Experimental Neurology 265 (2015) 160–170

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Regular Article

Gap junction blockers attenuate beta oscillations and improve forelimb function in hemiparkinsonian rats

Sujoy Phookan [a], Alexander C. Sutton [a], Ian Walling [a], Autumn Smith [a], Katherine A. O'Connor [a], Joannalee C. Campbell [a], Megan Calos [a], Wilson Yu [a], Julie G. Pilitsis [a,b], Jonathan M. Brotchie [c], Damian S. Shin [a,*]

[a] Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, NY USA
[b] Division of Neurosurgery, Albany Medical Center, Albany, NY USA
[c] Division of Brain Imaging and Behavioral Neuroscience Systems, Toronto Western Research Institute, Toronto Western Hospital, Toronto, ON, Canada

ARTICLE INFO

Article history:
Received 20 December 2014
Accepted 14 January 2015
Available online 23 January 2015

Keywords:
6-OHDA
Carbenoxolone
Globus pallidus externa
Parkinson's disease

ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease characterized by akinesia, bradykinesia, resting tremors and postural instability. Although various models have been developed to explain basal ganglia (BG) pathophysiology in PD, the recent reports that dominant beta (β) oscillations (12–30 Hz) in BG nuclei of PD patients and parkinsonian animals coincide with motor dysfunction has led to an emerging idea that these oscillations may be a characteristic of PD. Due to the recent realization of these oscillations, the cellular and network mechanism(s) that underlie this process remain ill-defined. Here, we postulate that gap junctions (GJs) can contribute to β oscillations in the BG of hemiparkinsonian rats and inhibiting their activity will disrupt neuronal synchrony, diminish these oscillations and improve motor function. To test this, we injected the GJ blockers carbenoxolone (CBX) or octanol in the right globus pallidus externa (GPe) of anesthetized hemiparkinsonian rats and noted whether subsequent changes in β oscillatory activity occurred using in vivo electrophysiology. We found that systemic treatment of 200 mg/kg CBX attenuated normalized GPe β oscillatory activity from 6.10 ± 1.29 arbitrary units (A.U.) (pre-CBX) to 2.48 ± 0.87 A.U. (post-CBX) with maximal attenuation occurring 90.0 ± 20.5 min after injection. The systemic treatment of octanol (350 mg/kg) also decreased β oscillatory activity in a similar manner to CBX treatment with β oscillatory activity decreasing from 3.58 ± 0.89 (pre-octanol) to 2.57 ± 1.08 after octanol injection. Next, 1 μl CBX (200 mg/kg) was directly injected into the GPe of anesthetized hemiparkinsonian rats; 59.2 ± 19.0 min after injection, β oscillations in this BG nucleus decreased from 3.62 ± 1.17 A.U. to 1.67 ± 0.62 A.U. Interestingly, we were able to elicit β oscillations in the GPe of naive non-parkinsonian rats by increasing GJ activity with 1 μl trimethylamine (TMA, 500 nM). Finally, we systemically injected CBX (200 mg/kg) into hemiparkinsonian rats which attenuated dominant β oscillations in the right GPe and also improved left forepaw akinesia in the step test. Conversely, direct injection of TMA into the right GPe of naive rats induced contralateral left forelimb akinesia. Overall, our results suggest that GJs contribute to β oscillations in the GPe of hemiparkinsonian rats.

Introduction

Parkinson's disease (PD) is a neurodegenerative disease characterized by akinesia, bradykinesia, resting tremors and postural instability. These motor impairments arise from the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNC) and the subsequent loss of dopamine in the striatum, which ultimately leads to dysfunction of the basal ganglia (BG) (Obeso et al., 2000; Wichmann and DeLong, 2003).

The recent awareness that PD patients and parkinsonian animals exhibit dominant beta (β) oscillations (12–30 Hz) in BG nuclei that coincide with motor dysfunction provides a novel way to describe BG dysfunction which focuses on akinetic "pathological" oscillations as a characteristic of PD (Avila et al., 2010; Kuhn et al., 2008; Levy et al., 2002; McCarthy et al., 2011; Sharott et al., 2005; Soares et al., 2004). In non-pathological states, β oscillations are normally present in rats (Hamada et al., 1999), monkeys (Lebedev and Wise, 2000; Lee, 2003; MacKay and Mendonca, 1995) and humans (Wheaton et al., 2005) and serve as a default, inactive state prior to the commencement of information processing and motor initiation (Brown and Williams, 2005; Courtemanche et al., 2003). However, β oscillations become dominant in the cortex, striatum, STN, substantia nigra reticulata

