Dopamine D1-Like Receptors Depress Excitatory Synaptic Transmissions in Striatal Neurons After Transient Forebrain Ischemia

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Background and Purpose—Spiny neurons in the neostriatum are highly vulnerable to ischemia. Despite an enormous body of research suggesting that dopamine is involved in ischemia-induced neuronal loss in the striatum, it remains unclear how dopamine interacts with the glutamatergic excitotoxicity that is widely accepted as a major cause of ischemic cell death. Our study was designed to investigate the effects of dopamine D1 receptor (D1R) activation on excitatory neurotransmission in posts ischemic striatal neurons.

Methods—We used the 4-vessel occlusion ischemia model and brain slice preparations. Whole-cell voltage-clamp recording was performed on striatal neurons to measure excitatory postsynaptic currents (EPSCs). Systemic administration of a D1R agonist after ischemia and hematoxylin/eosin staining were performed to evaluate the effects of D1R activation on ischemia-induced neuronal degeneration in the striatum.

Results—D1R activation depressed EPSCs in posts ischemic striatal neurons. The depression was attributable to inhibition of presynaptic release. An activator of cAMP-dependent protein kinase A (PKA) mimicked the depressive effects of D1R activation. Bath application of a PKA inhibitor blocked the depression of EPSCs, whereas intracellular postsynaptic application of the PKA inhibitor had no effect. The D1R agonist failed to reduce EPSC amplitude in the presence of an adenosine A1 receptor antagonist. Systemic administration of a D1R agonist after ischemia significantly attenuated ischemia-induced cell death in the striatum.

Conclusions—These results indicate that D1R activation presynaptically depresses excitatory synaptic transmission in striatal neurons after ischemia through activation of PKA and adenosine A1 receptors and thus demonstrate a novel mechanism of D1R-mediated protection against ischemia. (Stroke. 2008;39:2370-2376.)

Key Words: dopamine ■ ischemia ■ striatum ■ excitatory postsynaptic currents ■ adenosine
postischemic neuronal degeneration. Dopamine is able to modulate excitatory synaptic transmission in striatal neurons under control conditions and thus participates in motor learning and sensorimotor integration.11 However, it is largely unknown how dopamine modulates excitatory neurotransmission in striatal neurons after ischemia or how it influences glutamatergic excitotoxicity. The present study was designed to elucidate these questions.

Materials and Methods

Transient Forebrain Ischemia

Male Wistar rats (100 to 200 g; Charles River Laboratories, Wilmington, Mass) were subjected to transient forebrain ischemia via the 4-vessel occlusion method with modifications as described previously.13 The animals were anesthetized with a mixture of 1% to 2% halothane in 33% O2/66% N2. Bilateral vertebral arteries were electrocauterized. After craniotomy, a temperature probe was placed underneath the skull to maintain brain temperature at 37°C with a heating lamp via a feedback system. Silicone tubing was placed loosely around each common carotid artery to make bilateral common carotid arteries ready to be occluded. On the contralateral cranium, another hole was made to record the ischemic depolarization of striatal field potentials. The tip of the extracellular recording electrode was placed into the striatum with use of a stereotaxic instrument. The field potential was adjusted to zero before ischemia was instituted. During the occlusion procedure, bilateral common carotid arteries were clamped. Owing to the membrane depolarization induced by energy deprivation, the extracellular electrode detected the sustained field potential change, termed the ischemic depolarization (shift from 0 to −20 mV), within 2 to 3 minutes after occlusion. Occlusion was released when ischemic depolarization lasted for 20 minutes. The animals’ body temperature was maintained at 37°C with a temperature control system (VitaView 4.1, Mini Mitter Co, Bend, Ore). Experimental protocols were approved by the institutional animal care and use committees at the Indiana University School of Medicine.

Brain Slice Preparation and Whole-Cell Voltage-Clamp Recording

Brain slices were prepared from control and ischemic animals at different stages (3 and 9 hours) after reperfusion as described previously.13 For whole-cell recording, patch electrodes were filled with an intracellular solution containing (in mmol/L): 43 CsCl, 92 CsMeSO4, 5 TEA, 2 EGTA, 1 MgCl2, 10 HEPES, and 4 ATP (Sigma, St. Louis, Mo). Neurobiotin (2%; Vector Laboratories, Burlingame, Calif) was included in the solution to allow verification by passing depolarizing pulses after recording. The slice was then tonically immediately after ischemia.

