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Inhibition of rat brain mitochondrial electron transport chain activity by dopamine oxidation products during extended in vitro incubation: Implications for Parkinson's disease

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Abstract

Several studies on mitochondrial functions following brief exposure (5–15 min) to dopamine (DA) in vitro have produced extremely variable results. In contrast, this study demonstrates that a prolonged exposure (up to 2 h) of disrupted or lysed mitochondria to DA (0.1–0.4 mM) causes a remarkable and dose-dependent inhibition of complex I and complex IV activities. The inhibition of complex I and complex IV activities is not prevented by the antioxidant enzyme catalase (0.05 mg/ml) or the metal-chelator diethylenetriaminepentaacetic acid (0.1 mM) or the hydroxyl radical scavengers like mannitol (20 mM) and dimethyl sulphoxide (20 mM) indicating the non-involvement of ·OH radicals and Fenton's chemistry in this process. However, reduced glutathione (5 mM), a quinone scavenger, almost completely abolishes the DA effect on mitochondrial complex I and complex IV activities, while tyrosinase (250 units/ml) which catalyses the conversion of DA to quinone products dramatically enhances the former effect. The results suggest the predominant involvement of quinone products instead of reactive oxygen radicals in long-term DA-mediated inactivation of complex I and complex IV. This is further indicated from the fact that significant amount of quinones and quinoprotein adducts (covalent adducts of reactive quinones with protein thiols) are formed during incubation of mitochondria with DA. Monoamine oxidase A (MAO-A) inhibitor clorgyline also provides variable but significant protection against DA induced inactivation of complex I and complex IV activities, presumably again through inhibition of quinoprotein formation. Mitochondrial ability to reduce tetrazolium dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) in presence of a respiratory substrate like succinate (10 mM) is also reduced by nearly 85% following 2 h incubation with 0.4 mM DA. This effect of DA on mitochondrial function is also dose-dependent and presumably mediated by quinone products of DA oxidation. The mitochondrial dysfunction induced by dopamine during extended periods of incubation as reported here have important implications in the context of dopaminergic neuronal death in Parkinson's disease (PD).

Keywords: Dopamine; Mitochondria; Electron transport chain; Quinones; Oxygen radicals; Parkinson's disease

1. Introduction

Based on several lines of evidence, mitochondrial dysfunction has been implicated in the dopaminergic neuronal death in PD [1,2]. The analysis of post mortem brain tissue has indicated a decreased complex I activity in substantia nigra region of parkinsonian patients compared to that of normal controls [3,4]. The toxin 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) which produces parkinsonism in experimental animals and human beings has been shown to inhibit mitochondrial respiration and complex I (NADH-ubiquinone reductase) activity through its metabolite 1-methyl-4 phenyl pyridinum ion (MPP+) which is

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accumulated in dopaminergic neurons through dopamine reuptake system [5,6]. Rotenone, a specific inhibitor of NADH-ubiquinone reductase, causes degeneration of nigral dopaminergic neurons after intracerebral administration in experimental animals and the rotenone model is being used extensively to understand the pathogenesis of Parkinson's disease [7,8]. It is, however, not clear at this moment what leads to mitochondrial dysfunction in nigral dopaminergic neurons in idiopathic PD. Although mitochondrial genomic alterations may contribute to complex I defects in PD, it is generally accepted that oxidative damage mediated by DA oxidation products play a central role in this process [9–12]. Dopamine can undergo autoxidation or monoamine oxidase (MAO)-catalyzed oxidation to produce active oxygen species and reactive quinones, which are potential tissue damaging species [13]. It has been shown from in vitro studies that DA oxidation products can induce various forms of mitochondrial dysfunctions, such as mitochondrial swelling, decreased electron transport chain (ETC) activity and altered mitochondrial membrane potential during short-term (5–15 min) incubation in vitro [11,12,14]. However, in other studies with brief exposure of mitochondria to DA, the results have not been reproducible [15,16]. On the other hand, not many reports are available on the effects of DA on mitochondrial functions during extended periods of incubation. We have observed earlier that prolonged incubation (up to 2 h) of rat brain synaptosomal–mitochondrial fraction with DA results in the covalent cross-linking of membrane proteins and formation of quinoprotein adducts (protein–cysteinyl catechols) which are mediated by DA-derived quinones without any involvement of toxic oxygen radicals [17]. It was, therefore, thought interesting to investigate further the effect of DA on mitochondrial ETC activity during prolonged incubation (up to 2 h) and to elucidate the role of reactive quinones and active oxygen species in this process. The autoxidation of DA near the physiological pH of 7.4 is extremely slow and the extended period of incubation allows a sufficient accumulation of toxic quinone products.

