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Review Article

Role of Ion Channelopathy in Neurological Diseases; Role of Gabaergic System Connectivity and Dysfunction in Neurological Disorders

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Abstract:

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) is produced by GABAergic neurons and is essential in human neurodevelopmental stage. It has a variety of functions in the CNS and exerts its functions by binding to several GABA receptors including ionotropic and ligand-gated chloride channels. The CNS neurotransmitters endorphin, dopamine, serotonin or 5-hydroxytryptamine (5-HT), norepinephrine (NE), and acetylcholine (ACh) are all regulated by GABA. Additionally, GABA has a role in the pathogenesis of some CNS-related diseases. This study discusses the function of GABA in human neurodevelopmental stages and its interactions with other CNS neurotransmitters. The significance of Cl- homeostasis in GABA receptor activities, which regulate Cl--mediated neurotransmission in the CNS, is also stressed. Furthermore, the relationship between intracellular Cl- changes and CNS diseases such as Down syndrome (DS), epilepsy, schizophrenia, and autism spectrum disorders (ASD) is discussed. The study also highlights the potential application of bumetanide to regulate intracellular Cl- levels and treat CNS disease symptoms. The article comes to the conclusion that understanding the function of GABA and chloride homeostasis in clinical CNS diseases is beneficial in developing innovative treatment options, and the potential of bumetanide offers a new direction for research and clinical intervention.

Key words: gamma-aminobutyric acid; central nerves system; neurotransmitter

Introduction

This paper discusses the importance of Gamma-Aminobutyric Acid (GABA) as a neurotransmitter in human neurodevelopmental stages and its interaction with other neurotransmitters in Central Nerves System (CNS). The first section focuses on the importance of Cl- homeostasis in the functions of the GABAA receptors, which regulate Cl--mediated neurotransmission in the CNS. We discussed about how cation/chloride cotransporters (Na^+ /K^+/ $2Cl^-$ cotransporter) NKCC1 and (K^+ /Cl^- co-transporter) KCC2 of Cl^- cotransporters regulate the [Cl^-] i. The second and last part of this review article explores the dysregulation of intracellular Cl^- in CNS diseases, including Autism Spectrum Disorders (ASD), Epilepsy, Schizophrenia, and Down syndrome (DS). Our previous research has revealed the effectiveness of Bumetanide, a specific NKCC1 inhibitor, in reducing the seizure frequency in patient's resistant to Temporal lobe epilepsy (TLE), improving motor function in rats with spinal cord injury, and restoring some behavioral deficits in patients with DS. So, the paper emphasizes the potential use of Bumetanide to regulate intracellular Cl^- levels and improve symptoms of CNS diseases. The study also evaluates the safety of Bumetanide in patients with CNS disorders. The paper concludes that the understanding of the importance of GABA and chloride homeostasis in neurological diseases is useful for developing new therapies. The potential of Bumetanide offers a new avenue for research and clinical intervention in the cure of CNS disorders.

What is the Gaba System?

Gamma-Aminobutyric acid, or γ-aminobutyric acid (GABA) is an important inhibitory neurotransmitter, produced from amino acid and glutamate by glutamate decarboxylases (GADs). Gads genes include Gad1 and Gad2. They encode GAD67 (1) and GAD65 [2) proteins, respectively (3, 4). However, some alternative pathways may be involved in some situations (5). GABA-producing neurons, known as GABAergic

neurons, have suppressive effects on receptors in the adult vertebrates. A prominent illustration of neurons with inhibitory activity in the CNS, which is a particular kind of GABAergic neuron, is medium-sized-spiny neurons (MSNs) (5). The morphology and functional characteristics of GABAergic interneurons are widely diverse and to date, 20 cortical subtypes and 21 hippocampal subtypes have been identified (6). Additionally, many other GABAergic neurons are localized in other brain structures, such as amygdala (7). Mammal CNS contains a large number of GABAergic neurons, which together with other GABA related components, make up the GABAergic system. GABA initiated its function through binding to several ionotropic and ligand-gated chloride channels including GABAA and GABAC, as well as its metabotropic receptor, GABAB (5). Here, we will concentrate on GABAA receptors, because they have a profound effect on chloride flow in neurons, that is important for our further investigation of GABA. The post-synaptic mature neuron hyperpolarization is occurred following the GABA binding to GABAA receptors at the post-synaptic site, and subsequent opening of ion channel and diffusing chloride (Cl-) into the cell following its concentration gradient (8). GABA is also proposed to have multiple functions in different situations in the CNS, the peripheral nervous system (PNS), and in other non-neuronal tissues.

In present review, first, we will discuss the function of GABA in neurodevelopment, due to the important role of GABA in neurodevelopmental stages in human body. In second part, we will focus on chloride homeostasis in neurons and then we will illustrate the GABA mediation on neurotransmitter systems in CNS such as endorphin, dopamine (DA), serotonin (5-HT), norepinephrine (NE) and acetylcholine (ACh). Finally, the role of GABA in etiology of some neurological diseases and some therapeutic goals will be discussed.

1.GABA in Developmental Stages

GABAergic interneurons are crucial for maturation at different developmental stages in the brain of healthy individuals (6, 9). GABA is an important neurotransmitter expressed in embryonic stage and also whole life. It is discharged from GABAergic neurons and bound to its postsynaptic surface receptors (RSs) including GABAA or GABAC (ligand-gated chloride channels), and GABAB metabotropic receptors (G-protein coupled receptors), exerting its effects (5). Another predominant inhibitory neurotransmitter, which is influenced by changes in Cl^-, is glycine (10) which is not our focus in this review.

1.1. Cl- Homeostasis

1.1.1. Cl- Homeostasis in development

Cl- homeostasis is important in GABAA Rs activities in both embryonic stage and mature brain. GABA released from presynaptic neurons in mature brain and binds to its ion-coupled receptors -a pantamer channel, on the postsynaptic neurons. The ion channel open and the polarity of the current using GABAA RS is dictated according to content of intracellular chloride ([Cl-]i) (figure 1) (11, 12). When the intracellular [Cl-]i concentration is reduced in relation to the resting membrane potential (RMP), the reversal potential of Cl- (Ecl) turns into negative. In this situation, GABAARs mediate influx of Cl-, causing the cell membrane become hyperpolarized and inhibiting GABA to act via Ecl and preventing the cell from responding to external stimuli. On the other hand, a high content of [Cl-]i causes a positive shift in Ecl, an efflux of Cl- through GABAARs, and a depolarization that may culminate in the firing of an action potential. In developmental stage based on higher intracellular concentration, GABA works an excitatory neurotransmitter. This function back to the level of expression and activity of two chloride transporters, NKCC1 and KCC2 which will be discussed in Chloride homeostasis in neuron (1.1.2 section). Additionally, no net Cl- current via GABAA Rs will exist when Ecl changes to levels similar to RMP, indicating that Cl- is at its equilibrium condition (13-15).

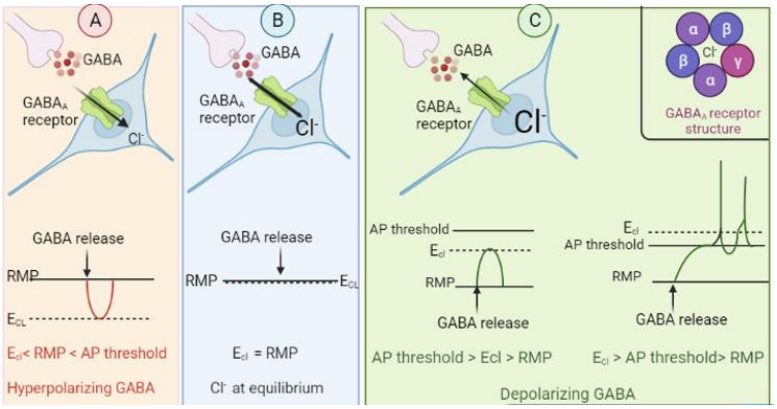

Figure 1: Cl- flow in neuronal cells and GABAA receptor's structure.

