Review Article
The Roles of GABA in Ischemia-Reperfusion Injury in the Central Nervous System and Peripheral Organs

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Ischemia-reperfusion (I/R) injury is a common pathological process, which may lead to dysfunctions and failures of multiple organs. A flawless medical way of endogenous therapeutic target can illuminate accurate clinical applications. γ-Aminobutyric acid (GABA) has been known as a marker in I/R injury of the central nervous system (mainly in the brain) for a long time, and it may play a vital role in the occurrence of I/R injury. It has been observed that throughout cerebral I/R, levels, syntheses, releases, metabolisms, receptors, and transmissions of GABA undergo complex pathological variations. Scientists have investigated the GABAergic enhancers for attenuating cerebral I/R injury; however, discussions on existing problems and mechanisms of available drugs were seldom carried out so far. Therefore, this review would summarize the process of pathological variations in the GABA system under cerebral I/R injury and will cover corresponding probable issues and mechanisms in using GABA-related drugs to illuminate the concern about clinical illness for accurately preventing cerebral I/R injury. In addition, the study will summarize the increasing GABA signals that can prevent I/R injuries occurring in peripheral organs, and the roles of GABA were also discussed correspondingly.

1. Introduction

Ischemia-reperfusion is a pathological process from an initial cutoff of blood supply to an organ to the subsequent blood perfusion and the accompanying reoxygenation [1]. Frequently, I/R injury occurs in various clinical settings (e.g., thrombolytic therapy, coronary angioplasty, organ transplantation, aortic cross-clamping, or cardiopulmonary bypass) [2]. In the process, I/R is followed by some biological variations in a wide range of conditions, including vascular leakage, cell death programs (apoptosis, necrosis, and autophagy-associated cell death), autoimmunity (autoantibody and complement activation), innate and adaptive immune activation, no reflow phenomenon, and transcriptional reprogramming [3]. These pathological variations cause I/R injuries of different tissues from one organ injury (e.g., acute coronary syndrome in the heart, acute kidney injury in the kidney, and intestinal I/R injury in the intestine) to multiorgan injuries (e.g., kidney and intestine injuries in trauma and resuscitation, kidney and brain injuries in circulatory arrest, and multiorgan injuries in major surgery). Multiple mechanisms are involved in the process, including free radicals, inflammation [4], the energy metabolism disorder of brain tissues, the toxicity of enhanced excitatory amino acid function, intracellular ion (Ca2+) overload [5], the nitric oxide (NO) cytotoxic effect [6], and increased opening of the blood-brain barrier [7]. Three therapeutic strategies for I/R injuries have been...
GABA is primarily synthesized from glutamate by glutamate decarboxylase (GAD) in the body. Two GAD isoforms have been identified: GAD65 and GAD67, which derive from two cDNAs of a single separate gene [36]. Apart from existing in the nervous system, GAD is also distributed in other tissues outside the nervous system including the liver, kidney, pancreas, testis, ovary, adrenals, sympathetic ganglia, gastrointestinal tract, and circulating erythrocytes [37]. Other sources of GABA are spermine, spermidine, ornithine and putrescine which are deaminated and decarboxylated to produce GABA. GABA packed in the synaptic vesicles by vesicular GABA transporters (VGAT) is released into the synaptic cleft under triggering of Ca\(^{2+}\) influx to combine GABA receptors before playing physiological roles [38]. In normal physiology, GABA has difficulties to cross the blood-brain barrier [39], despite some transporters (GABA transporters (GAT)/betaine-GABA transporter) being able of transporting GABA [40].

GABA has three types of receptors, including two ionotropic receptors (GABA\(_A\) and GABA\(_C\)) receptors) and a metabotropic receptor (GABA\(_B\) receptor). GABA\(_A\) and GABA\(_C\) receptors have various pentamer combinations derived from \(\alpha1-6, \beta1-3, \gamma1-3, \delta, \epsilon, \pi, \) and \(\theta\) subunits summarized in a review [41]. These subunits play different roles. The two ionotropic receptors mainly activate ligand-gated chloride (Cl\(^{-}\)) channels to produce hyperpolarization. Potassium (K\(^{+}\)) chloride cotransporter (KCC2) pulls Cl\(^{-}\) out of neurons [42] to keep balance of Cl\(^{-}\) contents between inside and outside the neurons. Moreover, GABA\(_B\) receptor is also the target of many substances (e.g., benzodiazepines, barbiturates, neuroactive steroids, intravenous and inhalational anesthetics, and ethanol [43]), in which benzodiazepines are well known for their applications as positive allosteric modulators of GABA\(_A\) receptor [44]. GABA\(_B\) receptor has two subunits (B1 and B2) and is a G protein-coupled receptor, mediating inhibitory effects by activating voltage-gated Ca\(^{2+}\) channels, K\(^{-}\) channels, and adenyl cyclase to induce cell hyperpolarization and inhibit neurotransmitter releases [45].

After producing biological effects, most GABA is reabsorbed from synaptic cleft and reused by GAT. GAT has four types: GAT1, GAT2, GAT3, and betaine-GABA transporter [46]. GAT1 and GAT3 are the major types in the brain, regulating the balance of GABA [47]. The GAT1 is especially important in neurons of the brain and can also inversely transport GABA out of neurons with uncertain mechanisms [48]. GAT3 is mostly distributed in glial cells [49]. GABA entry into cells is metabolized by two catalytic enzymes: GABA transaminase and succinic semialdehyde dehydrogenase (SSADH). The final metabolic product of GABA, succinic acid, enters into the tricarboxylic acid cycle for energetic supplements [50].

3. The Role of GABA in Cerebral I/R Injury in Adult

3.1. Variations of GABA Levels, Synthesis, and Metabolism during Cerebral I/R Injury. It is well known that cerebral ischemia injury can cause high mortality and disability [51].
Although the reperfusion is used to restore brain blood 
supplement, it can also induce further injuries involving  
a complex pathological progress (I/R injury) [52]. Many  
mechanisms have been proposed to explain the progress,  
including toxicity of high glutamate activity, Ca$^{2+}$  
overload, oxidative stress, inflammation and apoptosis,  
inflammation, breakdown of the blood-brain barrier, cerebral  
infarction, and edema [52–54]. The imbalance including  
the decreased transmission of GABA signal and the  
increased excretion of excitatory amino acid (glutamic  
acid) in I/R [55] indicated that GABA may play special  
roles in the process of I/R injury. Enhancing GABA  
actions by different enhancers of GABA transmission can  
partly restore the balance of GABA and glutamate trans-
missions, pointing out a potential preventive strategy for  
cerebral I/R injury. Even an enhanced GABA signal pathway  
can inhibit glutamate release induced by ischemia, such as  
treatment with GABA receptor agonists [56], promoting  
normalization of the two neurotransmitter pathways.  

