Unilateral Dopamine Denervation Blocks Corticostriatal LTP

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INTRODUCTION

The nigrostriatal dopaminergic projection plays a crucial role in the physiological activity of basal ganglia. Loss of dopamine (DA)-containing neurons of this pathway, in fact, is the main pathological characteristic of Parkinson’s disease (PD). The striatum also receives extensive glutamatergic projections from the cerebral cortex and thalamus (Calabresi et al. 1990, 1994). Briefly, corticostriatal synaptic transmission have been described, long-term depression (LTD) and long-term potentiation (LTP) (Calabresi et al. 1992c,d, 1994, 1996b; Lovinger et al. 1993; Walsh 1993). Although in both forms of synaptic plasticity the interplay between glutamate and DA receptors has been described (Calabresi et al. 1992c, 1997b; Choi and Lovinger 1997; Lovinger et al. 1993), the effects of DA-deafferentation has been investigated on corticostriatal LTD (Calabresi et al. 1992c) but not LTP. Morphological studies showed that DA-denervation causes major structural changes in the striatum that might profoundly interfere with the generation of this form of synaptic plasticity. In particular, after 6-hydroxydopamine (6-OHDA) denervation, a frequently used rat model of human PD, dendritic spines of striatal neurons are numerically reduced and present abnormal size and shape (Ingham et al. 1989; Nitsch and Riesenberg 1995). Similar data were also demonstrated in autopsy brains of PD patients (McNeill et al. 1988). Noticeably, dendritic spines of striatal neurons have been proposed to constitute the anatomic locus of the interaction between glutamate and DA and also the site of the expression of corticostriatal synaptic plasticity (Calabresi et al. 1996a, 1997a). Thus, the remodeling of these neuronal structures might cause an impairment of corticostriatal LTP. Accordingly, it has been shown that dendritic spines constitute the locus of long-term synaptic modifications associated with functional plasticity in other CNS areas (Desmond and Levy 1990).

In the present work we have investigated the effects of 6-OHDA–induced nigrostriatal lesion in the formation of corticostriatal LTP to study the role of DAergic pathway integrity in this form of synaptic plasticity.

METHODS

Adult male Wistar rats (150–250 g, n = 32) were used for all the experiments. The preparation and maintenance of coronal slices have been described previously (Calabresi et al. 1990, 1994). Briefly, corticostriatal coronal slices (200–300 μm) were prepared from tissue blocks of the brain with the use of a vibratome. A single slice was transferred to a recording chamber and submerged in a continuously flowing Krebs solution (35°C, 2–3 ml/min) gassed with 95% O2-5% CO2. The composition of the control solution was (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl2, 1.2 NaH2PO4, 2.4 CaCl2, 11 glucose, and 25 NaHCO3.

Intracellular recording electrodes were filled with 2 M KCl (30–60 MΩ), whereas extracellular electrodes were filled with 2 M NaCl (5–10 MΩ). Intracellular and extracellular potentials were recorded

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with the use of an Axoclamp 2A amplifier, displayed on an oscilloscope, and stored in a digital system. For synaptic stimulation, bipolar electrodes were used. These stimulating electrodes were located either in the cortical areas close to the recording electrode or in the white matter between the cortex and the striatum to activate corticostriatal fibers. During tetanic stimulation the intensity was increased to levels producing the maximal amplitude of the field potential or an action potential on the excitatory postsynaptic potential (EPSP; approximately twice the test intensity). The field potential amplitude was defined as the average of the amplitude from the peak of the early positivity to the peak negativity, and the amplitude from the peak negativity to the peak late positivity. Quantitative data on posttetanic modifications are expressed as percentage of the controls, the latter representing the mean of responses recorded during a stable period (15–30 min) before tetanic stimulation. Values given in the text and in the figures are means ± SE of changes in the respective cell populations. Student’s t-test (for paired and unpaired observations) was used to compare the means. Drugs were applied by dissolving them to the desired final concentration in the saline and by switching the perfusion from control saline to drug-containing saline. Drug solutions entered the recording chamber within 40 s after a three-way tap had been turned on.

6-OHDA (8 μg/4 μl of saline containing 0.1% ascorbic acid) via a Hamilton syringe through a cannula inserted just rostral to the substantia nigra under stereotaxic coordinates (Paxinos and Watson 1986): A, 3.7 mm anterior to the interaural line; V, 2.2 mm dorsal to the interaural line; L, 2.2 mm from the midline. Twenty days later, the rats were tested with 0.5 mg/kg sc apomorphine, and the contralateral turns were recorded with automatic rotometers for 3 h. Only those rats consistently making at least 200 contralateral turns were used for the electrophysiological studies. After brain dissection, we confirmed that the nigrostriatal pathway was lesioned. This was established by noting a >95% loss of DA neurons in the substantia nigra compacta and the almost complete absence of DA terminals in the striatum. This was detected by an immunoperoxidase technique, which utilized a monoclonal antibody for tyrosine hydroxylase. Most of the experiments were performed from rats killed 3–4 mo after the unilateral DA-denervation.

