In vivo imaging of mitochondrial function in methamphetamine-treated rats

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Abstract

Abuse of the powerfully addictive psychostimulant, methamphetamine, occurs worldwide. Recent studies have suggested that methamphetamine-induced dopaminergic neurotoxicity is related to oxidative stress. In response to nerve activation, the mitochondrial respiratory chain is rapidly activated. The enhancement of mitochondrial respiratory chain activation may induce oxidative stress in the brain. However, there is little experimental evidence regarding the mitochondrial function after methamphetamine administration in vivo. Here, we evaluated whether a single administration of methamphetamine induces ATP consumption and overactivation of mitochondria. We measured mitochondrial function in two different ways: by monitoring oxygen partial pressure using an oxygen-selective electrode, and by imaging of redox reactions using a nitroxyl radical (i.e., nitroxide) coupled with Overhauser-enhanced magnetic resonance imaging (OMRI). A single administration of methamphetamine to Wistar rats induced dopaminergic nerve activation, ATP consumption and an increase in mitochondrial respiratory chain function in both the striatum and cortex. Furthermore, antioxidant TEMPOL prevented the increase in mitochondrial oxidative damage and methamphetamine-induced sensitization. These findings suggest that energy-supplying reactions after dopaminergic nerve activation are associated with oxidative stress in both the striatum and cortex, leading to abnormal behavior.

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Introduction

Abuse of the powerfully addictive psychostimulant, methamphetamine, occurs worldwide. Chronic methamphetamine users show signs of psychiatric illness, including a psychotic state and an anxiety-like disorder (Akiyama, 2006; Grelotti et al., 2010; Sato, 1992). Repeated intermittent administration of methamphetamine produces an enduring hypersensitivity to the motor stimulant effect of methamphetamine in humans (Ujike and Sato, 2004) as well as in experimental animals (Shuto et al., 2006), which is termed behavioral sensitization. It is widely accepted that the behavioral sensitization caused by methamphetamine is a characteristic of drug dependence and addiction (Vanderschuren and Kalivas, 2000). Behavioral sensitization by methamphetamine is associated with dopaminergic nerve function (Kalivas and Stewart, 1991). Energy consumption and supply are tightly linked to neuronal activity in the brain. Depolarizations propagate from the dendritic spines to the dendrites, where they may cause further opening of voltage-gated sodium channels and activation of the Na+/K+ ATPases, leading to an increased demand for energy (ATP). In response, the mitochondrial respiratory chain is rapidly activated, causing a decrease in mitochondrial NADH content (Kasischke et al., 2004). Although this process is physiologically important, the enhancement of mitochondrial respiratory chain function after nerve activation may induce oxidative stress in the brain. Mitochondria are the major cellular source of oxygen free radicals because of the electron leakage from the respiratory chain; the electrons in turn react with molecular oxygen to give superoxide anion radicals (Chen et al., 2005). In fact, lipid peroxidation products accumulate in both the striatum and the prefrontal cortex in chronic methamphetamine users and in animal models (Ackigoz et al., 2000; Fitzmaurice et al., 2006).

During the mitochondrial respiratory chain reaction, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. To detect redox status in mitochondria, nitroxyl radicals (i.e., nitroxides) are powerful as a redox-sensitive contrast agent coupled with Overhauser-enhanced magnetic resonance imaging (OMRI) (Quintanilha and Packer, 1977; Yamato et al., 2009). OMRI is a double resonance technique that uses the presence of paramagnetic agents to enhance the signal intensity from nuclear spins by a process known as dynamic nuclear polarization, or the Overhauser effect (Krishna et al., 2002; Li et al., 2006; Lurie et al., 1988; Lurie et al., 2005). Recent studies have successfully expanded this technique to obtain functional information, including noninvasive pO2 maps (Krishna et al., 2002; Matsumoto et al., 2009) and simultaneous images of different redox reactions (Utsumi et al., 2006).
Here, we evaluated whether a single administration of methamphetamine induces ATP consumption and overactivation of mitochondria. We measured mitochondrial function using OMRI to monitor the reducing and oxidizing processes noninvasively. For a comprehensive investigation of the mitochondrial function, we also monitored oxygen partial pressure using an oxygen-selective electrode. We also examined the ability of the blood–brain barrier-permeable antioxidant, TEMPOL (4-hydroxy-2,6,6-tetramethylpiperidine-N-oxide) to alter methamphetamine-induced oxidative stress and examined its effect on behavior sensitization.

