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Research report

Pharmacological activation of the neurotensin receptor 1 abrogates the methamphetamine-induced striatal apoptosis in the mouse brain

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ABSTRACT

Methamphetamine (METH) is a widely abused psychostimulant displaying potent addictive and neurotoxic properties. METH induces neurotoxicity of dopaminergic terminals and striatal neurons in the striatum. Despite much information on neurotransmitters, the role of neuropeptides is poorly understood. In this study, we investigated the role of the neuropeptide neurotensin on the METH-induced apoptosis of some striatal neurons in mice. We observed that a single injection of METH (30 mg/kg, ip) induced the loss of approximately 15% of striatal neurons. An agonist of the neurotensin receptor 1 (PD149163, ip at various doses) attenuated the METH-induced striatal neuron apoptosis. Utilizing quantitative real time PCR, we showed that METH also up-regulated neurotensin gene expression with 96% increase in preproneurotensin mRNA levels in the striatum as compared to the control. Additionally, NTR1 agonist (ip injection) attenuated hyperthermia at 2 h post-METH injection; hyperthermia is a putative and significant component of METH-induced neurotoxicity. To investigate the role of neurotensin without affecting core body temperature, we performed stereotactic injection of PD149163 into the striatum and observed that this compound maintained attenuated the METH-induced apoptosis in the striatum, while leaving core body temperature unaffected. There was no effect of NTR1 agonist on METH-induced dopamine terminal degeneration, as evidenced by tyrosine hydroxylase levels determined by Western blot. These data indicate that the neuropeptide neurotensin modulates the striatal neuronal apoptosis induced by METH through diverse mechanisms that need to be investigated. Furthermore, due to its neuroprotective properties, neurotensin receptor agonists show potential as drug candidates for the treatment of METH abuse and some neurological disorders.

1. Introduction

Methamphetamine (METH) is a widely abused psychostimulant only second to cannabis in popularity (United Nations Office on Drugs and Crime, 2007). It poses major public health challenges and imposes high costs on nations combating this epidemic in the form of crime prevention and addiction recovery (United Nations Office on Drugs and Crime, 2013). Both chronic and acute treatment with METH induces neurotoxicity in several brain regions, including the striatum (Zhu et al., 2005; Bowyer et al., 2008; Fantegrossi et al., 2008; Deng et al., 2007; Sekine et al., 2008). Because METH is similar in chemical structure to the neurotransmitter dopamine, METH enters neurons through dopamine transporters evoking the leakage of vesicular dopamine ultimately causing the accumulation of excessively high levels of reactive oxygen and nitrogen species (Block, 2007; Perry et al., 2007). The dopamine overflow induced by METH is of long duration due to the long half-life of METH (Schepers et al., 2003) and the prolonged *de novo* synthesis of dopamine in the striatal terminals in the presence of METH (Larsen et al., 2002). This protracted state of oxidative stress in the presence of METH induces the apoptosis of some striatal projection and interneurons (Zhu et al., 2006a). Several laboratories have investigated the METH-induced loss of neurons in the rodent brain. For example, METH induces the loss of neurons in the cortex (Deng et al., 2001), hippocampus (Deng et al., 2001), olfactory bulb (Zhu et al., 2006b) and spinal cord (Samantaray et al., 2006). METH also induces the neurotoxicity of the dopaminergic dopamine terminals of the striatum in non-human primates (Weihmuller et al., 1992). Importantly, recent

Abbreviations: ip, intraperitoneal; METH, methamphetamine; NPY, neuropeptide Y; NTR1, neurotensin receptor 1; PBS, phosphate-buffered saline, pH 7.4; PD149163, N2-[(2S)-2,6-Diaminohexyl]-L-lysyl-L-prolyl-L-tryptophyl-3-methyl-L-valyl-L-Leucine ethyl ester tetrahydrochloride hydrate; SOM, somatostatin.

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studies utilizing imaging methodologies demonstrate the METH-induced toxicity of striatal dopamine terminals and the loss of cells in the human brain (Yuana et al., 2014; Chang et al., 2007). In the light of the above, it is indubitable that METH is highly damaging to nervous tissue and that its consumption poses a serious threat to human health.

