Endogenous Dopamine Amplifies Ischemic Long-Term Potentiation via D1 Receptors

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Background and Purpose—Several observations indicate that, during energy deprivation, endogenous dopamine may become neurotoxic. Accordingly, the nucleus striatum is a preferential site of silent infarcts in humans, and experimental ischemia caused by homolateral carotid occlusion selectively damages this dopamine-enriched brain area. In an attempt to clarify how dopamine takes part in ischemia-induced neuronal damage, we performed in vitro electrophysiological recordings from neurons of the nucleus striatum.

Methods—Intracellular recordings with sharp microelectrodes were performed from corticostriatal slices. Slices were obtained from both rats and wild-type and dopamine D1 receptor–lacking mice. In some experiments, the striatum was unilaterally denervated by injecting the dopamine-specific neurotoxin 6-hydroxydopamine in the homolateral substantia nigra. Dopamine agonists and antagonists, as well as drugs targeting the intracellular cascade coupled to dopamine receptor stimulation, were applied at known concentrations.

Results—Manipulation of the dopamine system failed to affect the membrane depolarization of striatal neurons exposed to combined oxygen and glucose deprivation of short duration, but it reduced the amplitude of postischemic long-term potentiation (LTP) expressed at corticostriatal synapses. In particular, pharmacological blockade or genetic inactivation of D1/cAMP/protein kinase A pathway prevented the long-term increase of the excitatory postsynaptic potential (EPSP) amplitude caused by a transient ischemic episode, while it failed to prevent the increase of the EPSP half-decay coupled to ischemic LTP.

Conclusions—The present data suggest that endogenous dopamine, via D1 receptors, selectively facilitates the expression of ischemic LTP on the AMPA-mediated component of the EPSPs, while it does not alter the expression of this form of synaptic plasticity on the N-methyl-D-aspartate–mediated component of corticostriatal synaptic potentials. Understanding the cellular and molecular mechanisms of ischemia-triggered excitotoxicity offers hope for the development of specific treatments able to interfere with this pathological process. (Stroke. 2002;33:2978-2984.)

Key Words: apoptosis ■ electrophysiology ■ energy metabolism ■ excitotoxicity ■ neuronal plasticity ■ synapses

Among the various neuronal subtypes of the brain, striatal spiny neurons are particularly vulnerable to ischemia. Abnormal release of excitatory amino acids is proposed to be a critical factor for neuronal death during ischemia, and, according to this hypothesis, in the ischemic striatum glutamate and aspartate concentrations increase significantly. Moreover, glutamate receptor antagonists prevent ischemic damage in this brain area.

Ischemia also causes a large increase in dopamine levels in the striatum, and considerable evidence supports the idea that monoamines or their metabolic by-products may become neurotoxic during metabolic impairment, either directly or from interplay with the glutamatergic system. In an in vitro model of striatal ischemia, for example, reduction of dopamine release has been found to be associated with a better histological outcome, and unilateral 6-hydroxydopamine (6-OHDA) lesion of the substantia nigra has been reported to reduce the volume of striatal necrosis induced by middle cerebral artery occlusion. Thus, the dopaminergic nigrostriatal pathway could be highly involved in the vulnerability of the striatum to ischemia, and glutamate-dopamine interactions may play a key role in striatal ischemic insult.

We have recently shown that in vitro ischemia (oxygen and glucose deprivation) induces a long-term potentiation (i-LTP) of excitatory glutamatergic transmission at corticostriatal synapses. This process depends on the stimulation of both N-methyl-D-aspartate (NMDA) and metabotropic glutamate receptors and includes the activation of mitogen-activated protein kinase ERK via protein kinase C.

In the present study, to investigate the molecular and cellular events associated with dopamine release during energy deprivation, we performed intracellular recordings.
from spiny neurons of the striatum. We demonstrate that an intricate sequence of intracellular signaling events is acti-
vated by the stimulation of D1 dopamine receptors in striatal
neurons during a brief in vitro episode of ischemia, which
leads to the amplification of i-LTP.

