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Parkinson's disease: pathological mechanisms and actions of piribedil

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Summary. The cause of the degeneration of dopamine-containing cells in the zona compacta of the substantia nigra in Parkinson's disease remains unknown. The ability of the selective nigral toxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) (via its metabolite MPP^+) to destroy nigral dopamine cells selectively by inhibiting complex I of the mitochondrial energy chain may provide a clue. Indeed, recent studies of post-mortem brain tissue have suggested the presence of an on-going toxic process in the substantia nigra in Parkinson's disease leading to excess lipid peroxidation. This appears also to involve a disruption of mitochondrial function since mitochondrial superoxide dismutase activity is increased and there is impairment of complex I. These changes may in turn relate to a selective increase in the total iron content of substantia nigra coupled to a generalised decrease in brain ferritin content. Piribedil is used in the symptomatic treatment of Parkinson's disease and is particularly effective against tremor. Piribedil (and its metabolites) acts as a dopamine D-2 receptor agonist. However, in our studies in contrast to other dopamine agonists, in vivo piribedil interacts with dopamine receptors in the substantia nigra and nucleus accumbens but not those in the striatum. In patients with Parkinson's disease the beneficial effects of piribedil may be limited by nausea and drowsiness. Indeed, in MPTP-treated primates piribedil reverses motor deficits but marked side-effects occur. However, pre-treatment with the peripheral dopamine receptor antagonist domperidone prevents the unwanted effects and piribedil produces a profound and longer-lasting reversal of all components of the motor syndrome. These results suggest that combined with domperidone piribedil could be used as an effective monotherapy in the treatment of Parkinson's disease.

Key words: Parkinson's disease – Post-mortem studies – Mitochondrial impairment – Piribedil – MPTP

Introduction

The motor deficits which characterise Parkinson's disease are due to the degeneration of pigmented dopamine-containing cells in the zona compacta of the substantia nigra [14]. This leads to a marked fall in the dopamine content in the putamen and a smaller decrease in the caudate nucleus [17]. The unequal losses of dopamine between caudate and putamen differentiate the biochemical changes characterising idiopathic Parkinson's disease from those occurring in the post-encephalitic form of the illness and those in progressive supranuclear palsy [42]. Other dopamine-containing brain-stem nuclei are also affected in idiopathic Parkinson's disease, particularly those cells in the ventral tegmental area, causing some loss of dopamine in the nucleus accumbens and frontal cortex [10]. How these losses of dopamine contribute to the symptoms of the illness is not known; they may relate to psychiatric or psychological changes occurring in some patients with Parkinson's disease (see Dubois et al., this issue). Again, the losses of dopamine in limbic areas differentiate the pathology of idiopathic Parkinson's disease from that occurring in multiple system atrophy.

The pathology and biochemistry of Parkinson's disease are not, however, limited to the dopamine-containing cells of the brain stem and there are changes in other nuclei, for example, the noradrenaline-containing cells of the locus coeruleus [2]. Alterations in the brain content of serotonin (5HT) and acetylcholine and gamma-aminobutyric acid (GABA)-related parameters have also been reported, together with selective changes in the neuropeptide content of the basal ganglia [1]. At present it is uncertain how any of these biochemical changes relate to the motor disturbances of Parkinson's disease. Wherever pathology occurs in Parkinson's disease it is accompanied by the presence of eosinophilic inclusions termed Lewy bodies, the exact nature of which remains unknown [14]. However, they serve to differentiate Parkinson's disease from other basal ganglia illnesses causing akinesia such as multiple system atrophy or progressive supranuclear palsy.

