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Research report

Opposite effects of acute and chronic amphetamine on Nurr1 and NF-κB p65 in the rat ventral tegmental area

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ABSTRACT

Dopamine neurons are overstimulated by drugs of abuse and suffer molecular alterations that lead to addiction behavior. Nurr1 is a transcription factor crucial for dopamine neurons survival and dopamine production, activating the transcription of key genes like tyrosine hydroxylase (TH). Interestingly, nuclear factor-kappa B (NF-κB) has emerged as a new Nurr1 partner in response to inflammatory stimulus. In this study we evaluated the effects of single and repeated amphetamine administration in the expression of Nurr1 and the NF-κB p65 subunit in the rat ventral tegmental area (VTA). We found that acute amphetamine treatment increased Nurr1, p65 and TH protein levels in the VTA. On the other hand, chronic amphetamine treatment decreased Nurr1 and p65 protein levels, but TH was unchanged. Mammalian reporter assays in cell lines showed that p65 represses Nurr1 transcriptional activity in an artificial promoter driven by Nurr1 response elements and in the native rat TH promoter. These results indicate that Nurr1 and NF-κB p65 factors are involved in the adaptive response of dopamine neurons to psychostimulants and that both transcription factors could be regulating Nurr1-dependent transactivation in the VTA.

1. Introduction

Drug addiction is one of the most serious public health problems that remain unresolved (Koob et al., 2010). There are different drugs of abuse, but all have a common target pathway: the mesocorticolimbic dopamine system, whose neurons arises in the ventral tegmental area (VTA) and project to the nucleus accumbens (NAc) and the prefrontal cortex (PFC) (Di Chiara and Imperato, 1988; Pierce and Kumaresan, 2006; Lüscher and Malenka, 2011). The psychostimulants cocaine and amphetamine raise dopamine extracellular levels with the dopamine transporter (DAT) as the molecular target. Cocaine acts as a competitive inhibitor of DAT, displacing dopamine while amphetamine operates as substrate, reverting DAT flow (Sulzer, 2011).

The nuclear receptor Nurr1 is crucial for the origin and survival of midbrain dopaminergic neurons and its expression is required from embryo to adult stages (Zetterström et al., 1997; Saucedo-Cárdenas et al., 1998; Perlmann and Wallén-Mackenzie, 2004; Kadkhodaei et al., 2009). This transcription factor is an immediate early gene that regulates the expression of key dopaminergic genes like tyrosine hydroxylase (TH) (Sakurada et al., 1999; Kim et al., 2003) and DAT (Sacchetti et al., 2001).

Nurr1 is broadly expressed in TH-positive dopaminergic neurons in the midbrain (Bäckman et al., 1999). Bannon et al. (2002) demonstrated that Nurr1 mRNA levels are decreased in dopaminergic neurons of human cocaine abusers. Later, they also showed that cocaine abusers have lower levels of mRNA for Pitx3, another transcription factor associated with Nurr1 in dopaminergic specification (Bannon et al., 2004). Heroin, another highly addictive drug, also decreases Nurr1 level in the midbrain of human abusers (Horvath et al., 2007). The reduction in Nurr1 levels after repeated drug consumption has also been observed in animal models. Chronic cocaine (Leo et al., 2007) and methamphetamine (Krasnova et al., 2011) administration reduces Nurr1 levels in rat midbrain. On the other side, mice with reduced Nurr1 levels exhibit a different behavior in response to drugs of abuse. Mice with a heterozygous deletion of Nurr1 has decreased ethanol preference (Werme et al., 2003). In addition, Nurr1 heterozygous mice show increased vulnerability to neurodegeneration after long-term methamphetamine exposure (Luo et al., 2010) and altered dopamine neurotransmission after combined exposure to amphetamine and social isolation (Moore et al., 2008), indicating that Nurr1 levels in dopamine neurons may protect against the harmful effects of drugs of abuse.

Interestingly, Saijo et al. (2009) reported that Nurr1 interacts with the nuclear factor-kappa B (NF-κB) in microglia and astrocytes in response to lipopolysaccharides. NF-κB is widely known for its role in inflammation and immune responses, but in the nervous system it has

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roles in neuroprotection, learning and memory. NF-κB has been associated with neuronal survival and plasticity (Mattson, 2005; Nestler, 2012). Drugs of abuse with strong inflammatory effects as methamphetamine increase NF-κB activity in mice striatum (Asanuma and Cadet, 1998). Meanwhile, repeated cocaine administration raises NF-κB protein levels in the rat NAc (Ang et al., 2001). Imam et al. (2005) describe an increase in NF-κB protein levels in a dose-dependent manner in PC12 cells. Russo et al. (2009) found that NF-kB signaling pathway is activated in mouse NAc in response to chronic cocaine administration, controlling neuron morphology and cocaine reward.

