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Tamoxifen protect against hydroxyl radical generation induced by phenelzine in rat striatum

Toshio Obata *

Department of Analytical Chemistry, Ohu University, School of Pharmaceutical Sciences, Koriyama, Fukushima 963-8611, Japan

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Abstract

The present study was examined whether tamoxifen, a synthetic nonsteroidal antiestrogen, could suppress antidepressant drug phenelzine can increase an active dopaminergic neurotoxin, 1-methyl-4-phenylpyridinium ion (MPP^+)-induced hydroxyl radical ($^\bullet OH$) generation in the extracellular fluid of rat striatum, using in vivo microdialysis system. Rats were anesthetized, and sodium salicylate (0.5 nmol/μl/min) was infused through a microdialysis probe to detect the generation of $^\bullet OH$ as reflected by the non-enzymatic formation of 2,3-dihydroxybenzoic acid (DHBA) in the striatum. Infusion of phenelzine (100 μM or 0.1 nmol/μl/min) into the striatum drastically increased dopamine (DA) efflux and the $^\bullet OH$ formation, trapped as 2,3-DHBA by the possible increased production of MPP^+. However, tamoxifen (100 μM) significantly suppressed phenelzine enhanced DA efflux and $^\bullet OH$ formation by MPP^+. These results in the present study is the first demonstration showing the protective effect of tamoxifen on $^\bullet OH$ generation induced by phenelzine enhanced MPP^+ by suppressing DA efflux.

Keywords: Tamoxifen; Phenelzine; 1-Methyl-4-phenylpyridinium ion (MPP^+); Hydroxyl radical; SSAO; Parkinson's disease

1. Introduction

Although a dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), produces a parkinsonian syndrome after its conversion to an active neurotoxin, 1 methyl-4-phenylpyridinium ion (MPP^+) by the type B monoamine oxidase (MAO; EC 1.4.3.4) in the brain (Chiba et al., 1984; Markey et al., 1984; Obata, 2002a), the etiology of idiopathic Parkinson's disease still remains obscure. Recent publications suggested a possibility that oxidative stress may be involved in the pathogenesis of Parkinson's disease (Youdim et al., 1989; Ben-Shachar et al., 1991; Smith and Bennett, 1997). Several reports have indicated that free radicals can be generated in the presence of MPTP or MPP^+ (Chiba et al., 1984; Markey et al., 1984; Obata, 2002a). The principle free radicals that can be produced by MPP^+ are superoxide and hydrogen peroxide (H_2O_2). Production of H_2O_2 as a by-product of the breakdown of active amines by the their metabolizing enzyme MAO has been hypothesized to greatly contribute to the formation of cytotoxic free radicals (Obata, 2002a). Semicarbazide-sensitive amine oxidases (SSAO) are a diverse group of enzymes within the classification EC 1.4.3.6. The term SSAO is nowadays most often used in a more narrow sense, excluding the semicarbazide-sensitive diamine oxidase and lysyl oxidase. With this more narrow definition, SSAO is used to describe the monoamine-oxidizing

Abbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, 1-methyl-4-phenylpyridinium ion; $^\bullet OH$, hydroxyl radical; 2,3-DHBA, 2,3-dihydroxybenzoic acid; MAO, monoamine oxidase; SSAO, semicarbazide-sensitive amine oxidase; HPLC-EC, high-performance liquid chromatographic-electrochemical; DA, dopamine

* Tel.: +81 24 932 9238; fax: +81 24 932 9238.
E-mail address: t-obata@pha.ohu-u.ac.jp.

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enzyme found its activity in the plasma membrane or circulating in plasma. Among two, the membrane-bound SSAO dose-dependently inhibited by carbonyl reagents, such as semicarbazide, probably by irreversibly binding to an enzyme co-factor. Tissue-bound SSAO (Andree and Clarke, 1981; Conforti et al., 1993; Deng and Yu, 1999; Obata, 2002b; Kinemuchi et al., 2004) differs from MAO in the subcellular localization, substrate preference and co-factor requirement. For example, SSAO primarily oxidizes only aliphatic and aromatic primary monoamines and it is largely associated with the plasma membranes of various mammalian tissues (Lyles, 1996).

