Biochemical mechanisms of pallidal deep brain stimulation in X-linked dystonia parkinsonism


Objective: Invasive techniques such as in-vivo microdialysis provide the opportunity to directly assess neurotransmitter levels in subcortical brain areas.

Methods: Five male Filipino patients (mean age 42.4, range 34–52 years) with severe X-linked dystonia-parkinsonism underwent bilateral implantation of deep brain leads into the internal part of the globus pallidus (GPe). Intraoperative microdialysis and measurement of gamma-aminobutyric acid and glutamate was performed in the GPi in three patients and globus pallidus externus (GPe) in two patients at baseline for 25/30 min and during 25/30 min of high-frequency GPi stimulation.

Results: While the gamma-aminobutyric acid concentration increased in the GPi during high frequency stimulation (231 ± 102% in comparison to baseline values), a decrease was observed in the GPe (22 ± 10%). Extracellular glutamate levels largely remained unchanged.

Conclusions: Pallidal microdialysis is a promising intraoperative monitoring tool to better understand pathophysiological implications in movement disorders and therapeutic mechanisms of high frequency stimulation. The increased inhibitory tone of GPi neurons and the subsequent thalamic inhibition could be one of the key mechanisms of GPi deep brain stimulation in dystonia. Such a mechanism may explain how competing (dystonic) movements can be suppressed in GPi/thalamic circuits in favour of desired motor programs.

1. Introduction

In dystonia, impaired cortical and subcortical surround inhibition is thought to be one of the most prominent pathophysiological features aside from maladaptive plasticity [1]. In isolated dystonia, in-vivo positron emission tomography and magnetic resonance spectroscopy studies revealed GABA_A receptors resulting in hyperpolarization of the postsynaptic membrane. Present knowledge on biochemical mechanisms in dystonia is, however, mainly exclusively based on animal models.

Invasive techniques such as in-vivo microdialysis provide the possibility to directly assess neurotransmitter levels in subcortical brain areas in humans [4,5]. Further, the combination of microdialysis and functional neurosurgical methods enables the study of subcortical areas [2,3]. The impaired GABAergic pathways may be one of the key mechanisms of sensorimotor system disinhibition in dystonia. Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the human central nervous system and binds to GABA_A receptors resulting in hyperpolarization of the postsynaptic membrane. Present knowledge on biochemical mechanisms in dystonia is, however, mainly exclusively based on animal models.

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neurochemical effects of high-frequency stimulation (HFS) in otherwise inaccessible human subcortical structures. In dystonia, HFS of the internal segment of the globus pallidus (GPi) is thought to "overwrite" pathological neuronal firing patterns by inhibiting local somatodendritic activity and promoting axonal activity. Animal studies suggest that HFS induces an increased GABA release by activating local GABAergic neurons whereas glutamate levels appear to remain unchanged in the vicinity of the stimulation electrode [6,7]. Similarly, an enhanced GABA release was also observed during HFS of human neocortical slices [8].

X-linked dystonia-parkinsonism (XDP) is an inherited neurodegenerative basal ganglia model disease which mainly affects male patients due to the X-linked mode of inheritance. The disease is characterized by adulthood onset of focal dystonia, which rapidly generalizes within a few years. In most patients, dystonic symptoms are replaced by parkinsonian features after several years [9]. Neuroimaging studies revealed varying degrees of caudate and putaminal atrophy in the combined and parkinsonian stages. Neuropathology shows a mosaic appearance of the caudate and putamen with patchy neuronal loss particularly involving the striosomes in the dystonic phase of the illness [10]. In contrast, both striosome and matrix components of the striatum degenerate in the parkinsonian phase [11]. Given the unique phenotypic pattern XDP can serve as an ideal model disease to study dystonia in patients sharing the same underlying genetic effect (i.e., an otherwise highly homogeneous group).

In the present study, we aimed at determining pallidal inhibitory (GABA) and excitatory (glutamate) neurotransmitter levels in a series of five Filipino patients with XDP who underwent GPI DBS and concurrent microdialysis of the GPI (n = 3) and the globus pallidus externus (GPe) (n = 2).

2. Materials and methods

The present study included five patients (male, mean age 42.4 years, range 34–52 years) with severe XDP who were refractory to pharmaceutical treatment and who underwent bilateral implantation of deep brain leads within the GPI. Subjects were recruited by the Filipino XDP study group in collaboration with the Institute of Neurogenetics and the Departments of Neurosurgery and Neurology, University of Luebeck, Germany. The study was conducted according to the ethical standards laid down in the 1964 Declaration of Helsinki and was approved by the local ethics committee. Before participation, informed written consent was obtained from all participants and their caregivers.

The clinical workup included detailed neurological investigations using the Burke—Fahn—Marsden Dystonia Rating Scale (BFMDRS) for dystonic symptoms. In the present paper, the change of the motor part of the BFMDRS is reported 1 week postoperatively compared to the preoperative status.

