-

garding differences in gene expression profiles in particular regions of brain. Finally, a clinical study has revealed that lithium treatment showed a decrease in the levels of global DNA methylation in lymphoblast of patient with bipolar disorder.³⁸⁾ Resolving the clinically significant effects of mood stabilisers on epigenetic mechanisms may be worthwhile as more common effects of these drugs are separated from effect which are limited to specific drugs and are therefore less likely to associate with their common effects on mood stability.

Epigenetic characteristics of antidepressant drugs

Antidepressant drugs have also been shown to affect epigenetic mechanisms, for example, chronic treatment of imipramine produces a selective hyper-acetylation of histone H3 at the BDNF III and BDNF IV promoters as well as de-methylation in histone H3 tri-methylation at lysine 4 (H3K4) at the BDNF III promoter in mice to chronic social defeat stress.³⁹⁾ These changes were associated with an enhancement of BDNF transcription in these mice, and this hyper-acetylation by chronic imipramine was associated with a selective down-regulation of HDAC5 whilst overexpression of HDAC5 inhibited the effect of imipramine in the social defeat paradigm. Thus, the observed histone and HDAC modifications with imipramine treatment support the therapeutic potential for histone methylation and deacetylation inhibitors in depression.³⁹⁾ Another study has reported that fluoxetine increased levels of MeCP2, which is thought to silence transcription of downstream gene by recruiting a HDAC, enhanced HDAC2 and reduced the acetylated forms of histone H3 in rat brain.⁴⁰⁾ Another potential of antidepressant drugs that included amitriptyline, venlafaxine and citalopram to affect epigenetic parameters in astrocytes showed amitriptyline reduced levels of genome-wide DNA methylation at CCpGG sites and inhibited enzymatic DNMT activity in primary astrocytes.³⁴⁾ Furthermore, the further study demonstrated this reduction of DNMT activity was neither due to reduced DNMT1 expression nor direct drug interference but decrease in levels of histone methyltransferase G9a, known modulator of DNMT1, indicating a functional impact of G9a on DNMT1.⁴¹⁾ However, the fact that this effect was limited to one of a number of antidepressant drugs suggests it is not a “class” effect and therefore may not be linked to the therapeutic efficacy of the amitriptyline. It has also been reported that treatment with escitalopram reduced the levels of methylation in P11 gene and levels of DNMT1 and DNMT3a mRNA in the prefrontal cortex of the flinders sensitive line (FSL)

rat; a genetic rodent model of depression.⁴²⁾ The P11 gene is associated with functional expression of 5-HT (1B) in both the human and rodent^{43,44)} and that receptor has been linked to the symptom of depression.⁴²⁾ Therefore, these findings suggest P11 could be one gene that could be affected by changes in epigenetic mechanisms during antidepressant treatment.

Epigenetic effects of poly-pharmacological treatments

Over the last several years, a variety of adjunctive treatments have been used to enhance the response to antipsychotics in preclinical and clinical studies⁴⁵⁾ that includes drugs such as VPA. As previously stated, VPA is a nonspecific HDCA inhibitor. The observation that VPA is efficacious when given in combination with antipsychotic drugs may suggest that the combined impact of both drug types on epigenetic mechanisms may give improved therapeutic benefit. Mechanistically, it has been shown that combined treatment with clozapine or sulpiride and VPA results in dose-related increases in de-methylation of hyper-methylated of reelin and GAD67 promoters in cortical and striatal mice pre-treated with L-methionine compared to normal brain.²⁴⁾ Another study also revealed, when co-administered with VPA, clozapine and sulpiride reverse the hyper-methylation of the reelin or GAD67 DNA that can be induced in mouse frontal cortex.⁴⁶⁾ In addition, the treatment with clozapine and VPA has been shown to increase growth arrest and DNA damage 45- β (GADD45- β) mRNA and protein expression in the frontal cortex of mice.⁴⁷⁾ It is therefore possible that GADD45- β is involved in the regulation of DNA-methylation after treatment with a combination of psychotropic drugs.

Intracellular Signalling Pathways

The regulation of gene expression involves intracellular signalling pathways and they are fundamental for the regulation of transcription of gene. A comprehensive understanding of neuronal functioning and the various mechanisms of drug action is a key factor for developing the future psychiatric pharmacotherapy. This is especially the case as psychotropic drugs target multiple sites that include neurotransmitter receptors, neurotransmitter transporters and specific molecules in signalling pathways. In this review, we specifically focused on intracellular signalling cascades, cyclic adenosine monophosphate (cAMP), glycogen synthase kinase-3 (GSK-3) and mitogen-activated protein kinase (MAPK) pathways which have been explored as common important mediators that may critically involve in the actions of psychiatry drugs in the cen-

tral nervous system (CNS).

Antipsychotics

The common pharmacological characteristic of all anti-psychotic drugs, including typical and atypical is to antagonise dopamine D2-like receptors.⁴⁸⁾ In addition, whilst first-generation antipsychotic drugs are antagonist of dopamine D2 receptors the second generation drugs target a number of other receptors with high affinity.^{49,50)} Finally, clozapine is considered a unique antipsychotics drug possessing superior therapeutic efficacy as demonstrated in particular with respect to patients not responding to first-generation typical antipsychotics such as haloperidol.^{51,52)} A unique action of clozapine seems to be that it has some receptor agonist activity and has a high affinity for four (M1-M4) of the five muscarinic receptors.^{53,54)} However, the particular mechanism underpinning the superior efficacy action of clozapine and its adverse side effects are poorly understood.^{52,55)} Accordingly, it may allow a more precise characterization of available drugs to establish differences not only between typical and atypical antipsychotics, but also within the groups of atypical antipsychotics.

