Electro-Acupuncture Stimulation Acts on the Basal Ganglia Output Pathway to Ameliorate Motor Impairment in Parkinsonian Model Rats

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The role of electro-acupuncture (EA) stimulation on motor symptoms in Parkinson’s disease (PD) has not been well studied. In a rat hemiparkinsonian model induced by unilateral transection of the medial forebrain bundle (MFB), EA stimulation improved motor impairment in a frequency-dependent manner. Whereas EA stimulation at a low frequency (2 Hz) had no effect, EA stimulation at a high frequency (100 Hz) significantly improved motor coordination. However, neither low nor high EA stimulation could significantly enhance dopamine levels in the striatum. EA stimulation at 100 Hz normalized the MFB lesion-induced increase in midbrain GABA content, but it had no effect on GABA content in the globus pallidus. These results suggest that high-frequency EA stimulation improves motor impairment in MFB-lesioned rats by increasing GABAergic inhibition in the output structure of the basal ganglia.

Keywords: electro-acupuncture, DA, GABA, Rota-Rod treadmill, Parkinson’s disease

Acupuncture is a popular alternative therapy in patients with Parkinson’s disease (PD) and might provide some benefit in the clinical setting (Rabinstein & Shulman, 2003). A survey of PD patients demonstrated that patients who received acupuncture reported improvement of their symptoms (Shulman et al., 2002). In addition, acupuncture is very safe and well tolerated, and it has no known interactions with medications (Eng, Lyons, Greene, & Pahwa, 2006).

PD is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). The behavioral deficits of PD, including resting tremor, rigidity, and bradykinesia, are expressed when striatal dopamine (DA) depletion exceeds 60–80% (Bernheimer, Birkmayer, Hornykiewicz, Jellinger, & Seitelberger, 1973). It has followed from these observations that the best course of therapy might be to increase the DA content of the striatum. However, increasing evidence suggests that a poor relationship exists between the dopamine content of the striatum and the improvement of motor symptoms during treatment (Gash et al., 1996; Tseng, Baetge, Zurn, & Aebischer, 1997). On the other hand, parkinsonian movement disorders are often associated with abnormalities in GABA neuron activity in the substantia nigra pars reticulata (SNr) (Zhou, Matta, & Zhou, 2008). Firing intensity and patterns are often altered in the SNr GABAergic output pathways in the parkinsonian brain (Hutchison et al., 2004). Inactivation of SNr in a primate model of PD has been shown to ameliorate parkinsonian motor symptoms, further demonstrating the importance of SNr in motor control (Wichmann, Kliem, & DeLong, 2001).

Previous work has demonstrated that high-frequency electro-acupuncture (EA) stimulation significantly reduced abnormal rotational behavior and enhanced the survival of degenerating dopaminergic neurons following medial forebrain bundle (MFB) axotomy (Liang et al., 2003). These effects might be attributable to enhanced synthesis and release of neurotrophic factors (Liang et al., 2002, 2003) and alleviation of inflammatory reactions in the SN (Liu et al., 2004). Furthermore, EA stimulation reversed the lesion-mediated decrease of Substance P content in the ventral midbrain (Jia et al., 2009). These studies suggested that EA stimulation may result in changes in neuronal activity in the basal ganglia (Zangen & Hyodo, 2002).

Therefore, the present study was conducted to evaluate the effects of different frequencies of EA stimulation (0, 2, or 100 Hz) on motor behavior and dopamine content in the striatum in MFB-lesioned rats. Changes in GABA content in the midbrain and the globus pallidus (GP) were also measured to assess the modulatory effect of EA stimulation on neuronal activity in the basal ganglia.

Method

Animal Care and MFB Axotomy

Adult male Wistar rats weighing 200–230 g were obtained from the laboratory animal center, Capital Medical University, and housed in a standard 12-hr light–dark cycle with ad libitum access to food and water. The rats were anesthetized with chloral hydrate (350 mg/kg ip) and then positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the mouth bar set at...
−3.3 mm. MFB lesions were performed using a retractable Scouten wire knife as described previously (Tseng et al., 1997). The experimental procedures were approved by the Committee on Animal Care and Usage, Capital Medical University, and all efforts were made to minimize animal suffering.