Histologic Examination

Histologic experiments were conducted to evaluate ischemic neuronal damage with or without D1R agonists. Twenty-four hours after ischemia, the rats were perfused transcardially with a mixture of 3.7% formaldehyde, 10% glacial acetic acid, and 80% methanol. Brain blocks containing neostriatum were dehydrated and embedded in paraffin. Serial coronal sections were cut into 10-μm-thick slices selected from 3 interaural planes in the neostriatum, corresponding to 10.6, 8.7, and 8.08 mm. Four consecutive sections were taken from each plane. The sections were subsequently stained with hematoxylin/eosin. Two areas in the dorsal neostriatum (lateral and medial) were randomly selected in each hemisphere of a section by light microscopy (×200) and digitized with an image analysis program (NIH Image 1.57). Dead cells show condensed nuclei and plasma membrane lysis, which can be readily separated from cells with normal morphology under a light microscope. Ischemic neuronal damage was evaluated by counting surviving neurons in each area. The experimenter conducting the counting was blinded to the experimental groups. For each rat, counting of surviving neurons was carried out in 48 microscopic fields (400×300 μm) in the dorsal part of the striatum in both hemispheres.

Drug Application

(−)-Bicuculline methiodide, (−)-2-amino-5-phosphonopentanoic acid, and 6-cyano-7-nitroquinoxaline-2,3-dione were purchased from Sigma. Drugs were applied via bath superfusion or recording pipette according to the experimental design. (−)-Bicuculline methiodide was used to block γ-aminobutyric acidA receptors at a concentration of 30 μmol/L throughout the experiment. Sodium metabisulfite (Na2S2O5, 50 μmol/L) was used to protect dopamine and dopaminergic agonists and antagonists from oxidation. For the histologic experiment, SKF38393 (5 mg/kg) was injected intrapectorally immediately after ischemia.

Results

Activation of D1Rs Suppresses EPSCs in Spiny Neurons After Ischemia

Whole-cell voltage-clamp recordings were performed on neurons in the dorsolateral striatum with medium-size somata in brain slices. Intrastriatal stimulation was delivered to evoke postsynaptic responses at a holding potential of −70 mV. After a stable whole-cell configuration was reached, we first determined the threshold of stimulus intensity. The threshold was defined as the stimulating current evoking the smallest detectable response from spiny neurons. Then a stimulation intensity of 2 times threshold was delivered to evoke EPSCs in the presence of the γ-aminobutyric acidA receptor antagonist (−)-bicuculline methiodide (30 μmol/L). As shown in Figures 1A and 1B, the recorded neuron possessed a medium-size soma (≈15 μm) with a large number of spines on its dendrites, indicating that the recorded neurons were medium spiny neurons.

To examine the effects of D1R activation on EPSCs in postischemic spiny neurons, we applied the specific D1R agonist SKF38393 (10 μmol/L) in the bath medium after a 5-minute baseline recording. SKF38393 reversibly suppressed EPSC amplitude at both 3 (76.1±4.6% of pre-SKF38393 application, \(P<0.05\), \(n=12\)) and 9 (75.8±4.3% of pre-SKF38393 application, \(P<0.05\), \(n=9\); Figure 1) hours after ischemia. In contrast, EPSCs were potentiated by SKF38393 in control neurons (132.2±15.1% of pre-SKF38393 application, \(P<0.05\), \(n=6\), which was consistent with a previous report.14 The rising slope and decay time constant were not significantly altered by SKF38393 (data not shown). Also, SKF38393 did not change the holding current during recording.
We also included the specific D1R antagonist SCH23390 (1 μmol/L) in the extracellular medium and found that subsequent application of SKF38393 failed to depress EPSC amplitude (102.3±2.9% of pre-SKF38393 application, P>0.05, n=5). Alternatively, we applied dopamine (50 μmol/L) after preincubation with sulpiride (10 μmol/L), a D2R antagonist. Dopamine decreased the EPSC amplitude by 26.7±4.6% (P<0.05, n=5; Figure 1D) in the presence of sulpiride. These results suggest that SKF38393 suppresses excitatory synaptic transmission in spiny neurons after ischemia.