In regard with D2, 5-HT2A and muscarinic receptors, these receptors are 7-transmembrane domain protein coupled to G proteins. The various functions of G protein coupled receptors have been associated with the regulation of cAMP and protein kinase A (PKA) via G protein dependent signalling. There is growing evidence that different way antipsychotics modulate the cAMP and PKA pathway may contribute to their differing clinical and/or side-effect profiles. For example, treatment with haloperidol has been shown to increase levels of PKA activity, mRNA and protein in the rat striatum whereas clozapine reduces these parameters in the striatum, hippocampus and frontal cortex of rats.⁵⁶⁾ In another study haloperidol was shown to increase levels of the PKA catalytic subunit in the mouse dorsal striatum whereas clozapine had the opposite effect in the medial prefrontal cortex.⁵⁷⁾ Thus, current data also suggests the different effects of typical and atypical antipsychotics on cAMP and PKA appeared to be complex and region-dependent, possibly due to their affinities for different G-protein coupled receptors that stimulate adenylyl cyclase signalling.^{56,57)} Dopamine- and cAMP-regulated phosphoprotein with molecular weight 32 (DARPP-32), a downstream to PKA, is another important mediator of PKA pathway, particularly in dopaminergic transmission.⁵⁸⁾ DARPP-32 phosphorylation catalysed by PKA at Thr 34 converts it into an inhibitor of

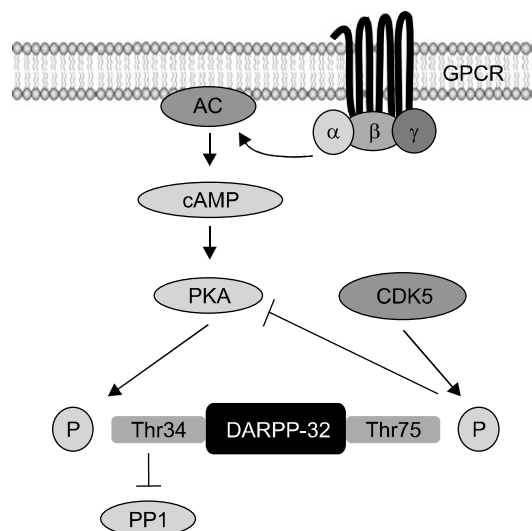


Fig. 2. Schematic of mechanism of DARPP32. GPCR, G protein coupled receptors; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; DARPP-32, dopamine- and cAMP-regulated phosphoprotein with molecular weight 32; CDK5, cyclin-dependent kinase 5.

proteinphosphates 1 (PP1), while phosphorylation at Thr 75 by cyclin-dependent kinase 5 (CDK5) converted into an inhibitor of PKA (Fig. 2). Importantly, DARPP-32 phosphorylation at Thr 34 has been shown to be increased in the mouse dorsal striatum after acute administration of haloperidol and clozapine,⁵⁹⁾ suggesting that both typical and atypical antipsychotics may modulate PKA downstream mechanisms. Another study has shown that both haloperidol and clozapine increase PKA dependent phosphorylation at Thr 32.⁶⁰⁾ However, another atypical antipsychotic drug, olanzapine, up-regulated the phosphorylation at Thr 75, which represented the specific site for the CDK5.⁶¹⁾ In addition, haloperidol has been shown to induce activation of PKA, and DARPP-32, which has been proposed to affect gene transcription by acting on nuclear targets, including the cAMP response element-binding (CREB) protein in mouse dorsal striatum.⁵⁹⁾ This has implications for the finding that haloperidol increases the phosphorylation of the ribosomal protein S6 (rpS6), a component of the small 40S ribosomal subunit, an effect exerted by promoting the c-AMP-DARPP-32 signalling.⁶²⁾ These findings suggest that regulation of protein synthesis through rpS6 may be a potential target of antipsychotic drugs (Table 1).

Another intracellular mechanism potentially relevant to antipsychotics is GSK-3 signalling. GSK-3 is a serine-threonine protein kinase that controls several cellular functions, negatively regulated by the phosphorylation of