Although it is well known that the excessively high levels of dopamine signaling and metabolism mediate much of METH's neuronal damage in the striatum, no pharmaceutical treatment has yet been approved for the treatment of METH-induced neurodegeneration. Thus, it is essential to study endogenous factors regulating the dopaminergic system in order to identify potential drug candidates for treatment. Neurotensin, a neuropeptide produced by striatal projection neurons that also synthesize enkephalin (Sugimoto and Mizuno, 1986), has emerged as a potential modulator of METH-induced striatal neurodegeneration mainly due to its intimate interactions with dopamine in the striatum and the substantia nigra of the midbrain (Quirion, 1983; Binder et al., 2001). For example, site-specific microinjection of neurotensin into the ventral tegmental area produces psychostimulant-like effects (Kalivas et al., 1983). However, neurotensin administration into the nucleus accumbens attenuates amphetamine-stimulated locomotor activity (Vadnie et al., 2014). These different effects most likely reflect differences in the neurons influenced by neurotensin signaling in these brain regions. METH increases neurotensin expression in striatal D1-positive neurons (Castel et al., 1996) and elevates extracellular neurotensin levels in the striatum through a mechanism involving both D1, D2 and NMDA receptor signaling (Wagstaff et al.1996; Singh et al. 1990), suggesting that neurotensin is released from D1-positive neurons. Moreover, low doses of METH reduced neurotensin-like immunoreactivity in the nucleus accumbens consistent with the hypothesis that METH induces the release and utilization of neurotensin (Alburges et al., 2014). Alternatively, neurotensin may be released from D2-positive neurons because neurotensin and enkephalin are co-localized in the striatum of the cat (Sugimoto and Mizuno, 1986). The neurotensin receptor 1 (NTR1) is the primary neurotensin receptor associated with dopamine neurons and also the only neurotensin receptor found on the membrane of striatal neurons (Brouard et al., 1992; Schotte et al., 1988). Therefore, most research on neurotensin has focused on NTR1 activity in the striatum. For example, systemic administration of the NTR1 agonist PD149163 or the neurotensin analog NT69L attenuated amphetamine-induced hyperactivity or METH self-administration in rodents (Hertel et al., 2001; Hanson et al., 2013). In addition to regulating dopamine neurotransmission, neurotensin also reduces body temperature via NTR1 (Wei et al., 2013). Elevation of body temperature induced by METH is a significant component of the METH-induced striatal dopamine terminal neurotoxicity (Riddle et al., 2006), as evidenced by the high correlation between the degree of changes in the dopamine system, or the degree of neurodegeneration, and the degree of hyperthermia (Davidson et al., 2001). However, despite these observations, there has been no direct evidence implicating neurotensin in the attenuation of the METH-induced striatal neurodegeneration. In the light of the above, we investigated the role of the neuropeptide neurotensin on the METH-induced striatal neurodegeneration.

2. Results

2.1. Effect of NTR1 agonist on METH-induced striatal apoptosis

To assess apoptosis in coronal sections of striatal tissue, we employed Terminal deoxynucleotidyl transferase dUTP nick end labeling (referred to as TUNEL) to visualize DNA fragmentation, which is a late-stage biomarker of apoptosis (Zhu et al., 2006). To determine the fraction of striatal neurons undergoing apoptosis, we labeled all striatal neurons with NeuN, a neuronal nuclear protein, using immunohistochemistry in all 52 mice. We observed METH-induced TUNEL staining in 15.24 ± 7% of striatal neurons, this value is significantly higher than 0.31% TUNEL-positive neurons in the striata of control animals (Fig. 1). It is notable that in mice pre-treated with 2 mg/kg of the NTR1 agonist PD149163 prior to METH (30 mg/kg), TUNEL staining was abrogated. One-way ANOVA reveals that these differences between different groups were significant [$F = 4.137$, $P = 0.0052$, $DF = 38$]. Tukey's HSD reveals significant differences in apoptotic neuron number after neurotensin agonist (2 mg/kg) treatment in the METH group. The NTR1 agonist failed to induce striatal apoptosis in the absence of METH (Fig. 1).

2.2. NTR1 agonist has no effect on METH-induced dopamine terminal degeneration

In addition to striatal neuron apoptosis, METH can also induce dopamine terminal toxicity. In 35 mice, we measured the level of tyrosine hydroxylase (TH), an enzyme that catalyzes the rate-limiting step of dopamine synthesis in dopamine terminals and serves as a reliable marker of dopamine terminal toxicity. We quantified the levels of TH in striatal homogenates by Western blot utilizing β-actin as the internal control and observed that METH (30 mg/kg) reduced TH levels by 42% compared to the control (Fig. 2). Injection of PD149163 (2 mg/kg, ip) prior to METH attenuated TH levels significantly with a change of 16% (Fig. 2). One-way ANOVA reveals these differences were significant [$F = 17.41$, $p < 0.0001$, $DF = 24$]. Tukey's HSD reveals no significant difference between METH and PD149163/METH groups. In addition, PD149163 alone did not alter TH levels significantly.

2.3. METH up-regulates preproneurotensin mRNA levels

Since delivery of the neurotensin receptor agonist PD149163 attenuated the apoptosis of striatal neurons, we decided to measure the levels of preproneurotensin mRNA in striatal tissue of 20 mice by Real-Time PCR six hours after a single injection of METH (30 mg/kg). The Taqman RT-PCR technique was employed to detect neurotensin mRNA levels within total RNA extracted from mouse striata. GAPDH was utilized as an endogenous control due to its ubiquitous and stable expression. We observed that striatal preproneurotensin mRNA levels reached 195 ± 30% relative to controls six hours after METH administration (Fig. 3).

2.4. The NTR1 agonist PD149163 attenuates the METH-induced hyperthermia

It has been shown that METH-induced hyperthermia plays a significant role in neurotoxicity (Albers and Sonsalla, 1995; Bowyer et al., 1994; Yuan et al., 2006; Farfel and Seiden, 1995; Miller and O'Callaghan, 1994). Due to the well-established role of hyperthermia in the METH-induced striatal neurodegeneration, we measured body core temperature of mice using a rodent probe at four time points from zero to six hours post-METH administration (30 mg/kg, ip). Previous studies in our laboratory showed that METH-induces hyperthermia at 2 h and subsides at 4 h post-injection (Zhu et al., 2005). In the present study with 35 mice, two-way ANOVA indicated that treatment and time have significant effects on temperature change [$F = 17.89$, $P < 0.0001$, $DF = 26$; $F = 17.15$, $P < 0.0001$, $DF = 26$, respectively]. In particular, we observed an increase of body temperature from 37.5 °C to 39.6 °C at two hours post-METH (Fig. 4). This increase is transient, as temperature drops back to 36.5 °C at four hours. Systemic