NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

C. D. Borkar et al. / Neuroscience xxx (2018) xxx–xxx

Cocaine- and Amphetamine-Regulated Transcript Peptide (CART) Alleviates MK-801-Induced Schizophrenic Dementia-Like Symptoms

Chandrashekhar D. Borkar,[a] Ashish P. Bharne,[a] B. Nagalakshmi,[b] Amul J. Sakharkar,[b] Nishikant K. Subhedar[c] and Dadasaheb M. Kokare[a]*

[a] *Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur 440 033, Maharashtra, India*

[b] *Department of Biotechnology, Savitribai Phule Pune University, Pune 411 007, Maharashtra, India*

[c] *Indian Institute of Science Education and Research, Dr Homi Bhabha Road, Pune 411 008, Maharashtra, India*

Abstract—Exaggerated thoughts, diminished mood and impaired cognition are the hallmarks of the schizophrenia-like condition. These symptoms are attributed to the dysregulation of dopamine and glutamate signaling in the brain. Since cocaine- and amphetamine-regulated transcript peptide (CART) modulates actions of dopamine as well as glutamate, we tested the role of this peptide in MK-801-induced schizophrenic dementia-like condition. MK-801-treated rats were allowed to interact with conspecific juvenile and tested for short-term (30-min) and long-term (24-h) social memory acquisition and recall. While MK-801 impaired the social interaction with a juvenile, the behavior was restored in CART (intracerebroventricular (icv) or intra-ventral tegmental area (VTA)) pre-treated animals. This action of CART was blocked by SCH23390 (dopamine D1 receptor antagonist) administered directly into the prefrontal cortex (PFC). Application of neuronal tracer Di-I in the PFC retrogradely labeled dopamine cells of the VTA, which in turn seem to receive CARTergic innervation. A significant increase in CART immunoreactivity was evidenced in the VTA, PFC and accumbens (Acbs) of the animals allowed to interact with a juvenile. However, MK-801 treatment attenuated the peptide expression and induced social memory deficits. The schizophrenic dementia-like symptoms following antagonism of glutamatergic receptors may be attributed to the reduced dopamine activity in the mesocortical system. We suggest that CART may, positively modulate the dopamine system to alleviate cognitive deficits associated with schizophrenia.

Key words: cocaine- and amphetamine-regulated transcript peptide, immunohistochemistry, MK-801, Schizophrenia, social recognition test.

INTRODUCTION

Schizophrenia is the most debilitating psychiatric disorder affecting more than 21 million people worldwide (World Health Organization, 2016). It is characterized by positive (hallucinations or delusions) and negative (flattening affect or social withdrawal) symptoms and cognitive deficits (impaired memory, attention and executive functions) (Hardingham and Do, 2016). The canonical dopamine hypothesis posits that the global dopaminergic hyperactivity is the principal etiological factor responsible for inducing schizophrenia (Weinberger, 1987). However, subsequent evidences prompted revision of the dopamine hypothesis. Accordingly, the hyperactive mesolimbic neurons engender positive symptoms, while hypoactive mesocortical dopaminergic systems give rise to negative symptoms (Davis et al., 1991; Baumeister and Francis, 2002).

The compromised cognitive function is an inescapable feature of schizophrenic pathology (Harrison, 2004). Neuroanatomical and neurophysiological findings implicate mesocortical system in the cognitive functions undertaken by the prefrontal cortex (PFC) (Goldman-Rakic, 1992; Gao and Goldman-Rakic, 2003). While dopamine regulates memory functions in the PFC, its depletion (as noted in schizophrenia) elicits severe cognitive deficits, akin to cortical ablation (Brozoski et al., 1979). Indeed, cognitive deficits are apparent prior to the onset of psychosis and do not respond to nootropic treatments currently in practice (Elvevag and Goldberg, 2000). In recent years, impairment of social and non-social cognition has been identified as a distinctive trait of schizophrenic dementia (Green and Harvey, 2014; Green et al., 2015). In schizo-

*Corresponding author. Address: Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Amravati Road, Nagpur 440 033, India. Fax: +91-712-2500355.
E-mail address: kokaredada@yahoo.com (D. M. Kokare).

Abbreviations: Acbs, accumbens; AcbSh, Acb, shell part; ARC, arcuate nucleus of hypothalamus; CART, cocaine- and amphetamine-regulated transcript peptide; i-VTA, intra-ventral tegmental area; LH, lateral hypothalamus; NMDA, N-methyl-D-aspartate; OFT, open field test; PBS, phosphate-buffered saline; PFC, prefrontal cortex; SEM, standard error of the mean; VTA, ventral tegmental area.

https://doi.org/10.1016/j.neuroscience.2018.01.056
0306-4522/

phrenic patients, social cognition deficits severely compromise daily activities (Fett et al., 2011). While the treatment strategies were mostly aimed at controlling the positive symptoms, the cognitive impairments have been ignored (Carpenter and Koenig, 2008; Wiescholleck and Manahan-Vaughan, 2013).

Studies in last decade suggest a role for cocaine- and amphetamine-regulated transcript peptide (CART) in the functions like learning, memory and cognition (Rogge et al., 2008; Subhedar et al., 2014). CART blocked voltage-gated Ca^{2+} channels in hippocampal slice preparations (Yermolaieva et al., 2001). CART treatment improved memory and synaptic structure in a mouse model of Alzheimer's disease (Jin et al., 2015). Earlier studies from our laboratory have revealed the pro-cognitive effect of CART in spatial and object recognition memory (Upadhya et al., 2011; Bharne et al., 2016).

