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DEFICITS OF GLUTAMATE TRANSMISSION IN THE STRIATUM OF EXPERIMENTAL HEMIBALLISM

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Abstract—Hemiballism (HB) is a quite rare disorder, generally secondary to stroke, neoplasms or demyelinating plaques, classically considered as almost pathognomonic of a lesion in the subthalamic nucleus (STN). This alteration causes involuntary movements in the chorea–ballism spectrum. One theory is that the output nuclei of the basal ganglia are overinhibited in HB, while little is known about the physiological state of the striatum, the major input structure of the basal ganglia. In the present study, we recorded spontaneous and miniature excitatory and inhibitory postsynaptic currents (sEPSCs, mEPSCs, sIPSCs, mIPSCs) from projection neurons of the striatum of experimental HB. We found a selective reduction of striatal sEPSC and mEPSC frequency following chemical lesion of the STN of the rat, suggesting that reduced synaptic excitation of the input structure of the basal ganglia represents a physiological correlate of HB.

Key words: electrophysiology, hyperkinetic disorders, mEPSC, sEPSC, subthalamic nucleus.

Hemiballism (HB) is a neurological disorder characterized by acute or subacute onset of involuntary movements of one side of the body, generally secondary to stroke, neoplasms or demyelinating lesions of the subthalamic nucleus (STN) (Postuma and Lang, 2003). Although a matter of debate, one theory is that in HB, as well as in other diseases causing involuntary movements, the output nuclei of the basal ganglia are overinhibited, with consequent increase of the motor thalamic activity and generation of hyperkinetic symptoms (DeLong, 1990; Graybiel, 1995; Mitchell et al., 1999; Obeso et al., 2000; Guridi and Obeso, 2001; Postuma and Lang, 2003). Whether hyperkinetic symptoms are associated with altered activity of the input nucleus of the basal ganglia is even more obscure.

Both glutamate- and GABA-mediated synaptic transmission governs the activity of the nucleus striatum, the input structure of the basal ganglia. GABAergic projection neurons, which account for the majority of striatal cells, in fact, are silent at rest and fire action potentials only when depolarized by glutamate released from corticostriatal terminals (Wilson and Kawaguchi, 1996; Stern et al., 1998). GABAergic inputs to these neurons, conversely, are essentially intrinsic and tend to contrast the excitatory action of glutamate. Accordingly, *in vivo*, blockade of ongoing GABAergic inhibition of medium spiny neurons significantly elevates their basal activity (Nisenbaum and Berger, 1992).

Touchon et al. (2004) reported a significant reduction of the extracellular levels of glutamate in the ipsilateral striatum of experimental HB, raising the possibility that the excitatory drive to striatal neurons is reduced in this pathological condition. However, a study addressing the effects of STN lesion on the physiological activity of glutamate inputs to striatal cells is still lacking. Furthermore, striatal GABA transmission in experimental HB has never been investigated. Thus, to identify a possible physiological correlate of HB symptoms, in the present study we measured both excitatory and inhibitory transmission in the striatum of rats with unilateral lesion of the STN. Our results indicate that experimental HB is associated with reduced synaptic excitation of striatal neurons, but normal activity of GABA transmission.

EXPERIMENTAL PROCEDURES

To mimic HB pathology, rats (Sprague–Dawley, Charles River, Marseille, France) (1–2 months) were anesthetized and secured in a Kopf stereotaxic apparatus to receive unilateral injection of ibotenic acid (9.4 μg/μl, i.e. 53 mM in phosphate buffer saline 0.1 M) into the STN (Baunez et al., 2002), a condition which mimics HB symptoms inducing postural impairment, rotation, and contralateral dyskinetic movements of the forepaw (Kafetzopoulos and Papadopoulos, 1983; Phillips et al., 1998; Henderson et al., 1999). All efforts were made to minimize animal suffering and to reduce the number of animals used, in accordance with the European Communities Council Directive of 24 November, 1986 (86/609/EEC). All experiments conformed to guidelines of the University of Rome Tor Vergata on the ethical use of animals.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP, anteroposterior; DV, dorsoventral; EGTA, ethylene glycol-bis (β-aminoethyl ether)-*N,N,N′,N′*-tetra-acetic acid; GTP, guanosine triphosphate; HB, hemiballism; HD, Huntington's disease; L, lateral; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature $GABA_A$-mediated inhibitory postsynaptic current; sEPSC, spontaneous excitatory postsynaptic current; sIPSC, spontaneous $GABA_A$-mediated inhibitory postsynaptic current; STN, subthalamic nucleus; TTX, tetrodotoxin.

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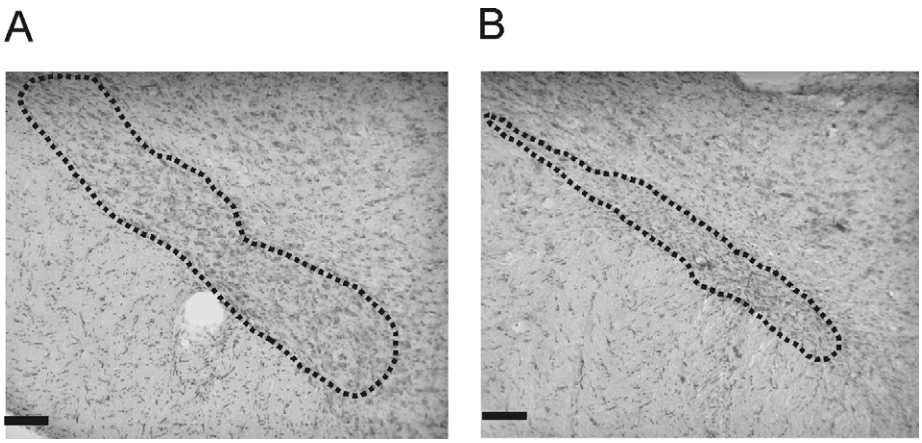

