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·Minireview·

The role and the mechanism of γ-aminobutyric acid during central nervous system development

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Abstract: γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter in adult mammalian central nervous system (CNS). During CNS development, the role of GABA is switched from an excitatory transmitter to an inhibitory transmitter, which is caused by an inhibition of calcium influx into postsynaptic neuron derived from release of GABA. The switch is influenced by the neuronal chloride concentration. When the neuronal chloride concentration is at a high level, GABA acts as an excitatory neurotransmitter. When neuronal chloride concentration decreases to some degree, GABA acts as an inhibitory neurotransmitter. The neuronal chloride concentration is increased by Na^+-K^+-Cl^--Cl^- cotransporters 1 (NKCC1), and decreased by K^+-Cl^- cotransporter 2 (KCC2).

Keywords: GABA; neurotransmitter receptor; central nervous system; development

1 Introduction

Amino acid, including glutamate, aspartic acid, γ-aminobutyric acid (GABA) and glycine, is the major neurotransmitter in the central nervous system. Glutamate and aspartic acid are excitatory neurotransmitters. GABA and glycine are inhibitory neurotransmitters. Excitatory neurotransmitters act at postsynaptic receptors and presynaptic receptors, which induce influx of Ca^{2+} into neuron, as a result of enhancing the excitability of neuron. On the contrary, inhibitory neurotransmitters inhibit Ca^{2+} influx into neuron, weakening the excitability of neuron. Glutamate and GABA play an important role in enhancing and weakening the excitability of neuron respectively.

It had been known for a long time that GABA is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS); however, recently a lot of studies have approved that the releasing of GABA in the early development of CNS can increase the Ca^{2+} concentration of postsynaptic neuron, and then induce the depolarization of postsynaptic neuron. GABA performs an excitatory function during the early period of CNS development[1,2].

This review focuses on the role that GABA plays in the different period of CNS development. Both the change of GABA in the different period of CNS development and the underlying mechanism will be discussed.

2 GABA and its receptors in the adult mammal CNS

GABA is one of the non-protein amino acids, which means GABA is not a common amino acid to compose protein but a functional amino acid. GABA is the most representative inhibitory neurotransmitter in the adult CNS, which distributes diffusely and nonuniformly in the mammalian CNS. In the brain, there are about 25%-40% of the synapses that use GABA as neurotransmitter, while only a very small amount of GABA is found in peripheral nervous system. GABA is synthesized principally with decarboxylation of glutamate by glutamate decarboxylase (GAD) which requires the cofactor pyridoxal 5'-phosphate (pyridoxal-P) for activity. When GABAergic neuron is active, GABA is released from nerve terminal to synaptic cleft and binds to receptors of GABA in the postsynaptic membrane, acting as a neurotransmitter.

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The unbound GABA is reuptaken to the neuron and gliocyte by Na^-/Cl^- dependent GABA transporter, and is degradated as a result of catalyzing into succinic semialdehyde (SSA) by GABA transaminase (GABA-T)[3].

The receptors of GABA can be divided into 3 subtypes: $GABA_A$ receptors, $GABA_B$ receptors and $GABA_C$ receptors. $GABA_A$ receptors are ligand-gated chloride ionotropic receptors, which are the most principal receptors in GABAergic synaptic transmission. When $GABA_A$ receptors in the postsynaptic membrane are activated, the ion channel of Cl^- opens up and Cl^- influx into the cells with lower density Cl^- through electrochemical gradient, which causes the hyperpolarization of postsynaptic membrane; consequently inhibitory postsynaptic potential (IPSP) is induced and the influx of Ca^{2+} is inhibited with the decrease of excitability of neuron as a result. Wu YM *et al*. found that the neuronal activity could be inhibited by potentiating $GABA_A$ receptor-mediated Cl^- current in hippocampal CA1 neurons[4]. $GABA_B$ receptors are G protein coupled receptors, which have lower concentration than $GABA_A$ receptors in CNS, and begin to react in the late development of CNS (the beginning in rodents is after birth)[5]. When $GABA_B$ receptors bind to GABA, the coupled G protein are activated and the ion channel of K^+ opens up. The efflux of K^+ hyperpolarizes the postsynaptic membrane, inhibits the influx of Ca^{2+}, causing the postsynaptic inhibition. GABA can also inhibit the releasing of excitatory amino acid by binding $GABA_B$-receptors of presynaptic membrane and result in a presynaptic inhibition. GABAc receptors are ligand-gated chloride ionotropic receptors, which are different from $GABA_A$ receptors and only exist in visual pathway.

