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Brain mitochondrial injury induced by oxidative stress-related events is prevented by tamoxifen

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Abstract

This study evaluated the effect of the synthetic, nonsteroidal antiestrogen drug tamoxifen on the function of brain mitochondria. We observed that tamoxifen concentrations above 30 nmol/mg protein induced a slight decrease on RCR and ADP/O ratio. However, only higher concentrations of tamoxifen ($\geq$ 70 nmol/mg protein) affected the phosphorylative capacity of mitochondria. Those effects were characterized by a decrease on mitochondrial transmembrane potential ($\Delta\Psi m$) and repolarization level and an increase on repolarization lag phase with a decrease in ATP levels. Moreover, our results also show that tamoxifen presented a potent capacity to inhibit hydrogen peroxide formation and reduced the extent of lipid peroxidation induced by the pro-oxidant pair ADP/Fe^{2+}. Tamoxifen also exerted some protection against mitochondrial permeability transition pore (MPT) opening, although in a smaller extension than that promoted by cyclosporin A, the specific inhibitor of the MPT. However, in the presence of tamoxifen plus cyclosporin A, the protection observed was significantly higher when compared with that induced by both agents alone. Furthermore, tamoxifen avoided the oxidation of thiol groups and GSH depletion promoted by Ca^{2+}.

These results show that tamoxifen can afford protection against brain mitochondrial injury promoted by several oxidative stress-related events such as hydrogen peroxide production, lipid peroxidation and the induction of the MPT. Since numerous neurodegenerative diseases are intimately related with mitochondrial dysfunction, future therapeutical strategies could be designed taking into account this protective role of tamoxifen.

Keywords: Brain; Mitochondria; Neuroprotection; Oxidative stress; Tamoxifen

1. Introduction

The synthetic nonsteroidal antiestrogen drug tamoxifen (TAM) is widely used in the chemotherapy of breast cancer (Richardson, 1988) and has been proposed as a prophylactic agent against this disease. In humans, TAM is extensively metabolised to give 4-hydroxytamoxifen (OHTAM) and *N*-desmethyltamoxifen (Fromson et al., 1973). Besides their chemopreventing function, TAM and OHTAM, which are extremely

Abbreviations: CsA, cyclosporin A; $\Delta\Psi m$, mitochondrial transmembrane potential; GSH, reduced glutathione; GSSG, oxidized glutathione; MPP^+, 1-methyl-4-phenyl-pyridinium ion; MPT, mitochondrial permeability transition pore; NAD^+, nicotinamide adenine dinucleotide ion; NADH, nicotinamide adenine dinucleotide; OHTAM, hydroxytamoxifen; $NADP^+$, nicotinamide adenine dinucleotide phosphate ion; NADPH, nicotinamide adenine dinucleotide phosphate; RCR, respiratory control ratio; TAM, tamoxifen; TPP^+, tetraphenylphosphonium ion.

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lipophilic (Custódio et al., 1991, 1993), induce multiple cellular effects, including antioxidant actions since they are strong intramembranous scavengers of peroxyl radicals (Custódio et al., 1994).

Tamoxifen can penetrate the blood–brain barrier and interact with several neuronal estrogen receptor-positive cell populations (Jordan et al., 1983). Mehta et al. (2003) observed that TAM dramatically reduced middle cerebral artery occlusion-induced ischemic damage of the affected cerebral hemisphere. The reduction of infarct size was primarily due to protection of two major structures, the cerebral cortex and striatum. In the same study, Laser Doppler analysis revealed that tamoxifen had no significant effect on cerebral blood flow either before or after ischemia, suggesting that tamoxifen protection is independent of cerebral blood flow changes. More recently tamoxifen has been shown to be markedly neuroprotective in rat models of reversible and permanent focal ischemia (Kimelberg et al., 2003; Feng et al., 2004). O'Neill and Brinton (2002) showed that both TAM and OHTAM attenuate the excitotoxic glutamate-induced intracellular Ca^{2+} rise. Furthermore, TAM suppresses the $HO^{\cdot}$ generation via dopamine efflux induced by MPP^{+} (Obata, 2002). A recent study shows that OHTAM attenuates methamphetamine-induced nigrostriatal dopaminergic toxicity in intact and gonadetomized mice (Kuo et al., 2003). However, the mechanisms of TAM-mediated neuroprotection independently of estrogen receptors are not yet clarified.

