Subcutaneous apomorphine increases regional cerebral blood flow in parkinsonian patients via peripheral mechanisms

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1 We have measured regional cerebral blood flow (rCBF) and motor function before and after the subcutaneous (s.c.) injection of apomorphine in parkinsonian patients deprived of their usual treatment for at least 48 h.

2 Nineteen patients, pretreated with domperidone (20 mg three times daily for 48 h), received a mean dose of 5.8 mg s.c. apomorphine. All patients switched ‘on’. The mean motor score was significantly improved (−65%, P < 0.01) but no significant change in rCBF was observed.

3 Seven other patients, not pretreated with domperidone, received a lower dose (0.3 mg) of s.c. apomorphine. No change in motor score was observed while the mean rCBF significantly increased (+12%, P < 0.05).

4 We conclude that s.c. apomorphine increases rCBF in parkinsonian patients. This effect is independent of the central therapeutic effects of the drug. It is mediated by the stimulation of dopaminergic receptors of the cerebral vessels. These receptors are located outside the cerebral blood brain barrier and can be considered as ‘peripheral’ ones.

Keywords apomorphine domperidone cerebral blood flow Parkinson’s disease single photon emission tomography

Introduction

A number of studies have been performed in order to investigate the regional cerebral blood flow (rCBF) changes induced by dopaminergic treatments (for review see Leenders et al., 1985; Montastruc et al., 1987). These studies generally concluded that levodopa and dopaminergic agonists increased rCBF in animals and humans. However, this effect has not been univocally observed and differences in doses or duration of treatments have been put forward to explain some discrepancies. The mechanism underlying this response is also confusing: some authors claim that the increase in rCBF seen after dopaminergic stimulation is solely caused by a local increase of glucose metabolism (McCulloch, 1984; McCulloch & Harper, 1977) while others conclude that dopaminergic drugs have a direct vasodilatory effect on cerebral blood vessels unrelated to any effect on the parenchymal nervous tissue (Leenders et al., 1985; Montastruc et al., 1987). Very little data are available about rCBF responses to dopaminergic stimulation when peripheral dopaminergic receptors are blocked. This aspect is however of importance because cerebral vessels could be relaxed through peripheral dopaminergic mechanisms. Such a mechanism has already been demonstrated in vivo for systemic blood vessels (Bogaert et al., 1978; Rascol & Montastruc, 1986) and in vitro for cerebral vessels (Edvinson et al., 1985; Oudart et al., 1981; Toda, 1976). In order to address these points, we studied the effects of subcutaneous (s.c.) injections of apomorphine, a mixed D1- and D2-dopamine receptor agonist with potent and rapid antiparkinsonian activity when given subcutaneously (Hardie et al., 1984; Hughes et al., 1990) on both the rCBF and the motor function of patients with Parkinson’s disease (PD) with or without blocking the peripheral dopaminergic receptors by domperidone (Brogden et al., 1982).
Methods

Patients

Twenty-seven patients entered this study (mean age = 64 (7) years; mean duration of PD = 7 (6) years; mean levodopa daily dose = 401 (346) mg day\(^{-1}\); mean levodopa treatment duration = 4 (6) years; mean Hoehn and Yahr (H & Y) scale = 2.6 (1.1)). All patients suffered from idiopathic Parkinson's disease according to the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria (Gibb & Lees, 1988). Six of these patients had never been treated before with antiparkinsonian dopaminergic drugs but a positive response to the apomorphine test was observed in all of them (Hughes et al., 1990). The 21 other patients were treated with levodopa. In these patients levodopa (and all other antiparkinsonian medication) was withheld for 48 h before testing in order to allow the reappearance of the parkinsonian symptoms and to study patients while 'off'. None of the patients was clinically considered as demented.

rCBF measurement

rCBF was assessed using a single photon emission tomograph (SPECT) (Tomomatic 64; Medimatic, Copenhagen) and intravenous injection of \(^{133}\)Xenon (2,220 mBeq, i.e. 60 mCi) (Celsius et al., 1981). The technique and the method for data analysis have been described in a previous paper (for details see Montastruc et al., 1987). Briefly, data were simultaneously collected from three transverse slices, each of 2 cm thickness, parallel and centred at 1, 5 and 9 cm above the canthomeatal plane respectively. A mean global rCBF value of the whole second slice was calculated. Thirteen regions of interest (ROI) of the same slice were also defined using a semi-automatic method. They corresponded to nine cortical and four subcortical brain areas (see Figures 1 and 2). Mean values of rCBF derived from these ROIs were calculated.

