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ASYMMETRICAL CHANGES OF EXCITATORY SYNAPTIC TRANSMISSION IN DOPAMINE-DENERVATED STRIATUM AFTER TRANSIENT FOREBRAIN ISCHEMIA

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Abstract—Spiny neurons in the neostriatum are highly vulnerable to cerebral ischemia. Recent studies have shown that the postischemic cell death in the right striatum was reduced after ipsilateral dopamine denervation whereas no protection was observed in the left striatum after dopamine denervation in the left side. In order to reveal the mechanisms of such asymmetrical protection, electrophysiological changes of dopamine-denervated striatal neurons were compared after ischemia between the left and right striatum using intracellular recording and staining techniques *in vivo*. No difference in cortically evoked initial excitatory postsynaptic potentials was found between the left and right striatum in intact animals after ipsilateral dopamine denervation. The initial excitatory postsynaptic potentials in the dopamine-denervated right striatum were suppressed after transient forebrain ischemia while no significant changes were found in the dopamine-denervated left striatum. Paired-pulse tests suggested that these changes involved presynaptic mechanisms. Although the incidence of a late depolarizing postsynaptic potential elicited by cortical stimulation increased after ischemia in both sides, the increase was greater in the left side. The analysis of current–voltage relationship of spiny neurons indicated that inward rectification in the left striatum transiently disappeared shortly after ischemia whereas that in the right side remained unchanged. The intrinsic excitability of spiny neurons in both sides were suppressed after ischemia, however, the suppression in the right side was stronger than in the left side. The above results demonstrate that after ipsilateral dopamine denervation, the depression of excitatory synaptic transmission and neuronal excitability in the right striatum is more severe than that in the left striatum following ischemia. The depression of excitatory synaptic transmission and neuronal excitability, therefore, might play an important role in neural protection after ischemic insult.

Key words: cell death, lateralization, excitatory postsynaptic potential, intracellular recording, *in vivo*.

Spiny neurons in neostriatum are highly vulnerable to transient forebrain ischemia (Pulsinelli et al., 1982; Ren et al., 1997). Spiny neurons are heavily innervated by nigrostiatal dopaminergic projections (Lindvall and Bjorklund, 1983) and by cortical and thalamic glutamatergic pathways (Spencer, 1976; Divac et al., 1977). Dopaminergic terminals formed synapses in close proximity to the glutamatergic terminals converging on the spines of spiny neurons (Kotter, 1994). Accumulating evidence indicates that dopamine acts as a modulator altering the efficiency of neuronal responses to excitatory glutamatergic transmission (Cheramy et al., 1986; Kiyatkin and Rebec, 1999). However, the neurophysiological outcomes of dopamine modulation are complicated and controversial. They are mediated by multiple receptor subtypes involving several transduction systems, and can be either excitatory or inhibitory (Calabresi et al., 1987; Cepeda et al., 1993; Kiyatkin and Rebec, 1999). Studies using *in vitro* preparations have shown that dopamine could have distinct effects on excitatory postsynaptic potentials (EPSPs) of spiny neurons depending on the subtype of dopamine receptors activated. D_2 receptor activation reduces α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated EPSPs (Cepeda et al., 1993; Hsu et al., 1995; Levine et al., 1996), whereas D_1 receptor activation enhances AMPA and *N*-methyl-D-aspartate (NMDA) receptor-mediated responses (Cepeda et al., 1993; Levine et al., 1996; Galarraga et al., 1997).

A massive increase of extracellular glutamate and dopamine has been reported in the neostriatum during cerebral ischemia (Globus et al., 1988; Obrenovitch et al., 1990). Glutamatergic excitotoxicity has been postulated as the major cause of cell death after cerebral ischemia (Choi and Rothman, 1990). The detrimental effects of excessive dopamine released during ischemia have also been suggested by the finding that the unilateral depletion of dopaminergic inputs by lesion of substantia nigra (SN) attenuates the degree of neuronal damage following ischemia (Globus et al., 1987a; Clemens and Phebus, 1988). However, other studies failed to reproduce the

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Abbreviations: 6-OHDA, 6-hydroxydopamine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; EPSP, excitatory postsynaptic potential; ISI, interstimulus interval; KPBS, potassium phosphate-buffered saline; L-PSP, late depolarizing postsynaptic potential; LTP, long-term synaptic potentiation; NMDA, *N*-methyl-D-aspartate; PPR, paired-pulse ratio; SN, substantia nigra; TH, tyrosine hydroxylase.

protective effects on striatal neurons after dopamine depletion (Pulsinelli and Block, 1987; Wieloch et al., 1990). Such controversy has been resolved by recent studies demonstrating the asymmetrical effects of dopamine depletion on striatal neurons following ischemia. Injection of 6-hydroxydopamine (6-OHDA) into the right SN significantly reduces the cell death in the right striatum following ischemia while the same treatment in the left SN offers no protection on neurons in the left striatum (Ren et al., 1997; Xu et al., 1999).

Neuroanatomical, neurochemical, and behavioral asymmetries have been reported in rat brain (Glick et al., 1974; Sherman and Galaburda, 1984). Asymmetrical differences in neurotransmitters such as GABA and dopamine have been demonstrated in the neostriatum. It has been shown that the number of [^{3}H]GABA binding sites in the left striatum is higher than those in the right striatum and the presynaptic activity of glutamic acid decarboxylase is also higher in the left striatum (Glick et al., 1974; Guarneri et al., 1985, 1988). However, little is known about the role of these lateralizations in neuronal injury following ischemia. To reveal the mechanisms of asymmetric protection after unilateral dopamine denervation, the synaptic transmission and membrane properties of spiny neurons in the left or right striatum were examined before and after severe transient forebrain ischemia using intracellular recording and staining techniques *in vivo*.

