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Cytochrome P450 and Parkinson's disease: protective role of neuronal CYP 2E1 from MPTP toxicity

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Summary. Elucidation of the biochemical steps leading to the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)-induced degeneration of the nigro-striatal dopamine (DA) pathway has provided new clues to the pathophysiology of Parkinson's Disease (PD). In line with the enhancement of MPTP toxicity by diethyldithiocarbamate (DDC), here we demonstrate how other CYP450 (2E1) inhibitors, such as diallyl sulfide (DAS) or phenylethylisothiocyanate (PIC), also potentiate the selective DA neuron degeneration in C57/bl mice. In order to provide direct evidence for this isozyme involvement, CYP 2E1 knockout mice were challenged with MPTP or the combined treatment. Here we show that these transgenic mice have a low sensitivity to MPTP alone, similarly to the wild type SVI, suggesting that it is likely that transgenic mice compensate for the missing enzyme. However, in these CYP 2E1 knockout mice, DDC pretreatment completely fails to enhance MPTP toxicity; this enhancement is instead regularly present in the SVI control animals. This study indicates that the occurrence of CYP 2E1 in C57/bl mouse brain is relevant for MPTP toxicity, and suggests that this isozyme may have a detoxificant role related to the efflux transporter of the toxin.

Abbreviations

DA dopamine; *PD* Parkinson's Disease; *DDC* diethyldithiocarbamate; *PIC* phenylethylisothiocyanate; *DAS* diallyl sulfide; *MPTP* 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine; *MPP*$^+$ 1-methyl-4-phenylpyridinium; *SN* substantia nigra; *TH* tyrosine hydroxylase; *SVI* Cyp 2e1+/+ (129S1/SvImJ); *GONZ* Cyp 2e1−/− (129/SV-Cyp 2e1^{tm1Gonz}).

Introduction

In 1985, a member of our group serendipitously achieved unexpected data on MPTP toxicity by demostrating, for the first time, that a compound, diethyldithiocarbamate (DDC), potentiates MPTP toxicity in the mouse model (Corsini, 1985). The DDC-induced enhancement of MPTP toxicity in mice has been extensively confirmed by numerous reports in which Authors give the results of their studies on MPTP metabolism in general and on MPP$^+$ kinetics (the toxic metabolite) in particular, pursuing the definition of the enzymes responsible for the neurotoxic versus neuroprotective pathways in the liver and brain as well.

More recently, the discovery of the occurrence of cytochrome P450 in the brain and in DA neurons as well, the function of which is still unknown (Warner et al., 1994), led to a

new hypothesis in this respect (Corsini et al., 2002). In particular, cytochrome P450 2E1 (CYP 2E1) was identified in the rat brain and in DA neurons of the substantia nigra as well (Watts et al., 1998) and, at the same time, DDC was found to be a fairly specific inhibitor of this isozyme (Stott et al., 1997).

In order to understand the role played by CYP 2E1 in the DDC-induced enhancement of MPTP toxicity in mice, we studied the effects of diallyl sulfide (DAS) and phenylethylisothiocyanate (PIC), two specific inhibitors of CYP 2E1 enzymatic activity (Brady et al., 1991; Nissbrandt et al., 2001), on MPTP toxicity. CYP 2E1 knockout mice were challenged with MPTP or the DDC-combined treatment.

Materials and methods

Animals

Male C57/bl mice (Harlan, Italy), 8 weeks old and weighing 20 to 24 gr, were kept under environmentally controlled conditions (12 hrs light/dark cycle with light on between 07.00 and 19.00 hrs; room temperature +21°C) with food and water *ad libitum*. The animals were treated in accordance with the Guidelines for Animal Care and Use of the National Institutes of Health. The experiments described in this article were formally approved by the Committee for Scientific Ethics of the University of Pisa.

Knockout mice

Male *Cyp 2e1* knockout mice (*129/SV-Cyp 2e1^{tm1Gonz}*) (*Cyp 2e1−/− Stock number: 002910*) and their wild type counterparts (*129S1/SvImJ*) (*Cyp 2e1+/+ Stock number: 002448*) were obtained from The Jackson Laboratory (Bar Harbor ME, USA). *Cyp2e1* (−/−) mice in 129/Sv-*Ter* background were generated in the Gonzalez laboratory (Lee et al., 1996) back-crossed four times into the wild type 129/Sv-*Ter* strain. These animals were screened for viral infection by Charles River Laboratories and all tests were negative. Confirmation of the *Cyp 2e1−/−* status was confirmed by the absence of CYP 2E1 as determined by liver DNA PCR phenotyping by Charles River Laboratories.

Experimental protocol

Twelve mice per group were treated i.p. with either MPTP hydrochloride (36 mg/kg) or distilled water. The animals were pretreated i.p. with DDC (400 mg/kg) or DAS (25 mg/kg) or PIC (25 mg/kg) or the vehicle, one hr before MPTP administration. DDC was easily dissolved in distilled water, whereas the liquid DAS was given already dissolved in a mixture of DAS plus tween 80 plus propylene glycol plus distilled water in 1, 1.5 and 10 volume proportions, respectively. The liquid PIC plus distilled water and one drop of tween 80 was made up to the appropriate volume and then homogenized with an ultrasonic disrupter prior to the injection. DA has been measured according to Vaglini et al. (2004). For statistical evaluation ANOVA with Sheffe-F analysis was used.

Results

Striatal modification of DA in C57-bl

The effect of the acute administration of CYP 2E1 inhibitors on the dopamine (DA) content in the mouse striatum one hour before a single exposure to MPTP is shown in Fig. 1.