Materials and methods

Chemicals

Methamphetamine was purchased from Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). 3-carboxy-2,2,5,5-tetramethylpyrrolidine-L-oxyl (carboxy-PROXYL), α-methyl-p-tyrosine (α-MT), and TEMPOL were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). ATP, ADP, AMP, lactate dehydrogenase (from pig heart), and NADH were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Methanol and acetonitrile (HPLC grade) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). EDTA was added. After mixing, the tubes were centrifuged at 10,000×g for 10 min at 0 °C. The supernatant fluid was neutralized by a solution containing 1.5 mol/L KOH, 0.4 mol/L imidazole, and 0.3 mol/L KCl (Folbergrova et al., 1972). Samples were stored at −80 °C until analyzed.

To estimate ATP, ADP, and AMP levels, a 20-μL sample was separated on a C18 reverse phase column (MC column 150×4.6 mm; MC Medical, Inc.) and detected with a UV system at 260 nm (JASCO, Inc., Tokyo, Japan). The mobile phase was 100 mmol/L potassium phosphate buffer (pH 6.0) and the rate of the mobile phase flow through the system was 1.0 mL/min. The energy charge rate was calculated according to method of Atkinson (Atkinson, 1968). Pyruvate and lactate were determined by methods previously described (Folbergrova et al., 1969; Goldberg et al., 1966).

Measurement of partial pressure of oxygen

Animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and mounted in a stereotaxic frame equipped with a mouse adapter. An oxygen-selective electrode (POE-10N, PO2-150S; Eikoukagaku, Tokyo, Japan) was implanted into the striatum (co-ordinates from bregma: anterior–posterior: +0.2; lateral–medial: +2.6; dorsal–ventral: +6.0 from the skull), or the cortex (co-ordinates from bregma: anterior–posterior: +0.2; lateral–medial: +2.6; dorsal–ventral: +2.5 from the skull). The currents were recorded on a data acquisition system (PowerLab 2/26; ADInstruments Pty, Castle Hill, NSW, Australia) connected to a computer (Chart software program, ver. 5.5.4; ADInstruments Pty). This electrode was calibrated in physiological saline at 37 °C under atmospheric pressure (partial pressure of oxygen, 150 mm Hg) (Schubert et al., 1978).

Phantom imaging

OMRI experiments were performed on a custom-built OMRI scanner (POEM; Philips Research Laboratories, Hamburg, Germany) with a surface-coil-type receiver coil for electron spin resonance (ESR) irradiation. The resonant circuit consisted of a single-turn loop coil, a parallel coaxial line formed by 50-ohm coaxial cables, a half-wave line balun, and trimmer capacitors for matching and tuning, as reported previously (Matsumoto et al., 2007). The operating frequency of the surface coil was designed to have an ESR irradiation frequency of 222.6 MHz for OMRI. The OMRI images were obtained using the following conditions: field of view, 32×32 mm; matrix, 64×64; slice thickness, 20 mm; repetition time/echo time/period of ESR irradiation (TR/TE/TESR), 1200/25/700 ms. To validate the ability of the imaging method to provide spatially resolved redox status information, a phantom consisting of identical tubes containing methoxy carbonyl-PROXYL (0.5 mmol/L) was used. The amount of methoxy carbonyl-PROXYL in the individual tubes was time-dependently reduced by the mitochondrial fraction (4.7 mg protein) in the presence of substrate. Substrate solution containing glutamate/malate (5 mmol/L) was used as a substrate for complex I, and succinic acid (20 mmol/L) for complex II.

