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Monoamine oxidase substrates in Parkinson's disease

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Platelet monoamine oxidase (MAO, EC 1.4.3.4) is a membrane bound mitochondrial enzyme that is involved in the metabolism of both endogenous and exogenous biogenic amines [1]. Human platelet MAO is classified as type B on its inhibitor and substrate specificity [2]. It was originally reported that MAO-B in platelets from Parkinsonian patients was slightly reduced compared with controls [3], when 4-hydroxy-phenylethylamine was used as substrate, and that on treatment of the patients with L-dopa, a further reduction of platelet MAO-B activity was observed. However, a more recent report suggested that platelet MAO-B activity using phenylethylamine, a different substrate, was elevated in treated Parkinson's disease (PD) patients compared to controls [4]. This work was recently repeated with untreated Parkinson's disease patients and the results were found to be in agreement [5]. The aim of this study was to investigate whether substrate specificities of platelet MAO-B activity are different in control and Parkinson's disease patients.

Methods

Patients with clinically defined untreated idiopathic Parkinsonism were selected as previously reported [5]. Healthy volunteers were used as controls. Platelets were isolated and assayed for MAO-B activity as described [6] but using 50 μM dopamine instead of 20 μM phenylethylamine as substrate. Deprenyl was used as an inhibitor of MAO-B activity [7] to provide blank results for the assay. 3,4-Dihydroxyphenylacetic acid (DOPAC) was determined by HPLC with electrochemical detection [8]. Protein concentration of platelets was determined by a colorimetric method [9]. Determinations were carried out in duplicate.

Results and Discussion

The result of the investigation can be seen in Table 1d. There was no age correlation between the control and PD population with regard to MAO-B activity nor was there a sex correlation in the PD population with MAO-B activity. There was a difference in MAO-B activity between the sexes in the control population but this was not significant ($P < 0.2$, two-tailed Student's t-test). It can be seen, though, that the MAO-B activity was clearly different in the two populations. A mean control platelet MAO-B activity of 476.2 nmol DOPAC/mg/hr was seen compared with a value of 230.6 nmol DOPAC/mg/hr in the PD population ($P < 0.005$, two-tailed Student's t-test).

These values show that the pattern of MAO-B activity in platelets of PD patients differs depending on which substrate is used; this can be seen when results from other workers are tabulated and compared with the data from this investigation (Table 1). When phenylethylamine was used as the substrate both treated PD (Table 1a) and untreated PD (Table 1b) platelets had a significantly higher

Table 1. The effects of varying substrates on platelet MAO-B activity in control and Parkinson's disease patients

	Group	MAO-B substrate	N	F/M	MAO-B (mean + SD)	Age (mean + SD)
1a*	Controls	Phenylethylamine (100 μM)	18	14/4	12.6 + 3.4 nmol/mg/hr	73.9 + 10.0
	PD (treated)	Phenylethylamine (100 μM)	18	14/4	16.5 + 6.1 nmol/mg/hr**	73.9 + 6.2
1b†	Controls	Phenylethylamine (20 μM)	20	10/10	5.1 + 0.2 nmol/mg/hr	57.9 + 15.2
	PD	Phenylethylamine (20 μM)	17	8/9	6.6 + 1.1 nmol/mg/hr¶	60.9 + 8.9
1c‡	Controls	4-Hydroxyphenylethylamine (30 μM)	18	8/10	39 + 3.7 nmol/10^9 platelets/hr§	—
	PD	4-Hydroxyphenylethylamine (30 μM)	15	10/5	31 + 4.2 nmol/10^9 platelets/hr§	—
	PD (treated)	4-Hydroxyphenylethylamine (30 μM)	34	9/25	20 + 2.7 nmol/10^9 platelets/hr§	—
1d	Controls	3,4-Dihydroxyphenylethylamine (50 μM)	27	13/14	476.2 + 107.3 nmol/mg/hr	52.2 + 23.4
	PD	3,4-Dihydroxyphenylethylamine (50 μM)	20	10/10	230.6 + 29.4 nmol/mg/hr‖	62.5 + 11.8

* Ref. 4.
† Ref. 5.
‡ Ref. 3.
§ Results expressed as mean + SE.
‖ $P < 0.005$ two-tailed Student's *t*-test.
¶ $P < 0.025$ two-tailed Student's *t*-test.
** $P < 0.050$ two-tailed Student's *t*-test.

MAO-B activity than their respective controls. However, with 4-hydroxyphenylethylamine (tyramine) as the substrate (Table 1c) both treated and untreated PD platelets had a lower (but not a significantly lower) MAO-B activity when compared to controls. In contrast, when 3,4-dihydroxyphenylethylamine (dopamine) was used as the substrate (Table 1d) a significantly reduced MAO-B activity was seen in the untreated PD platelets.

