Enhancement of Excitatory Synaptic Transmission in Spiny Neurons After Transient Forebrain Ischemia

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Zhang, Yuchun, Ping Deng, Yan Li, and Zao C. Xu. Enhancement of excitatory synaptic transmission in spiny neurons after transient forebrain ischemia. J Neurophysiol 95: 1537–1544, 2006. First published December 14, 2005; doi:10.1152/jn.01166.2005. Spiny neurons in the neostriatum are highly vulnerable to ischemia. Enhancement of excitatory synaptic transmissions has been implicated in ischemia-induced excitotoxic neuronal death. Here we report that evoked excitatory postsynaptic currents in spiny neurons were potentiated after transient forebrain ischemia. The ischemia-induced potentiation in synaptic efficacy was associated with an enhancement of presynaptic release as demonstrated by an increase in the frequency of miniature excitatory postsynaptic currents (mEPSCs) and a decrease in the paired-pulse ratio. The amplitude of inward currents evoked by exogenous application of glutamate did not show significant changes after ischemia, suggesting that postsynaptic mechanism is not involved. The ischemia-induced increase in mEPSCs frequency was not affected by blockade of voltage-gated calcium channels, but it was eliminated in the absence of extracellular calcium. Bath application of ATP P2X receptor antagonist pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) significantly reduced mEPSC frequency in ischemic neurons but had no effects on the control ones. Furthermore, the inhibitory effect of PPADS on ischemic neurons was abolished in Ca2+-free external solution. These results indicate that excitatory synaptic transmissions in spiny neurons are potentiated after ischemia via presynaptic mechanisms. Activation of P2X receptors and the consequent Ca2+ influx might contribute to the ischemia-induced facilitation of glutamate release.

INTRODUCTION

Excitotoxicity has been widely accepted as a major cause of ischemic neuronal damage. The high level of extracellular glutamate induced by ischemia overstimulates glutamate receptors with extensive influx of Ca2+, which initiates a wide variety of cytoplasmic and nuclear processes that promote posts ischemic cell death (Choi and Rothman 1990; Rothman and Olney 1986). Microdialysis studies have shown that extracellular glutamate concentration dramatically increases during ischemia and rapidly returns to the baseline levels after reperfusion (Benveniste et al. 1984; Globus et al. 1988). However, changes in extracellular glutamate might not faithfully reflect the changes in the synaptic cleft (Obrenovitch et al. 2000). Both in vivo and in vitro studies have shown that excitatory synaptic transmissions in the hippocampus are depressed during ischemia despite the extensive accumulation of extracellular glutamate (Gervitz et al. 2001; Sugahara et al. 2001; Tanaka et al. 2001). On the other hand, the excitatory synaptic transmission of CA1 neurons in the hippocampus is facilitated after reperfusion (Crépel et al. 1993; Gao et al. 1998b).

The neostriatum is one of the brain regions highly sensitive to cerebral ischemia. Most of the medium spiny neurons in the striatum die in 24 h after transient forebrain ischemia, whereas the large aspiny neurons in the same area survive the insult (Chesselet et al. 1990; Francis and Pulsinelli 1982). The mechanisms underlying this selective cell death remain to be elucidated. Excitatory synaptic transmission has attracted much attention due to its important roles in excitotoxicity. It has been shown in vitro that aglycemia induces depression of excitatory synaptic transmission in spiny neurons whereas oxygen and glucose deprivation (OGD) induces long-term potentiation (Calabresi et al. 1997a,b, 2002). Studies using intracellular recording in vivo indicate that the excitatory monosynaptic potentials are depressed but the excitatory polysynaptic potentials are facilitated after transient cerebral ischemia (Gajendiran et al. 2001). Interpretation and reconciliation of these results are complicated for several reasons. For in vitro preparations, the neuronal responses to OGD might differ from those to ischemia in vivo because of the dramatic difference in temperature, microenvironment, and homeostasis. In addition, the results from in vitro preparations can only reveal the changes during or shortly after OGD due to the limitations of experimental preparation. For in vivo preparations, the mixture of inhibitory and excitatory components of synaptic transmission and the difficulty in pharmacological manipulations make it very hard to clearly elucidate the changes of each component to the ischemic insults. In addition, the postsynaptic potential obtained in vivo using current-clamp recording makes it difficult to evaluate the synaptic efficacy because of the existence of somatodendritic voltage-dependent conductance (Magee and Johnston 1995; Stuart and Sakmann 1995). To circumvent these restrictions, Pang et al. (2002) used whole cell voltage-clamp recording on brain slices prepared at different intervals after ischemia in vivo and showed that excitatory synaptic currents are significantly reduced in ischemia-resistant large aspiny neurons after reperfusion, suggesting that the depression of excitatory transmission might contribute to the neuronal survival after ischemia. If this is the case, the excitatory synaptic transmission should be potentiated in ischemia-vulnerable medium spiny neurons after ischemia. To test this hypothesis, we examined the excitatory synaptic transmission in spiny neurons at 9 h after transient forebrain ischemia using whole cell voltage-clamp recording on striatal slices.

