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N-Methyltetrahydro-β-carboline analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin are oxidized to neurotoxic β-carbolinium cations by heme peroxidases

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Abstract

2-Methyl-1,2,3,4-tetrahydro-β-carboline (2-Me-THβC) and 2,9-dimethyl-1,2,3,4-tetrahydro-β-carboline (2,9-diMe-THβC) are naturally occurring analogs of the Parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), whereas their corresponding aromatic 2-methyl-β-carbolinium cations resemble 1-methyl-4-phenylpyridinium (MPP^+) and are considered potential toxins involved in Parkinson's disease (PD). To become toxicants, 2-methyltetrahydro-β-carbolines need to be oxidized (aromatized) by human metabolic enzymes to pyridinium-like (β-carbolinium) cations as occur with MPTP/MPP^+ model. In contrast to MPTP, human MAO-A or -B were not able to oxidize 2-Me-THβC to pyridinium-like cations. Neither, cytochrome P-450 2D6 or a mixture of six P450 enzymes carried out this oxidation in a significant manner. However, 2-Me-THβC and 2,9-diMe-THβC were efficiently oxidized by horseradish peroxidase (HRP), lactoperoxidase (LPO), and myeloperoxidase (MPO) to 2-methyl-3,4-dihydro-β-carbolinium cations (2-Me-DHβC^+, 2,9-diMe-DHβC^+) as the main products, and detectable amount of 2-methyl-β-carbolinium cations (2-Me-βC^+, 2,9-diMe-βC^+). The apparent kinetic parameters (k_{cat}, k_4) were similar for HRP and LPO and higher for MPO. Peroxidase inhibitors (hydroxylamine, sodium azide, and ascorbic acid) highly reduced or abolished this oxidation. Although MPTP was not oxidized by peroxidases; its intermediate metabolite 1-methyl-4-phenyl-2,3-dihydropyridinium cation ($MPDP^+$) was efficiently oxidized to MPP^+ by heme peroxidases. It is concluded that heme peroxidases could be key catalysts responsible for the aromatization (bioactivation) of endogenous and naturally occurring *N*-methyltetrahydro-β-carbolines and related protoxins to toxic pyridinium-like cations resembling MPP^+, suggesting a role for these enzymes in toxicological and neurotoxicological processes.

Keywords: MPTP; 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+; 1-Methyl-4-phenylpyridinium; β-Carbolinium; *N*-Methyltetrahydro-β-carbolines; Myeloperoxidase; Lactoperoxidase; Horseradish peroxidase; Cytochrome P450; CYP2D6; Monoamine oxidase (MAO); Parkinson's disease; Neurotoxins; Heme peroxidases; β-Carbolines

The causes of idiopathic Parkinson's disease (PD) are still unknown though the involvement of environmental and/or endogenous toxins is being increasingly considered. In fact, exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a contaminant firstly found in "synthetic heroin", induces Parkinsonism in humans and animals [1]. However, humans are not commonly exposed to MPTP neurotoxin and instead various structural analogs such as β-carboline and isoquinoline alkaloids are being considered [2,3]. These compounds are common in the diet and environment, occur in the human body and brain and exert a wide spectrum of psychopharmacological and behavioral effects [4–9]. For a toxicological outcome similar to MPTP, these alkaloids need to be bioactivated to *N*-methyl derivatives and oxidized (aromatized) to pyridinium-like β-carbolinium or isoquinolinium cations [10]. These cations share many functional and toxicological properties with 1-methyl-4-phenylpyridinium (MPP^+), the ultimate neuro-

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toxicant metabolite involved in MPTP neurotoxicity and could be slow-acting neurotoxins [11,12]. Remarkably, *N*-methyl-β-carbolinium cations such as *N*-methylnorharmanium (2-Me-βC$^+$) and 2,9-dimethylnorharmanium (2,9-diMe-βC$^+$) have been detected in post-mortem human brains [12–14] and found in higher proportion in cerebrospinal fluid of patients with PD [13].