**EA Stimulation**

Rats were randomly divided into five groups: the sham group, the MFB-lesioned group, and the MFB-lesioned group followed by 0-, 2-, and 100-Hz EA stimulation. The EA stimulation was administered on Day 2 following MFB lesioning as described previously (Liang et al., 2003). Two stainless steel needles 0.25 mm in diameter and 5 mm in length were inserted obliquely at the acupuncture point Dazhui (Du 14, just below the spinous process of the vertebra prominens) and horizontally at the Baihui point (Du 21, at the midpoint of the line connecting the two ears). For the 0-Hz group, the needles were placed into the acupoints, but no electrical stimulation was administered. Bidirectional square wave electrical pulses (0.2-ms duration, 2 Hz and 100 Hz), designated as EA, were given for a total of 30 min each day, 6 days per week. The duration of EA treatment was limited to 4 weeks. The intensity of the stimulation was increased stepwise from 1 to 2 mA and then to 3 mA, with each step lasting 10 min. EA was administered to awake, unrestrained animals while in their cages.

**Rota-Rod Test**

The motor coordination of rats was evaluated on Rota-Rod treadmills (Stoelting Company, Wood Dale, IL). The apparatus consisted of a base platform and a rotating rod with a diameter of 6 cm and a nonslippery surface. The accelerating speed of the Rota-Rod was set to increase from 6 rpm/min to 40 rpm/min within 2 min. The rats were placed on the treadmill, and the timers were set with acceleration programmed to automatically stop when the rat fell off. The maximal cutoff time was set to 600 s. Rats were tested on the Rota-Rod at 7, 14, 21, and 28 days following MFB lesioning.

**Tissue Collection and Processing**

Five rats from each group were randomly selected 28 days after MFB transection. After decapitation, the brain was quickly resected from the rat. The ventral midbrain (the section of the SN, bregma from −4.8 to −6.3 mm) and GP were dissected, rapidly frozen on dry ice, and stored at −80°C.

**High-Performance Liquid Chromatography (HPLC)**

Concentrations of DA were quantified by a modified method of HPLC combined with electrochemical detection (HPLC-ECD) as described elsewhere (Kotake, Heffner, Vosmer, & Seiden, 1985). The concentrations of GABA were determined using HPLC with laser-induced fluorescence detection. On the day of assay, tissue samples were weighed and then homogenized in 150 μl of 0.1 mM acetic acid as described previously (Carlson, Behrstock, Tobin, & Salamone, 2003). Briefly, samples or standards were derivatized with the same volume of the o-phthalaldehyde reagent solution (P0532 of Sigma Chemical, St. Louis, MO). The resulting mixture was automatically injected by a refrigerated autoinjector into a symmetry shield-C18 reverse-phase column (150 × 3.8 mm, 5 μm particle size; Waters Corporation, Milford, MA). The mobile phase consisted of 50 mM sodium acetate (pH was adjusted to 6.8 using acetic acid), methanol, and tetrahydrofuran. The flow rate was 1.0 ml/min, maintained with two Shimadzu LC (Agilent, Santa Clara, CA) pumps. Putative GABA peaks were identified by comparing retention times of GABA standard (Sigma–Aldrich) to retention times of the sample peak. The concentrations were estimated by rationing peak areas of GABA and its external standard (Agilent Chemstation analytical software). The running time for each determination was 40 min.

**Statistical Analysis**

Data were expressed as means ± SEM. Statistical significance was assessed using one-way analysis of variance followed by the Newman–Keuls post hoc test of difference between groups. A value of p < .05 was considered statistically significant.