Materials and Methods

Adult Wistar rats and wild-type and D1−/− mice were used.18 All
animal experiments were conducted in accordance with the 86/609/
EEC directive, and all efforts were made to minimize animal
suffering and to reduce the number of animals used. The preparation
and maintenance of coronal slices have been described previous-
ly.4,17,19,20 Briefly, corticostriatal coronal slices (200 to 300 μm)
were prepared from tissue blocks of the brain with the use of a
vibratome. Slices were incubated in continuously oxygenated saline
for 1 to 3 hours before they were placed in the recording chamber of
the electrophysiological setup. Only a single slice was transferred to
a recording chamber and submerged in a continuously flowing
Krebs’ solution (35°C, 2 to 3 mL/min) gassed with 95% O2/5% CO2.
To study ischemia in striatal neurons, slices were deprived of glucose
by totally removing glucose from the perfusate and by adding sucro-
sucrose to balance the osmolarity. This solution was gassed with a
mixture of 95% N2/5% CO2 instead of the normal gas mixture. In
some experiments the osmolarity was balanced by increasing the
NaCl concentration.4,20–22 Since experiments performed by using
these different procedures to replace glucose gave similar results,
all the data were pooled. Ischemic solutions entered the recording
chamber no later than 20 seconds after a 3-way tap was turned.
Complete replacement of the medium in the chamber was accom-
plished in approximately 90 seconds, as determined by the speed of
diffusion of a colored solution. The composition of the control
solution was as follows (in mmol/L): 126 NaCl, 2.5 KCl, 1.2
NaH2PO4, 2.4 CaCl2, 11 glucose, 25 NaHCO3. External magnesium
ions were omitted to maximize the contribution of NMDA receptors
in the induction of i-LTP.17 In all the experiments picrotoxin (200
μmol/L) was added to the external medium to avoid the contamina-
tion of the excitatory postsynaptic potentials (EPSPs) by a GABA-A-
mediated component.

Intracellular recording electrodes were filled with 2 mol/L KCl (30
to 60 MΩ). Signals were recorded with the use of an Axoclamp 2A
amplifier, displayed on a separate oscilloscope, and stored on a
digital system. For synaptic stimulation, bipolar electrodes were
plugged into an oscilloscope, displayed on a separate oscilloscope, and stored on a
digital system (Nicolet System 400,
Benchtop Waveform Acquisition System).

Drugs were applied by dissolving them to the desired final
concentration in saline and by switching the perfusion from control
saline to drug-containing saline. t-2-Amino-5-phosphonovalerate
(APV) was from Sigma, and 6-cyano-7-nitroquinoxaline-2,3-dione
(CNQX), quinpirole, and SKF 38393 were from RBI. Picrotoxin,
SCH 23390, t-sulpiride, and forskolin were from Tocris Cookson.
8-hydroxy-
3-(2H)-diazepine (8-OH-DPAT) was from Calbiochem.

Drugs were applied in the recording chamber for at least 10
minutes before the ischemic period and maintained throughout the
experiment. None of the tested drugs affected membrane potential
per se (at least n = 5 and P > 0.05 for each drug; data not shown) or
input resistance (at least n = 5 and P > 0.05 for each drug; data not
shown).

Results

Characterization of i-LTP

All the data were collected from 150 striatal spiny neurons
identified according to their electrophysiological properties.20
In control medium, a brief period (3 minutes) of oxygen and
glucose deprivation caused a membrane depolarization asso-
ciated with a decreased input resistance.17 In the present study
all neurons used for data analysis presented a complete
recovery to the preschismic resting membrane potential and
input resistance values 3 to 6 minutes after the ischemic
challenge. On return to control solution, the EPSP amplitude
recovered and developed an i-LTP expressed on both the
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)
and NMDA components of corticostriatal EPSP.17 This form
of synaptic plasticity started 4 to 6 minutes after the ischemic
insult and lasted throughout the period of observation (usu-
ally > 30 minutes and up to 3 hours). As expected, the
increased amplitude of the EPSP was coupled to an aug-
mented EPSP slope (n = 16; P < 0.001 at 20, 30, and 50
minutes for the 2 electrophysiological parameters). The EPSP
half-decay time increased as well, likely reflecting a differen-
tial potentiation of the 2 components of the EPSP, with
preferential facilitation of the slow NMDA-mediated part
(Figure 1).

