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ScienceDirect
NeuroToxicology 28 (2007) 672–678
NeuroToxicology

Biphasic mechanism of the toxicity induced by 1-methyl-4-phenylpyridinium ion (MPP^+) as revealed by dynamic changes in glucose metabolism in rat brain slices

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Received 6 August 2006; accepted 9 February 2007
Available online 23 February 2007

Abstract

1-Methyl-4-phenylpyridinium (MPP^+) is a well-known neurotoxin which causes a clinical syndrome similar to Parkinson's disease. The classical mechanism of MPP^+ toxicity involves its entry into cells through the dopamine transporter (DAT) to inhibit aerobic glucose metabolism, while recent studies suggest that an oxidative mechanism may contribute to the toxicity of MPP^+. However, it has not been adequately determined what role these two mechanisms play in the development of neurotoxicity after MPP^+ loading in the brain. To clarify this issue, MPP^+ was added directly to fresh rat brain slices and the dynamic changes in the cerebral glucose metabolic rate (CMRglc) produced by MPP^+ were serially and two-dimensionally measured using the dynamic positron autoradiography technique with [^{18}F]2-fluoro-2-deoxy-D-glucose as a tracer. MPP^+ dose-dependently increased CMRglc in each of the brain regions examined, reflecting enhanced glycolysis compensating for the decrease in aerobic metabolism. Treatment with DAT inhibitor GBR 12909 significantly attenuated the enhanced glycolysis induced by 10 μM MPP^+ in the striatum. Treatment with free radical spin trap α-phenyl-*N*-*tert*-butylnitrone (PBN) significantly attenuated the enhancement of glycolysis induced by 100 μM MPP^+ in all brain regions. These results suggest that the mechanism of the toxicity of MPP^+ is biphasic and consists of a DAT-mediated mechanism selective for dopaminergic regions at a lower concentration of MPP^+ (10 μM), and an oxidative mechanism that occurs at a higher concentration of MPP^+ (100 μM) and is not restricted to dopaminergic regions.

Keywords: 1-Methyl-4-phenylpyridinium; Neurotoxicity; GBR 12909; α-Phenyl-*N*-*tert*-butylnitrone; [^{18}F]2-fluoro-2-deoxy-D-glucose; Brain slice

1. Introduction

1-Methyl-4-phenylpyridinium (MPP^+), the pyridinium metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is a well-known neurotoxin which causes a clinical syndrome similar to Parkinson's disease (PD) in humans and in non-human primates and is used as a suitable drug in various experimental models to study new therapeutic strategies in PD (Adams and Odunze, 1991; Przedborski and Jackson-Lewis, 1998). MPTP, when administered systemically, is metabolized in the brain by astrocyte monoamine oxidase-B to MPP^+. The MPP^+ is then taken up specifically by dopaminergic neurons, where it exerts an intraneuronal toxic effect resulting in degeneration of the neurons (Langston et al., 1984; Markey et al., 1984). Therefore, MPP^+ is thought to be the ultimate mediator of the toxic effects of MPTP (Langston et al., 1984; Markey et al., 1984).

Although the neurotoxic action of MPP^+ is under discussion, it is thought that the mechanism of toxicity of this compound may involve uptake by the dopamine transporter (DAT) (Javitch et al., 1985), subsequent accumulation into the mitochondria (Ramsay and Singer, 1986) and inhibition of NADH: ubiquinone oxidoreductase in complex I of the electron transport chain (Przedborski and Jackson-Lewis, 1998; Tipton and Singer, 1993). The consequent depletion of the energy supply has been shown to be related to the cytotoxicity of MPP^+ in various cell cultures (Chalmers-Redman et al., 1999; Storch et al., 2000; Tipton and Singer, 1993). Supportive evidence indicating a role for mitochondrial involvement in PD is the fact

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0161-813X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2007.02.008

that exposure to rotenone, pesticides and other mitochondrial toxicants can lead to similar pathologies (Betarbet et al., 2000).

Other factors, such as generation of reactive-oxygen species, may contribute to the toxicity of MPP^+ (Akaneya et al., 1995; Cassarino et al., 1999; Cleeter et al., 1992; Fall and Bennett, 1999; Fallon et al., 1997; Hasegawa et al., 1990; Ramsay and Singer, 1992; Schulz et al., 1995). The involvement of oxidative stress in idiopathic Parkinson's disease has been extensively reviewed (Berg et al., 2001; Jenner, 1998; Schuman and Madison, 1994). On the other hand, several studies indicated that MPP^+ did not produce oxygen free radicals and antioxidants did not attenuate MPP^+ neurotoxicity (Fonck and Baudry, 2003; Lee et al., 2000; Sanchez-Ramos et al., 1988). Therefore, the involvement of the oxidative mechanism in the neurotoxicity of MPP^+ is less clear. Furthermore, it has not been adequately determined in what way these two mechanisms (i.e. the DAT-mediated and the oxidative mechanisms) contribute to the development of neurotoxicity after MPP^+ loading in the brain.