Brain imaging

The femoral vein was cannulated for injection of methoxy carbonyl-PROXYL after the induction of anesthesia by pentobarbital (Dainabot, Tokyo, Japan). The rat was then placed in the resonator. Immediately after the administration of methoxy carbonyl-PROXYL (1.3 mmol/kg), OMRI images were obtained (field of view, 32×32 mm; matrix, 32×32; slice thickness, 30 mm; TR/TE/TESR, 1200/25/700 ms), as described.
previously (Yamato et al., 2009). The dopamine synthetase inhibitor, α-MT (250 mg/kg) was intraperitoneally injected 4 h before recording, as described previously (Watanabe et al., 2005).

Semi-logarithmic plots of the time-course of the OMRI signal change in the region of interest were used for calculating the reduction rate. A region of interest in each cerebral hemisphere was selected for this determination. The region of interest analysis was carried out using MATLAB (The MathWorks, Natick, MA, USA).

Measurement of mitochondrial thiobarbituric acid reactive substance (TBARS)

Mitochondrial and cytoplasmic fractions were isolated from both striatum and cortex as described previously (Yamato et al., 2009). A 0.1-ml sample was mixed with reagents to give a final concentration of 2 mM EDTA, 7.5% acetic acid, and 0.4% SDS, and was then reacted with 0.3% thiobarbituric acid in a boiling water bath for 45 min. After cooling, the chromogen was extracted in n-butanol: pyridine (15:1, v/v). The fluorescence of the supernatant was measured at excitation and emission wavelengths of 512 and 553 nm, respectively, using an SH-900 grating microplate reader (Corona Electric Co., Ltd., Ibaragi, Japan). 1,1,3,3,-Tetraethoxypropane was used as the standard.

Statistical analysis

All the results are shown as the mean±SEM. Statistical significance was analyzed using the two-tailed t-test or the Tukey–Kramer test. A probability value of 0.05 was set as the minimum level of statistical significance.
Results

Methamphetamine-induced locomotor hyperactivity and dopamine release

A dose-dependent increase in locomotor activity was observed in methamphetamine-treated rats (Fig. 1). Administration of 5 mg/kg methamphetamine significantly affected locomotor activity, and therefore this concentration was chosen for subsequent experiments.

ATP consumption after methamphetamine administration

We examined whether ATP was consumed by methamphetamine-induced dopaminergic nerve activation. ATP levels in the brain were significantly decreased 30 min after methamphetamine administration (Fig. 2A). Simultaneously, ADP and AMP were increased, resulting in a decrease in the energy charge rate (Figs. 2B–D). ATP, ADP, and AMP levels in the brain normalized to control levels 24 h after methamphetamine administration (data not shown).

Activity of the glycolytic system after methamphetamine treatment

ATP can be produced by two main pathways in eukaryotic organisms: glycolysis and the mitochondrial respiratory chain reaction. To estimate ATP production, we measured the levels of lactate and pyruvate—metabolites of the glycolytic system. Fig. 2E shows that pyruvate levels in the brain tended to increase after methamphetamine administration. Lactate levels significantly increased in the brain of methamphetamine-treated rats (Fig. 2F).

Oxygen consumption as an index of mitochondrial respiratory chain function

We measured oxygen partial pressure using an oxygen-selective electrode in both the striatum and cortex of rats to estimate mitochondrial function. Oxygen partial pressure temporarily increased just after methamphetamine administration, and then reduced to a steady state in both brain areas (Figs. 3A and B).

![Fig. 3. Oxygen partial pressure in the striatum and cortex. Oxygen partial pressure was measured to estimate mitochondrial respiratory chain function in the brain of control (open circles) and methamphetamine (METH)-treated (closed circles) rats. Seven animals were used for each experiment. Each value represents the mean ± SEM. Measurement of oxygen partial pressure using oxygen-selective electrode was performed as described in “Materials and methods”.