Table 1. Summary of studies of intracellular signalling in antipsychotics

Signalling	Study	Antipsychotics	Results	Regions
cAMP/PKA	Dwivedi <i>et al.</i> , 2002 ⁵⁶⁾	Haloperidol and clozapine	Haloperidol increased PKA activity, mRNA and protein, while clozapine decreased PKA activity, mRNA and protein	Rat Striatum, hippocampus and frontal cortex
	Pozzi <i>et al.</i> , 2003 ⁵⁹⁾	Haloperidol and clozapine	Increased DARPP-32 (Thr 34) and CREB phosphorylation	Mice Striatum
	Turalba <i>et al.</i> , 2004 ⁵⁷⁾	Haloperidol and clozapine	Haloperidol increased PKA catalytic subunit levels, while clozapine decreased PKA catalytic subunit levels	Mice Striatum and medial prefrontal cortex
	Bateup <i>et al.</i> , 2008 ⁶⁰⁾	Olanzapine	Increased DARPP-32 (Thr 34 and Thr 75) phosphorylation	Striatonigral and striatopallidal neurons
GSK/Akt	Valjent <i>et al.</i> , 2011 ⁶²⁾	Haloperidol	Increased ribosomal protein S6 phosphorylation	Mice Striatum
	Emamian <i>et al.</i> , 2004 ⁶⁴⁾	Haloperidol	Increased Akt and GSK-3 β phosphorylation	Mice cerebral cortex and hippocampus
	Aubry <i>et al.</i> , 2009 ⁶⁵⁾	Clozapine and olanzapine	Increased Akt and GSK-3 β phosphorylation	SH-SY5Y human neuroblastoma cell
	Réus <i>et al.</i> , 2012 ⁶⁷⁾	Olanzapine	Increased Akt phosphorylation	Rat prefrontal cortex, hippocampus and striatum
GSK/Wnt	Kang <i>et al.</i> , 2004 ⁷¹⁾	Clozapine	Increased Dvl, beta-catenin protein and GSK-3 β phosphorylation	SH-SY5Y human neuroblastoma cell
	Alimohamad <i>et al.</i> , 2005 ⁶⁹⁾	Clozapine, haloperidol and risperidone	Increased Dvl-3, beta-catenin and GSK-3 β protein	Rat ventral midbrain, medial prefrontal cortex and striatum
	Sutton <i>et al.</i> , 2007 ⁷⁰⁾	Haloperidol and clozapine	Increased Wnt-5a, Axin, Dvl-3 protein levels, and GSK-3 phosphorylation	Rat the frontal cortex
	Roh <i>et al.</i> , 2007 ⁶⁶⁾	Haloperidol and clozapine	Haloperidol and clozapine increased Akt and GSK-3 β phosphorylation but only clozapine increased both phosphorylation and the amount of Dvl	Rat the frontal cortex
MAPK	Pozzi <i>et al.</i> , 2003 ⁵⁹⁾	Haloperidol and clozapine	Haloperidol increased ERK1/2, CREB and Elk1 phosphorylations, while clozapine decreased ERK1/2, CREB and Elk1 phosphorylations	Mice dorsal striatum
	Fumagalli <i>et al.</i> , 2006 ⁷³⁾	Clozapine, olanzapine and haloperidol	Olanzapine and clozapine but not haloperidol increased ERK1/2 phosphorylation	Rat prefrontal cortex
	Ahmed <i>et al.</i> , 2008 ⁷⁴⁾	Clozapine, olanzapine	Increased ERK1/2 phosphorylation	Rat prefrontal cortex
	Kim <i>et al.</i> , 2008 ⁷⁵⁾	Haloperidol	Increases followed by decreases in MEK, ERK1/2, and p90RSK phosphorylation and the dephosphorylation of MEK and ERK1/2 were regulated by PP2A	Rat prefrontal cortex
	Pereira <i>et al.</i> , 2012 ⁷⁷⁾	Clozapine, haloperidol and olanzapine	Increased ERK1/2 phosphorylation and the phosphorylation of ERK1/2 by clozapine was mediated by EGF receptor	Mouse prefrontal cortex and striatum
	Pereira <i>et al.</i> , 2009 ⁷⁸⁾	Clozapine and haloperidol	Increased ERK1/2 phosphorylation and the phosphorylation of ERK1/2 by clozapine was mediated by EGF receptor	Mouse prefrontal cortex
	Pereira <i>et al.</i> , 2013 ⁷⁹⁾	Clozapine, haloperidol and olanzapine	Increased ERK1/2 and the phosphorylation of ERK1/2 by clozapine modulates p90RSK phosphorylation	Mouse prefrontal cortex and striatum
	Kim <i>et al.</i> , 2013 ⁷⁶⁾	Clozapine	Increases followed by decreases in ERK1/2 phosphorylation and the dephosphorylation of ERK1/2 was regulated by MKP1	Rat prefrontal cortex

PKA, protein kinase A; DARPP-32, dopamine-and cAMP-regulated phosphoprotein with molecular weight 32; CREB, cAMP response element-binding; GSK-3, glycogen synthase kinase-3; Dvl, dishevelled; ERK1/2, extracellular signal-regulated kinases 1/2; MEK, mitogen-activated protein kinase kinase; p90RSK, p90 ribosomal S6 kinase; PP2A, protein phosphatase 2A; EGF, epidermal growth factor; MKP1, mitogen-activated protein kinase phosphatases 1.

N-terminal serine residues Ser 21 of GSK-3 α and Ser 9 of GSK-3 β .⁶³ It has been shown that the antipsychotic drugs haloperidol, risperidone, olanzapine, clozapine, quetiapine or ziprasidone act to increase the phosphorylation of GSK-3 β in different brain regions regardless of their class.⁶⁴⁻⁶⁶ Based on these data, Akt and GSK-3 signalling may represent a common pathway in these actions of antipsychotics. One study has revealed that treatment of mice with haloperidol induced the phosphorylation of Akt and led to an increased phosphorylation of GSK-3 β ⁶⁴ whilst it has also been shown that clozapine and olanzapine produced phosphorylation of Akt and GSK-3 β in SH-SY5Y human neuroblastoma cell and stimulate the expression of Akt-1 mRNA and protein.⁶⁵ Another study has shown that acute administration of olanzapine increased Akt levels in the prefrontal cortex, hippocampus and striatum of rats.⁶⁷ Hence, typical and atypical antipsychotics have similar effects regardless their class (Table 1).

GSK-3 is also mediated with the Wnt pathway (Fig. 3), a complex network of proteins with roles in embryogenesis and cancer as well as in adult physiological processes.⁶⁸ It has been shown that significant increases in the levels of dishevelled 3 (Dvl-3), beta-catenin and GSK-3 β protein occur after chronic treatment of clozapine, haloperidol or risperidone in rat ventral midbrain, medial prefrontal cortex and striatum.⁶⁹ Chronic treatment with clozapine or haloperidol also increased levels of Wnt-5a, Axin, Dvl-3, total and phosphorylation of GSK-3 β , and β -catenin in rat the frontal cortex.⁷⁰ In addition, both haloperidol and clozapine induced phosphorylation of Akt and GSK-3 β but, significantly, only clozapine increased both phosphorylation and the amount of Dvl in the rat frontal cortex.⁶⁶ Furthermore, clozapine has been shown to enhance GSK-3 β signalling via the activation of Wnt pathway but not via the PI3K/Akt pathway in SH-SY5Y cells.⁷¹ It is therefore possible that both first and second generation antipsychotics drugs differentially regulate PI3K/Akt and Wnt pathways depending on their relative D2- or 5HT2-receptor antagonism and that clozapine has unique effects that may be related to its increased therapeutic reach or its differential side-effects (Fig. 3, Table 1).