3 GABA and its receptors in the early development of CNS

Lin found that the Ca^{2+} concentration of newly born (postnatal days 0-5, P0-5) rat cortex neuron can be increased dramatically by being perfused with GABA, and the neuron of P0-2 had the largest extent in increase[1]. Ganguly confirmed in advance that using GABA to culture rat hippocampus neuron of P4-9 results in a quick and reversibly enhanced influx of Ca^{2+}. The rat hippocampus neuron of P4-6 showed an enhancement of GABA induced depolarization, however, the rat hippocampus neuron of P13 showed no difference[2]. This kind of Ca^{2+} influx increasing can be blocked by $GABA_A$ receptors antagonist bicuclline or L-type Ca^{2+} channel antagonist nimodipine, nevertheless, it can not be blocked by $GABA_B$ receptors antagonist baclofen, which clearly indicates that the increased Ca^{2+} influx is the result of the opening of L-type Ca^{2+} channel activated by the depolarization with $GABA_A$ receptors.

All the studies mentioned above indicate that in early stage of CNS development GABA raises the intracellular Ca^{2+} concentration of postsynaptic neuron via $GABA_A$ receptors and acts as an excitatory transmitter. With the developing progress going, the extent of intracellular Ca^{2+} concentration increasing decreases gradually and the role of GABA switches from excitatory transmitter to inhibitory transmitter. The switches can be reversed easily by various factors. That is why the nervous system in neonates is extremely vulnerable to various etiological factors and frequently subject to the damage.

4 The role of GABA changing from excitatory neurotransmitter to inhibitory neurotransmitter

In the early stage of CNS development, GABA acts as an excitatory transmitter. As the research kept moving on, the mechanism of this phenomenon begin to attract the researchers' attentions. During the development of CNS, $GABA_A$ receptors emerge earlier than $GABA_B$ receptors and $GABA_C$ receptors and distribute more widely, so that most of the GABAergic synaptic transmission is mediated via $GABA_A$ receptors. Because $GABA_A$ receptors are ligand-gated Cl^- ionotropic receptors, so it is presumed that the role of GABA switching is correlated with the concentration of intracellular Cl^-. In early development, the GABAergic reversal potential (E_{GABA}) is more liable to depolarize than resting membrane potential, indicating that in the prenatal and early postnatal days the concentration of intracellular Cl^- is higher than extracellular Cl^- concentration[6]. When $GABA_A$ receptors in the postsynaptic membrane are activated, the ion channel of Cl^- opens up and Cl^- effluxes through electrochemical gradient, and postsynaptic membrane is depolarized, facilitating the Ca^{2+} influxes of postsynaptic neuron[7]. Following the development, the intracellular Cl^- concentration decreases gradually. When the intracellular Cl^- concentration is lower than extracellular Cl^- concentration, the role of GABA switching ends[8]. In adult mammal CNS, the intracellular Cl^- concentration is lower than extracellular Cl^- concentration. When

GABA binds to $GABA_A$ receptors, the ion channel of Cl^- opens and Cl^- influxes through the electrochemical gradient, and postsynaptic membrane is hyperpolarized, resulting in the inhibition of the Ca^{2+} influx of postsynaptic neuron, which indicates that in the whole process GABA acts as a inhibitory neurotransmitter[9].

Further study found that the dominant factor responsible for the significant change of intracellular Cl^- concentration is cation-chloride cotransporter. To date, seven members of the cation-chloride cotransporters gene family have been reported, and they are designated by their ion selectivity as KCC (KCC1-4) for K^+ dependent Cl^- cotransporters, NCC for Na^+ dependent or NKCC (NKCC1 and NKCC2) for cotransporters that depend on the transmembrane gradients of Na^+ and K^+. Table 1 summarizes the key similarities and differences among these cation-chloride cotransporters.

4.1 NKCC1 The cation-chloride cotransporters transport Cl^- into cell, including Na^+-K^+-Cl^--Cl^- cotransporters (NKCC) and Na^+-Cl^--Cl^- cotransporters (NCC). NKCC maintains intracellular chloride concentration at levels above the predicted electrochemical equilibrium, which is used by epithelial tissues to promote net salt transport and by neural cells to set synaptic potentials. Two isoforms of the NKCC are currently known: NKCC1 and NKCC2. NKCC1, the "housekeeping" isoform, exists in the brain and other systems, and its expression changes as it develops. NKCC2 seems to be exclusively expressed in the kidney and mainly related with the urine concentration by mediating apical Na^+, K^+ and Cl^- entry into renal epithelial cells[10]. The NKCC1 isoform is the larger one with 1 200 amino acid residues and a transcript size of 7.4 kb. It has an overall 58% amino acid identity with NKCC2. The NKCC2 isoform is somewhat smaller than NKCC1, containing 1 100 amino acid residues with a transcript size of 5 kb. The difference in molecular size is almost entirely by an additional 80 amino acids at the amino terminus of the NKCC1. NCC is a Na^+-Cl^--Cl^- cotransporter, which also increases the intracellular Cl^- concentration[11].

