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Deep brain stimulation in Parkinson's disease patients: biochemical evidence

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Summary. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) in Parkinson's disease (PD) patients augments STN-driven excitation of the internal globus pallidus (GPi). However, other DBS-induced changes are largely unknown. Here we report the biochemical effects of STN–DBS in two basal ganglia stations (putamen – PUT – and GPi) and in a thalamic relay nucleus, the anteroventral thalamus (VA).

In six advanced PD patients undergoing surgery, microdialysis samples were collected from GPi, PUT and VA before, during and after one hour of STN–DBS. cGMP was measured in the GPi and PUT as an index of glutamatergic transmission, whereas GABA was measured in the VA.

During clinically effective STN–DBS, we found a significant decrease in GABA extracellular concentrations in the VA (−25%). Simultaneously, cGMP extracellular concentrations were enhanced in the PUT (+200%) and GPi (+481%).

DBS differentially affects fibers crossing the STN area: it activates the STN–GPi pathway while inhibiting the GPi–VA one. These findings support a thalamic dis-inhibition, as the main responsible for the clinical effect of STN–DBS. This, in turn, re-establishes a more physiological level of PUT activity.

Introduction

It has been recently shown that subthalamic nucleus (STN) deep brain stimulation (DBS) increases cGMP in the internal pallidus (GPi) while it induces a motor improvement similar to l-dopa treatment, in Parkinson's disease (PD) patients (Stefani et al., 2005). In the central nervous system, extracellular cGMP levels are considered as a marker of excitatory activity, being enhanced either by increased glutamatergic or by reduced GABA transmission (Fedele and Raiteri, 1999; Pepicelli et al., 2004). Paradoxically therefore, the recent report (Stefani et al., 2005) suggests that STN–DBS increases excitatory activity in the GPi; this finding is at odds with the expected effects, based on previous understanding of basal ganglia circuitry. If the pathways reported in the monkey models

of Parkinson's disease (Albin et al., 1989), also apply to human PD brain, then a motor improvement is expected when GPi activity is reduced and, in turn, motor thalamus activity increased. Thus, the cGMP increase, detected in GPi during STN–DBS seems at variance with basal ganglia changes expected to be related to motor improvement in PD.

DBS-induced motor improvement should be coupled to an GABA-decrease-driven increase in VA/VL activity. Hence, the assessment of GABA changes in the VA/VL during STN–DBS should be useful to clarify STN–DBS mechanism of action. Following VA/VL dis-inhibition, a cortical activation is expected which, in turn, could increase the activity of the cortico-putaminal pathway. Therefore, in the present study, we have measured the extracellular levels of GABA, the main inhibitory transmitter released by GPi fibers, in the VA and, simultaneously, cGMP in the PUT and GPi, before, during and after STN–DBS.

Methods

Six, out of ten, advanced PD patients were included in this study according to selection criteria previously reported (Peppe et al., 2004) to implant bilaterally permanent stimulating electrodes both in the STN and in the GPi. In each patient, microdialysis was performed only in one of the two hemispheres. Patient clinical characteristics are reported in Table 1. Written, informed consent was obtained from each patient who participated in the study. The Local Ethics Committee approved the protocol and consent form describing the risks and potential benefits of the study. Briefly STN and GPi target areas were identified preoperatively by means of ventriculography and intra-operatively by means of single unit recordings on two different trajectories, each performed with a multi-electrode holder, one aimed at the STN and the second aimed at the GPi (Peppe et al., 2004). The STN trajectory passed through the VA, the GPi trajectory passed through the PUT. After electrophysiological identification of the targets, the recording electrodes in the GPi, VA and PUT were replaced by microdialysis probes.

Probes were infused (5 μl/min) for stabilization (90 min) (Fedele et al., 2001). During stabilization, the permanent stimulating electrode (Medtronic mod 3389) was implanted in ipsilateral STN. Then, basal microdialysis and clinical data were collected for 50 minutes. Afterwards, STN–DBS was switched on for 60 minutes. Then, 60 minutes of recovery were performed. Clinical changes, contralateral upper limb rigidity and akinesia were continuously assessed by an expert neurologist utilizing selected items of UPDRS (Fahn et al., 1987) (rigidity 0–4, finger tapping 0–4, hand movement 0–4; total = 0 corresponds to "normal", 12 maximum score), while remaining blind to the stimulus intensity between 0 and 3 V. GABA and cGMP concentration were determined by high performance liquid chromatography and by radio-immuno assay (RIA), respectively (Stefani et al., 2005; Fedele et al., 2001).

