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Mechanisms of action of antidepressants: from neurotransmitter systems to signaling pathways

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Abstract

Antidepressants are commonly used in the treatment of anxiety and depression, medical conditions that affect ~17–20% of the population. The clinical effects of antidepressants take several weeks to manifest, suggesting that these drugs induce adaptive changes in brain structures affected by anxiety and depression. In order to develop shorter-acting and more effective drugs for the treatment of anxiety and depression, it is important to understand how antidepressants bring about their beneficial effects. Recent reports suggest that antidepressants can induce neurogenesis in the adult brain, although the mechanisms involved are not clearly understood. In this review, we describe the different neurotransmitter systems that are affected by anxiety and depression and how they are modulated by antidepressant treatment with a focus on signaling molecules and pathways that are activated during neurotransmitter receptor induced neurogenesis.

Keywords

Anxiety; Depression; Neurogenesis; SSRI; CRH; Cortisol

1. Introduction

Depression and anxiety are serious conditions that often require medical intervention. Depression is generally characterized by feelings of sadness accompanied by emotional and physical withdrawal, while anxiety is a fear-induced state not linked directly to a discernable stimulus [1]. Clinically, both disorders are treated with antidepressants coupled with counseling. However, not all patients respond to antidepressant treatment, the therapeutic effects take several weeks to manifest and these effects are often accompanied by unwanted side-effects. The development of more specific and faster-acting treatments requires the understanding of the mechanisms involved in the development of anxiety and depression.

Behavioral studies on anxiety and depression can be carried out in animals using the elevated plus maze or the light–dark box tests for anxiety, and the forced swim or conditioned suppression of motility tests for depression [2,3]. These studies helped in the elucidation of antidepressant targets and systems dysregulated in depressed or anxious states, which include the hypothalamic–pituitary–adrenal (HPA) axis, the monoaminergic system, the γ-aminobutyric acid (GABA) system, and adult hippocampal neurogenesis. In

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this review, we describe the changes that occur in these systems during anxiety and depression and upon antidepressant treatment. We also describe evidence obtained from different transgenic mouse models and how this may affect the development of new therapies for the effective treatment of anxiety and depression.

2. The hypothalamic–pituitary–adrenal (HPA) axis

HPA axis plays a major role in the response of organisms to stress and a hallmark of anxiety and depression is the malfunction of the HPA axis. Under stress conditions, the hypothalamus secretes corticotropin-releasing hormone/ factor (CRH/CRF), which in turn, stimulates the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary and ACTH stimulates the release of glucocorticoids from the adrenal cortex [4]. Since excessive amounts of glucocorticoids are damaging to the organism, the HPA axis is under tight control [4]. Negative feedback regulation occurs mainly through mineralocorticoid and glucocorticoid receptors [5]. In humans, cortisol is the principal glucocorticoid modulating metabolism, cognitive processes, and emotions, especially fear and anxiety [6].

Malfunction of the HPA axis in anxiety and depression may involve either increased CRH levels [7] or an impaired cortisol negative feedback mechanism [8]. These are described in the following sections.

2.1. Modulation by CRH

CRH has been implicated in the pathophysiology of a number of neuroendocrine, neurologic and psychiatric disorders, including chronic anxiety and melancholic and atypical depression [9]. CRH and related peptides mediate their effects by activating specific G-protein coupled receptors (GPCRs), CRH-R1 and CRH-R2 [10]. Activation of these receptors causes opposite effects in the modulation of anxiety, since CRH-R1 mediates anxiogenic effects while CRH-R2 mediates anxiolytic effects [11,12]. CRH-R1 binds CRH and urocortin I and is involved in the fight-or-flight response to stress. CRH-R2 binds urocortin I, II, and III and mediates the slower adaptive response to stress.