2. Materials and methods

2.1. Materials

All common chemicals were of analytical grade. Dopamine and bovine serum albumin (BSA) were obtained from E. Merck (Germany). Catalase, cytochrome *c*, clorgyline, deprenyl hydrochloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), EGTA., phenyl-methanesulphonyl fluoride (PMSF), diethylenetriaminepentacetic acid (DTPA) and tyrosinase were purchased from Sigma Chemical Co.(USA). NADH, GSH, mannitol, dimethyl sulphoxide (DMSO), sodium dodecyl sulphate (SDS), HEPES, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), dimethylformamide, trichloroacetic acid (TCA), nitroblue tetrazolium (NBT) and sucrose were from Sisco Research Laboratory (Bombay).

2.2. Animals

Albino rats of Charles–Foster strain kept on laboratory chow and water ad libitum were used in this study. The animals were maintained as per the guidelines of the Animal Ethical Committee of our institute.

2.3. Isolation of rat brain mitochondria

Rat brain mitochondria were isolated following the method published earlier [11]. Briefly, the brain from one adult rat was homogenized in 10 ml of buffer A (225 mM mannitol, 75 mM sucrose, 5 mM HEPES, 1 mM EGTA, 1 mg/ml BSA, pH 7.4). The homogenate was brought to 30 ml with the same buffer followed by centrifugation at $2000 \times g$ for 3 min at 4 °C. The supernatant was preserved and the pellet resuspended in 10 ml of buffer A followed by recentrifugation as earlier. The supernatants were pooled and centrifuged in 4 tubes at $12{,}000 \times g$ for 8 min. The pellet in each tube containing synaptosomes and mitochondria was treated with 10 ml of buffer A containing 0.02% digitonin to lyse the synaptosomes. The mitochondria were pelleted down by centrifugation at $12{,}000 \times g$ for 10 min. The mitochondrial pellet was washed again in buffer A without EGTA and BSA and resuspended in an appropriate buffer for further experimentation. For the measurement of complex I and complex IV activities and quinoprotein formation, mitochondria were resuspended in 50 mM phosphate buffer, pH 7.4, kept frozen at −20 °C in several aliquots and used within 3 days. In case of MTT reduction assay, mitochondria were resuspended in isotonic buffer B (145 mM KCl, 50 mM sucrose, 5 mM NaCl, 1 mM EGTA, 1 mM magnesium chloride, 10 mM phosphate buffer, pH 7.4) and used immediately in the experiments.

2.4. Assay of citrate synthase for mitochondrial integrity

The mitochondria suspended in buffer B were checked for membrane integrity by assaying citrate synthase activity before and after treatment with 0.1% Triton X-100 to obtain the latency value of citrate synthase and ratios exceeding 10 were considered indicative of good membrane integrity [18]. The reaction measured the release of free coenzyme A from acetyl CoA by citrate synthase and DTNB was used to react with free thiol groups of coenzyme A.

2.5. Measurement of quinones and quinoprotein adducts

Dopamine was incubated in 50 mM phosphate buffer, pH 7.4 for 2 h in the presence or absence of rat brain mitochondria with or without GSH (5 mM) or clorgyline (10 μM). Quinone formation was monitored by absorbance change at 480 nm at the end of the incubation after correcting for