EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 3
121-123
Year: 2020

ROLE OF DOPAMINE D2 RECEPTOR IN CHOLINERGIC INTERNEURON IN RESPONSE TO COCAINE

Elmira Mofid MD*, Sukhrajwinder Sekhon MD, Casem Ballouk MD, Olha Huzo MD

Department of Microbiology and Molecular Genetics, University of California, Irvine.

***Corresponding Author: Dr. Elmira Mofid**
Department of Microbiology and Molecular Genetics, University of California, Irvine.

Article Received on 09/01/2020 **Article Revised on 30/01/2020** **Article Accepted on 20/02/2020**

INTRODUCTION

Dopamine (DA) is the predominant catecholamine neurotransmitter in the human center nervous system, where it controls a variety of functions including cognition, emotion, locomotion activity, hunger and endocrine system regulation. The effects of the DA are brought about when DA interacts with the membrane bound dopamine receptors that belong to the G-protein coupled receptor family. There are five different types of DA receptors which are subdivided into two subfamilies; first, the D1-like that includes D1R, D5R and second, the D2-like that includes D2R, D3R and D4R. Dopamine is synthesized by the neurons of the substantial nigra and ventral tegmental area (VTA), and by hypothalamic neurons of the arcuate and periventricular nuclei (8). Projections originating from brain areas that synthesize this neurotransmitter give rise to four axonal pathways which are (i) nigro-striatal; (ii) mesolimbic; (iii) mesocortical; and (iv) tuberoinfundibular (7). The mesolimbic pathway originates from the midbrain VTA and innervates the ventral straitum, the olfactory tubercle (OT) and parts of the limbic system (7). This pathway has been implicated in the control of the reward mechanisms and in the psychomotor effects generated by drugs of abuse, including cocaine.

Cocaine is a psychostimulant that acts by blocking the activity of DA transporter (DAT), and thereby elevates DA levels in nucleus accumbens (NAc), dorsal striatum (DS) and frontal cortex. The nucleus accumbens (NAc) is a brain region involved in functions ranging from motivation and reward to feeding and drug addiction. It consists of two functionally and anatomically distinct subregions, the central "core" and the surrounding "shell". Striatum is a heterogeneous structure composed of >95% medium sized spiny neuron and 5% interneurons. The interneurons may be either cholinergic or GABAergic which release acetyl choline (Ach) or GABA respectively in the striatum. In the presence of cocaine, DA levels in the synapse rise up and it activates the dopamine receptors. Activation of dopamine receptors, in turn activate several kinases which induce the expression of several immediate early genes (IEG) for example c-fos. These IEGs have been used as marker of cellular response to cocaine in brain. The activation of the D1Rs is important for cellular and behavioral reactions to cocaine to be produced. Research shows that if D1R- mediated signaling is impaired, either by genetic ablation of D1R or by blocking of D1R by pharmacological agents, it strongly decreases the behavioral and cellular response to cocaine. In such a case, therefore, it is expected that because the D2Rs are not involved, then the effects of cocaine would be amplified *in vivo* in genetically engineered mice devoid of D2R. However, this is in contrary to the observations that have been made (10). Results have shown that the D2R-/- mice responded in an impaired manner to cocaine and other drugs of abuse. It means that an intact D2R-mdiated signaling is required to show the rewarding effects of drugs. Also, cocaine fails to induce c-fos in D2R -/- mice. This leads researchers to speculate that the absence of the inhibitory circuit that is normally controlled by the D2R is responsible for suppression of c-fos in MSN. It is also well documented that D2R mediates the control of release of Ach and GABA from cholinergic and GABAergic interneurons respectively.

Hypothesis

We aim to observe the expression of c-fos, an early immediate gene, which has been used as a marker to study cellular responses exerted by cocaine in brain of ChAT Cre mice. In this line of mice, exon 2 of D2R has been floxed by LoxP sites and Cre has been put under the control of ChAT (Choline Acetyl Tranferase) promoter. ChAT is expressed only in the neurons that synthesize Ach. In this way, D2R is genetically ablated specifically in cholinergic interneurons. We hypothesize that since release of acetylcholine is regulated by D2R-mediated mechanism, the loss of D2R in cholinergic neurons will lead to overflow of acetyl choline in the striatum leading to suppression of c-fos expression, and thereby reducing effects exerted by cocaine in ChAT Cre

mice.

Procedure

In this experiment, mutant mice, whose D2R is genetically ablated only in cholinergic interneurons, were tested for c-fos expression and were compared to wild type mice. Total of 10 mice, 5 mutant and 5 wild types, were injected with saline (control) or cocaine (20 mg/kg *i.p.*). Mice were sacrificed 60 minutes after injection, and their brains were taken out. The brains were occluded within OCT solution to freeze the brain. The brains were sectioned with cryostat machine into sections of brain, which were mounted onto slides (10 sections per slide). Double in situ hybridization was performed on the sections to look for c-fos expression and enkaphelin expression (ENK). ENK is expressed in D2R expression neurons, so it is used as marker of D2R expression neurons in striatum and NAc. Three pictures from dorso-medial striatum and nucleus accumbens (shell) were acquired from each side of brain. In each brain slice, the number of green (c- fos) and orange (c-fos-ENK) neurons were manually counted and expressed as the average.