First, accumulating data had shown that GABA levels  
and the function status of its metabolism are affected differ-
ently in different periods of cerebral I/R injury, reflecting that  
the GABA system can be an important index of cerebral I/R  
injury and also monitor the restoration of cerebral I/R injury.  
The most prominent feature in these phenomena is the rise of  
evacellular GABA levels in most cerebral I/R injuries, while  
in transient cerebral ischemia, GABA levels return to the nor-
mal at the start of reperfusion, or in repeated ischemia, it  
decreases to the undetected level [57]. Recent studies showed  
that in animals [58, 59] and humans [60] suffering ischemia  
attacks, GABA contents in the cerebral hemisphere actually  
decreased. Although the efflux transport of GABA out of the  
brain via the blood-brain barrier increased in I/R injury  
[61], reduced GABA contents may mainly result from the  
variations of GABA synthesis, release, and metabolism. In  
the early stage of ischemia, GABA release and the inhibition  
of its metabolism may mainly be responsible for increased  
GABA levels. Allen et al. found that GABA releasing was  
induced sequentially by the exocytosis due to the entering  
of extracellular Ca$^{2+}$ into neurons during the anoxic depo-
larization, and then, reversed uptake of GABA by GAT1  
occurring after the anoxic depolarization [48]. However,  
upregulated GAT1 expressions under ischemic conditions  
after a long time started the removal of GABA from the syn-
aptic cleft [62], reducing GABA metabolism. In the aspect of  
GABA synthesis, the variation of GAD after cerebral I/R has  
a striking similarity. Moreover, increased expressions of  
GAD in a short time after I/R (e.g., large aspiny neurons  
[63]) can produce more GABA and provide the basis for  
GABA release and reversed uptake of GABA by GAT1 in  
neurons to increase the survival. After that, the loss of  
GABAergic neurons [64] and the decrease of GAD protein  
expression [65] occurred (e.g., in substantia nigra of chronic  
ischemia rats). At this point, although increased protein  
expression of GAD (GAD67) may compensate the neuron  
injury induced by hypoxia-ischemia, elevated GAD expres-
sion is also associated with cell death [66], and the compen-
sation is even weaker in the immature brain compared with  
the mature brain. Another study showed that truncated  
cleavage product (tVGAD) from VGAD by calpain following  
focal ischemia had the lower ability in the GABA synthesis-
packaging coupled with VGAT, leading to reduced GABA  
transmission despite its increased activity compared with  
that of VGAD in the focal cerebral ischemic rat brain [67].  
Further, the decreased mRNA level of the VGAT [68] after  
I/R indicated faster eliminations and slower packages of  
GABA. However, the protein levels of VGAT in substantia  
nigra pars reticulate [65] and other brain regions [69] were  
stable after ischemia by using immunohistochemistry.  
It may be due to the rapid formation of a stable truncated cleav-
age product (tVGAT) from VGAT under ischemia by calpain  
which cannot be discriminated by immunohistochemistry  
and may further decrease GABA release after a long time  
[70]. The reason may be that tVGAT is redistributed out of  
synaptosomes with an unclear mechanism, probably explain-
ing reduced GABA release with more production and redis-
tribution of tVGAD in ischemia [67]. In addition, the  
protein levels of SSADH and SSAR were reduced in some  
regions after ischemia [71], indicating that GABA metabo-
lism was disturbed, and due to the downregulated SSADH  
level, the less supplement of GABA shunt to produce adeno-
sine triphosphate (ATP) in pyruvate dehydrogenase into the  
tricarboxylic acid cycle aggravated the neuron damages.  

3.2. Expressions, Binding Abilities, and Signal Transmissions  
of GABA Receptors during Cerebral I/R. Apart from the nor-
mal GABA level in the brain necessary for maintaining neu-
ral functions in the cerebral I/R process, GABA receptors and  
GABA signal transmissions are also very vital. An interesting  
study had well proved the view of point that only blocking  
GABA synthesis is not enough to alter neuron functions. A  
study [72] showed that reduced GABA production by a  
GAD inhibitor did not alter dendrite growth in vitro. So,  
GABA receptors and their transmissions may be involved in  
nearl I/R injury. Thus far, data about variations of GABA  
receptors showed the characteristic of time dependence  
resulting from I/R injury of the mature neuron. A previous  
study had demonstrated that the level of GABA$\alpha_2$ receptor  
in the hippocampus and cerebral cortex was downregulated  
within 30 min after I/R [73]. In mechanism, the reduced den-
sity of GABA$\alpha_2$ receptor probably accounted for this event. It  
can result from the internalization of GABA$\alpha_2$ receptor medi-
dated by dephosphorylation of its $\beta_3$ subunit (serine 408/409)  
[74], which occurred under increased recycling of GABA$\alpha_2$  
receptor by huntingtin-associated protein 1 [75] and  
decreased GABA$\alpha_2$ receptor clustering by downregulating a  
protein ( gephyrin ) responsible for the transport and synaptic  
anchoring of GABA$\alpha_2$ receptor after oxygen-glucose depriva-
tion [76]. Differently reduced GABA$\alpha_2$ receptor density was  
also demonstrated in the optic lobe of an embryonic chicken  
[77]. Meanwhile, the mRNA levels of GABA$\alpha_2$ receptor sub-
units (a1 and a2) in the hippocampus and dentate gyri were  
also downregulated in another study [78]. Besides, at 48 h  
after transient global ischemia, there were different variations  
of GABA$\alpha_2$ receptor subunit (a1, $\beta_2$, and $\gamma_2$) mRNA in cere-
bral cortices (no change) and caudate putamens (upregu-
lated both subunits) in the rat ischemia model [79],  
indicating different injuries in different brain regions in the  

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same time. However, despite the confirmed upregulated mRNA levels of GABA<sub>A</sub> receptor α1 and α2 subunits [80], protein contents of the α1 subunit in rats with brain ischemia induced by the manmade thrombus decreased at 7 days after I/R [81]. In other cases, subunits (e.g., α1) of GABA<sub>A</sub> receptors in the perilesional cortex were highly expressed, which may induce functional reorganization (increase in dendritic-like structures [82]) or other dysfunctions of the nervous system (e.g., epileptogenesis [83]) in cerebral I/R injury. In addition, although the mRNAs of GABA<sub>B2</sub> receptor subunits (B1 and B2) were detected coexisting with parvalbumin in gerbil hippocampus neurons at 4 days after transient global ischemia [84], these neurons surviving the ischemia insult were just a few. In vitro, a brief episode of oxygen and glucose deprivation can decrease GABA<sub>B2</sub> receptor contents in the cell surface through activating endoplasmic reticulum stress [85]. Therefore, the fluctuating process of GABA receptor expressions hinted the decline of neuron functions or even death in cerebral I/R injury. In contrast, GABA receptors in young individuals were more vulnerable and caused functional compliments. For instance, the mRNA levels of GABA<sub>A</sub> receptor α1, β2, and γ2 subunits significantly decreased in the cerebral cortex and caudate putamen in young ischemia rats [79]. These variations in neonatal individuals can directly induce seizure occurrence [86].