Two kinds of Cl- cotransporters regulate [Cl-]i in human neurons. SLC12 gene family or cation-chloride cotransporter gene family (CCC) (14), are two well-studied electrically neutral cation/chloride cotransporters termed (Na^+ /K^+/ 2Cl^- cotransporter) NKCC1 and (K^+ /Cl^- co-transporter) KCC2 (14), which significantly impact on neurotransmission through Cl- in CNS. The NKCC1 uses the electrochemical gradient of Na^+ to absorb Cl^- in mature brain. The accumulation of the intracellular Cl^-, causes hyperpolarization of neurons which not response to any stimulation signals. Whereas, the neuron-specific KCC2 drives Cl^- extrusion using the K^+ gradient, subsequently charge of inside the neuron will be less negative and depolarization and responding to the signals will be possible (8, 11). Expression of these co-transporters are regulated by gene activity and post-translational modification (16-18).

In functional regulation stage in human, WNKs (With No lysine = K), which is a serine–threonine kinases, act as an Osmo sensor (19). It has been crystal clear that not a single "chloride/volume sensitive kinase" controls the Na–K–2Cl and K–Cl cotransporters and it is much more complex. But within 0.5 minutes, endogenous WNK1 is activated by extracellular hyperosmotic circumstances (such as sorbitol, a salt like NaCl and KCl), which causes its phosphorylation at Ser382 regulatory sites. WNK kinase (s) are activated by hypertonic stress and perhaps lowered [Cl^-] i through autophosphorylation or stimulation by an unidentified upstream kinase. In turn, phosphorylated WNKs bind to, activate, and phosphorylate OSR1 and SPARK (STE20/SPS1-related proline/alanine-rich kinase and oxidative stress responsive kinase, respectively) (19). SPARK then binds to, activates, and phosphorylates NKCC1 at specific amino-terminal residues in its cytoplasmic tail (20). WNKs, in a direct or indirect manner, phosphorylate and block the K-Cl

cotransporters, by inactivating a phosphatase under comparable circumstances (21). However, it is generally unknown how Wnk kinase is activated in particular tissues and situations.

What is the net effect of NKCC1 and KCC2 phosphorylation?

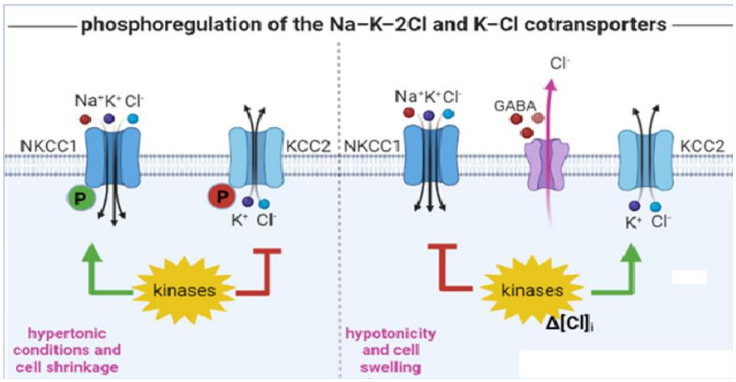

Figure 2: Changes in the [Cl−]i or extracellular tonicity cause the NKCC1 and KCC2 cotransporters to reciprocally phosphorylate one another. Hypertonia and cell shrinking promote cotransporter phosphorylation (P), which activates NKCC1 and inhibits K-Cl cotransporter. On the contrary side, NKCC1 and K-Cl cotransporter are inhibited by cotransporter dephosphorylation, which is promoted by hypotonicity and cell swelling. When NKCC1 activity is elevated and KCC2 activity is down, GABA signaling in GABAergic neurons is excitatory; in contrary, when NKCC1 activity is low and KCC2 activity is high, the signaling is inhibitory (21).

During embryonic stages, the expressed and activated form of NKCC1 is high, whereas KCC2 is remarkably low, therefore GABA excitatory manner is witness. During cortical maturation, interneurons have various roles including discharging of depolarizing GABA, boosting reproduction and migration, promoting synaptic maturation, and causing the immature networks to synchronize. During delivery, some factors like oxytocin secretion can significantly rise the activity and expression of KCC2. Additionally, in postnatal development, some genetic alterations such as decreased expression of NKCC1 and enhanced expression of KCC2, beside activity of WNKsm, make the GABA function as a hyperpolarizing response (22). So, as illustrated above, when interneurons developed, GABA becomes more hyperpolarizing, and a synchronized alteration happens in the whole GABAergic system, that involve the maturity of firing patterns, GABA discharge, GABA decomposition and absorption. These features, when combined, allow cortical networks to generate fluctuations with increased amplitude and frequency, and ultimately fluctuations in the fast gamma band, which is a property of mature circuits (6).

Still, some other features can play pivotal role in Cl^- homeostasis. Firstly, GABA extracellular concentration is regulated by high-affinity and specific transporters which are Na^+/Cl^- dependent. They (GAT^{-1}, GAT^{-2}, GAT^{-3}, and BGT^{-1}) are produced by four genes, among which two genes (GAT-1 and -3) are cerebral cortex-specific. The expression level of GATs is low at the time of birth. Rodents achieve adult levels gradually over the first postnatal month (23). Second, GABA is produced by two different isoforms of glutamic acid decarboxylase (GAD), including GAD65 and GAD67, which are found in the majority of $GABA^-$ containing neurons. GAD67, on the other hand, is found within the cytoplasm, while GAD65 is only found in membranes and nerve terminals. GAD67 is thought to largely create cytoplasmic GABA, whilst GAD65 ensures vesicular GABA synthesis (1). GAD65/67 expression development serves as a reliable hallmark of neuronal capacity maturation to generate and discharge of GABA. In rodents, GAD immunoreactivity steadily increases in the visual cortex until puberty (24). Overall, each environmental or genetic modifiers could dysregulate GABA functions and Cl^- homeostasis.

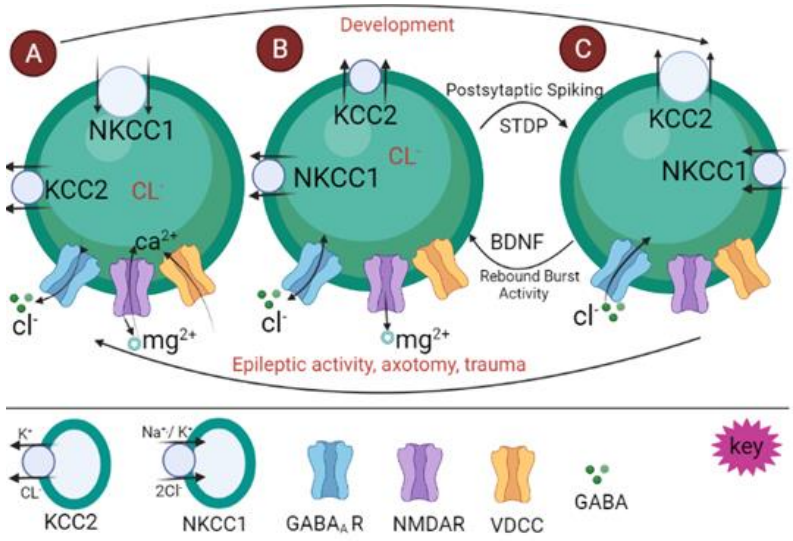

Figure 3: GABA in neuronal developmental stage and disorders.