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METHODS

Male young adult Wistar rats (100–180 g; Charles River Laboratories, Wilmington, MA) were used in the present study. Experimental protocols were institutionally approved in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize both the suffering and number of animals used.

Transient forebrain ischemia

Transient forebrain ischemia was induced using the four-vessel occlusion method (Pulsinelli and Brierly 1979) with modifications (Ren et al. 1997). The animals were fasted overnight to provide uniform blood-glucose levels. For surgical preparation, the animals were anesthetized with a mixture of 1–2% halothane, 33% O2, and 66% N2 via a gas mask placed around the nose. A silicon tube loop was placed loosely around each common carotid artery to allow subsequent occlusion of these vessels. The animal was then placed on a stereotaxic frame, and the vertebral arteries were electrocauterized. A tiny temperature probe (0.025-in diam; Physitemp, Clifton, NJ) was inserted beneath the skull in the extradural space, and the brain temperature was maintained at 37°C with a heating lamp using a temperature-control system (BAT-10; Physitemp). Glass microelectrodes (5–8 μm tip diameter) filled with 2 M NaCl were used to record ischemic depolarization, which is an indication of complete ischemia (Ren et al. 1997). A burr hole was drilled at 9.5 mm anterior to the interaural line, 3.0 mm from the midline. The microelectrode was advanced 3.0 mm below dura into the neostriatum. The record-to-interaural line, 3.0 mm from the midline. The microelectrode was advanced 3.0 mm below dura into the neostriatum. The recording chamber. The slice was submerged beneath the fluid surface and superfused continuously with oxygenated ACSF. The flow rate was adjusted to 2–3 ml/min. Recordings were performed at 33–35°C.

For whole cell recording, patch electrodes were prepared from borosilicate glass (Warner Instruments, Hamden, CT) using a horizontal electrode puller (P-97; Flaming/Brown; Sutter, Novato, CA) to produce tip openings of 1–2 μm (3–5 MΩ). Electrodes were filled with an intracellular solution containing (in mM) 130 NaCl, 3 KCl, 2 CaCl2, 2 MgCl2, 1.25 NaH2PO4, 26 NaHCO3, and 10 glucose. For Ca2+-free medium, Ca2+ was replaced by Mg2+, and 3 mM EGTA was included. Transverse striatal slices of 300 μm thickness were cut using a vibratome (VT 1000S; Leica, Nussloch, Germany) and incubated in ACSF for ≥1 h at 35°C before being transferred to the recording chamber. The slice was submerged beneath the fluid surface and superfused continuously with oxygenated ACSF. The flow rate was adjusted to 2–3 ml/min. Recordings were performed at 33–35°C.

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Brain slices were prepared from animals before ischemia and at 9 h after reperfusion as described previously (Pang et al. 2002). The brains were quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF), which was composed of NaH2PO4, CaCl2, KCl, NaCl, MgCl2, and glucose. Transverse striatal slices of 300 μm thickness were cut using a vibratome (VT 1000S; Leica, Nussloch, Germany) and incubated in ACSF for ≥1 h at 35°C before being transferred to the recording chamber. The slice was submerged beneath the fluid surface and superfused continuously with oxygenated ACSF. The flow rate was adjusted to 2–3 ml/min. Recordings were performed at 33–35°C.

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Drug application

(−)-Bicuculline methiodide (BIC), (−)-2-amino-5-phosphonopentanoic acid (APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PDADS) were purchased from Sigma. Antagonists were applied via bath superfusion. BIC was used to block GABAA receptors at a concentration of 30 μM, and 50 μM APV was used for N-methyl-D-aspartate (NMDA) receptor (NMDAR) blockade. Exogenous glutamate was applied through a Y-tube system (Pang et al. 2002). The tip of the Y-tube had a diameter between 100 and 150 μm and was placed close to the recorded neuron.