On the other hand, the functional status of GABA receptors is also responsible for normal GABA signal transmission. It had been demonstrated that the binding abilities of GABA receptors with ligands declined after ischemia and the binding activity is often characterized by the binding value of special agonists with them. For instance, muscimol, a GABA receptor agonist, had a lower binding value in the ischemic cortical penumbra after transient focal cerebral ischemia I/R [87]. Even after transient oligemia, a significant reduction of GABA<sub>A</sub> receptor binding values can last for a long time [88]. Conversely, GABA<sub>B1</sub> receptor in surviving neurons of the gerbil hippocampus against I/R had upregulated binding abilities with muscimol according to a research [89]. However, the tolerance of neurons was just limited in a certain period (within 48 h after cerebral I/R).

Moreover, the intracellular signal transmission of GABA receptors also disrupts. Inhibitory postsynaptic potentials induced by GABA had disappeared ex vivo [90] or weakened in vitro [91] in the short term after ischemia. GABA signal transmission also declined with reduced chloride ion influx in vivo [91], in vitro [92], and ex vivo [90]. Downregulation of KC2 may be responsible for the results [93], which are unable of restoring Cl<sup>-</sup> gradient on both sides of the neuron membrane. In a research about long-term injuries after ischemia, the dysfunction of GABA signal transmission was remarkable, despite the normal presynaptic neurons which had survived the I/R injury releasing a normal amount of GABA [94]. This result indicated that GABA transmission after GABA receptors was more fragile. On the other hand, high-resistant neurons still exhibited the normal response ability of the GABA receptor. Zhan et al. found that miniature inhibitory postsynaptic currents and GABA-evoked currents in high-resistant CA1 interneurons after transient forebrain ischemia did not decrease [95], which might delay the I/R injury. Liang et al. further proved that the enhancement of GABA synaptic transmission in CA1 neurons following forebrain ischemia was attributed to the postsynaptic signal dysfunction rather than disrupted GABA releasing in the presynaptic membrane [96]. The research had proved that adenosine A1 receptor activation was a potential reason for the phenomenon. Other factors after ischemia had been proposed accounting for the dysfunction of postsynaptic GABA transmission in a comprehensive review [97], including increase in intracellular chloride ion, accumulation of some signal substances (e.g., Ca<sup>2+</sup> and eicosanoids), production of reactive oxygen species, and reduction of ATP. In addition, the degradation of tumor suppressor phosphatase and tensin homolog after ischemia had double effects according to a recent research [98]. The molecule can enhance GABA-evoked current to protect neural functions by increasing the expression of GABA<sub>B2</sub> receptor γ2 subunit on the one hand and has the converse action of damaging astrocyte functions on the other hand.

### 3.3. The Protective Effects of GABA and Its Enhancers on Cerebral I/R Injury

In view of the importance of GABA in cerebral I/R injury and the difficulty of GABA crossing the blood-brain barrier, some GABAergic drugs had been used for attenuating I/R injury in clinical and animal tests. Early in 2001, Schwartz-Bloom and Sah had reviewed some GABAergic drugs used in I/R animals and few human subjects [97], including GABA agonists (e.g., muscimol, thip, and baclofen), GABA modulators (e.g., benzodiazepines), GABA uptake inhibitors (e.g., tiagabine), and GABA metabolism inhibitors (γ-vinyl GABA). However, due to the narrow therapeutic window, only short-term protective effects (about 1 month) against cerebral I/R injury were demonstrated in most researches. Moreover, adverse effects of some drugs aggravating neuronal deaths were frustrating. The discussion of further mechanisms was still limited. Most importantly, selective applications of these drugs and clinical investigations were not discussed due to the shortage of data. In recent years, much broader and deeper studies were illuminating to develop preventive and therapeutic strategies for cerebral I/R through targeting the GABA signal pathway. In this section, an updated review from several aspects of new progresses was made.

#### 3.3.1. The Clinical Effects of GABA on Cerebral I/R Injury

For a long time, barbiturates had clinically been used to control the intracranial hypertension in transient ischemia events in the operating room; some potential side effects (having a depressant effect on the cardiovascular and respiratory systems) might affect the applications (e.g., needing intensive care) [99]. Meanwhile, another system review [100] summarized that GABA receptor agonists (e.g., chlormethiazole) had better effects on patients with acute ischemic (within 12 hours) or hemorrhagic stroke. Some adverse events (e.g., somnolence and rhinitis) also occurred. Recently, the long-term intrathecal treatment of baclofen can improve the functional independence in the traumatic brain injury and stroke groups [101]. However, adjustments of doses were complex and needed further investigations. In addition, GABA
enhancer treatments, such as rac-haptenonic acid (pantogam active) [102, 103] and adaptol [104], remarkably improved psychopathological, psychometric, and detailed somatic functions (e.g., cognitive functions, but also emotional state) with reduced adverse events (e.g., drowsiness and headaches) in patients with chronic cerebral ischemia. Therefore, developing new drugs of GABA enhancers with less adverse effects may be the next focus of clinical studies.

3.3.3. More Mechanisms Based on or beyond GABA Signal Transmission. With the broader researches of using GABAergic drugs preventing cerebral I/R injury, some strategies are emerging and may produce better effects (e.g., coadministrations of GABA_A and GABA_B receptors having better effects than given alone in the in vitro ischemia model [111]) and less adverse effects (e.g., special activations of GABA subunits: β1, α1, and γ1; coadministrations of etomidate and propofol; and coadministrations of zolpidem and diazepam [112]).

Moreover, multiple mechanisms had been presented in exploring the effects of different GABAergic enhancers in cerebral I/R (Table 1), including improved expressions of GABA receptors (or subunits); increased NO synthase and postsynaptic density 95 ( PSD95 ) interaction; activated α2-δ subunit of the voltage-dependent Ca^{2+} channel and Ca^{2+}/calmodulin-dependent protein kinase II; upregulated B-cell lymphoma-2 (Bcl-2) expressions; decreased Bcl-2-like protein 4 (Bax)/Bcl-2 ratio; downregulated expressions of procaspase-3 and caspase-3 through glutamate receptor 6 (GluR6)/PSD95/mixed lineage kinase 3 (NLK3)/c-Jun N-terminal kinase 3 (JNK3) and protein kinase M zeta (PKMζ)/KCC2; reduced glutamate and lactate dehydrogenase (LDH) levels; inhibited N-methyl-d-aspartate acid (NMDA) receptor/Src-mediated signal; suppressed autophagy by activating protein kinase B (Akt), glyogen synthase kinase-3 beta (GSK-3β), and extracellular regulated protein kinases (ERK) and neutrophil-directed migration in a phos- phoinositide 3-kinase-(PI3K-) dependent manner; attenuated oxidative stress; decreased intracellular Ca^{2+} contents; and restored contents of brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), tyrosine kinase B (TrkB), neural cell adhesion molecule (NCAM), and G protein-activated inwardly rectifying potassium (GIRK, Kir3). Among them, most played the roles which may be related to improved GABA transmission. However, individual drugs played the protective effect through some pathways which were unrelated to GABA signals. For instance, diazepam acted on the peripheral benzodiazepine receptor in the outer mitochondrial membrane [108]. Therefore, more potential mechanisms related to GABA signal or other effects in or outside the ischemia regions needed further investigations, and systemic classifications of different GABA enhancers are necessary in the long run.