1.1.2. Chloride Homesostasis in Neurons

As earlier discussed, [Cl^-]i has a crucial effect on GABA function and consequently on excitability of neurons, action potential firing patterns, and coordination of motion in neuronal cells (14). In this section we will discuss mechanisms and factors which have effect on Cl^- homeostasis in cells.

Generally, the homeostatic concentrations of Cl^- in different cells is characterized by two main regulatory mechanisms, the first via plasma membrane Cl^- channels and the second, through co transporters. Mechanisms that act as Cl- channels are categorized in three major classes including MP alterations (including ClC channels), intracellular Ca^{2+} signaling (including anoctamin channels), and ligand gated channels. Cation Cl^- co-transporters which include Na^+-K^+-Cl^-. cotransporter ($NKCC^1$), and K^+-Cl^- cotransporters (KCCs) are discussed earlier as the most important members. The pH sensitive Cl^- transporters (SLC4 and SLC26) are the second group that we will discuss.

1.1.2.1. Intracellular Cl^- Regulation Mechanisms

1.1.2.1.1. Voltage-Gated Cl^- Channels (CLC Family)

Chloride-conducting ion channels or CLCs are voltage-gated Cl^- channels and categorized in two main classes: 1) ClC^{-1} and ClC^{-2} are found in plasma membrane, and 2) ClC^{-3} to ClC^{-7} in intracellular organelles and vesicles. Plasma membrane ClCs function as stabilizer of MP and/or Cl- level, whilst intracellular ones (ClC^{-3} to ClC^{-7}) serve as an electrogenic Cl^-/H^+ exchangers, facilitating the endosomal and vesicular acidification (25-27).

1.1.2.1.2. Ca^{2+}-Activated Cl^- Channels (Anoctamins)

For a long time, it has been demonstrated that concentration of Ca2+ has an important function in cell signal transduction pathways, the release of neurotransmitter, and cellular excitability (28, 29). In a nutshell, by membrane depolarization, voltage-gated Ca2+ channels open and, in the same time, stored Ca2+ inside the cell causes elevating [Ca2+] i, which makes Ca2+-activated ion channels to be activated too (28). The TMEM16 family (also referred to Anoctamins) is one of the most important families of Ca2+-activated Cl- channels, with 10 members (Ano1-10) and attributed in neuronal excitability (30). Based on the Cl- gradient along the cell membrane, opening anoctamins causes Cl- efflux or influx (14, 31). Cl- outflow via anoctamins increases sensory signal transduction in neurons with high [Cl-]i through depolarizing the cell. Anoctamins causes hyperpolarization in low [Cl-]i cells via regulating Cl- inflow. Anoctamins begin gating Cl- fluxes in response to an increase in [Ca2+] i through the opening of voltage-gated Ca2+ channels and the discharge of Ca2+ from its internal storages. Based on the gradient of Cl- within the cell membrane, anoctamins open, resulting in Cl- outward or inward current (14, 31). Cl− efflux by anoctamins improves sensory signal transmission via cell depolarization in neurons with increased [Cl-]i. In low [Cl-]i level, Anoctamins promote hyperpolarization by influx of Cl-. Anoctamins begin gating Cl- fluxes in response to an increase in [Ca2+] i through opening the voltage-gated Ca2+ channels and discharge from internal Ca2+ storages (14).

1.1.2.1.3. Ligand-Gated Cl^- Channels

GABA-activated Cl- channels (13), glycine-activated Cl^- channels (32), and glutamate-activated Cl^- channels (33) are receptors that act as an ion-gated channels. As earlier mentioned, GABAA Rs is selective ion-gated channel which is permeable to both Cl^- and HCO^{-3} and has function in fast postsynaptic current. However, since GABAA Rs exhibits a 5-fold greater permeability to Cl^- than HCO^{-3} and a 4-fold elevated content of extracellular Cl^- than HCO^{-3}, Cl- plays a critical role here (34, 35). Another ion-gated channel is glycine-activated Cl^- channel. Gly Rs modulates excitatory or inhibitory reactions through increasing the inward or outward current of Cl^-, respectively (36, 37). The membrane potential (MP) and E_{cl} determine the polarity. The last group of this category are glutamate-activated Cl^- channels or excitatory amino acid transporters (EAATs). EAATs uptake of glutamate from the synaptic cleft to terminate glutamatergic transmission in order to prevent glutamate receptor overstimulation (33, 38).

Beside this, EAATs may also operate as glutamate-activated, Na+-dependent Cl- channels (39, 40). Electrophysiological researches in Purkinje cells (a type of neuron which is specific to cerebellar cortex (41) demonstrate that after glutamate discharge and elevated frequency action potential firing, Cl- transport through EAAT4 enhances, which could act as a further force to restrict excessive PC excitation (14, 38).

1.1.2.1.4. Cation-Chloride

Co-Transporters (Slc12 Family)

The electroneutral cation-chloride-coupled cotransporter gene family SLC12, also referred to cation-chloride cotransporter gene family (CCC) (14) has 9 member and consists of two major subdivisions. The first comprises two bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporters and the second is thiazide-sensitive Na^+-Cl^- cotransporter (42). Other than two SLC12 isoforms (A8 and A9), there are other transporters which transfer Cl-, accompanied with Na^+ and/or K^+ in as electroneutral manner (42). SLC12A2 (NKCC1) and SLC12A5 (KCC2) are the most studied members in neuron-related studies. NKCC1 and KCC2 are discussed in 1.2 in detail.

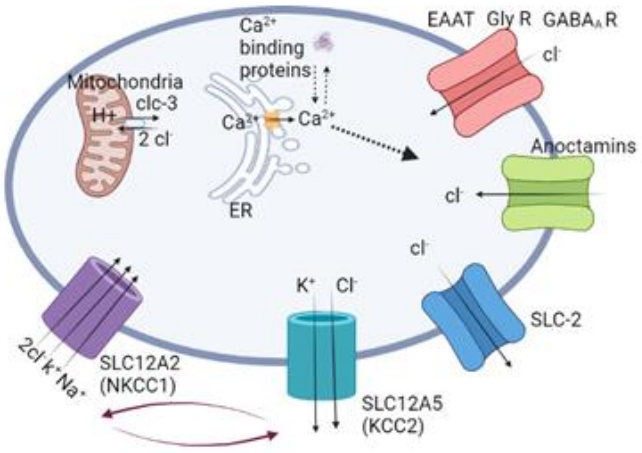

Figure 4: Chloride channels and two transporters in the olivocerebellar neurons (14).