Fig. 1. Histology of STN following ibotenic acid lesion. (A, B) Cresyl Violet staining on a frontal section at the level of the STN (outlined by the tight dotted line): in control condition (A) and after the ibotenic acid lesion (B). An important structural damage is induced in the STN after the ibotenic lesion. Lesions were restricted to this structure, and in most cases few neurons were spared in the very lateral tip. (Scale bars=100 μm.)

The volume injected was 0.5 μl infused over 3 min using a 10-μl Hamilton microsyringe fixed on a micropump (CMA1000, Sweden). The injection coordinates were taken as the average of interaural and bregma coordinates from the atlas of Paxinos and Watson (1986) (from the bregma): anteroposterior (AP), −3.8 mm; lateral (L), ±2.4 mm; dorsoventral (DV), −8.35 mm (from skull) and from the interaural point: AP, +5.2 mm; L, ±2.4 mm; DV, +1.65 mm, incisor bar set at −2.2 mm. When killed for the electrophysiological experiments, blocks of tissue were kept fresh and frozen to be cut with a cryostat. The 40-μm-thick sections were then fixed and stained with Cresyl Violet to detect the extent and location of the STN lesions (Baunez et al., 1995, 2002).

Rats were recorded 1–2 months following STN lesion (n=14) or sham operation (n=14). Corticostriatal coronal slices (200 μm) were prepared from tissue blocks of the brains with the use of a vibratome. A single slice was transferred to a recording chamber and submerged in a continuously flowing artificial cerebrospinal fluid (ACSF, 34 °C, 2–3 ml/min) gassed with 95% O_2–5% CO_2. The composition of the ACSF solution was (mM): NaCl (126), KCl (2.5), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), $CaCl_2$ (2.4), glucose (11), $NaHCO_3$ (25).

The striatum could be readily identified under low power magnification, whereas individual neurons were visualized *in situ* using a differential interference contrast (Nomarski) optical system. This employed an Olympus BX50WI (Japan) non-inverted microscope with ×40 water immersion objective combined with an infrared filter, a monochrome CCD camera (COHU 4912), and a PC compatible system for analysis of images and contrast enhancement (WinVision 2000, Delta Sistemi, Italy). Recording pipettes were advanced toward individual striatal cells in the slice under positive pressure and, on contact, tight GΩ seals were made by applying negative pressure. The membrane patch was then ruptured by suction and membrane current and potential monitored using an Axopatch 1D patch clamp amplifier (Axon Instruments, Foster City, CA, USA). Whole-cell access resistances measured in voltage clamp were in the range of 5–30 MΩ. The few neurons with access resistances between 20 and 30 MΩ were not considered for the analysis of the kinetic properties of the cells.

Whole-cell patch clamp recordings were made with borosilicate glass pipettes (1.8 mm o.d.; 3–5 MΩ). Striatal neurons were clamped at −80 to −85 mV, close to their resting membrane potential. One to six neurons per animal were recorded.

To study spontaneous excitatory postsynaptic currents (sEPSCs) and miniature excitatory postsynaptic currents (mEPSCs), the recording pipettes were filled with internal solution of the following composition (mM): K^+-gluconate (125), NaCl (10),

Table 1. Membrane properties of striatal neurons from control and STN-lesioned rats

Property	Spiny neurons	
	Control	STN lesion
Membrane potential (mV)	−82 (±3)	−84 (±4)
Input resistance (MΩ)	186 (±14)	191 (±16)
Firing elicitated by depolarizing current	Tonic	Tonic
Action potential amplitude (mV)	73 (±6)	76 (±5)
Width at half amplitude (ms)	1.08 (±0.07)	1.04 (±0.05)

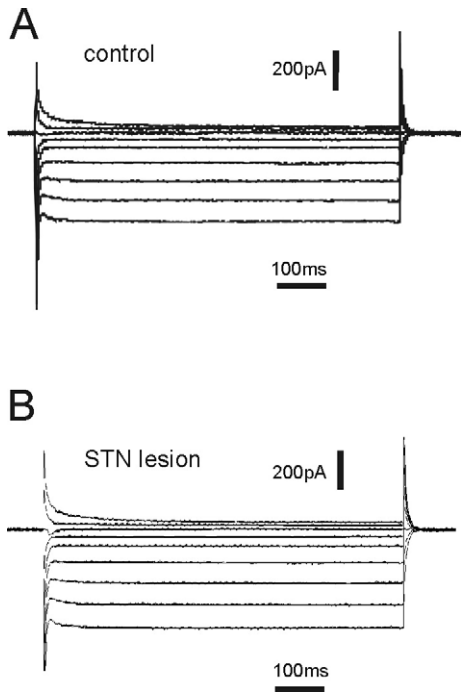

Fig. 2. Intrinsic properties of striatal spiny neurons. (A, B) Examples of current–voltage relationships obtained in voltage-clamp mode by injecting nine voltage steps of progressively increasing amplitude (from −140 mV to −60 mV) in striatal neurons of control (A) and STN lesioned rats (B). Resting membrane potentials of both A and B=−84 mV.