In agreement with previous data (Corsini et al., 1985), 7 days after the combined treatment (DDC + MPTP), striatal DA content fell to 8.9% of controls (10.3 ± 2.8 and 115.2 ± 4.5 ng/mg protein, respectively). The animals treated with DDC alone did not show any change compared with control values. Figure 1 also shows the effects of pretreatment with DAS. The combined treatment

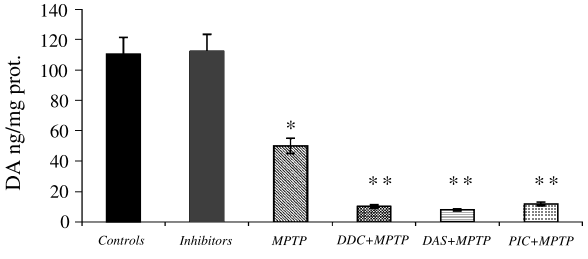

Fig. 1. Effect of DDC (400 mg/Kg), DAS (25 mg/kg) and PIC (25 mg/kg) on striatal tissue levels of dopamine (DA) in MPTP-treated mice. The results are the mean ± s.e. of 3 experiments (n = 6–8 mice for each experiment). C57-bl were treated with MPTP (30 mg/kg i.p.) or saline 60 min. after DDC, DAS or PIC. The animals were sacrified 7 days later. Respective controls received saline instead of inhibitors or MPTP. $^{*}p < 0.05$ in comparison with control mice; $^{**}p < 0.05$ in comparison with the animals treated with MPTP

Table 1. Effect of DDC on striatal tissue levels of dopamine (DA) in wild type (SVI) and knockout (GONZ) (Cyp 2e1−/−) male mice treated with MPTP. The results are the mean ± s.e. of N(10–20) animals for each group. Wild type and knockout were treated with MPTP (30 mg/kg i.p.) or saline solution 60 min after DDC at the dose of 400 mg/Kg i.p. The animals were sacrified 7 days later

Treatments	SVI (CYP 2E1+/+) DA (ng/mg prot.)	%	GONZ (CYP 2E1−/−) DA (ng/mg prot.)	%
Controls	114.1 ± 8.3		109.4 ± 9.8	
DDC	105.8 ± 9.0		108.8 ± 4.1	
MPTP	80.2 ± 4.1*	29	76.5 ± 6.3*	30
DDC + MPTP	48.7 ± 5.1**	57	73.7 ± 5.7***	32

*$p < 0.05$ in comparison with control mice; **$p < 0.05$ in comparison with MPTP-treated animals; ***n.s. in comparison with MPTP-treated animals

(DAS + MPTP) reduced the striatal DA content to 7.2% whereas MPTP alone caused a reduction of only 45.0% compared with controls. (Control values were 110.2 ± 8.2 ng/mg protein; 7.9 ± 1.0, 49.5 ± 4.4 ng/mg protein DAS + MPTP and MPTP respectively). As shown in Fig. 1, PIC also potentiated MPTP toxicity. The combined treatment significantly reduced the striatal DA content in comparison with MPTP alone ($p < 0.05$).

MPTP toxicity in CYP 2E1 knockout mice (GONZ)

The effect of MPTP treatment on the striatal DA content was studied in *CYP 2E1−/−* (GONZ) and wild type (SVI) male mice. As shown in Table 1, the single usual dose of 30 mg/kg i.p. produced a significant reduction in GONZ (30%) and SVI (29%) as well animals. The DDC pretreatment, at the usual time and dose schedule, completely failed to potentiate the DA fall in knockout mice (32%) whereas in wild type animals the combined treatment significantly enhanced the DA fall up to 57%.

Discussion

In this study we have demonstrated that, similarly to DDC, the CYP 2E1 inhibitors, such as DAS and PIC, markedly enhance MPTP toxicity, as measured by the dramatic fall in striatal DA content. This finding cannot be considered a fortuitous event, since for many years a great number of compounds have been tested as 'enhancers' but none of them have proved to increase MPTP toxicity, except for ethanol and acetaldehyde.

In order to provide direct evidence of toxicity, we have also performed the usual procedure of tyrosine-hydroxylase immunoreactivity in midbrain coronal slices of our treated mice (not reported here). Our results clearly indicate that while MPTP, at the dose we used, produced a minimal loss of DA perikaria in the SNpc (about 10%), the combined treatments induced at least 50% damage of the DA neurons, as previously observed with DDC (Vaglini et al., 2004). All these data confirm that DAS and PIC also strongly potentiate MPTP toxicity in this animal species, suggesting a specific role of CYP 2E1 in this toxic event.

In order to provide direct evidence for CYP 2E1 involvement, CYP 2E1 knockout mice (GONZ) and their respective wild type animals (SVI) were challanged with MPTP or the combined DDC + MPTP treatment. GONZ mice revealed a sensitivity to MPTP neurotoxicity similar to that one of SVI animals, but significantly lower than C57/bl strain. This suggests that it is likely that transgenic mice compensate the lack of CYP 2E1 with other isozyme. A similar compensation among

different P450 enzymes in this strain was observed for acetominophene toxicity (Lee et al., 1996). However in these knockout mice, DDC completely failed to enhance MPTP toxicity; this effect was instead regularly observed in the wild type animals. This further confirms the direct role of CYP 2E1 in DDC-induced enhancement of MPTP toxicity.

This study adds new insight into the role of CYP isozymes in MPTP-induced lesions of the nigro-striatal DA pathway, drawing attention more towards the metabolism in the brain than in the liver.

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