1.1.2.1.5. PH-Sensitive Transporters

Commonly, acid regulation in all type of cells ranging from glial cells to neurons is regulated by three transmembrane transport mechanisms containing Cl-/HCO-3 exchangers, Na+/H+ exchangers, and Na+/HCO-3 cotransporters. These transporters are localized in cell membrane and membrane of intracellular organelles (43). Neuronal excitability and intracellular pH have been shown to be correlated, so that, following increased intracellular pH, the neuronal excitability can increase and vice versa (44, 45). Cl-/HCO-3 exchangers have been shown to be essential for maintaining intracellular pH at rest and for accelerating the recovery after pH fluctuations (14). Two main groups of pH-sensitive Cl- channels and transporters are SLC4 family of anion transporters and SLC26 family of anion transporters (14) which are important in controlling of intracellular pH and Cl-. SLC4 family encode ten genes (46) which are broadly expressed in the body. There are 10 members of this family (SLC4A1-5 and A7-11). Nine SLC4 members encode HCO-3 transporting proteins, among which, 9 proteins are categorized in two main groups: 3 proteins for Cl- /HCO3 exchangers (AE1 – 3) and 5 proteins for Na+-coupled HCO-3 transporters (NBCe1, NBCe2, NBCn1, NBCn2, NDCBE) (14, 46). Cl- is transported inside the cell by AE transporters, which also mediate HCO-3 extrusion. In contrast, NCBE and NDCBE mediate the influx of HCO-3 and efflux of Cl- NA (14). The other family, SLC26, comprises ten members (SLC26A1-A11), which exchange Cl- with some other molecules (14, 47). Although several studies have investigated the expression and role of this family in all part of the body (14), there is no much study about wide expression of SLC26 family in the brain.

1.2 GABA-Glutamate Interactions

GABA depolarization cooperates with stimulation of N-methyl-D-aspartate (NMDA) receptor, a subtype of ionotropic l-glutamate receptor, to regulate excitatory synapse formation. As part A of figure 2 shows, at early developmental stage, high concentration of Cl- as a result of GABA binding on its GABAA receptors and an efflux of Cl-, leads to a stimulating reaction that removes the Mg2+ block from the NMDA receptor and allows Ca2+ inward through opening voltage-dependent Ca2+ channels (VDCCs) and NMDA receptors. During normal development, elevated KCC2 expression, as indicated in section C, decrease [Cl-]i, which inhibits GABAA receptor activation. Inhibitory reactions are incapable of opening VDCCs or unblocking Mg2+ from NMDARs. As shown in section A, a neuron, following epileptic activity, axotomy, or neuronal damage, reverts (regarding Cl- homeostasis) to an immature state with excitatory GABA reactions. In reaction to increased physiological activity or when neurotrophic compounds are present, a reduction in the Cl--extruding capability of KCC2 results in an increase in EGABA. GABAergic neurotransmission may stimulate VDCCs and remove the Mg2+ blockage from the NMDAR according to the amount of Cl- buildup in the cell. Positive EGABA modulation may lead a postsynaptic current that is depolarizing. This current does not exceed the action potential threshold but still has an inhibitory effect through diverting excitatory current (11). Ca2+ may also influence the WNK-OSR1 pathway as well as NKCC1 function (48). Hypotonic stimulation causes a rise in intracellular Ca2+ concentration ([Ca2+]i), and this response is timed with the phosphorylation of OSR1 (48). In addition, any CNS insult, such as ischemia (49), secondary injury following trauma (50), or age-related neurodegenerative diseases (51-53), can result in excitotoxicity of glutamate and cell death (49). Excitotoxicity is caused by glutamate receptor over-activation, which leads to Na+ and Ca2+ influx and the opening of NMDA receptor channels and voltage dependent Ca2+ channels. Elevated [Ca2+]i can activate OSR1 (48) and WNKs kinase together with a change in NKCC1 and KCC2 function(21), resulting in the excitatory switching of GABA(54).

2. Interaction Between Gaba System and Other Brain's Neurotransmitters

Except GABA-glutamate interactions which discussed in section 1.2, GABA has various interactions with other neurotransmitters in brain which will be explained in the following.

2.1. Dopamine

Overall, dopamine is one of the catecholamine neurotransmitters in body (55) playing a crucial role in motor controlling, mood, and motivational functions such as rewarding sense (56). At first, we will review all types of dopamine (DA) receptors, because dopamine exhibits its function through binding and activating its surface membrane receptors (57).

2.1.1 Dopamine Receptors

The best investigated dopamine signaling pathway involves the control of cyclic AMP (cAMP) synthesis, with D1-like receptors increasing cAMP synthesis via Gs/olf and D2-like receptors reducing AC activities by Gi/o proteins (56). Overall, mammalian bodies have two primary kinds of dopamine receptors, D1-like receptor subtypes (D1 and D5) activate adenylyl cyclase via coupling to the G protein Gs which results in opening of sodium channels, so it can have excitation effect on other cells. Another group have inhibition activity and include the D2-like subfamily (D2, D3, and D4), which is G protein-coupled receptors and binds to Gi/Go, blocking adenylyl cyclase while activating K+ channels. Hence, it is not true to consider dopamine as merely excitatory or inhibitory. However, its impact on a target neuron is regulated by the kinds of receptor existing on the neuron membrane and its internal reaction to the second messenger cAMP. In addition, there are some differences in DA receptor's structures as transmembrane receptors. Most brain regions express multiple DA receptor subtypes (57, 58).

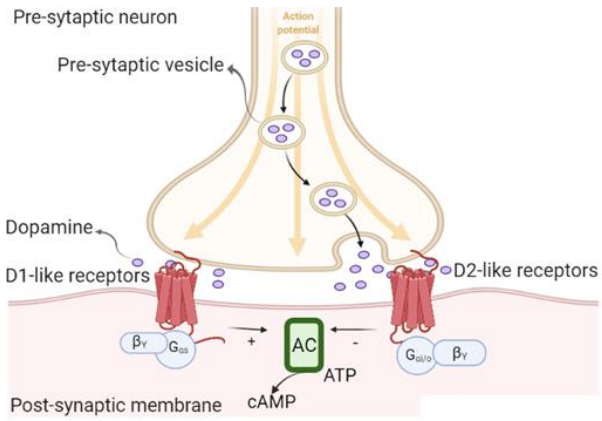

Figure 5: Regulation of adenylyl cyclase by D1-like and D2-like dopamine receptors.

Family	Receptor	Gene	Type	Mechanism
D1-like	D1	DRD1	Gs-coupled.	Increase intracellular levels of cAMP by activating adenylate cyclase.
	D5	DRD5		
D2-like	D2	DRD2	Gs-coupled.	Decrease intracellular levels of cAMP by inhibiting adenylate cyclase.
	D3	DRD3		
	D4	DRD4		

Table 1: Primary targets of dopamine in the human brain (20)

2.1.2 GABA-Dopamine Interactions

One of the most important and largest sources of dopamine in the vertebrate brain is in VTA (ventral tegmental area) which has function in a mesocorticolimbic dopamine system (projecting to the prefrontal cortex from the VTA (58). and drives reward-related behavior (59). VTA is made of three main cell types: GABAergic neurons, DA neurons (60) and glutamate neurons (61). By neurotransmission with GABAA rs, GABA neurons of the VTA suppress DA neurons (62). It has been demonstrated that stimulating function of VTA GABA neurons significantly reduces the spontaneous excitation of DA neurons (62). In contrast, following GABA neuron inhibition by activating halorhodopsin proton pumps in GABAergic neurons in VTA and influx of Cl-, a disinhibition or increase of dopamine was witnessed (63). GABA neurons also provide extended inhibitory effects on projection regions in nucleus accumbens (64).

GABA interneurons regulate the function of DA cells which discharge DA in the NAc using their extended projections. MSNs which lie below the cortex in the brain (56) respond to dopamine and expressing dopamine receptor type 1 (D1R-MSNs) of the NAc project to the VTA (65), make projections back to the VTA, selectively suppressing GABA cells and subsequently remove the inhibiting DA neurons. Additionally, certain GABA neurons make extended projections to the NAc, that specifically suppress CINs (cholinergic interneuron) (63). By activating of GABAA receptors, local GABA cells regulate their target DA neurons, which reduce their excitement and balances firing from glutamatergic inputs (63, 66).