Results from targeted disruption studies have further supported a role for CRH-R1 in mediating normal responses to stress [11] and CRH-R2 in fine-tuning these responses [12]. Since CRH has been shown to be anxiogenic and urocortin II and III to be anxiolytic, it has been suggested that an imbalance between CRH and urocortin peptides is involved in the pathogenesis of anxiety and depression [13]. At the same time, evidence also points to hyperfunction of CRH-R1 or hypofunction of CRH-R2 in anxiety disorders [14].

Since activation of CRH-R1 receptors gives rise to anxiogenic and possibly depressogenic behavior, it is possible that treatment with receptor antagonists might elicit anxiolytic and non-depressogenic responses. Studies have shown that treatment of rats or monkeys with non-peptidic CRH-R1 receptor antagonists prevented the establishment of fear, and the expression of already established fear responses, as well as decreased the general anxiety and increased the exploratory activity of test animals [14].

2.2. Modulation by mineralocorticoid and glucocorticoid receptors

Impaired corticosteroid receptor function plays a key role in the pathophysiology of stress-related disorders such as anxiety and depression. Both glucocorticoid and mineralo-corticoid receptors are involved in negative feedback regulation of the HPA axis [5] at the level of the paraventricular nucleus of the hypothalamus and of the anterior pituitary [6], and are co-expressed in hippocampus, septum and amygdala–brain regions implicated in anxiety and fear [15].

Studies have shown that treatment with antidepressants and electroconvulsive shock upregulates the levels of mineralocorticoid and glucocorticoid receptors [16]. It has been reported that hippocampal mineralocorticoid receptors may be dysfunctional in human depression [17,18]. However, a study examining the effect of administration of the antagonist, spironolactone, to depressed patients found that the receptor activity was not altered in these patients [17]. The total number of glucocorticoid receptors does not appear to be altered during depression although some studies have reported a decrease [19]. Recent evidence suggests that the receptor activity (ability of the glucocorticoid receptor to bind to its ligand or translocate to the nucleus), rather than a change in receptor number, is more likely to play a role in mood disorders [16]. Of the many theories for the primary cause of reduced glucocorticoid receptor function, the most compelling is that it is altered by ligand-independent mechanisms, i.e., signal transduction pathways activated by compounds unrelated to corticosteroids such as interleukin 1 and protein kinase A, both of which have been implicated in the pathophysiology of major depression [16].

Transgenic animals with specific genomic alterations in glucocorticoid or mineralocorticoid receptors have been generated to study the regulatory mechanisms of the HPA axis as well as the behavioral consequences of genetic manipulation. Mice with decreased glucocorticoid receptor gene expression (by transgenic expression of antisense mRNA directed against the receptor) exhibit reduced hypothalamic CRH expression [20], enhanced stress-associated ACTH response [21–23], and impaired efficiency of glucocorticoid-mediated negative feedback [21,23–25]. These mice exhibit deficits in spatial learning, enhanced responses to novelty and decreased locomotion in familiar environments [6]. These mice also exhibit changes in mesolimbic dopaminergic [26] and raphehippocampal serotonergic neurotransmission [27,28], as well as disrupted hippocampal long-term potentiation [29]. In addition, they show differences in binding to 5-HT_1 and 5-HT_2 receptors in hippocampal areas containing both glucocorticoid and mineralocorticoid receptors [30]. Mice lacking glucocorticoid receptor (GR k/o) die shortly after birth due to severe impairment of lung development and respiratory failure [31]. Approximately 10% of the mice survive and they have high plasma ACTH and corticosterone levels [31,32]. These mice exhibit impaired long-term spatial memory and increased activity in the open field test [33]. Mice with a nervous system-specific knockout of the glucocorticoid receptor (GR^{NesCre}) have increased hypothalamic CRH expression, reduced plasma ACTH levels, and increased plasma cortisol levels [34,35]. These animals exhibit reduced anxiety-like behavior [34,35]. Mice with conditional knock-in of a DNA-binding defective glucocorticoid receptor (GR^{dim}) express glucocorticoid receptors that cannot dimerize or activate glucocorticoid response element (GRE)-driven genes [36,37]. These mice have normal hypothalamic CRH expression, increased pituitary proopiomelanocortin (POMC) expression, normal plasma ACTH levels, and increased plasma corticosterone levels. They also exhibit impaired spatial memory but normal anxiety-like behavior [38]. Mice that overexpress the glucocorticoid receptor (YGR mice) have a marked decrease in hypothalamic CRH and pituitary POMC expression, increased plasma ACTH levels, and decreased plasma corticosterone levels [39]. To date, there are no reports on behavioral studies carried out in these mice. Finally, mice with brain-specific conditional overexpression of glucocorticoid receptors have normal basal plasma ACTH and corticosterone levels. These animals show increased anxiety behavior in a dark–light box paradigm but normal locomotor activity [6]. Mice lacking the mineralocorticoid receptor (MR k/o) usually die 10 days after birth due to severe dehydration and pseudohypoal-dosteronism [40]. They have increases in plasma aldosterone and corticosterone levels. Interestingly, they exhibit reduced density of hippocampal granule neurons and reduced hippocampal neurogenesis upon exogenous administration of NaCl to correct for the salt loss syndrome [6,35].

