A Brief Overview of Molecular Mechanisms of Depression and its Treatments

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INTRODUCTION

Major depressive disorder (MDD) is a widespread, chronic, and debilitating mood disorder with a lifetime prevalence of up to 20% in the US adult population (Kessler et al., 2003). Its symptoms include loss of motivation, deleterious changes in sleep and appetite, anhedonia, feelings of despair, and suicidal thoughts. MDD may have lethal consequences such as suicide and cardiovascular disease (Bernard et al., 2011). This disorder, historically treated by exorcisms, lobotomies, and electroshock therapies (Nemade, Staats Reiss, & Dombeck, 2015), involves changes in brain chemistry. Biomolecules such as serotonin and norepinephrine have been implicated in its etiology (Papakostas & Ionescu, 2015). Despite the known molecular underpinnings, MDD is still very poorly understood and its mechanisms remain elusive. Current treatments which target noradrenergic neurotransmission produce remission in only 37% of all patients and in most cases take several weeks to come into effect (Bernard et al., 2011). Moreover, many experience severe side effects including nausea, loss of appetite, decrease in libido, fatigue, insomnia, dizziness, and increased anxiety (Ferguson, 2001). Although the exact etiology of depression is unclear, environmental and oxidative stress, epigenetics, and genetics have been shown to contribute to the diathesis of depression. Recent findings suggest the involvement of neuroendocrine, immuno-inflammatory, and metabolic pathways, revealing that depression may be a much more fundamental disturbance in physical function than previously assumed. This paper focuses on some of the current research on the molecular and cellular mechanisms underlying depression and the action of its treatments.

ETIOLOGY

Oxidative Stress

The central nervous system (CNS) is capable of quickly adapting to normal or severe stressors. Under MDD conditions the brain is unable to react normally and thus its ability to adapt is compromised. It has been shown that in crisis situations the dopaminergic reward system increases its activity to maintain mental stability and well-being. However, stress mediators
downregulate this system, resulting in depressive moods (Phillip W. Gold, Machado-Vieira, & Pavlatou, 2015; P. W. Gold & Chrousos, 2002). Additionally, environmental stress has been shown to affect hippocampal neurogenesis, glucocorticoid release, and the function of 5-HT (serotonin) receptors (Mahar, Bambico, Mechawar, & Nobrega, 2014; Lupien, McEwen, Gunnar, & Heim, 2009), all of which have been associated with the molecular and cellular mechanisms of depression.

Several links between stress and genetics in the pathology of depression have been made, including polymorphisms in glucocorticoid receptor-coding genes in mice (El Hage, Powell, & Surguladze, 2009; Mahar et al., 2014). Animals subjected to chronic unpredictable mild stress, a common animal model of depression (Czéh, Fuchs, Wiborg, & Simon, 2015), have increased glucocorticoid levels and decreased expression of hippocampal genes linked to synapse formation (Mahar et al., 2014). Similar effects were also observed in human patients (Brown, Varghese, & McEwen, 2004; Christiansen, Bouzinova, Palme, & Wiborg, 2012; Duric et al., 2013; Goldstein et al., 2000; Henningsen et al., 2012; Mahar et al., 2014).

Stressors such as inflammation, glucocorticoids, and metabolic stress also frequently contribute to the shortening of telomeres in mononuclear cells, increasing likelihood of apoptosis or senescence observed in MDD (Blackburn, 2000; Szebeni et al., 2014). Shortened telomeres can serve as a biomarker for acceleration of cells aging due to exposure to said stressors. Although the exact mechanisms of telomere shortening are unknown, oxidative stress resulting from inflammation or stress hormones has been implicated. Reactive oxygen species (ROS) produced by the brain, usually mediators of the intracellular stress responses, with age play an increased role in cellular damage. ROS react with DNA, among other biological targets, attacking guanine nucleotides which are especially sensitive to oxidation. Because the telomere region of DNA is especially guanine rich, oxidative stress contributes to its shortening (Szebeni et al., 2014).

**Epigenetics**

Stress is widely accepted as a causal factor in depression and has been shown to induce changes in gene expression through mechanisms such as the methylation or acetylation of DNA (Dalton, Kolshus, & McLoughlin, 2014), (Heim & Binder, 2012). However, there is still an absence of predictors for anti-depressant response in patients, and effective treatments for each individual have to be determined via trial and error. It has been suggested that there are several distinct molecular mechanisms of MDD, depending on whether the disorder is “endogenous,” meaning it has no identifiable external cause, or “reactive,” meaning it is in response to a specific stressor.
Moreover, the timing of the stressor, distal or proximal, has also been shown to have an effect on antidepressant responses in patients (Malki et al., 2014).