Pharmacological Manipulation of Dopamine
Receptors on i-LTP

The first step of the study was the analysis of the effects of
selective D1- and D2-like dopamine receptor antagonists on
i-LTP. The D1-like dopamine receptor antagonist SCH 23390
(10 μmol/L; n = 15) fully prevented the potentiation induced
by ischemia on EPSP amplitude (P > 0.05 at 20, 30, and 50
minutes) but failed to prevent the increase of the half-decay
time associated with a control i-LTP (P < 0.001 at 20, 30, and
50 minutes). SCH 23390 not only failed to increase EPSP
slope observed in control medium but caused an opposite
effect on this electrophysiological parameter, ie, a significant
reduction (P < 0.01 at 20, 30, and 50 minutes). In contrast,
t-sulpiride (3 μmol/L; n = 15), a D2-like receptor antagonist,
failed to affect EPSP amplitude, slope, and half-decay time
t potentiation induced by ischemia (P < 0.001 for the 3 param-
eters) (Figure 1A to 1D). These receptor antagonists, applied
by bath for at least 10 minutes before the ischemic insult, did
not modify the changes of membrane potential and input
resistance (Figure 1E and 1F) measured before, during, and
after the ischemic period (P > 0.05; n = 15 for each drug).
Moreover, neither antagonist altered the EPSP amplitude,
slope, and half-decay time measured before the ischemic insult ($P>0.05$; $n=15$) (data not shown).

As previously shown, i-LTP was dependent on the activation of NMDA glutamate receptors. Accordingly, bath application of APV (50 μmol/L), an NMDA receptor antagonist, fully prevented the induction of i-LTP in striatal spiny neurons ($n=5$; $P>0.05$ for EPSP amplitude, slope, and half-decay time) (Figure 2).

**Effect of SCH 23390 on i-LTP Recorded in the Presence of AMPA Receptor Antagonism**

Both AMPA and NMDA components of the corticostriatal EPSPs are augmented by transient ischemia. Given the differential kinetic properties of the 2 ionotropic glutamate receptors, it is conceivable that the selective augmentation of the half-decay time of the EPSP observed in the presence of SCH 23390 is mainly attributable to the facilitation of NMDA-mediated responses. The delayed peak amplitude of the posts ischemic EPSP also corroborates this hypothesis. Accordingly, we found that SCH 23390 failed to affect i-LTP when this form of synaptic plasticity was induced in the presence of 10 μmol/L CNQX to block AMPA-mediated transmission ($n=6$ and $P<0.01$ for amplitude and slope increase in CNQX-bathed slices; $n=5$ and $P<0.01$ for amplitude and slope potentiation in CNQX plus SCH 23390–bathed slices) (Figure 2). Interestingly, although i-LTP of isolated NMDA receptor–mediated EPSPs had amplitude and slope changes comparable to those of i-LTP recorded on combined NMDA- and AMPA-mediated EPSPs (control

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**Figure 1.** Pharmacological blockade of D1-but not of D2-like receptors alters ischemic LTP. A, Traces show averages of EPSPs recorded from single experiments under control conditions (a), in the presence of the D1-like receptor antagonist SCH 23390 (10 μmol/L) (b), and in the presence of 3 μmol/L L-sulpiride, a D2-like receptor antagonist (c). The 2 superimposed traces show EPSPs before (pre-) the ischemic episode and 30 minutes after (post-ischemia) the insult. B through D, Time course of changes induced by 3 minutes of ischemia (black bar) on EPSP amplitude (B), slope (C), and half-decay time (D) in control conditions in the presence of SCH 23390 and L-sulpiride. E and F, Time course of membrane potential (E) and input resistance changes (F) caused by 3 minutes of ischemia in control conditions and after pharmacological manipulations.