RESULTS

Potentiation of AMPAR-mediated EPSCs after ischemia

Whole cell voltage-clamp recordings were performed on the striatal neurons with medium-sized somata in brain slices. To ensure the recorded neurons being medium spiny neurons, we labeled the neurons randomly with neurobiotin after recording. As shown in Fig. 1A, the labeled neuron had a medium-sized cell body (~15 μm) and the large numbers of spines on their dendrites. Intrastriatal stimulation evoked both EPSCs and inhibitory postsynaptic currents (IPSCs) from medium spiny neurons (Kita 1996). To isolate EPSCs, bicuculline (30 μM) was added to the bath solution to block GABAA receptors. Both AMPA receptor (AMPAR)- and NMDAR-mediated components contribute to EPSCs in spiny neurons. Inward EPSCs were evoked at a holding potential of −70 mV. Bath application of APV (50 μM) slightly decreased the peak amplitude of...
EPSCs, whereas CNQX (30 μM) completely blocked the rest part in a reversible manner (Fig. 1B), indicating that eEPSCs in spiny neurons in our experimental condition (2 mM MgCl₂ in external solution and holding potential of −70 mV) were mediated largely by AMPA receptors. Therefore the present study focused on changes in AMPAR-mediated EPSCs in spiny neurons after ischemia. APV (50 μM) and bicuculline (30 μM) were applied throughout the experiments unless otherwise noted.

To examine the efficacy of synaptic transmission in medium spiny neurons, we first determined the threshold of stimulus intensity, which was defined as the stimulating current evoking the smallest detectable response from spiny neurons. Then a step increase in stimulus intensity, which was normalized as one to five times of the threshold intensity (1–5T), was performed to evoke postsynaptic responses. In both control and posts ischemic neurons, the amplitude of AMPAR-mediated EPSCs increased with step increment of stimulus intensities accordingly. The EPSCs amplitude was significantly enhanced at 9 h after a 20-min ischemia compared with control neurons across the stimulus intensities (Fig. 1, C and D). At a stimulus intensity of three times the threshold intensity (3T), the amplitude was 171.9 ± 13.6 pA in controls (n = 10) and 257.8 ± 29.5 pA (n = 12) after ischemia (P < 0.05). The rise time and decay time constant of EPSCS were not affected by ischemia (data not shown). In control animals, the threshold stimulus intensity for inducing EPSCs was 210 ± 31 μA (n = 10) in the present experimental conditions. The threshold stimulus intensity decreased to 135 ± 18 μA at 9 h after ischemia (P < 0.05, n = 12), suggesting an increase in the sensitivity of synaptic transmission. The current-voltage relationships of AMPAR-EPSCs were also investigated in control and ischemia groups. There was no significant change in the I-V relationship of AMPAR after ischemia (Fig. 1E). To determine whether the potentiation of synaptic transmission in spiny neurons after ischemia is specific for EPSCs or simply a general phenomenon for all synaptic transmissions, we examined the changes in evoked IPSCs (eIPSCs). We found that eIPSCs were significantly depressed at 9 h after reperfusion. At 3T stimulus intensity, the amplitude of eIPSCs was 104.1 ± 18.1 pA in controls (n = 11) and 54.1 ± 9.6 pA (n = 10) at 9 h after ischemia (P < 0.05). These results indicate that transient ischemia specifically poten tiates EPSCs in spiny neurons at 9 h after reperfusion.

Presynaptic mechanism is involved in the posts ischemic potentiation of EPSCs

Enhancement of evoked synaptic transmission after ischemia could be due to the alterations in pre- or/and postsynaptic function. To examine the locus of ischemia-induced enhancement, we analyzed mEPSCs in the presence of 1 μM TTX. mEPSCs in the spiny neurons were measured under voltage clamp at a holding potential of −70 mV. The mEPSCs can be completely blocked by bath application of CNQX (30 μM), confirming them to be AMPA/kainate receptor-mediated events. As shown in Fig. 2, in control neurons (n = 16), mEPSCs had a mean frequency of 5.82 ± 0.66 Hz and a mean amplitude of 11.51 ± 0.57 pA. In the spiny neurons subjected to ischemia (n = 45), the frequency and amplitude of mEPSCs was increased to 8.83 ± 0.63 Hz, and 14.46 ± 0.31 pA, respectively (P < 0.05). Analysis of kinetics of mEPSCs before and after ischemia showed that there were no changes in rising time (1.02 ± 0.06 ms in control; 0.98 ± 0.07 ms after ischemia; P = 0.73) and decay time constants (3.31 ± 0.18 ms in control; 3.57 ± 0.29 ms after ischemia; P = 0.54). No detectable changes in spontaneous EPSCs frequency were observed before and after TTX (1 μM) application in either control or posts ischemic spiny neurons (data not shown), suggesting no spontaneous action potential mediated event in spiny neurons.