All patients were included in a larger study focusing on short- and long-term outcome of GPI DBS in XDP.

Surgery was performed in conscious sedation in patient A and general anaesthesia (propofol, 5–7 mg/kg/h; remifentanil, 0.2 µg/kg/min) in patients B-E due to severe dystonic movements of the head, neck or trunk. Preoperatively a stereotactic MRI on a 3 T Philips Achieva scanner was performed with a axial oriented 3D-T1-weighted turbofieldecho-sequence after intravenous administration of 0.2 ml/kg/bw (TR 6.6 m s, TE 3 m s, flip angle 9°, 1 × 1 × 1 mm, matrix 288 × 288, 160 slices covering the whole brain) for navigation purpose and additionally coronal T2 weighted turbospin echo images (TR 2500 m s, TE 100 m s, flip angle 90°, 20 × 3 mm slices, 576 × 576 mm matrix) and also coronal T1 IR images (TR 2000 m s, TE 20 m s, TI 800 m s, flip angle 90°, 20 × 3 mm slices, 512 × 512 matrix) in equal geometric orientation were performed to visualize the optic tract. The pre-calculated coordinates were 20 mm lateral of the midline; 2 mm anterior of the midpoint of AC-PC and 4 mm below the AC-PC plane. The lateral coordinates were adjusted according to the coronal MRI, aiming for a placement immediately dorsolateral to the optic tract.

Microelectrode recordings (MER) were performed using a Ben-Gun Microdrive (Inomed, Teningen, Germany) with an interelectrode distance of 2 mm. Raw signals were amplified, filtered (between 1 and 100 Hz for local field potentials and between 300 and 5 kHz for unit data) and digitized at a sampling rate of 20 kHz (ISIS MER system, Inomed, Teningen, Germany). Usually three microelectrodes (combined micro-macroelectrodes with 1 mm distance between micro-tip and macroring for stimulation), Inomed, Teningen, Germany) were advanced simultaneously according to the surgeons’ preference either in the anterior-central-posterior or medial-central-lateral positions. Micro-tip impedances were 0.8 ± 0.1 MΩhm (range 0.5–1.0). Starting at 10 mm above the surgical target, electrodes were advanced in steps of 500 µm and ongoing activity was recorded at each site for at least 20 s. In agreement with previous observations [12], under general anaesthesia with propofol/remifentanil, unit activity in both pallidal segments was often indistinguishable and characterized by a multitude of different activity levels and patterns, respectively. Therefore, the occasional recording of highly regular “border cell” discharges at 20–30 Hz proved to be especially helpful for a delineation of peri-pallidal white matter layers and for an on-line differentiation between the GPe and GPI, respectively. Transition to the sub-pallidal fibre field was indicated by a decline of background activity and a near-complete cessation of unit activity, respectively.

When the task was reached, one of the microrecording electrodes were replaced by the microdialysis probe. After calibration for 5 min, baseline measurements with sampling for 25/30 min (velocity 2 µl/min) were performed. The second sample was collected during a stimulation period of 25/30 min with the macroring (amplitude 2 mA, frequency 130 Hz and pulse width 60 µs). The distance between site of stimulation and dialysis sampling was 2 mm (Fig. 1).

A custom-made microdialysis probe from M Dialysis AB
(Hammarby, 12030 Sweden) was used (PA membrane, cut-off of 20 kDa, 260 mm shaft length, 300 mm in- and outlet; outer diameter 1 mm and membrane length 2 mm). The microdialysis probe was inserted at the target depth through one of the micro-guiding tubes replacing one of the microrecording electrodes. HPS was performed at the pallidal base (as delineated by MER) through the central channel. The left globus pallidus was perfused with a pyrogen free PBS solution supplied by the manufacturer and contained as perfusate (in grams/liter): NaCl 8.8KCl 0.2, CaCl$_2$ 0.132, MgCl$_2$ 0.1, Na$_2$HPO$_4$ 1.15, K$_2$HPO$_4$ 0.2 at a pH of 7.4.

From each patient, two dialysis samples of a 25 (Patient A-B) or 30 min (Patient C-E) sampling period each were collected before and during DBS and subsequently stored at −80 °C until analysis. GABA and glutamate were analysed by means of HPLC with electrochemical detection after pre-column derivatisation.

The location for the microdialysis probes of patients A-C were 2 mm rostral (anterior) and of patient D-E 2 mm lateral to the stimulation electrode.

