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Research Report

Differential effects of methamphetamine and SCH23390 on the expression of members of IEG families of transcription factors in the rat striatum

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ABSTRACT

Methamphetamine (METH) is a psychostimulant that can cause long-lasting neurodegenerative effects in humans and animals. These toxic effects appear to occur, in part, via activation of dopamine (DA) D1 receptors. This paper assessed the possibility that the DA D1 receptor antagonist, SCH23390, might inhibit METH-induced changes in the expression of several members of immediate early genes (IEGs) which are known to control more delayed expression of other genes. We found that injections of METH (4×10 mg/kg, given at 2 h intervals) caused significant increases in c-fos and fra-2 expression which lasted from 30 min to 4 h. Pre-treatment with SCH23390, given 30 min before each METH injection, completely blocked METH-induced expression of c-fos, but only partially inhibited fra-2 mRNA expression. These results were confirmed by Western blot analysis which showed METH-induced changes in c-Fos protein expression that were blocked by pretreatment with SCH23390. There were also delayed METH-induced DA D1 receptor-dependent effects on fosB mRNA expression. Even though fra-1 expression was not affected by pretreatment with METH alone, the repeated injections of SCH23390 caused substantial decreases in fra-1 mRNA expression in both the presence and absence of METH. The repeated injections of METH caused no changes in the mRNAs for c-jun, junB or junD. However, there were significant increases in the phosphorylation of c-Jun protein (ser63). Phosphorylation of c-Jun occurred in a delayed fashion (16 and 24 h after the last METH injections) and was attenuated by SCH23390 pretreatment. Interestingly, SCH23390 given alone caused significant decreases in phospho-c-Jun at all time-points. The METH injections also caused delayed induction in the expression of members of the Egr family of transcription factors in a DA D1 receptor-dependent fashion. Repeated injections of SCH23390 caused substantial suppression of basal striatal egr-1 and egr-2 mRNA expression but not of that of egr-3. Both crem and arc mRNA levels were induced by METH in a SCH23390-sensitive fashion. Moreover, multiple injections of SCH23390 given alone caused marked inhibition of basal arc expression. These results show that multiple injections of METH can differentially affect the expression of several IEGs, some of which occurred in a DA D1

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receptor dependent fashion. The SCH23390-mediated suppression of basal fra-1, egr-1, and egr-2 mRNA levels suggests that their basal expression in the striatum might be dependent on tonic stimulation of the DA D1 receptor.

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1. Introduction

Methamphetamine (METH) is an addictive drug that causes long-term motor and cognitive abnormalities (Volkow et al., 2001; Gold et al., 2009). Imaging studies using positron emission tomography and post-mortem studies of the brains of METH-abusers have provided evidence for loss of striatal dopamine (DA) nerve terminals (Wilson et al., 1996; Volkow et al., 2001). Studies in rodents have shown that administration of toxic doses of METH, either as single or multiple injections, results in long-term changes in the brain (Krasnova and Cadet, 2009). These abnormalities include decreases in the levels of striatal DA and serotonin (5-HT) and of their metabolites as well as decreases in the activity of their biosynthetic enzymes (Kita et al., 2003). The use of toxic doses of METH can also cause neuronal apoptosis in the striata and cortices of rodents (Deng et al., 2002; Jayanthi et al., 2005; Thiriet et al., 2005; Cadet et al., 2005).