To further test the possibility that presynaptic mechanism contributes to the posts ischemic potentiation of evoked AMPAR-EPSCs, we examined the effects of ischemia on paired-pulse ratio (PPR). As shown in Fig. 3, A and B, the magnitude of PPR was decreased from 1.49 ± 0.12 in control neurons (n = 8) to 1.09 ± 0.10 after ischemia (P < 0.05, n = 9). These
results suggest that presynaptic mechanism is involved in the postischemic potentiation of excitatory neurotransmission in striatal spiny neurons after ischemia.

In addition to the increase in mEPSCs frequency after ischemia, the mEPSCs amplitude distribution shown in Fig. 2B also argues against the involvement of postsynaptic mechanism. If the changes in amplitude distribution resulted from the postsynaptic mechanism, such as an increase in receptor conductance, the amplitude of all events would be increased equally, there would be a global shift of amplitude distribution to the large-amplitude direction, and the small-amplitude events would have disappeared after ischemia. However, it was not the case. The small-amplitude events in postischemic neurons were clearly distinguishable from the baseline noise (Fig. 2B), indicating that they were not derived from the events that were undetectable and masked by baseline noise before ischemia. These results suggest that postsynaptic mechanism is not involved in the ischemia-induced potentiation of EPSCs in spiny neurons.

To further rule out the possible contribution of postsynaptic mechanism to the potentiation of eEPSCs after ischemia, postsynaptic responses were examined by focal application of exogenous glutamate in the presence of 50 μM APV. Application of glutamate induced an inward current at a holding potential of –70 mV. The AMPA receptors rapidly desensitize (Trussell et al. 1988), preventing us from accurately measuring the peak amplitude in slices. Therefore we measured the steady-state currents in control and postischemic neurons. To eliminate the impact of cell size, currents were normalized by cell capacitance and expressed as current densities. Glutamate (100 μM) induced the inward currents of 19.4 ± 5.0 and 23.7 ± 4.0 pA/pF in control (n = 7) and postischemic neurons (n = 7), respectively (P = 0.51. Fig. 3, C and D). Likewise, no significant differences in current densities were found between control (128.6 ± 16.6 pA/pF, n = 7) and ischemia groups (151.4 ± 18.2 pA/pF, n = 7) in response to 500 μM glutamate (P = 0.36. Fig. 3, C and D).

Postischemic increase in mEPSCs amplitude do not result from the increased mEPSCs frequency

It is worthwhile to point out that despite the lack of involvement of postsynaptic mechanism, the amplitude of mean mEPSCs was increased after ischemia. This is an unconventional phenomenon since mEPSCs amplitude reflects the postsynaptic responses to single vesicle release. Interestingly, several previous studies have also shown the similar results and suggested that the increase in mEPSCs amplitude may arise from a random superimposition of the more frequent mEPSCs (Sharma and Vijayaraghavan 2003; Shigetomi and Kato 2004).
If the increase in mEPSCs amplitude was caused by random superimposition of mEPSCs, more frequent mEPSCs should create more large-amplitude events, which in turn increase the mean amplitude of mEPSCs. Consequently, we should observe a positive correlation between the increase in frequency and mean amplitude of mEPSCs (Shigetomi and Kato 2004). However, there was no correlation between the relative frequency and mean amplitude in posts ischemic spiny neurons (Fig. 4A). Examination of rise time of the mEPSCs after ischemia and their relation to amplitude also suggests that the increase in mEPSCs amplitude did not arise from random superimposition of mEPSCs. If the increase in mEPSCs amplitude was caused by the spatial summation of mEPSCs at distinct sites, the rise time of large-amplitude events would be longer than that of small-amplitude events because of the weak synchronization of release (Sharma and Vijayaraghavan 2003; Shigetomi and Kato 2004), and there should be a positive correlation between the rise time and amplitude of mEPSCs after ischemia as well. However, as shown in Fig. 4B, there was no such a correlation. These results suggest that the increase in mEPSCs amplitude do not arise from random superimposition of events.