EXPERIMENTAL PROCEDURES

Male adult Wistar rats (220–350 g, Charles River, MA, USA) were used in the present study. Experimental protocols were institutionally approved in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

Dopamine depletion

The depletion of dopamine was performed by unilateral administration of 6-OHDA into the SN (Ungerstedt, 1968). The animals were anesthetized with 1–2% halothane. Desipramine (25 mg/kg, i.p.) was injected 30 min before 6-OHDA infusion to protect the noradrenergic pathways (Breese and Traylor, 1971). 6-OHDA was delivered stereotaxically into the SN region with two injections. One injection site was at the rostrolateral SN (AP, 4.2 mm; ML, 2.0 mm; and DV, 8.0 mm, interaural) and the other was at the caudomedial SN (AP 3.2 mm; ML, 1.5 mm, and DV, 8.0 mm). The 6-OHDA solution was freshly prepared as 2 μg/μl calculated as a free base dissolved in sterile distilled water containing 0.1% ascorbic acid and 0.9% NaCl. The solution was adjusted to pH 5.5 and kept at 4°C. The volume of each injection was 2 μl. The 6-OHDA solution was slowly infused for 5 min for each site through a 10-μl Hamilton syringe. The needle of the Hamilton syringe remained in place for an additional 10 min after injection. Seven to ten days after SN lesion, the rats were injected with apomorphine (0.5 mg/kg, s.c.) and observed for rotational behavior (Ungerstedt, 1968). Only the rats that exhibited consistent contralateral turning (10–20 turns/min) for at least 20 min, a behavior indicating the depletion of more than 90% of dopamine in the neostriatum, were used for the recording. All reagents were obtained from Sigma (St. Louis, MO, USA). Immunocytochemistry was performed on some animals to verify the dopamine depletion using monoclonal antibodies against tyrosine hydroxylase (TH, Chemicon, CA, USA).

Transient forebrain ischemia

Transient forebrain ischemia was induced using the four-vessel occlusion method (Pulsinelli and Brierley, 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% O_2 and 66% N_2 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 core body temperature was maintained with a heating pad through a temperature control unit (TC-120, Medical System, NY, USA). The vertebral arteries were electrocauterized. A very small temperature probe (0.025″ D, Physitemp, NJ, USA) 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, NJ, USA). Severe forebrain ischemia was induced by occluding both common carotid arteries to induce ischemic depolarization for ~21 min. Cerebral blood flow resumed immediately upon release of the carotid artery clasps.

In vivo intracellular recording and staining

In vivo intracellular recording was performed as described in previous studies (Xu, 1995; Gajendiran et al., 2001). In brief, the animals were anesthetized with a mixture of 1–2% halothane, 33% O_2 and 66% N_2 and the skull was opened to expose cortex above the recording site and for placement of stimulus electrodes. One pair of bipolar stimulating electrodes was made from 000 stainless steel insect pins and insulated except for within 1.0 mm of the tips. The electrodes were separated by 1.0 mm and placed into the ipsilateral medial agranular cortical field at an angle of 30° to the vertical, 2.2 mm from the dural surface. Stimuli were constant current pulses of 0.1 ms duration ranging in amplitude from 0.1 to 3 mA. Zero to five times the threshold stimulus intensity (0–5*T*) was used for corticostriatal stimulations. Recording electrodes were pulled from glass capillaries with glass filament (A-M Systems, WA, USA) using a vertical electrode puller (Kopf 750, CA, USA). The impedance of the electrodes was 50–70 MΩ when filled with a solution of 3–5% neurobiotin (Vector, CA, USA) in 2 M potassium acetate. Cerebral spinal fluid was drained via a cisternal puncture at the posterior atlanto-occipital membrane to reduce brain pulsation. After placement of a microelectrode in the cortex above the neostriatum for recording, the exposed surface of the brain was covered with soft paraffin wax. In order to record neurons in the dorsal striatum, the electrodes were advanced no more than 4.0 mm from the surface. After impalement, only neurons with stable membrane potentials of −50 mV or greater were selected for the study. Recordings were digitized with the data acquisition program Axodata (Axon Instruments, CA, USA) and stored on Macintosh computers for off-line analysis.

In some experiments, paired-pulse stimulation was used to compare the changes of paired-pulse ratio (PPR). Two pulses of stimulation were delivered with an interval of 20 or 40 ms. The PPR was defined as the ratio of the slope of testing EPSP (EPSP1) to the slope of conditioning EPSP (EPSP2).

After each successful recording neurobiotin was iontophoresed into the cell by passing depolarizing current pulses (2 Hz, 300 ms, 0.5–1.3 nA) for 10–30 min. At the end of the experiment, the animals were deeply anesthetized and perfused transcardially with 0.01 M phosphate-buffered saline (pH 7.4) followed by 4% paraformaldehyde. The brains were removed and stored in fixative overnight. The brains were trimmed and parasagittal sections were cut at 50 μm thickness using a vibratome (Ted Pella, CA, USA). The sections were incubated in 0.1% horseradish peroxidase-conjugated avidin-D (Vector, CA, USA) in 0.01 M potassium phosphate-buffered saline (KPBS, pH 7.4) with 0.5% Triton X-100 for 6–8 h at room temperature. After detection of peroxidase activity with 3′,3′-diaminobenzidine as the chromogen, the sections were examined in KPBS. Those sections containing labeled neurons and stimulation sites

were mounted on gelatin-coated slides and counterstained with Cresyl Violet for light microscopy. In some cases, sections containing intracellularly stained neurons were processed for dopamine fiber staining using anti-TH antibodies (Chemicon, CA, USA).

Statistics

Data were presented as means ± S.E.M. Student's *t*-test was used for two-group comparison and analysis of variance (ANOVA) followed by post hoc Scheffe's test was used for multi-group analysis using Statview 4.1 (Abacus Concepts). Differences were considered significant when $P < 0.05$.