**Results**

**EA Stimulation Improves Motor Coordination**

The Rota-Rod treadmill test was performed following EA stimulation of all experimental rats to investigate the effects of different frequencies of EA stimulation on motor coordination. The MFB-lesioned rats demonstrated a decreased treadmill occupancy time compared with the sham group under all conditions (see Figure 1A). From Week 2, the rats receiving 100-Hz EA stimulation demonstrated significantly increased treadmill occupancy time compared with MFB-lesioned rats (p < .05). These improvements in motor coordination were retained for the duration of the high-frequency EA treatment. These data demonstrate that 100-Hz EA stimulation can enhance motor coordination in MFB-lesioned rats.

**EA Stimulation Did Not Increase DA Content in the Striatum**

Wire knife lesions of the MFB dramatically decreased the DA content in the ipsilateral striatum in hemiparkinsonian rats compared with the sham group (p < .01). In contrast, MFB lesions did not result in significant changes in DA levels on the contralateral side (see Figure 1B). The HPLC-ECD analysis data demonstrate that neither low- nor high-frequency EA stimulation could enhance the DA level in the ipsilateral striatum. These results suggest that the improvement in motor coordination is not fully dependent on the restoration of the striatal DA content, as seen in our EA-treated hemiparkinsonian rats.

**Effect of EA on GABA Content in the GP and the Midbrain**

To investigate the effects of MFB lesioning and EA treatment on the output structures of the basal ganglia, we examined the GABA content in the GP and the ventral midbrain area. Our data show that the GABA content of the ipsilateral GP was higher in MFB-lesioned rats than in sham groups, but no changes were observed on the contralateral side (see Figure 2A). This result is consistent with previous reports that ipsilateral DA lesions induce...
a marked increase in the GABA levels in the output nuclei (the GPi and the SNr) of the basal ganglia (Windels, Carcenac, Poupard, & Savasta, 2005). No significant differences in the GABA content were detected between rats with the MFB lesion and hemiparkinsonian rats receiving EA stimulation of any frequency. In contrast to the results from the GP, 100-Hz EA stimulation normalized midbrain GABA from the increased levels observed in MFB-lesioned rats. This effect was not seen in hemiparkinsonian rats subjected to 0- or 2-Hz EA stimulation (see Figure 2B).

Discussion

The present study demonstrates that high-frequency EA stimulation can improve the motor coordination of MFB-lesioned rats through a mechanism that involves the potentiation of GABAergic inhibition in the basal ganglia output structure (SNr).

Previous studies have confirmed that a slower rate of degeneration and progression of motor impairment occurs following MFB axotomy (Brecknell, Dunnett, & Fawcett, 1995; Knusel et al., 1992). In this model, 100-Hz EA stimulation (but not 2 Hz or 0 Hz) significantly improves motor coordination as manifested by performance on the Rota-Rod test. However, the mechanisms underlying this improvement are not fully understood.

The striatum is the primary projection target of SNc dopaminergic neurons. Movement disorders can result when striatal DA depletion exceeds 70%. The restoration of DA levels in the striatum is thought to be critical for treatment of PD. However, many studies have demonstrated motor improvement without restoration of striatal DA content. For example, intracerebral injection of glial

Figure 1. (A) Effects of electro-acupuncture (EA) stimulation on motor coordination of rats measured by the Rota-Rod treadmill test. The time spent on the treadmill among the five groups was recorded at the end of 1, 2, 3, and 4 weeks post-transection. Values are represented as means ± SEM; n = 7–15; * p < .05, ** p < .01 versus the medial forebrain bundle (MFB)-lesioned group. (B) Effect of EA stimulation on striatum dopamine (DA) content of MFB-transected rats 28 days after MFB transection. The striatum dopamine content was measured by high-performance liquid chromatography with electrochemical detection. The results are given as means ± SEM; n = 6–10; * p < .01 versus sham lesioned side.