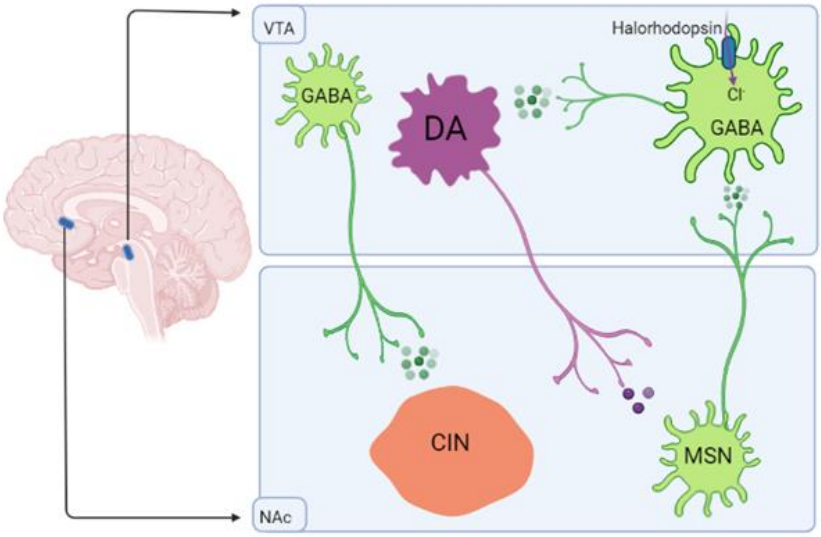

Figure 6: regulation of DA in VTA and NAc by GABA.

However, by deep look at VTA DA receptors in this region of the brain (shown in fig 5), it can conclude that D1 receptors regulate transmitter release from afferents to the dopamine perikarya, while D2 receptors are classified as dopamine autoreceptors that directly regulate the firing speed of dopamine cells (67). As a result, any dysfunction in GABA at VTA has an impact on sense of patients rewarding.

2.2 Serotonin

Serotonin or 5-hydroxytryriptamine (5-HT) is one of the indolamines neurotransmitters family derived from tryptophan amino acid (68), which most of them are produced by enterochromaffin cells in the GI tract, where it controls bowel motions (69). However, serotonin has wide function in CNS not as a "classical" neurotransmitter but as a neuromodulator (70). Serotonin in CNS has numerous functions as fallow: hormone release (71), sleep and wake cycle (72), motor control (73), immune system function (74), nociception (75), food intake (76) and energy balancing (77). Additionally, 5-HT influences major brain tasks, including cognition and emotional state, through regulation synaptic plasticity (78) and, as currently revealed, neurogenesis (79, 80). Serotonin-secreting neurons are classified into three groups: 1) raphe nuclei (81), 2) central gray, and 3) reticular formation (82-84). These neurons have been divided into nine groups, designated B1 to B9(85).

2.2.1 Serotonin Receptors

5-HT receptors are categorized in seven major groups of 5-HT1 and 5-HT2(86), 5-HT3(87), 5-HT4(88), 5-HT5, 5-HT6 and 5-HT7 (89, 90), each of them has their own subtypes. 5-HT3 receptor is a single ligand-gated ion channel in these receptors (70).

Family	Potential	Type	Mechanism of action
5-HT1	Inhibitory	G_i/G_o-protein coupled	Decreasing intracellular concentration of cAMP
5-HT2	Excitatory	G_{q11}- protein coupled	Increasing intracellular concentration of IP3 and DAG
5-HT3	Excitatory	Ligand-gated Na^+/K^+ channel	Depolarization of cell plasma membrane
5-HT4	Excitatory	G_i/G_o-protein coupled	Increasing intracellular concentration of cAMP
5-HT5	Excitatory	G_i/G_o-protein coupled	Decreasing intracellular concentration of cAMP
5-HT6	Excitatory	G_i/G_o-protein coupled	Increasing intracellular concentration of cAMP
5-HT7	Excitatory	G_i/G_o-protein coupled	Increasing intracellular concentration of cAMP

Table 2: Families of 5-HT receptors. IP3 stands for inositol triphosphate. DAG stands for diacylglycerol and cAMP stands for cyclic adenosine monophosphate (91).

2.2.2 GABA-Serotonin Interactions

Serotonergic neurons remain spontaneously functioning, like other monoamines, however excitatory amino acids (EAA) and GABA affect how they release (84). Numerous interneurons and local circuits are believed to be associated with GABA functioning in the brain that is relevant to mood disorders, and could potentially have a facilitative influence on serotonergic activity (92). The effect has been detected in nociception, sensory processing, motor control and learning-memory (70). According to complexity and wide-spreading of serotonergic system and its various types of receptors in CNS, we discuss some types of GABA-serotonin interactions.

Occasionally, GABA result in serotonin release. As a prime instance, a GABA agonist, (y-vinyl) has been shown to facilitate basal discharge of serotonin in the prefrontal cortex of rodents (IO) (92) or to stimulate the GABA Rs in suprachiasmatic nucleus (a region in hypothalamus, responsible for controlling circadian rhythms) of the rat brain, inducing a raise in serotonin discharge (92).

In has been shown in rat brain that GABA Rs my function as heteroreceptors on serotonergic endings in limbic region. Also, progabide (93) and baclofen (94) have been demonstrated to increase the 5-HT RS-mediated head twitch reactions and the concentration of cortical 5-HT RS in rodents (95). Additionally, GABA exerts a potent inhibitory effect on 5-HT discharge, which is accomplished partially by inhibition of glutamatergic transmission 5-HT which contributes to mood stabilization (96). There have been reports of 5-HT-GABA interactions in structures related to motor control. Lugaro cells (inhibitory interneurons), which are broadly located in cerebellar cortex of cerebellum (97, 98), are typically silent until 5-HT is present. Actually, GABA is released by Lugaro cells in response to 5-HT as a upstream to Purkinje neurons (99,100), which then hyperpolarized by an GABAA-mediated inhibiting current (101). 5-HT decreases the excitability of Purkinje neurons and its impact on motor performance, in an indirect manner (99). In the following we look more closely at the functions of serotonin receptors in response of GABA.

Stimulation of 5-HT1A RS in the red nucleus (RN) increases GABA reactions in neurons that are mainly situated in the rostral part of the nucleus. Conversely, stimulation of 5-HT2A RS suppresses GABA-mediated suppression of rural neurons primarily situated in the caudal part of the nucleus (102). 5-HT2 Rs in pyramidal neurons, primarily excitatory neurons (103), from prefrontal cortex activate protein kinase C (PKC) and its anchoring protein RACK1, finally decreases GABAA–mediated Cl- currents (104). In thalamic nuclei, 5-HT2 increases GABA release from interneuron dendrites by activating phospholipase C and increasing [Ca2+] i on the short transient receptor potential channel 4 (TRPC4) (105). 5-HT can affect thalamic processing of sensory inputs by boosting GABA release from the dendrites of thalamic interneurons (105). In neurons of the spinal dorsal horn, stimulation of the 5-HT2 RS augments the GABAA-induced Cl- current via a protein kinase-dependent mechanism (106-108). Moreover, stimulation of 5-HT3 receptors induces GABA discharge (109), with both impress strengthening the inhibition mediated by GABA (70). According to the level of protein kinase A (PKA) activation which can be low or high, 5-HT4 receptors in pyramidal neurons can have opposing impacts, that is enhancing or decreasing, on the GABAA current mediated by these receptors. It has been suggested as a potential explanation that different PKA activity levels could encourage the phosphorylation of different GABAA receptor subunits, particularly a 3 subunit (decreased GABAA receptor activity) at lower PKA level and a 1 subunit (enhanced GABAA-mediated current) at elevated PKA level (104). Phosphorylation through PKA is elevated with neuronal depolarization and serves as an instance of how 5-HT4 RS may actively control GABAergic transmission in an activity-based approach. A rise in neuronal excitation boost GABAA-mediated suppression, serving as a negative feedback regulation. However, when 5-HT4 RS are likewise stimulated, 5-HT4- regulate the GABAA current shifts from inhibition to facilitation (110). Based on these descriptions, in DRN, GABA has an effect on serotonergic system, while in all other regions (which are discussed here), serotonin has an either inhibitory or excitatory effect on GABA. Collectively, these illustrations show how complex the connections between GABA and serotonin are. Studies have shown significant role of GABA concentration in major depressive disorder (MDD) patients which have connections between serotonergic and GABAergic neurotransmission (111).