RESULTS

The experiments were performed on 79 rats. The animals were divided into two groups. One group of animals was subjected to left SN lesion ($n = 25$) and the electrophysiological recordings were performed in the left striatum. The other group was subjected to right SN lesion ($n = 54$) and the recordings were performed in the right striatum. Recordings were made before ischemia (left: $n = 6$; right: $n = 13$) and at different intervals (0–3 h, 3–6 h, 6–9 h, 9–12 h) after reperfusion (left: $n = 19$; right: $n = 41$). Ischemia with an ischemic depolarization of ∼21 min consistently produced more than 90% cell death in dorsal striatum under halothane anesthesia (Ren et al., 1997). In this paper, the neurons in the left or right striatum refer to the neurons recorded in the left or right striatum after ipsilateral dopamine depletion unless otherwise noted.

Intracellular recordings were performed on 146 neurons in either left or right striatum, of which two were excluded from the present study because they were morphologically identified as aspiny neurons. A total of 144 neurons were selected, of which 57 were successfully stained and identified as spiny neurons. All recovered neurons were located in the dorsal half of the neostriatum. They had round or oval somata with dendrites radiating in all directions. The dendrites were heavily loaded with spines except in their most proximal parts (Fig. 1, insert). Unidentified cells were considered as spiny neurons based on the characteristic spontaneous fluctuation of membrane potential between −60 and −90 mV, the electrode tracks in the dorsal striatum or the stereotaxic coordinates. The recording at 9–12 h after reperfusion became extremely difficult and only a few cells were recovered after intracellular staining. TH immunocytochemistry was performed in five rats after recovery of the intracellular recorded neurons. Dense TH-immunoreactive fibers were found throughout the contralateral striatum in animals after unilateral SN lesion. On the contrary, few TH-immunoreactive fibers were found in the ipsilateral striatum, especially in the dorsolateral parts (Fig. 1).

Changes of EPSPs following reperfusion

EPSPs in spiny neurons were evoked by stimulation of the ipsilateral medial agranular cortex. The EPSPs were elicited with stimulus intensities ranging from two to five times as strong as the threshold stimulus intensity (2–5*T*). The initial EPSPs were followed by a long-lasting hyperpolarization and a subsequent rebound excitation

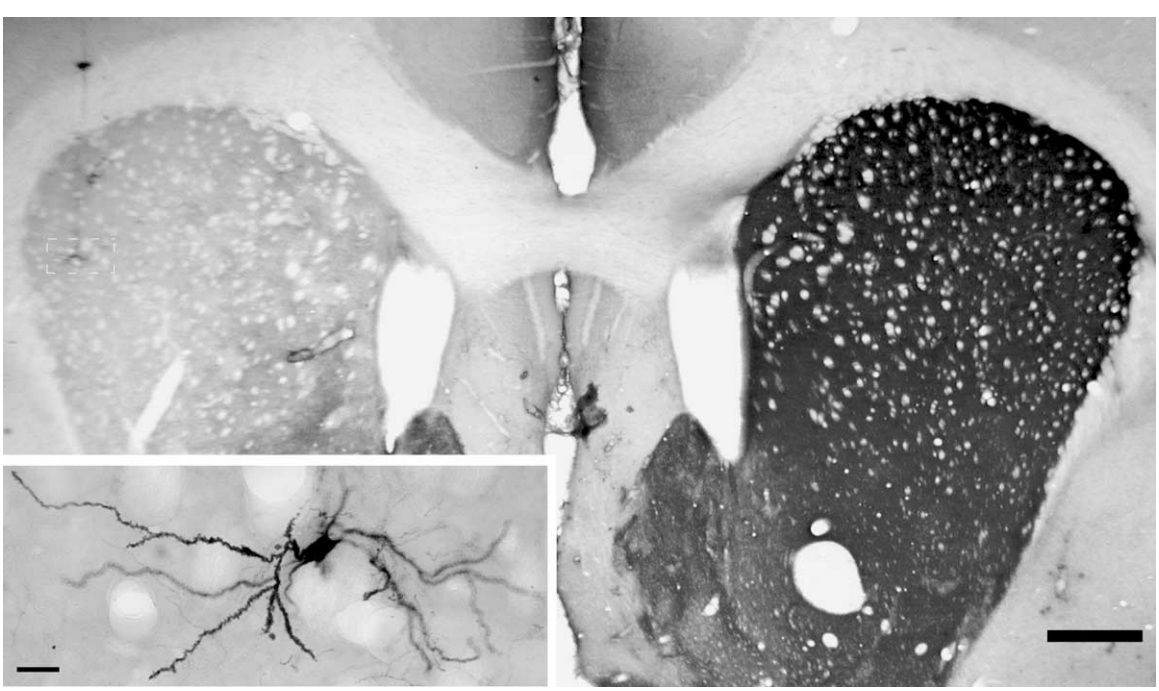

Fig. 1. Photomicrograph of a TH-immunocytochemical staining section containing an intracellularly stained spiny neuron from an animal after unilateral dopamine denervation. Ten days after unilateral 6-OHDA injection into SN, very few TH-immunoreactive fibers were observed in the striatum of lesion side, while in the intact side, dense TH-immunoreactive fibers were found throughout the striatum. The insert is the higher magnification of the dash-squared area in the figure. The track of the recording electrode can be observed above the cell. Intracellularly stained spiny neuron has dense spines on its dendrites except the most proximal portions. Scale bar = 1 mm; 20 μm for the insert.