General procedure

All patients were evaluated for the first time (motor score and rCBF), while 'off', without treatment. Their motor status was assessed using the global score of the motor examination of the Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn et al., 1987). Then, patients received an acute s.c. dose of apomorphine and a second evaluation (UPDRS motor score and rCBF) was performed afterwards. A plasma sample for apomorphine assay was collected at the end of the second rCBF measurement. The method for assaying apomorphine in plasma was high performance liquid chromatography (Bianchi et al., 1986). Two different protocols were conducted in two different groups of patients:

Protocol 1: high doses of s.c. apomorphine with domperidone pretreatment In this protocol, each patient received a s.c. dose of apomorphine which was known, from individual preliminary tests, to be sufficient to switch him 'on'. In order to avoid the peripheral dopaminergic side effects which are encountered with such doses, all the patients of protocol 1 were pretreated with oral domperidone (20 mg three times daily for at least 48 h) (Agid et al., 1979; Corsini et al., 1979). The second motor and rCBF evaluations were performed 10 min after the patients had fully switched 'on', i.e. when apomorphine was supposed to exert its central 'metabolic' effects.

Protocol 2: low doses of s.c. apomorphine without domperidone pretreatment In protocol 2, patients received a low standard dose of s.c. apomorphine (0.3 mg). This dose was insufficient to modify clinically the motor status of the patients but has been proved to be sufficient to induce a peripheral vascular relaxation in dogs (Bogaert et al., 1978). As no clinical motor change was supposed to occur, the moment of the second rCBF measurement was arbitrarily chosen 10 min after the s.c. injection of apomorphine. According to published data, this time was believed to correspond to the \(t_{\text{max}}\) of the drug (Gancher et al., 1989).

Chronically treated patient without domperidone We had the opportunity to study the rCBF of a severely fluctuating patient chronically treated for 'on-off' phenomenon with self injections of s.c. apomorphine (Frankel et al., 1990; Stibie et al., 1989). He was a 61 year old man (PD evolution = 10 years; levodopa daily dose = 2 g day\(^{-1}\) for 7 years, H & Y stage while 'off' = 4) adding to his levodopa treatment 3 to 4 daily injections of s.c. apomorphine (10 mg each) in order to reduce or avoid 'off' periods when needed. After 3 months of s.c. apomorphine treatment, this man had developed tolerance to the peripheral side effects of the drug while the 'off' periods continued to be effectively improved by the injections. He was therefore able to withhold domperidone and to continue s.c. apomorphine without discomfort. In this patient, a first rCBF was assessed during an 'off' period. A second evaluation was assessed 10 min after he had switched 'on' with his usual 10 mg s.c. apomorphine injection. No drug holiday was performed.

Informed consent was obtained from all patients. The project was approved by the local ethics committee.

Statistical evaluation

All values are expressed as mean (1 s.d.). Differences were assessed using the non parametric Mann-Whitney U test and Wilcoxon signed-rank test. Significance was accepted for \(P < 0.05\).

Results

Nineteen patients (12 F, 7 M) entered protocol 1, seven patients (5 F, 2 M) entered protocol 2. At baseline, there was no significant difference between the two groups of patients (mean age = 62 (8) years vs 66 (6) years, NS; mean PD duration = 8 (7) years vs 6 (3) years, NS; mean levodopa dose = 413 (372) mg day\(^{-1}\) vs 371 (293) mg day\(^{-1}\), NS; mean H & Y score = 2.5 (1.2) vs 2.9 (0.7), NS; mean UPDRS global score = 31 (17) vs 27 (6), NS; mean blood pressure = 133 (13)/84 (8) mm Hg vs
142 (16)/88 (16) mm Hg, NS; mean global rCBF value of slice 2 = 58 (5) ml 100 g⁻¹ min⁻¹ vs 59 (6) ml 100 g⁻¹ min⁻¹, NS).

Protocol 1

All patients switched ‘on’ within 10 to 20 min. The mean dose of s.c. apomorphine used to obtain this result was 5.8 (2.6) mg (range 3–10 mg). The second evaluation (motor score and rCBF measurement) was performed 32 (4) min after the s.c. apomorphine injection. In two patients, rCBF could not be compared before and after s.c. apomorphine, one because of a different position of the head between the two evaluations and the other because of too large dyskinetic movements preventing to keep the head motionless in the apparatus. Therefore, these two patients were excluded from analysis.