RESULTS

Electrophysiological experiments were conducted on corticostriatal slices obtained from 6-OHDA–denervated striata and contralateral naive striata. Intracellular recordings showed that intrinsic membrane properties of striatal neurons were similar in the naïve and dopamine (DA)-denervated corticostriatal slices. A: tonic action potential discharge is induced by depolarizing current steps (+1.1 nA) both in naïve (a, resting membrane potential = −84 mV) and DA-denervated (b, resting membrane potential = −85 mV) neurons. B: current-voltage plots obtained during single microelectrode voltage-clamp experiments from naïve (left) and DA-denervated neurons (right). In both experiments the cell was held at −85 mV (see text for further details). C: in 1.2 mM magnesium, the blockade of glutamate N-methyl-D-aspartate (NMDA) receptors by 30 μM DL-2-amino-5-phosphovaleric acid (APV) affected neither the excitatory postsynaptic potential (EPSP) recorded from a naïve striatum (a) nor the potential obtained from a DA-denervated striatum (b). In both experiments the resting membrane potential was −85 mV. Conversely, in both experimental groups, the EPSP was fully suppressed by coadministration of 30 μM APV plus 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). In both experiments the resting membrane potential was −86 mV. In Mg-free medium, an APV-sensitive component was present both in a naïve slice (c) and in a slice obtained from a DA-denervated striatum (d). In both experiments the resting membrane potential was −86 mV.
two groups and closely resembled the electrical activity described previously for both naive (Calabresi et al. 1990, 1996b; Cepeda et al. 1994; Jiang and North 1991; Kita et al. 1984) and DA-denervated rat striatal neurons (Calabresi et al. 1993). The resting membrane potential was \( -86 \pm 4 \) (SE) mV in unlesioned striata \((n = 37)\) and \( -85 \pm 4 \) in DA-lesioned striata \((n = 40; P > 0.05)\). In both groups, neurons were silent at rest, and the injection of positive current \((0.6–1.0 \text{nA})\) through the recording pipette induced a tonic firing discharge. Voltage-clamped neurons, at membrane potentials close to the resting level \((-85 \text{ mV})\), from DA-denervated and naive striata displayed similar responses to voltage steps \((0.5–3 \text{s duration})\) of increasing amplitude shifting the membrane in depolarizing and hyperpolarizing directions \((-120 \text{ to } -40 \text{ mV}; n = 10 \text{ for each experimental group}; \text{Fig. 1} B)\). Membrane rectification was present in both groups \((n = 8 \text{ for each experimental group})\). Also synaptic potentials evoked by stimulation of corticostriatal glutamatergic fibers were similar in naive and DA-denervated slices. In fact, in control medium extracellular field potentials (FPs) and intracellularly recorded EPSPs were not affected by the \( N \)-methyl-\( \delta \)-aspartate (NMDA) glutamate receptor antagonist APV \((30 \mu \text{M}, n = 8 \text{ for each experimental group})\) but were almost completely abolished by coadministration of 30 \( \mu \text{M} \) APV plus 10 \( \mu \text{M} \) CNQX, an \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor antagonist, in both groups of animals \((n = 8 \text{ for each experimental group}; \text{Fig. 1}, C_a \text{ and } C_b)\). In magnesium-free solution, a procedure that deactivates NMDA receptors, both FPs and EPSPs increased, unmasking an APV-sensitive component that was similar in naive and DA-denervated striata. In fact, under this condition the pharmacological application of 30 \( \mu \text{M} \) APV produced a significant reduction of the EPSP amplitude and duration. The amplitude of this pharmacological effect was similar in both naive and DA-denervated striata \((n = 8 \text{ for each experimental condition})\). The coadministration of APV and CNQX fully blocked corticostriatal synaptic transmission in both the experimental groups \((n = 8 \text{ for each experimental group}; \text{Fig. 1}, C_c \text{ and } C_d)\).