3.4. The Protective Effect of Preconditioned and Delayed Treatments with GABA Transmission Inhibitors on Adult Cerebral I/R Injury. However, in some circumstances, preconditioned and delayed treatments with GABA transmission inhibitors can produce protective effects on adult cerebral I/R injury according to limited studies. In fact, an early study had already some kind of hint. A benzodiazepine receptor partial agonist (PNU-101017) intriguingly exhibited the better effect of preventing hippocampal neuronal degeneration in I/R injury than a full benzodiazepine receptor agonist [129] in preconditioning. The difference was more obvious when the two drugs were given after ischemia. The result was probably due to GABA receptor overactivations after I/R, triggering the outward of bicarbonate over the inward of Cl^{-} and a further depolarization. Recently, some reports further confirmed the effect. First, the treatment with GABA_A receptor antagonist (bicuculline) at 1-2 days before oxygen-glucose deprivation made the cultured cortical neurons more tolerant to the insult [130]. The mechanism was related to calmodulin-dependent phosphorylations of ERK1/2 and cAMP-response element binding protein (CREB). Interestingly, alike results were also obtained in vivo. In a study, in contrast to attenuating I/R injury by activating GABA transmissions in adults, the treatment with a GABA_A receptor α5 subunit inverse agonist (L-655,708) at a low dose of 1.5 mg by subcutaneous implantation at 1 week after the focal ischemia induced by cortical microinjection of endothelin-1 can significantly restore motor skills of ischemia stroke rats by inhibiting GABA signal [131]. Recently, L-655,708 treatment (1, 5 mg/kg) only after 3 days after middle cerebral artery occlusion can trigger neurogenesis [132]. It hinted that the targeted inhibition of special GABA receptor subunits may be the key. In another research, Alia et al. found that hampering GABA_A transmission after photothrombotic ischemia by a benzodiazepine inverse agonist (DMCM: methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate, i.p., 1.5 mg/kg) can significantly improve motor function [133]. The effect of DMCM may remodel GABAergic inhibitory networks controlling motor function. Although limited reports were presented, more mechanisms about status of GABA receptors and its signals during the period of I/R injury may be responsible for the results.
### Table 1: Protective effects of GABA and its agonists on several cerebral I/R injuries and the mechanisms.

<table>
<thead>
<tr>
<th>Drugs and dose</th>
<th>Treatments</th>
<th>Effects and mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA, γ-vinyl GABA (30-300 mol/L) <em>in vitro</em></td>
<td>At 90 min after oxygen-glucose deprivation and reperfusion in rabbit brain slices</td>
<td>Reducing the release of glutamate and LDH and tissue water gain (GAT inhibitors)</td>
<td>[106]</td>
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<tr>
<td>Diazepam (10 mg/kg)</td>
<td>Injection (i.p.) at 30 and 90 min after transient gerbil brain ischemia</td>
<td>Reducing excitotoxic and oxidative stress by the peripheral benzodiazepine receptor in the outer mitochondrial membrane (GABA&lt;sub&gt;A&lt;/sub&gt; receptor agonist)</td>
<td>[108]</td>
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<tr>
<td>Zolpidem (0.5, 1.0 mg/kg)</td>
<td>Injection (i.p.) following ischemic stroke in rats</td>
<td>Increasing numbers of cells containing BDNF (GABA&lt;sub&gt;A&lt;/sub&gt; receptor agonist)</td>
<td>[113]</td>
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<tr>
<td>Propofol (20 mg/kg/h, 2 h)</td>
<td>Injection (i.p.) at the onset of reperfusion in the rat model of middle cerebral artery occlusion</td>
<td>Increasing the number of survival neurons and the expression of KCC2, extruding Cl⁻ by upregulating the activity of the PKM/KCC2 pathway (GABA&lt;sub&gt;A&lt;/sub&gt; receptor agonist)</td>
<td>[114, 115]</td>
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<tr>
<td>JM-1232 (500 μM) <em>in vitro</em></td>
<td>During rat oxygen-glucose deprivation and reoxygenation</td>
<td>Reducing cell mortality in pyramidal neurons by GABA&lt;sub&gt;A&lt;/sub&gt; receptor/decreased intracellular Ca&lt;sup&gt;2+&lt;/sup&gt; and other mechanisms</td>
<td>[116]</td>
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<tr>
<td>Tramadol (10, 20 mg/kg)</td>
<td>Injection (i.p.) before forebrain I/R in rats</td>
<td>Attenuating postischemic motor impairment by reducing the lipid peroxidation (GABA&lt;sub&gt;A&lt;/sub&gt; receptor agonist)</td>
<td>[117]</td>
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<tr>
<td>Topiramate (80 mg/kg/day, twice daily)</td>
<td>Injection (i.p.) after occlusion of the bilateral carotid arteries in gerbils</td>
<td>Decreasing neurological deficit, attenuating neuronal loss by decreasing the expressions of procaspase-3, caspase-3, Bax/Bcl-2 ratio, GABA&lt;sub&gt;A&lt;/sub&gt; receptor α1 and γ2 subunits, and KCC2</td>
<td>[118]</td>
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<tr>
<td>Muscimol (1 mg/kg) and baclofen (20 mg/kg) alone</td>
<td>Injection (i.p.) at 6 hours and 1 day after rat I/R</td>
<td>Protecting neurons against death induced by I/R by enhancing NO synthase (GABA&lt;sub&gt;A&lt;/sub&gt;,B receptor agonists)</td>
<td>[119]</td>
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<tr>
<td>Muscimol (1 mg/kg) and baclofen (20 mg/kg) together</td>
<td>Injection (i.p.) at 30 min before I/R in rats</td>
<td>Inhibiting NMDA receptor/Src-mediated signal amplification (GABA&lt;sub&gt;A&lt;/sub&gt;,B receptor agonists)</td>
<td>[120]</td>
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<td>[121]</td>
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<td>[122]</td>
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<td>[119]</td>
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<tr>
<td>R-Phenibut (10, 50 mg/kg)</td>
<td>Injection (i.p.) at 2 h and 7 days following reperfusion in transient middle cerebral artery occlusion in rats</td>
<td>Improved histological outcome and reduced brain volume; increase of BDNF and VEGF gene expressions (by activating GABA&lt;sub&gt;B&lt;/sub&gt; receptor and α2-δ subunit of the voltage-dependent Ca&lt;sup&gt;2+&lt;/sup&gt; channel)</td>
<td>[123]</td>
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<td>Baclofen (50 mg/kg)</td>
<td>Injection following (i.p.) ischemic insult in the ischemic gerbils</td>
<td>Preventing the loss of hippocampal CA1 pyramidal cells and calmodulin-dependent protein kinase II but not memory deficits (GABA&lt;sub&gt;B&lt;/sub&gt; receptor agonist)</td>
<td>[124]</td>
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<td>Baclofen (1.25, 2.5 mg/mL)</td>
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<td>Increasing Bcl-2/Bax ratio and activating Akt, GSK-3β, and ERK which suppressed autophagy (GABA B receptor agonist)</td>
<td>[125]</td>
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<tr>
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<td>Injection (i.p.) at 17 d after permanent occlusion of the bilateral common carotid arteries in rats</td>
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<td>[126]</td>
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<tr>
<td>Baclofen (0.5, 2, and 5 μmol/L) in vitro</td>
<td>At 1 hour from the beginning of I/R in rat hippocampal slice cultures</td>
<td>Neuroprotection (GABA B receptor agonist)</td>
<td>[127]</td>
</tr>
<tr>
<td>Baclofen (500 mg/L)</td>
<td>Pretreatments (i.p.) before rat I/R</td>
<td>Stimulating neutrophil-directed migration by PDK (GABA B receptor agonist)</td>
<td>[128]</td>
</tr>
</tbody>
</table>
Preconditioned and delayed treatments may also be the key of the effect. Therefore, more accurate effects concerning GABAergic inhibitory drugs need further investigations.