Compared with baseline, no change in mean blood pressure (133 (13)/84 (9) mm Hg vs 135 (17)/83 (7) mm Hg, NS) and PaCO₂ (38 (2) mm Hg vs 38 (2) mm Hg, NS) was observed. Conversely, the global score of the UPDRS motor score was significantly improved (31 (17) vs 11 (9), P < 0.01). At the same time no significant change of the mean global rCBF was observed (58 (5) ml 100 g⁻¹ min⁻¹ vs 59 (5) ml 100 g⁻¹ min⁻¹). No significant change occurred in any ROI (see Figure 1). Mean apomorphine plasma level was 45 (28) pmol ml⁻¹. Nausea was reported by four patients and mild sedation by nine patients.

Protocol 2

No patient switched ‘on’. The dose of s.c. apomorphine used was significantly lower than in protocol 1 (0.3 mg vs 5.8 (2.6) mg). The second evaluation of the patients was performed 13 (3) min after the s.c. apomorphine injection. This delay was significantly shorter than in protocol 1 (P < 0.01). At this time, there was no change in the global score of the UPDRS motor examination (27 (6) vs 27 (6)). On the contrary the mean global rCBF was significantly increased (59 (6) ml 100 g⁻¹ min⁻¹ vs 66 (6) ml 100 g⁻¹ min⁻¹, P < 0.05). Mean rCBF increased in most ROIs (see Figure 2) without any significant difference between any ROI. No difference in mean blood

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Figure 1 Mean values of rCBF in the different ROIs of the 17 patients of Protocol 1 before (left) and 32 ± 4 min after (right) the s.c. apomorphine injection (mean dose = 5.8 ± 2.6 mg). All patients were pretreated with oral domperidone (20 mg three times daily for 48 h). All patients switched ‘on’. Notice that, after s.c. apomorphine, the UPDRS global motor score was significantly improved (31 ± 17 vs 11 ± 9, P < 0.01) but that no significant rCBF change was seen in any ROI.

Figure 2 Mean values of rCBF in the different ROIs of the seven patients of protocol 2 before (left) and 10 min after (right) the s.c. apomorphine injection (0.3 mg). Patients were not pretreated with domperidone. No patient switched ‘on’. Notice that, after s.c. apomorphine, the UPDRS global motor score remained unchanged (27 ± 6 vs 27 ± 6) while, at the same moment, rCBF mean values were significantly increased in most ROIs (* = P < 0.05, ** = P < 0.02).
pressure (142 (16)/88 (16) mm Hg vs 140 (14)/90 (17) mm Hg, NS) and paco2 (37 (5) mm Hg vs 36 (4) mm Hg, NS) was observed. Mean apomorphine plasma level was 31 (2) 5 pmol ml−1, a value not significantly different from that of protocol 1. Vomiting was observed in one case and nausea in two others.

Chronically treated patient

The patient fully switched ‘on’ 20 min after the s.c. injection of 10 mg apomorphine. The second evaluation was assessed 30 min after the injection. No side effect occurred; no change in blood pressure was observed (150/90 mm Hg vs 140/90 mm Hg). UPDRS motor score improved from 60 to 31. No change in mean global rCBF (62 (10) ml 100 g−1 min−1 vs 60 (11) ml 100 g−1 min−1) or mean rCBF in any ROI (data not shown) was observed. Apomorphine plasma concentration was 53 pmol ml−1.

Discussion

Using SPECT, we observed that s.c. apomorphine was able to switch ‘on’ parkinsonian patients pretreated with domperidone without any detectable change in rCBF; conversely, in the absence of domperidone, lower doses of s.c. apomorphine induced a significant increase in rCBF without any change in motor status. We explain these results by a direct vasodilatory effect of s.c. apomorphine on cerebral vessels independent of therapeutically or central metabolic effects. The present study did not consider any normal subjects as a control population. Several reasons explain this choice. First, in this work we intended to study the reactivity of rCBF to dopaminergic stimulation and not to compare baseline rCBF in parkinsonian patients and normal subjects. This last question has been considered many times and it is generally recognized that, compared with normals, a global and moderate rCBF decrease, roughly correlated to the severity of the disease, is observed in Parkinson’s disease (Marc-Vergnes et al., 1988). As the range of normal rCBF values is quite wide, a large number of subjects needs to be investigated to demonstrate statistically a moderate group mean difference (Frackowiak et al., 1980). Second, previous works from our (Montastruc et al., 1987) and other groups (Leenders et al., 1984, 1985) showed that levodopa effects on rCBF are similar in parkinsonian patients and normal subjects. Finally, in a previous experiment, one of our normal controls presented after an acute dose of a dopaminergic agonist a severe hypotension with angor pectoris. Therefore, we and our ethics committee felt it was not totally safe and not essential to study the effects of s.c. apomorphine in normal subjects. The two protocols were not performed in the same patients because we and our ethics committee considered that the total radioactive dose would have been too large if the same patients had received four doses of 133Xenon.