A Presynaptic Mechanism Is Responsible for the Depressive Effects of D1Rs
We recorded mEPSCs without adding tetrodotoxin, which has no effect on spontaneous EPSCs in postischemic spiny neurons.10 We found that mEPSC frequency decreased by 23.3±5.9% (P<0.05, n=10; Figures 2A and 2B) in the presence of SKF38393 while mEPSC amplitude was unchanged under the same conditions (99.5±1.5% of pre-SKF38393 application, P>0.05, n=10; Figures 2A and 2B). We also examined the effects of D1R activation on paired-pulse ratio. Figures 2C and 2D show that the magnitude of the paired-pulse ratio was increased in 6 of 6 cells tested, with an average 29.1±9.1% increase (P<0.05). These results suggest that a presynaptic, but not a postsynaptic, mechanism is responsible for the depressive effects of D1R activation on excitatory inputs of postischemic spiny neurons.

The Depressive Effect of D1R Activation Is Dependent on PKA
D1R activation triggers a G protein–mediated signal cascade that stimulates adenylyl cyclase and subsequently, an activator of cAMP-dependent protein kinase A (PKA). We therefore tested whether this pathway was involved in the SKF38393-induced EPSC depression. 8-Br-cAMP (100 μmol/L), a membrane-permeable PKA activator, reversibly reduced the evoked EPSC to 81.9±3.8% of baseline (P<0.05, n=7; Figures 3A and 3C), an effect comparable to that of SKF38393. Preincubating the slices with 10 μmol/L Rp-cAMP, a membrane-permeable PKA inhibitor, blocked the depressive effects of SKF38393 on EPSC amplitude (98.2±3.4% of pre-SKF38393 application, P>0.05, n=6; Figures 3B and 3C). H89 (5 μmol/L), a structurally distinct
PKA inhibitor, had a comparable lack of effect in blocking the SKF38393-induced EPSC depression (96.8±2.7% of pre-SKF38393 application, \(P<0.05, n=5\); Figure 3C).

Electron microscopy study has shown that presynaptic D1Rs are very rare under normal conditions, although the expression pattern of D1Rs after ischemia is unclear. Our data, however, suggest that D1R activation presynaptically depresses excitatory neurotransmission in spiny neurons after ischemia. To further examine the locus of D1R effects, we intracellularly applied PKI 6-22 (20 \(\mu\)mol/L), a PKA inhibitor, to postsynaptic spiny neurons through a recording pipette. Unlike bath-applied PKA inhibitors, SKF38393 (10 \(\mu\)mol/L) still depressed the EPSCs to 80.7±6.2% of baseline (\(P<0.05, n=6\); Figure 4) in the presence of intracellular PKI 6-22, an extent comparable to that of SKF38393 alone. To confirm that PKI 6-22 intracellularly applied through a recording pipette can inhibit PKA in postsynaptic neurons, we performed another experiment to serve as a control. Activation of D1R is known to potentiate EPSCs by PKA under control condition. We tested the effect of SKF38393 on EPSC amplitude in control spiny neurons with an internal solution containing PKI 6-22 and found that SKF38393 failed to potentiate EPSCs (95.8±3.1% of pre-SKF38393 application, \(P<0.05, n=4\)), suggesting that intrapipette PKI 6-22 was functional.

If D1R activation depresses EPSCs after ischemia without the involvement of PKA in postsynaptic neurons, then pre-activation of PKA in postsynaptic neurons should not occlude the SKF38393 effect on EPSC depression. We tested our prediction by intracellular application of 8-Br-cAMP (100 \(\mu\)mol/L) and found that SKF38393 still depressed EPSCs in the presence of intracellular 8-Br-cAMP (68.4±5.5% of pre-SKF38393 application, \(P<0.05, n=5\); Figures 4B and 4C).

Figure 2. A presynaptic mechanism was responsible for the D1R-induced depression of EPSCs in posts ischemic spiny neurons. A, Sample traces of mEPSCs recorded from posts ischemic spiny neurons before, during, and after SKF38393 application. B, Box plot showing the group data of SKF38393-induced changes in mEPSC frequency and amplitude. C, Sample traces of paired-pulse stimulation before and during SKF38393 application. D, Paired-pulse ratio changes in individual neuron before and during SKF38393 application.