4. The Role of GABA in Cerebral I/R Injury in Neonate

As stated above, GABA exhibits excitatory effects in the immature nervous system compared with inhibitory effects in the mature nervous system. Therefore, its effect on cerebral I/R injury of neonate was specially discussed in this section. Thus far, limited studies showed that GABA transmission inhibitors played the protective effects on neonatal cerebral I/R injury. The effects may be due to increased GABA levels and the losses of GABA receptors (subunits) or its pathway expressions [86, 134, 135] occurring in immature I/R injury, hinting possible overactions of the GABA transmission pathways. This point can be demonstrated in a study that a GABA_A receptor antagonist (bicuculline) can further enlarge the intracellular Ca^{2+} accumulation under reduction in CI gradient across the membrane induced by oxygen-glucose deprivation in neonatal rat neocortex [136]. Therefore, activating GABA_A receptor may aggregate the I/R injury in the neocortex of neonatal rats. Moreover, treatments with two GABAB receptor antagonists (CGP 35348 and CGP 55845) can remarkably induce the motor function and inflammatory response of hypoxic-ischemic brain of neonatal rats (over 7-day age), except for protective effects (topiramate) generated more damage to the brain and the cognitive functions in the mature nervous system. Therefore, its effects may be different in the immature nervous system compared with inhibitory effects in the mature nervous system.

4.1. GABA and Gastric I/R Injury. Gastric ischemia-reperfusion is a common clinical problem associated with various physiopathological stress conditions such as surgery, hemorrhagic shock, peptic ulcer bleeding, vascular rupture, and ischemia gastrointestinal disease, which may cause the damage of the gastric mucosa [148]. In these processes, the dysfunction occurring in the neuroendocrine system can induce increased catecholamine levels, vasoconstriction, decreased cardiac output, reduced blood volume, and the release of proinflammatory factors [149] which promote the flow of blood into the heart, brain, and muscles from the gastrointestinal tract and skin [150]. So, the central nervous system may play a vital role in gastric I/R injury. Nowadays, fastigial nucleus (FN), hypothalamic paraventricular nucleus (PVN) [151, 152], and lateral hypothalamic area (LHA) [153] are thought as major regions regulating gastric activity and gastric mucosal injury in gastric I/R injury. The GABA system, abundant in the central nervous system, was proved playing a vital role in attenuating gastric I/R injury. Zhu et al. [154] found that microinjecting glutamate into the interpositus nucleus (IN) of the cerebellum can attenuate gastric I/R injury, decrease discharge frequency and intensity of greater splanchnic nerve (GSN), and improve blood flow, apoptosis, and proliferation of rat gastric mucosa. The effect of GABA on the gastrointestinal tract can be reversed by the pretreatments of microinjecting 3-mercaptopropionic acid (GAD inhibitor) into IN or bicuculline into LHA. The result suggested that GABAergic neuron in IN acted on its receptors in the lateral hypothalamic area and subsequently inhibited the activity of the GSN innervating stomach. Its dysfunction was considered as an important promoter of gastric I/R injury progression. Also, GABAergic neurons in FN of the cerebellar also participated in attenuating gastric I/R injury through the cerebellar-hypothalamic circuit [155]. Another study [156] further proved that high levels of GABA_A receptor in the LHA of rats could be responsible for significantly alleviated gastric I/R injury by the overexpression of the receptor induced by adenosine vector. Reduced norepinephrine and angiotensin II levels in plasma and peripheral GSN activity marked multiple ways of GABA preventing against gastric I/R injury in the study. A recent investigation [157] showed that the GABA_B receptor in the LHA was involved in the protective effects on the gastric I/R injury caused by clamping the celiac artery. The administration of GABA_B receptor agonist (baclofen) can significantly attenuate gastric I/R injury. Interestingly, significantly reduced oxidative stress indexes (e.g., nicotinamide adenine dinucleotide phosphate-oxidase (NOX2, NOX4)) and attenuated inflammation (IL-1β) in the gastric mucosa by baclofen treatment were observed in model rats. Although the pathway of baclofen attenuating gastric I/R injury was probably through decreasing expressions of NOX2/NOX4, the neuroendocrine effect mediated by neurotransmitter calcitonin gene-related peptide also played the important role.
because the neurotransmitter is released by neurons innervating the stomach from the dorsal root ganglion under the injection of baclofen into the LHA. Therefore, GABA or its agonists may have the prospect of curing gastric I/R injury. However, the direct effect of GABA in gastric I/R is still unclear.

6.2. GABA and Intestinal I/R Injury. Intestinal ischemia-reperfusion (IIR) injury occurs in numerous clinical events following intestinal ischemia, such as superior mesenteric artery occlusion, hemorrhagic shock, intestinal and liver transplantation [158, 159], neonatal necrotizing enterocolitis [160], volvulus [161], trauma [162], and cardiopulmonary disease [163]. IIR injury can induce the damage and dysfunction of multiple organs [164]. Although improved treatments were recently applied to clinical tests, the morbidity and mortality still remained high [165]. Studies about the effect of GABA on IIR injury had just begun. A recent study [166] showed that IIR resulted in a significant decrease of intestinal innate immunity indexes (immunoglobulin A, alpha-defensin-5, and antioxidative enzyme) in Peyer’s patch cells of the rat small intestine, and GABA pretreatments (oral administration, 30 mg/kg) significantly produced the protective effects on IIR injury in which mRNA levels of R alpha-defensin-5 and superoxide dismutase 1, 3 together with immunoglobulin A levels were increased in vitro. In addition, GABA receptor activations in rat focal cerebral I/R injury seem to affect intestinal flora [167], suggesting that indirect effect of GABA on IIR may exist. Therefore, GABA’s indirect effect and clinical data about applying GABA or its agonists to prevent or cure IIR injury should be focused in the future.