Whereas the *N*-methylation of β-carbolines by *N*-methyltransferases has been already established [10], a biochemical way catalyzing the oxidation (aromatization) of *N*-methyltetrahydro-β-carbolines to *N*-methyl-β-carbolinium cations resembling MPP$^+$ has not been explored. By analogy with the paradigmatic model of MPTP, monoamine oxidase (MAO) and cytochrome P450 could be the key enzymes involved in this oxidation [15–17]. As shown in Fig. 1, MPTP is oxidized by MAO-B within the glial cells of the brain to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP$^+$) and then to 1-methyl-4-phenylpyridinium (MPP$^+$) [1], which is selectively uptaken by dopaminergic neurons resulting in mitochondria inhibition and cell death. MPTP is metabolized by cytochrome P450 enzymes (mainly CYP2D6) through *N*-demethylation and aromatic *p*-hydroxylation to products devoid of toxicity [15,18]. Alternatively, P450s oxidize MPTP to traces of MPDP$^+$ and MPP$^+$ toxins [15,19].

Heme peroxidases are oxidoreductases widely distributed in nature that also participate in the oxidation of endogenous substrates, drugs, and xenobiotics [20]. Mammalian peroxidases such as myeloperoxidase (MPO), eosinophil peroxidase (EPO), and lactoperoxidase (LPO) are found in neutrophils, eosinophils, and secretory cells of the exocrine glands, respectively. They oxidize halides to produce hypohalous acid and participate in antimicrobial and anti-inflammatory processes. MPO occurs in activated microglia and neuroinflammation at sites of degenerative diseases [21,22] and certain peroxidases in substantia nigra produce toxic substances such as dopaminochrome and might be involved in PD and neurodegeneration [23,24].

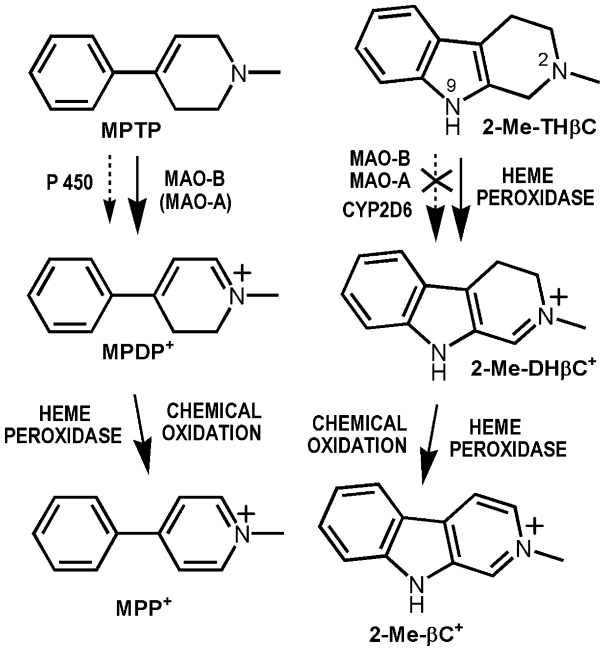

Fig. 1. Aromatic oxidation (*i.e.* bioactivation) of MPTP neurotoxin to MPP$^+$ and that proposed for *N*-methyltetrahydro-β-carbolines (2-Me-THβC) to β-carbolinium (pyridinium-like) cations. Same oxidation for 2,9-dimethyltetrahydro-β-carboline (2,9-diMe-THβC).

