Neuroscience 317 (2016) 12–22

CHOLINERGIC EXCITATION FROM THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS TO THE DENTATE NUCLEUS IN THE RAT

F. VITALE,[a†] C. MATTEI,[a†] A. CAPOZZO,[a] I. PIETRANTONI,[a] P. MAZZONE[b] AND E. SCARNATI[a*]

[a] *Department of Biotechnological and Applied Clinical Sciences (DISCAB), University of L'Aquila, Via Vetoio, Coppito 2, I-67100 L'Aquila, Italy*

[b] *ASL RM2 Regional Center for Functional Neurosurgery and DBS, Operative Unit of Stereotactic and Functional Neurosurgery, CTO Alesini Hospital, Via San Nemesio 21, 00145 Rome, Italy*

Abstract—In spite of the existence of pedunculopontine tegmental nucleus (PPTg) projections to cerebellar nuclei, their nature and functional role is unknown. These fibers may play a crucial role in postural control and may be involved in the beneficial effects induced by deep-brain stimulation (DBS) of brainstem structures in motor disorders. We investigated the effects of PPTg microstimulation on single-unit activity of dentate, fastigial and interpositus nuclei. The effects of PPTg stimulation were also studied in rats whose PPTg neurons were destroyed by ibotenic acid and subsequently subjected to iontophoretically applied cholinergic antagonists. The main response recorded in cerebellar nuclei was a short-latency (1.5–2 ms) and brief (13–15 ms) orthodromic activation. The dentate nucleus was the most responsive to PPTg stimulation. The destruction of PPTg cells reduced the occurrence of PPTg-evoked activation of dentate neurons, suggesting that the effect was due to stimulation of cell bodies and not due to fibers passing through or close to the PPTg. Application of cholinergic antagonists reduced or eliminated the PPTg-evoked response recorded in the dentate nucleus. The results show that excitation is exerted by the PPTg on the cerebellar nuclei, in particular on the dentate nucleus. Taken together with the reduction of nicotinamide adenine dinucleotide phosphate-diaphorase-positive neurons in lesioned animals, the iontophoretic experiments suggest that the activation of dentate neurons is due to cholinergic fibers. These data help to explain the effects of DBS of the PPTg on axial motor disabilities in neurodegenerative disorders.

Key words: pedunculopontine tegmental nucleus, cerebellum, acetylcholine, electrophysiology, neurodegenerative disorders.

INTRODUCTION

The cerebellum and basal ganglia have been long considered as anatomically separated neuronal systems that perform distinct functional operations in the control of motor, cognitive, and behavioral tasks. According to this view, the cerebral cortex is the main locus of interactions between the two systems. This traditional view has been challenged in recent years by studies using transneural transport of the rabies virus in monkeys, which proved the existence of two disynaptic pathways, linking, respectively, the dentate nucleus to the striatum by way of thalamic nuclei (Hoshi et al., 2005), and the subthalamic nucleus (STN) to the cerebellar cortex by way of pontine nuclei (Bostan et al., 2010, 2013).

The discovery of these connections has, on the one hand, shown that the basal ganglia communicate with the cerebellum, and on the other hand, provided a basis to better consider the role of the cerebellum in basal ganglia-related motor disorders. For instance, the loss of dopamine (DA) neurons in the substantia nigra that occurs in Parkinson's disease (PD) causes a profound disruption and increased activity of STN neurons (Rodriguez-Oroz et al., 2001; Wichmann and Delong, 2006). Such abnormal activity is consistent with functional changes occurring in the absence of DA in the indirect pathway that links the striatum to STN neurons (Albin et al., 1995; Obeso et al., 1997; Nambu, 2009). Given the existence of the STN-cerebellum pathway, disruption of STN activity would also affect the cerebellum. Another structure worthy of attention in investigating the relationships between the basal ganglia and cerebellum is the pedunculopontine tegmental nucleus (PPTg). Several aspects of motor and non-motor functions in which the PPTg is involved have been the subject of many reviews (Garcia-Rill, 1991; Inglis and Winn, 1995; Scarnati and Florio, 1997; Winn et al., 1997; Lee et al., 2000; Pahapill and Lozano, 2000; Takakusaki et al., 2003; Winn, 2006, 2008; Takakusaki, 2009; Garcia-Rill, 2015). In spite of some controversy (Gut and Winn, 2015), the motor nature of the PPTg is supported by solid evidence: it is part of the mesencephalic locomotor region (Skinner and Garcia-Rill, 1984; Sherman et al., 2015), exhibits connections with basal ganglia (Edley and Graybiel, 1983; Jackson and Crossman, 1983; Sugimoto and Hattori, 1984; Rye et al., 1987; Lee et al., 1988; Woolf and Butcher, 1989; Lavoie and Parent, 1994a,c; Futami et al., 1995; Dautan et al., 2014), deep cerebellar nuclei, and cerebral cortex (Woolf and Butcher, 1989; Hazrati

*Corresponding author.
E-mail address: scarnati@univaq.it (E. Scarnati).
† These authors equally contributed to this work.
Abbreviations: ACh, acetylcholine; DA, dopamine; DBS, deep brain stimulation; GLU, glutamate; NADPH-diaphorase, nicotinamide adenine dinucleotide phosphate-diaphorase; PD, Parkinson's disease; PETH, peri-event time histogram; PPTg, pedunculopontine tegmental nucleus; STN, subthalamic nucleus.

http://dx.doi.org/10.1016/j.neuroscience.2015.12.055
0306-4522/

and Parent, 1992; Ruggiero et al., 1997; Aravamuthan et al., 2007), sends fibers to brainstem reticulospinal neurons (Rye et al., 1988; Skinner et al., 1990a,b; Grofova and Keane, 1991), integrates vestibular, proprioceptive, and reward-related signals which trigger goal-directed movements (Krauthamer et al., 1995; Reese et al., 1995a,b; Kobayashi and Isa, 2002; Aravamuthan and Angelaki, 2012; Hong and Hikosaka, 2014), and has been recently reported to be activated in locomotor imaginary tasks (Tattersall et al., 2014; Lau et al., 2015). In PD, PPTg neurons degenerate (Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989), hence it is reasonable to hypothesize that cerebellar functions may be compromised to some extent in PD patients.

