NEUROBIOLOGICAL MODULATORS OF ANXIETY

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INTRODUCTION

Anxiety is a state of excessive fear and is characterized by motor sympathetic hyperactivity, apprehension and vigilance syndromes. The most common observation is an acute stress response characterized by a state of abnormal or exaggerated arousal or fear 1. Anxiety is the most common psychiatric disease seen in patients irrespective of nations, societies, and religions. Anxiety, or learned fear, is not necessarily harmful to everyday life but, rather, is a natural ability that may have evolved to evade unnecessary dangers. However, excessive anxiety is debilitating or disadvantageous for life as it reduces behavioral activities necessary for adaptation. Moreover, anxiety can be a core symptom of various mental/behavioral disorders, such as major depressive disorders, obsessive-compulsive disorders, panic disorder, adaptive disorder, post-traumatic stress disorder, social withdrawal disorder, and various phobias. The neuroanatomic circuits that support fear and anxiety behavior are modulated by a variety of neurochemicals, including peptides, neuropeptides, neurotransmitters, and neurohormones. These molecules play a role in modulating anxiety by interacting with receptors that are located in the brain and periphery. The neuroanatomic circuits that support fear and anxiety behavior are modulated by a variety of neurochemicals which provides knowledge about the pathophysiology of specific anxiety disorders and the neural pathways involved in anxiety are as follows.

Acetylcholine

Two different lines of evidence exist regarding cholinergic modulation of anxiety state. Cholinergic input to hippocampus is enhanced in response to anxiogenic and stressful stimuli, wherein, muscarinic M1 receptors mediate induction of anxiety states through noradrenergic pathways 6. On the other hand, nicotine facilitate GABAergic neurons and induce anxiolysis and anxiolysis is also being observed after increasing acetylcholine levels on administration of acetylcholinesterase inhibitor phystostigmine in doral or the ventral hippocampus 7.

Adenosine

Adenosine is formed by hydrolysis of 5-adenosine monophosphate and is transformed to inosine, which is then stored as adenosine triphosphate 8. Adenosine through A1 and A2A receptors exert anxiolysis through its facilitatory influence on GABA release in the septum and hippocampus 9.

Arginine vasopressin (AVP)

This nonapeptide regulates Hypothalamus-pituitary-adrenal system by enhancing the effects of corticotrophin releasing hormone (CRH) on adrenocorticotrophic hormone (ACTH) release. AVP elicits its effects through G protein-coupled receptors viz. V1A and V1B. SSRI49415, selective and orally active non-peptide antagonist of vasopressin V(1B) receptors produced anxiolytic-like activity 10.

Atrial natriuretic peptide (ANP)

Atrial natriuretic factor is produced by heart and released into the circulation. Intracerebroventricular (i.c.v.) administration of ANP elicit anxiolytic activity in the open field, the social interaction, and the elevated plus maze tests 11. Central and
peripheral administration of atriopeptin II, an amino acid residue peptide of ANP also produce anxiolysis in elevated plus maze test12.

**Cannabinoids**

They suppress flow of glutamate, norepinephrine and dopamine in hippocampus and cortex also interfere with GABAergic transmission in the amygdale, hippocampus and frontal cortex13. Due to complex pattern of influence of cannabinoids on release of neurotransmitters, both anxiolytic as well as anxiogenic profile has been observed for cannabinoids14.

**Cholecystokinin**

Cholecystokinin (CCK ) is an anxiogenic neuropeptide present in both the brain and the gastrointestinal tract. CCK-containing neurons are found in high density in the cerebral cortex, amygdala, hippocampus, midbrain PAG (periaqueductal gray), substantia nigra, and raphe. CCK has depolarizing effects on pyramidal neurons and stimulates action potential formation in the dentate gyrus of the hippocampus. Stimulation of CCK-receptor by CCK-4, is anxiogenic, whereas CCK-receptor antagonists exert anxiolytic effects15. CCK has important functional interactions with other systems implicated in anxiety and fear (noradrenergic, dopaminergic, BZD)16. CCK-4 is more anxiogenic in Panic disorder (PD) and Posttraumatic stress disorder (PTSD)17-18.