RESULT

In situ hybridization studies reveal that the c-fos expression is suppressed in ChAT Cre mice in response to cocaine as opposed by what was observed in WT mice. In the WT mice treated with cocaine (20mg/kg), c-fos was found to be induced in both in striatum (Fig. 1a) and in NAc (Fig. 1b). When looked for co-expression of c-fos and ENK, we found that c-fos largely expressed in ENK negative neurons (Fig. 2a and 2b).

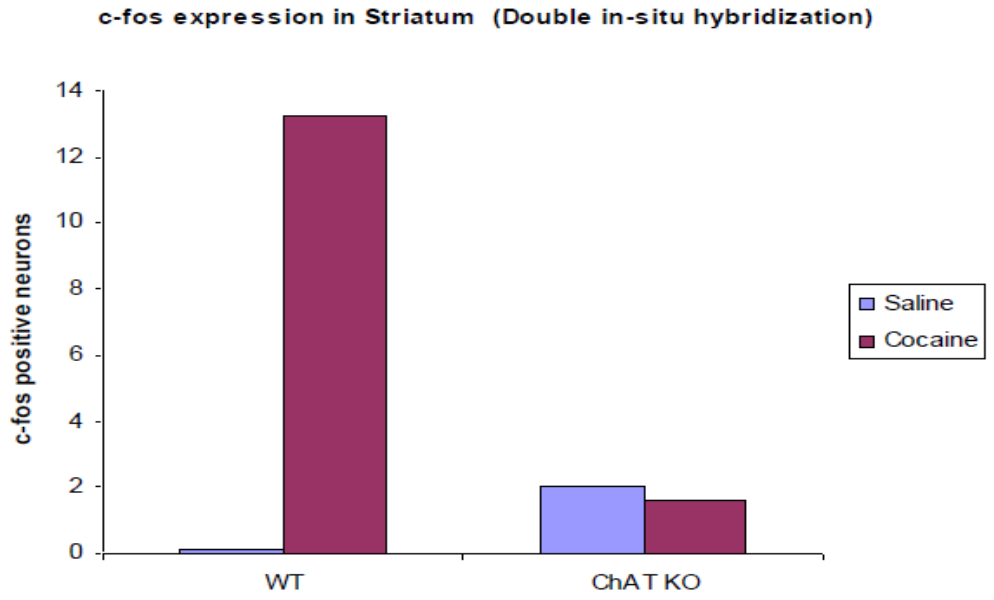

Figure 1a

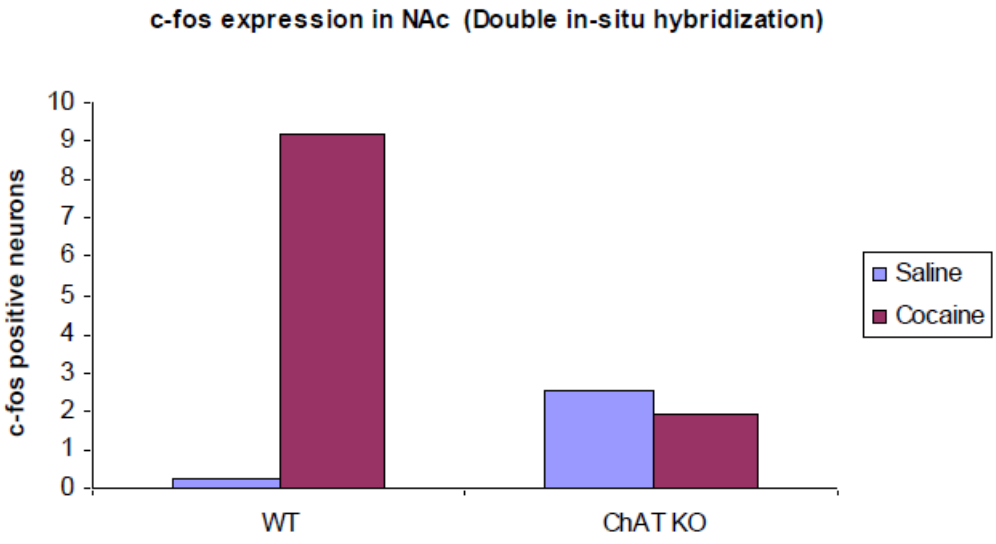

Figure 1b

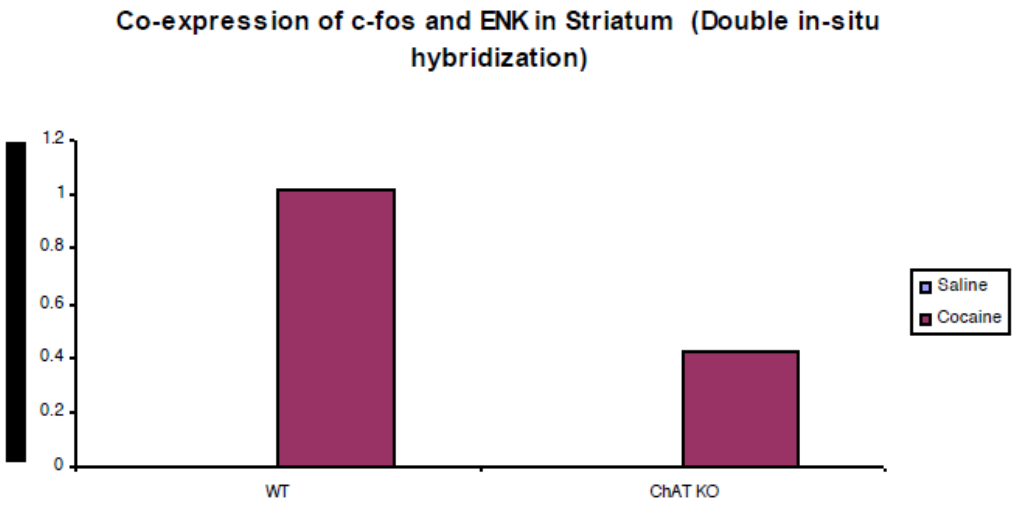

Figure 2a

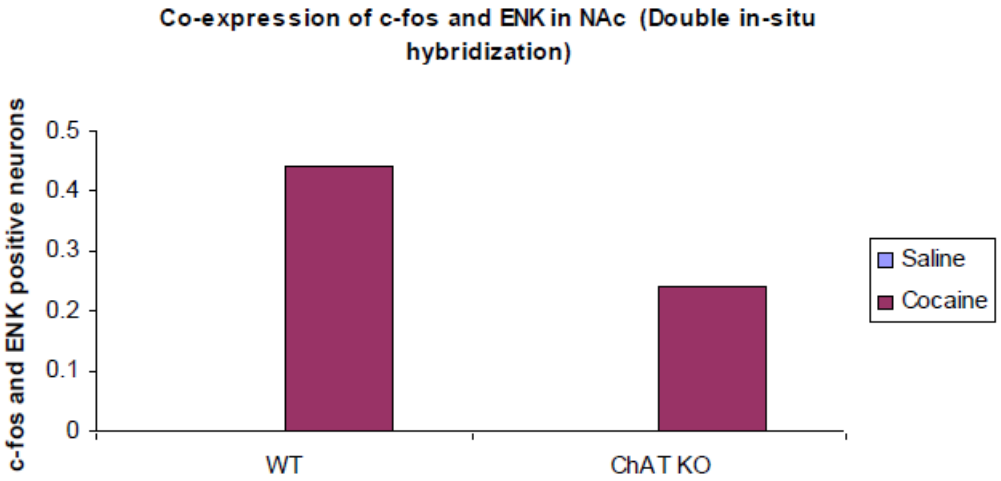

Figure 2b

CONCLUSION

Results obtained from in-situ hybridization analysis in ChAT Cre mice suggest that cholinergic interneurons of striatum might play an important role in eliciting the rewarding effect of cocaine, and D2Rs is key player in regulating Ach release from cholinergic internuerons. Also, it seems in the light of our results that the c-fos is expressed in the ENK negative neurons verifying indirectly the well documented report that says c-fos in induced in D1R expression neurons.