Recently, it has become clear that mitochondria have a significant role to play not only in energy production, but also in cell death (Bossy-Wetzel and Green, 1999). A potentially central factor in cell death during neurodegeneration is the mitochondrial permeability transition pore (MPT) (Kim et al., 1999). The MPT is a phenomenon that is characterized by the opening of pores in the inner mitochondrial membrane and by its sensitivity to a very low concentration of cyclosporin A (CsA). Ca^{2+} and oxidative stress have long been known to favour the permeability transition (Zoratti and Szabo, 1995). The MPT possesses at least two redox-sensitive sites that increase the probability of opening after oxidation: (1) the "S-site", a dithiol in apparent redox equilibrium with matrix glutathione and (2) the "P-site", in apparent redox equilibrium with the pyridine nucleotides pool (Chernyak and Bernardi, 1996).

Previous studies indicate that TAM (Custódio et al., 1998) and OHTAM (Cardoso et al., 2002a) are potent inhibitors of the MPT in liver mitochondria. Hoyt et al. (2000) reported that TAM might be an inhibitor of the MPT in intact neurons. However, MPT phenomenon in intact neurons has proven difficult to establish since other cellular components may contribute to misinterpretations, such as calcineurin that is also inhibited by cyclosporin A (CsA), the specific inhibitor of MPT. With these evidences, we decided to study the effect of TAM directly in isolated brain mitochondria. Our first task was the evaluation of the impact of increasing concentrations of TAM (15, 30, 70, 100 nmol/mg protein) on the respiratory chain and oxidative system. For that purpose we analysed the respiratory indexes (RCR and ADP/O), mitochondrial transmembrane potential ($\Delta\Psi m$), repolarization level, repolarization lag phase and ATP levels. The second part of this study consisted in the evaluation of the protective effect of TAM against hydrogen peroxide production, lipid peroxidation and Ca^{2+}-induced MPT. The lipid peroxidation was evaluated by analysing $\Delta\Psi m$, TBARS formation and oxygen consumption while the induction of MPT was characterized by analysing the $\Delta\Psi m$, calcium fluxes, GSH content and protein thiol groups oxidation.

2. Materials and methods

2.1. Materials

Tamoxifen and Protease type VIII (Subtilisin Carlsberg) were obtained from Sigma Chemical Co. (St. Louis, MO). Digitonin was obtained from Calbiochem (Merck Biosciences Ltd., Nottingham). All the other chemicals were of the highest grade of purity commercially available.

2.2. Animals

Male Wistar rats (3 months old) were housed in our animal colony (Laboratory Research Center, University Hospital, Coimbra, Portugal). They were maintained under controlled light (12 h day/night cycle) and humidity with free access to water (except in the fasting period) and powdered rodent chow (URF1-Charles River, France). Adhering to procedures approved by the Institutional Animal Care and Use Committee, the animals were sacrificed by cervical displacement and decapitation.

2.3. Isolation of brain mitochondria

Brain mitochondria were isolated from male Wistar by the method of Rosenthal et al. (1987), with slight modifications, using 0.02% digitonin to free mitochondria from the synaptosomal fraction. In brief, one rat is decapitated, and the whole brain minus the cerebellum was rapidly removed, washed, minced, and homogenised at 4 °C in 10 ml of isolation medium (225 mM mannitol, 75 mM sucrose, 5 mM Hepes, 1 mM EGTA, 1 mg/ml bovine serum albumin, pH 7.4) containing 5 mg of the bacterial protease. Single brain homogenates were brought to 30 ml and then centrifuged at 2500 rpm (Sorvall RC-5B Refrigerated Superspeed Centrifuge) for