**Figure 2.** Pharmacological blockade of D1-like receptors does not alter posts ischemic potentiation of the NMDA receptor–mediated component of EPSPs. The effects of the NMDA receptor antagonist APV (30 μmol/L), the AMPA receptor antagonist CNQX (10 μmol/L), and CNQX (10 μmol/L) plus 10 μmol/L SCH 23390 on the various parameters of posts ischemic (30 minutes) EPSPs are shown.
Role of cAMP and Protein Kinase A in Dopamine-Stimulated i-LTP

Since D1-like receptors increase cAMP levels by stimulating adenyl cyclase activity, we tested whether forskolin, a selective activator of this enzyme, was able to restore control i-LTP also in the presence of SCH 23390 and dopamine deafferentation, 2 experimental conditions in which D1-like receptors are not stimulated by endogenous dopamine. As expected, bath application (5 to 10 minutes) of forskolin was able to generate full i-LTP even in the presence of SCH 23390 or dopamine denervation (n = 5 and P < 0.001 in the 2 experimental conditions and for amplitude, slope, and half-decay time potentiation) (Figure 4A).

The D1-like receptor–triggered elevation of cAMP results in the stimulation of protein kinase A (PKA). PKA is involved in several forms of synaptic plasticity in various brain areas. Thus, we tested the effect of the selective PKA inhibitor H89 on i-LTP. As seen with SCH 23390 and dopamine-denervated rats, intracellular injection of H89 (100 μmol/L; n = 8) blocked the postschismic increase of the EPSP amplitude (P < 0.05) but caused a significant reduction of the slope (P < 0.001) and an increased half-decay time (P < 0.001) (Figure 4B). In addition, intraelectrode application of H89 failed to affect the ischemia-induced changes in membrane potential and input resistance (Figure 4C and 4D).

Analysis of i-LTP in Mice Lacking D1 Receptors

The D1-like dopamine receptor family includes 2 distinct subtypes of receptors, D1 and D5 receptors. Since the available pharmacological tools do not allow discrimination between these 2 subtypes of receptors, we performed electrophysiological experiments in slices obtained from D1-receptor–lacking (D1/−/−) mice. In D1/−/− striatal neurons, transient ischemia reduced the EPSP slope and enhanced the EPSP half-decay time (n = 9; P < 0.001 for the 2 parameters) without causing significant changes of the amplitude (n = 9; P > 0.05). As expected, incubation with 10 μmol/L SKF 38393 failed to restore i-LTP in D1/−/− slices (n = 4; P > 0.05). This suggests, therefore, that endogenous dopamine selectively binds to D1 receptors to produce i-LTP of the EPSP amplitude. Conversely, in normal (wild-type) mice, the potentiation induced by ischemia was expressed on amplitude, slope, and duration of corticostriatal EPSP, as seen in rats (n = 8; P < 0.001 for the 3 parameters) (Figure 5A and 5B). In both these experimental groups the ischemia-induced changes of membrane potential and input resistance were comparable to those measured in rats (Figure 5C and 5D).

Discussion

To our knowledge, this study represents the first demonstration that endogenous dopamine plays a pivotal role in amplifying postschismic enhancement of excitatory transmis-
D1-like receptors, controls physiological excitatory transmission selectively damages this dopamine-enriched brain region. Ischemia caused by homolateral carotid occlusion selectively damages this dopamine-enriched brain region.29 After energy deprivation and in vivo ischemia. The nucleus accumbens is a preferential site of silent infarcts in humans,29 and experimental ischemia caused by homolateral carotid occlusion selectively damages this dopamine-enriched brain area.30

It has been reported that endogenous dopamine, acting on D1-like receptors, controls physiological excitatory transmission in both cortical11,12 and striatal13 neurons. However, our study provides evidence supporting the involvement of D1 dopamine receptors in a pathological form of synaptic plasticity as well.

Although i-LTP requires the activation of NMDA but not AMPA receptors for its induction, it is expressed on both NMDA- and AMPA-mediated components of corticostriatal EPSPs.37 Interestingly, activation of D1 receptors by endogenous dopamine is necessary for the facilitatory and long-lasting effects on the AMPA component but not for the potentiation of the NMDA component of the EPSP. This conclusion is supported by the differential effects produced by the blockade of D1/cAMP/PKA pathway in inhibiting i-LTP. Intracellular application of the PKA inhibitor H89 (100 μmol/L) mimics the effects of D1-like receptor blockade in inhibiting i-LTP. Intra-electrode H89 alters neither the membrane potential (C) nor the input resistance changes (D) induced by 3 minutes of ischemia in striatal neurons.