2.3 Acetylcholine

Acetylcholine (Ach) is a first neurotransmitter which has been discovered. Ach neurotransmitters are in all autonomic ganglia, at several autonomically innervated organs, at the neuromuscular junction, and at numerous synapses in CNS, Autonomic Nervous System (ANS), and PNS (112). In cholinergic neurons ACh is synthesized by one step reaction via choline acetyltransferase (CAT) enzyme (112). Acetylcholine influences synaptic transmission, fosters synaptic plasticity, and coordinates the excitation of clusters of neurons in the brain (113). Despite excitatory role of Ach neurotransmitters in periphery region of body, it has a neuromodulator role in the brain (113). Tonically active ACh neurons of the striatum and nucleus accumbens (NAc) are examples of cholinergic projection neurons and cholinergic interneurons (113).

2.3.1 Receptors of Acetylcholine

Cholinergic signaling occurs via ionotropic nicotinic and metabotropic muscarinic receptors (113, 114). Nicotinic receptors (nAChRs) are excitatory ion channels that are permeable cations like sodium, potassium, and calcium with a pantamer structure and typically are found in neocortex and hippocampus (115). The other groups of receptors are muscarinic, recognized as G-protein-coupled receptors. Gαq is phospholipase C activation (M1 type) and Gαi/o inhibition of adenylate cyclase (M2 type) (116). The brain location of these RS and their proximity to presynaptic discharge sites have a significant impact on their activities (115).

Family	Type	Localization
Nicotinic Receptors	N1 or Nm	Neuromuscular junctions
	N2 or Nn	Autonomic gangelia CNS, Adrenal medulla
Muscarinic Receptors	M1	Striatum, Cortex, Hippocampus
	M2	Forebrain, Thalamus, Heart, Pupil, Spinal Cord, Exocrine
	M3	Brain, Hypothalamus, Pupils, Exocrine, Peripheral Arteries
	M4	Striatum, Cortex, Hippocampus, Spinal Cord
	M5	Dopaminergic neurons, Basal gangelia, Brain vascularture

Table 3: Subtypes of muscarinic and nicotinic receptor (86)

2.3.2 GABA-Acetylcholine Interactions

Based on the preview's studies, a single neuron can discharge several fast-acting neurotransmitters coincidentally (117). Ach neurons frequently discharge multiple neurotransmitters. Releasing inhibitory neurotransmitter GABA from cholinergic neurons in the retina and cortex (118), hippocampus (119) and other brain areas (120-123) are reported. Ach is a single neurotransmitter which co-transmitted with GABA. It is worth mentioning that it is not a co-release but is a co-transmit via different vesicles (119). Presynaptic auto receptors mediate the mutual regulation of GABA and acetylcholine transmissions, which are regulated by separate calcium channels (119). Release of Ach as an excitatory and GABA as an inhibitory neurotransmitter by the same axons could take place in parallel, if a single postsynaptic cell gets both ACh and GABA at the same time, and depending on which concentration is dominant, it will have its effect. GABA may function to limit or shunt the Ach-provided excitement level. In addition, releasing one neurotransmitter can be utilized to regulate second neurotransmitter's secretion or reaction to another stimulation. For example, nicotine acetylcholine receptors (nAChRs) can have profound effect on GABA neurons either by increasing elicited GABA discharge or lowering following inhibition through Ca2+-mediated phosphorylation of GABA postsynaptically (124). On the other hand, co-transmission and non-transmission of GABA and Ach are proved in brain, discussed at the follow.

2.3.2.1 co-transmission of GABA and Ach

One function of ACh and GABA co-transmission is in layer1 interneurons in cerebral cortex (125). Both GABA- mediated prohibitive postsynaptic currents (IPSCs) and ACh-mediated excitatory postsynaptic currents (EPSCs) are derived by preferential activation of cholinergic axons in the cortex employing channel rhodopsin (ChR2) (116, 126).

2.3.2.2 Non co-transmission of GABA and Ach

GABA and Ach not necessarily co-transmit together. The postsynaptic component at cholinergic axon synapses in the cortex (Synaptic) is often a dendrite cholinergic. Receptors of Nicotinic (nAChR) and muscarinic (mAChR) are found on dendritic branches and spines, as well as noncholinergic axons (GABA, glutamate), controlling the releasing of transmitters. Cholinergic receptors are able to be stimulated synoptically (through quick synaptic (or wired) transmission) with a 1-to-1 interaction between the transmitter and the recipient, as well as nontypically via volume transmission (115).

2.4. Norepinephrine

Norepinephrine (NE), or noradrenaline (NA), is another member of catecholamine family which is a neurotransmitter and hormone in the brain and body. It is a precursor of adrenaline (127) and produced from phenylalanine, tyrosine and dopamine through several enzymatic reactions (128). In 1999, Nestler et al. suggested that in animal models, the activation of central NE systems is triggered by both acute and chronic stress (129).

2.4.1 GABA-Norepinephrine Interactions

One of the significant regions of NE system in the brain is locus coeruleus (LC). As figure 7 shows, ascending noradrenergic neurons project to four different parts of the rat brain as follow: hippocampus, bed nucleus of the stria terminalis (BST), amygdala and, to a lesser extent, the lateral septum (LS).

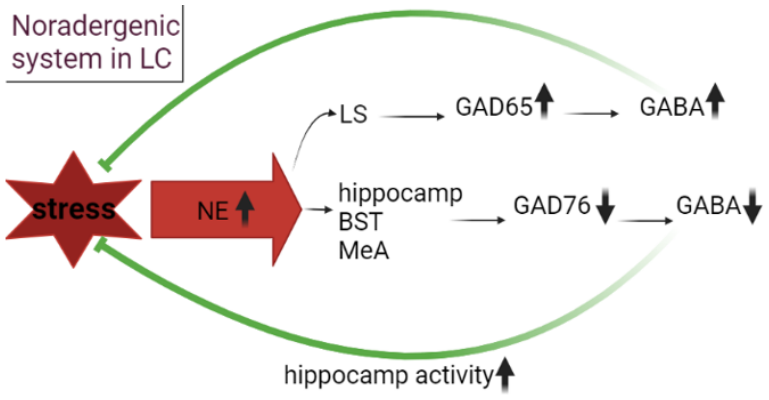

Figure 7: Diagram depicting putative NE GABA interactions in limbic stress circuitry (94). HPC stands for hippocampus; LC stands for locus coeruleus; and HPA stands for hypothalamic-pituitary-adrenocortical axis.