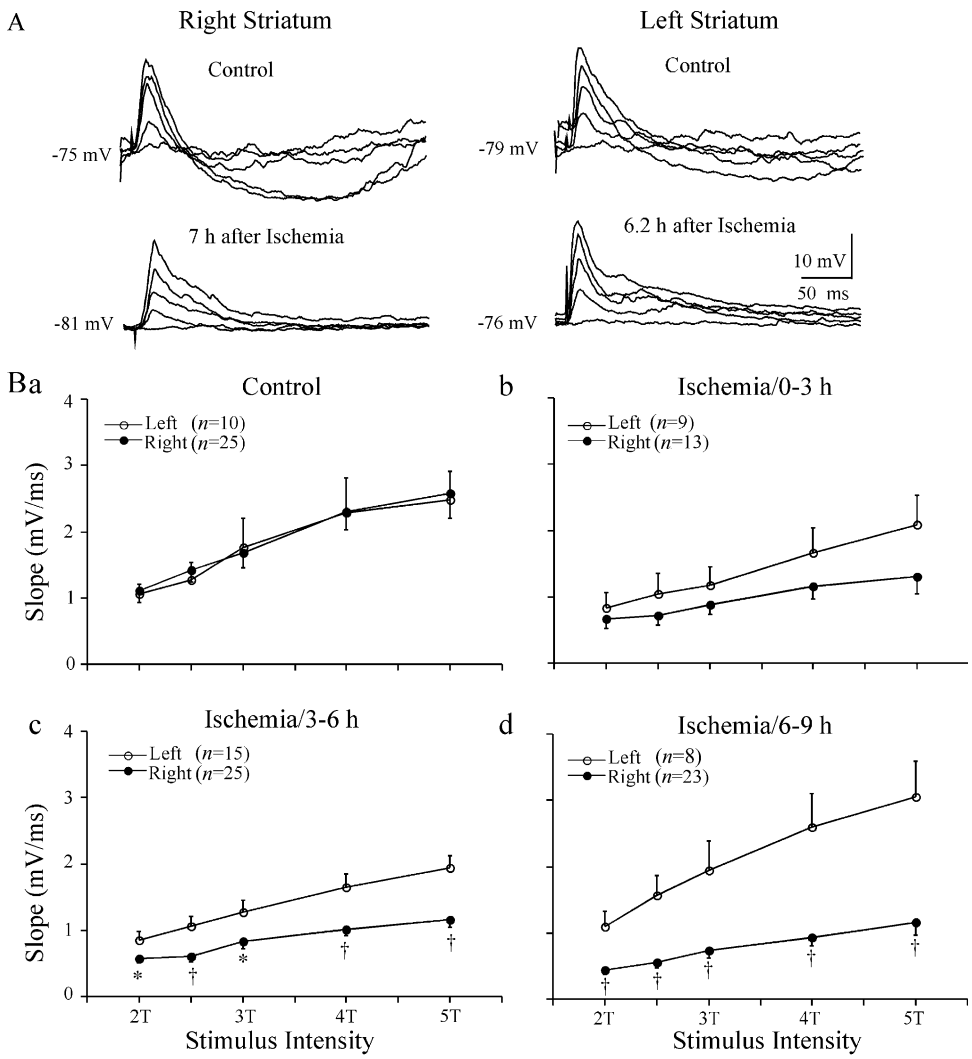

Fig. 2. Comparison of initial EPSPs in spiny neurons between dopamine-denervated left and right striatum after ischemia. (A) Representative recordings of cortically evoked postsynaptic potentials of spiny neurons before (upper panels) and after transient forebrain ischemia (lower panels). The amplitude of initial EPSPs increases according to the increasing stimulus intensities. The stimulus intensity ranges from two to five times of the threshold stimulus intensity (2–5*T*) for inducing the initial EPSPs. The traces are the average of four consecutive recordings. The scales apply to all traces. (B) The plot shows the relationship between stimulus intensity and the slopes of initial EPSPs. The slopes of the EPSPs in the right striatum were about the same as that in the left side before ischemia (a) but significantly depressed after ischemia as compared with the left side (b–d). The values are means ± S.E.M. The number of neurons recorded is shown in parentheses. $^{*}P < 0.05$, $^{\dagger}P < 0.01$.

similar to those observed in naive animals (Fig. 2A). No significant difference in stimulus threshold and latency of initial EPSPs was found between the left and right striatum in control animals. The slopes of the initial EPSPs were used to compare the strength of synaptic transmission. The slopes of the initial EPSPs in both sides were increased according to the increase of stimulus intensity. The slopes were about the same between the left and right striatum before ischemia (Fig. 2Ba). The slopes of the initial EPSPs in the right striatum were significantly smaller than those in the left striatum at 3–9 h after ischemia (Fig. 2Bc, d). The temporal profiles of the initial EPSPs before and after ischemia were compared at a stimulus intensity of 2.5*T* (Fig. 3A). In the right striatum, the slope of initial EPSPs was 1.43 ± 0.19 mV/ms before ischemia ($n = 25$) and decreased to 0.72 ± 0.14 mV/ms shortly after ischemia ($n = 13$). It was further decreased to 0.60 ± 0.08 mV/ms ($P < 0.01$, $n = 25$) at 3–6 h and 0.55 ± 0.09 mV/ms ($P < 0.01$, $n = 23$) at 6–9 h after ischemia, respectively. The changes in amplitudes of initial EPSPs in the right striatum exhibited a similar pattern as those of slopes (Fig. 3B). These results indicated a decrease in the synaptic strength in the right striatum after ischemia. In contrast, both the slope and amplitude of initial EPSP remained unchanged in the left striatum after ischemia and even showed a slight increase at 6–9 h following reperfusion (Fig. 3A, B). The initial EPSPs of spiny neurons were also compared between the intact right striatum and dopamine-denervated right striatum before and after ischemia. At 6∼9 h after ischemia, the slope of initial EPSPs in intact right striatum decreased to 74.2 ± 17.8% of control level (Fig. 3C, con-

