INTRODUCTION

It has been proposed that an imbalance between the “direct” and “indirect” pathways, caused by alterations of excitatory and inhibitory neurotransmission in the internal globus pallidus/substantia nigra pars reticulata (GPi/SNr), underlies Parkinson’s disease (PD) physiopathology (1-3). Therapy with dopaminergic drugs is supposed to restore a quasi-physiological balance. Electrophysiological and clinical perioperative data show that acute administration of apomorphine causes a significant decrease in the cell firing activity of GPi neurones (4-6) while it also produces an amelioration of PD hypokinesia as assessed by perioperative UPDRS scale (7). Also, resting activity of subthalamic nucleus (STN) cells appears to be high in PD patients, as observed in the MPTP-treated monkey (8). These alterations could explain the imbalance between GABA and glutamate in the above-mentioned basal ganglia (BG) output nuclei. To verify this hypothesis, microdialysis studies were performed under clinical and electrophysiological conditions.
microdialysis measurements of neurotransmitter release from output BG nuclei could be very helpful.

In line with this view, a first microdialysis study was performed some years ago during stereotaxic thalamic surgery for PD tremor (9). The aim was to test the reliability of this technique for neurochemical characterisation of the target area. The study was performed by inserting microdialysis probes in the same trajectory as the electrodes, thus avoiding additional damage to the tissue.

In the present study, during stereotaxic GPe/GPi or STN neurosurgery in drug-free PD patients, we used the microdialysis technique, replacing damaged or inefficient tungsten recording microelectrodes with microdialysis probes. Perioperative administrations of therapeutic doses of the dopaminergic agonist apomorphine were performed. Our aims were to investigate both microdialysis safety/reliability and whether the clinical effects of apomorphine were coupled with changes in the extracellular concentrations of neuroactive excitatory and inhibitory amino acids (glutamate and GABA) in some BG regions.

MATERIALS AND METHODS

Subjects

Six advanced PD patients were enrolled in the study. The basis for selection was poor quality of life under pharmacological treatment, resulting from the well-described “on-off” events and levodopa-induced dyskinesias (LID) (10,11). In accordance with the type of clinical symptoms found to be prevalent, three patients (two males and one female) were selected for GPi surgery, and three (two females and one male) for STN surgery (Table I).

Other inclusion/exclusion criteria were as follows:

– Inclusion criteria for PD patients: long and clear history of idiopathic PD; clear clinical response to levodopa and/or to apomorphine administration, as measured by section III of the UPDRS (7); good general and mental conditions; under 70 years old; good tolerance of therapy withdrawal in the presurgery period.

– Exclusion criteria for PD patients: over 70 years old; cerebral atrophy on MRI; mental deterioration (Mental Deterioration Batteries) (12); presence of psychiatric disorders; uncertain clinical response to dopaminergic treatment; prominent vegetative dysfunction (clear orthostatic hypotension or bladder dysfunction) suggesting multisystem atrophy; any severe illness other than PD.

Therapy was withdrawn at least 15 days before surgery in order to obtain clear responses to perioperative drug administration, which would confirm the choice of target area.

The study was approved by the ethics committee of our university and the patients gave their informed consent.

Surgical procedure

The two hemispheres were implanted in two surgical sessions at least one month apart. The surgical technique was a modification of the one originally reported by Limousin et al. (13). Electrode trajectories were aimed at the posteroventral portion of the GPi or at the centre of the STN. Both nuclei were localised by means of high resolution MRI axial, coronal and sagittal views, T2-weighted or T1-weighted inversion recovery. These images, when drawn on the GPi or STN Talairach diagram (14), derived from coronal and lateral contrast ventriculography, correspond in location to the GPi or STN area in atlases (1). The GPi target coordinates in the three subjects were found to be between 2-3 mm anterior to the midcommissural point, 19-22 mm lateral to the midline of the third ventricle, and 6 mm below the intercommissural line. STN target coordinates were between 1.5-0 mm posterior to the midcommissural point, 12 mm lateral to the midline of the third ventricle, and 4 mm below the intercommissural line. The standard
trajectories pass through the putamen and GPe to reach the GPi and through the thalamus to reach the STN. In each of these nuclei, both electrodes and probes may be positioned by inserting them in different trajectories; this procedure allows simultaneous microdialysis and extracellular recordings from the GPe, GPi, or STN. Taking the extension of each nucleus into account, a 4-mm membrane probe was placed in the GPi and GPe, and a 2-mm membrane probe was positioned in the STN.