**Facilitated presynaptic release is dependent on the extracellular Ca**

Calcium plays important roles in neurotransmitter release. To investigate the calcium-dependence of enhanced presynaptic release after ischemia, we compared the frequency of mEPSCs in ischemic neurons with and without calcium. After 5-min baseline recording of mEPSCs in posts ischemic neurons, we switched the standard ACSF to Ca**2+**-free ACSF by removing the calcium from the external medium and adding EGTA (3 mM) in the presence of TTX (1 μM). Treatment of Ca**2+**-free ACSF for 5–10 min reversibly decreased the mEPSCs frequency by 44.5 ± 5.9% (P < 0.05, n = 8, Fig. 5). Interestingly, Ca**2+**-medium only slightly reduced the mEPSCs frequency in control neurons (86.5 ± 8.1% of pre-Ca**2+** removal, P = 0.15, n = 6). To reveal the involvement of voltage-gated calcium channels (VGCCs) in this process, we bath applied CdCl2 (100 μM) in posts ischemic slices to block the VGCCs and did not find any significant changes in the frequency of mEPSCs (96.6 ± 3.6% of pre-Cd**2+** application, P = 0.48, n = 5, Fig. 5C). These results suggest that the increase of glutamate release after ischemia depends on extracellular Ca**2+**, and VGCCs do not participate in this event.

**Activation of P2X receptor is responsible for the increased glutamate release after ischemia**

The preceding data indicate that the excitatory synaptic transmission onto spiny neurons is presynaptically potentiated after ischemia, resulting from Ca**2+** influx into presynaptic terminals through the channels other than VGCCs. Previous studies have shown that extracellular ATP concentration is increased after brain ischemia through multiple mechanisms (Braun et al. 1998; Dutta et al. 2004), and the increased ATP can facilitate glutamate release in CNS through activation of presynaptic P2X receptors (Khabib et al. 2003; Shigetomi and Kato 2004). To examine whether activation of P2X receptors is responsible for the increased glutamate release after ischemia, we applied P2X receptor antagonist PPADS (50 μM) after 5-min stable recording of mEPSCs in either control or ischemic spiny neurons. Bath application of PPADS did not change the holding current and input resistance, suggesting its deficiency in postsynaptic function. As shown in Fig. 6, PPADS reliably reduced the mEPSCs frequency of ischemic neurons in a reversible manner (63.8 ± 4.7% of pre-PPADS application, P < 0.05, n = 9), but it had no detectable effects in control neurons (99.6 ± 3.2% of pre-PPADS application, P = 0.73, n = 7, Fig. 6B). Furthermore, we tested the effects of PPADS (50 μM) in Ca**2+**-free external solution. Removal of extracellular Ca**2+** consistently reduced the mEPSCs frequency in

![Fig. 4](http://jn.physiology.org/)

**Fig. 4.** The correlation between the amplitude and frequency of mEPSCs after ischemia. A: no correlation was found between the amplitude and frequency of mEPSCs after ischemia (r = 0.04, P > 0.05), indicating that the increase in mEPSCs amplitude do not result from random superimposition of mEPSCs. B: relationship between rise time and amplitude of mEPSCs. The amplitude of mEPSCs (0–60 pA) from 5 posts ischemic neurons (total of 9,803 events) was binned in 1-pA bins. The events >60 pA were binned together. Mean rise time and amplitude were calculated for each bin and plotted. No correlation was detected between the rise time and amplitude of mEPSCs (r = 0.12, P > 0.05). Inset: the superimposed traces of mEPSCs in posts ischemic neurons. - - - -, the beginning and the peak of the EPSCs.

![Fig. 5](http://jn.physiology.org/)

**Fig. 5.** The effects of extracellular Ca**2+** on the frequency of mEPSCs in posts ischemic neurons. A, 1 and 2: representative traces from control and posts ischemic neurons before and after Ca**2+** removal. B: cumulative probability of the interevent interval before and during Ca**2+** removal for the same neuron as recorded in A2. Ca**2+** removal decreased the frequency of mEPSCs in posts ischemic neurons. C: group data showing that the increase of mEPSCs frequency in posts ischemic neurons is dependent on extracellular Ca**2+**. Application of Cd**2+** does not alter the frequency of mEPSCs after ischemia, suggesting that the voltage-gated calcium channel is not involved in this process. The frequency after Ca**2+** application and Ca**2+** removal was normalized to that of before Cd**2+** application and Ca**2+** removal, respectively. *, P < 0.05.
ischemic neurons. However, PPADS failed to further change the mEPSCs frequency in postischemic spiny neurons in the absence of external Ca\(^{2+}\) (96.5 \pm 3.8% of pre-PPADS application, \(P = 0.26, n = 7\)). These results suggest that activation of P2X type of ATP receptor resulted in enhanced glutamate release in ischemic neurons through a Ca\(^{2+}\)-dependent manner.