The selective modulation exerted by endogenous dopamine on the AMPA-mediated component of the i-LTP may have profound pathophysiological implications. Striatal neurons oscillate in vivo between a depolarized “up” state and a hyperpolarized “down” state.35 It is conceivable that in the “up” state both AMPA and NMDA receptors are activated by glutamate released from corticostriatal terminals, while in the “down” state AMPA receptors are mainly operating. We can assume that a brief episode of ischemia would perturb both states of striatal neurons. In particular, endogenous dopamine, via D1 receptors, would greatly amplify AMPA transmission, transforming the normal oscillatory behavior in a persistent depolarized condition.

Ischemia causes acute necrotic death in the “core” of the ischemic area by leading to resting membrane potential disruption and neuronal swelling.6,36 Conversely, it activates apoptotic processes in the ischemic “penumbra,” where sublethal energy substrate deprivation occurs, or when a short ischemic episode is followed by restoration of energy substrate supply.37,38 Interestingly, central neurons that display the morphological and biochemical features of the apoptotic process die several hours and even days after an ischemic episode,38 suggesting that an irreversible enhancement of glutamate-mediated transmission may be involved in this pathological process. Delayed ischemia-induced neuronal death, in fact, is blocked, as is i-LTP, by NMDA-glutamate receptor antagonists37,39 and can be replicated by exposure to glutamate receptor agonists.6

We hypothesize that the D1-like dopamine receptors necessary for the persistent potentiation of AMPA transmission...
observed in i-LTP are located postsynaptically on striatal spiny cells. Accordingly, the intracellular injection of the PKA blocker H89 fully prevented, as did SCH 23390 applied by bath, the ischemic potentiation of AMPA transmission. Further support of this conclusion was provided by a previous electrophysiological study that demonstrated that postsynaptic stimulation of the D1/PKA pathway facilitates AMPA transmission in isolated striatal spiny neurons.40

D1- but not D5-like receptors seem to be involved in i-LTP, as supported by the experiments performed with the use of D1 -/− mice. The specificity of this receptor may be useful to design specific treatments aimed at countering the injurious effects of dopamine receptor stimulation during ischemia, minimizing the side effects caused by the simultaneous blockade of D1 and D5 receptors by available pharmacological agents.

We recently reported that i-LTP is selectively expressed is striatal projection neurons. Striatal cholinergic interneurons, conversely, do not undergo long-lasting changes in the efficacy of excitatory transmission after transient in vitro ischemia.25 Interestingly, the differential neuronal vulnerability between spiny neurons and cholinergic interneurons is a striking feature of in vivo ischemia1–3 and has also been observed in Huntington’s disease, a pathological condition in which energy metabolism is impaired.41 This synaptic phenomenon, therefore, may represent the electrophysiological correlate of different pathological events that injure the striatum. Several other factors, however, might account for the differential vulnerability among striatal neuronal populations, such as a different distribution of ionotropic glutamate receptor subunits and of intracellular calcium-binding proteins.42

In the present study we have demonstrated that dopamine facilitates pathological synaptic plasticity via D1 receptors located on striatal spiny neurons. This mechanism may represent a critical step of the vicious cycle initiated by ischemia in the vulnerable regions of the brain. It is well known, in fact, that a massive release of dopamine occurs during ischemic events in the striatum and that endogenous dopamine amplifies the neuronal damage caused by excitotoxicity and energy deprivation.13–10 Endogenous dopamine, however, can facilitate both acute necrotic and delayed apoptotic neuronal death by additional mechanisms, such as the control of intracellular calcium levels and the production of free radicals.33,44 The ischemia-induced abnormal release of endogenous dopamine in vulnerable brain areas, such as striatum, cerebral cortex, and hippocampus, may represent the critical factor that transforms a transient ischemic attack into an ischemic episode causing irreversible consequences for brain tissue and synaptic circuits. In fact, all of these vulnerable areas receive a dense dopaminergic innervation and express D1 receptors.45 Further studies are required to elucidate whether the mechanisms triggered by activation of dopamine receptors play a role in i-LTP in dopamine-enriched areas other than the striatum and in the clinical consequences of transient ischemic episodes in other brain areas and neuronal subtypes.

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