To our knowledge, it is the first time that the effects of s.c. apomorphine on rCBF are reported in humans. S.c. apomorphine is a specially suitable tool for pharmacological tests because of its unique pharmacokinetic (very short tmax, very short half-life) (Gancher et al., 1989) and pharmacodynamic (one of the most potent and rapidly acting D1-D2 dopaminergic agonists usable in man) (Hardie et al., 1984; Hughes et al., 1990) profile. A large number of studies has investigated the effects of various dopaminergic drugs on rCBF using various techniques in animals and humans; two major controversies still persist: (1) why did some studies, mostly in humans, fail to observe the increase in rCBF usually reported by the others? (2) is this increase due to a direct vasodilatory effect or is it secondary to central metabolic effects of the drug?

In protocol 2, we observed that a low (0.3 mg) dose of s.c. apomorphine was able to diffusely increase rCBF in parkinsonian patients deprived of their usual treatment for 48 h. A non specific test-retest effect can be ruled out because in similar conditions apomorphine + domperidone (protocol 1) and tropatepine (Celsis et al., 1989) failed to modify rCBF. The mean increase (+12%) observed with apomorphine in the present study is remarkably comparable with what has been reported with levodopa or bromocriptine using positron emission tomography (PET) and SPECT (Celsis et al., 1988; Leenders et al., 1985; Montastruc et al., 1987). However, other studies did not reproduce these results (Melamed et al., 1986; Perlmutter & Raichle, 1985) and it has been suggested that low doses of levodopa were unable to modify rCBF. In fact, ‘low’ (200 mg) acute doses of levodopa have been reported to increase rCBF (Montastruc et al., 1987) while ‘high’ chronic doses (1080 mg day−1) (Melamed et al., 1986) failed to do so. In the present study low doses of s.c. apomorphine (0.3 mg) increased rCBF while higher doses (mean = 5.8 mg) did not. Therefore, the dose cannot explain the discrepancies. Time dependent differences in drug administration could be a more suitable explanation. In man, the acute administration of dopaminergic drugs increased rCBF in several trials (Bès et al., 1983; Celsis et al., 1988; Leenders et al., 1985; Montastruc et al., 1987). In these positive studies, the dopaminergic treatment had been withheld for a rather long delay (48 h or more) while this delay was shorter (less than 24 h) in negative studies (Perlmutter & Raichle, 1985; Henriksen & Boas, 1985). Moreover, no rCBF changes were observed in patients chronically treated with levodopa (Leenders et al., 1985; Melamed et al., 1986) and in our patient chronically treated with s.c. apomorphine. It is then conceivable that drug holidays could favour the dopaminergic response of cerebral vessels. Tolerance to peripheral dopaminergic gastrointestinal and vascular effects is a well known phenomenon (Clarke, 1990) while it is generally accepted (Frankel et al., 1990; Stibe et al., 1988), despite some controversies (Grandas & Obeso, 1989), that tolerance to s.c. apomorphine does not develop at the central level. The development of tolerance to the dopaminergic reactivity of cerebral vessels would therefore suggest that peripheral mechanisms are probably involved in the response of rCBF to antiparkinsonian drugs. This conclusion is in fact supported by the results of protocol 1 and 2.

S.c. apomorphine had opposite effects in protocol 1 and 2. Four main reasons can explain this difference: (a) the dose of apomorphine, (b) the delay between the s.c. apomorphine injection and the second rCBF measurement, (c) the effectiveness of the drug on the parkinsonian symptoms and (d) the domperidone pretreatment.
The difference in apomorphine doses is not a satisfactory explanation because the dose of apomorphine was higher in the protocol where no rCBF change was observed. In monkeys, a dose-response relationship between the rCBF increase and apomorphine has been observed (McCulloch & Harper, 1977).