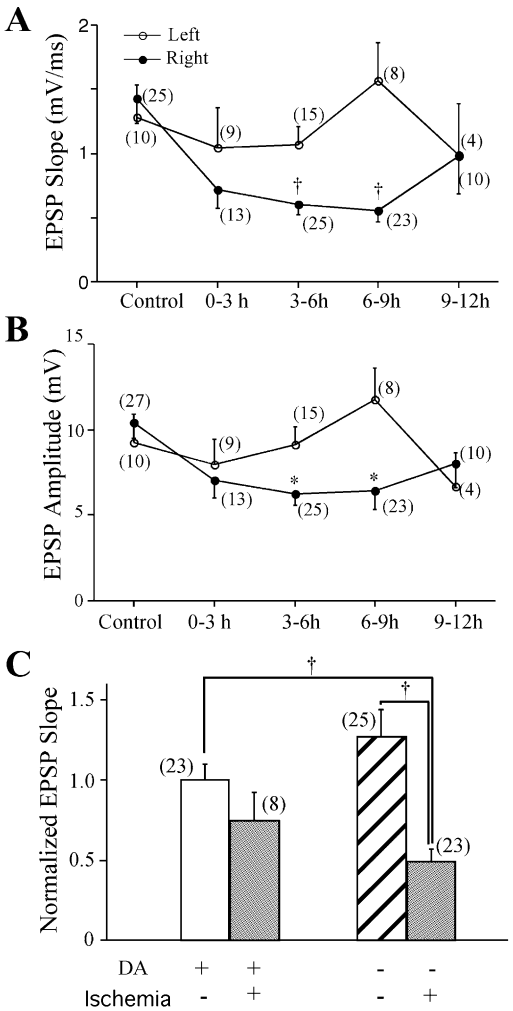

Fig. 3. Temporal profiles of initial EPSPs in dopamine-denervated left or right striatum after ischemia. The slope and amplitude of initial EPSPs were measured from EPSP evoked by a stimulus intensity of 2.5*T*. Both the slope (A) and amplitude (B) of spiny neurons were significantly decreased after ischemia in the right striatum while those in the left striatum were unchanged and even slightly increased at 6–9 h after ischemia. $*P<0.05$; $\dagger P<0.01$. (C) Comparison of the slope of initial EPSPs of spiny neurons between the intact and dopamine-denervated right striatum. In intact right striatum, the slope of initial EPSPs reduced to $74.2\pm17.8\%$ of control levels at 6–9 h after ischemia. Comparing to the control values, the slope of initial EPSPs in dopamine-denervated right striatum increased to $126.8\pm17.1\%$ and significantly decreased to $49.1\pm7.6\%$ at 6–9 h after ischemia. DA+: intact right striatum; DA−: dopamine-denervated right striatum; Ischemia−: without ischemia; Ischemia+: 6–9 h after ischemia. The number of neurons is indicated in parentheses. $\dagger P<0.01$, unpaired *t*-test.

trol: $n=23$, ischemia: $n=8$). In comparison to that of control values, the slope of initial EPSPs in dopamine-denervated right striatum increased to $126.8\pm17.1\%$ before ischemia ($n=25$) but significantly decreased to $49.1\pm7.6\%$ ($P<0.01$, $n=23$) at 6–9 h after reperfusion (Fig. 3C).

In an attempt to determine whether the depression of initial EPSP after ischemia in the right striatum is mediated by a presynaptic mechanism, paired-pulse stimulation was conducted in some neurons. A change in the PPR is correlated with the change of release probability of the presynaptic terminals. At an interstimulus interval (ISI) of 20 ms, control neurons showed a paired-pulse depression with a PPR of $65\pm5\%$ ($n=22$). The paired-pulse depression changed to paired-pulse facilitation in neurons after ischemia. The PPR increased to $168\pm15\%$ shortly after reperfusion ($n=8$, $P<0.01$). It remained at that level up to 9 h after ischemia (Fig. 4). At an ISI of 40 ms, the PPR also significantly increased after ischemia (Fig. 4B). These results suggested that the releasing probability of excitatory neurotransmitter was decreased after ischemia in the right striatum.

In a subpopulation of spiny neurons in naive animals, a late depolarizing postsynaptic potential (L-PSP) was evoked by cortical stimulation (Gajendiran et al., 2001). The latency of L-PSPs decreased with increasing stimulus intensities indicating that they are polysynaptic events (Fig. 5A). The amplitude of L-PSPs was dramatically increased by paired-pulse stimulation in dopamine-denervated animals after ischemia (Fig. 5B) but the extent of the potentiation varied among neurons. The incidence of L-PSP was increased after dopamine denervation and further increased following ischemia. However, the increase of the proportion of neurons exhibiting L-PSPs was different between the left and right striatum. Compared to naive animals, a 500% increase was found in the left striatum after dopamine denervation, only 275% increase was found in the right striatum. After ischemia, an 1175% increase was detected in the left striatum whereas only 812% increase was found in the right (Fig. 5C).

Changes of membrane properties following reperfusion

The membrane properties of spiny neurons in dopamine-denervated animals before ischemia were about the same between the left and right striatum (Table 1). However, some differences in postischemic change were detected between the left and right striatum. The resting membrane potential of spiny neurons in the right striatum became more hyperpolarized (-85.9 ± 1.9 mV, $n=17$) than that in the left striatum (-77.3 ± 2.8 mV, $n=8$, $P<0.05$) 0–3 h after reperfusion. The input resistance of neurons in the right striatum (28.6 ± 2.4 MΩ, $n=17$) was significantly lower than that in the left side (37.8 ± 3.1 MΩ, $n=8$, $P<0.05$) at 0–3 h after ischemia. The spike threshold of spiny neurons in the right striatum significantly increased after ischemia while that in the left striatum remained at the same level. The rheobase, another indication of neuronal excitability, in the right striatum (1.04 ± 0.09 nA for 0–3 h, 0.96 ± 0.07 nA for 3–6 h) was higher than that in the left side (0.61 ± 0.11 nA for 0–3 h, 0.66 ± 0.12 nA for 3–6 h, $P<0.05$). These data indicated that the excitability of spiny neurons in the right striatum was more depressed than that in the left striatum after transient forebrain ischemia. The current–voltage (*I–V*) relationship of dopamine-denervated spiny neurons was also compared before and after ischemia. As shown in Fig. 6B, the *I–V* curves of spiny neurons in the right striatum