2.3. Modulation by atrial natriuretic peptide

Atrial natriuretic peptide (ANP) inhibits the HPA axis at all its regulatory levels. ANP is not only synthesized by atrial myocytes but is also found in neurons of brain regions like the hypothalamus, locus coeruleus and amygdala, where ANP receptors have been identified. ANP inhibits CRH-induced ACTH and cortisol secretion [41]. Intracerebroventricular, intra-amygdalar or intraperitoneal administration of ANP or related peptides produce anxiolytic effects [41]. Isatin, an ANP receptor antagonist, has anxiogenic effects when administered to animals and is able to reverse the anxiolytic effects of intraventricularly administered ANP [42].

3. GABAergic system

The GABA system is another system critical to our understanding of anxiety and depression. The neurotransmitter GABA acts on ionotropic $GABA_A$ and $GABA_C$ receptors as well as metabotropic $GABA_B$ receptors [43]. Emrich et al. [44] proposed the involvement of GABAergic dysfunction in mood disorders based on studies with the mood stabilizer, valproate, which effectively treats bipolar patients. Since valproate causes an enhancement in the concentration of GABA in the brain, the authors postulated that the pathophysiology of mood disorders involved GABAergic deficiency [44,45]. Data obtained on the levels of GABA in plasma and cerebrospinal fluid are conflicting, with some studies reporting decreases and others no change [44]. However, neuroimaging studies suggest the involvement of reduced GABAergic neurotransmission in major depressive disorder [46]. In addition, benzodiazepenes, anxiolytics used in the treatment of depression, directly enhance GABA function by interacting allosterically with the α_2 and α_5 subunits of the $GABA_A$ receptor [44]. Furthermore, the selective GABA reuptake inhibitor, tiagabine, which targets the GABA transporter GAT-1, has been effectively used in the treatment of anxiety-related behaviors in mouse and human studies [47]. Additional evidence for the involvement of the GABAergic system in anxiety and depression comes from $GABA_{B(1)}$ subunit knockout mice which exhibit antidepressant-like activity in the forced swim test [48].

An interesting observation is the reduction in the number of GABA neurons detected in the orbitofrontal cortex of postmortem cases of major depression [49]. The mechanisms involved in this phenomenon are not known, although it could be due to a stress-mediated decrease in brain derived neurotropic factor (BNDF), a molecule involved in neurogenesis [50]. Another possibility involves a reduction in functional genes such as bcl-2, or decreased neurogenesis of GABA neurons if they undergo substantial turnover [51].