6.3. GABA and Renal I/R Injury. I/R injury is primarily responsible for acute kidney injury, which can cause high mortality and morbidity and elevated cost of treatment [168, 169]. Renal I/R injury is the restoration of blood reperfusion after the blocking of renal blood flow, which often occurs in surgeries of vascular and cardiac, trauma, and kidney transplantation [170, 171]. GABA may play important roles in renal physiology and pathology [172]. For instance, GABA has been reported to regulate blood pressure by increasing excretion of water and sodium ion (Na+) in the kidney ex vivo [173]. Recent studies showed that GABA can attenuate renal I/R injury from two pathways of the central nervous system and the peripheral nervous system. Kobuchi et al. found that intravenous injection of GABA (10 and 50 mmol/kg) can dose-dependently suppress the elevated activity of renal sympathetic neuron and the venous norepinephrine overflow in the rat renal I/R model [33]. Selective treatment using GABA_R receptor antagonist (CGP52432) can reverse the protective effect of GABA on renal I/R injury, hinting that GABA_R receptor is the targets of GABA action. Then, GABA_A receptor antagonist picrotoxin had been found aggravating the acute kidney injury induced by I/R in investigating the mechanism of sodium valproate (reducing GABA degradation) protecting against renal I/R injury [174], suggesting that GABA_A receptor directly plays the protective effect of GABA on renal I/R injury compared with the little, no, or opposite effect of GABA_R receptor in the condition of GABA shortage. But a recent study [175] showed that GABA_A receptor antagonist (bicuculline) treatment by the intracerebroventricular administration was unable to prevent the preventive effect of GABA on renal I/R injury. Conversely, renal I/R injury could be prevented by GABA_A receptor agonist (baclofen), indicating that activated GABA_A receptor in the central nervous system was responsible for the protective effect. In addition, the effect of GABA on renal I/R injury exhibited gender-related difference. GABA treatment can attenuate pathological renal parameters (blood creatinine and urea nitrogen, kidney weight, and renal level of malondialdehyde) in female rats rather than male rats [176]. However, it was intriguing that GABA was unable to protect renal I/R injury in ovariectomized rats whether estradiol was administered or not [177]. The result suggested that variations of the internal environment in different physiological states may interrupt the effect of GABA on renal I/R injury. Therefore, more complex internal environments need considerations.

6.4. GABA and Hepatic I/R Injury. Hepatic I/R injury is a systemic pathological process which induces not only hepatic function and irreversible injury but also cascade of dysfunctions of other organs [178]. Hepatic I/R injury occurs in some clinical scenarios, including liver resection (e.g., the Pringle maneuver and the hepatic vascular exclusion in liver surgery), liver transplantation, and trauma [179–181]. Two types of hepatic I/R injuries have been proposed. The preservation and storage of the liver before transplantation can cause cold I/R injury [182]. Liver surgery, trauma, setting of transplantation, etc. can induce warm I/R injury due to transient blockage of blood flow [183]. A lot of mechanisms are involved in hepatic I/R injury, including oxidative stress, inflammation, respiratory chain dysfunction of mitochondria, Kupffer cell activation, upregulated vascular cell adhesion molecule, and polymorphonuclear neutrophil injury [184]. Some substances with antioxidative and anti-inflammatory activities had demonstrated protective effects against hepatic I/R injury [178]. Recently, novel targets for attenuating hepatic I/R injury were also proposed (e.g., peroxisome proliferator-activated receptor gamma and several noncoding RNAs) [185]. However, the mechanism remained unclear. Present data showed that GABA was associated with hepatic I/R injury. First, it had shown that GABA may play an important role in monitoring hepatic I/R injury. According to a clinical study about 18 patients, GABA levels in the grafts for liver transplantation immediately after the reperfusion with an isotonic solution for 48 hours were remarkably decreased and then stabilized at baseline values [186]. Thus, GABA can be an index predicting the changing process of a normal functioning liver graft and may participate in protecting hepatic I/R injury. Further studies showed that GABA agonists can attenuate hepatic I/R injury. An animal research showed that pretreatment of donor rats in vivo by GABA_A receptor agonist (muscimol) before the liver graft surgery significantly reduced the liver cold ischemia/warm reperfusion injury and oxidative stress after the orthotopic liver transplantation [187]. At the end of 6 hours, after the orthotopic liver transplantation with the same treatment on recipient
rats according to the donor rats, PI3K, Akt, antioxidant enzymes (superoxide dismutase), ataxia-telangiectasia mutated kinase (ATM), and phosphorylated histone H2AX (gamma H2AX) were also greatly enhanced. The final index as the markers of DNA damage indicated the potential pathway that GABA attenuated hepatic I/R injury. In vitro, the treatment to the harvested graft of donor rats with GABA receptor agonists for 4 h of cold ischemia cannot prevent hepatic I/R injury in the orthotopic liver transplantation model or shear stress in the split orthotopic liver transplantation model [188], suggesting that GABA had no direct effect on hepatic I/R injury. In addition, a noteworthy study indicated that flumazenil, an antagonist of benzodiazepine receptors, can improve hepatic encephalopathy produced by hepatic I/R injury [189]. Benzodiazepines are a class of drugs targeting GABA_\_A receptor and enhancing GABA to produce effects which are sedative, hypnotic, anxiolytic, anticonvulsant, etc. [190]. The result that flumazenil attenuates the cerebral complication of hepatic I/R injury may be due to its direct interaction with GABA receptor. A recent research had confirmed that a1-y2 interface of GABA_\_A receptor may be the action site of flumazenil [45]. But it was still uncertain whether the effect of flumazenil was related to inhibited GABA pathway mediated by blocking the benzodiazepine signal pathway. In the meanwhile, the effect of flumazenil on hepatic I/R injury had not been explored. Therefore, it was unclear whether it is consistent concerning effects of flumazenil on hepatic I/R injury and its complications (e.g., hepatic encephalopathy). The difference between the probably inhibited GABA pathway and other GABA receptor agonists in attenuating hepatic I/R injury needs further investigations.