Using hippocampal tissues from three animal models for “reactive,” “endogenous-distal,” and “endogenous-proximal” depression, Malki et al. (2014) identified unique sets of genes involved in each animal model of depression in addition to a small group of genes that were involved in all three models. The gene network associated with the early (distal) stressors is primarily related to neurodevelopmental disorders, and the gene network associated with the late (proximal) stressors is involved in cell signaling and stress response. Moreover, the overlap in altered gene expression between the two reactive models was much smaller than that between either early or late stress and endogenous (9%, 19%, and 11%, respectively). This points to the existence of several significantly different mechanisms of depression which could account for the heterogeneity in responses to treatment (Malki et al., 2014).

A small group of genes was also identified that was differentially regulated across the three models, which could represent a common pathway to major depressive disorder. Identified genes include Ppm1a, Ywhaz, Nfkb, and Mapk. Ppm1a expression has been shown to be significantly altered by nortriptyline (a tricyclic antidepressant), and the human analog of the gene exhibited similar responses to the drug. Ywhaz (tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein) is associated with neurogenesis and cell proliferation, which is consistent with the neurogenesis hypothesis for MDD. Nfkb plays a role in peripheral inflammation, which is in agreement with the inflammation theory of depression. Thus, despite the varying etiological stressors and their effects, there seems to be a commonality which could serve as a drug target to eliminate the heterogeneity in antidepressant responses among MDD patients (Malki et al., 2014).

Genetics

To date, MDD does not have any single causative risk factor and is hypothesized to be a composite polygenic phenotype (Sarosi et al., 2008). Single nucleotide polymorphisms (SNPs) play an important role in the etiology of depression, but genome-wide association studies (GWAS) have failed to determine significant genome-wide associations between MDD and SNPs (Lubke et al., 2012; Sullivan et al., 2009; Wray et al., 2012). However, alternative methods focusing on the additive effects of SNPs and phenotypic variance determined that SNPs explain a significant fraction of the heritability of MDD phenotype and the variance in treatment responses (Lubke et al., 2012).

The most prominent SNP site associated with the therapeutic response to selective serotonin reuptake inhibitors (SSRIs) in MDD patients occurs in the transcriptional control
region of the serotonin transporter gene (Tsai et al., 2009). Sarosi et al. (2008) investigated the STin2 (serotonin transporter intron 2) polymorphism, which has been hypothesized to have an effect on the serotonin transporter expression levels. The researchers found that the presence of one or two copies of the STin2.10 allele was linked to suicide completion and cognitive impairment in MDD. This polymorphism affected attention, verbal learning and memory, but it did not affect executive function and visual memory. Individuals with the STin2.12 allele, which is linked to higher expression levels of the serotonin transporter, performed significantly better than STin2.10 carriers on cognitive assessment tests. Moreover STin2.10/STin2.12 genotype has been shown to be less responsive to SSRI treatments than the STin2.12/STin2.12 genotype (Sarosi et al., 2008).

One more SNP affected gene related to cognitive impairment is AMST (N-acetylserotonin O-methyltransferase), which has been shown to be downregulated in MDD patients. ASMT catalyzes the conversion of N-acetylserotonin to melatonin and is the limiting enzyme in melatonin formation (Sugden, Ceña, & Klein, 1987), (Reiter, Tan, Terron, Flores, & Czarnocki, 2007). Melatonin is involved in sleep induction, circadian rhythms, memory, metabolic regulation, antioxidative defense, and memory (Pagan et al., 2011). Because melatonin is involved in neuroplasticity and enhances cell survival, the downregulation of ASMT has been linked to cognitive impairment (Gałecki, 2014). Thus, it seems that melatonin misregulation is consistent with the neuroplasticity, inflammation, and metabolic theories of depression.

KEY PLAYERS IN THE MECHANISMS OF DEPRESSION
Locus coeruleus

In the mammalian brain, locus coeruleus (LC) has the highest levels of norepinephrine (NE)-producing neurons, which project throughout the entire CNS. Among many other areas, the LC is connected with the hippocampus, hypothalamus, thalamus, cerebral cortex, and 5-HT-producing raphe neurons. Together, the LC and raphe neurons regulate serotonergic and noradrenergic systems and modulate circadian rhythms, memory, and responses to stress (Bernard et al., 2011; Loughlin, Foote, & Bloom, 1986; Loughlin, Foote, & Fallon, 1982). Because all of these systems and functions have been implicated in the etiology of depression, the LC is an attractive research target.