3.1. Interaction with the serotonergic system

There is strong evidence for interactions between the GABAergic system and the serotonergic system. Experimental data suggest that $GABA_B$ receptor antagonists display antidepressant-like properties via an interaction with the serotonergic system [43]. $5\text{-}HT_{1A}$ receptors are found on GABA inhibitory interneurons [52], and $GABA_A$ and $GABA_B$ receptor activation inhibits the firing of serotonergic neurons in the dorsal raphe nucleus [53]. Also, in 5-HT transporter (5-HTT) knockouts, the $GABA_B$ agonist, baclofen, causes lower hyperpolarization and inhibition of neuronal firing of the dorsal raphe nucleus serotonergic neurons [53]. Furthermore, inactivation of the $5\text{-}HT_{1A}$ gene leads to the downregulation of $GABA_A$ receptor alpha subunits, reduction in $GABA_A$ receptor binding and benzodiazepine-resistant anxiety [54].

3.2. Interactions with the noradrenergic system

Studies have described interactions between the GABAergic and the noradrenergic systems; the latter has been implicated in anxiety and depression. GABA can induce the activity of

norepinephrine in the rat brain [44]. Activation of $GABA_A$ receptors increases the release of norepinephrine in the rat hippocampus and cortex [55]. However, activation of $GABA_B$ receptors causes a decrease in norepinephrine release in the same brain regions [55]. Treatment with baclofen leads to a reduction in adrenergic binding sites [55], while treatment with norepinephrine increases GABA inhibitory transmission, probably through interactions with alpha adrenergic receptors in the human cerebral cortex and rat cerebellar cortex [56,57].

3.3. Modulation of HPA axis

Verkuyl et al. [58] conducted a study to examine how the GABAergic system modulates negative feedback regulation of the HPA axis during stress. They measured the miniature inhibitory postsynaptic currents (mIPSCs) in parvocellular neurons of unpredictably stressed rats and observed a reduced mIPSC frequency as well as changes in the expression of α_5 and δ $GABA_A$ subunits. This suggests that alterations or downregulation of GABAergic innervation in this region of the brain are responsible for HPA axis dysfunction during stress [58].

3.4. Interaction with neuroactive steroids

Rupprecht et al. [59] have examined the interactions between the GABAergic system and neuroactive steroids, such as 3α-reduced metabolites of progesterone and deoxycorticosterone, in neuropsychological disorders. The 3α-reduced neuroactive steroids exhibit antidepressant and anxiolytic properties via interactions with the GABAergic system [59]. Neuroactive steroids are potent positive allosteric modulators of $GABA_A$ receptors as they increase the frequency or duration of opening of GABA-gated chloride channels [60,61]. In turn, $GABA_A$ chloride channels influence gene expression via intracellular progesterone receptors [62].

4. The monoaminergic system

The first effective antidepressants, monoamine oxidase inhibitors and tricyclic antidepressants, augmented serotonin and noradrenaline levels in the synapse [63]. This led to the monoamine hypothesis for the pathophysiology of depression, which postulated a deficit in serotonin and noradrenaline in key areas of the brain in affected patients. However, this hypothesis does not account for the effectiveness of antidepressants in the treatment of anxiety disorders, and does not explain why drugs such as tianeptine, that increase serotonin reuptake are effective antidepressants [63]. In addition, a study conducted by Delgado et al. [64] showed that depletion of 5-HT or NE did not induce clinical depression in healthy subjects or worsen depression in unmedicated symptomatic patients with major depression. In the following sections, we describe the involvement of serotonergic and noradrenergic systems in anxiety and depression.

4.1. The noradrenergic system

In parallel with the serotonergic system, the noradrenergic system has been a valuable target for antidepressants. Norepinephrine is found throughout the brain and its functions include acting as a general regulator of mood and responses to stimuli such as stress [65]. As with the serotonergic system, the noradrenergic system consists of a complex circuitry with many connections to other neurological systems. Depression seems to be associated with a hypofunction of the noradrenergic system [65], and some antidepressants act by increasing the synaptic availability of norepinephrine [66].