Recent studies have reported that estrogen showed neuroprotection against neuronal degeneration (Tang et al., 1996; Birge, 1997). In postmenopausal women who have declined estrogen levels, the risk of Alzheimer's disease is considered to increase, and replacement therapy reduces its risk (Tang et al., 1996; Birge, 1997; Arvin et al., 2000; Obata and Kubota, 2001). Tamoxifen, a synthetic nonsteroidal antiestrogen, is currently used in treatment and prevention of breast cancer. However, its effects on brain are not fully understood. Further, tamoxifen and its metabolite, 4-hydroxytamoxifen, have pleiotropic function as follows. They inhibit both metal ion-dependent enzymatic and non-enzymatic lipid peroxidation in a number of model membranes (Wiseman et al., 1993; Thangaraju et al., 1994), and induce glutathion peroxidase, catalase and superoxide dismutase in postmenopausal women (Wiseman et al., 1993). Based on the properties of tamoxifen, I hypothesized that tamoxifen would have neuroprotective effects against MPP^+-induced hydroxyl radical ($^\bullet OH$) generation. To probe this hypothesis, I have investigated whether tamoxifen could suppress the formation of $^\bullet OH$ products induced by MPP^+ in the striatum.

In the present study, I examined the effect of tamoxifen on MPP^+ and antidepressant drug phenelzine (Parent et al., 2002) induced dopamine (DA) release and $^\bullet OH$ production in rat striatum. The $^\bullet OH$ generated after MPP^+ was measured by the hydroxylated derivative of salicylic acid by a high-performance liquid chromatographic-electrochemical (HPLC-EC) procedures as reported previously (Chiueh et al., 1992; Obata, 1999).

2. Materials and methods

2.1. Animal preparation

Adult male Wistar rats, body weights 300–400 g, were housed in an environmentally controlled room (20–25 °C, 50–60% humidity) with available food and water ad libitum for 4 days prior to our experiments. The rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and prepared for intracranial microdialysis brain perfusion by the methods previously reported (Chiueh et al., 1992; Obata, 1999). This study was approved by the Ethical Committee for Animal Experiments, Oita Medical University, Japan.

2.2. Assay of SSAO activity

The SSAO activity was measured using the radioactively labelled [^{14}C]-benzylamine (Bz) as a substrate. Rat brain homogenates were used as sources of SSAO activity. The 10% (v/v) homogenates of these were prepared in 0.32 M sucrose plus 10 mM phosphate buffer, pH 7.4. SSAO activity was assayed radiochemically as reported previously (Fowler et al., 1979). In inhibition studies, enzyme preparations were preincubated with various concentrations of phenelzine (from 10^{-3} M to 10^{-13} M) for 30 min at 37 °C before adding [^{14}C]-Bz for assay of remaining SSAO or MAO activity, since benzylamine (1 μM) is a common substrate for both SSAO and MAO. Protein concentrations of the enzyme preparations were measured by the method of Lowry et al. (1951) with bovine serum albumin as standard.

2.3. Microdialysis experiments

In the preliminary experiments, the recovery of 0.1 μM DA was 20.8 ± 0.9% ($n = 8$) at a flow rate of 1 μl/min, used here for other experiments. The drugs were dissolved in Ringer's solution containing 147 mM NaCl, 2.3 mM $CaCl_2$ and 4 mM KCl, pH 7.4 for perfusion (1 μl/min) through a microdialysis probe into the striatum. The microdialysis probe was pre-washed with Ringer's solution for at least 30 min prior to stereotaxical implantation in the striatum (stereotaxic coordinates: AP: 1.0, R/L: 2.5, H: −7 mm from dura matter) (Paxinos and Watson, 1982). Thereafter for trapping $^\bullet OH$ radicals (Chiueh et al., 1992; Obata, 1999) in the striatum, sodium salicylate in Ringer's solution (0.5 nmol μl/min) was perfused by a microinjection pump (Carnegie Medicine CMA/100 Stockholm, Sweden) and basal levels of DHBA during a definite period of time were determined. Brain dialysate (1 μl/min) was collected every 15 min into small collecting tubes containing 15 μl of 0.1 N $HClO_4$ to prevent amine oxidation and assayed immediately for DHBA, as described by an HPLC-EC procedure (Chiueh et al., 1992; Obata, 1999).

2.4. Materials

MPP^+ was purchased from Research Biochemicals Inc. (Natic, MA, USA). Bz hydrochloride, tamoxifen, sodium salicylate and its hydroxylated metabolites were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium salicylate and its hydroxylated metabolites were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phenelzine was purchased from ICN Pharmaceuticals Inc. The radioactive substrate [7-^{14}C]-Bz hydrochloride (1.85–2.29 Gbq/mmol) was