There is significant evidence supporting the hypothesis that MPP^+ toxicity corresponds to parallel changes in glycolytic activity. For example, in neuronal cells and astrocytes, MPP^+ toxicity occurs in parallel to reductions in phosphocreatine and ATP (Marini and Nowak, 2000; Storch et al., 2000; Wu et al., 1992), with a simultaneous increase in lactic acid and glucose utilization, and the subsequent depletion of extracellular glucose (Fall and Bennett, 1999; Marini and Nowak, 2000; Mazzio and Soliman, 2003; Wu et al., 1992). Moreover, preserving the cellular ATP concentration through sustaining cellular glucose metabolism is a preeminent factor in deterring MPP^+-induced toxicity (Chalmers-Redman et al., 1999; Storch et al., 2000), whereas a glucose-deficient environment exacerbates MPP^+-induced toxicity in vitro (Wu et al., 1992). These data indicate a paramount role of the impairment of glucose metabolism in the development of MPP^+-mediated toxicity in the brain. However, since MPP^+ itself does not cross the blood–brain barrier, most previous studies have been conducted on cell preparations (Marini and Nowak, 2000; Mazzio and Soliman, 2003; Wu et al., 1992), and it is difficult to directly map the effects of MPP^+ itself on cerebral glucose metabolism. As an imaging technique in living brain slices, we have developed the ''dynamic positron autoradiography technique'' (dPAT), which utilizes positron emitter-labeled ligands as probes and a radioluminography plate as the detector (Murata et al., 1999; Omata et al., 2000). Serial two-dimensional images of radioactivity in the slices can be constructed quantitatively with a short exposure time while the brain tissue is still alive in the incubation solution because of the high specific radioactivity of the radiotracers, high energy of positrons, and high sensitivity of the radioluminography plate. dPAT is especially useful for examining the effect of MPP^+ itself on cerebral glucose metabolism because MPP^+ and/or other agents can be applied directly to brain slices at precisely controlled concentrations for specific time intervals.

In the present study, MPP^+ was added directly (rather than being generated from MPTP) to fresh rat brain slices and the dynamic changes in the cerebral glucose metabolic rate (CMRglc) produced by MPP^+ were serially and two-dimensionally measured using dPAT with [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) as a tracer. In addition, to clarify how the classical mechanism of toxicity of MPP^+, i.e. entry through the DAT (Javitch et al., 1985) and blockade of complex I of the respiratory chain (Przedborski and Jackson-Lewis, 1998; Tipton and Singer, 1993), and the oxidative mechanism are involved in the promotion of the neurotoxicity, the effects of the addition of GBR 12909 as a specific DAT inhibitor and α-phenyl-*N*-*tert*-butylnitrone (PBN) as a free radical scavenger on the MPP^+-induced changes in glucose metabolism were quantitatively evaluated.

2. Materials and methods

2.1. Materials

MPP^+ iodide, GBR 12909 and PBN were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were from Nacalai Tesque Inc. (Kyoto, Japan).

2.2. Dynamic positron autoradiography technique

All animal procedures were approved by the Animal Care and Use Committee of the University of Fukui in accordance with the Guidelines for Animal Experiments, University of Fukui. Male Wistar rats (250–300 g) were decapitated under diethyl ether anesthesia and their brains were removed. Sagittal brain slices (300 μm in thickness) were prepared with a microslicer (DTK-2000, Dosaka EM, Kyoto, Japan), and incubated as previously described (Murata et al., 1999; Omata et al., 2000). The system is shown schematically in Fig. 1. The outer chamber was filled with Krebs-Ringer solution and the inner chamber was immersed in it. The bottom of the inner

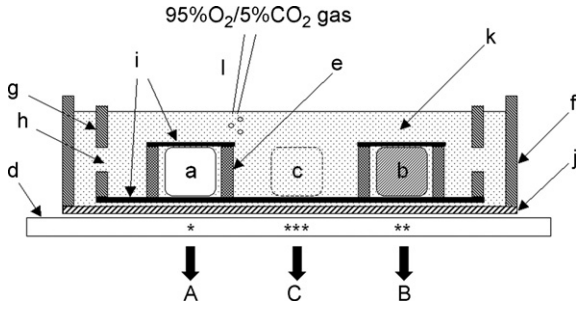

Fig. 1. Schematic side view of the apparatus for incubation and the radioactivity signal detected on the radioluminography plate. (a) brain slice (300 μm thick); (b) nylon sheet (300 μm thick) as a slice phantom for measuring the background noise; (c) 300-μm-thick bathing solution layer; (d) radioluminography plate (replaced every 20 min); (e) stainless steel ring; (f) outer chamber; (g) inner chamber; (h) hole on the side wall of the inner chamber; (i) nylon net (80 μm thick); (j) polyvinylidene chloride film (10 μm thick); (k) Krebs-Ringer solution containing [^{18}F]FDG [36 °C, pH 7.3–7.4, and bubbled with 95% O_2/5% CO_2 gas (oxygen partial pressure 650–700 mmHg)]; (l) polytetrafluoroethylene catheter for bubbling. A (*), B (**) and C (***) were defined as the radioactivity signal [photostimulated luminescence (PSL)/mm^2] on the radioluminography plate detected beneath the brain region of interest and the bathing medium solution, respectively. Images were obtained in a dark environment at 36 °C.