**Corticotropin-releasing Factor (CRF)**

Corticotropin-releasing factor (CRF), a 41 amino acid containing peptide, appears to mediate not only the endocrine but also the autonomic and behavioral responses to stress. Increased level of CRF increases anxiogenic behavior19-20. Conversely, either a CRF antisense oligodeoxynucleotide or a CRF receptor antagonist produce anxiolytic effects19,21,22. Similar anxiolytic action has recently been reported in transgenic mice lacking CRF1 receptors23-26. A recent study by Heinrichs and coworkers (1997) using CRF1 and CRF2 receptor antisense oligonucleotides provides evidence that the anxiogenic actions of CRF are mediated by CRF1 rather than CRF2 receptors23. The anxiogenic effects of CRF have been hypothesized to be mediated through actions of CRF on the locus coeruleus (LC) noradrenergic systems. The activity of the norepinephrine (NE) neuronal system has been observed to be increased during stress and anxiety in several animal species, and states of anxiety and fear appear to be associated with an increase in NE release in humans26. There is anatomical evidence for direct synaptic contact between CRF terminals and dendrites of NE cells in the LC, and both acute and chronic stress increases CRF-like immunoreactivity in the LC27,28. The anxiogenic effect of CRF may be mediated through its ability to increase the activity of the LC noradrenergic system. Both acute and chronic stress as well as stress in early life increase CRF levels in the LC.

**Estrogen**

Low estrogen is probably a risk for depression, though estrogen out of balance is probably a risk for anxiety. But it can get confusing! Estrogen affects all the big neurotransmitters. The most straightforward connection is with serotonin. Estrogen boosts serotonin29 by both making more of it and keeping it around after it's made30. So that's likely be the reason that estrogen for most people helps to fight depression and helps with sleep. Estrogen has a mixed effect on norepinephrine29 but probably boosts it in most women, which for most would mean feeling better, more alert. But for some, high norepinephrine might be the cause of the anxiety and panic attacks that estrogen seems to produce in some women. Estrogen probably lowers dopamine29 more than it boosts it30,31. Since low dopamine can be depressing, this might be one of the reasons that some women feel more depressed on estrogen. Estrogen definitely boosts acetylcholine29. And high acetylcholine has been linked with depression, so there's another possibility. Estrogen has receptors all through the "mouse" and "lizard" parts of the brain that control physical responses to emotions and moods. In particular, the hippocampus in this "old" part of the brain connects emotions and memory; we're more likely to remember something with an emotion attached to it.

**Gammaminobutyric acid (GABA)**

GABA is the central nervous system's most abundant inhibitory neurotransmitter. The presence of GABA in neural tissue tends to hyperpolarize neurons. This hyperpolarization occurs when GABA neurotransmitter binds to GABA-A receptors on neurons. Negatively charged chloride ions are allowed to flow down chemical gradient and into the neuron’s cell body. This electrochemical negativity inhibits the neuron and decreases the likelihood of its firing further electrical impulses. As GABA levels and GABA activity rises, neuronal firing and activity lowers2. Physiologically, GABA is a sedative and muscle relaxant. Preclinical and clinical evidence exist for dysregulation of the central GABA-ergic tone in anxiety disorders. Tiagabine, a selective GABA reuptake inhibitor exerts anxiolytic effect via GAT-1 transporter blockade thus facilitating GABA neurotransmission33.

**Galanin**

Galanin suppress the noradrenergic, serotonergic and dopaminergic neurons24. Endogenous galanin exert anxiolysis in amygdale in response to stressful conditions35. However, exogenous galanin has produced variable effects in anxiety states. Intracerebroventricular administration of galanin reduced anxiety-like behavior, whereas, injection into amygdala produced an anxiogenic effect36.