One intracellular mechanism by which antipsychotic drugs have been shown to act is the MAPK pathway. This pathway is important in a number of neuronal functions, including the regulation of gene expression, protein synthesis, and receptor modulation, which contribute to synaptic plasticity and adaptive behaviours such as learning

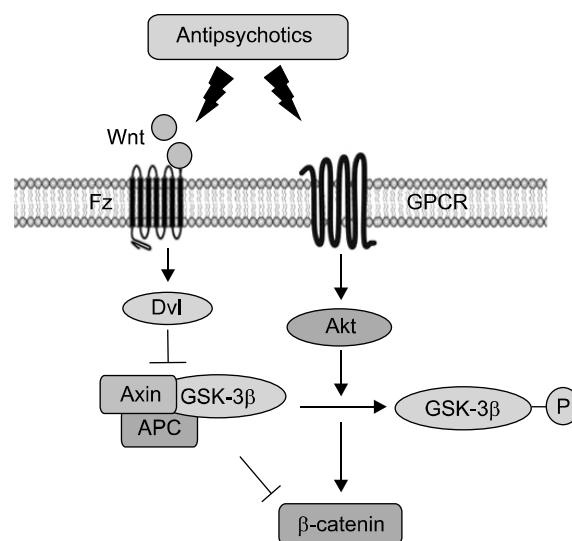


Fig. 3. Schematic showing the pathways of Wnt and Akt in antipsychotics. Fz, frizzled; GPCR, G protein coupled receptors; Dvl, dishevelled; APC, adenomatous polyposis coli.

and memory.⁷² Several studies have reported different impacts of typical and atypical antipsychotics on extracellular signal-regulated kinases 1/2 (ERK1/2) phosphorylation in the brain. In particular, while a single injection of haloperidol increased phosphorylations of ERK1/2 and Elk1 in mice dorsal striatum, these phosphorylations were decreased following clozapine administration.⁵⁹ Chronic administration of the olanzapine and clozapine enhanced ERK1/2 activation in rat prefrontal cortex,^{73,74} whereas haloperidol does not produce any significant in ERK1/2 phosphorylation.⁷³ Antipsychotic drugs may modulate the MAPK pathway in different ways. For example, a single injection of haloperidol has been to induce an acute increase followed by a decrease in mitogen-activated protein kinase kinases (MEK), ERK1/2, and p90 ribosomal S6 kinase (p90RSK) phosphorylation in the rat frontal cortex; this occurs without changes in Raf-1 phosphorylation, and these dephosphorylation of MEK and ERK1/2 were regulated by protein phosphatase 2A (PP2A).⁷⁵ Clozapine also induces an initial increase followed by a decrease ERK1/2 phosphorylation.⁷⁶ Interestingly, the dephosphorylation of ERK1/2 was shown to be mediated by mitogen-activated protein kinase phosphatases 1 (MKP1), which regulates ERK1/2 through direct binding.⁷⁶ These results provided critical information on the dephosphorylation mechanism of MAPK in the typical and atypical antipsychotics. In addition, a study examining a novel mechanism of action of clozapine demonstrated clozapine but not olanzapine or haloperidol induced initial in-

Table 2. Summary of studies of intracellular signalling in mood stabilizers

Signalling	Study	Mood stabilizers	Results	Regions
cAMP/PKA	Liang <i>et al.</i> , 2008 ⁸¹⁾	Lithium	Increased PKA and CREB phosphorylation	Neuron-enriched cerebral cortical cultures of rats
	Cechinel-Recco <i>et al.</i> , 2012 ⁸²⁾	Lithium	Increased CREB phosphorylation	Rat hippocampus, prefrontal, amygdala and striatum
	Gallagher <i>et al.</i> , 2004 ⁸³⁾	Valproic acid	Inhibited forskolin-induced increase in cAMP levels	C6 rat glioma cells
	Montezinho <i>et al.</i> , 2007 ⁸⁴⁾	Valproic acid	Inhibited forskolin-induced increase in cAMP levels	Cultured cortical neurons and prefrontal cortex of rats
	Chang <i>et al.</i> , 2010 ⁸⁵⁾	Valproic acid	Inhibited forskolin-induced increase in cAMP levels	Rat prefrontal cortex of rats
GSK/Akt	Grimes and Jope, 2001 ⁸⁶⁾	Lithium	Increased GSK-3 β phosphorylation	SH-SY5Y cells
	De Sarno <i>et al.</i> , 2002 ⁹¹⁾	Valproic acid	Increased Akt and GSK-3 β phosphorylation	SH-SY5Y human neuroblastoma cell
	Roh <i>et al.</i> , 2005 ⁹²⁾ Liang <i>et al.</i> , 2008 ⁸¹⁾	Valproic acid Lithium	Increased GSK-3 β phosphorylation Increased Akt and CREB phosphorylation	Mouse cerebral cortex Rat hippocampus, prefrontal, amygdala and striatum
GSK/Wnt	Gould <i>et al.</i> , 2004 ⁸⁹⁾	Lithium	Increased beta-catenin levels	Rat prefrontal cortex
	Wexler <i>et al.</i> , 2008 ⁹⁰⁾	Lithium	Increased beta-catenin levels	Primary cells of hippocampal progenitors of rat
MAPK	Mai <i>et al.</i> , 2002 ⁹³⁾	Carbamazepine	Increased ERK1/2 phosphorylation	SH-SY5Y human neuroblastoma cell
	Di Daniel <i>et al.</i> , 2005 ⁹⁴⁾	Lamotrigine and carbamazepine	No change in levels of ERK1/2 phosphorylation	SH-SY5Y human neuroblastoma cell
	Yuan <i>et al.</i> , 2001 ⁹⁵⁾	Lithium and valproic acid	Increased ERK1/2 and Elk phosphorylation	SH-SY5Y human neuroblastoma cell
	Einat <i>et al.</i> , 2003 ⁹⁶⁾	Lithium and valproic acid	Increased ERK1/2, RSK1, CREB phosphorylation	Rat hippocampus and frontal cortex
	Casu <i>et al.</i> , 2007 ⁹⁷⁾	Valproic acid	Increased ERK1/2 phosphorylation	Rat amygdala and nucleus accumbens
	Young <i>et al.</i> , 2008 ⁹⁸⁾	Lithium	Increased ERK1/2 phosphorylation	Mouse infant caudate putamen
	Longoni <i>et al.</i> , 2011 ⁹⁹⁾	Lithium	Increased ERK1/2 phosphorylation	Mouse bed nucleus of stria terminalis and central and basolateral amygdala
	Kim <i>et al.</i> , 2013 ¹⁰⁰⁾	Lithium	Increased ERK1/2, Elk, Egr1 phosphorylation and levels of PER2	SH-SY5Y human neuroblastoma cell

PKA, protein kinase A; CREB, cAMP response element-binding; cAMP, cycline adenosine monophosphate; GSK-3, glycogen synthase kinase-3; ERK1/2, extracellular signal-regulated kinases 1/2; RSK1, ribosomal S6 kinase; PER2, clock gene period 2.

hibition and subsequent activation of cortical and striatal ERK1/2^{77,78)} and p90RSK⁷⁹⁾ phosphorylations in mice, and uniquely, the clozapine-induced ERK1/2 activation was mediated by the epidermal growth factor (EGF) receptor (Table 1).⁷⁷⁻⁷⁹⁾ It showed a unique action of clozapine which differently modulates the phosphorylation of ERK1/2 compared to other antipsychotics.