Works Cited

1. Chiara G: Dopamine in the CNS. New York: Springer, 2002.
2. Jenner P: Dopamine receptor subtypes: from basic sciences to clinic application. Amsterdam: IOS Pres, 1998.
3. Neve K: Function selectivity of G-Protein receptor ligands: new opportunities for drug discovery. New York: Humana press, 2009.
4. Neve K, Neve R: The dopamine receptors .new York: Springer, 2009.
5. Niznik H: Dopamine receptors and transporters: pharmacology, structure and function. New York: M. Dekker, 1994.
6. Vallone D, Picetti R, Borrelli E: Structure and function of dopamine receptors. Neurosci. Biobehav Rev., 2000; 24: 125-32.
7. Bozzi Y, Borrelli E: Dopamine in neurotoxicity and neuroprotection: What do D2 receptors have to do with it? 2006; 9: 167-74.
8. De Mei C, Ramos M, Iitaka C, Borrelli E: Getting specialized: Presynaptic and postsynaptic dopamine D2 receptors: 2009; 9: 53-8.
9. Marc W, Daniela V, Tarek A.S, Hamid M, Alessandro U, and Borrelli E: Absence of Dopamine D2 receptors unmasks an inhibitory control over the brain circuitries activated by cocaine: PNAS 2007; 104: 6840–6845.