**Ghrelin**

Ghrelin is a peptide hormone, mediator of both behaviors linked to food intake and body weight and behaviors associated with psychosocial stress, mood, and anxiety. Ghrelin has effects on mood-related and anxiety-related behaviors as well as there is stress-induced elevations in ghrelin. It has been reported that rises in ghrelin occur not only during periods of energy insufficiency but also following either acute or chronic stress. Investigations into the ramifications of these stress-associated ghrelin increases are only in their early stages. Further it is suggested that these raised ghrelin levels may help to minimize the deleterious, depression-like behaviors often associated with stress, but perhaps at the expense of a worsened metabolic profile37.

**Glutamatergic transmission**

Glutamate levels are profoundly increased upon exposure to aversive stimuli and stress18. Antagonism of endogenous excitatory amino acid neurotransmission in the dorsolateral Periaqueductal gray (DLPAG) reverse behavioral suppression. Glutamate antagonists show an anxiolytic-like profile in the elevated plus maze39.
Glucagon-like peptide 1

Glucagon-like peptide-1 is widely present in brain stem neurons, which innervate locus ceruleus, hippocampus and amygdala. Injection of Glucagon-like peptide-1 into amygdala produced anxiogenic effect. The KF-1, a ubiquitin ligase

Ubiquitination plays an essential regulatory role in all critical eukaryotic cellular processes. Proteasomal degradation through the Endoplasmic Reticulum Associated Degradation (ERAD) pathway is not an exception. It has been well established that these processes play an important role in a variety of human somatic diseases, ranging from cancer, viral infection, diabetes, and inflammation to muscle wastage and neurodegenerative disorders. The KF-1, a ubiquitin ligase located on the endoplasmic reticulum (ER), may prevent excessive anxiety. KF-1 degrades some target proteins, responsible for promoting anxiety, through the ER-associated degradation pathway, similar to Parkin in Parkinson’s disease (PD).

Melatonin

Melatonin controls sleep and rhythm, which are generally disturbed in anxiety. Melatonin produce anxiolysis, which is blocked by Flumazenil, a GABA-A receptor antagonist. Neuroepinephrine Y

Activation of Y1 and Y5 receptors of NPY in the basolateral amygdala produces dose-dependent anxiolytic-like effects, which is reversed by a2- adrenergic receptor antagonists. Moreover, mutant mice lacking NPY show increased anxiety-related behavior.

Neuroactive steroids (Neurosteroids)

They are steroids synthesized from cholesterol in glial cells and neurons and has capability to alter neuronal excitability. They exert anxiolysis through GABA-A receptors. Deoxycorticosteroid derivatives like 3α, 5α-tetrahydroprogesterone (3α,5α-THP) and 3α,5α-tetrahydrodeoxy corticosterone (3α,5α-THDOC) bind at GABA-A receptors to enhance GABA-induced chloride currents, similar to benzodiazepines. Neurosteroids may be tested as therapeutic target for the treatment anxiety disorders with improved efficacy without motor and cognitive side effects.

Norepinephrine (NE)

Majority of noradrenergic neurons are found in the locus ceruleus. Altered noradrenergic signaling is linked to anxiety disorders. Sustained stimulation of locus ceruleus result in manifestation of anxiety symptoms. Stress-induced release of NE facilitates a number of anxiety-like behavioral responses. Norepinephrine transporter-deficient mice have increased circulating catecholamines and elevated heart rate and blood pressure. Blockers of adrenergic β receptors have also been utilized clinically for treatment of performance anxiety.

Progesterone

Progesterone may be even more unpredictable than estrogen. It can convert to estrogen and testosterone, and no one's quite sure what the progesterone receptors scattered throughout the brain are doing. And it can be turned into different chemicals (metabolites). One metabolite (allopregnanolone) is very depressing and another (pregnenolone) is just the opposite. It's clear though that progesterone helps with anxiety,
probably because it helps with GABA. Its metabolites bind with the GABA receptors (which are also throughout the brain). But it's not clear whether progesterone helps with depression. Some people feel more depressed when GABA is high because it’s a sedative, but some people are less depressed when it's high. It’s possible that progesterone might help with depression by helping with dopamine levels (the pleasure neurotransmitter).