Mood stabilizers

Mood stabilizers also modulate the cAMP-PKA signalling pathway. It has been shown lithium increased activation of PKA leading to enhance the phosphorylation of GSK-3 β ⁸⁰⁾ and CREB in neuron-enriched cerebral cortical cultures from rats⁸¹⁾ and in the intact rat brain.⁸²⁾ Conversely, VPA has been suggested to reduce forskolin-induced increase in cAMP levels in C6 rat glioma cells⁸³⁾ in the cultured cortical neurons⁸⁴⁾ and in the pre-

frontal cortex of rats.⁸⁵⁾ It remains unclear whether these mood stabilizers affect cAMP directly or indirectly by inhibiting the formation of cAMP. Furthermore, the roles of the mood stabilizers in cAMP and PKA have yet to be explored (Table 2).

The archetypal mood stabilizer, lithium, has been shown to directly inhibit GSK-3 β activity, possibly by competing with magnesium which constitutively required for GSK-3 β to function optimally. Alternatively, it is possible that lithium acts indirectly by increasing Akt-mediated inhibition of GSK-3 β ;^{86,87)} this mechanism is supported by data showing that lithium activates Akt signalling with a subsequent inhibition of GSK-3 β .^{81,88)} Lithium acts on multiple signalling pathways, hence it acutely affects the Wnt signalling to increase levels of β -catenin in the rat brain⁸⁹⁾ and in rat hippocampal progenitor cells.⁹⁰⁾ By affecting this pathway it can also down-regu-

late GSK-3 β activity, a key translator of the activation of Wnt pathway. Considering the notion that drugs mechanism of therapeutic importance should constitute a drug class effect it is significant that there is some data to suggest that VPA inhibits GSK-3 β .^{91,92} Thus, *in vitro* studies revealed that VPA elevated levels of phosphorylation in Akt and GSK-3 β in SH-SY5Y cells,⁹¹ and a single VPA treatment to mice prevented hypoxia-induced reduction in phosphorylation of GSK-3 β ,⁹² suggesting VPA has a role to inhibit the activity of GSK-3 (Table 2).

In addition, carbamazepine has been reported to increase the phosphorylation of ERK1/2 in SH-SY5Y cells⁹³; however, this finding was not replicated using lamotrigine or carbamazepine in another study.⁹⁴ *In vivo* studies have shown that lithium and VPA increases the phosphorylation of ERK1/2, Elk, and RSK1^{95,96} whilst it

has also been shown that these two drugs cause widespread ERK1/2 phosphorylations across rat amygdala, nucleus accumbens,⁹⁷ and caudate putamen of infant mouse brains,⁹⁸ bed nucleus of stria terminalis and central and basolateral amygdala of mouse.⁹⁹ One interesting study now links an action of lithium to circadian rhythms by showing the drug enhances the ERK1/2-Elk-Egr1 cascade which in turn increases levels of the clock gene period 2 (PER2) in SH-SY5Y cells and the mouse frontal cortex. Furthermore, lithium-induced PER2 expression was inhibited by depletion of Egr1 by siRNA in SH-SY5Y cells and Egr1 knockout mice, and ERK1/2-Elk pathway also regulated lithium-induced Egr1 and PER2 expression, thus it indicated the PER2 expression was regulated by ERK-Elk-Egr1 pathway (Table 2).¹⁰⁰ This mechanism may prove to be significant in understanding how

Table 3. Summary of studies of intracellular signalling in antidepressants

Signalling	Study	Antidepressants	Results	Regions
cAMP/PKA	Thome <i>et al.</i> , 2000 ¹⁰³	Desipramine, fluoxetine and tranylcypromine	Increased CREB phosphorylation	Mouse limbic brain regions
	Tiraboschi <i>et al.</i> , 2004 ¹⁰⁴	Desipramine, fluoxetine and tranylcypromine	Desipramine and reboxetine, but not fluoxetine, increased the activity of PKA	Rat hippocampus and prefrontal cortex
	Wang <i>et al.</i> , 2006 ¹⁰⁵	Fluoxetine	Increased PKA and CREB phosphorylations	Rat hippocampus
	Takano <i>et al.</i> , 2012 ¹⁰⁶	Imipramine	Increased BDNF and the BDNF was suppressed with inhibitors for PKA	Cultured rat brain astrocytes
GSK/Akt	Li <i>et al.</i> , 2004 ¹⁰⁷	Imipramine and fluoxetine	Increased GSK-3 β phosphorylation	Mouse prefrontal cortex, hippocampus, and striatum
	Basta-Kaim <i>et al.</i> , 2005 ¹⁰⁸	Imipramine and fluoxetine	Increased Akt phosphorylation	Neuro-2A cells
	Huang <i>et al.</i> , 2013 ¹⁰⁹	Fluoxetine	Increased Akt phosphorylation	Neural stem cell of rat embryonal brain tissue
GSK/Wnt	Mostany <i>et al.</i> , 2008 ¹¹⁰	Venlafaxine	Increased beta-catenin levels	Rat hippocampus
	Pinnock <i>et al.</i> , 2010 ¹¹¹	Fluoxetine	Increased Wnt3a expression	Rat hippocampal dentate gyrus
MAPK	Pilar-Cu��llar <i>et al.</i> , 2012 ¹¹²	Fluoxetine	Increased beta-catenin levels	Rat hippocampus
	Fumagalli <i>et al.</i> , 2005 ¹¹³	Fluoxetine	Decreased ERK1/2 phosphorylation	Rat hippocampus and frontal cortex
	Hisaoka <i>et al.</i> , 2007 ¹¹⁷	Amitriptyline	Increased ERK1/2 phosphorylation and GDNF expression	C6 rat glioma cells
	Hisaoka <i>et al.</i> , 2008 ¹¹⁸	Amitriptyline	Increased ERK1/2 and CREB phosphorylation	C6 rat glioma cells
	Qi <i>et al.</i> , 2008 ¹¹⁶	Fluoxetine	Increased ERK1/2 phosphorylation	Rat hippocampus and frontal cortex
	Di Benedetto <i>et al.</i> , 2012 ¹¹⁴	Fluoxetine and desipramine	Decreased only neuronal ERK1/2 activity not astrocytic ERK1/2 activity	Rat frontal cortex
	First <i>et al.</i> , 2011 ¹¹⁵	Fluoxetine	Increased ERK1/2 phosphorylation	Rat hippocampus and frontal cortex
	Di Benedetto <i>et al.</i> , 2012 ¹¹⁴	Reboxetine	Increased ERK1/2 phosphorylation and GDNF expression	C6 rat glioma cells
Huang <i>et al.</i> , 2013 ¹⁰⁹	Fluoxetine	Increased ERK1/2 phosphorylation	Neural stem cell of rat embryonal brain tissue	