Figure 2. Effect of electro-acupuncture (EA) stimulation on (A) globus pallidus (GP) and (B) ventral midbrain GABA content in medial forebrain bundle (MFB)-transected rats. The GABA content was measured by high-performance liquid chromatography. The histograms represent the mean content on the unlesioned side and the lesioned side of MFB-lesioned rats. The values are expressed as percentages ± SEM of the sham values; n = 5; * p < .05 versus sham rats and * p < .05 versus MFB rats.
cell-derived neurotrophic factor (GDNF) has significantly improved parkinsonian behavioral symptoms but does not affect DA levels in the caudate nucleus or putamen of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (Gash et al., 1996). Moreover, continuous release of low levels of GDNF near the SN has also been reported to protect nigral dopaminergic neurons from MFB axotomy-induced degeneration and to significantly improve apomorphine-induced rotational behavior. However, GDNF infusion did not prevent the loss of DA in the striatum (Tseng et al., 1997). Our previous experiments (Jia et al., 2009; Liang et al., 2002, 2003) and present study demonstrate that 100-Hz EA stimulation can significantly improve movement disorders in MFB-lesioned rats without affecting DA levels in the striatum (Liang et al., 2003). Analysis of the DA content in the striatum shows that there is no significant difference between the MFB-lesioned group and the groups treated with 2-Hz or 100-Hz EA. However, rats treated with high-frequency EA stimulation showed a slightly higher striatal DA content than those treated with low-frequency EA stimulation. These effects of 100-Hz EA stimulation might be attributable to enhanced synthesis and release of neurotrophic factors (Liang et al., 2002, 2003) and alleviation of inflammatory reactions in the SN (Liu et al., 2004). L-dopa-induced rotational behavior has suggested that DA levels in the striatum are only elevated for a short time, whereas DA levels in the SN remain elevated until the behavioral effects of L-dopa have subsided. This suggests that elevated DA levels in the SN maintain circling behavior when striatal DA levels have begun to decline (Robertson & Robertson, 1989). Morphological evidence also revealed that EA at 100 Hz protected axotomized dopaminergic neurons from degeneration in the SN (Jia et al., 2009). Therefore, a SN-based mechanism potentially involved in the improvement of motor disorders should be investigated.

SNc dopaminergic neurons release DA not only via axonal terminals that affect neurotransmission within the striatum but also via dendrites, some of which densely protrude into the SNr (Nieoullon, Cheramy, & Glowinski, 1977). This anatomical arrangement makes it possible for dendritically released DA to interact with SNr neurons, thus affecting the activity of this key output structure of the basal ganglia (Windels & Kiyatkin, 2006b). In MFB-lesioned rats, we found that high-frequency EA stimulation prevented the degeneration of both dopaminergic cell bodies in the SNc and dopaminergic neurons that arborize in the SNr (Jia et al., 2009). These data indicate that high-frequency EA stimulation may induce an increase in DA release within the SNr, which may result in significant alterations in the output pathways of SNr neurons. However, it remains unclear how increased dendritic DA release might affect SNr GABAergic projection neurons (Gonzalez-Hernandez & Rodriguez, 2000).

The SNr is usually considered a major output nucleus of the basal ganglia, and striato-nigral GABAergic projections are primary inputs to SNr neurons. However, D1 DA receptors were identified on GABA (Yung et al., 1995) and glutamate terminals (Rosales et al., 1997) synapsing on SNr neurons. Therefore, the effects of DA on SNr neurons may be indirect, resulting from presynaptic modulation of GABA or glutamate (GLU) inputs, the two opposing forces regulating the activity of these cells. Windels and Kiyatkin (2006a) have studied the effects of iontophoretic DA and amphetamine on SNr neurons in awake, unrestrained rats. Their results showed that the activity state of GABAergic SNr neurons may be modulated by dendritic release of DA through activation of D1 DA receptors on the terminals of striato-nigral efferents. It has also been suggested that increased somatodendritic DA release can decrease the activity of SNr neurons by increasing GABA input. On the other hand, in vitro work also suggests that DA may affect SNr activity via modulation of glutamatergic inputs (Wittmann, Marino, & Conn, 2002). Somatodendritically released DA may play an additional role in regulating GLU release from subthalamic terminals in the SN (Hatzipetros & Yamamoto, 2006). It indicated that somatodendritically released DA in the SN provides negative feedback control on overstimulated or phasic GLU release from subthalamic nucleus (STN) terminals within the SN by activation of D2 dopamine heteroreceptors. Moreover, lesion or high-frequency stimulation of the STN reduces most of the motor impairments associated with PD (Parkin et al., 2001; Benazzouz et al., 2000). This effect is presumably mediated by a reduction in the activity of the subthalamo-nigral pathway.