James P et al. indicated that elevated NE availability diminished the mRNA level of glutamic acid decarboxylase (GAD67) in dentate gyrus, posteromedial BST, and medial amygdaloid nucleus (MeA), and lower GAD65 mRNA in the lateral septum. Since the hippocampus normally inhibits stress effector systems, lower GABA in the hippocampus implies increased hippocampal reactivity; the overall outcome is lower stress

response. Reduced GABA production in MeA and BST would largely affect projection neurons, leading to decreased GABAergic outflow from these areas. The overall outcome is a decrease in stress stimulation (or stress disinhibition). Enhanced GAD65 production in the LS is associated with greater firing of GABAergic neurons, which are thought to block stress effectors (130).

We would like to briefly describe role of amygdala controlling of the hypothalamic-pituitary-adrenal (HPA) activity which accompany with GABAergic interneurons. The HPA axis is an important neuroendocrine system which governs numerous physiological systems and affects stress reactions (131). Almond shaped amygdala (adjacent to the hippocampus) contains subnucleis including the BLA (basolateral amygdala), CeA (central amygdala), and MeA (medial amygdala), and LA (lateral amygdala). Amygdala regulated the HPA axis activity mainly by several intermediate nuclei (132) which send efferent fibers to the paraventricular nucleus of the hypothalamus (PVN), one of the brain's major autonomic control centers (133). In presence of stress from thalamus and cortex, secreted norepinephrine is increased and firstly, result in activation of BLA. Then CeA and MeA are activated by glutamatergic neuron from BLA. CeA and MeA have inhibitory effect on pBNST (posterior BNST) in intermediate stage. In normal circumstance, pBNST has an inhibitory effect on hypothalamus which result in activation of HPA axis. Then by activation of GABAergic interneurons which have projections to HPA axis, (adrenocorticotropic hormone), ACTH release from PVN and stimulates releasing corticosterone from adrenal cortex (134).

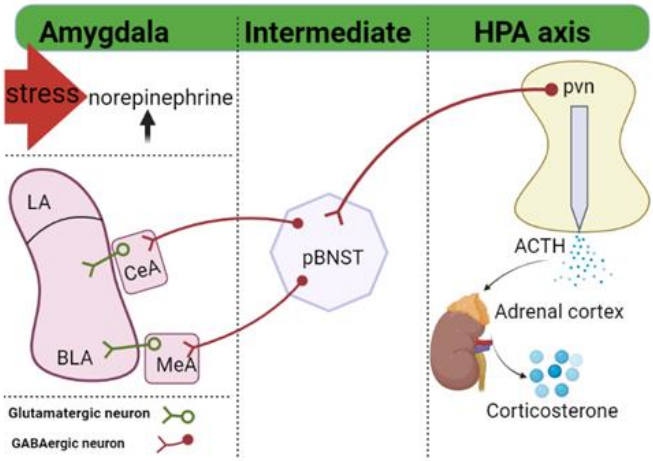

Figure 8: Circuit basis for amygdala regulation of the HPA axis activity.

By increasing suppression and reducing the firing of limbic system pathways that regulate neuroendocrine, autonomic, and behavioral stress effectors, NE reabsorption blockade has the potential to modify GABAergic tone (130). By considering all interactions between GABA and some other neurotransmitters mentioned in section 3, and how Cl- homeostasis works and importantly has impact on output of neurons, it can be concluded that any disturbance in GABA system may have pivotal influence on other neurotransmitter systems in our body. In fact, of chemoneurological study of brain by having a network outlook in neurons would have deep and efficient impact in etiology of CNS's diseases. Therefore, understanding GABA system in human body has a significant importance both clinically and molecularly. In the last part of the article, we are going to discuss etiology of some complicated neurological disorders which have association with Cl- homeostasis in human brain.

3. Clinical aspects of GABA dysregulation

Dysregulation of GABA-mediated inhibition is a common hallmark of many brain illnesses caused by abnormal intracellular chloride homeostasis resulting from abnormalities in the expression or activity of chloride transporters. In particular, in animal models of these illnesses and in certain human clinical trials, pharmacological blockage of the chloride importer NKCC1 can cure brain-related core impairments. Interestingly, changes in neuronal chloride homeostasis caused by differences in NKCC1 and/or KCC2 expression or transport activity are a typical feature of several kinds of neurological disorders (135). Several clinical and experimental studies prove that control of Ecl can be beneficial for treating or relieving some brain disorders. Manipulation of NKCC1 and KCC2 by clinically pharmacological approach is our aim in next section.

3.1. Autism Spectrum Disorders (ASD)

ASD is a set of neurodevelopmental problems which influences 1 in 160 children globally (136). It is distinguished by prolonged deficiencies in social interaction and participation.as well as the expression of repeated and stereotypical behavioral patterns (137). Both genetic mutations (a heterogeneous disorder (138-145) and environmental factors during pregnancy and delivery (146-149) play curtail rules in ASD. Since autism is a spectrum condition, each patient may experience distinct symptom and signs. It is estimated that1003 genes are correlated to autism, among which 418 genes have been determined as strong candidate ASD risk genes (150). ASD is a neurodevelopmental disorder and as discussed in section 1, GABA system has an important role in neurodevelopment stage of the CNS. It is demonstrated that excitatory/inhibitory switching of the GABA does not occur in ASD patients. One possible explanation for this abnormality is that expression level of KCC2, as a Cl- exporter is not up-regulated during neurodevelopment stage. Consequently, excitatory/inhibitory switching of GABA does not occur in ASD patients and GABA has not inhibitory function in mature brain (151).

Furthermore, several heritable genetic variants have been linked to abnormalities in GABAA subunits receptors, which may have a particular impact on inhibitory signaling of GABA (152). This evidence suggests that lowering the intracellular chloride content could be an effective treatment for autism. By controlling NKCC1, scientists could reduce the level of intracellular chloride. Bumetanide, a diuretic, is a high-affinity selective chloride-importer NKCC1 antagonist which blocks NKCC1, decreases [Cl-]i, and strengthens GABAergic inhibition (136, 153-155). Autism symptoms did not significantly correlate with glutamate and GABA gene set (156).

3.2. Epilepsy

A theoretical description of "seizure" and "epilepsy" was developed by the International League Against Epilepsy (ILAE) in 2005. It is defined as follows: a transitory episode of signs or symptoms associated with abnormally excessive or synchronized neuronal activities in the brain. Epilepsy is a brain disease characterized by a lasting propensity for causing epileptic seizures as well as its consequences from the cognitive, social, neurobiological and psychological aspects. The happening of at least one epileptic episode is necessary for the diagnosis of epilepsy. Epilepsy is thought to be addressed by taking into consideration a few specific indicators and thus is not a lifelong condition (157). By considering short-term synaptic plasticity in seizures, two main culprits maybe proposed for this abnormality. A) excess of excitation or B) decrease of the inhibitory system. Some molecular candidate for ictogentic mechanisms in epileptic patients is as fallow (158): suppressive GABAergic synapses on primary neurons (159), and glutamatergic synapses on inhibitory neurons are depressed (160), glutamatergic synapses between main cells (161) and inhibitory GABAergic connections onto inhibitory interneurons (162) are facilitated.