Toxin involvement in Parkinson's disease

While the biochemical and pathological changes occurring in Parkinson's disease have been well documented, there has been relatively little progress in understanding the cause of the illness or the mechanisms underlying dopamine cell death. Parkinson's disease does not ap-

pear to be a primary hereditary disorder and advancing age is the only accepted predisposing factor. Recent interest in the cause of the illness has centred on the involvement of environmental factors [48]. This concept largely originates from epidemiological studies apparently indicating a higher incidence of Parkinson's disease in western industrialised societies than in newly industrialised countries such as Nigeria or China. Indeed, within these latter countries there appears to be a greater occurrence of Parkinson's disease associated with the industrialised cities compared with that in the rural surroundings. In contrast, in North America other studies have suggested that early exposure to a rural environment coupled to the drinking of well water might lead to the early onset of the disease.

There is, however, no conclusive evidence to suggest that there are marked differences in the global incidence of Parkinson's disease. Epidemics of Parkinson's disease do not appear to occur and pockets of the illness are not evident within communities. There is also no evidence to support the involvement of any one environmental factor. Thus it appears unlikely that environment alone could be responsible for the majority of the cases of Parkinson's disease. However, there have been suggestions that patients developing Parkinson's disease might show a genetic susceptibility to the actions of environmental toxins, due to a deficiency of some xenobiotic metabolising enzymes or of sulphydryl radicals, which are important in the inactivation of toxic species [47, 50]. Therefore, a combination of genetic susceptibility and environment might indeed explain how Parkinson's disease occurs, but even so this does not give any clear indication as to the agents involved or the mechanism by which dopamine cells are destroyed.

The neurotoxic actions of MPTP

The only clue to the cause and mechanism underlying Parkinson's disease has come from the discovery of the ability of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to destroy selectively dopamine-containing cells in substantia nigra in man and other primate species [3, 8, 20–22]. The administration of MPTP to primates produces the major signs of Parkinson's disease, with the possible exception of tremor, and mimics the major biochemical and pathological changes occurring in the disorder, namely the loss of dopamine-containing neurons in the nigro-striatal pathway. It is not a perfect model of Parkinson's disease since other biochemical and pathological changes do not occur in most instances and because, with the passage of time, the animals show recovery from some of the motor symptoms induced by MPTP [18]. However, it is a highly effective model in which to test potential treatments for Parkinson's disease and so far has proved predictive of drug effects in man.

The mechanism by which MPTP kills brain dopamine cells has been studied extensively [18]. MPTP is metabolised within brain by monoamine oxidase B located in glial cells to produce the corresponding pyridinium species 1-methyl-4-phenylpyridinium ion (MPP^+). MPP^+ is accumulated within dopamine neurons, either by active uptake or by direct passage through the neuronal membrane and once within dopamine neurons is actively taken up by mitochondria where it reaches high concentrations. Within the mitochondria MPP^+ acts at the level of complex I to inhibit the mitochondrial respiratory chain, so initiating cell death [30, 49]. Precisely how MPP^+ induces inhibition of mitochondrial respiration is not known, but one suggestion has been that a redox reaction might occur between MPP^+ and an intermediate in its formation, $MPDP^+$, to generate toxic superoxide radicals [41].

The important question is whether either MPP^+ or the mechanism by which it acts is of any relevance to processes occurring in Parkinson's disease. Certainly MPTP itself is not the cause of Parkinson's disease, since it is not commonly found in the environment. However, there are many toxic analogues of MPTP, and some endogenous molecules such as tetrahydroisoquinolines and beta-carbolines also contain the MPTP structure [29, 32]. Thus, compounds such as these may be important in the initiation of the pathological process causing Parkinson's disease.

To determine whether there is any relationship between the mechanisms by which MPTP produces dopamine cell death and that occurring in Parkinson's disease, we and others have examined post-mortem tissue from patients dying with Parkinson's disease in an attempt to detect a similar toxic process.