As previously reported (Calabresi et al. 1992d), in the absence of magnesium ions from the external medium, high-frequency stimulation (HFS; 3 trains, 3 s duration, 100-Hz frequency, 20-s interval) of corticostriatal fibers produced, in the intact striata, an APV-sensitive LTP of extracellularly recorded FPs \((n = 15, P < 0.001; \text{Fig. 2} A_a \text{ and } A_b)\). Similar data were obtained by using intracellular recordings from intact striata \((n = 15, P < 0.001; \text{Fig. 2} B_a \text{ and } B_b)\). Conversely, in DA-denervated slices the same stimulation failed to induce LTP. In these slices, in fact, no significant changes in the amplitude of FPs \((n = 15, P > 0.05; \text{Fig. 2} A_c \text{ and } A_d)\) and EPSPs \((n = 15; \text{Fig. 2} B_c \text{ and } B_d)\) were observed after the conditioning HFS \((P > 0.05)\).
DISCUSSION

In the present study we provide evidence for the absence of LTP in the striatum of DA-denervated striata. Noticeably, the absence of post-tetanic LTP is coupled neither to changes of intrinsic membrane properties of the recorded striatal neurons (resting membrane potential and current-voltage relationship) nor to alterations in the pharmacology of the corticostriatal synaptic potentials evoked by single cortical activation.

A critical role of DA in the long-term regulation of the efficacy of excitatory transmission in the striatum has been demonstrated by previous electrophysiological findings. Coactivation of D1-like and D2-like receptors, in fact, is required for the induction of corticostriatal LTD (Calabresi et al. 1992b,c; Choi and Lovinger 1997), whereas D2-like receptors modulate corticostriatal LTP (Calabresi et al. 1997b). In mice lacking D2 DA receptors, the amplitude of HFS-induced LTP is higher than in control condition, closely resembling the values obtained following the acute blockade of D2-like receptors in slices prepared from control animals (Calabresi et al. 1997). The latter observation strengthens the idea that the integrity of DAergic pathway, and not simply the expression of DA receptors, allows the induction of LTP. The permanent disruption of D2 DA receptor encoding gene, in fact, is not sufficient to mimic the chronic DA-denervation condition.

The integrity of DAergic input from the substantia nigra to the striatum is crucial for the physiological activity of basal ganglia and various pre- and postsynaptic short-term effects of DA have been described in striatal neurons (Calabresi et al. 1987, 1992a, 1993; Cepeda et al. 1998; Surmeier et al. 1992, 1995). Accordingly, the loss of DAergic modulation of striatal synaptic plasticity has been proposed to represent the cellular substrate for parkinsonian symptoms (Calabresi et al. 1996; 1997b) and abnormal synaptic plasticity, in the absence of changes of other electrophysiological parameters, has been found in the striatum of mice lacking D2 receptors (Calabresi et al. 1997b), which present a parkinsonian-like phenotype (Baik et al. 1995). However, the lesion of nigrostriatal DAergic pathway by 6-OHDA injection is the most widely used animal model of PD and causes major morphological and functional changes in the striatum that closely resemble alterations described in parkinsonian patients. Both an increased turnover of DA have been described in striatal neurons (Calabresi et al. 1993; Hornykiewicz 1993; Zigmund et al. 1990). In addition, an increased concentration and release of glutamate from corticostriatal terminals has been reported in the striatum following DA-denervation (Lindefors and Ungerstedt 1990), and morphological and electrophysiological findings strongly support this idea. In 6-OHDA–treated rats and also in PD (Calabresi et al. 1993; Hornykiewicz 1993; Zigmund et al. 1990). In 6-OHDA–treated rats (Ingham et al. 1993) and also in PD (Anglade et al. 1996), in fact, a significant increase in the length of the postsynaptic densities of corticostriatal synapses has been found, suggesting a hyperactivity of these synapses. Furthermore, in vitro electrophysiological recordings showed after nigral lesion a prominent enhancement of spontaneous depolarizing postsynaptic potentials in rat striatal neurons that are blocked by the glutamate receptor antagonist CNQX (Calabresi et al. 1993). Thus the increased release of glutamate in the striatum following DA-deafferentation might represent a plastic compensatory adaptation to the observed loss of long-term facilitation of corticostriatal glutamatergic transmission exerted by DA. Accordingly, in both 6-OHDA–treated animals and PD, motor symptoms become apparent when a great proportion of nigrostriatal neurons is lost.

In conclusion, two different hypotheses might explain the absence of LTP after DA-denervation. First, the loss of this form of synaptic plasticity might be due to the absence of endogenous DA after unilateral 6-OHDA–induced nigral lesion. Alternatively, it is also possible that the profound morphological adaptive synaptic changes observed in the striatum following chronic denervation play a role in the disruption of this form of synaptic plasticity. We are planning future experiments using exogenous DA and DAergic agonists in DA-denervated slices to investigate whether the activation of DA receptors might restore post-tetanic LTP. These future experiments might provide conclusive data concerning this issue.

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