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The role of the neuropeptide somatostatin on methamphetamine and glutamate-induced neurotoxicity in the striatum of mice

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ABSTRACT

A large body of evidence shows that methamphetamine (METH) causes sustained damage to the brain in animal models and human METH users. In chronic users there are indications of cognitive and motor deficits. Striatal neuropeptides are in a position to modulate the neurochemical effects of METH and consequently striatal neural damage. Somatostatin (SST) is an intrinsic striatal neuropeptide that has been shown to inhibit glutamate transmission; glutamate is integral to METH toxicity and contributes to nitric oxide (NO) synthesis. We hypothesize that SST will protect from METH by inhibition of NO synthesis and thus reducing oxidative stress. To this end, the SST analogue octreotide (OCT) was microinjected into the striatum prior to a systemic injection of METH (30 mg/kg). We then assessed 3-nitrotyrosine (3-NT), an indirect index of NO production, tyrosine hydroxylase (TH) protein levels (dopamine terminal marker) and Fluoro-Jade C positive cells (degenerating cells). The SST agonist OCT dose dependently attenuated the METH-induced accumulation of striatal 3-NT. Moreover, pretreatment with OCT effectively mitigated cell death but failed to protect dopamine terminals. Next we co-infused OCT and NMDA and measured 3-NT and Fluoro-Jade C staining. Treatment with OCT had no effect on these parameters. The data demonstrate that SST attenuates the METH-induced production of NO protecting the striatum from the METH-induced cell loss. However, SST failed to prevent the toxicity of the dopamine terminals suggesting that pre- and post-synaptic striatal damage occur via independent mechanisms.

1. Introduction

The illicit psychostimulant methamphetamine (METH) is a longer lasting and more potent derivative of the stimulant amphetamine. METH exerts its effects on the brain through its influence on the monoaminergic system. Its efficacy is governed by the resemblance of its chemical structure to the neurotransmitter dopamine allowing it to enter the dopamine terminal, cause the excessive release of dopamine, and prevent its reuptake (Sulzer et al., 1995; Jones et al., 1998; Krasnova and Cadet, 2009; Logan, 2002; Sulzer,

Abbreviations: 3-NT, 3-nitrotyrosine; ICR, Institute for Cancer Research; ip, intraperitoneal; METH, (+)-methamphetamine hydrochloride; NO, nitric oxide; NOS, nitric oxide synthase; OCT, octreotide; SST, somatostatin

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2011). Exposure to METH results in a plethora of neurochemical dysfunctions including but not limited to the prolonged decrease in the enzyme tyrosine hydroxylase (Hotchkiss and Gibb, 1980), tissue dopamine and metabolite levels (Fumagalli et al., 1998; Villemagne et al., 1998), reduction in dopamine transporters (Baucum et al., 2004) and cell death in some regions of the brain including the striatum and the cortex (O'Dell et al., 1992; Deng et al., 2001; Eisch and Marshall, 1998; Pu et al., 1996; Zhu et al., 2005). The METH-induced dopamine overflow may be the triggering event but it is not the sole causative factor of METH toxicity, compelling evidence implicates glutamate transmission in METH neurotoxicity (Sonsalla et al., 1989; Stephans and Yamamoto, 1994). For example, pharmacological inhibition of the NMDA receptor diminishes the damage caused by METH (O'Dell et al., 1992; Riddle et al., 2006; Sonsalla et al., 1991). It is postulated that the generation of reactive oxygen species from NMDA-mediated excitotoxicity and oxidation of excessive cytoplasmic dopamine may serve as the mediator of damage in METH neurotoxicity through oxidative stress (Yamamoto and Zhu, 1998; Thomas et al., 2008). Several experiments have shown that free radical scavengers prior to METH attenuate METH neurotoxicity (Kawasaki et al., 2006; Yamamoto and Zhu, 1998).

Excessive activation of the NMDA receptor has been linked to activation of nitric oxide (NO) synthesis (Dawson and Dawson, 1996). During pathophysiological conditions excessive NO synthesis can result in the production of peroxynitrite, which can serve as both a reactive nitrogen and oxygen species (Beckman, 1996; Boje, 2004; Bruckdorfer, 2005). NO is synthesized by three different isoforms of the enzyme nitric oxide synthase (NOS), neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) (Boje, 2004; Bruckdorfer, 2005). nNOS is considered the primary source of NO in the toxic cascade set-off by METH since several studies have shown that nNOS expression can be dynamically regulated by toxic insults including METH (Dawson et al., 1998; Deng and Cadet, 1999; Desaiah et al., 2000). Also, treatment with METH elevates the expression of nNOS (Dawson et al., 1998; Deng and Cadet, 1999; Desaiah et al., 2000), which contributes to METH toxicity since pharmacological and genetic inhibition of nNOS attenuated striatal dopamine terminal toxicity (Desaiah et al., 2000; Itzhak and Ali, 1996; Itzhak et al., 2000).