Anatomical distribution of CART suggests possibilities of its interaction with the dopaminergic system. CART is distributed in the striatum, ventral tegmental area (VTA) and PFC (Koylu et al., 1998), the areas enriched in dopamine and implicated in psychosis. CART neurons in the nucleus accumbens (Acbs) are innervated by mesolimbic dopamine terminals (Hubert et al., 2008). Intracerebroventricular (icv) administration of CART elevates extracellular dopamine metabolites, presumably via dopamine release in the Acb (Jaworski et al., 2003; Yang et al., 2004; Shieh and Yang, 2008). The elevated dopamine levels in the animals with schizophrenia-like condition were shown to up-regulate CART gene expression via activation of intracellular cAMP response element-binding protein signaling (Jones and Kuhar, 2006; Vicentic and Jones, 2007). While cocaine withdrawal resulted in a transient increase in CART gene transcription in the PFC (Freeman et al., 2008), phosphorylation of dopamine receptors in Acb was inhibited by CART (Fu et al., 2016). However, the involvement of CART in the manifestation of cognitive deficits in schizophrenia-like condition has not been studied.

Herein, we attempt to understand the role of CART in MK-801-induced schizophrenic dementia-like symptoms and the underlying mechanisms. Unlike dopamine facilitators, which produce only positive symptoms (Krystal et al., 2005), a single injection of non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801-induced positive, negative psychotic and cognitive symptoms in humans (Neill et al., 2010) and the rats (Tiedtke et al., 1990; Rung et al., 2005; Boulay et al., 2013). The application of MK-801 is also recommended since (a) it does not produce oral stereotypies like gnawing, biting, licking, as induced by D-amphetamine, and (b) MK-801, but not D-amphetamine- or apomorphine-, induced schizophrenia-like condition could be reversed by clozapine (Tiedtke et al., 1990). Indeed, NMDA receptor antagonism induced metabolic dysregulation of glutamatergic as well as dopaminergic systems (Bubeníková-Valesová et al., 2008), similar to that encountered in acute psychotic patients (Soyka et al., 2005). In the present study, the animals were administered with a single injection of MK-801 (at doses 0.01, 0.025 or 0.05 mg/kg; intra-peritoneal, ip) and 30-min later they were subjected to the social recognition test. The animals were screened for social interaction with either novel or familiar juvenile in the test cage. Similar strategy has been used in several studies (Chadman et al., 2006; Neill et al., 2010). Since social cognition deficits were evidenced in schizophrenia-like rats as well as humans (Fett et al., 2011), we employed social recognition task to evaluate the memory functions. Increased hyperactivity (positive symptom) in the open-field test (OFT), along with dementia in social recognition task confirmed the induction of the psychotic symptoms. The animals with schizophrenia-like condition were treated with CART [via icv or intra-VTA routes] and the effect on social recognition memory was tested. Dopamine D1 receptor antagonist SCH23390 (intra-PFC) was co-administered to test the involvement of dopamine receptor in CART's action. The PFC, Acb, VTA, arcuate nucleus of hypothalamus (ARC) and lateral hypothalamus (LH) of the animals were subjected to immunohistochemistry to track the changes in the expression of CART, if any. To find out if the VTA dopamine neurons with projections to the PFC receive CARTergic inputs, Di-I was injected in the PFC and the fibers were traced retrogradely in the VTA and the sections were co-labeled with CART-antibody.

EXPERIMENTAL PROCEDURES

Animals

Twelve weeks old, adult male Wistar rats (considered as residents) and 3- to 4-week-old juveniles (considered as intruders), were group housed in separate polypropylene cages to avoid familiarity. Fresh bedding was provided to the rats in a climate-controlled room maintained on a 12:12-h light/dark cycle (lights on 07:00-h), temperature (25 ± 1 °C) and relative humidity (50–70%). Food (Trimurti feeds, Nagpur, India) and water were available *ad libitum*. Animals were handled individually, 5 min each, for 2 days prior to the commencement of the experiments in order to reduce handling-related stress. Since rats exhibit more social behaviors during light phase (Moura et al., 2009), all experiments were conducted between 8:00 and 11:00 am. Throughout the experiments, resident and intruder rats were kept in separate cages. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India.

Stereotaxic cannulae implantation

Adult rats were anesthetized with thiopental sodium (45 mg/kg, ip route]. The surgical procedures and post-surgical care have been standardized in our laboratory (Dandekar et al., 2008). Briefly, anesthetized rats were immobilized in the stereotaxic apparatus and implanted with stainless steel guide cannulae fabricated in-house (Kokare et al., 2011). The cannulae were targeted at the right lateral cerebral ventricle (−0.8 mm posterior from bregma, +1.3 mm mediolateral, −3.5 mm ventral from

Please cite this article in press as: Borkar CD et al. Cocaine- and Amphetamine-Regulated Transcript Peptide (CART) Alleviates MK-801-Induced Schizophrenic Dementia-Like Symptoms. Neuroscience (2018), https://doi.org/10.1016/j.neuroscience.2018.01.056