α2-adrenergic and β-adrenergic receptors present in the frontal and prefrontal cortex appear to be closely associated with depression. Studies have demonstrated a downregulation of

α2-adrenergic receptors in depression [66]. The antidepressants mirtazapine and robexetine mediate at least some of their effects through α2-autoreceptors [66]. Norepinephrine transporters, which are responsible for norepinephrine reuptake in the synapse, are also a target of antidepressants. Administration of the norepinephrine reuptake inhibitor (NRI), desipramine, to rats shows that a decrease in norepinephrine transporter function is a result of decrease in transporter binding sites and not in gene expression [67].

The study of the interconnectedness between the serotonergic and norepinephrine systems has proven fruitful since serotonin/norepinephrine reuptake inhibitors (SNRIs) exhibit higher efficacy than SSRIs or NRIs alone [68]. Studies with knockout mice that are unable to synthesize norepinephrine and epinephrine show that these mice are unresponsive not only to the NRIs, desipramine and reboxetine, the monoamine oxidase inhibitor (MAOI) pargyline and the norepinephrine/dopamine reuptake inhibitor bupropion, but also to the SSRIs fluoxetine, sertraline and paroxetine [69]. These results demonstrate the important role norepinephrine plays in antidepressant therapy.

4.2. Serotonergic system

The serotonergic system has long been implicated in the pathogenesis of anxiety and depression. Some of the most compelling evidence involves the alleviation of depression caused by serotonin selective reuptake inhibitors (SSRIs), which increase the availability of serotonin at the synapse [70]. Tryptophan depletion studies also confirmed the relationship between serotonin and anxiety and depression [2]. Numerous studies have been conducted in an effort to uncover how antidepressants operate on the serotonergic system to alleviate mood disorders.

To date, over 15 different serotonin receptors have been identified. The receptors are divided into seven families, 5-$HT_{1–7}$, and exist in a number of subtypes such as 5-HT_{1A}, 5-HT_{2A}, 5-HT_{1B}. 5-HT_3 is a ligand-gated ion channel, while the other receptors belong to the superfamily of GPCRs [71]. Serotonin receptors are found throughout important fear structures in the brain, namely the hippocampus, cortex, and raphe nuclei, and their activation leads to both immediate and long-term changes [72,73].

5-HT_{1A} is the most extensively studied serotonin receptor. It is an important part of the fear circuit that regulates motor and autonomic stress responses. Anxiety and depression are often associated with a downregulation of the 5-HT_{1A} receptor in the hippocampus and in the temporal lobe [72]. Presynaptic autoreceptors negatively regulate serotonin activity on serotonergic neurons in the raphe nuclei, while postsynaptic heteroreceptors in the forebrain mediate the effects of serotonin in this target tissue [2]. Both receptor types work by hyperpolarizing the membrane and decreasing neuronal excitability [72]. Agonists of both receptor types are usually anxiolytic in mice [72].

Various 5-HT receptor knockout mouse strains have been developed to further study the role of serotonin in modulating the stress response. 5-HT_{1A} knockout mice exhibit anxiety-like behaviors, lower HPA response rates, and reduced adrenal gland weight [72]. Studies involving tissue-specific rescue of the 5-HT_{1A} knockout showed that recovery of the wildtype phenotype required the expression of 5-HT_{1A} receptors in the hippocampus and cortex, and not in raphe nuclei, during early postnatal development and not during adulthood [73]. Studies carried out to ascertain how chronic stress affects learning and memory mediated via 5-HT_{1A} receptors, showed that these mice exhibit a deficit in hippocampal-dependent learning and memory, impaired paired-pulse facilitation in the dentate gyrus, and higher limbic excitability [52]. This demonstrated the involvement of 5-HT_{1A} receptors in the decreased cognitive function that is often associated with mood disorders [52].