There are several factors to control the operation of NKCC, including ATP, intracellular ions and cytoskeleton. ATP plays a fundamental role in the operation of the NKCC. The majority of available evidence supports the present view that the effects of ATP on the NKCC are mediated via a protein phosphorylation/dephosphorylation mechanism[12]. Nevertheless, the NKCC is an example of secondary active transport that derives it energy from the combined chemical gradients of the three cotransported ions. So the change of intracellular ions concentrations is the most critical factor. Furthermore, the changes of cell volume will be "sensed" by the cytoskeleton. In turn, the resultant cytoskeletal changes will be transmitted to the membrane ion transporters in the plasmalemma through the cortical cytoskeletal network of actin and associated proteins.

Tab. 1 Comparison of general properties of NKCC, KCC and NCC

Property	NKCC	KCC	NCC
Cotransport ions	Na^+, K^+, Cl^-	K^+, Cl^-	Na^+, Cl^-
Direction of net cotransport	Influx	Efflux	Influx
Loop diuretic sensitive	Bumetanide > furosemide	Furosemide > bumetanide	No effect
Thiazide sensitive	No effect	?	No effect
Isoforms: tissue distribution	NKCC1: kidney, stomach, heart, lung, brain, skeletal muscle NKCC2: kidney	KCC1: brain, colon, heart, kidney, liver, lung, spleen, stomach KCC2: brain	Urinary bladder, distal convoluted tubule
Effects of inhibiting intracellular Cl^-	Inhibit	Stimulate	?
Electrically silent	Yes	Yes	Yes

In the internally dialyzed squid giant axon, elevation of $[Cl^-]_i$ inhibits the unidirectional fluxes of all three cotransported ions in both the influx and efflux[13]. These findings grew out of an early observation that the removal of intracellular Cl^- had the surprising effect of increasing Cl^- influx. Since then, the role of NKCC1 on the intracellular Cl^- concentration began to be recognized. So it was postulated that immature neurons depolarize in response to $GABA_A$ receptor activation as a consequence of active Cl^- accumulation via NKCC1. By using *in situ* hybridization technology, Clayton found that only NKCC1, instead of NKCC2 and NCC, exists in the brain, and the expression of NKCC1 reaches the highest level at the third postnatal weeks, then decreases significantly, and stays at low level until adult[14]. Indeed, NKCC1 transcripts were detected in the developing CNS as early as embryonal day 12.5, when expression was observed in scattered cells of the neuroepithelium[15]. By studying in Wistar rats at postnatal 1-21 d, Yamada found that in the early CNS development, the expression of NKCC1 mRNA is much higher than that in the later development, with the expression at the highest level in Wistar rats at postnatal 0-3 d[16]. The functional importance of NKCC1 in GABA signaling has been showed by targeted deletion[17].

It is clear that only NKCC1 exists in the immature brain and there is robust expression of NKCC1 in immature neurons, with subsequent downregulation in mature neurons, which is correlated with the role change of GABA. Moreover, the antagonist of NKCC1 can inhibit the excitatory activity of GABA by decreasing the intracellular Cl^- concentration. Dzhala VI made substantial findings by researching into rats at postnatal 6-23 d[18]. The NKCC1 antagonist bumetanide can control the seizure of rats at postnatal 6-12 d, but the traditional anti-epileptic drug phenobarbital can not; Phenobarbital can control the seizure of rats at postnatal 21-23 d, but bumetanide is invalid. The generation of epilepsy is related with the unbalanced releasing of excitatory neurotransmitters and inhibitory neurotransmitters. The traditional anti-epileptic drugs inhibit the postsynaptic Ca^{2+} influx by imitating the inhibitory activity of GABA and increasing the intracellular Cl^- concentration. The Cl^- concentration of neonatal mammal CNS is higher than the extracellular Cl^- concentration. When the $GABA_A$ receptors are activated by traditional anti-epileptic drugs, the postsynaptic neuron Cl^- effluxes and Ca^{2+} influxes, which leads to the increase of neuronal excitability. That is why the traditional anti-epileptic drug phenobarbital can not control the seizure of rats at postnatal 6-12 d. The NKCC1 antagonist bumetanide controls the seizure of neonate by decreasing the intracellular Cl^- concentration and inhibiting the excitatory activity of GABA. It indicates that intracellular Cl^- concentration is at high level during the period of postnatal 6-12 d, when GABA acts as an excitatory neurotransmitter, and the intracellular Cl^- concentration is at low level during the period of postnatal 21-23 d, when GABA acts as an inhibitory neurotransmitter. NKCC1 has an influence on the transition process.