Pathological mechanisms occurring in Parkinson's disease

In post-mortem tissues taken from patients with Parkinson's disease a global index of cell death, namely lipid peroxidation, was examined by the measurement of the substrate, polyunsaturated fatty acids (PUFAs), and an intermediate in the lipid peroxidation process, namely malondialdehyde (MDA) formation [9]. Studies in a variety of brain areas showed that there was no difference between the levels of PUFAs or MDA in most regions when comparing parkinsonian tissue with those from age-matched controls. However, within substantia nigra there was a decrease in the PUFA content coupled to an increase in MDA formation. Therefore, these data might suggest that some on-going toxic process can be detected in substantia nigra in Parkinson's disease up to the time of death. Recently these findings have been confirmed by the demonstration of a marked increase in substantia nigra of an early component of the lipid peroxidation chain, namely lipid hydroperoxides (unpublished observation). Such results are in agreement with recent morphological data showing a reactive microgliosis in substantia nigra in Parkinson's disease [26], again suggesting that some continuous toxic process occurs. The question, however, remains as to whether this toxic process involves either impairment of mitochondrial function or superoxide radicals, as has been suggested for MPTP.

Table 1. Comparison of respiratory chain enzyme activities from substantia nigra of controls and patients dying with Parkinson's disease. *** $P < 0.01$; ** $P < 0.02$; * $P < 0.05$; $n = 9$ unless otherwise stated. Statistical analysis by unpaired t-test. Enzyme activities expressed as nmol/min/mg total protein, mean ± 1 SD. Taken from [44]

	Control	Parkinson's disease
Age (years)	66.1 ± 23.0	64.3 ± 21.3
Death to refrigeration (min)	107.4 ± 52.2	128.9 ± 72.5
Death to brain removal (h)	19.0 ± 5.9	17.7 ± 11.1
Substantia nigra protein concentration mg/g wet weight	109.0 ± 8.9	109.7 ± 17.8
Citrate synthase	110.4 ± 42.1	105.8 ± 50.0
NADH cytochrome *c* reductase (rotenone sensitive) [a]	4.36 ± 1.41	2.68 ± 1.01***
Succinate cytochrome *c* reductase [b]	9.46 ± 3.01	9.59 ± 3.15
[a]:[b]	0.48 ± 0.16	0.30 ± 0.11**
NADH CoQ reductase	3.36 ± 0.44	2.34 ± 0.76*

Mitochondrial function in Parkinson's disease

The levels of the cytosolic and mitochondrial forms of superoxide dismutase in the parkinsonian brain were compared with activity in control tissues. In our own study there was no difference in the total activity of superoxide dismutase in the substantia nigra and cerebellum comparing parkinsonian tissues with controls or in the levels of the cytosolic form of the enzyme [43]. However, although in the cerebellum there was no difference in the levels of mitochondrial superoxide dismutase activity between controls and parkinsonians, there was an increase in the activity of this isoenzyme in the substantia nigra in Parkinson's disease. This might suggest that the mitochondria are responding to an increased superoxide radical load by altering enzyme activity. It should be noted, however, that in another study by Martilla and colleagues [25] an increase in superoxide dismutase activity in the substantia nigra was found in the cytosolic, but not in the mitochondrial, form of the enzyme. So, although there is agreement that superoxide dismutase activity changes in Parkinson's disease, there is dispute over the isoenzyme involved.

Measurement of markers of mitochondrial function in substantia nigra in Parkinson's disease showed that there was no alteration in the levels of succinate cytochrome *c* reductase activity, a marker of complexes II and III, but that there was a decrease in the rotenone-sensitive NADH cytochrome *c* reductase activity, a marker of complexes I-III [44]. Hence, by subtraction there would appear to be a deficit in complex I (Table 1). Indeed, measurement of a specific marker of complex I NADH-Co Q reductase, showed this was also decreased in substantia nigra in Parkinson's disease. There thus appears to be the same fault in mitochondrial function in substantia nigra in Parkinson's disease as occurs in the presence of MPTP.