Figure 3. PKA mediated D1R depression of EPSCs in posts ischemic spiny neurons. A, 8-Br-cAMP reduced the amplitude of EPSCs in a sample neuron. B, SKF38393 had no effect on EPSC amplitude with Rp-cAMP in the bath medium in a sample neuron. C, Group data showing the percentage change in EPSC amplitude by the drugs indicated in each bar.

Figure 4. Inactivation of PKA in postsynaptic neurons did not block the D1R depression of EPSCs in posts ischemic spiny neurons. A1, Intracellularly applied PKI 6-22 did not block the SKF38393-induced EPSC depression in a sample neuron. A2, Representative traces taken from the time indicated by numbers in A1. B, Intracellularly applied 8-Br-cAMP did not occlude the SKF38393-induced EPSC depression in a sample neuron. C, Group data showing the percentage change in EPSC amplitude by the drugs indicated in each bar.
Activation of A1Rs Is Required for D1R-Mediated Depression of EPSCs

Presynaptic depression could be either homosynaptic or heterosynaptic. Adenosine A1 receptors (A1Rs) are known to inhibit synaptic glutamate release and are reported to act downstream of D1R activation.17 To test whether this was true in our case, we preincubated brain slices with the A1R antagonist 1,3-dipropyl-8-cyclopentylxanthine (0.5 μmol/L) and found that both SKF38393 and 8-Br-cAMP failed to depress EPSCs in postischemic spiny neurons (96.1 ± 3.5% for SKF38393, P > 0.05, n = 6; Figures 5A and 5E; 100.8 ± 5.7% for 8-Br-cAMP, P > 0.05, n = 5; Figures 5B and 5E). Pretreatment with cyclopentyladenosine (0.1 μmol/L), an A1R agonist, also occluded SKF38393-induced EPSC depression (101.0 ± 1.9% of pre-SKF38393 application, P > 0.05, n = 5; Figures 5C and 5E), suggesting that A1R activation mediated the effects of D1Rs. We also tested the effects of AM251 (1 μmol/L), an endocannabinoid CB1 receptor antagonist, because CB1 receptors have been shown to induce presynaptic depression. As shown in Figure 5, AM251 did not block the effects of SKF38393 (80.1 ± 8.3% of pre-SKF38393 application, P < 0.05, n = 5; Figures 5D and 5E).

Systemic Administration of a D1R Agonist Attenuated Ischemic Neuronal Injury

To test whether depression of EPSCs can protect striatal neurons against ischemia, we intraperitoneally injected SKF38393 (5 mg/kg) immediately after ischemia, and histologic slides were prepared to evaluate ischemic damage. It is worthwhile to point out that previous studies regarding the effects of a D1R agonist on ischemic injury are controversial. A substantial source of the debate is the variation in brain temperature after ischemia,6,7 because it is well known that hypothermia can dramatically alleviate ischemic neuronal injury. To reduce the variation in brain temperature and make the results more consistent, we maintained the animals’ body temperature at 37°C after ischemia until they were euthanized. Survival of striatal neurons was assessed 24 hours after termination of ischemia. Normal striatal neurons are characterized by a round soma and clear intact nuclei by hematoxylin/eosin analyses (Figure 6B), whereas dying neurons appear shrunken with pyknotic nuclei (Figure 6A). In animals that received saline injection after ischemia, the number of surviving neurons in each image was 252 ± 51 (n = 6, see Methods), which was consistent with our previous study in the same ischemia model.4 The number of surviving neurons in
animals that received SKF38393 injection after ischemia showed a dramatic increase, to 381±30 (n=10, P<0.05; Figure 6C).

Discussion

Our results have demonstrated that D1R activation depresses excitatory neurotransmission in postischemic striatal neurons through activation of PKA and adenosine A1Rs, and such depression might contribute to D1R-induced neuroprotection (Figure 6D). Excitatory synaptic transmission has received much attention in ischemic cell death because of its important role in excitotoxicity. It is therefore reasonable to consider that facilitation of synaptic glutamate release would potentiate synaptic activity and neuronal injury after ischemia. This idea is supported by both in vivo and in vitro studies. BAPTA-AM protects cultured neurons against oxygen-glucose deprivation (OGD) by inhibiting glutamate release rather than buffering calcium in postsynaptic cells.18 Our previous studies also showed that synaptic glutamate release is facilitated in striatal spiny neurons after transient global ischemia,10 whereas it is inhibited in ischemia-resistant cholinergic interneurons.19 It is thought that the differential changes in synaptic glutamate release of these 2 types of neurons are associated with their distinct sensitivity to ischemia. In this regard, modulation of excitatory synaptic transmission may be a factor that influences ischemic outcome.