2.1. HPLC analysis

After precolumn derivatization with o-phthalaldehyde and sodium sulphide for 10 min, GABA and glutamate values were measured using HPLC with electrochemical detection [6,13,14]. The HPLC system consisted of a C18 column (Europhaser 100, 5 µm, column size 250 mm × 4 mm) and a precolumn (30 mm × 4 mm). The isocratic mobile phase (0.1 M sodium phosphate buffer, pH 4.5, containing 0.5 mM EDTA and 25% methanol) was previously degassed by helium and pumped at a flow rate of 1.0 ml/min and temperature of 30 °C. The compounds were detected electrochemically using a glassy carbon electrode set at a potential of 900 mV relative to an Ag/AgCl reference electrode (Waters 460 electrochemical detector, Millipore Corporation, Eschborn/Ts. Germany).

The detection limits were 1 nM and 2 nM for GABA and glutamate, respectively. Mean GABA and glutamate values were expressed in nM ± standard error. Changes were expressed in percent. The existence of differences within patients was assessed with a one-way analysis of variance (ANOVA) with post-hoc analysis for pairwise comparisons as indicated. Alpha level was set at 0.05.

3. Results

Bilateral GPi DBS resulted in an improvement of dystonia severity in all five operated XDP patients one week after surgery (reduction on the BFMDRS motor part of 49.6% (range 34–56), table).

The intraoperative microelectrode recordings provided valuable clues concerning the precise mediolateral position of the probe. In the lateral tracks (used for microdialysis sampling from the GPe), putaminal activity was encountered just above the GPe — confirming a lateral plane of >20 mm [15]. While border cell activity — verifying the position of the lamina medullaris interna (separating GPe from GPi) — could subsequently be mapped in the medial and central (but not lateral) trajectories, we concluded that the lateral tracks traversed GPe, but not GPi. Accordingly, DBS electrodes were assumed to be localized in the lateral aspect of the GPe.

Baseline GABA values of all five patients ranged from 6.7 to 100 nM (mean 34.3 ± 14.0 nM) prior to GPi DBS, whereas glutamate values ranged from 4 to 1000 nM in the dialysates (356 ± 84 nM). Due to the high interindividual differences and the broad ranges, baseline values were normalized and set to 100% (baseline) (Table 1).

In patients A-C, microdialysis probes were located rostral of the stimulation electrode corresponding to a more anteriorly located area within the GPe. In these patients GABA values increased during HFS to 231 ± 102% in comparison to baseline values (Fig. 2). The slight HFS-related increase of glutamate levels was not different from baseline (128 ± 8%).

In patients D-E, microdialysis was performed lateral of the stimulation electrode. Postoperative MR imaging gave evidence that the intraoperative sampling probes were located in the GPe. In these patients GABA levels decreased (22 ± 10%) (Fig. 2). HFS-related glutamate levels in the second group were inconsistent (mean 71 ± 45%).

4. Discussion

The present study is the first one to report intraoperative microdialysis during GPi HFS in dystonia in humans. In keeping with recently published case reports, pallidal neurostimulation clearly improved dystonic signs in the operated XDP patients [16].

A recent study in PD patients reported findings of intraoperative microdialysis of the GPi and motor thalamus during STN HFS. They demonstrated increased glutamate levels in the GPi and putamen as well as reduced GABA levels in the motor thalamus during active HFS [5]. Their data confirm that STN HFS results in increased glutamateergic projections from STN to GPi and, subsequently, GABAergic projections from the GPi to the motor thalamus, thus normalising thalamic inhibition and reducing symptoms of the hyperkinetic state.

In this study, intraoperative microdialysis revealed that GPi HFS enhanced local GABA release, whereas glutamate release largely remained unchanged. Moreover, GPi HFS also appeared to have

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Table 1 Demographic and surgery-related data of the five operated XDP patients. Glutamate and GABA levels of microdialysis samples are given in nM prior and during deep brain stimulation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Disease duration (y)</th>
<th>Predominant pheno-type</th>
<th>GPi target coordinates</th>
<th>Trajectory – microdialysis</th>
<th>Anesthesia</th>
<th>Change of BFMDRS M'-M (pretreat-1 week postop)</th>
<th>HFS-related glutamate change</th>
<th>HFS-related GABA change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A L7992</td>
<td>52</td>
<td>3</td>
<td>D/P</td>
<td>18 3 – 2</td>
<td>Central</td>
<td>Anterior</td>
<td>LA (−/−) −56%</td>
<td>+18%</td>
<td>+273%</td>
</tr>
<tr>
<td>B L7673</td>
<td>43</td>
<td>3</td>
<td>D</td>
<td>20 3.5 – 2</td>
<td>Central</td>
<td>Anterior</td>
<td>GA (6.1/0.2) −56%</td>
<td>+35%</td>
<td>+80%</td>
</tr>
<tr>
<td>C L7153</td>
<td>36</td>
<td>6</td>
<td>D/P</td>
<td>20 3 – 2</td>
<td>Medial</td>
<td>Anterior</td>
<td>GA (7.0/0.2) −34%</td>
<td>+33%</td>
<td>+40%</td>
</tr>
</tbody>
</table>

Note: D = dystonia, P = parkinsonism.