The deficiency of complex I has not been found in other areas of the parkinsonian brain, including the caudate and putamen [45]. It does not occur in other disease states such as multiple system atrophy and it does not appear to be due to levodopa treatment of the patients. A similar deficiency in complex I has also been reported in the platelets of patients with Parkinson's disease [34], but this has not been confirmed (unpublished observation). Interestingly, it has been suggested that some-subunits of complex I are specifically affected in patients with Parkinson's disease [28], although these studies were carried out in striatum where no overall deficiency of complex I was detected and where pathology does not occur.

The discovery of the selective impairment of complex I provides for the first time a specific mechanism by which cell death might occur in Parkinson's disease.

Iron involvement in Parkinson's disease

Despite new evidence on basic mechanisms involved in cell death in Parkinson's disease there is still no clue to the nature of the causative agent. One possibility lies in the involvement of brain iron content, which was suggested to be altered by MRI studies in patients with Parkinson's disease [33]. Iron is a potent initiator of free radical mechanisms and in early studies of formalin-fixed tissue Earle showed that the iron content of the midbrain in Parkinson's disease was increased [13]. For these reasons changes in iron and other metals have been studied in selected regions of the brain [10, 39, 46].

In most areas of the brain in Parkinson's disease there was no difference in the iron content compared with controls. Indeed, in the globus pallidus iron levels were reduced compared with those in control subjects. However, in the substantia nigra there was a 35% increase in the total iron content compared with control tissues [10] (Fig. 1). This might suggest an increase in free and reactive iron capable of stimulating free radical mechanisms. However, iron is normally inactivated in brain by binding to ferritin. The brain levels of ferritin were therefore measured using a monoclonal antibody technique. There was no increase in ferritin in substantia nigra; rather there was a marked decrease which was also found in other areas of the brain [11]. This generalised decrease in ferritin might suggest an alteration in iron metabolism occurring in Parkinson's disease. Hence, an alteration in brain iron content might indeed be part of the pathological process associated with Parkinson's disease. Altered

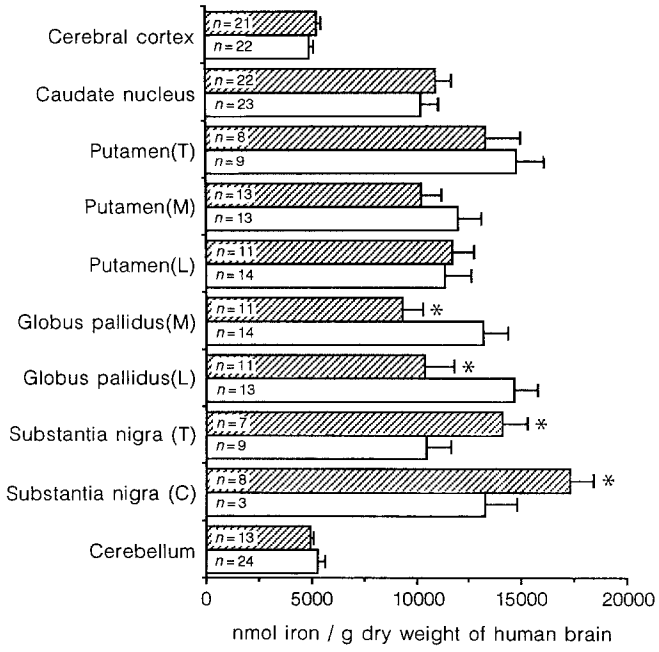

Fig. 1. Total levels of iron (nmol/g dry weight) in Parkinson's disease and age-matched control human autopsy brains. Values represent means ± 1 SEM. $^{*}P < 0.05$ compared to controls (Student's *t*-test). ▨, Parkinson's disease patients; □, control subjects; *C,* compacta; *L,* lateral; *M,* medial; T, total. Taken from [9]

iron metabolism is unlikely to be the primary cause of degeneration, since increases in brain iron content have also been demonstrated in other degenerative disorders of basal ganglia such as Huntington's chorea, multiple system atrophy and progressive supra-nuclear palsy [12]. Thus, wherever pathology occurs an increase in iron content is observed. One difference between Parkinson's disease and these conditions is that there is either no change or an increase in ferritin content in diseases other that Parkinson's disease. It therefore seems that iron may play a role in neuronal degeneration, but this appears to be secondary to some primary cause of dopamine cell death such as an inhibition of complex I.