Although previous studies have indicated that GAD67 mRNA levels are increased in the GP after dopaminergic neuronal degeneration (Billings & Marshall, 2004; Soghomonian & Chesselet, 1992), this increase in GAD67 mRNA is believed to be secondary to activation of excitatory subthalamo-pallidal projections. In this study, we found that EA stimulation has no effect on the increased GABA content in GP in MFB-lesioned rats. This suggests that EA stimulation does not entirely modulate the activity of GP neurons, which is affected by the two processes of degeneration of dopaminergic neurons: activation of STN inputs to GP neurons and the removal of nigro-pallidal DA transmission (Billings & Marshall, 2004; Soghomonian & Chesselet, 1992).

The GABA content in the midbrain also increased in hemiparkinsonian rats, which is consistent with other reports (Boulet et al., 2006). Some of this nigral GABA may result from an increase in GP neuronal activity from GABAergic pallido-nigral projections to the SNr (Celada, Paladin, & Tepper, 1999). In anesthetized hemiparkinsonian rats, GP lesions abolish the increase in SNr GABA levels induced by STN high-frequency stimulation (Windels et al., 2005). However, SNr inhibition may also result from activation of the intranigral axon collateral network of GABAergic SNr cells (Mailly, Charpier, Menetrey, & Deniau, 2003). According to the “classical” model, direct striatonigral GABAergic pathway activation inhibits the tonically active GABAergic neurons of the SNr, abolishing target nucleus inhibition in premotor structures in the thalamus and brainstem (Chevalier & Deniau, 1990). Thus, the inhibition of SNr neurons may be important in mediating movement hyperactivity. Here, we show that high-frequency EA stimulation decreases the content of GABA in the midbrain of MFB-lesioned rats. This implies that EA results in increased somatodendritic DA release, resulting in inhibition of the activity of SNr neurons. In keeping with this, high-frequency EA stimulation was found to antagonize the MFB lesion-induced increases in GAD67 mRNA levels in the midbrain (Jia et al., 2009). Previous studies (Guridi et al., 1996; Salin, Manrique, Forni, & Kerkerian-Le Goff, 2002) have shown that STN high-frequency stimulation or subthalamotomy reverses the DA lesion-mediated increase in GAD67 mRNA levels in SNr neurons. The observed reduction of GAD67 mRNA levels in the midbrain indicates that EA stimulation may reduce inhibitory excitatory efferent activity from the output pathways of the basal ganglia. Therefore, EA...
treatment may affect GABA transmission to target brain regions, especially to SNr neurons in the basal ganglia output structure.

Rats receiving 0-Hz EA stimulation exhibited no improvement in motor coordination. This observation indicates that electrical stimulation, and not just needle placement, is necessary for the positive effects of EA therapy. In addition, low-frequency stimulation (2 Hz) showed no significant change compared with the MFB-lesioned group. This suggests that different frequencies of EA stimulation may affect different brain regions or that certain basal ganglia structures may be less sensitive to the low-frequency stimulation.

Electrostimulation at the Dazhui (Du 14) and Baihui (Du 21) acupoints produces a neuroprotective effect on dopaminergic neurons in the SN. Park et al. (2003) also demonstrated that acupuncture at the Yanglingquan (GB 34) and Taichong (LR 3) acupoints reduces the degeneration of dopaminergic neurons. However, acupuncture at nonacupoint or nondirectly related acupoints does not halt the degeneration of dopaminergic neurons (Kim et al., 2005). These results provide evidence for the existence of acupoint specificity. This is consistent with evidence from fMRI studies (Zhang et al., 2004), although additional research is necessary to further corroborate this phenomenon.

The GDNF-induced changes in DA levels seen in rhesus monkeys were limited to the SN, ventral tegmental area, and GP. MPTP-induced depletion of DA in the caudate nucleus and puta-

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