Another extensively studied serotonin receptor is the 5-HT_{1B} receptor. 5-HT_{1B} receptors are located on axon terminals of retinal ganglion cells in the superior colliculus and on the septal terminals in the hippocampus. Serotonin regulates acetylcholine and glutamate release via 5-HT_{1B} receptors [74]. Like 5-HT_{1A} receptors, they play a role in cognitive behavior. Studies found that knockouts have an increased tendency towards impulsive [74] or aggressive [75] behavior. Studies investigating the relationship between 5-HT_{1A} and HT_{1B} receptors showed that 5-$HT_{1A/1B}$ knockouts had increased extracellular serotonin in the hippocampus, suggesting that the pairing of SSRI with a 5-$HT_{1A/1B}$ antagonist might prove to be a potent treatment for anxiety and depression [70].

Treatment with antidepressants causes a downregulation of 5-HT_2 receptors [71]. In agreement with these findings, 5-$HT_{2A/2C}$ receptor agonists are anxiogenic [76]. Quantification of 5-HT_2 receptors in the postmortem frontal cortex of patients suffering from major depression indicated that untreated patients had increased receptor binding compared to normal controls. Smaller increases in 5-HT_2 receptor binding were seen in the case of medicated patients while individuals that had recovered from depression exhibited decreased receptor binding when compared to controls [77].

Studies with 5-HT_3 knockouts show that these animals display decreased levels of anxiety, lower ACTH response to stress, lower vasopressin levels in the hypothalamus, and higher CRH mRNA in the amygdala [78]. Additionally, the 5-HT_3 agonist, mCPP, appears to be anxiogenic [2].

Care should be taken in the interpretation of data obtained from knockouts of the serotonergic system because of the possible compensatory effect of other serotonergic receptors or other closely linked receptors. For example, the firing rate of 5-HT_{1A} neurons in dorsal raphe of 5-HT_{1B} knockout mice is almost doubled although it is not accompanied by changes in 5-HT_{1D}, 5-HT_{1B}, or α2-adrenergic receptors, nor there are changes in α2-adrenoceptor on norepinephrine terminals [79]. This suggests that the 5-HT_{1A} receptors are altered by the absence of 5-HT_{1B} receptors.

A number of studies have demonstrated interactions between the serotonergic system and the HPA axis. In the depressed state, elevated cortisol levels may lower *l*-tryptophan availability, decrease 5-HT turnover, down-regulate presynaptic 5-HT_{1A} receptors, and upregulate 5-HT_2 receptors. Conversely, serotonin stimulates the secretion of CRH and ACTH and may modulate negative feedback of the HPA axis by glucocorticoids [80]. It has been observed that a lack of CRH-R1 leads to an increase in serotonin levels under basal and stressful conditions [14]. In addition, chronic stress causes a downregulation of 5-HT_{1A} receptor mRNA and binding in the hippocampus [81].

All the studies described in this section confirm the importance of the serotonergic system in regulating mood. They also reveal that no receptor subtype is singularly responsible for diseases like anxiety and depression. Even the paradoxical role of serotonin as both anxiolytic and anxiogenic highlights the complexity of the brain response to stress [2]. If the pathogenesis of anxiety and depression lies in the serotonin system, more work needs to be done to uncover the molecular mechanisms of signal transduction and receptor trafficking in healthy as well as diseased states.

5. Molecular genetic approaches

In this era of enormous genetic capability, researchers have searched for the source of anxiety and depression at the genetic level. This approach is logical as first degree relatives of depressed individuals are at a threefold risk of becoming depressed [82]. Indeed, subjects who have never suffered from a psychiatric disorder, but belonged to a family with a high

incidence of depression, display an abnormal response to the combined dexamethasone-CRH-challenge test, a measure of HPA axis function [83]. Although it is becoming clear that there is no bdepression geneQ or banxiety gene, Q biochemical evidence paired with genetic technology have provided new insights into the etiology of anxiety and depression.