These findings seem to indicate that as the number of hydroxyl substituents on the substrate increases, from zero through mono- to di-, the enzyme found in PD platelets changes its relative activity with respect to the values found for platelets from control subjects. Since the enzyme MAO-B binds the FAD co-factor in its active site [10] while the substrate is bound outside the active site, these results suggest that as the number of aromatic substitutes increases the platelet activity to the substrate decreases in Parkinson's Disease. This may have wide reaching implications if Parkinson's disease patients have different MAO-B activity to MPTP than controls, especially since MPP^+ cannot readily cross the blood–brain barrier and cause neurotoxicity [11]. Alternatively, reduced catabolism of dopamine itself would increase its intracellular concentration which may in itself be toxic [12].

These observations, however, may also be the result of different levels of endogenous MAO-B inhibitors, like tribulin [13] but it has recently been reported [14] that there is no significant difference in tribulin or basic MAO-B inhibitor urinary excretion in Parkinson's disease or controls making this explanation seem unlikely, whereas a more detailed investigation of monoamine oxidase substrates in Parkinson's disease may throw new light on the interaction between the substrate and the enzyme.

In summary, platelet MAO-B activity was determined in control and PD patients using dopamine as the substrate and was found to be significantly reduced in PD platelets compared to controls. These results have been discussed in relation to published results using non-hydroxylated, mono-hydroxylated and di-hydroxylated aromatic amines as substrates for platelet MAO-B. They indicate a substrate variation relative activity defect in platelet MAO-B from PD patients.

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Protection against iron-induced uroporphyria in C57BL/10ScSn mice by the peroxisome proliferator nafenopin

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Administration of some polyhalogenated aromatic chemicals to C57BL/6J and C57BL/10ScSn mice causes a depression of uroporphyrinogen decarboxylase activity in the liver resulting in uroporphyria perhaps by an oxidative process [1]. Depletion of hepatic iron stores or iron overload significantly modulate the response of mice to chemicals of this type such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [2–4], polychlorinated and polybrominated biphenyls [5] and hexachlorobenzene [6, 7]. This experimental uroporphyria closely resembles the iron-linked human disorder porphyria cutanea tarda, which may occur in some patients with moderate liver damage prompted by excessive intake of alcoholic beverages or other drugs [8]. The connection between the experimental and human uroporphyrias has been strengthened by the demonstration that some non-halogenated chemicals and drugs cause hepatic uroporphyria in iron-loaded C57BL/6J or C57BL/10ScSn mice [9–11]. In fact, the most recent experiments demonstrate that in these strains of mice, inhibition of hepatic uroporphyrinogen decarboxylase and moderate uroporphyria can eventually occur under the influence of iron overload alone or in conjunction with 5-aminolaevulinic acid and be maintained for many months [1, 5, 11–13]. Thus in mice, the influence of the drugs and chemicals could be viewed as potentiation of a malfunction of iron metabolism. All of these compounds are inducers of the microsomal cytochrome P450 system and most of them of the P450IA isoenzymes [1, 9, 10]. In the present work, we have investigated the effects of nafenopin which like other peroxisome proliferators [14–16] induces a novel form of cytochrome P450 termed P450IVA1. This isoenzyme is very active in the metabolism of endogenous substrates of cytochrome P450 including fatty acids and steroids [14–16]. In contrast to the other chemicals so far studied, nafenopin protected mice against developing uroporphyria during iron overload.

Materials and Methods

Chemicals. Iron–dextran solution (Imferon; 50 mg/mL Fe, 200 mg/mL dextran) was purchased from Fisons plc (Loughborough, U.K.). Dextran C (the 5000 mol. wt dextran used in the manufacture of Imferon) was a gift from Fisons. Nafenopin (2-methyl-2-[*p*-(1,2,3,4-tetrahydro-1-naphthyl)phenoxy]propionic acid) was a gift from Ciba Geigy Ltd (Basel, Switzerland). Ethoxy-, pentoxy- and benzyloxyresorufins (more correctly the alkoxyphenoxazones) were prepared as described previously [17]. Pentacarboxyporphyrin I, uroporphyrins and other porphyrin standards were from Porphyrin Products (Logan, U.S.A.). [1-^{14}C]Lauric acid (26 mCi/mmol 98% pure) was from Amersham International plc (Amersham, U.K.).

Animals and treatments. Male C57BL/10ScSn mice (7–10 weeks old) were bred on site. Mice received Imferon (12 mL/kg 600 mg Fe/kg) or the equivalent volume of dextran solution by s.c. injection and then were fed powdered Breeder diet No. 3 (Special Diet Services, Witham, U.K.) with or without 0.025% nafenopin as indicated. Experiment A: one week after receiving iron–dextran or dextran mice were fed nafenopin for 15 weeks. Experiment B: mice were fed nafenopin-containing diet from week 16 to week 32 after receiving iron–dextran or dextran. Animals were killed by cervical dislocation. Samples of urine were obtained from individual mice onto plastic Petri dishes and pooled for analysis.

Biochemical assays. Porphyrins in urine samples were analysed by reverse phase HPLC as described previously [9] and are expressed relative to creatinine content which was estimated using Sigma Diagnostic kit 555A. Livers were homogenized in 0.25 M sucrose (1:5 w/v) and total liver porphyrins were determined by spectrofluorimetry and expressed in terms of uroporphyrin [18]. Non-haem