The enzymatic bioactivation of naturally occurring analogs of MPTP neurotoxin is a matter of current scientific interest because of its toxicological implications. It may help to explain the toxicological features of these compounds. This research was aimed to determine the enzymatic outcome of 2-methyltetrahydro-β-carboline (2-Me-THβC and 2,9-diMe-THβC) protoxins (Fig. 1), as metabolized by MAO, P450s, and heme peroxidases. These compounds are naturally occurring tetrahydropyridine analogs of MPTP proposed as environmental and/or endogenous neurotoxic agents [3]. Tetrahydro-β-carbolines (THβC) are *N*-methylated by brain *N*-methyltransferases to 2-Me-THβCs [25]; 2-Me-THβC has been found in rat brain [26] whereas its fully aromatic product *N*-methylnorharmanium cation (2-Me-βC$^+$) produces irreversible striatal lesions resembling those of MPP$^+$ [27]. Similarly, the *N*,*N*-dimethyl-β-carbolinium cation 2,9-diMe-βC$^+$ (oxidized product of 2,9-diMe-THβC) is a potent neurotoxin [28,29], and both 2-Me-βC$^+$ and 2,9-diMe-βC$^+$ were found in post-mortem human Parkinsonian brains [12–14]. The results presented below point out to heme peroxidases but not MAO or P450 enzymes as the key bioactivators of endogenous and/or environmental *N*-methyltetrahydro-β-carbolines to pyridinium-like toxins.

Material and methods

Chemicals and enzymes

MPTP hydrochloride (caution: MPTP is a neurotoxin and should be handled with appropriate precautions), MPDP$^+$ perchlorate, MPP$^+$ iodide and other chemicals were obtained from Sigma–Aldrich (St. Louis, MO). 2-Methyl-β-carbolinium (or 2-methylnorharmanium) iodide (2-Me-βC$^+$); 2,9-dimethyl-β-carbolinium (or 2,9-dimethylnorharmanium) iodide (2,9-diMe-βC$^+$); 2-methyl-1,2,3,4-tetrahydro-β-carboline hydrochloride (2-Me-THβC), 2,9-dimethyl-1,2,3,4-tetrahydro-β-carboline (2,9-diMe-THβC), and *2*-methyl-3,4-dihydro-β-carbolinium chloride (2-Me-DHβC$^+$) were prepared by described standard synthetic procedures [15]. Horseradish peroxidase (HRP) (type II) and bovine lactoperoxidase (LPO) obtained from Sigma; human myeloperoxidase (MPO) was from Calbiochem (Merck); two preparations of human cytochrome P450 (plus P450 oxidoreductase): CYP2D6*1 and Supermix™ expressing CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 enzymes were obtained from BD Gentest Co. (Woburn, MA, USA); human monoamine oxidase (MAO-A and -B) were obtained from BD Gentest Co (Woburn, MA, USA). All enzymes were prepared in phosphate buffer and used as supplied.

Enzymatic metabolism

MAO and P450 enzymes. A 0.2 ml reaction mixtures in buffer phosphate (pH 7.4) containing MAO-A or -B (0.01–0.2 mg/ml) and 2-Me-THβC (50–500 μM), MPTP (300 μM), or 250 μM kynuramine plus 2-Me-THβC (50–250 μM) as substrates, were incubated (37 °C, 40 min) and treated as previously [6]. Similarly, 0.2 ml phosphate buffer, pH 7.4,

containing CYP2D6 (7 pmol P450) or a mixture of six P450s (14 pmol P450) and 2-Me-THβC (0–600 μM) or MPTP (0–500 μM) as substrates, were added with 50 μl of 4 mM NADPH, incubated (37 °C, 25 min) and treated as elsewhere [15]. The oxidation products were determined by RP-HPLC-DAD. Kynuramine deaminated by MAO cyclizes to give 4-hydroxyquinoline determined at 320 nm [30]; 2-Me-DHβC$^+$ and MPDP$^+$ were determined at 355 nm, 2-Me-βC$^+$ at 254 nm and MPP$^+$ at 280 nm.

Heme peroxidases. Fresh solutions of peroxidases were prepared to make 0.27 μM HRP, 0.178 μM LPO, and 0.0126 μM MPO in 50 mM phosphate buffer, pH 7, (0.5 ml final volume for MPO or 1 ml for HRP or LPO), containing 2-Me-THβC (0–1000 μM), 2,9-diMe-THβC (250 μM), MPTP (100–500 μM) or MPDP$^+$ (0–1000 μM) as substrates. Reactions were also carried out in presence of ascorbic acid, sodium azide, hydroxylamine and excess H_2O_2, as inhibitors. The reaction was initiated by addition of H_2O_2 solution (25 μM for LPO and MPO and 50 μM for HRP) and incubated at 37 °C, 40 min. Following addition of $HClO_4$ + methanol (1:1) (50 or 100 μl, 10% v/v), the tubes were centrifuged at 10,000 rpm, 10 min, and reaction products analyzed by HPLC and HPLC-MS. The formation of 2-Me-DHβC$^+$, 2,9-diMeDHβC$^+$, MPDP$^+$ or MPP$^+$ was determined as μM/min.