Previous work in our lab provides experimental evidence that the striatal neuropeptide substance P participates in the METH-induced striatal injury. Histological observation showed that exposure to METH resulted in a robust internalization of the neurokinin-1 receptor in the nNOS-expressing interneuron (Wang and Angulo, 2011b; Wang et al., 2008) and infusion of a substance P agonist by itself into the striatum resulted in increased 3-nitrotyrosine (3-NT) immunoreactivity, an indirect index of NO production (Wang and Angulo, 2011a; Ayata et al., 1997; Schulz et al., 1995). Moreover, suppression of substance P signaling reduced METH-induced NO synthesis and afforded protection from METH-induced striatal injury (Wang et al., 2008; Yu et al., 2004; Zhu et al., 2006). Recently, our group demonstrated utilizing selective agonists and antagonists of the neuropeptide Y Y1 and Y2 receptors that this neuropeptide modulates the METH-induced striatal production of NO (Yarosh and Angulo, 2012). In addition to substance P and neuropeptide Y, somatostatin (SST) is an intrinsic striatal neuropeptide well placed to modulate NO production in the presence of METH. In the striatum, SST is synthesized and stored in the SST/NPY/nNOS interneuron (Kawaguchi et al., 1995). SST is a neuroprotectant in pathologies attributed to glutamate-induced excitotoxicity (Cervia et al., 2008; Forloni et al., 1997). For example, following middle cerebral artery occlusion, infusion of SST or an agonist, reduced infarct volume (Rauca et al., 1999).

SST appears to exert an inhibitory influence on glutamatergic release and transmission in addition to intracellular calcium influx, which have been attributed as the means by which it protects from excitotoxic insults (Forloni et al., 1997). SST's influence on striatal signaling and growing evidence of neuroprotection in several excitotoxic models makes it a compelling candidate of further investigation in METH toxicity. It was the aim of the present study to test the hypothesis that SST is neuroprotective during METH toxicity. Moreover, we aimed to test that the influence of SST on NO synthesis and its possible neuroprotection is attributable to its inhibition on glutamate transmission, particularly via the NMDA subtype receptor.

2. Results

2.1. SST attenuates the METH-induced NO production and cell loss but not terminal degeneration.

Animals received a 1 μl (rate of 0.1 μl per minute) intrastriatal infusion of the SST analogue OCT (0.1, 1.0 and 10 nM) in one hemisphere and aCSF in the other hemisphere. Fifteen minutes after the surgery both the control and experimental group were injected IP, the control group (aCSF) received saline and the experimental condition METH (30 mg/kg). We measured 3-NT immunoreactivity in striatal tissue sections by confocal microscopy. The SST agonist OCT dose dependently attenuated the METH-induced production of 3-NT (Fig. 1). Based on these results the 10 nM dosage was then chosen for the subsequent series of experiments (METH and NMDA).

Degeneration of neurons native to the striatum was measured 24 h post-METH by staining with Fluoro-Jade C and utilizing stereological cell counts. Dopamine terminal damage was determined by quantifying striatal TH protein levels 72 h after METH by Western blot analysis. TH is the rate-limiting enzyme necessary for the production of catecholamines such as dopamine (Fibiger and McGeer, 1971). The presence of TH in the striatum is used as an indicator of DA terminal viability. As seen in Fig. 2, pretreatment with OCT showed a significant protection that almost reached control baseline levels. The agonist by itself showed no effect whereas METH as expected had a substantial and significant increase in cell loss (Fig. 2). Alternatively, animals pretreated with OCT did not demonstrate protection of dopamine terminals (Fig. 3), TH levels remained almost equivalent to METH levels. Treatment with METH showed the expected and significant reduction in TH levels and the OCT alone group's TH levels were comparable to baseline levels (Fig. 3).

2.2. SST has no influence on NMDA-induced NO synthesis or cell death

To investigate the possible connection between SST and striatal glutamate transmission, OCT was administered as described above with the exception that in the experimental condition, NMDA (20 nM) was dissolved in aCSF and infused into one