It is demonstrated that some anticonvulsants drugs like pregabalin and levetiracetam are fail to relieve all seizures, therefor, dysfunction in temporary synaptic plasticity mechanisms are neither required nor sufficient in seizures and epilepsy (163). Beside mutations in each of elements of the molecular mechanisms that mentioned above (164-170), activity-dependent changes in ion contents involves in ictogenesis as well. Dysregulation of ionic concentrations like potassium, calcium, protons and chloride in the intracellular or extracellular spaces have been seen in seizures (171-174). Additionally, proteins acted in a transmembrane channel's structures and transporters both in the cytoplasm membrane of neurons and membrane of organelles undergo wide dysfunctions and mutations in seizures (175-177). With a comprehensive look, the lack of balance between neural excitement and neural suppression is the main mechanism of epileptogenesis (178). It is demonstrated that expression of NKCC1, which is broadly express in different tissue of the body, and expression of KCC2, as a neuron-specific manner (179), undergo a dysregulation in both animal models and epileptic patients which result in alteration of [Cl-]i (180-185). Specifically, GABAA blockers such as bicuculline and picrotoxin are widely used as an epileptogenic agent in experimental studies (186). However, during treatment, about 40% of the patients show types of drug-resistant epilepsy like Temporal lobe epilepsy (TLE) which is among the most prevalent ones (187).

it is revealed that decreased KCC2 and enhanced NKCC1 expression can cause GABA response to shift towards excitement and cause seizures (188). So, some studies put their attention on novel therapeutic potential of controlling activity of NKCC1 and KCC2 in treatment of epilepsy. Bumetanide, as an inhibitor of NKCC1, had antiepileptic effects (189, 190). In our pilot study, we demonstrated that utilizing bumetanide in the treatment regimen of individuals with TLE, who are drug-resistant, resulted in a decrease in seizure frequency (191). A clinical trial phase 1 and 2 carried out for efficiency assessment of bumetanide add-on therapeutics in patients demonstrating drug-resistant TLE. A significant improvement was reported and 70% of patient's response to this add-on treatment (192).

3.3. Schizophrenia

Schizophrenia is a neurodevelopmental disorder identified by structural brain changes (193), genetic mutations (194, 195), and perinatal risk factors (196). It is suggested that alterations in cortical GABAergic neurons is essential for cognitive deficits in schizophrenia (197, 198). Also, reduction of GABA activity, hyper-dopamine, and hypo-NMDA receptor are reported. Changed microcircuitry involves disruption in GABA-dopamine inputs and/or interaction. Dysregulation of GAD67/65 (199), diminishes the free GABA in frontal cortex (FC), anterior cingulate cortex (ACC) (200), and dorsolateral prefrontal cortex (201). While, reduced concentration of neuron GAT-1 mRNA is limited to prefrontal cortex layers 1–5 in subset of neurons (202). Also, significantly lower α5-GABAA Rs levels in the hippocampus and their mRNAs (203), are reported in this neurological disorder (204). In schizophrenia patients, the function of the chloride transporters NKCC1 and KCC2 are changed, resulting in greater than normal intracellular chloride levels, so that stimulation of GABAA RS leads to reduced chloride influx and a lesser rise in hyperpolarization (205). Treatment of schizophrenia using Diuretic Bumetanide is reported (206, 207). The roles of receptor subtypes such as dopamine D2 (208, 209) and serotonin 5HT-2 receptor (210, 211) are also acknowledged. Due to complicated interaction between GABA and DA, it is demonstrated that polytherapy with both of them, like GABA agonists (benzodiazepines and valproate (212) and antipsychotics inhibition of dopamine hyper innervation of the GABA interneuron, could be more beneficial for treatment (204).

3.4. Spinal Cord Injury (SCI)

One of the most frequently symptoms (up to 70%) in spinal cord injury patients is neuropathic pain (NP) (213). Management of NP is a priority in current SCI research. Therefore, understanding molecular pathways of pain is critical to reveal it. In the processing course of spinal nociceptive signals, GABA has an essential function (214). Administration of a GABAA receptor agonist, Muscimol, hindered hyperalgesia and lowered the level of the GABA transporter GAT-1(215). It is widely suggested that alteration of the GABA, NKCC1, and KCC2 expressions play a role in inflammatory or NP (216-219). By controlling the activity of the transporters, we can raise intracellular Cl- and enhancement of GABAergic hypersensitivity. In our previews study NKCC1, KCC2 and their alteration in response to bumetanide was tested. Since that baseline expression of KCC2 was reduced in SCI patients, treatment by bumetanide resulted in increased protein level of KCC2 and subsequently the pain was reduced (220).

In our subsequent investigation, the effectiveness and safety of bumetanide were also assessed (192). One of our early research projects demonstrated the privilege of early bumetanide therapy in patients with spinal cord damage (SCI). Bumetanide 4 mg/day-treated rats showed enhanced recovery of locomotor function and decreased expression of the NKCC1 gene (221).

3.5. Down syndrome (DS)

Down syndrome, a birth defect, is characterized by a trisomy of chromosome 21. It is the most prevalent genetic disease worldwide and appears in about 1 in 400-1500 birth (222). Besides, multiple DS-associated phenotypes risk of early onset of neurological problems are recognized (223). It has been demonstrated that the hippocampus and temporal cortex of DS-effected individuals have less GABA (224). Also, the reversal potential for GABAARs-driven Cl- currents (ECl) was moved towards greater positive potentials in the hippocampi of mature DS mice, indicating that GABAARs signaling was excitatory instead of inhibitory. As a result, both trisomic mice as well as individuals with DS showed enhanced hippocampus expression of the cation Cl- cotransporter NKCC1(225). The GABAARs signaling is switched from hyperpolarizing to depolarizing, which results in a rise in neuronal [Cl-]i and a reduction in the effectiveness of GABAA Rs-mediated suppression (226). In particular, ECl, synaptic plasticity, and hippocampus-dependent memory in adult DS mice were recovered following NKCC1 suppression with the FDA-approved medication bumetanide (225, 227, 228). Also, down-regulation of NKCC1 by RNA interference due to restoring neuronal chloride homeostasis in mouse models was successful (226). In our pervious case-control study, bumetanide, a selective NKCC1 inhibitor prescribed for 18 months, showed encouraging results in reversing certain behavioral abnormalities in a fourteen-year-old boy with genetically proven mosaic Down's syndrome (229).

Conclusion

GABA function in the brain and its interactions with other neurotransmitters are essential for the CNS to operate normally. Numerous CNS disorders, such as Autism Spectrum Disorders, Epilepsy, Schizophrenia, and Down syndrome have been linked to dysregulation of intracellular Cl- homeostasis and, as a result, dysfunctionality of the GABAA receptors. For the therapy of these conditions, the use of NKCC1 and KCC2 transporters to control intracellular Cl- levels has showed promise. A particular NKCC1 inhibitor called bumetanide has demonstrated considerable promise as a CNS disease therapy. Bumetanide, in one of our prior research projects, has been shown to enhance locomotor performance in rats with spinal cord injuries, decrease seizure frequency in patients with drug-resistant TLE, and correct certain Behavioural abnormalities in people with Down syndrome. These findings and our own investigation, which assessed the safety of bumetanide, imply that employing bumetanide to target intracellular Cl- homeostasis may be a secure and efficient strategy for treating several CNS conditions. Overall, a network and comprehensive understanding of brain chemistry as well as knowledge of the significance of GABA and chloride homeostasis in clinical CNS illnesses are essential for the creation of novel therapeutic alternatives. More research is required to evaluate the effectiveness of bumetanide in bigger patient groups. But this strategy's potential for treating CNS ailments opens up a fresh field for investigation and therapeutic therapy, with the potential to benefit countless individuals with these crippling conditions.

Declaration

The authors declare that they have no competing interests.

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