Electrophysiological recordings

F.H.C. tungsten recording electrodes (500-1000 Kohm; 0.4 mm external diameter), inserted into each trajectory, recorded single and multi-unit activity at different depths. The same electrodes allowed perioperative stimulation (monopolar, cathode, 180-200 Hz, 80-200 µs pulse width, 0-120 Volts) of the GPi/STN sites with an external stimulator (Grass model 8800) coupled to a stimulus isolation unit (SIU 8) to assess the acute clinical effectiveness of a given site. Electrophysiological data were displayed on oscilloscopes (up to eight traces displayed on four different oscilloscopes) and recorded on a digital system (National LabView) for off-line analysis. A multiple digital window discriminator allowed off-line selection of single unit activity and a home-made software allowed counting of firing frequency (time base adjustable between 1 ms and 10 s) and statistical analysis of the data. In each session, a subcutaneous apomorphine injection was performed while extracellular recordings from functionally identified GPi or STN neurons were being performed.

Intracerebral microdialysis measurements

Concentric microdialysis probes (external probe diameter 0.6 mm, membrane PES with 20 kDa molecular weight cut-off; 4 mm dialysing zone for GPe, GPi and 2 mm for STN; Metalant AB) were introduced directly into the target point via pre-positioned guide cannulae when one (or more) of the tungsten electrodes used for the electrophysiological identification of the target site was not properly working. Microdialysis probes were then connected to a CMA100 microinjection pump (CMA Microdialysis) set at 5 µl/min. Perfusion was started immediately with sterile PBS solution containing (g/l): NaCl 8, KCl 0.2, CaCl₂·2H₂O 0.132, MgCl₂·6H₂O 0.1, Na₂HPO₄ 1.15, KH₂PO₄ 0.2, pH 7.4. The dialysates were collected in plastic vials situated in a stand fitted to the stereotaxic frame. Complete sterility of all the materials used was assured. Samples

### Table I - Clinical characteristics of PD patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at surgery (in yrs)</th>
<th>Age at onset/therapy duration (in yrs)</th>
<th>Age at dysk. onset (in yrs)</th>
<th>UPDRS III Best ON</th>
<th>UPDRS III OFF</th>
<th>UPDRS IV (32-34)*</th>
<th>H&amp;Y ON</th>
<th>H&amp;Y OFF</th>
<th>Mean daily l-dopa</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPI 1</td>
<td>M</td>
<td>51</td>
<td>38/13</td>
<td>45</td>
<td>29</td>
<td>71</td>
<td>10</td>
<td>2.5</td>
<td>5</td>
<td>900</td>
</tr>
<tr>
<td>GPI 2</td>
<td>M</td>
<td>49</td>
<td>35/14</td>
<td>42</td>
<td>24</td>
<td>63</td>
<td>9</td>
<td>2</td>
<td>4</td>
<td>750</td>
</tr>
<tr>
<td>GPI 3</td>
<td>F</td>
<td>56</td>
<td>40/16</td>
<td>48</td>
<td>30</td>
<td>66</td>
<td>10</td>
<td>2.5</td>
<td>5</td>
<td>950</td>
</tr>
<tr>
<td>STN 4</td>
<td>F</td>
<td>69</td>
<td>45/24</td>
<td>60</td>
<td>25</td>
<td>58</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>1050</td>
</tr>
<tr>
<td>STN 5</td>
<td>F</td>
<td>63</td>
<td>52/11</td>
<td>59</td>
<td>45</td>
<td>78</td>
<td>6</td>
<td>2.5</td>
<td>5</td>
<td>1250</td>
</tr>
<tr>
<td>STN 6</td>
<td>M</td>
<td>55</td>
<td>45/11</td>
<td>52</td>
<td>30</td>
<td>57</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>1050</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>57.2</td>
<td>42.5/14.7</td>
<td>51.0</td>
<td>30.5</td>
<td>65.5</td>
<td>8.0</td>
<td>2.3</td>
<td>4.5</td>
<td>991.7</td>
</tr>
<tr>
<td>S.D.</td>
<td></td>
<td>7.5</td>
<td>6.1/5.0</td>
<td>7.4</td>
<td>7.6</td>
<td>8.0</td>
<td>1.9</td>
<td>0.3</td>
<td>0.5</td>
<td>168.6</td>
</tr>
</tbody>
</table>

* items for dyskinesia evaluation.
were collected every 10 min (50 µl) and immediately frozen. Amino acid content in each fraction was determined by HPLC analysis following automatic derivatization and resolution through a C18 reverse-phase chromatographic column (Chrompack; 10 X 4.6 mm, 3 µm; 30 °C) coupled with fluorimetric detection. The buffers and gradient programme used in the present study are those reported by Fedele et al. (16). Detection limits for all the amino acids were approx. 80-100 fmol/40 µl. In order to ascertain that all the probes used sampled amino acids with the same efficiency, at the end of the operation they were tested in vitro in standard solutions to determine the relative recovery of the amino acids. No significant differences as regards in vitro recovery were observed among the probes used in the different patients.

