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Inhibition of brain mitochondrial monoamine oxidases by the endogenous compound 5-hydroxyoxindole

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Abstract

5-Hydroxyoxindole is a recently identified endogenous compound. Its physiological role remains unclear but certain evidence exists, that it may share some regulatory properties with isatin, a known endogenous inhibitor of monoamine oxidase (MAO) type B (MAO-B). In this study several oxidized indoles were tested for their *in vitro* inhibition of MAO type A (MAO-A) and B of rat brain non-synaptic mitochondria. 5-Hydroxyoxindole was less potent MAO-A inhibitor (IC_{50} 56.8 μM) than isatin (31.8 μM) and especially 5-hydroxyisatin (6.5 μM), but it was the only highly selective MAO-A inhibitor among the all compounds studied (IC_{50} MAO-A:IC_{50} MAO-B = 0.044). Thus, the *in vitro* data suggest that MAO-A may represent potential target for 5-hydroxyoxindole.

Keywords: Brain MAO; 5-Hydroxyoxindole; Isatin; Endogenous regulation; Oxidized indoles

1. Introduction

There is increasing evidence that endogenous oxidized indoles, oxindole and isatin, may play a regulatory role in the brain and peripheral tissues, however, mechanisms underlying their effects remain poorly understood [1–3]. Recently, we identified the other oxidized indole, 5-hydroxyoxindole, to be present in rat tissues and also in serum of four mammalian species [4]. Although 5-hydroxyoxindole (Fig. 1) was identified long time ago as a urinary metabolite of [^{14}C]indole exogenously administered to rat [5] nothing is known about its physiological and/or pharmacological properties. Studying antiproliferative properties of the endogenous oxindoles, we found that 5-hydroxyoxindole and isatin but not oxindole were antiproliferative and proapoptotic in some cell cultures [6]. This suggests that 5-hydroxyoxindole and isatin may share some structure-functional properties. Isatin was originally identified in body fluids and tissues as a component of the endogenous MAO inhibitor tribulin [2,7]. Although isatin itself selectively inhibits MAO-B, some synthetic isatin analogues including 5-hydroxyisatin are selective MAO-A inhibitors [8,9]. So we have suggested that 5-hydroxyoxindole might also act as MAO inhibitor.

Thus, in the present study we have compared MAO inhibitory activity of 5-hydroxyoxindole, oxindole, isatin and also its synthetic analogue, 5-hydroxyisatin.

2. Materials and methods

5-Hydroxyoxindole was provided by Valbiofrance. 5-Hydroxyisatin was synthesized as described previously [8]. The radiolabeled substrates 5-[1,2-^{3}H(*N*)]-hydroxytryptamine (5-HT) creatinine sulfate (24 Ci/mmol) and β-[ethyl-1-^{14}C]-phenylethylamine (PEA) hydrochloride (52 mCi/mmol) were purchased from New England Nuclear Life Science Products. All other chemicals were obtained from Sigma-Aldrich.

The fraction of rat brain non-synaptic mitochondria was isolated using Ficoll procedure described by [10]. The final

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Abbreviations: MAO, monoamine oxidase; MAO-A, monoamine oxidase type A; MAO-B, monoamine oxidase type B.

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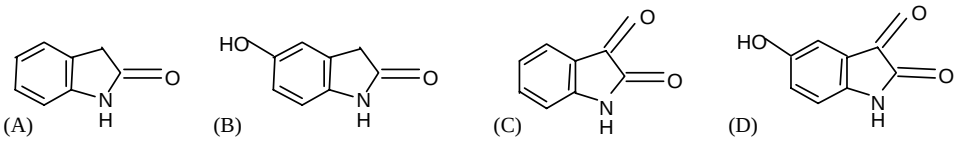

Fig. 1. Structural formulas of oxindole (A), 5-hydroxyoxindole (B), isatin (C), and 5-hydroxyisatin (D).