It has remained unknown whether Nurr1 and NF-κB functionally interact in midbrain dopamine neurons during adaptive response to addictive drugs. Our main goal is to find a relationship between Nurr1 and NF-kB in dopaminergic neurons of the VTA area during acute and chronic exposure to amphetamine. Here, we report that Nurr1 and the NF-κB subunit p65 are basally expressed in dopamine neurons of VTA in adult male rats. Acute amphetamine administration increased Nurr1, p65 and TH protein levels in the VTA meanwhile repeated amphetamine treatment decreased Nurr1 and p65 protein levels, leaving TH unchanged compared to saline controls. Mammalian reporter gene assays in PC12 cells showed that p65 represses Nurr1-induced transcription in the TH promoter. Our results suggest that Nurr1 and p65 could mediate a common adaptive pathway of dopamine neurons to psychostimulants.

2. Results

2.1. Nurr1 and p65 are expressed in dopaminergic neurons of rat VTA

In our interest to learn about Nurr1 and p65 localization in the rat

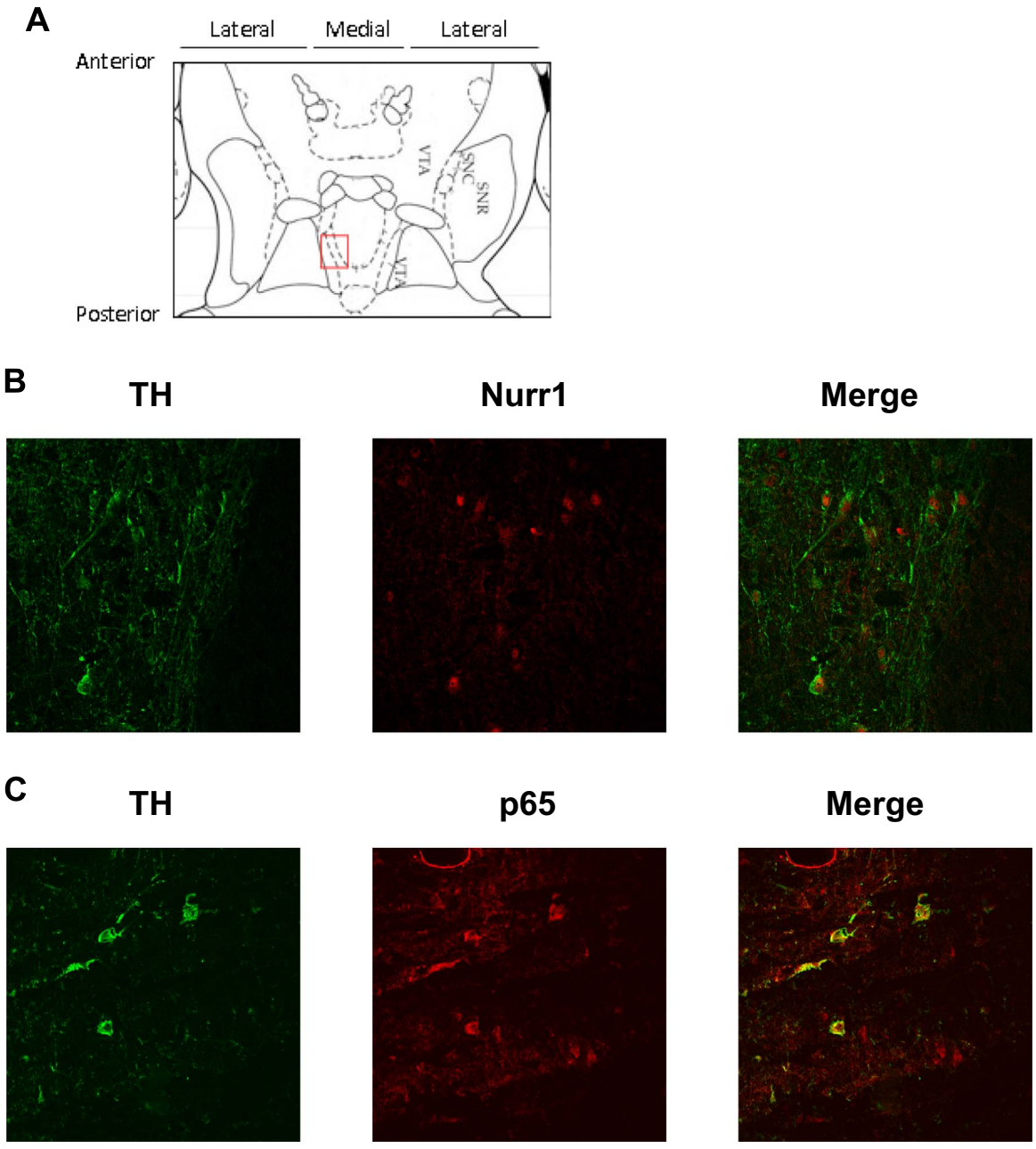

Fig. 1. Nurr1 and p65 are expressed in rat VTA TH+ cells. (**A**) Schematic diagram of VTA with the region showed in (**B**) and (**C**) (red box), adapted from the rat brain atlas (Paxinos and Watson, 2007). Adult rat brain horizontal slices were incubated with (**B**) mouse anti-TH and rabbit anti-Nurr1 or (**C**) mouse anti-TH and rabbit anti-p65 antibodies. Twenty four hours later, slices were incubated with fluorescent secondary antibodies. Labeling was visualized by indirect immunofluorescence. The images show the area in a 40× magnification.