CREB, cAMP response element-binding; PKA, protein kinase A; BDNF, brain derived neurotrophic factor; GSK-3, glycogen synthase kinase-3; ERK1/2, extracellular signal-regulated kinases 1/2; GDNF, glial cell-derived neurotrophic factor.

treating with lithium can modulate sleep patterns in people with bipolar disorder.¹⁰¹⁾

Antidepressant drugs

A number of studies reported the cAMP pathway is up-regulated by antidepressant treatment, for example, long-term treatment with citalopram increased the adenylyl cyclase (AC) type 1 mRNA in the hippocampus increasing cAMP signalling.¹⁰²⁾ Unfortunately, antidepressant drugs do not seem to have consistent effects on the cAMP pathway. Thus, desipramine, fluoxetine and tranylcypromine significantly increase the phosphorylation of CREB in several mouse limbic brain regions, including the cerebral cortex, hippocampus, amygdala, and hypothalamus regardless their class.¹⁰³⁾ However, desipramine and reboxetine, but not fluoxetine, increase the activity of PKA in rat hippocampus and prefrontal frontal cortex;¹⁰⁴⁾ these latter data suggest that PKA does not seem to account for increase of CREB induced by fluoxetine, a selective serotonin reuptake inhibitors (SSRI).¹⁰⁴⁾ This conclusion must be tempered by the findings from another study which show that fluoxetine activates both PKA and CREB phosphorylations in the rat hippocampus.¹⁰⁵⁾ In addition to cAMP-CREB pathway, a recent study demonstrated that imipramine increased BDNF mRNA expression in cultured rat brain astrocytes and the imipramine-induced BDNF increase was suppressed with inhibitors for PKA (PKI 14-22 amide), suggesting imipramine induced BDNF expression through PKA (Table 3).¹⁰⁶⁾

Much like antipsychotics and mood stabilisers, GSK-3 pathway is also affected by antidepressant drugs. A single treatment with imipramine and fluoxetine increases GSK-3 β phosphorylation in mouse brain.¹⁰⁷⁾ Both imipramine and fluoxetine also enhanced the phosphorylation of Akt but, did not affect total Akt levels in the differentiated neuro-2A cells¹⁰⁸⁾ or neural stem cells (NSCs) from rat embryonal brain tissue.¹⁰⁹⁾ In addition, on Wnt pathways, it has been reported that venlafaxine elevated nuclear translocation of β -catenin protein in the rat hippocampus¹¹⁰⁾ and fluoxetine induced Wnt3a expression improving neurogenesis in the hippocampal dentate gyrus¹¹¹⁾ and increased levels of β -catenin protein in the hippocampus of rat (Table 3).¹¹²⁾

Altered MAPK activity has been also observed in the intracellular mechanism of antidepressant drugs. Interestingly, the various intracellular effects of antidepressant drugs on MAPK are reported. Chronic treatment with fluoxetine inhibited ERK1/2 phosphorylation in hippo-

campus and frontal cortex of rat brain,¹¹³⁾ and a recent study also revealed that acute treatments with fluoxetine and desipramine decreased neuronal, but not astrocytic, ERK1/2 activity in the frontal cortex.¹¹⁴⁾ These data highlight a complexity in understanding drug action in the CNS because of the potential for cell-type specific effects. Another study has reported chronic treatment with fluoxetine reversed the reduced ERK1/2 phosphorylation caused by chronic mild stress (CMS) in hippocampus and frontal cortex of rats in an animal model of depression, suggesting ERK1/2 may have a role in mediating the neural stress response and the mode of action of fluoxetine.^{115,116)} In addition, a further study to investigate upstream of ERK1/2 reported upregulated ERK1/2 phosphorylation by fluoxetine in neural stem cells were blocked by both PI3-K inhibitor (LY294002) and MEK inhibitor (PD98059), showing a crosstalk mechanism between Akt and ERK1/2 in the action of antidepressant drugs.¹⁰⁹⁾ More interestingly, it has been also demonstrate a relationship between ERK1/2 and glial cell line-derived neurotrophic factor (GDNF) by antidepressants. Treatment with amitriptyline increased both ERK1/2 phosphorylation and levels of GDNF mRNA in rat C6 glioma cells (C6 cells), and this ERK1/2 activation was correlated with antidepressant-induced GDNF production.¹¹⁷⁾ In addition, it has revealed that CREB was also activated by amitriptyline in ERK1/2 dependent manners, and it indicated ERK1/2-CREB activations might contribute to gene expression in GDNF in glial cells.¹¹⁸⁾ In the similar manner, recent data have shown in vitro that reboxetine can activate the ERK1/2 and GDNF expression in C6 cells and pre-treatment with a ERK inhibitor reversed GDNF expression.¹¹⁴⁾ It suggests that ERK1/2 is a critical role in GDNF expression by antidepressant drugs (Table 3).