Obviously, in the early development of CNS, the high concentration of intracellular Cl^- is mainly caused by NKCC1, and with the development, the decreased expression results in the intracellular Cl^- concentration reduced to lower level to some degree.

4.2 KCC2 KCC is one of the superfamily of cation-chloride cotransporter, and cotransports K^+ and Cl^- out of the cell through the electrochemical gradient of K^+, which decreases the intracellular Cl^- concentration. KCC family includes KCC1, KCC2, KCC3 and KCC4. KCC2 only exists in the nervous system and is the only one who can cotransport K^+ and Cl^- under isotonic conditions[19]. KCC2 increases the rate of Cl^- extrusion, thus leading to a reduction of $[Cl^-]_i$ and a consequent negative shift in E_{GABA}. Indeed, Li has already found that the change in the KCC2 mRNA level correlates with the ontogenic switch in GABAergic transmission from depolarization to hyperlarization[20]. Furthermore, Clayton found that KCC2 expressed prenatally at very low levels which increased dramatically after the first week of postnatal life[14]. Thus, the expression level of the neuronal-specific K^+-Cl^- cotransporter KCC2 is a major determinant of whether neurons will respond to GABA with a depolarizing, excitatory response or a hyperpolarizing, inhibitory response.

When the expression of KCC2 is at a lower level, GABA tends to play the excitatory role. Some researchers presumed that the down regulation of KCC2 resulted in role switch of GABA, which is the reason for the hypoxia induced seizure. It has already found that the mutation of K^+/Cl^- cotransporter gene *kazachoc* can increase seizure susceptibility of flies[21]. Mercado found that the expression inhibition of KCC2 can lead to the death of the neonatal rats for epileptic seizure[22].

During the development of nervous system, the expression of KCC2 increases gradually. Upon the birth of a rat,

KCC2 barely expresses in the brain stem. KCC2 expresses in the hippocamp us about a week after birth, and expresses in the cortex between 1-2 weeks after birth[23]. At present, it is figured that the inducing factor of KCC2 expression is GABA itself. Ganguly found that $GABA_A$ receptors antagonist bicuculline and picrotoxin can noticeably inhibit the expression of KCC2 mRNA, and the antagonist of glutamate receptors showed no effect to the expression of KCC2 mRNA[2]. Those results indicate that GABA, as a signal, activates the intracellular cascade for KCC2 expression regulation. That is how GABA changes its role in the synaptic transmission by controlling the intracellular Cl^- concentration indirectly.

But the absence of expression of the known Cl^- cotransporters in neuroepithelium and glia suggests that other as yet unidentified members of this gene family may be involved in chloride homeostasis in immature neuronal precursors and neuroglia.

5 Conclusion

In the CNS development, GABA changes from an excitatory neurotransmitter in the early phase to an inhibitory transmitter in the maturation phase. The main reason for the role switch of GABA is the expression change of cation/chloride cotransporter NKCC1 and KCC2. More investigation into the mechanism of the expression change of NKCC1 and KCC2 in the CNS development is needed in further study.

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γ- 氨基丁酸在中枢神经系统发育中的作用及机制

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摘要: γ-氨基丁酸 (γ-Aminobutyric acid, GABA) 是成年哺乳动物中枢神经系统内的抑制性神经递质。在中枢神经系统发育过程中，GABA 由兴奋性神经递质转变为抑制性神经递质。其转变过程主要表现为 GABA 的释放由促进突触后神经元的 Ca^{2+} 内流变为抑制突触后神经元的 Ca^{2+} 内流。中枢神经元内 GABA 作用的转变受细胞内 Cl^- 浓度的影响：当细胞内 Cl^- 浓度处于高水平时 GABA 发挥兴奋性神经递质的作用，当细胞内 Cl^- 浓度降低到一定程度后 GABA 发挥抑制性神经递质的作用。升高中枢神经元内 Cl^- 浓度的是 Na^+-K^+-Cl^--Cl^- 同向转运蛋白 1 (Na^+-K^+-Cl^--Cl^- cotransporters 1, NKCC1)，而 K^+-Cl^- 协同转运蛋白 2 (K^+-Cl^- cotransporter 2, KCC2）则使中枢神经元内 Cl^- 浓度降低。

关键词： γ- 氨基丁酸；神经递质受体；中枢神经系统；发育