Significance of GABA and cGMP changes was assessed by performing the mean of single determinations obtained under basal conditions, during STN–DBS and during recovery conditions. Mean values were compared by non-parametric Friedman ANOVA followed by Wilcoxon test. To assess significance of variations in the single fractions after switching DBS on or off, single fraction means were compared to the mean of the previous section (i.e. DBS vs. basal or recovery

Table 1

Patient no.	Age (years)	Disease duration (years)	LD therapy (years)	LTTS duration (years)	LD therapy before DBS (mg)
1	54	7	7	3	800
2	45	6	5	3	1200
3	54	11	6	5	800
4	65	13	9	6	1100
5	62	10	8	3	750
6	68	8	5	3	800
Mean	**58.00**	**9.17**	**6.67**	**3.83**	**908.33**
SD	**8.56**	**2.64**	**1.63**	**1.33**	**190.83**

vs. DBS). All these comparisons were performed by Wilcoxon test. Clinical scores were compared using similar non parametric methods.

Results

Extracellular cGMP concentration was examined in ten subjects. In four patients, cGMP basal values (in the first 5 fractions), either in the GPi or in the PUT, were below the RIA detection limit (<1 fmol) and, therefore, data were eliminated. In the other six patients, cGMP basal concentrations were clearly detectable and were relatively similar among patients (see Table 2).

Clinical data

In all the six selected patients with detectable basal cGMP levels, STN–DBS produced a significant (Friedman ANOVA, $Chi^2 = 14.25$, $p < 0.001$) and clear decrease of the UPDRS items score in the contralateral part of the body. The mean basal score (8.27 ± 1.05) significantly decreased to 5.67 ± 0.70 (Wilcoxon test, $Z = 2.52$, $p < 0.05$) after only ten minutes of STN–DBS. Once STN stimulation was switched off, the UPDRS score increased significantly to 8.06 ± 0.88 just 10 minutes after DBS (Wilcoxon test, $Z = 2.52$, $p < 0.05$).

Microdialysis data

STN–DBS produced a significant change of GABA extracellular concentrations in the VA ($n = 6$, mean -24.5%, Friedman ANOVA $Chi^2 = 9.75$, $p < 0.05$). The decrease in GABA levels was significant (Wilcoxon test $Z = 2.52$, $p < 0.05$) from the first fraction following STN–DBS in comparison to the mean basal value (Table 2). During STN–DBS, the mean value of GABA concentration, was significantly lower than mean basal value (Wilcoxon test $Z = 2.52$, $p < 0.05$). These changes paralleled the amelioration of clinical performance. After cessation of DBS, GABA levels remained decreased for at least

Table 2

	Basal					Mean	STN–DBS						Mean	Recovery					Mean
Fractions	1	2	3	4	5		1	2	3	4	5	6		1	2	3	4	5	6	
Patient no.																				
GABA-VA																				
1	4.30	4.00	4.50	4.90	4.30	**4.40**	3.90	3.70	4.00	4.00	3.80	3.90	**3.88**	3.70	3.70	3.90	3.50	3.40	3.70	**3.65**
2	4.90	4.30	4.10	4.50	4.70	**4.50**	4.10	4.20	3.90	3.80	4.10	4.10	**4.03**	1.50	2.30	3.00	3.50	4.00	3.40	**2.95**
3	4.00	3.90	3.70	4.30	4.10	**4.00**	3.00	4.00	3.50	3.90	3.20	3.00	**3.43**	3.00	3.30	4.10	5.40	6.30	5.00	**4.52**
4	5.10	4.70	4.50	4.80	5.30	**4.88**	3.20	3.10	3.30	3.50	3.40	3.10	**3.27**	3.70	3.80	4.00	4.00	4.20	4.50	**4.03**
5	4.20	4.10	4.00	4.70	4.50	**4.30**	3.90	4.10	3.90	4.10	3.80	3.80	**3.93**	3.40	4.00	5.80	6.20	5.30	5.70	**5.07**
6	5.10	5.60	5.40	5.30	5.50	**5.38**	3.50	2.09	2.00	2.04	2.13	1.70	**2.24**	5.00	5.00	5.50	5.30	6.30	6.00	**5.52**
Mean	**4.60**	**4.43**	**4.37**	**4.75**	**4.73**	**4.58**	**3.60**	**3.53**	**3.43**	**3.56**	**3.41**	**3.27**	**3.46**	**3.38**	**3.68**	**4.38**	**4.65**	**4.92**	**4.72**	**4.29**
SD	**0.49**	**0.64**	**0.59**	**0.34**	**0.56**	**0.49**	**0.44**	**0.81**	**0.75**	**0.77**	**0.70**	**0.89**	**0.67**	**1.14**	**0.88**	**1.06**	**1.14**	**1.24**	**1.05**	**0.94**