6.5. GABA and Myocardial I/R Injury. Myocardial ischemia refers to coronary blood flow reduction which cannot meet their metabolic requests. Myocardial ischemia is characterized by loss of oxygen, accumulations of hydrogen ions and lactate, and ion changes (the decline of K\(^+\) and the increase of Na\(^+\)) in the myocardial extracellular fluid [197]. Myocardial ischemia can lead to arrhythmias, sudden death, myocardial infarction, aneurysms, ruptures, and valvular dysfunction of the heart [198]. Reperfusion is the only effective treatment to prevent the pathological process. However, reperfusion has some deleterious consequences including oxygen radicals, Ca\(^{2+}\) loading, and inflammations, which further caused reperfusion-induced arrhythmias, myocardial stunning, microvascular obstruction, and lethal myocardial reperfusion injury [199]. Thus, it is necessary to develop new strategies to prevent the harmful events. GABA has been detected in sympathetic premotor neurons of the mediulary raphe controlling sympathetic tone to the heart [200]. Thus, GABA may provide a potential outlet to attenuate myocardial I/R injury through the nervous system. Thirty years ago, Meerson et al. had found that GABA accumulation in the brain by administring the inhibitor of GABA transaminase (sodium valproate) prevented the total duration of arrhythmias and cardiac fibrillation in acute I/R of conscious rats with closed chests [201]. Recently, two studies [202, 203] indicated that the GABA pathway in the nervous system was involved in the cardioprotection against myocardial I/R injury induced by the acute sleep deprivation. Bicuculline, a GABA_\_A receptor antagonist, can abolish the effects, and the mechanisms were related to repressing oxidative stress, NO production, and inflammation. In another hand, how GABA can directly affect myocardial I/R injury should also receive special attentions due to confirmed GABA receptors in the heart [204]. Several other studies (Table 2) showed that different benzodiazepine receptor agonists able of enhancing GABA signals remarkably improved indexes of myocardial I/R injury in several ways, including reducing Ca\(^{2+}\) influx, oxidation, ATP-dependent potassium (K\((ATP)\)) channels, and protein kinase C. However, it was likely that these benzodiazepines attenuated myocardial I/R injury not through the GABA signal pathway or at least not directly through acting

Table 2: Protective effects of GABA receptor enhancers on myocardial I/R injuries and the potential mechanisms.

<table>
<thead>
<tr>
<th>Species</th>
<th>I/R model</th>
<th>Drugs and dose</th>
<th>Effects and mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar rats: in vivo and in vitro</td>
<td>Ischemic 20 min, reperfusion 5 min</td>
<td>Phenazepam preconditioning 1 mg/kg</td>
<td>Anticonvulsant and antiarrhythmic effect by central nervous system</td>
<td>[191]</td>
</tr>
<tr>
<td>Chick cardiomyocyte in vitro</td>
<td>Ischemia 1 h, reoxygenation 3 h</td>
<td>Flumazenil preconditioning 10 μmol/L</td>
<td>Inhibiting ROS/mitochondrial K(ATP) channel</td>
<td>[192]</td>
</tr>
<tr>
<td>Chick cardiomyocyte in vitro</td>
<td>Ischemia 10 min, reoxygenation 10 min</td>
<td>Flumazenil preconditioning 10 μmol/L</td>
<td>Inhibiting protein kinase C/mitochondrial K(ATP) channel</td>
<td>[193]</td>
</tr>
<tr>
<td>Chick embryonic cardiomyocyte in vitro</td>
<td>Hypoxia 1 h, reoxygenation 3 h</td>
<td>Diazepam preconditioning (100 μmol/L)</td>
<td>Inhibiting protein kinase C epsilon</td>
<td>[194]</td>
</tr>
<tr>
<td>Isolated rat heart ex vivo</td>
<td>LPS-induced ischemia 5 h, reoxygenation 20 min</td>
<td>Diazepam in reperfusion (3.0 μg/mL)</td>
<td>Myocardial functional parameters and coronary flow</td>
<td>[195]</td>
</tr>
<tr>
<td>Rat cardiomyocytes in vitro</td>
<td>Hypoxia 1 h, subsequent reoxygenation</td>
<td>Clonazepam in reoxygenation (100 μmol/L)</td>
<td>Ca(^{2+}) accumulation by reducing Ca(^{2+}) influx and preserves mitochondrial membrane potential</td>
<td>[196]</td>
</tr>
</tbody>
</table>
on cardiac muscle because it was irreproducible in the isolated heart according to a study [191]. Whether other benzodiazepine receptor agonists targeted peripheral GABA receptors in myocardial cells needs further investigations. In addition, direct effects of GABA on myocardial I/R injury had not been explored.

6.6. Similarities and Differences about the Potential Effects of GABA on I/R Injury in Different Peripheral Organs. Due to microcirculatory dysfunction by I/R potentially triggering multiple organ injuries [3], it is vital to sum up characteristics about the effects of GABA on I/R injury in different peripheral organs (stomach, intestine, kidney, liver, and heart) before appropriately applying GABA or GABA-related drugs in defending I/R injuries. First, in the similarity, the results about the protective effect of GABA on I/R injuries of peripheral organs from the central nervous pathway were consistent, but the role difference between GABA receptors was not utterly identified, needing further investigations. Further, the direct effects of GABA or GABA-related drugs on different peripheral organs were diverse. For instance, there were no direct protective effects of GABA or GABA-related drugs on I/R injuries of the stomach and liver, compared with direct protective effects on kidney I/R. However, it was particularly noteworthy that flumazenil, an antagonist of benzodiazepine receptors, can aggregate hepatic I/R injury with the utterly unclarified mechanism which differed from the protective effects of GABA enhancers. In addition, the effects of GABA on I/R injuries of the intestine and heart needed to be further certified.

7. The Linkage of GABA Molecular Pathway between Central Nervous System and Peripheral Organs in Affecting I/R Injury

In applying GABA-related drugs to attenuate I/R injury, both roles of GABA in the central nervous system and peripheral organs should be carefully considered in order to avoid inconsistent consequences. According to what was mentioned above, some common molecular pathways in the two regions by GABA enhancers had been identified, including increased signal pathways (e.g., PI3K and AKT), reduced signal pathways (e.g., ROS and inflammation), and restored calcium balance. Although there was still shortage of data in peripheral organs, promising prospects with further researches would be possible for simultaneously improving I/R injuries of the central nervous system and peripheral organs by GABA-related drugs. However, more GABA molecular pathways linking the two regions need to be clarified. Besides, the effects that would happen to these common GABA molecular pathways of peripheral organs after applying GABA transmission inhibitors to attenuate adult cerebral I/R injury should also be paid attention in next investigations.

8. The Necessity to Combine GABA-Related Drugs and Molecular Pathways

Due to many GABA-related drugs able to cross the blood-brain barrier resulting in possible dual effects on I/R injuries of the central nervous system and peripheral organs by peripheral administrations (e.g., possible effects of some GABA-related drugs by i.p. on peripheral organs in Table 1), it is essential to combine the drug therapy with molecular pathways and the effects in applying GABA-related drugs to I/R injuries of the two regions. Based on this, it was equally important to further confirm accurate common molecular pathways in protective roles on the two regions and exclude inconsistent effects for other molecular pathways in applying GABA-related drugs on I/R injuries of the two regions in the future.