Several proteins that play a role in the disturbances of NE production levels in the LC have been identified. Postmortem studies in MDD patients have revealed increased expression of presynaptic alpha 2 adrenoreceptors, which inhibit firing of neurons and NE secretion. Additionally, tyrosine hydroxylase (a rate limiting enzyme in NE
synthesis) is upregulated. Expression of glutamate signaling genes was also altered (Bernard et al., 2011).

Because the LC locally expresses mRNA subunits for several different metabotropic glutamine receptors (GRM) and subunits for alpha-amino-3-hydroxyl-5-methyl-4-isoxazole (AMPA) receptors, which elicit norepinephrine release, it has been postulated that glutamate drives norepinephrine turnover in the LC. Bernard et al. (2011) found that the glutamate signaling pathway was the most significantly altered in the LC of MDD patients. Moreover, only glial cells exhibited expression changes of glutamate signaling genes including the downregulation of a high affinity glutamate transporter (SLC1A3). SLC1A3 mediates glial glutamate reuptake and maintains synaptic glutamate concentrations to protect neurons from excitotoxicity. The expression of GLUL, an enzyme that converts glutamate to glutamine, was also decreased in the MDD locus coeruleus, inferior frontal gyrus, anterior cingulated and dorsolateral prefrontal cortex (Bernard et al., 2011).

Glial cells regulate neurotransmitter density and reuptake, and have been shown to be involved in the molecular mechanisms of depression. Postmortem studies have found a decrease in glial cell number and density in MDD cortical and limbic brain regions (Bernard et al., 2011). Glial cells also play a significant role in pathology of bipolar disorder (Dong & Zhen, 2015). Beneddeto et al. postulated that glial cells may serve as substrates for antidepressants’ restructuring of the neuronal network, which is mis-wired as a result of MDD (Di Benedetto, Rupprecht, & Czéh, 2013). Additionally, glia have been shown to respond to non-pharmacological therapies in animal models of depression, such as chronic electroconvulsive shock, which has been shown to increase CNS glial cell proliferation in the hippocampus, piriform cortex, and prefrontal cortex (Rajkowska & Stockmeier, 2013).

The reduction of glial cells, particularly astrocytes, in the postmortem prefrontal cortex (PFC) has been widely reported in subjects with MDD. Histological analyses of MDD anterior cingulate cortex white matter have also revealed a hypertrophy (i.e. inflammation) of astrocytic processes and cell bodies, supporting the theory of the involvement of neuro-inflammatory system in depression (Rajkowska & Stockmeier, 2013). Among numerous other functions, astrocytes mediate the connection between vasculature and neuronal tissue through projections dubbed “endfeet”. Astrocytic endfeet are also a major component of the blood brain barrier (BBB), which is frequently impaired in MDD. AQP4 (a protein mainly expressed in astrocytic endfeet) helps maintain the BBB through water distribution and ion transportation. Rajkowska et al. (2013) used immunofluorescent techniques to
track the presence of AQP4 in the PFC and found that only the orbitofrontal gray matter expressed a significant reduction in AQP4 as a result of MDD. A decrease in the number of endfeet could reduce the overall exchange of nutrients with the circulatory system, thus implicating the circulatory system as yet another component of MDD pathology (Rajkowska, Hughes, Stockmeier, Miguel-Hidalgo, & Maciag, 2013).

The role of astrocytes in glutamate neurotransmission has also been extensively studied in MDD pathology. It has been determined that glutamate transporters and glutamine synthetase are disregulated in postmortem MDD brain tissue, specifically in the anterior cingulate, dorsolateral prefrontal cortex, premotor cortex, locus coeruleus, and the amygdala (Rajkowska & Stockmeier, 2013), (Bernard et al., 2011). The degeneration of astrocytes as a result of depression results in glutamate/GABA imbalance in the affected structures in addition to an excess of glutamate in the synaptic cleft (Śmialowska, Szewczyk, Woźniak, Wawrzak-Wlecia, & Domin, 2013), thus leading to excitotoxicity. Together, these findings suggest a global dysfunction in astrocyte-mediated glutamate signaling in MDD.