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distant effects on GPe GABA concentrations. The present results on HFS-related neurotransmitter changes corroborate recent animal studies in which concordant changes were observed [6].

In dystonia, the direct pathway (cortico-striato-GPi projections) mediating voluntary movements is thought to be overactive giving rise to a hyperkinetic state. Accordingly, electrical motor cortex stimulation of a DYT1 dystonia mouse model and a patient with cervical dystonia who underwent GPi DBS resulted in long-lasting GPi inhibition [17,18]. It is currently unknown whether a disinhibited direct pathway per se or a reduced inhibitory indirect pathway via cortico-striato-GPe-STN-GPi projections result in the reduced inhibitory activity of the GPi. The GPi is the major output structure of the basal ganglia modulating thalamic and consequently cortical neuronal activity. A reduced thalamic inhibition as a consequence of reduced GABAergic GPi outflow would lead to an increased (disinhibited) glutamatergic thalamo-cortical state. The results of the present study support the assumption that GPi HFS does not generally suppress neuronal activity, but may increase the firing rate of certain neuronal populations [21]. GPi HFS is also an effective therapeutic option in advanced Parkinson’s disease (PD), which seems paradoxical at first sight. Interestingly, parkinsonism is also a part of the phenotypic spectrum of XDP. The neuronal activity in the GPi differs between dystonia and PD with regard to firing rates and patterns [22]. Thus, GPi DBS could selectively suppress pathological firing characteristics at the pallidal level in both disorders and may thereby normalise the disturbed GABAergic output.

Secondly, GPi HFS could affect GPe-STN fibres traversing the GPi causing a possible blockade of the GABAergic GPe projections. Microdialysis studies in primates revealed that the ambient GABA

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Fig. 2. Changes of glutamate and GABA levels of patients A–C (sampling presumably in the GPi) during DBS are given as percent of the values prior to stimulation (100% = dotted line) (A) and of patient D–E (sampling presumably in the GPe).

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Fig. 3. Hypothetical effects of GPi HFS on pallidal activity. DBS was performed in the central GPi in all the patients. A. In patients A–C microdialysis probes were located rostral (A) and in patients D–E lateral (B) to the stimulation electrode. B. Direct effects on GABAergic output neurons. c. Strengthening of the indirect pathway by antidromic inhibition of GABAergic GPe-STN projections. D. Reduction of direct pathway input by antidromic inhibition of GABAergic striato-GPi projections. Inhibitory GABAergic neurons are shown in blue; excitatory glutamatergic neurons in red. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)  

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levels in the GPe are relatively high with concentrations of approximately 0.5 µM under resting conditions [23,24]. The GPe itself receives GABAergic inhibitory inputs from the striatum via the indirect cortico- striato-GPe pathway (Fig. 3c). An increased GPe inhibition would result in a reduced inhibition of the subthalamic nucleus (STN), which in turn sends more glutamatergic projections to the GPi leading to a higher GPi activation and subsequently thalamic inhibition. Increased GPi glutamate levels, however, were not observed during GPi HFS. Evidence for a reduced activation of the indirect pathway in dystonia including a reduced surround inhibition at the level of the basal ganglia derives from several multimodal neuroimaging and electrophysiological studies for review [25].

Lastly, GPi HFS could block presynaptic GABAergic striato-GPi projections corresponding to an inhibition of the direct pathway leading to a higher pallidal GABAergic output (Fig. 3d). It may be hypothesized that the increased GABA levels derive from local collaterals of the GABAergic GPi neurons.

4.1. Strengths and limitations

Apart from the small sample size and the lack of statistical analysis the most critical point in the present study is the highly homogeneous patient population with the same genetic and environmental background, the same gender (male) and the same genotype.

5. Conclusions

Palidal microdialysis is a promising intraoperative monitoring tool to better understand pathophysiological implications in movement disorders and therapeutic mechanisms of DBS.

Intraoperative microdialysis sampling in XDP patients revealed an increase of GABA levels in the GPi and a decrease in the GPe during GPi HFS. The increased GABAergic inhibitory tone of GPi neurons to the ventral thalamus could be one of the key mechanisms of DBS in dystonia. Such a mechanism could explain how competing (dystonic) movements can be suppressed on the GPe /thalamic level in favour of desired motor programmes.

Future studies with concurrent sampling of GPe and GPi in larger sample size are warranted to further study the consequences of GPi HFS on the direct and indirect cortico-subcortical pathways.

Conflict of interest

The authors do not have any conflict of interest.

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Dr. Tronnier received honoraria from St. Jude, Medtronic, EISAI and Codman for scientific presentation. He is member of the advisory boards of EISAI and Medtronic.

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