(continued)

Table 2 (*continued*)

	Basal					Mean	STN–DBS						Mean	Recovery						Mean
Fractions	1	2	3	4	5		1	2	3	4	5	6		1	2	3	4	5	6	
cGMP PUT																				
1	1.10	1.50	1.90	1.60	1.20	**1.46**	2.00	3.50	5.40	4.40	5.50	4.70	**4.25**	4.30	1.40	1.00	1.90	1.40	1.50	**1.92**
2	1.90	1.70	1.20	1.30	1.30	**1.48**	1.50	1.60	1.40	1.70	2.10	2.10	**1.73**	2.20	1.50	1.70	1.10	1.20	1.50	**1.53**
3	1.40	1.10	1.40	1.30	1.20	**1.28**	1.70	2.20	2.70	2.50	2.80	2.30	**2.37**	2.90	2.10	1.60	1.10	1.40	1.70	**1.80**
4	1.70	1.50	1.70	1.40	1.30	**1.52**	1.40	2.30	2.50	2.30	3.20	3.50	**2.53**	3.20	1.40	1.50	1.70	1.70	1.60	**1.85**
5	1.20	1.50	1.70	1.30	1.70	**1.48**	1.30	2.90	3.20	2.90	3.20	3.10	**2.77**	2.50	1.50	1.20	1.70	1.60	1.40	**1.65**
6	1.40	1.50	1.60	1.30	1.50	**1.46**	1.40	5.60	6.40	5.90	6.90	6.30	**5.42**	6.10	2.10	1.40	1.50	1.00	1.50	**2.27**
Mean	**1.45**	**1.47**	**1.58**	**1.37**	**1.37**	**1.45**	**1.55**	**3.02**	**3.60**	**3.28**	**3.95**	**3.67**	**3.18**	**3.53**	**1.67**	**1.40**	**1.50**	**1.38**	**1.53**	**1.84**
SD	**0.30**	**0.20**	**0.25**	**0.12**	**0.20**	**0.08**	**0.26**	**1.42**	**1.90**	**1.57**	**1.84**	**1.59**	**1.38**	**1.45**	**0.34**	**0.26**	**0.33**	**0.26**	**0.10**	**0.26**
cGMP GPi																				
1	2.40	2.20	1.90	2.40	2.10	**2.20**	4.40	9.70	24.50	22.10	29.70	30.10	**20.08**	28.50	2.40	1.30	1.10	2.10	2.30	**6.28**
2	1.70	1.30	1.70	1.50	1.40	**1.52**	2.70	2.30	2.50	3.20	3.10	3.40	**2.87**	3.90	2.10	1.50	1.40	1.20	1.30	**1.90**
3	2.50	1.90	2.40	1.60	2.00	**2.08**	3.00	2.70	2.30	2.30	2.50	2.90	**2.62**	3.20	2.00	1.50	1.10	2.10	2.30	**2.03**
4	1.00	1.50	1.10	1.00	1.00	**1.12**	2.50	9.40	10.70	12.60	10.90	11.20	**9.55**	12.10	1.90	1.10	1.40	1.10	1.40	**3.17**
5	1.30	1.10	1.70	1.30	1.20	**1.32**	4.40	8.20	16.40	15.50	17.20	16.10	**12.97**	15.20	2.20	1.20	1.10	1.30	1.40	**3.73**
6	1.90	2.10	2.80	2.30	2.40	**2.30**	3.10	2.80	3.50	5.90	6.40	5.10	**4.47**	13.40	2.70	1.80	1.50	2.20	1.50	**3.85**
Mean	**1.80**	**1.68**	**1.93**	**1.68**	**1.68**	**1.76**	**3.35**	**5.85**	**9.98**	**10.27**	**11.63**	**11.47**	**8.76**	**12.72**	**2.22**	**1.40**	**1.27**	**1.67**	**1.70**	**3.49**
SD	**0.59**	**0.45**	**0.60**	**0.56**	**0.56**	**0.50**	**0.84**	**3.60**	**9.05**	**7.81**	**10.41**	**10.47**	**6.89**	**9.21**	**0.29**	**0.25**	**0.19**	**0.52**	**0.47**	**1.60**
UPDRS																				
1	7.0	8.0	7.0	7.0	8.0	**7.40**	6.0	5.0	5.0	5.0	5.0	5.0	**5.17**	5.0	8.0	8.0	8.0	8.0	9.0	**7.67**
2	8.0	7.0	7.0	8.0	8.0	**7.60**	7.0	6.0	5.0	5.0	5.0	5.0	**5.50**	5.0	8.0	7.0	8.0	8.0	8.0	**7.33**
3	9.0	10.0	10.0	9.0	11.0	**9.80**	9.0	7.0	7.0	6.0	6.0	6.0	**6.83**	6.0	9.0	9.0	10.0	11.0	11.0	**9.33**
4	8.0	7.0	8.0	8.0	8.0	**7.80**	6.0	6.0	5.0	5.0	5.0	5.0	**5.33**	6.0	8.0	8.0	7.0	8.0	9.0	**7.67**
5	9.0	9.0	10.0	10.0	9.0	**9.40**	8.0	7.0	6.0	6.0	5.0	5.0	**6.17**	5.0	9.0	9.0	10.0	11.0	10.0	**9.00**
6	7.0	8.0	7.0	8.0	8.0	**7.60**	5.0	5.0	5.0	5.0	5.0	5.0	**5.00**	5.0	8.0	7.0	8.0	8.0	8.0	**7.33**
Mean	**8.00**	**8.17**	**8.17**	**8.33**	**8.67**	**8.27**	**6.83**	**6.00**	**5.50**	**5.33**	**5.17**	**5.17**	**5.67**	**5.33**	**8.33**	**8.00**	**8.50**	**9.00**	**9.17**	**8.06**
SD	**0.89**	**1.17**	**1.47**	**1.03**	**1.21**	**1.05**	**1.47**	**0.89**	**0.84**	**0.52**	**0.41**	**0.41**	**0.70**	**0.52**	**0.52**	**0.89**	**1.22**	**1.55**	**1.17**	**0.88**