Table 1
MAO-A and MAO-B inhibitory activity of 5-hydroxyoxindole and related compounds

Compound	IC_{50} MAO-A (M)	IC_{50} MAO-B (M)	MAO-A:MAO-B IC_{50}
5-Hydroxyoxindole	$(56.8 \pm 6.4) \times 10^{-6}$	$(1.3 \pm 0.06) \times 10^{-3}$	0.04
Oxindole	$(163.4 \pm 71.0) \times 10^{-6}$	$\gg 1000$	$\ll 0.16$[a]
Isatin	$(31.8 \pm 6.7) \times 10^{-6}$	$(9.2 \pm 1.0) \times 10^{-6}$	3.46
5-Hydroxyisatin	$(6.5 \pm 1.1) \times 10^{-6}$	$(20.2 \pm 1.5) \times 10^{-6}$	0.32
Clorgyline	$(0.24 \pm 0.04) \times 10^{-9}$	$(23.5 \pm 1.8) \times 10^{-6}$	1×10^{-5}
Deprenyl	$(2.57 \pm 0.47) \times 10^{-6}$	$(2.5 \pm 0.45) \times 10^{-9}$	1.028.0

The IC_{50} values were determined from concentration–response curves by fitting experimental data to a sigmoidal curve with variable slope using GraphPad Prism 2.01 for Windows 95 (GraphPad Software Inc.). Data are means ± SEM of three independent experiments performed in triplicate.
[a] Oxindole failed to produce 50% inhibition at the highest concentration used (1×10^{-2} M).

pellet of brain non-synaptic mitochondrial fraction was resuspended in the medium containing 0.32 M sorbitol, 0.1 mM EDTA, 0.1% fatty acid-free BSA and 5 mM potassium phosphate, pH 7.8, and stored at −80° until use.

Protein concentration was determined by the method of [11] using BSA as standard.

The MAO activity was determined radiometrically by the method of Wurtman and Axelrod [12], using 100 μM [^{3}H]-5-HT creatinine sulfate (final radioactivity 5 mCi/mmol) and 20 μM [^{14}C]-PEA hydrochloride (final radioactivity 20 mCi/mmol) as the selective substrates of MAO-A and MAO-B, respectively. The inhibitors used in this study were added right before initiation of the reaction by adding substrates.

The inhibitory efficiency of the compounds tested in the range of concentrations from 10^{-2} to 10^{-12} M was evaluated by their IC_{50} values (concentration required for 50% inhibition of enzymatic activity). They were determined by fitting experimental data to a sigmoidal curve with variable slope using GraphPad Prism 2.01 for Windows 95 (GraphPad Software Inc.).

3. Results

Table 1 shows that MAO-A and MAO-B of rat brain non-synaptic mitochondria exhibited standard sensitivity to diagnostic acetylenic inhibitors, clorgyline and deprenyl, which preferentially inhibit MAO-A and MAO-B, respectively.

Table also shows that in term of IC_{50} values, 5-hydroxyoxindole was 23-fold more potent inhibitor of MAO-A than MAO-B. In agreement with previous studies [7–9] isatin was 3-fold more potent inhibitor of MAO-B than MAO-A whereas its synthetic analogue, 5-hydroxyisatin, was 3-fold more potent inhibitor of MAO-A than MAO-B. Oxindole inhibited MAO-A at higher concentrations than three other compounds studied, and failed to produce 50% inhibition of MAO-B over the whole range of concentrations used.

4. Discussion

Results of the present study demonstrate that recently identified endogenous compound, 5-hydroxyoxindole, is a rather selective inhibitor of MAO-A. Its MAO-A inhibitory activity is somewhat lower than that of isatin, another endogenous oxidized indole, and its synthetic analogue, 5-hydroxyisatin. However, selectivity of the 5-hydroxyoxindole effect, evaluated by the ratio of IC_{50} values for MAO-A and MAO-B, was much higher than for 5-hydroxyisatin, the other selective MAO-A inhibitor among the compounds studied. In accordance with previous studies oxindole was much less active MAO inhibitor. This is also consistent with other studies demonstrating that 5-hydroxyoxindole and isatin but not oxindole share some (MAO-independent) regulatory properties on cell cultures [6].

It should be noted that 5-hydroxyoxindole concentration in the blood does not exceed a middle nanomolar range, and in the whole brain 5-hydroxyoxindole concentration is 2-fold higher [4]. However, in contrast to isatin itself [13] and isatin-binding sites [14], which are widely distributed in the rat brain, just a few spots of 5-HT specific binding were found in several rat brain regions.[1] So it is possible that within particular brain cells local concentrations of the newly discovered oxidized indole may be much higher than those found for the whole brain. If our consideration is

[1] Crumeyrolle-Arias *et al.*, unpublished observation.

correct the inhibition of brain mitochondial MAO-A by 5-hydroxyoxindole may represent physiologically important mechanism of the endogenous regulation of brain MAO-A activity.

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