The pharmacological and neurotoxic effects of METH depend on the release of DA from striatal DA terminals and stimulation of DA receptors in the striatum which contains high densities of DA D1 and D2 receptors (Russell et al., 1992; O'Dell et al., 1991, 1993). The toxic effects of the drug depend, in part, on activation of D1 receptors because inhibition of DA D1 receptors protects against METH-induced toxicity in the striatum (Jayanthi et al., 2005; Xu et al., 2005). These protective effects of DA D1 antagonism might occur through inhibition of DA D1 receptor-mediated induction of genes that might be involved in pro-toxic cascades (Cadet et al., 2005; Jayanthi et al., 2005, 2009). To test the idea, we measured the effects of multiple METH injections on the expression of immediate early genes (IEGs), including several members of AP-1 and Egr families of transcription factors, in the absence and presence of the DA D1 receptor antagonist, SCH23390 (Iorio et al., 1983). We also measured the effects of SCH23390 on METH-induced changes in arc and crem mRNA levels because Arc is an effector gene involved in synaptic plasticity (Bramham et al., 2008) and because Crem participates in the regulation of neuroadaptive processes (Hughes and Dragunow, 1995). Our experiments show that multiple injections of METH doses which are known to cause long-term DA depletion and neuronal apoptosis (Deng et al., 1999; Ladenheim et al., 2000; Jayanthi et al., 2005) are associated with marked increases in the expression of several IEGs in a SCH23390-sensitive fashion.

2. Results

2.1. *Multiple injections of METH caused differential changes in the expression of fos and jun families of IEGs*

Fig. 1 shows the effects of METH and SCH23390 on members of the fos family of transcription factors. Repeated injections of SCH23390 alone caused no changes in c-fos expression (Fig. 1A). METH injections caused rapid and substantial increases in c-fos expression which were apparent at 30 min and lasted for the 4 h duration of the study. Injections of saline before each of the four METH injections gave identical results to the injections of METH alone (data not shown). Injections of SCH23390 before each METH administration caused total inhibition of METH-induced c-fos expression (Fig. 1A). mRNA levels were measured according to a standard curve for each gene. We used six replicates for each reaction. These reactions yielded a standard curve with a slope of –3.3 and the efficiency value was ≈2. The amplification curve for a replicate of c-fos mRNA is shown in Fig. 1B. Similar curves were generated for each gene at all time points and are available on request. The protein level of c-Fos was also assessed and showed also increased at 30 min, 2 h and 4 h (Fig. 2). In contrast to the rapid METH-induced changes in c-fos expression, METH caused somewhat more delayed increases in fosB expression occurring at the 4 h time point (Fig. 1C). Pretreatment of the animals with SCH23390 also blocked the METH effects of fosB expression (Fig. 1C). Unexpectedly, repeated injections of METH caused no changes in fra-1 expression in the rat striatum (Fig. 1D). In contrast, repeated injections of SCH23390 caused significant decreases in fra-1 expression at the 4 h time point in both the absence and presence of METH, suggesting that the latter effects were due solely to DA D1 receptor antagonism. Fig. 1D shows that the METH-induced effects on fra-2 expression are somewhat similar to those observed for c-fos expression. Specifically, fra-2 expression was rapidly induced by METH, peaked at 2 h then started to revert towards normal by the 4 h time point (Fig. 1E). Interestingly, SCH23390 pretreatment blocked the effects of METH on fra-2 expression only at the 30-min time point, suggesting that METH might cause the more delayed increases via mechanisms other than stimulation of DA D1 receptors.

The repeated injections of METH caused no significant changes in the expression of c-jun (Fig. 3A), junB (Fig. 3B) and junD (Fig. 3C). Repeated injections of saline before each METH injection did not affect these results (not shown). Injections of SCH23390 alone caused no significant decreases in basal expression of these genes when compared to controls. As previously reported, c-Jun protein exerts its action via phosphorylation at serine-63 or -73 through the actions of c-Jun N-terminal kinases (JNKs) (Raivich, 2008), a process that seems to be involved in METH-induced toxicity since a single large toxic dose of METH can cause increased c-Jun phosphorylation at ser-63 (Jayanthi et al., 2002). Similarly, repeated injections of METH used in the present study also caused c-Jun phosphorylation at 16- and 24-h after the last METH injection (Fig. 4). In contrast, SCH23390 caused significant decreases in the basal levels of phosphorylated c-Jun. These changes are not due to increased c-Jun protein expression since, similar to the PCR results, METH caused no changes in total c-Jun protein expression.