Spectral measurements

UV–vis spectra of enzymatic incubations were obtained in a Beckman spectrophotometer. Concentration of peroxidases (HRP, LPO, MPO) was determined by absorbance using the known extinction coefficients of the respective Soret bands (403, 412, and 430 nm) [31]. H_2O_2 concentration was calculated at 240 nm ($\varepsilon = 39.4\ M^{-1}\ cm^{-1}$).

RP-HPLC and HPLC-MS (electrospray ionization) analysis

The chromatographic analysis of enzyme reaction products was done by RP-HPLC coupled to diode array and fluorescence detectors and by HPLC-MS (electrospray, cone voltage 70–100 V), using a C18 column, as reported elsewhere [6,7,15,30].

Kinetic studies

Peroxidase-catalyzed oxidations were studied as a function of substrate concentration and the apparent K_M and V_{MAX} were determined from non-linear regression fitting to Michaelis–Menten curves. Peroxidases have not true V_{MAX} and k_{cat}, and a pseudo-first order rate constant k_4 corresponding to the oxidation leading to 2-Me-DHβC$^+$ was calculated from K_M^{app} and k_{cat} as k_{cat}/K_M^{app} [32].

Results

It has been generally assumed that structural analogues of MPTP such as 2-methyl-1,2,3,4-tetrahydro-β-carboline (2-Me-THβC) would be metabolized (*i.e.* dehydrogenated) with the participation of MAO enzymes [16,17] (Fig. 1). This enzymatic conversion would produce 2-methyl-3,4-dihydro-β-carbolinium cation (2-Me-DHβC$^+$), which is subsequently oxidized to 2-methylnorharmanium cation (2-Me-βC$^+$), a toxin resembling MPP$^+$. However, human MAO-A or -B (0.01–0.2 mg/ml recombinant protein) were unable to significantly convert 2-Me-THβC (50–500 μM range) to the corresponding 2-Me-DHβC$^+$ cation or the fully aromatic 2-Me-βC$^+$. In contrast, under the same conditions, MAO efficiently deaminated kynuramine (23.3 ± 2.7 nmol of 4-hydroxyquinoline/min × mg recombinant protein for MAO-A and 15.7 ± 1.0 nmol/min × mg recombinant protein for MAO-B) in incubation media containing kynuramine (250 μM) along with 2-Me-THβC (50 μM). MPTP neurotoxin (300 μM) was also oxidized by MAO to MPDP$^+$ and disproportionated to MPP$^+$, with a rate of 6.4 and 4.5 nmol MPDP$^+$/min × mg recombinant protein for human MAO-B and MAO-A, respectively. On the other hand, cytochrome P450 2D6 was unable to significantly catalyze the dehydrogenation of 2-Me-THβC (10-600 μM) to 2-Me-DHβC$^+$/2-Me-βC$^+$. The mixture of six P450 enzymes gave only traces of dehydrogenation of 2-Me-THβC to account for 0.25 ± 0.02 nmol 2-Me-DHβC$^+$/min × mg recombinant protein.