Abbreviations: GJ, Gap Junctions; CBX, Carbenoxolone; A.U., Arbitrary Units; TMA, Trimethylamine; LFP, Local Field Potential; TH, Tyrosine Hydroxylase; LAT, Limb Asymmetry Test; DAB, Diaminobenzidine; I_{Nap}, Persistent Sodium Channel; HCN, Hyperpolarization-Activated Cyclic Nucleotide-Gated Ion Channel; NaF, Fast, Transient Voltage-Dependent Sodium Channels

* Corresponding author at: Center for Neuropharmacology & Neuroscience, Albany Medical College, 43 New Scotland Ave., Albany, NY 12208, USA. Fax: +1 518 262 5799.
E-mail address: shind@mail.amc.edu (D.S. Shin).

http://dx.doi.org/10.1016/j.expneurol.2015.01.004
0014-4886/

(SNR), GPi and globus pallidus externa (GPe) in PD patients (Kuhn et al., 2008; Levy et al., 2002; Silberstein et al., 2003) and parkinsonian animals (Avila et al., 2010; McCarthy et al., 2011; Sharott et al., 2005; Soares et al., 2004) with akinesia and/or bradykinesia.

While the function of β oscillations in the BG in PD remains uncertain, there is compelling, but indirect evidence to allege that modulating β oscillations can influence motor function in PD patients. For instance, levodopa treatment or DBS in PD improves motor function while concurrently decreasing the dominance of these oscillations (Avila et al., 2010; Gaynor et al., 2008; Hammond et al., 2007; Sutton et al., 2013a; Sutton et al., 2013b). In non-PD individuals with recording electrodes in the GPi, voluntary movement coincided with bilateral de-synchronization of β oscillations (Tang et al., 2005), while healthy volunteers undergoing cortical transcranial stimulation at the β frequency (20 Hz) exhibited slower voluntary movement (Pogosyan et al., 2009). Furthermore, PD patients stimulated at the β frequency in the STN exhibited worsened elbow movements (Chen et al., 2013) or increased rigidity of the wrist (Little et al., 2012). Lastly, optogenetic stimulation of STN at 20 Hz worsened parkinsonian symptoms in PD rats (Gradinaru et al., 2009).

Limited experimental and computational studies showed that synaptic interaction and the activity of certain ion channels that exist between BG nuclei contribute to the formation and/or propagation of β oscillations in PD. While these studies provided important information about these processes, it is still unclear how these oscillations form and/or propagate or what their explicit role is in PD. In this study, we assess whether gap junctions (GJs) can underlie the formation and/or propagation of these akinetic β oscillations in PD since GJs are expressed in many BG nuclei of normal and parkinsonian preclinical models such as the striatum (Adermark and Lovinger, 2008; Hjorth et al., 2009; Onn and Grace, 1999), SNC (Berretta et al., 2001; Kawasaki et al., 2009; Lin et al., 2003; Vandecasteele et al., 2005), GPe (Rash et al., 2000; Vis et al., 1998) and SNR (Kawasaki et al., 2009). GJs can synchronize neuronal activity in the hippocampus (Draguhn et al., 1998; Traub et al., 2000) and neocortex (Beierlein et al., 2000) to produce neuronal oscillations and they are involved in β oscillations in the rat somatosensory cortex in vitro (Roopun et al., 2006). Lastly, a loss of dopamine in parkinsonian animals coincides with increased GJ activity (Cepeda et al., 1989; Onn and Grace, 1994, 1999) and it was recently reported that PD patients have higher expression levels of GJs in the BG (Schwab et al., 2014).

In this study, we recorded local field potentials (LFPs) from the GPe of anesthetized hemiparkinsonian rats with and without GJ modulators. To show proof-of-concept that modulating GJs can alter motor function in these animals, we tested forelimb akinesia in these animals using the self-adjusting step test (Olsson et al., 1995) and the limb-use asymmetry test (LAT) (Schallert et al., 2000).