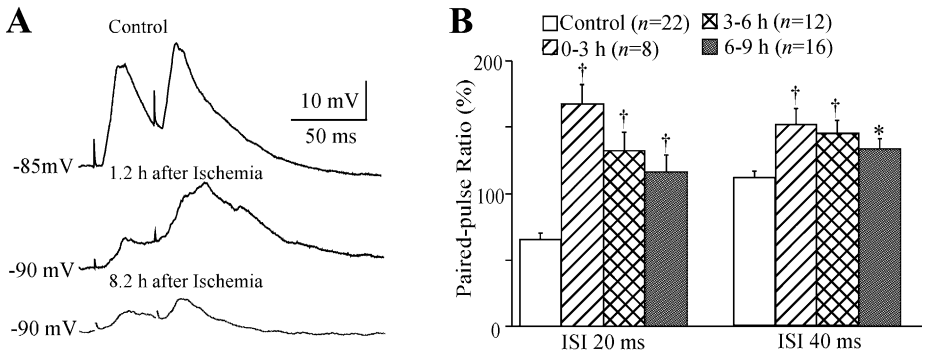

Fig. 4. PPR changes in dopamine-denervated right striatum before and after ischemia. (A) Examples of traces showing the paired-pulse test of spiny neurons before and after ischemia. Paired-pulse stimulation was delivered with an ISI of 40 ms, the traces are the average of four consecutive recordings. PPR is defined as the ratio of testing EPSP slope to the conditioning EPSP slope. (B) Histograms of pooled data showing that the PPR increased after ischemia at an ISI of 20 and 40 ms, respectively. $*P < 0.05$, $\dagger P < 0.01$ compared to control values.

remained unchanged after ischemia whereas those in the left side were significantly altered following reperfusion. The inward rectification of spiny neurons in the left striatum disappeared at 0–3 h after ischemia (Fig. 6B).

DISCUSSION

Using an *in vivo* preparation, the present study compared the cortically evoked EPSPs in dopamine-denervated left and right striatum after forebrain ischemia that induced ~21 min ischemia depolarizaion in rats. Such ischemia consistently produces more than 90% cell death in dorsal striatum 24 h after reperfusion (Ren et al., 1997). No significant difference in electrophysiological properties of spiny neurons was found between the left and right striatum before ischemia. However, the excitatory synaptic transmission in the right striatum was depressed after ischemia whereas that in the left side was slightly enhanced. The PPR of EPSPs in the right striatum was increased after ischemia indicating a decrease of releasing probability of excitatory neurotransmitter from the corticostriatal pathways.

Synaptic transmission in dopamine-denervated spiny neurons after ischemia

In the present study, the amplitude and slope of initial EPSPs in the right striatum were decreased after ischemia, indicating a suppression of excitatory synaptic transmission. Such changes of initial EPSPs in the right striatum are not due to the alterations of membrane properties after dopamine denervation because the membrane properties of spiny neurons remain unchanged after dopamine denervation (Onn and Grace, 1999; Pang et al., 2001). The difference in amplitude and slope of initial EPSPs is not likely due to the changes of resting membrane potentials after ischemia either. Because the resting membrane potentials in the right striatum became more hyperpolarized than those in the left after ischemia (Table 1), a change should increase the amplitude of the EPSPs in the right striatum. Taking the

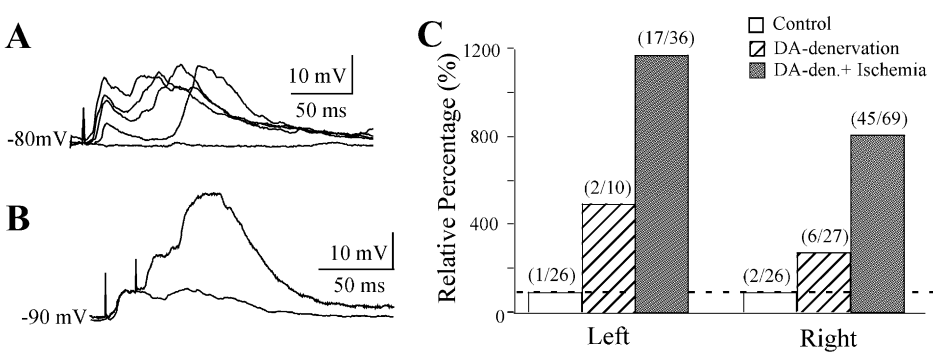

Fig. 5. Comparison of L-PSPs between left and right striatum at different experimental conditions. (A) An example of L-PSPs recorded from dopamine-denervated animals after ischemia. The stimulus intensity increased from 0 to 5*T*. The latency of the peak of the L-PSP became shorter upon stronger stimulus intensities indicating its polysynaptic nature. (B) Superimposed traces of a paired-pulse stimulation at an ISI of 20 ms and a single pulse stimulation from a dopamine-denervated spiny neuron after ischemia. The L-PSP was greatly potentiated by the second pulse stimulation. Traces in A and B are averages from four consecutive recordings. (C) Plot showing the relative proportion of cortically evoked L-PSPs in the left or right striatum at different experimental conditions. The values are normalized to the proportion of L-PSPs neurons in the left or right striatum in naive animals. The increased proportions of L-PSP neurons in the right striatum are less than those in the left after dopamine denervation and following ischemia. The numbers in parentheses are number of L-PSP neurons over the number of recorded neurons.