**Protein kinase C epsilon (PKCe)**

Corticotropin-releasing factor (CRF), its receptors, and signaling pathways that regulate CRF expression and responses are areas of intense investigation for new drugs to treat affective disorders. It is reported that reduced protein kinase C epsilon (PKCe) shows reduced anxiety-like behavior, have reduced levels of CRF messenger RNA and peptide in the amygdala. In primary amygdala neurons, a selective PKCe activator, C epsilon Receptor for Activated C-Kinase (ceRACK), increases levels of pro-CRF, whereas reducing PKCe levels through RNA interference blocks increase in CRF. Local knockdown of amygdala PKCe by RNA interference reduces anxiety-like behavior. In short PKCe increases anxiety-like behavior.

**Serotonin (SHT)**

Serotonin's cell bodies are located in the midbrain raphe, and its axons project to frontal cortex where they may have important regulatory functions in mood, basal ganglia where limbic areas where they may modulate emotions, particularly.

Serotonergic neurons are implicated in the alteration of appetite, energy, sleep, mood and cognitive function in anxiety. Its role in anxiety is supported by its modulating effect on the locus ceruleus and its projections to the amygdala; anatomical structure almost conclusively implicated in anxiety. Fear and stress activate serotonergic pathways.

**Tachykinins**

Tachykinins throughout the brain, spinal cord, and peripheral nervous system are implicated in the pathophysiology of anxiety. Pre-clinical studies suggest anxiolytic effects of NK1 receptor Antagonists. Further, disruption of the NK1 receptor by knockout techniques results in reduced anxiety in response to stress.

**Testosterone**

Almost nothing is really known about testosterone and mood. There are receptors throughout the brain. It probably increases a sense of more energy and well-being for someone who is depressed. But some think that the main effect of testosterone even in men may be that it converts to estrogen. Testosterone may increase anger and aggression.

**Tryptophan And Related Compound**

Modulatory roles have been proposed for various nutrients, including the amino acid tryptophan (Trp) and its catabolite 5-hydroxytryptamine (serotonin, 5-HT), and also for several enzymes in Trp metabolism. Tryptophan 2,3-dioxygenase (TDO) plays an essential role in the homeostasis of systemic and brain Trp metabolism, including the dominant regulation of serotonergic pathway, under the physiological conditions. TDO also play a role in the maintenance of brain morphology via regulating adult neurogenesis in the hippocampus and subventricular zone. Furthermore, TDO modulates anxiety-related behavior, indicating a role of TDO in higher brain functions. Tryptophan (Trp), the initial rate limiting enzymes for the kynurenic pathway of tryptophan metabolism are tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). Inhibition or deficiency of TDO leads to increased plasma levels of Trp and its metabolites 5-hydroxyindoleacetic acid (5-HIAA) and kynurenine, as well as increased levels of Trp, 5-HT and 5-HIAA in the hippocampus and midbrain and anxiolytic modulation and increased neurogenesis.

**CONCLUSION**

The neuroanatomic circuits that support fear and anxiety behavior are modulated by a variety of neurochemicals. The agents like Adenosin, GABA, KF-1 A ubiquitin ligase, Atrial natriuretic peptide, LYNX2 a prototoxin genes, melatonin and neurosteroids are anxiolytics. Cholecystokinin, CRF, Estrogen, Arginine vasopressin, Glutamatergic transmission, Glucagonlike peptide-1, melatonin concentrating hormone, Norepinephrine, progesterone, PKCe, serotonin and Tachykinins are Anxiogenic. Whereas Cannabinoids, Neuropeptide Y and Galanin have dual action i.e. anxiogenic as well as anxiolytic. This detailed knowledge about the pathophysiology of specific anxiety disorders and the neural pathways involved in anxiety and fear processing helps in the development of therapeutic strategies that may ultimately provide the optimal means for reducing the morbidity of anxiety disorders.

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