Subsequently, three heme peroxidases, one plant (HRP) and two mammalian peroxidases (MPO, LPO) were assessed. Incubations of 2-Me-THβC with HRP and H_2O_2 showed that this protoxin was efficiently oxidized by HRP to 2-Me-DHβC$^+$ as a main product, and in a minor extend to 2-Me-βC$^+$ (1–2% of that of 1-Me-DHβC$^+$) (Fig. 2). The metabolic products of this reaction were identified by co-injection with authentic standards, UV spectra, and confirmed by HPLC-MS (ESI) that provided mass spectra with ions at *m/z* 185 (2-Me-DHβC$^+$), and *m/z* 183 (2-Me-βC$^+$). LPO and MPO carried out the oxidation of 2-Me-THβC to give 2-Me-DHβC$^+$ and 2-Me-βC$^+$, as well. This enzymatic oxidation was followed by spectrophotometry as shown for LPO (Fig. 2), showing that the native enzyme (Soret band at 412 nm, trace 1), upon addition of H_2O_2, shifted to compound II (band at 430 nm, trace 2), whereas the subsequent addition of 2-Me-THβC

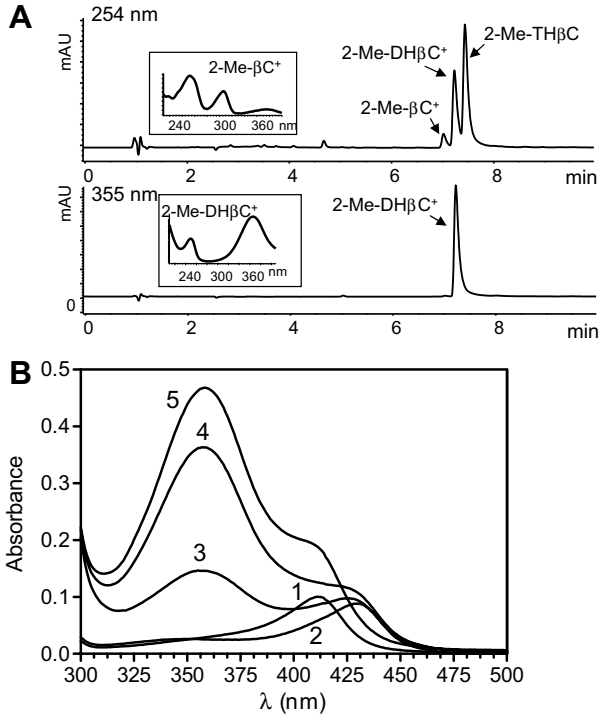

Fig. 2. (A) HPLC chromatogram (254 and 355 nm) of 2-Me-THβC (250 μM) oxidized with HRP (0.27 μM), H_2O_2 (50 μM) (37 °C, 40 min) with UV-spectra of 2-Me-DHβC$^+$ and 2-Me-βC$^+$ included. (B) Spectral changes during oxidation of 2-Me-THβC (250 μM) with LPO, 0.89 μM and H_2O_2, 25 μM. LPO (1), LPO + H_2O_2 (2), LPO + H_2O_2 + 2-Me-THβC, initial (3), LPO + H_2O_2 + 2-Me-THβC, 5 min (4), LPO + H_2O_2 + 2-Me-THβC, 30 min (5).

as an electron donor produced 2-Me-DHβC$^+$ (band at 355–360 nm, traces 3–5).

The formation of 2-Me-DHβC$^+$ was evaluated as a function of the concentration of substrate (Fig. 3), allowing the calculation of apparent K_M and V_{MAX} (Table 1). Under the experimental conditions used, a pseudo-first order rate constant (k_4) [32] was calculated from $K_M{}^{app}$ and k_{cat}. The calculated k_4 was similar for HRP and LPO and higher (about 6 times) for MPO. Peroxidase-catalyzed oxidation of 2-Me-THβC to dihydro-β-carbolinium cation (2-Me-DHβC$^+$) was highly inhibited in presence of peroxidase inhibitors (Fig. 3). Thus, sodium azide, ascorbic acid, hydroxylamine and excess of H_2O_2 reduced oxidation between 50% and 98% with hydroxylamine and ascorbic acid being the best inhibitors. Inhibition (40–95%) was also observed for the fully aromatic metabolite (2-Me-βC$^+$) (not shown).

HRP, LPO, and MPO were also able to oxidize 2,9-dimethyltetrahydro-β-carboline (2,9-diMe-THβC), a *N*,*N*-dimethylated analog of MPTP (Fig. 1). Incubation media with 2,9-diMe-THβC (250 μM) produced 6.6 ± 0.9, 3.45 ± 0.2, and 12.3 ± 3.5 μmol of 2,9-diMe-DHβC$^+$/min × μmol enzyme for HRP, LPO, and MPO, respectively. The fully aromatic compound 2,9-diMe-βC$^+$ also appeared in the reaction media.