Glial cells also have been implicated in depression in a number of other ways, including abnormal morphology and function of astrocytic gap junctions in MDD brains, upregulation of connexin proteins in response to SSRIs, and the presence of S100B (a calcium binding protein produced by astrocytes) in cerebrospinal fluid during major depressive episodes. Oligodendrocytes, too, have been shown to play a role in the mechanisms of MDD due to the significant shortening of oligodendrocyte telomere lengths compared with astrocytes, neurons, and other brain cells in MDD patients (Szebeni et al., 2014).

**Hippocampus**

The hippocampus is one of the few brain areas that facilitates adult neurogenesis, and disruption of that process has been implicated in many mental disorders, including Alzheimer’s, bipolar disorder, post-traumatic stress disorder, and major depressive disorder (Ge et al., 2015). It is involved in learning, memory, motivational control, and emotional control, and it connects to the PFC, thalamus, basal ganglia, hypothalamus, and amygdala, which form the mood regulation network. Hippocampal reduction in volume has also been well-documented in MDD patients (Cao et al., 2012), and it has been shown that greater severity of hippocampal size reduction corresponds with poorer responses to treatments (Lai, 2014). The hippocampus also mediates the hypothalamic-pituitary-adrenal (HPA) axis and expresses a high number of 5-HT and GABAergic receptors (Ge et al., 2015), making it a popular therapeutic target for antidepressant action.
Antidepressant treatments have been shown to stimulate the proliferation of neural progenitor cells in the dentate gyrus (a substructure of the hippocampal formation). Moreover, anti-depressive and anti-anxiety effects of fluoxetine (a widely used anti-depressant also known as Prozac) have been shown to fail in the absence of hippocampal neurogenesis (David et al., 2009). The stunted neurogenesis and hippocampal cell apoptosis associated with depression therefore suggest that long term depression may be more resistant to SSRI treatment. Patients with chronic depression not only exhibit higher symptom severity, but also lower remission rates in response to treatment. However, the phenomena was not in any way affected by differences in pharmacological treatments, suggesting that, even if the stunted neurogenesis hypothesis of long term depression is true, there are other factors at play (Köhler et al., 2015).

The importance of the hippocampus in the etiology of depression also stems from its susceptibility to stressors. The HPA axis controls mammalian stress response by controlling glucocorticoid production, and its hyperactivity has been linked to MDD and other mood disorders (Medina et al., 2013). In the hippocampus, glucocorticoids, mediated by the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR), play a role in plasticity and neuronal excitability. MR and GR have been hypothesized to affect the dysregulation of HPA axis function in MDD. Medina et. al (2013) demonstrated significantly different levels of MR expression in the hippocampus. Interestingly, they found that MR expression decreased in the anterior but not posterior hippocampus of MDD patients while GR expression remained unaltered. The researchers hypothesized that normal HPA axis function depends on a delicate balance between MR and GR receptors and their relative abundance in different hippocampal regions. The imbalance between the two likely contributes to stress-related dysfunction in MDD.

**Neuro-inflammatory pathways**

Numerous studies have demonstrated that major depressive disorder is also an inflammatory disease. Increased levels of proinflammatory cytokines (small proteins secreted by immune system cells that play a role in cell communication) have been found in patients with symptoms of depression and neurodegeneration. Several hypotheses exist about the role cytokines play in MDD pathogenesis. One theory is that cytokines stimulate the conversion of tryptophan to kynurenine by indoleamine 2, 3-dioxygenase (IDO) in glial cells (Miller, Haroon, Raison, & Felger, 2013; Noto et al., 2014). Kynurenine may then be transformed into quinolinic acid, which binds to NMDA receptors and depletes tryptophan (a precursor of serotonin). This leads to an increase in glutamatergic activity and
neurotoxicity that is likely responsible for the neuronal cell death and loss of plasticity characteristic in MDD.

Among the proinflammatory cytokines believed to be at the top of the stress-induced inflammatory cascade is the proinflammatory cytokine interleukin-1 beta (IL-1β), which has been shown to be involved in inflammatory responses leading to stress-related cellular damage (Gądek-Michalska, Tadeusz, Rachwalska, & Bugajski, 2013; Pan, Chen, Zhang, & Kong, 2014). IL-1β expression is significantly upregulated in MDD PFCs in both rats and humans, and antidepressants block its inflammatory effects (Pandey et al., 2012; Pan et al., 2014; You et al., 2011). NLRP3 inflammasome (nucleotide binding receptor) is involved in IL-1β synthesis and regulates transcription and function of IL-1β in the nuclear factor kappa B inflammatory pathway, which regulates proinflammatory cytokine production and cell survival (Lawrence, 2009; Pan et al., 2014). NLRP3 inflammasome and its associated proteins are also over-expressed in MDD patients with inflammation as well as in rats subjected to chronic unpredictable mild stress. Pan et al. (2014) demonstrated that IL-1β likely plays a role in initiating the inflammatory cascade in response to stress.

