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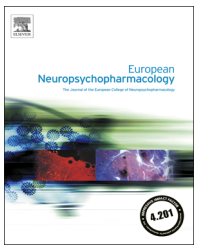

Identification of a developmentally-regulated and psychostimulant-inducible novel rat gene *mrt3* in the neocortex

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Abstract
The psychotomimetic effects of stimulant drugs including amphetamines and cocaine are known to change during the postnatal development in humans and experimental animals. To obtain an insight into the molecular basis of the onset of stimulant-induced psychosis, we have explored the gene transcripts that differentially respond to methamphetamine (MAP) in the developing rat brains using a differential cloning technique, the RNA arbitrarily-primed PCR. We identified from the rat neocortex a novel and developmentally regulated MAP-inducible gene *mrt3* (MAP responsive transcript 3) that is transcribed to a presumable non-coding RNA of 3.8 kb and is located on the reverse strand of the F-box/LRR-repeat protein 17-like gene mapped on the rat chromosome Xq12. The *mrt3* mRNAs are predominantly expressed in the brain and lung. Acute MAP injection upregulated the *mrt3* expression in the neocortex at postnatal day 50, but not days 8, 15 and 23, in a D1 receptor antagonist-sensitive manner. This upregulation was mimicked by another stimulant, cocaine, whereas pentobarbital and D1 antagonist failed to alter the *mrt3* expression. Moreover, repeated treatment with MAP for 5 days inhibited the ability of the challenge dose of MAP or cocaine to increase the neocortical *mrt3* expression without affecting the basal *mrt3* mRNA levels on day 14 of withdrawal. These late-developing, cocaine-cross reactive, D1 antagonist-sensitive and long-term regulations of *mrt3* by MAP are similar to those of stimulant-induced behavioral sensitization, a model of the onset and relapse of stimulant-induced psychosis and schizophrenia, and therefore may be associated with the pathophysiology of the model.

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1. Introduction

It has well been described that chronic or sustained use of psychostimulants, such as amphetamines, cocaine and methylphenidate, after adolescence causes a hallucinatory-paranoid state indistinguishable from that schizophrenia (Lieberman et al., 1987) and dependence on these drugs (Paus et al., 2008). These disturbances bring us not only pressing threats against metal health, but also excellent clues to clarify the pathophysiology of schizophrenia type psychosis and drug abuse. The drug-induced psychotic state has been known to be easily relapsed by the reuse of a stimulant in a small dose, which does not elicit psychotomimetic effects in the first experience, or by a non-specific stress even after a long symptom-free period following cessation of any kind of drug of abuse (Sato et al., 1983). The craving for drugs due to the dependence also appears to be progressively augmented during the drug abuse. Similar sensitization phenomenon is recognized in experimental animals as behavioral sensitization that the repeated treatment of the animals with a psychostimulant keeps them highly sensitive to the abnormal behavior eliciting effects of a psychostimulant or stressor even long after abstinence of the drug (Lieberman et al., 1987; Nishikawa et al., 1993; Sato et al., 1983). The behavioral sensitization has been considered to be a useful model for investigation of the neural mechanisms underlying schizophrenia-type psychosis as well as drug-dependence in order to develop their novel therapeutic and prophylactic methodologies, because a group of schizophrenic patients has been observed to be much more sensitive to the psychotomimetic effects of stimulants than normal volunteers (Lieberman et al., 1987).