In contrast to 2-Me-THβC or 2,9-diMe-THβC; HRP, LPO or MPO were unable to significantly oxidize (dehydrogenate) MPTP to MPDP$^+$ or MPP$^+$. Indeed, negligible oxidized products were observed at the highest concentration of MPTP (*e.g.* 0.02 μmol MPDP$^+$/min × μmol HRP at 250 μM MPTP, and undetectable in lower concentrations). However, heme peroxidases were good catalyzers of the oxidation of MPDP$^+$ to MPP$^+$ (Fig. 4). Disproportionation (chemical oxidation of MPDP$^+$ to MPP$^+$) was also produced especially in high concentrations of MPDP$^+$ (500 and 1000 μM). However, the oxidation of MPDP$^+$ to MPP$^+$ increased substantially with heme peroxidases. Thus, formation of MPP$^+$ in low concentrations of MPDP$^+$ (50 μM) increased up to 30- and 18-folds with HRP and LPO, respectively. MPO also increased oxidation of MPDP$^+$ (250 μM) a 48.5% (not shown). Peroxidase-catalyzed oxidation of MPDP$^+$ (250 μM) to MPP$^+$ was inhibited by peroxidase inhibitors. Thus, oxidation by LPO was

Table 1
Apparent kinetics values for the oxidation of 2-Me-THβC by heme peroxidases to 2-Me-DHβC$^+$

	$V_{MAX}{}^{app}$ (μM/min)	$K_M{}^{app}$ (μM)	k_{cat} (min^{-1})	k_4 ($\mu M^{-1}\ min^{-1}$)
HRP	7.22 ± 0.67	1088 ± 165	26.7 ± 2.4	0.025 ± 0.0023
LPO	2.08 ± 0.19	488 ± 95	11.68 ± 1.0	0.024 ± 0.0022
MPO	0.64 ± 0.05	325 ± 49	50.8 ± 3.87	0.156 ± 0.012

k_4 is calculated from ($k_{cat}/K_M{}^{app}$) [32]; k_{cat} is calculated as $V_{MAX}{}^{app}$/[enzyme]; HRP, 0.27 μM; LPO, 0.178 μM; and MPO, 0.0126 μM. H_2O_2, 25 μM for LPO and MPO and 50 μM for HRP.