Calculations and statistical analysis

Microdialysis results are expressed as pmol/50 µl; values are not corrected for the in vitro recovery. Data were collected from both hemispheres in five and from one hemisphere in one patient, due to lack of a free cannula for the microdialysis probe in the latter patient. Since one patient had only one measurement, the data from the two hemispheres of the other five patients were averaged in each subject for each nucleus. Data were analysed by two-way ANOVA with two within factors: “treatment” (with two levels: control vs apomorphine) and “nucleus” (with two levels: GPi vs GPe). Measurements in the STN were studied using a one-way ANOVA. When appropriate, post-hoc analysis was carried out using Tukey’s test. Differences were considered significant at p<0.05, at least. Given the small number of subjects, mean GPi amino acid concentrations in basal conditions (calculated from the 7th, 8th and 9th fractions) were compared to those observed in the GPe by means of the Mann-Whitney non parametric test. Comparison between STN and GPi or GPe values were precluded by the different dialytic membrane lengths.

RESULTS

Clinical findings

As shown in Table II (see over) and Fig. 1, the selected apomorphine doses produced a clear clinical improvement in all patients. The effect appeared within 10-15 minutes in all patients, peaked within 20 minutes and disappeared progressively within 45-55 minutes.

Fig. 1 - Mean clinical scores assessed during surgery before and after apomorphine administration. The upper part reports the mean clinical score in the three patients implanted in the GPi. The black bars represent the score before apomorphine, while the open bars the score during apomorphine effect. The lower part reports the score in the three patients implanted in the STN. Abbreviations: R = right; L = left; UP = upper limb; Low = lower limb.
Electrophysiological recordings

Fifteen GPi cells, recorded from the three patients implanted in the GPi (between -2 and -6 mm below the intercommisural line), were selected for this study on the basis of their stable firing rate. In six different surgical sessions, six out of the 15 recorded cells responded to apomorphine (1.5-2.0 mg s.c.) with a 25-55% decrease in spontaneous firing activity (Fig. 2, see over). A complete recovery was observed in two cells after 50 and 55 minutes, whereas in the other four cells, where the recovery was not complete, the recording deteriorated after 30-45 minutes. Ten cells recorded from the three patients implanted in the STN were included in the present study. Their firing pattern (average frequency of 27±8 Hz; Fig. 2) was irregular, presenting prolonged (100-300 ms) trains of high frequency spikes separated by intertrain periods of lower frequency activity. Apomorphine administration (2.0-2.5 mg s.c.) was performed in four out of the 10 cells recorded in the three patients and invariably reduced their firing activity (Fig. 2). However, the percentage of inhibition varied greatly among subjects (10-75%) without the emergence of a correlation with the clinical improvement. Other features of the apomorphine responses in the parkinsonian subthalamus were the relative rapidity of the observed inhibition (significant 6-10 minutes after s.c. administration) and the slowness and incompleteness of electrophysiological recovery.

Microdialysis

Figures 3 and 4 (see over) show the STN, GPi and GPe extracellular levels of glutamate (GLU) and GABA in our Parkinson’s disease patients. As normally observed in in vivo microdialysis studies on laboratory animals, the various amino acids displayed high concentrations in the first 10-min sample collected immediately after insertion of the probes in the different brain nuclei. Afterwards, these levels declined and reached stable and reproducible baseline values within 60 min of the beginning of the infusion.
Mean basal values for the two amino acids, computed by averaging the content of fractions 7-9 obtained from each nucleus of each patient, are reported in Table III (see p. 64).

We observed a significantly higher concentration of GABA in the GPi in comparison to the GPe. Glutamate concentrations in these two nuclei did not differ significantly. Following administration of a single dose of apomorphine (1.5-2.5 mg, s.c.), we further monitored amino acid levels for 70 min. Despite the clear clinical and electrophysiological effects, apomorphine failed to cause any significant change in the extracellular levels of the two amino acids in the STN, GPi and GPe (Figs 3 and 4).

DISCUSSION

Microdialysis: safety, reliability and stability

In recent years, in vivo intracerebral microdialysis in humans has been used to monitor neu-
rochemical events in ischaemia (17,18), head injury (19-22), subarachnoid haemorrhage (23,24), epilepsy (25) and brain energy metabolism alterations (18, 23).

Similarly to Meyerson et al. (9), we found that stable amino acid levels were achieved within 50-60 minutes. Moreover, the basal levels of amino acids in different patients exhibited a 10-20% range of variability as observed in in vivo microdialysis experiments carried out on laboratory animals. In view of this reliability of results and of the small inter-subject variability, our findings suggest that microdialysis may be utilised in humans, even for prolonged periods of time.