MicroRNA

MicroRNAs (miRNAs) are a class of small non-coding RNAs with relatively few nucleotides (an average of 22) that recognise binding sites located in the three prime untranslated region (3'UTR) of an mRNA target.¹¹⁹⁾ In miRNA biogenesis, most of the miRNAs have the same transcription machinery as mRNA. The miRNA genes are transcribed as primary miRNAs (pri-miRNAs), with 5'Cap and 3'poly (A) tails, in the nucleus by RNA polymerase II. The pri-miRNAs are further processed into stem loop precursor miRNAs (pre-miRNAs), about 70 nucleotides long, by the microprocessor complex containing RNase III-type enzyme Droscha and its partner protein DiGeorge syndrome critical region gene 8 (Dgcr8) in

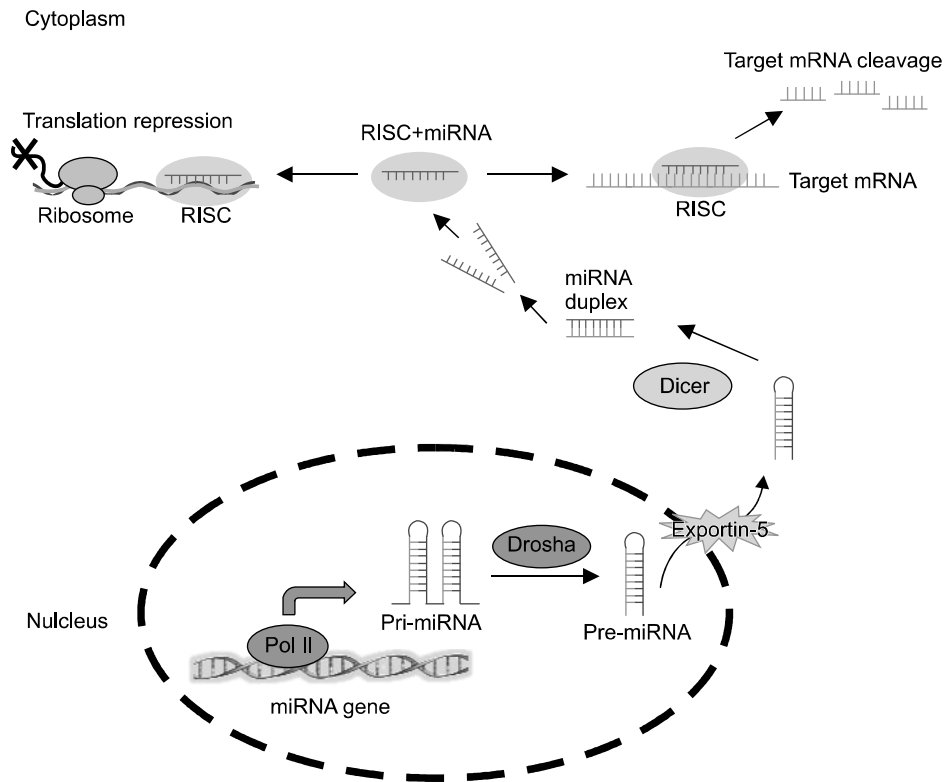


Fig. 4. Schematic showing biogenesis of microRNAs (miRNAs). Pri-miRNAs, primary miRNAs; Pre-miRNAs, precursor miRNAs; RISC, RNA-induced silencing complex; mRNA, messenger RNA.

human. These pre-miRNAs are then exported to the cytoplasm via Exportin-5. In the cytoplasm, pre-miRNAs are further cleaved into the ~22 nucleotides mature miRNA duplexes by another RNase III-type enzyme, Dicer1. Only one strand of miRNA duplex, referred to as the mature miRNA, assembles into the RNA-induced silencing complex (RISC) while the opposite strand is generally not active and is degraded. The miRNA-associated RISC binds to the target mRNA to inhibit its translation or cause the degradation of the target mRNA (Fig. 4). Nowadays, the sequences of 1872 pre-miRNAs and 2578 mature miRNAs have been identified (miRBase 20, released in June 2013). The particular miRNAs expression and regulation have been found to be important for a wide range of biological processes and have subsequently been reported to be involved in the pathophysiology of psychiatric disorders.^{120,121)}

There are still only a few studies investigating the effects of the current psychotropic drugs on miRNA expression and subsequent gene expression.

Antipsychotics and miRNA

Whilst there are still few studies on the ability of antipsychotic drugs to modulate miRNA these effects may be profound as one study has shown that treatment with hal-

operidol affected levels of 179 miRNA including miR-199a, miR-128a and miR-128b which were expressed at higher levels in the frontal cortex of treated rats.¹²²⁾ It has also been shown that pre-treatment with antipsychotic drugs haloperidol or clozapine can prevent the reduction of miR-219 caused by the phencyclidine-like NMDA-R antagonist (MK-801), dizocilpine, in prefrontal cortex of rat.¹²³⁾ This suggests antipsychotic drugs may interfere with neurotransmitter mediated changes in gene expression. However, a recent study using a miRNA microarray followed with quantitative real-time PCR (qPCR) found that only 3 miRNAs were down-regulated following treatment with olanzapine (n=1; mmu-miR-193) or haloperidol (n=2; mmu-miR-434-5p and mmu-miR-22) in whole brain of mice.¹²⁴⁾ These data would suggest that antipsychotic drug treatment minimally, but differentially, affect levels of miRNAs. Another study has used microarray technology to investigate the effects of chlorpromazine, haloperidol and clozapine on miRNA levels in human T-lymphocyte cells.¹²⁵⁾ By contrast to earlier studies, this study reported that both haloperidol and clozapine appeared to regulate the expression of a large number of miRNAs which are thought to be related to oxidative stress and metabolism.¹²⁵⁾ Significantly, the data suggested that 73 haloperidol-

modulated mRNA and miRNA pairs are involved in a wide variety of metabolic pathways including 'carbohydrate metabolism', 'lipid metabolism' and 'small molecule biochemistry'.¹²⁵⁾ In addition, it was argued that, overall, the antipsychotics driven changes associated with oxidative stress and metabolic pathways were more relevant to the drug side-effect profiles that include weight gain, metabolic syndrome, dyslipidemia and insulin resistance.¹²⁵⁻¹²⁷⁾