The PPTg has a complex and heterogeneous cytoarchitecture, being composed of different types of neurons, some of which are cholinergic (Mesulam et al., 1983; Manaye et al., 1999). Other neurons have been reported to express glutamate (GLU), GABA or co-localization of different neurotransmitters (Lavoie and Parent, 1994b; Wang and Morales, 2009; Martinez-Gonzalez et al., 2012). Acetylcholine (ACh) and GLU neurons are involved in PPTg efferents in different ways; namely, the former is present mainly in neurons from which ascending fibers are directed to thalamic structures and the latter is present in the brainstem and spinal cord (Grofova and Keane, 1991; Sherman et al., 2015). A GLU PPTg input to the substantia nigra has been also reported (Scarnati et al., 1986; Futami et al., 1995; Charara et al., 1996).

PPTg fibers directed to the cerebellum have been reported (Woolf and Butcher, 1989; Hazrati and Parent, 1992; Newman and Ginsberg, 1992; Ruggiero et al., 1997), but not mentioned in others (Martinez-Gonzalez et al., 2011). Today, the nature of these fibers needs to be investigated, given the demonstration of a PPTg cerebellum tract in the human brain (Aravamuthan et al., 2007), and considering that freezing of gait and postural instability in advanced PD are refractory to dopaminergic medication, while patients benefit from deep-brain stimulation (DBS) of the PPTg (Ferraye et al., 2010; Moro et al., 2010; Ostrem et al., 2010; Thevathasan et al., 2011; Mazzone et al., 2013, 2014). These latter results have led some authors to consider gait and axial disturbances in PD as possible consequences of a disruption of ACh mechanisms in the brainstem rather than of the nigrostriatal DA system, and the severity of these motor disabilities has been directly related to the degree of ACh neuronal loss in the PPTg (Karachi et al., 2010; Bohnen et al., 2013). Thus, the present study investigated how electrical microstimulation of the PPTg modulates the activity of neurons in deep cerebellar nuclei, and to provide insights into the neurotransmitter involved in such modulation. This was accomplished by recording changes in PPTg-evoked responses in intact rats as well as in rats in which a loss of ACh-containing neurons was induced. The involvement of ACh in PPTg-evoked responses of cerebellar neurons was further investigated by iontophoretic application of ACh antagonists on dentate nucleus neurons, since these neurons were the most responsive to PPTg-stimulation in the course of the study.

EXPERIMENTAL PROCEDURES

Animals

Experiments were carried out on 30 male Wistar albino rats (240–280 g) anesthetized with chloral hydrate (400 mg/kg i.p.) supplemented with additional i.m. injections as required. The animals were fixed in a stereotaxic frame according to Paxinos and Watson (1998). Body temperature was kept at 37–38 °C by a recirculating hot water pad controlled by an intracolonic telethermometer. Heart rate was continuously monitored and recorded. All the experimental procedures were conducted in accordance with the European Union Directive (2010/63/UE) and supervised by the University Veterinary Service. The experiments were also planned to minimize the number of animals and their sufference.

Electrophysiology

Single-unit activity of cerebellar nuclei neurons was recorded using a glass micropipette filled with sodium acetate containing 2% Pontamine Sky Blue (resistance 4–8 MΩ, tip 1–2 μm), vertically mounted on a micro manipulator. Recordings were taken through 2–3 parallel tracks made in the fastigial (five rats, AP from −2.5 to −2.8 mm posterior to the interaural line; L from 0.4 to 1.6 mm lateral to the midline; DV from 4.0 to 4.4 mm above the interaural line); interpositus (five rats, AP from −2.0 to −2.8 mm posterior to the interaural; L from 2.0 to 3.4 mm lateral to the midline; DV from 3.4 to 4.2 mm above the interaural line); or dentate nucleus (ten rats, AP from −2.0 to −2.6 mm posterior to the interaural line; L from 2.6 to 4.2 mm lateral to the midline; DV from 3.6 to 4.6 mm above the interaural line), according to the Paxinos and Watson's stereotaxic atlas (Paxinos and Watson, 1998).

A bipolar concentric stainless steel stimulating electrode manufactured by FHC (Bowdoin, ME, USA) (325 μm external diameter, 125 μm inner pole diameter, 200 μm non-insulated exposures, 200 μm tips separation) was stereotaxically positioned in the caudal part of the PPTg at a final target 1 mm anterior, 2 mm lateral and 3 mm above the interaural line (7.6 mm below the dura). This region was chosen after considering the relative abundance of ACh neurons in this subregion of the PPTg compared to other types of neurons (Martinez-Gonzalez et al., 2011). The electrode was inclined 10°–15° both in the lateral and anteroposterior plane to gain optimal surface for inserting the recording pipette and avoiding bleeding from vessels in the dura overlying the PPTg region.

Neuronal activity was amplified, filtered and discriminated from baseline through a Digitimer Neurolog system (Digitimer, Welwyn Garden City, Hertfordshire, England), displayed on storage oscilloscope, and analyzed online and offline by the BrainWave Workbench ver.6.1 and SciWorks ver.8 systems (DataWave Technologies, Berthoud, CO, USA). Only neurons with a signal-to-noise ratio of at least 3:1 were studied. Impulses were converted into digital pulses and analyzed online and offline in order to