**Observations in “off” therapy PD patients**

In PD patients, GABA concentration is higher in the GPi than in the GPe, and the difference is statistically significant.

STN concentrations cannot be directly compared to those observed in the two pallidal segments since the length of the dialytic membrane of the STN probe was half the length of those used in the GPi and GPe. Yet, considering that the recovery of the dialytic probe is dependent upon its length, we might conclude that GABA levels in the STN are similar to those found in the GPe, and almost half those measured in the GPi. There are several possible physiological explanations for the differences in GABA observed. First, GABA is released in the GPi both from the putaminal fibres of the “direct pathway” and from the “indirect pathway” via collaterals of the GPe fibres directed to the STN (26-29). Second, GPi cell density appears to be much higher than GPe cell density. Both of these facts could account for a larger physiological content of GABA in the GPi. Considering the current view on basal ganglia physiopathology, a pathological origin of the higher GABA release in the GPi would appear quite paradoxical. In fact, both GABAergic pathways to the GPi should be less active in off-state PD patients, thus producing the apomorphine-sensitive GPi hyperactivity. Finally, if an abundant network of intranuclear axon collaterals were found to be present in the GPi, similarly to that which has been described in the striatum (30), the GPi cell hyperactivity might account per se for the larger GABA release observed in our patients. Yet, the failure of apomorphine to induce changes in this higher basal GABA concentration renders all these “pathological” hypotheses unlikely.

**Observations in PD patients following acute apomorphine administration**

Dopaminergic receptor stimulation by apomorphine is supposed to improve clinical symp-
toms by decreasing the exaggerated glutamatergic excitation from the STN to the GPi and/or increasing GABAergic inhibition from the putamen to the GPi. The expected amino acid variations would be glutamate decrease in the GPi/GPe and an increase of GABA in the STN/GPi. The clinical symptoms improved after apomorphine administration in our PD patients and the electrophysiological data also changed, in accordance with previous findings (4,5). Indeed, GPi and STN showed an apomorphine-induced firing decrease; although the amplitude of the STN firing decrease differed rather from subject to subject (ranging from -10 to -50%), increases, or changes, in firing were never observed. Thus, the dopaminergic agonist was indeed effective at brain level, as evidenced by our clinical and electrophysiological observations during collection of microdialysis samples.

Unfortunately, we were not able to confirm the expected apomorphine-induced amino acid variations in the STN, GPi and GPe, as no changes in glutamate or GABA extracellular levels occurred. The relatively higher GABA concentration in GPi was also unaffected by apomorphine. These data appear to suggest that the acute effects of the dopaminergic therapy are not due to modulation of neuroactive amino acid release. However, several methodological considerations need to be taken into account. Microdialysis probes sampling from the extracellular environment may fail to detect physiologically important synaptic events, not leaking out of the synaptic cleft. In addition, microdialysis data obtained from laboratory animals cast serious doubts on the neuronal origin of these amino acid pools, as recently discussed by Timmerman and Westerink (31). This might be the case in the human brain, too, although this has yet to be proven. These considerations do not exclude the possibility of using the high GABA extracellular concentration in GPi as a potential marker of that area, regardless of the origin of this high concentration.

Having said this, we could alternatively hypothesise that the acute apomorphine challenge is able to improve the clinical symptoms without modifying amino acid release. This finding could be explained by the activation – not involving a cascade of amino acid release changes – of dopaminergic receptors located postsynaptically in the GPi and STN. This, at least as far as acute drug administration is concerned, would reduce the importance of both the “direct” and the “indirect” pathways in regulating GPi activity. However, this does not imply that the effects of long-term administration would be mediated by similar mechanisms. Indeed, differences in acute and long-lasting levodopa effects have been described in PD patients (32,33). Possible different basal concentrations during long-term dopaminergic stimulation could be assessed by comparing the amino acid levels observed in untreated patients with those measured in patients under chronic dopaminergic therapy.

Table III - Amino acid basal levels in the basal ganglia of Parkinson’s disease patients assessed by intracerebral microdialysis.

<table>
<thead>
<tr>
<th></th>
<th>GPe</th>
<th>GPi</th>
<th>STN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>55.04 ± 0.170</td>
<td>45.32 ± 1.811</td>
<td>25.56 ± 0.439</td>
</tr>
<tr>
<td>GABA</td>
<td>4.64 ± 0.252</td>
<td>9.88 ± 0.178</td>
<td>2.83 ± 0.185</td>
</tr>
</tbody>
</table>

Values are expressed as pmol/50 µl and represent the mean (± S.E.M.; no. = 3) of amino acid content in fractions 7-9. GABA basal levels in the GPi were significantly (p<0.05) higher than those measured in the GPe.
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