**DISCUSSION**

The main finding of the present study is that the excitatory synaptic transmission of striatal spiny neurons is potentiated 9 h after transient forebrain ischemia. Such potentiation results from the facilitated glutamate release in ischemic neurons through a Ca\(^{2+}\)-dependent manner.

Accumulating evidence has shown that excitatory synaptic transmission in ischemia-vulnerable and ischemia-resistant neurons are differentially disturbed by ischemia. In the hippocampus, in vivo intracellular recording showed that evoked excitatory postsynaptic potentials (eEPSPs) in CA1 neurons are potentiated after ischemia (Gao and Xu 1996; Gao et al. 1998). In contrast, the slope and amplitude of EPSPs are reduced or unchanged in CA3 neurons and dental granule cells subjected to the same insult (Gao et al. 1998a). In the striatal cholinergic interneurons, which are resistant to ischemia, excitatory synaptic transmission is depressed after ischemia because of activation of presynaptic adenosine A1 receptors and inhibited glutamate release (Pang et al. 2002). Studies using in vitro ischemia model demonstrated that ischemia is capable of inducing long-term synaptic potentiation (LTP) in spiny neurons but not in large aspiny interneurons (Calabresi et al. 2002). It is hypothesized that ischemia-induced LTP may, at least in part, account for the cell type-specific vulnerability after ischemia. The animal model of ischemia in the present study results in \(>80\%\) cell death in the striatum 24 h after reperfusion (Pulsinelli et al. 1982; Ren et al. 1997). Therefore the potentiation of EPSCs at 9 h after ischemia as shown in the present study is probably associated with the cause rather than the result of ischemic cell death.

Both pre- and postsynaptic mechanisms affect the synaptic strength. Based on the studies in the hippocampus, the potentiation of excitatory neurotransmission results from the enhancement in postsynaptic responsiveness (Tsubokawa et al. 1995). For instance, exogenous application of glutamate receptor agonist evokes larger current and higher intracellular Ca\(^{2+}\) in postsynaptic brain slices than those in normal slices, suggesting that postsynaptic mechanism is involved (Mitani et al. 1998). More detailed studies have shown the upregulation of postsynaptic glutamate receptor function by protein kinase phosphorylation in CA1 pyramidal neurons after transient global ischemia. For example, ischemia induces tyrosine phosphorylation of NR2B subunit by Src-family kinases, and serine phosphorylation of NMDA NR2A subunit by cyclin-dependent kinase 5, leading to the enhancement of NMDAR currents (Takagi et al. 2003; Wang et al. 2003). Blockade of NMDAR phosphorylation, in turn, protects the neurons from ischemic injury (Wang et al. 2003). Increased phosphorylation of GluR1 subunits of AMPA receptors by calcium/calmodulin-dependent kinase II were also observed in the hippocampus (Takagi et al. 2003). In addition, ischemia is also capable of inducing the changes in subunits composition of AMPA receptor, particularly, downregulation of GluR2 subunits that limits Ca\(^{2+}\) entry (Gorter et al. 1997; Pellegrini-Giampietro et al. 1992). Taken together, postsynaptic mechanism has been widely accepted as a major factor enhancing excitatory synaptic transmission in ischemic neuronal injury.