20–30 minutes in all the subjects and then returned to pre-stimulation values in the subsequent fractions.

STN–DBS produced also a significant change in cGMP concentration in the PUT ($n = 6$, mean +219%, Freidman ANOVA $Chi^2 = 14.25$, $p < 0.001$). In each patient, the rise in cGMP was significant from the second fraction following STN–DBS (Table 2). During STN–DBS, the mean value of cGMP was significantly (Wilcoxon test, $Z = 2.52$, $p < 0.05$) higher than mean basal and recovery values. After cessation of DBS, cGMP concentration remained elevated for only 10 minutes in all the six subjects and, like clinical amelioration, returned to pre-stimulation values in the following fractions.

In the same subjects, STN–DBS produced a significant increase of cGMP concentration in the GPi ($n = 6$, mean +497%. Friedman ANOVA $Chi^2 = 14.25$, $p < 0.001$). In each patient, the rise in cGMP was significant (Wilcoxon test, $Z = 2.52$, $p < 0.05$) from the first fraction following STN–DBS (Table 2). During STN–DBS, the mean value of cGMP was significantly (Wilcoxon test, $Z = 2.52$, $p < 0.05$) higher than basal and recovery values.

Discussion

It has been postulated that stimulation leads to inhibition of STN – hence, of basal ganglia output – because STN–DBS alleviates parkinsonian symptoms as does STN lesion (Gill and Heywood, 1997) or STN inactivation caused by local injection of lidocaine or muscimol (Levy et al., 2001).

In the present study, we show that clinically effective STN–DBS induced (i) a significant decrease of GABA extracellular levels in the VA and (ii) a significant increase of cGMP extracellular concentrations in the PUT; (iii) moreover, we confirm our previous report (Stefani et al., 2005) of a significant increase in cGMP extracellular levels in the GPi.

In line with the view that increased glucose metabolism in afferent inhibitory fibers from GPi occurs in VA–VL of MPTP-lesioned monkeys (Mitchell et al., 1989), the present study shows that motor improvement by STN–DBS is paralleled by a net decrease of GABA in motor thalamus. Indeed, it has been shown that GPi firing activity, far from decreasing, is increased during STN–DBS (Hashimoto et al., 2003). In addition, a recent PET study in humans has reported a STN–DBS-induced elevation of blood flow in the GPi, suggesting an increased metabolic activity in this area (Hershey et al., 2003). Accordingly, our previous and present findings, showing an increase of excitatory neurotransmission in the GPi, may represent the biochemical counterpart of these electrophysiological and PET data.