The recent data on neuropathological changes in Parkinson's disease have started to suggest a mechanism by which dopamine cells may degenerate in this disorder. The object of such studies is to identify the mechanism which causes Parkinson's disease and then to design agents capable of slowing or stopping degeneration of brain dopamine cells. However, at the present time, with the possible exception of Selegiline [35], there are no compounds known to be effective in reducing pathological change in Parkinson's disease. Therefore for the time being clinicians will have to rely on the symptomatic treatment of the illness and cope with the inability to control the underlying progression of disease.

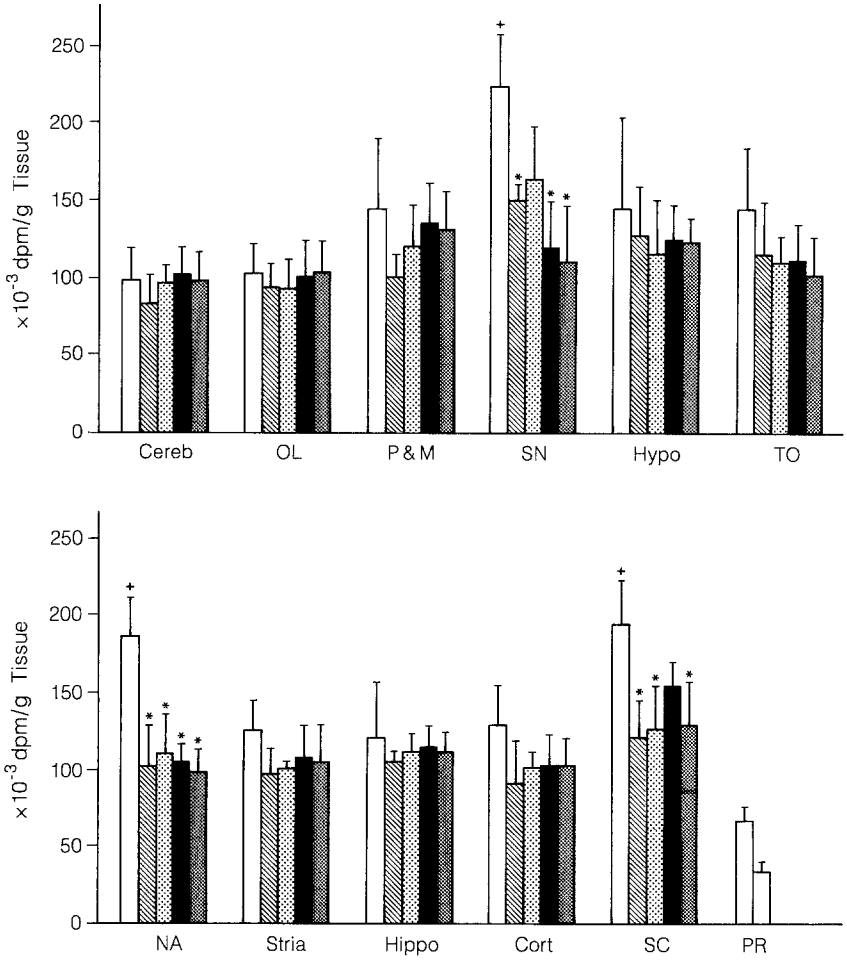

Fig. 2. The accumulation of radioactivity derived from [^{3}H]piribedil (25 μCi i.v.) in various areas of rat brain and prevention of accumulation by piribedil (40 mg/kg i.p., 20 min previously), S 3608 (40 mg/kg i.p., 20 min previously (+)-butaclamol (5 mg/kg i.p., 30 min previously) or apomorphine (0.5 mg/kg s.c., 15 min previously). $^{+}P < 0.05$ compared with cerebellar levels. $^{*}P < 0.05$ compared with the accumulation occurring in the same area in the absence of unlabelled drug. Each value is the mean (± 1 SEM) of the radioactivity measured in tissue from at least ten animals. □, Ligand alone; ▨, piribedil (40 mg/kg i.p.); ▩, S 3608 (40 mg/kg i.p.); ■, (+)-butaclamol (5 mg/kg i.p.); ▩, apomorphine (0.5 mg/kg s.c.). *Cereb,* cerebellum; *OL,* olfactory lobes; *P & M,* pons and medulla; *SN,* substantia nigra; *Hypo,* hypothalamus; *TO,* tuberculum olfactorium; *NA,* nucleus accumbens; *Stria,* striatum; *Hippo,* hippocampus; *Cort,* cortex; *SC,* spinal cord; *P,* plasma; *R,* red cells. Taken from [16]

Basic mechanisms of action of piribedil in Parkinson's disease

Piribedil was introduced into the treatment of Parkinson's disease in the early 1970s. However, it was largely employed in conjunction with levodopa in patients exhibiting "wearing-off" effect or other long-term problems associated with therapy [23, 40]. As such, it did not find a place as monotherapy in the treatment of Parkinson's disease and was thought to be mainly effective against the tremor component of the disorder rather than akinesia. Its usage was also hampered by the side-effects experienced at higher doses, namely nausea and drowsiness. Piribedil produces its effects in Parkinson's disease by acting as a dopamine agonist, but it is uncertain whether this is due to the effects of the parent compound or to one of its major metabolites, the catechol S 584 [19].