Materials and methods

Animals and surgery

All animal use was conducted with approval from the Institutional Animal Care and Use Committee at Albany Medical College. We used the 6-hydroxydopamine (6-OHDA) hemiparkinsonian rat, which is a well-accepted animal model of PD (Blandini et al., 2008; Ungerstedt, 1968). Male Sprague Dawley rats weighing 225–300 g were anesthetized with 2% isoflurane using an inhalant system (Harvard Apparatus, MA, USA) in a stereotaxic frame (David Kopf Instruments, CA, USA). Rats were injected intraperitoneally (IP) with desipramine (25 mg/kg) and pargyline (50 mg/kg) 20–30 min prior to craniotomy and body temperature was maintained at 37 °C throughout the surgery (Homeothermic Monitor, Harvard Apparatus, MA, USA). Lubrifresh (Major Pharmaceuticals, MI, USA) was applied to the eyes to prevent dehydration and 2% lidocaine gel (Akorn Pharmaceuticals, IL, USA) was applied to the ear bars to minimize discomfort. An injectable 2% lidocaine solution (Hospira Inc., IL, USA) was administered subcutaneously (SC) under the shaved scalp to minimize discomfort. After, a burr hole was made in the cranium such that 4 μl of 6-OHDA (3 μg/μl, made up in 0.1% ascorbic acid) or saline (0.9% NaCl) could be injected into the right medial forebrain bundle (from Bregma: 4.8 mm posterior, 1.2 mm lateral, and 7.7 mm ventral from dura). After injection of 6-OHDA or saline, rats had their incisions stapled shut and were given penicillin (80 μg/kg), buprenorphine (0.12 g/kg) and saline (10 ml/kg) SC, as well as topical bacitracin post-operatively; buprenorphine was also given every 12 h for 72 h post-surgery for pain management.

We confirmed animals as PD in a similar manner as before (Sutton et al., 2013a; Sutton et al., 2013b); first, we tested for forelimb akinesia 2–3 weeks after craniotomy and 6-OHDA injection and then we verified the loss of DAergic terminals in the striatum in paraformaldehyde-fixed brain sections using tyrosine hydroxylase (TH) immuno-reactivity after experiments were completed and rats were sacrificed. Quantification was done with stereology using Image J software (NIH, MD, USA) and represented as TH immuno-reactivity in the right lesioned hemisphere/left un-lesioned hemisphere.

Behavioral tests

The LAT was used to assess forepaw motor function and akinesia (Schallert et al., 2000). In brief, naive, sham-lesioned or hemiparkinsonian rats were placed individually in an upright plexiglass cylinder (20 cm in diameter, 30 cm high) and video recorded for 5–15 min while it explored and touched the glass with their forepaws. Forepaw contacts were noted by two evaluators and later calculated as # right contacts/# total contacts × 100%; one evaluator was experimentally-blinded and the other was not. A hemiparkinsonian rat will display akinesia-like immobility in the forepaw (left side) contralateral to the 6-OHDA lesion, while forepaw use in the intact, ipsilateral side (right side) will remain unimpaired. Therefore, a value of 50% is characterized as normal, while an animal that touched more than 80% with its right forepaw correlated with >90% striatal dopamine depletion on the ipsilateral side (Schallert et al., 2000).

We also employed the self-adjusting step test (Olsson et al., 1995) as another means to test for changes in forelimb akinesia. Briefly, this test requires the experimenter to restrain both hindlimbs and the non-tested forelimb while allowing the tested forelimb freedom of motion. The rat is then moved forward and backward horizontally for 10 s along a surface 100 cm long. During this test, the un-restrained forepaw is allowed to contact the surface. The presence of forelimb akinesia is noted when unequal forepaw contacts are made onto the surface. Ipsilateral (right forepaw) step percentage is calculated by ipsilateral steps/total steps × 100% and averaged from three trials for each animal. Both the step test and LAT were performed 21 days after 6-OHDA or saline injection for hemiparkinsonian or sham-lesioned rats, respectively.

To determine whether decreasing or disrupting β oscillations in hemiparkinsonian rats improved motor function, these animals were tested with the step test to assess forelimb akinesia. After, PD rats were IP-injected with CBX (200 mg/kg, 1 ml/kg) and the step test was repeated every 30 min post-treatment for 2 h to evaluate any alteration in forelimb performance. As a control, a separate group of hemiparkinsonian and naive rats were IP-injected with saline (1 ml/kg) and evaluated for forelimb akinesia in the same manner. We also directly injected 1 μl trimethylamine (TMA, 500 nM) (Medina-Ceja and Ventura-Mejia, 2010; Nassiri-Asl et al., 2008) into the GPe of naive non-parkinsonian rats to determine whether forelimb akinesia could manifest by increasing GPe synchrony and inducing β oscillatory activity; TMA is a chemical that increases oscillatory synchrony in anesthetized rats by opening GJs (Nassiri-Asl et al., 2008) and increasing GJ activity (Bocian et al., 2011). Since TMA is not blood–brain-barrier (BBB) permeable, this chemical was injected intracerebrally (Juszczak and Swiergiel, 2009). We also employed the step test for this experiment, instead of the LAT, since