To obtain an insight into the molecular basis of the behavioral sensitization, we focused our attention on its establishment after a specific postnatal period that is similar to the post-adolescent onset of drug-induced psychosis and dependence of schizophrenia (American Psychiatric Association, 2000). Thus, a robust and long-lasting sensitization is caused by the postnatal repeated administration of a psychostimulant only after a critical period around postnatal week 3 in the rodents. The prenatal repeated exposure to cocaine or methamphetamine (MAP) has also been reported to fail to alter the behavioral response to the first postnatal challenge dose of a stimulant in the adult period (Crozatier et al., 2003; Miller and Seidler 1994; Sato and Fujiwara, 1986), although some papers indicated that the *in utero* exposure enhanced the behavioral effects of a psyshostimulant (Glatt et al., 2000; Bubenikova-Valesova et al., 2009). Such a late-developing property suggests that the onset of these psychiatric disorders or behavioral sensitizations may require the maturation or critical change in a specific human information processing system in the brain or its rodent homolog. The hypothetical system should include the molecular cascade that responds differently to a stimulant before and after the critical period. In agreement with this view, our preliminary experiments (Nishikawa et al., 1993) have shown the distinct patterns of the brain activity as revealed by c-*fos* gene expression mapping after an acute injection of the stimulant, MAP, in the neocortex and striatum, but not in the piriform cortex, before the critical period (Nishikawa et al., 1993) as compared to the post-critical period (Nishikawa et al., 1993: Umino et al., 1995). Accordingly, we have further isolated from the neocortex a developmentally regulated MAP-responsive gene, *mrt1*, that has been pharmacologically shown to be related to the behavioral sensitization (Kajii et al., 2003). We now report another candidate as a member of the sensitization-associated molecular cascade, a novel transcript *mrt3* whose expression is unchanged and enhanced following an acute MAP injection before and after the critical period, respectively, in the rat neocortex.

2. Experimental procedures

2.1. Animals

The present animal experiments were performed in strict accordance with the guidance of the Tokyo Medical and Dental University, and were approved by the Animal Investigation Committee of the institute. Male Wistar rats (ST strain, Clea Japan, Tokyo, Japan) at postnatal days 8, 15, 23 and 50 were used. In our experiments, we used only male rats to study the effects of MAP because the onset of the menstrual cycle in females has been suggested to modify the behavioral and biochemical responses to a variety of drugs.

2.2. Chemicals

MAP hydrochloride, cocaine hydrochloride, MK801 hydrogen maleate (dizocilpine hydrogen maleate) ((5R,10S)-(+)-5-methyl-10,11-dihydro-5Hdibenzo (a,d) cyclohepten-5,10-imine) and SCH23390-hydrochloride were dissolved in physiological saline for subcutaneous (s. c.) or intraperitoneal (i.p.) injection. Nomifensine hydrochloride was kindly donated by Hoechst Pharmaceutical, Inc. (presently Sanofi-Aventis, Paris). An ampoule solution of pentobarbital sodium salt was diluted with physiological saline and injected i.p. The control animals received only the vehicle. All the chemicals used were of ultrapure quality and were commercially available except for nomifensine. Doses were always referred to the free bases. The use of MAP and cocaine was officially permitted by the Tokyo Metropolitan Bureau of Public Health.

2.3. Selection of the dose and time course for methamphetamine or cocaine injection and developmental time points of gene expression analyses

We selected the dose and time course for the acute MAP injection and the developmental time points of the gene expression analyses in terms of formation of the behavioral sensitization and stimulant-induced abnormal behavior as models of the psychotic symptoms or their onset or relapse (see Sato et al. (1983) for a review).

As described in our previous reports (Kajii et al., 2003; Murata et al., 2001), we injected 4.8 mg/kg of MAP and removed the brain regions 1 h later in the present experiments because 1) we clarified based upon the dose-related experiments that an acute injection of 4.8 mg/kg of MAP caused typical and robust stereotyped behavior, a useful model for psychotic symptoms, without ataxic movements around 1 h thereafter (Nishikawa et al., 1983), 2) behavioral sensitization occurs in the young adult rats repeatedly treated with the dose of MAP (Nishikawa et al., 1983, 1993), and 3) the purpose of this study is to obtain information about the initial or early step of the molecular cascade for behavioral sensitization by exploring hypothetical molecules that show a development-dependent response to MAP (Kajii et al., 2003). We chose the dose of 30 mg/kg for cocaine according to our previous studies (Fujiyama et al., 2003; Hiraoka et al., 2010; Kajii et al., 2003) as a moderate dose (Aliane et al., 2009; Reith, 1986; Tilley and Gu, 2008).

The temporal features of the postnatal development of behavioral sensitization induced by repeated administration of MAP have well been characterized in the rodents by a series of studies (Fujiwara et al., 1987; Kolta et al., 1990, Murata et al., 2001; Nishikawa et al.,