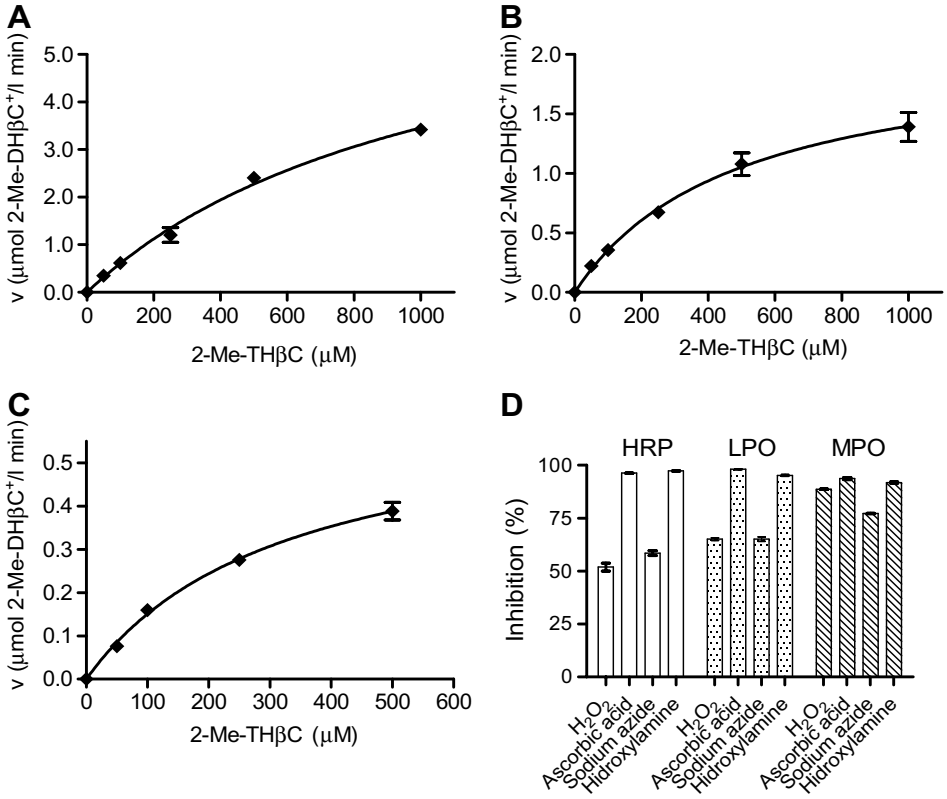

Fig. 3. Formation of 2-Me-DHβC$^+$ (μM/min) from 2-Me-THβC by (A) HRP, 0.27 μM; (B) LPO, 0.178 μM, and (C) MPO, 0.0126 μM in presence of 25 μM H_2O_2 (LPO and MPO) and 50 μM H_2O_2 (HRP). (D) Inhibition (%) of the oxidation of 2-Me-THβC (250 μM) in presence of ascorbic acid (1 mM), sodium azide (1 mM), hydroxylamine (1 mM) and excess of H_2O_2 (2 mM for HRP and LPO, and 4 mM for MPO). Results are average of duplicates.

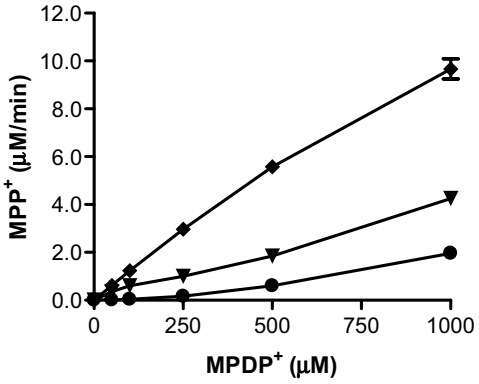

Fig. 4. Oxidation of $MPDP^+$ to MPP^+ by HRP 0.27 μM, H_2O_2 50 μM (♦) and LPO 0.178 μM, H_2O_2, 25 μM (▼) and without peroxidases (similar results for 0, 25, 50 μM H_2O_2) (●). Results are average of duplicates.

inhibited a 55%, 88%, and 67% by 1 mM concentrations of ascorbic acid, hydroxylamine, and sodium azide, respectively.

Discussion

MPTP is a chemical inducer of Parkinsonism in animals and humans and their naturally occurring and endogenous tetrahydro-β-carboline or isoquinoline analogs could be causative agents in PD. Indeed, *N*-methyl-β-carbolinium ions exhibit common characteristics with MPP^+, the ultimate toxic metabolite involved in MPTP neurotoxicity. They are cytotoxic pyridinium-like cations which are uptaken by dopaminergic cells, inhibit ATP synthesis and induce apoptosis and oxidative stress to cause mitochondrial respiratory inhibition and cell death [3,11,28,29]. β-Carbolines are common in the environment and diet, occur and accumulate in biological tissues and brain and exert a wide spectrum of pharmacological effects [4–9,14]. An involvement of tetrahydro-β-carbolines and/or β-carbolines as proneurotoxins in PD is based on the fact that they can be *N*-methylated and also oxidized to produce toxic *N*-methyl-β-carbolinium cations [3,10,11,25]. In this regard, *N*-methylation has been established [10,25] but little was known about the oxidation of these compounds.