Several anti-inflammatory agents have been investigated as treatments for MDD, including aspirin, celecoxib, and TNF-α (tumor necrosis factor) antagonists. One study demonstrated that patients who are non-responsive to SSRI treatments exhibit an accelerated response after a course of aspirin. Celecoxib has been shown to have anti-depressive effects on its own and to be very effective in combination with fluoxetine in decreasing depressive symptoms (Noto et al., 2014).

**TREATMENTS**

Traditional treatments for depression typically feature tricyclic anti-depressants, selective serotonin reuptake inhibitors (SSRIs), and, more recently, selective norepinephrine reuptake inhibitors (SNRIs). Additionally, glutamatergic neurotransmission has been hypothesized to be involved in the therapeutic action of many antidepressant drugs, such as phenelzine (an irreversible non-selective inhibitor of monoamine oxidase) (DrugBank, 2013; Śmiałowska et al., 2013). However, the exact mechanisms of action of these compounds are not very well understood and are current subject of intense research.

One of the current therapeutic research targets are mood altering agents that promote neuronal survival and alter energy levels. For example, creatine is an ergogenic compound that has been shown to possess anti-depressant action in treatment-resistant patients. The creatine-phosphocreatine system stores energy and controls its availability in tissues that have fluctuating energy needs, such as the brain, thus
creating a metabolic barrier to prevent energy exhaustion and cell death. Chronic administration of creatine resulted in diametrically opposed effects in male and female rats, with male rats more vulnerable to the negative effects of creatine. Conversely, creatine had antidepressant-like effects in female rats, which is consistent with prior findings (Brotto, Barr, & Gorzalka, 2000; Drossopoulou et al., 2004; Lifschytz, Shalom, Lerer, & Newman, 2006) that responses to antidepressant treatments are sex-dependent (Allen, D’Anci, Kanarek, & Renshaw, 2010). This difference between sexes poses yet another question about the mechanisms of depression and demonstrates the difficulties in regulating the neural function.

Another research target is oleamide, an endogenous bioactive lipid signaling molecule which has been shown to evoke anti-anxiety and anti-inflammatory effects. Oleamide (OLE) treatment of rats subject to chronic mild stress (CMS) revealed several key hippocampal proteins that were differentially expressed between the CMS and CMS + OLE groups. For example, GSTA4, a protein involved in oxidation pathways, was upregulated in the CMS group and downregulated following oleamide treatment. The increase in GSTA4 expression is likely part of a protective mechanism against oxidative damage. The fact that GSTA4 is downregulated following oleamide treatment suggests that oleamide reverses or hinders oxidative damage caused by CMS. Several other differentially expressed proteins have been identified that were involved in metabolic and cell communication pathways as well. However, those proteins were either only altered by CMS and unaffected by oleamide and vice versa, making it difficult to establish links to the antidepressive actions of oleamide (Ge et al., 2015). Nonetheless, potential new therapeutic targets have been identified.

**CONCLUSIONS**

Contrary to prior beliefs, depression is not simply an emotional disorder, nor can it be solely explained by the decrease of serotonin levels. Major depressive disorder is a common malfunction affecting inflammatory, neurogenesis, and neuroendocrine systems. Scientists have gained a deeper understanding of the etiology of depression through epigenetics and genetics and a deeper understanding of the cellular and molecular mechanisms underlying the disorder. Ultimately, the goal is to use this knowledge to develop treatments, or even preventative measures, for major depressive disorder. In addition to being a wide-spread mental disorder, MDD also has a high rate of comorbidity other afflictions, including panic disorder, alcoholism, addictions, cardiovascular disease, diabetes, dementia, and osteoporosis (Ferentinos et al., 2015; Lai, 2014; Szelenyi et al., 2014; Wolkowitz, Reus, & Mellon, 2011). Increased knowledge of depression mechanisms can increase the understanding of its comorbid
diseases. Moreover, because MDD is primarily a cognitive disorder, knowledge of its mechanisms will provide insight into the mystery of human cognition.
References:


