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Research report

Inhibitory effect of melatonin on cerebral endothelial cells dysfunction induced by methamphetamine via NADPH oxidase-2

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ABSTRACT

Melatonin is a hormone that mostly produced from the pineal gland, and it performs as a strong neuroprotectant to both neuron and glial cells against methamphetamine (METH)-induced neurotoxicity. Recently, it has been found that METH also damages the blood brain barrier (BBB) structure and function. However, the protective mechanism of melatonin on the BBB impairment caused by METH has not been investigated. In this study, the primary rat brain microvascular endothelium cells (BMVECs) isolated from neonatal rats was used to investigate the protective effect of melatonin on METH-induced BBB impairment and the underlying mechanism. The results demonstrated that melatonin decreased the level of reactive oxygen species (ROS), reactive nitrogen species (RNS), and apoptosis induced by METH via NADPH oxidase (NOX)-2 since apocynin, a NOX-2 inhibitor abolished those changes. In addition, melatonin was found to improve cell integrity by increasing the transendothelial electric resistance (TEER) values, and up-regulate the tight junction proteins ZO-1, occludin, and claudin-5, thereby decreasing the paracellular permeability caused by METH mediated by NOX-2. Our data suggest that METH induces BBB impairment by mediating NOX-2 activity, and then induces oxidative and nitrative stress, as well as apoptosis, which causes the impairment of cell integrity, and that melatonin reduces these negative effects of METH by mediating via MT1/2 receptors.

1. Introduction

Methamphetamine (METH) is a potent neurotoxin that causes neurologic and psychiatric disorders (Hsieh et al., 2014; Halpin et al., 2014). Short- and long-term METH administration causes neurodegeneration by inducing dysfunction not only in neuron and glial cells but also in the blood-brain barrier (BBB) (Panenka et al., 2013; Taylor et al., 2013; Loftis and Janowsky, 2014; Northrop and Yamamoto, 2015). The BBB is a cellular complex interface between the blood circulation and the brain parenchyma, forming a strong tight junction that functions as a protective barrier which shields the central nervous system (CNS) from neurotoxic substances circulating in the blood (Abbott, 2013). Previous studies have demonstrated that BBB impairment caused by METH leads to overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and pro-inflammatory cytokines and chemokines such as inducible nitric oxide synthase (iNOS), nitric oxide (NO), interleukin (IL)-1, and tumor necrosis factor (TNF)-α (Zhang et al., 2009; Ramirez et al., 2009; Banerjee et al., 2010; Northrop and Yamamoto, 2012; Toborek et al., 2013; Martin et al., 2013; Coelho-Santos et al., 2015). NADPH oxidase 2 (NOX-2) is an important enzyme responsible for producing ROS, and it has been closely associated with the inflammatory responses in various cell types including brain endothelium before impairing the BBB integrity (Park et al., 2012; Choung-Nguyen et al., 2015).

The BBB integrity is composed of many adhesive molecules, such as zonula occludin (ZO)-1, occludin, claudin-5 protein etc., which form strong tight junction causing very high transendothelial electric resistance (TEER), and low paracellular permeability (Liu et al., 2012). Previous studies have demonstrated that exposure to METH induces BBB impairment by reducing the expression of adhesive molecules, which then leads to an increase in the paracellular permeability and a reduction in the TEER values both in vivo and in vitro, finally inducing apoptosis which is the common cause of progressive BBB impairment (Mahajan et al.,

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2008; Kiyatkin and Sharma, 2009; Abdul-Muneer et al., 2011; Ma et al., 2014; Parik et al., 2015). In addition, METH causes cell death in various cell types including brain endothelium by inducing the Bcl-2 protein imbalance and caspase-3 activation (Cadet et al., 2005; Ramirez et al., 2009; Jumnongprakhon et al., 2014; Yu et al., 2015). Additionally, the inhibition of the caspase cascade caused by METH could promote cell survival and increase the BBB integrity in brain endothelium (Ma et al., 2014).

Melatonin (*N*-acetyl-5-methoxytryptamine) is mostly secreted by the pineal gland and has been reported to have pharmacological potential as a protective agent in neuron, glial cells, and BBB (Shaikh et al.,1997; Chen et al., 2006; Acuna-Castroviejo et al., 2014; Garcia et al., 2014; Manchester et al., 2015). Previous studies have demonstrated that melatonin protects the central nervous system (CNS) after exposure to METH, both in vivo and in vitro, by reducing both inflammation and death-signaling responses but not found in BBB (Kaewsuk et al., 2009; Permpoonputtana and Govitrapong, 2013; Jumnongprakhon et al., 2014; Hutchinson et al., 2014; Parameyong et al., 2015; Jumnongprakhon et al., 2015). Considering the beneficial effects of melatonin on the CNS, we propose that melatonin may protect the BBB against METH toxicity.

Primary rat brain microvascular endothelial cells (BMVECs) were used as the BBB model to investigate whether melatonin could protect METH-induced BBB impairment. We then investigated the role of melatonin on METH-induced oxidative and nitrative stress, apoptosis, and impairment of BBB integrity. We also investigated the effect of melatonin on METH-toxicity as to whether it is dependent on its receptor-mediated signaling for ameliorating these negative effects of METH.

2. Results

2.1. Melatonin improved cell viability in METH treatment

To determine the toxicity of METH on BMVECs, various concentrations of METH (10, 20, 50, 100, 200, and 500 μM) were treated to the cultured BMVECs for 24 h. After treating with METH, the cell viability was determined by the MTT assay. These data showed that there were differences induced by METH [($F=188.07$, df (7,16), $p<0.0001$)] such that 100 μM METH significantly induced cell death of approximately 50% cells ($p<0.001$) compared to the control group (Fig. 1A). In accordance with these results, METH at this concentration was used in the subsequent studies. To investigate the protective effect of melatonin on METH-induced toxicity in BMVECs, various concentrations of melatonin (0.1, 1, 10, and 100 nM) were pretreated for 2 h prior to treatment with 100 μM METH for 24 h. The results showed that melatonin at concentrations of 1, 10, and 100 nM significantly increased cell viability in a dose-dependent manner to $72.0\pm1.7\%$, $86.8\pm5.2\%$, and $95.9\pm5.7\%$, respectively [($F=57.83$, df (5,12), $p<0.0001$)] (Fig. 1B).

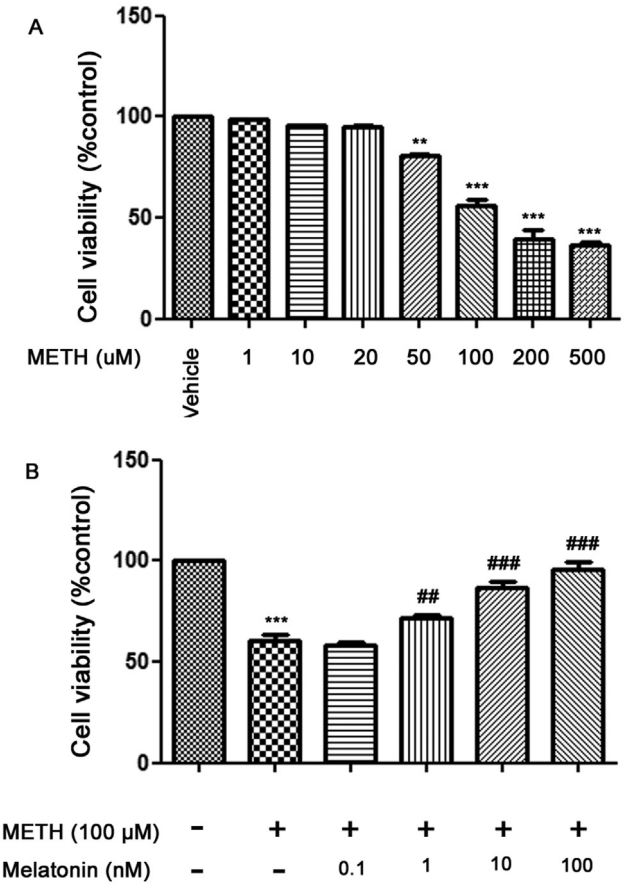

Fig. 1. - The effect of melatonin on cell viability in the presence of METH in BMVECs. (A) The cells were treated with METH in various concentrations (1, 10, 20, 50, 100, 200, and 500 μM) for 24 h. The toxicity of METH on the BMVECs was evaluated as regards the cell viability by the MTT assay. (B) The cells were pretreated with various concentrations of melatonin (0.1, 1, 10, and 100 nM) for 2 h prior to the treatment with METH (100 μM) for 24 h. $^{**}P<0.01$, and $^{***}P<0.001$ vs. control group. $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ vs. only METH treatment group. $n=3$.

2.2. Melatonin protects against METH-induced oxidative and nitrative stress via NOX2

Previous studies have demonstrated that the BBB impairment that is caused by METH is closely implicated with excessive ROS and RNS formation involved with NADPH oxidase activation. To elucidate the role of melatonin on METH-induced oxidative stress mediated by NOX-2, a well-known NOX-2 inhibitor, apocynin, was used in this study. As shown in Fig. 2, the only- METH (100 μM) treatment resulted in significant increase in both ROS ($788.3\pm10.6\%$) [($F=226.68$, df (8, 18), $p<0.0001$)] and RNS ($396.6\pm26.1\%$) [($F=132.7$, df (8,18), $p<0.0001$)], which could be markedly inhibited by melatonin in a concentration-dependent manner. A similar result was observed in apocynin-treated cells. Moreover, co-treatment with apocynin and melatonin was also found to inhibit ROS production induced by METH in the same range as observed in apocynin-treated cells ($p<0.001$). These results clearly indicate that melatonin inhibits METH-induced ROS and RNS production through the inhibition of NOX-2 activation.

2.3. Melatonin reduces METH-impaired tight junction via NOX2

Next, we investigated whether melatonin could protect METH-induced BBB disruption mediated by NOX-2 activation. First, the direct effect of METH on the integrity of the endothelial monolayers was analyzed by TEER measurements and paracellular permeability by determining the flux of fluorescein across brain endothelial layers. Upon using 100 μM METH, it was observed that the TEER value had significantly reduced ($100\pm5.2\ \Omega\ cm^2$) [($F=390$, df (8,18), $p<0.0001$)] and significantly increased of paracellular permeability ($1100.6\pm15\%$) [($F=106.83$, df (8, 18), $p<0.0001$)] compared with untreated control ($p<0.001$). Melatonin significantly increased the TEER values ($101.6\pm4\%$, $150.6\pm5.6\%$, $191.3\pm4.7\%$, respectively) (Fig. 3A) and decreased the paracellular permeability ($1063.3\pm55\%$, 780 ± 26.4, $466\pm21.6\%$, respectively) (Fig. 3B), compared to the only-METH treatment in a dose-dependent manner. Additionally, the expressions of tight junction proteins ZO-1 [($F=152.24$, df (5,12),