![Fig. 4. OMRI images of methoxycarbonyl-PROXYL phantoms. (A) Images of methoxycarbonyl-PROXYL and its hydroxylamine form. (B) The molecular structure of methoxycarbonyl-PROXYL and its reversible one-electron reduction/oxidation interconversion. (C) Time-dependent OMRI image of methoxycarbonyl-PROXYL in the presence of mitochondria. The three tubes contained 0.5 mmol/L methoxycarbonyl-PROXYL (P), with the mitochondrial fraction (Mit) or with the mitochondrial fraction and substrates (+S). Substrate solution containing glutamate/malate (5 mmol/L) was used as a substrate for complex I, and succinic acid (20 mmol/L) for complex II. OMRI was performed as described in “Materials and methods”.](image-url)
Redox reactions as an index of mitochondrial respiratory chain function

Fig. 4A shows three tubes, containing the methoxycarbonyl-PROXYL radical solution (paramagnetic, enhanced), its hydroxylamine (diamagnetic, not enhanced), and water. Although the image obtained without electron spin resonance (ESR) irradiation had a low signal/noise ratio, ESR irradiation preceded each phase-encoding step of the MRI data acquisition, resulting in an increased NMR signal intensity of the tube containing methoxycarbonyl-PROXYL, but not of its hydroxylamine or water. Subtraction of the image obtained without ESR from that with ESR irradiation yielded a difference image that showed the pure distribution of the free radical (Fig. 4A). The addition of mitochondrial enzyme reduces the nitroxyl radical to its corresponding hydroxylamine (Fig. 4B). To assess the ability of our system to monitor the redox reaction, we used a redox phantom, consisting of methoxycarbonyl-PROXYL and mitochondrial samples (Fig. 4C). The intensity of the methoxycarbonyl-PROXYL in the mitochondrial fraction gradually decreased as a function of time after mixing it with the substrates. Fig. 4C also shows the rate constants in the mitochondrial fractions, which were calculated by assuming first-order kinetics for the time-dependent decrease in contrast.

We visualized the reduction reaction in mitochondria, using methoxycarbonyl-PROXYL as a redox-sensitive contrast agent for brain imaging because of its high permeability for the blood–brain barrier (Anzai et al., 2003; Yamato et al., 2009). Fig. 5A shows an OMRI redox image of the brain of a live rat, 30 min after methamphetamine administration. The redox reaction was enhanced in the brain of the methamphetamine-treated rat (yellow-red area) compared with that in the control rat (blue area). Enhancement of the redox reaction was observed throughout the brain. Methamphetamine produces its effect by increasing the synaptic levels of dopamine, resulting in ed increase in locomotor activity, similar to control levels. However, methamphetamine/TEMPOL-treated rats demonstrated an attenuated increase in locomotor activity, similar to control levels.

To examine the association between amelioration of sensitization and antioxidant effects induced by TEMPOL, we measured the accumulation of lipid peroxidation products in mitochondria using a TBARS assay. Fig. 6C shows the increase in TBARS level in mitochondria from methamphetamine-treated rats in both the striatum and cortex. Importantly, the TBARS level was decreased in mitochondria from methamphetamine/TEMPOL-treated rats.

Discussion

In the present study, we demonstrated that a single administration of methamphetamine reduced dopaminergic nerve activation, ATP consumption and an increase in the mitochondrial respiratory chain reaction. In particular, we detected the reduction of nitroxyl radical in the brain as index of mitochondrial function in vivo. Nitroxyl radical is reduced to the corresponding hydroxylamine form in the mitochondrial respiratory chain reaction, and then the reduction can be visualized by OMRI. Thus, this technique should enable the visualization of in vivo mitochondrial function non-invasively. Therefore, we could clarify that mitochondrial respiratory chain activation occurred in the whole brain of methamphetamine-treated rat.

In neurons, a large amount of ATP is consumed to maintain a resting membrane potential and to recover after an action potential (Hall, 1992). When energy charge is low, ATP is produced by cellular processes such as the glycolytic pathway in the cytosol or the citric acid cycle/respiratory chain reaction in mitochondria. Glucose is metabolized to pyruvate via the glycolytic pathway, resulting in the generation of two molecules of ATP. If a large amount of ATP is needed, pyruvate is supplied to the citric acid cycle; however, when the citric acid cycle reaches its capacity, excess pyruvate is converted...
into lactate (Mathews et al., 2000). Our data showed that locomotor activity was increased in methamphetamine-treated rats, indicating nerve activation (Fig. 1). In parallel with these responses, ATP in the brain was significantly decreased 30 min after methamphetamine administration, and ADP and ATP were increased (Fig. 2). In addition, lactate significantly accumulated in the brain of methamphetamine-treated rats (Fig. 2F), indicating an enhancement of the glycolytic pathway.