This research has assessed whether 2-Me-THβC and 2,9-diMe-THβC, which are close analogs of MPTP, are bioactivated to dihydro-β-carbolinium (2-Me-$DH\beta C^+$, 2,9-diMe-$DH\beta C^+$) and β-carbolinium cations (2-Me-βC^+, 2,9-diMe-βC^+) by MAO, P450, and heme peroxidases. MAO-A or -B did not catalyze this oxidation, ruling out a similar bioactivation route for these compounds, as for MPTP (Fig. 1). Cytochrome P-450 2D6 was not able either to significantly dehydrogenate 2-Me-THβC, whereas the mixture of six P450s enzymes gave only minor traces of 2-Me-$DH\beta C^+$. Consequently, a role for cytochrome P-450 in the aromatic oxidation (bioactivation) of *N*-methyltetrahydro-β-carbolines is unlikely. In contrast, CYP2D6 metabolizes these compounds through aromatic hydroxylation to give 6- and 7-hydroxy-*N*-methyltetrahydro-β-carbolines [15].

Oxidation of 2-Me-THβC and 2,9-diMe-THβC to dihydro-β-carbolinium and β-carbolinium cations was efficiently catalyzed by heme peroxidases (MPO, LPO and HRP) (Figs. 1 and 3). Dihydro-β-carbolinium cations were the main products although β-carbolinium cations also appeared in the reaction media. Peroxidases seemed less efficient in oxidizing dihydro-β-carbolinium to β-carbolinium cations than using 2-Me-THβCs. The latter compounds were good electron donors in the peroxidase cycle. This agrees with the ability of tetrahydro-β-carbolines to participate in one-electron redox reactions against radicals [9,33] and with the fact that indoles reduce redox intermediates of peroxidases [31]. These results can explain how 2-Me-THβC and 2,9-diMe-THβC protoxins are bioactivated to toxic pyridinium-like cations, suggesting a new enzymatic way for the formation of endogenous *N*-methyl-β-carbolinium or *N*-methyl-dihydro-β-carbolinium cations (Fig. 1). These *N*-methyl-β-carbolinium cations are potent neurotoxicants which might be sequestered inside the cells triggering degenerative processes [27–29,34]. Remarkably, these products (2-Me-βC^+ and 2,9-diMe-βC^+) have been found in human brains, and in higher proportion in brains of PD patients [12,13].

MPTP is oxidized by MAO and detoxified by CYP2D6 [15]. Peroxidases were not able to oxidize MPTP to its dihydropyridinium cation ($MPDP^+$). In contrast, they did oxidize $MPDP^+$to MPP^+. This agrees with recent results on this oxidation catalyzed by HRP [24]. It suggests that heme peroxidases including MPO may accelerate the flow from $MPDP^+$ (*i.e.* produced by MAO) to the ultimate toxicant MPP^+, resulting in an increase of MPTP toxicity (Fig. 1). This may also stimulate the hypothesis that peroxidases are involved in PD and neurodegeneration [23,24]. MPO occurs at sites of degenerative diseases and neuroinflammation and increases in Alzheimer's disease [21], whereas, interestingly, the ablation of MPO mitigates PD produced by MPTP neurotoxin in animals [22]. Taken together these results suggest a relevance of heme peroxidases in bioactivation processes and neurodegeneration.

In conclusion, 2-Me-THβC and 2,9-diMe-THβC protoxins and structural analogs of MPTP neurotoxin are oxidized by heme peroxidases to 2-Me-$DH\beta C^+$/2-Me-βC^+ and 2,9-diMe-$DH\beta C^+$/2,9-diMe-βC^+, which are purported neurotoxins [11,28,29]. These results suggest a novel bioactivation way to give pyridinium-like cations resembling MPP^+. Hypothetically, an extended and continuous (perhaps years) exposure to tetrahydro-β-carbolines (or *N*-methyltetrahydro-β-carbolines) might increase their presence in the body and brain, where they could be *N*-methylated [10,25] and oxidized by heme peroxidases, with the β-carbolinium cations produced potentially involved in neurodegeneration as slow-acting neurotoxicants. Noticeably, these β-carbolinium toxins have been detected in normal and Parkinsonian human brains [12,13]. If heme peroxidases are involved in bioactivation and toxicity, inhibitors of these enzymes may become neuroprotectants.

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