Changes in synaptic strength, eg, long-term potentiation, were observed in striatal spiny neurons after OGD and have been proposed as a putative mechanism of selective cell death in the striatum because it was not observed in other ischemia-resistant striatal neurons.20 Study of brain slices subjected to OGD have demonstrated that ischemic long-term potentiation in spiny neurons is amplified by endogenous activation of dopamine D1Rs.21 Excitatory postsynaptic potentials failed to increase after OGD in the presence of a D1R antagonist or in D1R−/− mice,21 demonstrating an endogenous role for D1Rs in ischemia-induced synaptic plasticity. However, our studies, based on an in vivo ischemia model, found that ischemia-induced enhancement of excitatory neurotransmission10 can be suppressed by exogenous activation of D1Rs. The result is not surprising, because our study revealed an exogenous effect of D1Rs on posts ischemic synaptic activity. Furthermore, a previous study from this laboratory revealed a similar discrepancy. For example, excitatory neurotransmission in cholinergic interneuron is presynaptically depressed after in vivo ischemia,19 but it does not show significant changes after in vitro OGD.20 We speculate that the difference may result from the distinct experimental conditions, eg, the ischemia model and different stages after insult.

It is well known that D1R activation potentiates EPSCs in normal striatal neurons through PKA phosphorylating postsynaptic N-methyl-D-aspartate (NMDA) receptors.22 However, we demonstrated an alternative pathway of D1R signaling in postischemic striatal neurons, ie, D1R-PKA-A1R-EPSC (Figure 6D). It is interesting to explore why D1Rs activate a different pathway after ischemia. It has been known that a similar mechanism underlies D1R depression of EPSCs in normal nucleus accumbens neurons, a process that is N-methyl-D-aspartate (NMDA) receptor dependent.22 Indeed, calcium influx through N-methyl-D-aspartate receptors mediates multiple dopamine effects in the striatum.23 On the basis of these previous studies, we speculate that other posts ischemic changes in striatal neurons, eg, elevated calcium concentrations, promote D1R-induced adenosine formation and thus depress EPSCs. It should also be pointed out that our recordings were performed in brain slices taken from rats subjected to in vivo ischemia, and there was no ischemic insult during recording. Thus, any observed difference in D1R effects between control and ischemic conditions can be attributed to the actions of ischemia that occurred in vivo.

Dopamine exacerbates ischemic cell death, as indicated by the evidence that depletion of dopaminergic neurons in the
substantia nigra alleviates neuronal degeneration after ischemia. It has been shown that dopamine-mediated toxicity might also involve glutamate. This was deduced initially from studies in which dopamine denervation attenuated glutamate-induced striatal lesions. Elevated levels of dopamine inhibit glutamate uptake, perhaps via the action of dopamine-derived reactive oxygen species. This could cause an elevation of synaptic glutamate levels, which leads to overactivation of glutamate receptors and cell death. Aside from the ability of dopamine to produce reactive oxygen species, dopamine can also be involved in ischemic injury through receptor-dependent mechanisms. Dopamine can protect cultured striatal neurons against glutamate-induced cell death through D1R-mediated increases in intracellular cAMP. Studies with in vivo ischemia model have shown comparable results.

In conclusion, our study not only confirmed previous reports that dopamine is involved in glutamate excitotoxicity but also, more important, demonstrated that a subtype of dopamine receptor, D1Rs, can inhibit synaptic glutamate release after ischemia and might underlie the neuroprotective mechanisms of D1R activation. Our study described a correlation between EPSC depression and alleviated cell death by D1R activation. Obviously, correlation does not mean causation. To test for functional causality, we need to prevent the D1R-induced EPSC depression in vivo and then examine ischemic injury. Unfortunately, such experiments are not feasible owing to technical restrictions. It is extremely difficult, if not impossible, to selectively manipulate synaptic receptors without interfering with extrasynaptic receptors in intact brains. However, as advances in genetic manipulation and molecular biology techniques continue, we believe that our concern could be resolved in the near future.

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Disclosures
None.

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