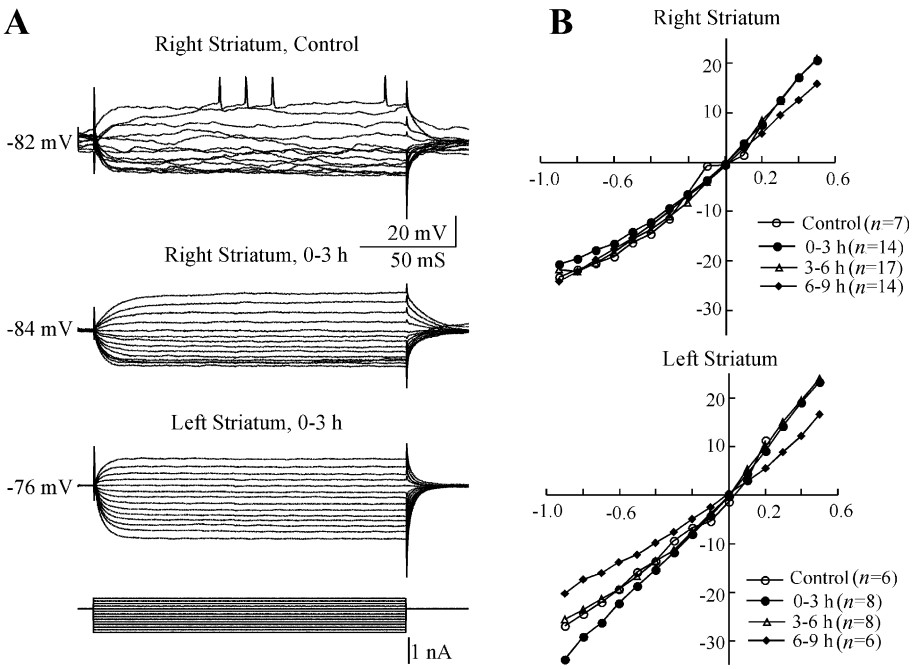

Fig. 6. Current–voltage (*I–V*) relationship of spiny neurons in dopamine-denervated striatum before and after ischemia. (A) Representative recordings of voltage deflection caused by the current pulses. The upper panel is the recording from the right striatum before ischemia. The middle panel is from the right striatum and the lower panel is from the left striatum 0∼3 h after ischemia, respectively. Scale applies to all three recordings. The lowest panel is the intracellularly applied current pulses. The traces recorded before ischemia showed a noisy voltage deflection due to the spontaneous membrane potential fluctuations of spiny neurons. (B) Plots of *I–V* curve showing the changes at different intervals after ischemia. The *I–V* curves from the right striatum (upper panel) remain unchanged after ischemia while those from the left striatum (lower panel) are altered after reperfusion. The inward rectification that is evident in the hyperpolarizing direction disappears in the left striatum 0–3 h after ischemia.

changes in resting membrane potential into consideration, the depression of EPSPs in the right striatum actually was underestimated. The decrease of input resistance and increase of rheobase of spiny neurons in the right striatum indicate that the neuronal excitability of these neurons is also depressed after ischemia. The notion that the postischemic changes in membrane properties differ between the dopamine-denervated left and right striatum is further supported by the fact that the *I–V* curves of spiny neurons in the right striatum remained unchanged while those in the left striatum were altered after ischemia (Fig. 6).

It has been shown that striatal neurons in the right side are resistant to ischemia while those in the left are sensitive to ischemia after ipsilateral dopamine denervation (Ren et al., 1997). It is possible that the depression

Table 1. Membrane properties of spiny neurons in left and right neostriatum after ischemia following ipsilateral DA-depletion

	RMP (mV)	H_{spk} (ms)	W_{spk} (mV)	T_{spk} (mV)	R_{in} (MΩ)	T_{con} (ms)	Rheobase (nA)	fAHP (mV)
Control								
Left (8)	−73.7 ± 2.0	77.6 ± 3.9	1.21 ± 0.11	−50.1 ± 1.2	31.4 ± 4.7	7.03 ± 1.01	0.31 ± 0.04	7.1 ± 0.9
Right (20)	−75.6 ± 1.7	78.3 ± 2.1	1.21 ± 0.05	−52.9 ± 0.8	31.1 ± 2.7	6.39 ± 0.61	0.41 ± 0.05	6.4 ± 0.7
0–3 h								
Left (8)	−77.3 ± 2.8	82.3 ± 5.0	1.27 ± 0.07	−48.5 ± 2.1	37.8 ± 3.1	7.35 ± 0.74	0.61 ± 0.11	11.3 ± 1.2
Right (17)	−85.9 ± 1.9**, ***	90.2 ± 3.1**	1.45 ± 0.10	−48.5 ± 1.3**	28.6 ± 2.4***	6.75 ± 0.67	1.04 ± 0.09**, ***	14.2 ± 1.0**
3–6 h								
Left (10)	−80.4 ± 2.5	82.5 ± 3.2	1.53 ± 0.19	−48.2 ± 2.2	29.8 ± 3.7	7.31 ± 0.68	0.66 ± 0.12	10.3 ± 1.6
Right (19)	−84.9 ± 1.7*	86.3 ± 1.9	1.49 ± 0.09	−49.0 ± 0.8	31.0 ± 2.1	5.60 ± 0.46***	0.96 ± 0.07**, ***	13.2 ± 0.7**
6–9 h								
Left (8)	−82.4 ± 1.8	88.7 ± 2.3	1.36 ± 0.13	−49.0 ± 1.3	30.1 ± 2.1	6.46 ± 0.61	0.81 ± 0.17	11.8 ± 1.3
Right (19)	−79.3 ± 1.8	78.3 ± 1.6***	1.32 ± 0.06	−50.2 ± 0.9	28.4 ± 2.7	4.81 ± 0.51	0.77 ± 0.08**	11.0 ± 0.7**

Values are means ± S.E.M.; with number of neurons in parentheses. RMP, resting membrane potential. H_{spk}, spike height is measured from the resting membrane potential. W_{spk}, spike width is measured at the half of the action potential peak. T_{spk}, spike threshold is measured at the beginning of the upstroke of the action potential. R_{in}, input resistance is derived from the linear portion of *I–V* curve (0–0.5 nA). T_{con}, time constant is derived from transients of hyperpolarizing pulses (−0.3 nA, 200 ms). fAHP, fast after-hyperpolarization is measured as deviation from the beginning of the upstroke of an action potential within 5 ms after the peak of a single spike. *$P < 0.05$; **$P < 0.01$ as compared ipsilaterally before ischemia. ***$P < 0.05$ as compared to left striatum.