Piribedil interacts with D-2 receptors, while the metabolite S 584 is able to interact with D-1 sites as judged by adenylate cyclase assays [16, 27]. The dopamine agonist activity of piribedil can also be demonstrated in vivo by its ability to induce contraversive rotation in 6-hydroxydopamine lesioned rats, to induce stereotypy and motor activity in rodents and to prevent tremor induced by lesions of the brain stem in primates [4–7, 15, 36, 37]. The effects of piribedil in the 6-hydroxydopamine lesioned rat can be prevented by prior treatment with α-methylparatyrosine [38], which suggests some dependence of its actions on endogenous dopaminergic tone.

Piribedil is differentiated from other dopamine agonists in that it is structurally unrelated to other compounds of this class. It may also be differentiated by some of its pharmacological effects. For example, in vivo there appear to be differences in the dopamine receptor populations with which it interacts compared with other dopamine agonists. Thus, the administration of ^{3}H-piribedil led to an accumulation in the substantia nigra and the nucleus accumbens but not in the striatum or elsewhere (Fig. 2). The accumulation of ^{3}H-piribedil in the substantia nigra and in the nucleus accumbens could be displaced by treatment of the animals with unlabelled piribedil or *N,n*-propylnorapomorphine or (+)-butaclamol. In contrast, administration of ^{3}H-*N,n*-propylnorapomorphine produced an accumulation in the substantia nigra, the striatum and the nucleus accumbens but not elsewhere. The administration of unlabelled *N,n*-propylnorapomorphine or (+)-butaclamol caused a displacement of the ligand from all three brain regions. In contrast, the administration of unlabelled piribedil only displaced ^{3}H-*N,n*-propylnorapomorphine from the substantia nigra and the nucleus accumbens and not significantly from the striatum and the cortex.

These studies suggest differences in the location of the receptor populations within basal ganglia with which piribedil interacts, at least compared with an aporphine derivative. However, the nature of these dopamine receptors in terms of D-1 and D-2 characteristics remains to be determined. The alternative explanation is that the localisation of piribedil in the substantia nigra and nucleus accumbens represents a preferential penetration of the drug into these brain regions and so is more related to physicochemical properties of the compound than its interaction with receptor sites.