9. Conclusion

I/R insults different tissues and organs so that it is still difficult to protect against I/R injuries according to present data. Developing and perfecting potential protective targets against I/R injury are a more effective strategy. GABA, one of the most abundant neurotransmitters, plays inhibitory effect by targeting its receptors (A, B, and C) to generate hyperpolarization potentials in the adult brain compared with excitatory effects in the immature brain. The increasing number of researches concerning reduced GABA transmission compared with increased glutamate signals occurring in cerebral I/R indicated that GABA could play the key role in the pathology of cerebral I/R injury. Therefore, most researches about GABA and I/R injury focus on the nervous system. In this review, the variations of GABA levels, synthetases, releases, mechanisms, receptors, and signal transmissions occurring during the cerebral I/R process were firstly reviewed from a comprehensive aspect. Alike variations from elevation to reduction in these indexes of the mature brain during I/R injury and the confirmed mechanisms probably provided evidences for the treatment window period. Therefore, strengthening the GABA transmission was a common scheme to prevent cerebral I/R injury in adult. Blocking of the GABA transmission was the method to attenuate cerebral I/R injury of the immature brain. However, new problems are gradually emerging in accumulating studies applying the enhancers of GABA transmission to prevent against cerebral I/R injury, such as dose-effect relationships, beneficial effects of applying antagonists, and multiple mechanisms. First, the relationship between the dose and the effect was a traditional issue and may be resolved by the adjustment of different doses or cotreatments of several lower doses of different drugs. Moreover, some mechanisms in applying different GABA transmission enhancers to attenuate the cerebral I/R injury were found, including improved expressions of GABA receptors; increased NO synthase and PSD95 interaction; activated α2-δ subunit of the voltage-dependent Ca2+ channel and Ca2+/calmodulin-dependent protein kinase II, enhanced proliferation (upregulated Bcl-2 expressions and Bcl-2/Bax ratio); inhibited apoptosis (downregulated expressions of procaspase-3 and caspase-3) through different pathways (GluR-PSD95-MLK3-JNK3 and PKM2/KCC2); reduced glutamate and LDH levels, inhibiting NMDA receptor/Src-mediated signal; suppressed autophagy by activating Akt, GSK-3β, and ERK; neutrophil-directed migration by PI3K; attenuated oxidative stress; decreased intracellular...
Ca\textsuperscript{2+} contents; and restored contents of BDNF, VEGF, TrkB, NCAM, and Kir3. However, more mechanisms and the accurate application of these drugs in different statuses may be the next challenges for investigators. In contrast, pretreatments or delayed treatments with antagonists to block GABA transmission instead restored motor functions \textit{in vivo} and increased the tolerance to oxygen-glucose deprivation \textit{in vitro}. These results are obviously different from the results of previous studies in applying enhancers of GABA transmission. The mechanisms are related to calmodulin-phosphorylation of ERK1/2 and targeting GABA\textsubscript{A} receptor α5 subunit, respectively. These researches indicated that the adjustment to the status of different steps in GABA transmission may immunize the cerebral tissue or later repair neurons. In spinal cord I/R injury, the role of GABA had bidirectional effects, making the application more complex and arousing more attentions in the future.

In addition, the roles of GABA in peripheral organs had also been unearthed, including the stomach, intestine, kidney, liver, and heart. As a whole, enhancing GABA functions by GABA or its agonist can prevent I/R injury despite insufficient data (especially in the clinic) through different pathways. The protective effects of GABA were proved dependent on activated nervous functions, such as the stomach, kidney, and heart. In the intestine and liver, the treatment of GABA agonists can directly attenuate I/R injury. However, the corresponding mechanisms in these organs were seldom reported, whether in test models or in the clinic. Besides, during the I/R injury of multiple organs, the applications of drugs (agonists or antagonists based on GABA transmission) should be considered in the whole body. Consequently, in the view of this point, present data are far from enough for applying GABA to clinic treatments.

**Abbreviations**

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
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<tbody>
<tr>
<td>Akt</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>Bax</td>
<td>Bcl-2-like protein 4</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>B-cell lymphoma-2</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP-response element binding protein</td>
</tr>
<tr>
<td>ERK1/2</td>
<td>Extracellular regulated protein kinases 1/2</td>
</tr>
<tr>
<td>FN</td>
<td>Fastigial nucleus</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-Aminobutyric acid</td>
</tr>
<tr>
<td>GAD</td>
<td>Glutamate decarboxylase</td>
</tr>
<tr>
<td>GAT</td>
<td>GABA transporters</td>
</tr>
<tr>
<td>GIRK, Kir3</td>
<td>G protein-activated inwardly rectifying potassium</td>
</tr>
<tr>
<td>GluR</td>
<td>Glutamate receptor</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>Glycogen synthase kinase-3 beta</td>
</tr>
<tr>
<td>GSN</td>
<td>Greater splanchnic nerve</td>
</tr>
<tr>
<td>IIR</td>
<td>Intestinal ischemia-reperfusion</td>
</tr>
<tr>
<td>IN</td>
<td>Interpositus nucleus</td>
</tr>
<tr>
<td>I/R</td>
<td>Ischemia-reperfusion</td>
</tr>
<tr>
<td>JNK</td>
<td>Jc-Jun N-terminal kinase 3</td>
</tr>
<tr>
<td>KCC2</td>
<td>Potassium chloride cotransporter</td>
</tr>
<tr>
<td>LDH</td>
<td>Glutamate and lactate dehydrogenase</td>
</tr>
<tr>
<td>LHA</td>
<td>Lateral hypothalamic area</td>
</tr>
<tr>
<td>NCAM</td>
<td>Neural cell adhesion molecule</td>
</tr>
<tr>
<td>NLK3</td>
<td>Mixed lineage kinase 3</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-Methyl-d-aspartic acid</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PKM</td>
<td>Protein kinase M zeta</td>
</tr>
<tr>
<td>PSD95</td>
<td>Postsynaptic density 95</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
</tr>
<tr>
<td>SSADH</td>
<td>Succinic semialdehyde dehydrogenase</td>
</tr>
<tr>
<td>tGAD</td>
<td>Truncated glutamate decarboxylase</td>
</tr>
<tr>
<td>TrkB</td>
<td>Tyrosine kinase B</td>
</tr>
<tr>
<td>tVGAT</td>
<td>Vesicular GABA transporters</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VGAT</td>
<td>Vesicular GABA transporters</td>
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</table>

**Disclosure**

Xiang Zhou was the co-first author.

**Conflicts of Interest**

The authors declare that they have no conflict of interest.

**Authors’ Contributions**

Xiang Zhou contributed equally to this work as Chaoran Chen.

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**References**


[41] S. Schipper, M. W. Aalbers, K. Rijkers et al., "Erratum to: Tonic GABA 