On the other hand, studies have shown that abnormal synaptic transmissions after ischemia are ascribed to the presynaptic intervention. In vitro studies have demonstrated that excitatory synaptic transmissions are presynaptically depressed during ischemia/hypoxia, conferred by unchanged response to exogenous application of glutamate (Calabresi et al. 1997b; Hershkowitz et al. 1993) and increase in paired-pulse facilitation (Calabresi et al. 1997a; Tanaka 2001). These effects can be antagonized by pretreatment with adenosine A1 receptors antagonist 8-CPT, suggesting that presynaptic A1 receptors are activated during ischemia and subsequently inhibit synaptic glutamate release (Calabresi et al. 1997a; Fowler et al. 2003; Tanaka 2001). Coincided with these observations, presynaptic depression of excitatory neurotransmission through adenosine A1 receptors has also been reported in large cholinergic interneurons in the striatum after ischemia in vivo (Pang et al. 2002). Presynaptic mechanism has also been implicated in the potentiation of excitatory synaptic transmission in CA1 pyramidal neurons \(\leq 48\) h after reperfusion (Gao et al. 1998b). To further explore the role of presynaptic mechanism in ischemic injury, Abdel-Hamid and Tymianski (1997) enhanced neuronal Ca\(^{2+}\) buffering with BAPTA-AM to protect neurons against oxygen glucose deprivation. The protection by Ca\(^{2+}\) buffering appears to be presynaptic because BAPTA-AM is ineffective when endogenous transmitter release is bypassed by directly applying NMDA to the cultures (Abdel-Hamid and Tymianski 1997). Similarly, Monyer and co-workers (1992) found that tetanus toxin, presumably acting as a presynaptic blocker, attenuated neuronal injury after combined oxygen and glucose deprivation. In consistent with the preceding observations, the present provides direct evidence indicating that facilitated presynaptic release strengthens the excitatory neurotransmission in spiny neurons after reperfusion. These results offer new insights into the mechanisms of enhancement of excitatory neurotransmission in ischemia-vulnerable neurons after ischemia.
We have shown that blockade of P2X receptors almost completely reverses the increase of mEPSC frequency induced by ischemia, suggesting that the enhancement of P2X receptor function is responsible to the increased glutamate release from presynaptic terminals after ischemia. In the CNS, ATP is not only an energy source of the cell but also a potent extracellular signaling molecule eliciting a wide variety of physiological effects through activating ionotropic P2X and metabotropic P2Y receptors (Volonte et al. 2003). In physiological condition, ATP is present at a low concentration in extracellular space (Dubay and el-Moatassim 1993). However, mounting evidence indicates that extracellular ATP concentration is increased in the pathophysiological conditions, such as ischemia, tissue injury and hypoxia (Braun et al. 1998; Lutz and Kabler 1997; Volonte et al. 2003). ATP may be released from swollen cells through ATP-conductive anion channels in response to ischemia or hypoxia (Dutta et al. 2004; Nieber et al. 1999), or co-released with glutamate from presynaptic terminals (Mori et al. 2001). Extracellular ATP is rapidly hydrolyzed to ADP and adenosine by a surface-located enzyme chain. Loss of ecto-ATPase activity in the pathological conditions provides additional evidence for the accumulation of extracellular ATP (Robson et al. 1997; Zimmermann 1994). P2X receptors are widely expressed in the CNS as indicated by immunocytochemistry and in situ hybridization (Deuchars et al. 2001; Kanjan et al. 1999). Electron microscopic studies have revealed that the upregulation of P2X receptors after ischemia is located on the presynaptic appositions of synapses (Franke et al. 2004). Activation of presynaptic P2X receptors depolarizes presynaptic terminals and activates voltage-gated calcium channels to enhance neurotransmitter release (Khakh and Henderson 1998). Calcium could enter the terminal through presynaptic P2X receptors directly and facilitate transmitter release (Shigetomi and Kato 2004). Our data suggest that the enhancement of presynaptic release of glutamate in the striatum after ischemia is probably due to the calcium influx directly through the P2X receptor rather than the voltage-gated calcium channels.

The present study indicates that activation of ATP P2X receptor potentiates excitatory synaptic transmission in medium spiny neurons while activation of adenosine A1 receptor depresses excitatory synaptic transmission in large aspiny neurons after ischemia (Pang et al. 2002). Given the facts that hydrolyzing extracellular ATP can form adenosine (Zimmermann 1994), we speculate that such differential changes in excitatory input to medium spiny and large aspiny neurons presumably result from the distinct activities of ectoATPase after ischemia. It has been well established that high level of ATP is neurotoxic (Amadio et al. 2002; Neary et al. 1996; Ryu et al. 2002). Application of ATP receptor antagonists has the ability to prevent excitotoxicity (Cavaliere et al. 2001; Raslevic 2002). The detrimental mechanisms of ATP are not fully understood. Our results demonstrated a novel putative mechanism mediating neurotoxic effects of ATP.

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GRANTS

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