Mitochondrial respiration is crucial in energy production, and mitochondria account for most oxygen consumption in the majority of cells. Thus, oxygen consumption serves as a direct indicator of mitochondrial function. Hussain et al. (2008) estimated the oxygen concentration to measure mitochondrial function in live cells. We showed that the oxygen partial pressure was transiently elevated and then decreased below the control level in both the striatum and cortex after methamphetamine administration (Fig. 3). This result indicates that ATP synthesis may activate using oxygen.

In the mitochondrial respiratory chain, redox reactions occur using oxygen as a substrate to generate ATP. Here, we detected the reduction of nitroxyl radicals in the brain as index of in vivo mitochondrial function. However, nitroxyl radicals can also be changed to the corresponding hydroxylamine form by ascorbic acid (Mehlhorn, 1991) or free radicals (Krishna et al., 1992; Kudo et al., 2008). In this study, the concentration of ascorbic acid in the brain was the same between control and methamphetamine-treated rats (Supplemental Fig. 1A). The enhanced reduction rate in methamphetamine-treated rats was not changed by treatment with the antioxidants both in the in vitro respiratory chain reaction and the in vivo OMRI experiments (Supplemental Figs. 1B and C). Therefore, the methamphetamine-induced enhancement of nitroxyl radical reduction may primarily reflect enzymatic reduction in the mitochondrial respiratory chain. Importantly, the reduction in nitroxyl radicals, as well as the decrease in oxygen partial pressure, was observed throughout the whole brain (Fig. 5). We also confirmed that ATP levels recovered to control levels at 24 h after methamphetamine administration. These data suggest that the mitochondrial respiratory chain reaction becomes activated to compensate for ATP consumption after dopaminergic nerve activation.

Although the mitochondrial respiratory chain reaction is vital to metabolism, it produces reactive oxygen species such as superoxide and hydrogen peroxide, which leads to the propagation of free radicals that damage cells. If energy-supplying reactions are associated with oxidative stress in the brain of methamphetamine-treated rats, treatment with antioxidants may prevent not only the development of behavioral sensitization but also mitochondrial oxidative damage.
damage. In fact, our data showed that simultaneous administration of TEMPO prevented the development of behavioral sensitization and the accumulation of lipid peroxidation products in mitochondria (Fig. 6). In this study, the TBARS level was lower in the cortex than in the striatum, the latter of which is high in dopamine (Fig. 6C). Dopamine induces oxidative stress through quinone formation (Miyazaki et al., 2006), a decrease in the glutathione level (Moszczynska et al., 1998), and the formation of 6-hydroxydopamine (Maharaj et al., 2005). These mechanisms, including the enhancement of energy-supplying reactions in mitochondria, might be inter-related, and exacerbate the oxidative stress in our model.

Mitochondrial oxidative stress plays a crucial role in the progression of diseases (Ide et al., 1999; Zhu et al., 2005). The vicious circle between dopaminergic nerve activation, enhancement of the energy-supplying reaction, and mitochondrial oxidative stress may cause damage to the brain and result in further drug dependence and addiction.

Supplementary materials related to this article can be found online at doi:10.1016/j.neuroimage.2011.05.041.

List of abbreviations

- carboxy-PROXYL: 3-carboxy-2,2,5,5-tetramethylpyrroline-1-oxyl
- ECD: electrochemical detector
- ESR: electron spin resonance
- METH: methamphetamine
- methoxycarbonyl-PROXYL: 3-methoxy carbonyl-2,2,5,5-tetramethylpyrroline-1-oxyl
- α-MT: α-methyl p-tyrosine
- OMRI: Overhauser-enhanced magnetic resonance imaging
- SOD: superoxide dismutase
- TBARS: thiobarbituric acid reactive substance
- TEMPO: 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl

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References