A first hypothesis to explain how STN–DBS may promote a reduced GABA release in the VA/VL complex together with an increased cGMP within the GPi would involve the possible activation of presynaptic glutamate receptors increasing GABA release in the GPi during STN–DBS (Bruet et al., 2003) and thus inhibiting GPi–VA/VL transmission. This explanation is conflicting with the clear increase of firing activity in the GPi during clinically efficacious STN–DBS reported by Hashimoto et al. (2003).

Recent electrophysiological findings in humans during surgery may provide alternative hypotheses. The Toronto group reported STN cell inhibition by STN–DBS (Filali et al., 2004) and Welter et al. (2004) described analogous firing changes but associated with the appearance of a ''burst-like'' firing. Actually, a DBS-induced STN firing decrease should result in a reduced excitatory transmission to the GPi possibly accounting for the reduced GABA release in the VA/VL. However, reduced STN activity should produce a decrease of cGMP in the GPi, which is the opposite of what we observed. As acknowledged by the authors themselves

(Welter et al., 2004), it is possible that, STN–DBS stimulation may simultaneously excite the thin fibers (0.8–1.6 μm) (Kita et al., 1983) originating from the STN and innervating GPi, while inhibiting spontaneous firing from STN cell bodies recorded during surgery. This ''decoupling'' between somatic and axonal firing is based on the different sensitivity to a cathodal stimulation of large elements like cell body in comparison to thin fibers, according to their different diameter (Rattay, 1999; Plonsey and Barr, 2000; Basser and Roth, 2000). Alternatively, the increased cGMP extracellular concentrations in GPi could be induced by the shift of STN firing pattern to a stimulus-driven ''burst firing'' mode (Welter et al., 2004) coupled to inhibition of the spontaneous inter-stimulus firing. Nevertheless, we find that increased GPi excitation (i.e. increased cGMP concentration in the GPi) does not produce an increased GABA release in the target VA–VL complex, as one would expect on the basis of basal ganglia circuitry, but instead a fall in thalamic GABA levels.

A possible explanation for this paradox is that the electric field around the STN–DBS electrode may preferentially affect the large (1.5–5.0 micron) GPi fibers (Sidibé et al., 1997) directed to the thalamus and passing along the dorsal border of the STN, where the active contact is often located, inhibiting action potentials propagation along this pathway, similarly to the effect on large cell somata in the STN. Alternatively the ''burst firing'' induced by DBS in the STN may driven GPi firing into a pattern antagonist to the pathological ''high frequency'' dominating basal ganglia in off condition (Brown et al., 2004).

Thalamic nuclei relay form a crucial link between the basal ganglia and cortex by transmitting basal ganglia output to specific cortical areas. Once VA–VL is dis-inhibited by STN–DBS, the activation of the thalamo-cortical pathway is likely to produce an increased excitatory drive to the cortex at rest and/or during movement. PET studies, performed before and following effective STN stimulation, reported a re-activation of fronto-temporo-parietal cortex at rest (Hilker et al., 2004) or of dorsolateral prefrontal cortex and supplementary motor area during movement (Limousin et al., 1997). Several electrophysiological studies have pointed out that cortical functions, impaired in PD patients, are improved during STN–DBS (Pierantozzi et al., 1999, 2002). A direct STN–DBS-induced activation of the thalamo-cortical pathway, producing cortical potentials, has been recently demonstrated for low frequency stimulation (MacKinnon et al., 2005). This finding reinforces the theory that STN–DBS activates the thalamo-cortical pathway.

There are few reports concerning cortico-putaminal pathway changes following pharmacological treatments in PD state. During clinical improvement we found an increase of cGMP in the PUT, an effect that is reminiscent of the l-dopa-induced striatal glutamate increase reported in animals rendered parkinsonian (Jonkers et al., 2002). The cGMP increase we observed is likely due to an increased glutamatergic excitatory input. Large glutamate inputs to PUT come from the cortex, as well as from the centromedian (CM)-parafascicular (Pf) thalamic complex and from the VA/VL complex itself. Both these possibilities are in line with the classical view of the basal ganglia circuitry.

We conclude that: (i) DBS-induced motor improvement in PD is linked to a disinhibition of the thalamo-cortical pathway; (ii) that the second event is a re-activation of putaminal activity, marked by cGMP level increase in putamen, probably linked to increased glutamatergic cortico-putaminal or thalamo-putaminal activity.

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