We have already discussed various knockout animals as they pertain to the appropriate physiological systems believed to be involved in depression and anxiety. Described here are studies that more directly address the genetics and heredity of anxiety and depression. A study compared the short and long variations of a functional polymorphism in the promoter region of the 5-HT transporter gene [84]. The short variation of the gene, *s* allele, correlates with a lower transcriptional efficacy of the 5-HT transporter gene when compared to the *l* allele. The *s*/*l* or *s*/*s* allele combinations were predictive of a more depressive response to life stress in individuals with this genotype [84]. Thus the study showed that the genotype only predisposes towards disease, which is only uncovered by stressful life events [84]. Additional studies have reported greater neuronal activity in response to fearful stimuli in individuals with a copy of the *s* allele as compared to individuals with a copy of the *l* allele [85]. In addition, six of seven family members with a missense Ile-425-Val substitution in the same gene had obsessive–compulsive disorder [86].

Microarray analysis can be used to detect genes that are upregulated or downregulated by treatment with antidepressants. This technique has been used to measure gene expression changes in the brains of unstressed mice treated with mirtazapine or paroxetine. Mirtazapine downregulated genes involved with cell cycle and brain development while paroxetine affected genes involved in metabolism and cell structure [87]. This technique could be used to investigate the roles of receptors such as, adenosine 2A, 5-HT_{1A} and 5-HT_{2A} which have been implicated in panic disorder [88] and 5-HT_{1B}, 5-HT_{2A}, dopamine D4, subunit 2B of ionotropic *N*-methyl-*d*-aspartate receptors implicated in obsessive–compulsive disorder [88].

In an interesting study, Alfonso et al. [89] used polymerase chain reaction amplification to measure mRNA for hippocampal genes in stressed and unstressed animals. The study identified four sequences: nerve growth factor, membrane glycoprotein 6a, CDC-like kinase 1, and $G_{\alpha q}$ that were reduced in the stressed animals. These genes are all presumed to be involved in cell differentiation. Interestingly, treatment with the antidepressant, clomipramine, restored normal expression in stressed animals for every gene except nerve growth factor [89]. The results of this study are especially promising because they provide support for the relatively new idea that neurogenesis is affected during anxiety and depression as well as recovery.

6. Neurogenesis

Recent studies suggest that neurogenesis is involved in the action of antidepressants. Neurogenesis is the process of neuronal stem cell proliferation, differentiation, and survival [90]. In the hippocampus, neuronal progenitor cells of the subgranular zone migrate into the granule cell layer, where they extend processes and mature into granule cells. This process has also been thought to occur in humans at a slow but detectable rate [91].

6.1. Neurotransmitter systems in neurogenesis

A possible mechanism by which antidepressants mediate their effects on neurogenesis is described by Duman et al. [92]. SSRIs or NRIs increase serotonin and/or NE levels, allowing these neurotransmitters to stimulate the cAMP cascade or the Ca^{2+} cascade, leading to an increase in CREB and BDNF [92]. Studies indicate that the CRH receptor antagonist reverses the decrease in neurogenesis observed in stressed mice [93]. This

suggests the involvement of multiple receptor systems and signal transduction cascades in neurogenesis. Other molecules postulated to play an important role in neurogenesis are fibroblast growth factor [90,92], Sonic hedgehog (Shh), Noggins [90], epidermal growth factor [92], insulin-like growth factor 1 [92,94] and transforming growth factor-α [94]. In addition, glutamate, GABA, and opiates are believed to play an inhibitory role in neurogenesis [94].

Initial evidence demonstrating the importance of neurogenesis in the treatment of depression and anxiety was the observation of reduced hippocampal volume in stressed individuals. Posttraumatic stress disorder (PTSD) patients have 8% smaller right hippocampal volume compared to healthy controls [95]. In another study, a 12% decrease in left hippocampal volume in survivors of child abuse (that met the criteria for PTSD) was also detected [96]. In addition, women that were victims of sexual abuse in childhood had a 5% decrease in left hippocampal volume [97]. In contrast, one study found no significant differences in hippocampal volume of unmedicated depressed versus undepressed individuals [98].