of excitatory synaptic transmission might be associated with the protection of striatal neurons against ischemia in dopamine-denervated rats. In hippocampus, CA1 pyramidal neurons die two to four days after transient forebrain ischemia while the CA3 neurons and dentate granule cells survive the same insult (Pulsinelli et al., 1982). The synaptic transmission of ischemia-vulnerable CA1 neurons, ischemia-resistant CA3 and granule cells display completely different responses to ischemic insult. Potentiation of excitatory synaptic transmission has been observed in CA1 neurons after ischemia or hypoxia (Urban et al., 1989; Crepel et al., 1993; Hori and Carpenter, 1994; Gao and Xu, 1996). In contrast, the neuronal activities and synaptic transmission in CA3 and granule cells was transiently depressed following ischemia of the same severity (Chang et al., 1989; Gao et al., 1998). Recent studies have shown that the incidence of L-PSPs in ischemia-vulnerable spiny neurons was increased after ~21 min ischemia induced by four-vessel occlusion in rat (Gajendiran et al., 2001). The appearance of L-PSPs in spiny neurons after ischemia resembles the polysynaptic EPSPs evoked by thalamic stimulation in decorticated animals (Wilson et al., 1983). The increase of L-PSP in spiny neurons after ischemia might be one form of synaptic plasticity induced by ischemia/hypoxia. Long-term plasticity, i. e. long-term synaptic potentiation (LTP) and long-term synaptic depression, has been observed in corticostriatal synapses (Calabresi et al., 2000). Ischemic LTP has been induced in spiny neurons after hypoxia/hypoglycemia without tetanic stimulation (Calabresi et al., 2002). The ischemic LTP, therefore might be associated with the increase of L-PSPs in striatum following ischemia. However, the synaptic response is counterbalanced by excitatory and inhibitory components. Another possible mechanism underlying the increased L-PSP occurrence is the reduced inhibition in the striatum. The incidence of L-PSPs was increased after dopamine denervation, which might be due to the removal of endogenous tonic inhibition by dopamine (Pang et al., 2001). The proportion of the neurons exhibiting L-PSPs was further increased after ischemia because the impairment of inhibitory postsynaptic potentials (Gajendiran et al., 2001). However, the increase in the proportion of L-PSPs neurons in the right striatum was smaller than that in the left striatum, suggesting a stronger inhibition in the right striatum in comparison to that in the left after ischemia (Fig. 5C). Therefore, it is conceivable that the enhancement of excitatory synaptic transmission is associated with the postischemic cell death while the synaptic depression might be related to the neural protection against ischemia. In support of this notion, CA1 neurons in hippocampus and spiny neurons in striatum also exhibited a transient depression of excitatory synaptic transmission after ~5 min ischemia induced by four-vessel occlusion that will not cause cell death (Xu and Pulsinelli, 1994; Xu, 1995; Xu and Pulsinelli, 1996). The depression of excitatory synaptic transmission in spiny neurons of dopamine-denervated right striatum after ischemia coincides with the changes of CA3 and granule cells after severe ischemia and CA1 neurons and spiny neurons after mild ischemia. It strongly indicates that the depression of synaptic transmission might play an important role in asymmetrical protection in the striatum against ischemia after unilateral dopamine depletion.

Asymmetrical protection after dopamine denervation

Cerebral ischemia induces a dramatic increase of extracellular dopamine concentration (Globus et al., 1988; Obrenovitch et al., 1990). Removal of dopamine inputs to neostriatum attenuates the degree of cell death following ischemia indicating that the dopamine is involved in neuronal damage in striatum after ischemia (Weinberger et al., 1985; Globus et al., 1987a,b; Clemens and Phebus, 1988). One of the dopamine functions is to modulate glutamate-mediated synaptic transmission (Nicola et al., 2000). Dopamine has different effects on EPSPs of spiny neurons depending on the subtype of dopamine receptors activated. Among the five types of identified dopamine receptors, D_1 and D_2 receptors have been confirmed to exist predominantly in the mesostriatal and mesocortical systems (Le Moine et al., 1990; Weiner et al., 1991; Sibley and Monsma, 1992). D_2 receptor activation reduces AMPA receptor-mediated EPSPs (Cepeda et al., 1993; Hsu et al., 1995; Levine et al., 1996), whereas D_1 receptor activation enhances AMPA and NMDA receptor-mediated responses (Cepeda et al., 1993; Levine et al., 1996; Galarraga et al., 1997). It is possible that D_1 receptor activation could potentiate and D_2 receptor activation could reduce the glutamate excitotoxic effects after ischemia. In support of this notion, systemic administration of dopamine D_2 receptor agonist has been shown to eliminate neurodegeneration in hippocampus after global cerebral ischemia (O'Neill et al., 1998). However, D_1 receptor antagonist offers no protection on striatal neurons against ischemia (Hashimoto et al., 1994; O'Neill et al., 1998).

Because the residual dopamine levels in the left or right striatum following ipsilateral 6-OHDA lesion were about the same during ischemia (Xu et al., 1999), the dramatic difference in ischemic outcome between the left and right striatum might stem from the lateralization of dopamine receptors in neostriatum. Asymmetrical distribution of dopamine receptors in striatum has been demonstrated in naive animals. Studies have shown that the D_2 receptors in the left striatum were 23% greater than those in the right striatum of male rats (Schneider et al., 1982) whereas the lateralization of D_2 receptors was reversed in female animals, being 40% greater in the right striatum than in the left (Drew et al., 1986). It has also been shown that after a lesion of SN with 6-OHDA, the D_2 receptor binding sites were significantly increased in striatum while those of D_1 receptors remained unchanged (Joyce, 1991a,b). A recent report has indicated that after ipsilateral dopamine denervation, the D_2 receptor binding in the right striatum is 14% higher than that in the left side (Ling et al., 2001). It is possible that the increase of dopamine release during ischemia through the residual dopamine fibers after SN lesion (Song and Haber, 2000) causes the depression of EPSPs by activation of up-regulated D_2

receptors in the right striatum, which in turn reduces the glutamate excitotoxicity. This might be one of the mechanisms of asymmetrical protection on striatal neurons against ischemic insult in animals after dopamine denervation.

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