INTRODUCTION

In 1985, André Barbeau, in an elegant lecture delivered at the world congress on Parkinson’s disease held in New York City, focused attention on the lack of some hepatic metabolic enzymes as a factor favouring the degeneration of the dopaminergic nigro-striatal pathway induced by neurotoxic agents (1). In particular, Barbeau emphasized that the majority of parkinsonian patients were “slow metabolizers” of debrisoquine, and therefore presented a reduced capacity to hydroxylate this substance in the liver. On this basis, Barbeau suggested that these subjects were unable to metabolize, and therefore eliminate, toxins likely to be responsible for the disease, assumed in rural environments. The “toxic hypothesis” of Parkinson’s disease was, at that time, being widely tested, especially after the accidental discovery of 1-methyl, 4-phenyl 1,2,3,6 tetrahydropyridine (MPTP), a chemically simple molecule present in the synthesis of several compounds. MPTP was responsible for a large cluster of parkinsonisms presenting in a Californian population of drug addicts (2). Numerous studies have been performed since then on the gene association between the expression of metabolic enzymes (and in particular CYP2D6) and Parkinson’s disease. From a mass of contrasting data, the general idea has emerged of a lack of significant correlation between these parameters (3). However, some authors recently criticized
this negative conclusion, proposing that definitive data need to be founded on the association of metabolic enzymes with the "toxic" form of Parkinson's disease, whose nosographic identity is unfortunately still unclear (4), rather than with the "idiopathic" form of the disorder. It is now known that, besides the liver, the dopaminergic neurons themselves are equipped with cytochrome P450 enzymes, and in particular CYP2E1 and CYP2D6 (3,5,6). The latter enzymes, which occur in both neuronal somata and synaptic terminals, in all likelihood subdend the metabolism of endogenous and exogenous compounds potentially harmful to the neurons (7). Numerous substances, carried by the dopamine transporter, can eventually enter the dopaminergic terminal. These substances remove dopamine from its binding and storing sites, causing a massive intraneuronal release of the neurotransmitter (8). High concentrations of dopamine outside the storage structures (the vesicles), where pH, protein binding sites, and di-hydroascorbic acid can maintain it in its reduced form, are oxidized to quinone, resulting in a reactive species that interacts with the -SH groups of numerous molecules besides proteins (Fig. 1) (9). Therefore, all false neurotransmitters that enter the dopaminergic neuron play a role as neurotoxic agents (10,11). Several endogenous and exogenous environmental substances bear these characteristics, which make them potentially noxious agents for the nigro-striatal dopaminergic system, and hence potential causes of parkinsonism in humans. These substances include the following agents: 1) catecholamines, such as 6-OH dopamine (12); 2) phenyl-ethyl-amines, such as amphetamine and its derivatives (12); 3) indolamines, such as tryptamine and its derivatives (12); 4) β-carbolines (13); 5) tetra-hydro-quinolines (14); and 6) tetra-hydro-pyridines, such as MPTP and its derivatives (15).

Most of these substances are metabolized by the enzymes CYP2E1 and CYP2D6 (7), which could therefore play a crucial role in the protection of the dopaminergic neuron. Precise knowledge of this possible intraneuronal role is a fundamental pre-requisite for an understanding of parkinsonisms and of the extrapyramidal disturbances observed clinically in some instances after accidental or voluntary exposure to substances or drugs that inhibit selectively these enzymes. In particular, parkinsonian syndromes caused by di-thio-carbamates (16), by other potent CYP2E1 inhibitors, as well as by selective inhibitors of serotonin re-uptake (17) that inhibit CYP2D6 (18), have been described. On this basis, the definition of the detoxifying and neuroprotective role of these cytochromes could provide an important contribution to understanding of the pathogenesis and potential causes of Parkinson's disease, as well as to new therapeutic approaches.

CYP2E1 AND MPTP-INDUCED TOXICITY IN EXPERIMENTAL PARKINSONISM

In 1985 our group produced some important data on MPTP toxicity, demonstrating, for the first time, that a substance, di-ethyl-dithyo-
carbamate (DDC), potentiates the MPTP toxic effects in a murine model (Fig. 2). Our study pointed out that an MPTP dose of 30 mg/kg, which in C57/black mice normally induces a 50% loss of dopaminergic terminals, measured as long-term decrease of striatal dopamine, following DDC treatment caused, instead, the selective death of up to 90% of nigral neurons (19). In order to unravel the mechanism responsible for this effect, and after having tested several substances, we demonstrated that acetaldehyde and ethanol, like DDC (Fig. 3), also potentiated MPTP toxicity in mice (19,20). These combined treatments led to an experimental model, almost as effective as the MPTP intoxication in the monkey for the study of preventive treatments and/or cell transplantation in Parkinson’s disease (21). Several research groups have since advanced hypotheses on the mechanisms of potentiating agents (22-24). The hypotheses that take into account the kinetics of MPP+, the toxic metabolite responsible for MPTP toxicity (25), merit particular attention. MPTP is metabolized in the liver, where it is inactivated by cytochromes, including CYP2D6 (26). MPTP is liposoluble but crosses the blood-brain barrier and enters the CNS, where it is metabolized to MPP+ by monoamine-oxidases, especially the type B ones (25,27). Once formed, MPP+ can be taken up by the dopamine transporter, thus entering the dopaminergic terminal where it exerts its toxic effect (28,29). Like other toxins, MPP+ binds the vesicular transporter VMAT (30), removing dopamine from its vesicular binding sites (31), thus causing a massive dopamine release (32). MPP+ toxicity, considered a mitochondrial poison, has also been described (33). Therefore, the kinetics of MPP+ in the target area, i.e., the striatum, is of primary importance, and the variability in the persistence of this metabolite in the striatum could be responsible for inter-species differences in susceptibility to MPTP toxicity (34). In the monkey, the most susceptible species, the MPP+ half-life in the striatum is longer than 10 days, whereas in the mouse, a

Fig. 2 - Structure of diethyldithyocarbamate.

Fig. 3 - DDC induces dopaminergic neuronal death in the substantia nigra in mice. Thyrosine hydroxylase immunostaining of ventral mesencephalon from (A) control, (B) MPTP, (C) DDC + MPTP.
species with lower susceptibility, the MPP+ has a half-life of a few hours, and of some minutes in the rat, a resistant species (35). MPP+ metabolism in the target area of its toxicity represents, therefore, a parameter of crucial importance. Irwin (22) reported that DDC increases the MPP+ half-life in the mouse striatum and that this effect is responsible for the potentiating effect (Fig. 4). However, through the analysis of the striatal kinetics of MPP+ following different treatments, our group found that the increased striatal half-life of MPP+ does not correspond consistently to increased toxicity (36). More recently, following the discovery of the occurrence of CYP2E1 and CYP2D6 in the dopaminergic neuron, a more convincing hypothesis has been put forward (37). According to this hypothesis, MPTP toxicity may be highly correlated with MPP+ persistence, and therefore with its half-life, in the dopaminergic neuron, and not in the striatum where other cell types, i.e., both glial and neuronal cells, could take part in the metabolism. DDC, as acute ethanol administration and acetaldehyde, are potent CYP2E1 inhibitors (38,39), and this enzyme could therefore play a selective detoxifying role (Table I).

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