A number of studies have tried to establish a relationship between depression, neurogenesis, and the pharmacology of antidepressants. Gould et al. [99] demonstrated a suppressive effect of corticosteroids on neurogenesis in the adult rat dentate gyrus. However, reduced hippocampal volume in PTSD patients may represent a predisposition for developing the disease rather than a result of the neurotoxic effect of corticosteroids. An elegant study by Santerelli et al. [100] demonstrated the involvement of antidepressants in adult hippocampal neurogenesis. Investigators blocked the neurogenic and behavioral effects of antidepressants in mice by X-irradiation of the hippocampus. In addition, this study showed that 5-HT_{1A} knockout mice did not exhibit neurogenesis or respond behaviorally to SSRI treatment, implicating these receptors in antidepressant-mediated neurogenesis [100]. These studies suggest that treatment with antidepressants leads to adaptive changes such as neurogenesis over a period of weeks which may account for the delayed therapeutic effect of these drugs. In rats that were given the antidepressants venlafaxine or fluoxetine, 33 proteins were modulated by drug treatment including proteins involved in neurogenesis, outgrowth, maintenance of neuronal process, and neural regeneration/axonal guidance systems [101]. Very little information is available about signal transduction cascades involved in adult neurogenesis, although some studies have implicated the cAMP-CREB cascade in this process [93]. It is quite likely that additional signaling pathways and cellular networks are involved and they need to be explored further.

6.2. Cellular signaling molecules and pathways in neurogenesis

As described above, the 5-HT_{1A} receptor has been implicated in antidepressant mediated cell proliferation and neurogenesis [100]. A few studies have begun to examine the pathways and molecules involved in GPCR mediated neurogenesis. It appears that activation of a subset of $G_{\alpha i}$ coupled receptors leads to neuronal survival and neurite outgrowth. In fact, a recent study has shown that the expression of the activated form of one of the members of the $G_{\alpha i/o}$ family ($G_{\alpha o}$ Q205L) leads to increase in neurite outgrowth via a STAT-3 dependent pathway [102]. We have initiated studies to examine the signaling pathways critical for neurite outgrowth and neuronal survival mediated by a variety of $G_{\alpha i}$ coupled receptors including 5-HT_{1A} receptors. We find that $G_{\alpha i}$-Rap1-Src-STAT-3 pathway plays a major role in neurite outgrowth induced upon activation of a number of these receptors [102–104]. This is consistent with studies in PC12 cells where Rap1 and STAT-3 have been implicated [105,106]. In addition, muscarinic receptor induced neurogenesis of neural stem/progenitor cells involves CREB signaling which is mediated by Src phosphorylation [107]. Taken together with our studies, these data support the notion that STAT-3 is activated via Src phosphorylation and this plays a critical role in receptor-

mediated neurogenesis. It would be of interest to identify the proteins that are regulated by STAT-3 activation during neurite outgrowth and investigate their role in neuronal survival.

7. Future research

In this review, we have described the different systems involved in the pathophysiology of anxiety and depression. It is apparent that the molecules, pathways, and systems thought to be involved in anxiety and depression are interconnected. Moreover, these processes seem to play an important role in neurogenesis. It is important to note that patients with depression and anxiety display an array of neurological abnormalities. Therefore, anxiety and depression should be regarded as global neurological disorders.

There are several areas to be pursued in the search for effective antidepressant and anxiolytic drugs. An exciting area of research has been the use of CRH receptor antagonists as antidepressants. Administration of CRH-R1 antagonist reduces chronic stress [108] and induces neurogenesis [93] in a manner similar to that of the antidepressant fluoxetine suggesting that CRH-R1 antagonists have the potential to become a novel class of antidepressants.

Another area of research involves investigations into the molecular mechanisms of action of SSRIs. The identification of signaling cascades activated by SSRIs as well as other neurotransmitter systems (serotonin, adrenergic, GABA receptor) and the molecules, pathways and networks involved in neurogenesis will be critical to the development of more effective and fast-acting antidepressants.

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