Mood stabilizers and miRNA

A few studies have investigated miRNA expressions in response to mood stabilizers, such as lithium or VPA. Thus, a study in Wister rats showed treatment with lithium changed levels of 37 miRNAs whereas treatment with VPA caused changes in 31 miRNAs.¹²⁸⁾ Again, looking for drug-class effects lithium and VPA significantly decreased levels of let-7b, let-7c, miR-128a, miR-24a, miR-30c, miR-34a and miR-221 and up-regulated expression of miR-144. These changes will have functional consequences because it has been shown that a VPA associated down-regulation of miR-34a leads to an increase in expression of one of its target gene, metabotropic glutamate receptor 7 (mGlu7).^{128,129)} These findings are the first to demonstrate that miRNAs and their predicted effectors are targets for the action of psychotherapeutic drugs. Expanding this notion, it has been shown that chronic treatment with lithium increased levels of muscarinic M1 receptor expression and this occurs at least in part through by a down-regulation of lithium-sensitive miRNA, let-7b.¹³⁰⁾

Returning to more generalised effects of mood stabilizers, studies using a human lymphoblastoid cell lines (LCLs) showed that levels of 7 miRNAs were altered in response to lithium at treatment day 4 with 4 miRNAs being up-regulated (miR-34a, miR-152, miR-155, and miR221) at a longer treatment day 16.¹³¹⁾ Using a microarray technique and qPCR confirmation, lithium and VPA treatment was reported to down-regulation of miR-34a, miR-495 and miR-690 and up-regulation of miR-147b, miR-182, and miR-222 after lithium and VPA treatment in neuron cultured from rat cerebellar granule cells.¹³²⁾ It is therefore clear that mood stabilisers have a varied effect on miRNA and further work is required to better understand these effects and their likely consequences.

Antidepressant drugs and miRNA

MiRNA may play a role in modulating depressive neurophysiology via the regulation of serotonergic signalling.

¹³³⁾ It is shown that miR-16 is involved in fate determination of serotonergic and noradrenergic cells in 1C11 neuroectodermal cells, which can differentiate into either serotonergic or noradrenergic neuronal cells by the adaptation of serotonin transporter (SERT). In 1C11 cells, the basal levels of miR-16 expressed at higher levels in the noradrenergic locus coeruleus than in serotonergic raphe nuclei that suppresses translation of SERT mRNA within the locus coeruleus. However, after fluoxetine treatment, cells within the locus coeruleus showed decreased miR-16.¹³⁴⁾ Further, the exposure of raphe to fluoxetine released the neurotropic factor, S100 β , which acts on down-regulation of miR-16, and S100 β promoted the reduction in miR-16 and turned on the expression of serotonergic functions in locus coeruleus.¹³⁴⁾ In the similar line, recent data has revealed that treatment with fluoxetine also induced a decrease in the levels of miR-16 in hippocampus.¹³⁵⁾ This study investigated whether the fluoxetine-induced secretion of S100 β protein by the raphe acts on the hippocampus and found that the fluoxetine-induced changes in hippocampal miR-16 and SERT were partly reversed (40-50%) upon si-RNA-mediated knockout of S100 β mice in the raphe.¹³⁵⁾ Given the data that the action of fluoxetine on the locus coeruleus is mediated by the secretion of S100 β by the raphe,¹³⁴⁾ it may suggest the down-regulation of miR-16 in the hippocampus is relayed, in part, by S100 β via the noradrenergic neurons of the locus coeruleus.¹³⁵⁾ Another study indicated treatment with paroxetine increased BDNF expression and these effects were potentially limited by up-regulation of miR-30e-5p in the human glioblastoma-astrocytoma cells (U87).¹³⁶⁾ Finally, a clinical study investigated the levels of miRNA in the blood of patients with major depression before and after chronic treatment with escitalopram.¹³⁷⁾ It has been found 28 miRNAs up-regulated and 2 miRNAs (miR-34c-5p and miR-770-5p) down-regulated after escitalopram treatment. Taken together, these studies suggest there are several pathways mediating the differential expression of certain miRNAs in action of psychiatric drugs and it could contribute to the pathophysiology of clinical depression.

CONCLUDING REMARKS

This review clearly highlights the fact that psychotropic drugs affect many pathways that modulate cell signalling and gene expression. The challenge is that these effects are extremely diverse and complex. Based on the hypothesis that "class actions" by different drug types may be an

important guide to which changes may result in therapeutic outcomes. It would appear the effects of antipsychotic drugs on schizophrenia, mood stabilisers on bipolar disorder and antidepressant drugs on major depressive disorder may be of particular interest. However, the growing use of drug types outside of their classically defined areas, for example second generation antipsychotic drugs in mood disorders, may suggest that using drug “class actions” as a primary divider does not reflect their usefulness in clinical practice. Therefore there is a clear need to better understand the molecular mechanisms that are target by psychotropic drugs and to try and relate these findings to how the drugs improve symptom severity. Unfortunately, current data suggest psychotropic drugs have both widespread and localised effects on different molecular mechanisms. Hence, it will be necessary to dissect out what are CNS wide, region specific or cell type-specific effects of different drugs to gain a comprehensive understanding of their potential mechanisms of action. This is a challenging goal for the field moving forward but could produce great rewards in underpinning the development of drugs that may have a broader therapeutic reach with lesser unwanted side-effects.

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