One could also argue that we missed a rise in rCBF in protocol 1 because the second measurement was performed too late. However, this hypothesis can be refuted because of the following reasons: 32 min after the injection of apomorphine, the drug was still present (as demonstrated by the clinical response and the plasma assays). The second examination was more delayed in the first protocol but the dose of apomorphine was higher. According to the interindividual plasma concentrations variations and the pharmacokinetic features of the drug (Gancher et al., 1989; Montastruc et al., 1991), plasma concentrations were then not statistically different during the second rCBF assessment in the two protocols. Moreover, in monkeys, rCBF was reported to be still significantly increased 40 min after the i.v. injection of apomorphine (McCulloch & Harper, 1977).

Could the changes which occurred in the parkinsonian motor status of the patients explain the difference in rCBF responses? It is obvious, using SPECT, that we did not measure the cerebral metabolism. However, the presence of a substantial clinical improvement in the patients of protocol 1 strongly suggests that the drug was acting at the central level and was inducing its central metabolic effects. Therefore, if the rCBF changes induced by s.c. apomorphine were secondary to brain metabolism changes, one would have expected to observe them in the patients clinically improved. On the contrary, rCBF remained unchanged in protocol 1 and increased in protocol 2 where no antiparkinsonian efficacy was clinically noticed. The relationship between levodopa, regional blood flow and brain energy expenditure appears complex and confusing. In animals, metabolic or autoradiographic studies reported that dopaminergic stimulation increases glucose utilisation in certain regions related to basal ganglia but also in regions without any known dopaminergic innervation (McCulloch & Harper 1977; Porrino et al., 1987). Based on these observations, some authors claimed that the increase in rCBF seen after dopaminergic stimulation is solely caused by a local increase of glucose metabolism (McCulloch & Harper, 1977; McCulloch, 1984). However, other data contradict this assumption. In animals, a vasodilatory effect unrelated to energy metabolism has also been observed (Ekström-Jodal et al., 1974; Ingvar et al., 1983). In isolated cerebral vessels, dopaminergic drugs induce vascular relaxation (Edvinsson et al., 1985; Oudart et al., 1981; Toda, 1976). In parkinsonian patients, no correlation has ever been demonstrated between the clinical improvement and the rCBF changes induced by levodopa (Leenders et al., 1985; Melamed et al., 1986; Montastruc et al., 1987). Moreover, in the present and in other studies (Leenders et al., 1985; Montastruc et al., 1987), levodopa induced a global increase in rCBF roughly similar in cortical and subcortical areas; no topographic relationship has been demonstrated between rCBF changes and central dopaminergic pathways. Finally, in man, after levodopa treatment, PET studies failed to demonstrate a significant increase in glucose metabolism or oxygen utilisation in all (Leenders et al., 1985; Rougemont et al., 1984) but one (Raichle et al., 1984) parkinsonian patients and control subjects.

Therefore, the pretreatment with the peripheral dopamine receptor antagonist domperidone appears to be the more suitable explanation for the opposite results of protocol 1 and protocol 2. This suggests that the rCBF increase observed after s.c apomorphine was secondary to the stimulation of dopaminergic receptors located on cerebral blood vessels outside the blood brain barrier. We found very little data about the effects of domperidone pretreatment on the dopaminergic responses of rCBF. To our best knowledge, Leenders et al. (1984) reported, using PET in five parkinsonian patients, that the rCBF increase observed after levodopa was blocked by domperidone. This is in agreement with our results. Conversely, in five migraineous patients, Bès et al. (1984) did not observe any difference in the rCBF response to pirebidil before and after domperidone. However, these authors probably used too low doses of domperidone (one oral dose of 20 mg before the pirebidil test) to effectively block all the peripheral dopaminergic receptors.

In conclusion, our results confirm that the acute administration of dopaminergic agents increases rCBF in parkinsonian patients. This response, as detected with SPECT, is independent of the central therapeutic effects of the drugs and is mediated by peripheral dopaminergic vascular receptors. This result does not refute however the possibility that more subtle rCBF changes, undetectable using SPECT, and possibly related to central metabolic effects can occur. Autoradiographic data obtained in MPTP-treated animals suggest that such changes may exist (Porrino et al., 1987). Techniques more sensitive than SPECT could perhaps bring to the fore these putative effects in patients pretreated with domperidone or chronically treated with antiparkinsonian drugs in order to avoid the peripheral vascular dopaminergic effects.

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