Actions of piribedil in MPTP-treated common marmosets

To explore further the potential antiparkinsonian activity of piribedil, its actions were examined in the MPTP-treated common marmoset. These animals respond to all known antiparkinsonian agents and have identified the clinical antiparkinsonian actions of compounds such as (+)-PHNO or N-0437 [24, 31]. Piribedil was incorporated into a series of studies designed to clarify further the predictive nature of the model (unpublished data). It was thought that the compound would not be highly effective in MPTP-treated animals, since they show relatively little tremor and since it is this component of the disease which piribedil is thought to control most effectively.

Piribedil caused a reversal of the motor deficits induced by MPTP, but this was of relatively short duration and the animals exhibited a high incidence of nausea and retching (Fig. 3). Thus, although akinesia was reversed the side-effects were marked, as has been found with high doses of piribedil in patients with Parkinson's disease.

In subsequent experiments the animals were pre-treated with the peripheral D-2 dopamine receptor antagonist domperidone to alleviate the nausea and retching. Piribedil now caused a rapid and profound dose-dependent reversal of all aspects of motor disability which was of longer duration than that seen following piribedil alone. Indeed, the animals showed a well-controlled increased

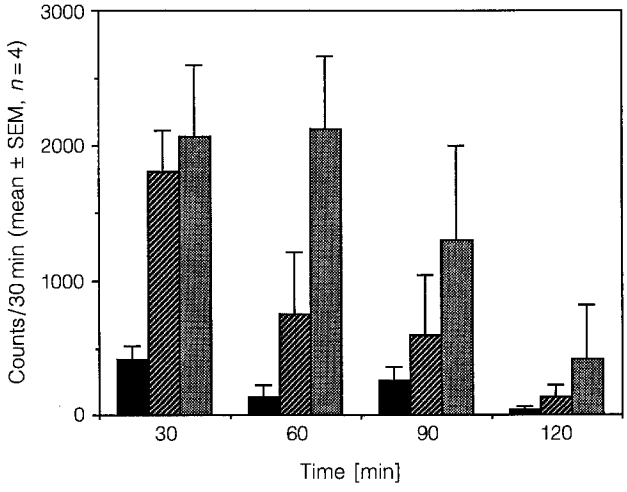

Fig. 3. The reversal of motor deficits in MPTP-treated common marmosets produced by the administration of piribedil (5.0 mg/kg p.o.) with and without prior treatment with domperidone (2 mg/kg p.o.). Values are the mean activity counts (± 1 SEM) for groups of four animals. Activity counts were accumulated over 30 min periods for up to 2 h. Domperidone was administered 30 min prior to treatment with piribedil. ■, Vehicle; ▨, piribedil (5 mg/kg p.o.); ▩, piribedil (5 mg/kg p.o.) plus domperidone (2 mg/kg p.o.)

in normal motor function with no occurrence of stereotyped behaviours or other abnormal movements.

There may be a variety of reasons why piribedil appears effective in the MPTP model. First, there may be species differences in the metabolism of piribedil in the marmoset compared with man, rendering it a more effective antiparkinsonian drug in the monkey. Second, the use of domperidone to prevent nausea and vomiting may allow the administration of higher and more effective doses of piribedil. It appears that to date there have been no studies of the use of domperidone treatment on the antiparkinsonian efficacy of piribedil. Whatever the reasons, the present data would suggest that in conjunction with domperidone piribedil should be an effective anti-parkinsonian agent in monotherapy if the predictive nature of the MPTP model is confirmed (see also, Rondot and Ziegler, this issue). Indeed, with the production of a longer-acting form of piribedil or some other means of drug administration such as transdermal patches, piribedil could be an effective first-line treatment for the symptoms of Parkinson's disease.

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