Press Release

Title

Phospho-proteomics of the dopamine pathway enables discovery of Rap1 activation as a reward signal *in vivo*

Key Points

- ○ Phospho-proteomic analysis identified more than 100 candidate substrates of PKA downstream of D1R, including Rap1 GEF (Rasgrp2)
- ○ PKA-mediated Rasgrp2 phosphorylation enhances its GEF activity on Rap1
- ○ Rap1 activated by PKA-Rasgrp2 regulates neuronal excitability and cocaine reward-related behavior

Summary

Prof. Kozo Kaibuchi (Department of Cell Pharmacology) and Dr. Taku Nagai (Neuropsychopharmacology and Hospital Pharmacy) in Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi, M.D., Ph.D.) identified more than 100 candidate substrates of PKA downstream of dopamine receptor D1R by a kinase-oriented phospho-proteomic analysis, and demonstrated a novel DA-PKA-Rap1-MAPK intracellular signaling mechanism in D1R-MSNs which increases neuronal excitability to enhance reward-related behaviors. This work was supported by the “Bioinformatics for Brain Sciences” performed under the SRPBS from AMED.

Research Background

It is well known that dopamine (DA) is necessary for motor function, motivation, working memory and reward. The principal target of DA is medium spiny neurons (MSNs), which are a special type of GABAergic inhibitory cell that comprise 95% of the neurons within the striatum, including the nucleus accumbens (NAc). There is a distinct class of spatially intermixed MSNs that express DA type 1 or 2 receptors (D1R-MSNs or D2R-MSNs, respectively). The D1R activates protein kinase A (PKA), whereas the D2R inhibits. DA acts to increase the excitability of D1R-MSNs and their response to glutamatergic synaptic input from the cerebral cortex, hippocampus and amygdala. Conversely, DA appears to reduce the excitability of D2R-MSNs. Based on pharmacological observations, PKA is thought to regulate not only the excitability of MSNs but also the synaptic plasticity that controls reward-related behaviors. However, whether and how D1Rs and PKA regulate neuronal excitability and behavior remains largely unknown.

Research Results

The research group developed a phospho-proteomic analysis method to identify known and novel PKA substrates downstream of the D1R and obtained more than one hundred candidate

substrates, including Rap1 GEF (Rasgrp2). They found that PKA phosphorylation of Rasgrp2 activated its guanine nucleotide exchange activity on Rap1. Cocaine exposure activated Rap1 in the nucleus accumbens in mice. The expression of constitutively active PKA or Rap1 in accumbal D1R-expressing medium spiny neurons (D1R-MSNs) enhanced neuronal firing rates and behavioral responses to cocaine exposure through MAPK. Knockout of *Rap1* in the accumbal D1R-MSNs was sufficient to decrease these phenotypes.

The phospho-proteomic analysis method enabled to comprehensively identify PKA substrates downstream of D1Rs, including Rasgrp2. Based on their observations, they propose the following mechanism for DA-dependent reward signaling *in vivo* (Figure 1). The binding of DA to D1Rs activates PKA to phosphorylate Rasgrp2. The phosphorylation of Rasgrp2 leads to Rap1 activation, followed by recruitment of the MAPK pathway, which increases the excitability of accumbal D1R-MSNs. The enhancement of D1R-MSN excitability increases spike firing in response to excitatory glutamatergic input from the cortex and/or thalamus. The D1R-MSN pathway is subsequently activated, which eventually results in reward-related behaviors. The increase in the excitability of D1R-MSNs through the novel DA-PKA-Rap1-MAPK intracellular signaling pathway is the initial and crucial step that promotes accumbal processing of excitatory glutamatergic input.

Research Summary and Future Perspective

In summary, this study provides a novel DA-PKA-Rap1-MAPK intracellular signaling mechanism in D1R-MSNs that increases neuronal excitability to enhance reward-related behaviors. DA signaling dysfunction has been implicated in various neuropsychological diseases, including Parkinson's disease, drug addiction, compulsive behavior, attention-deficit/hyperactivity disorder, autism spectrum disorders and schizophrenia. We believe that their phosphoprotein screening is a powerful and useful tool to increase molecular-level understanding of these diseases by elucidating the function of the DA.

Article

Nagai T, Nakamuta S, Kuroda K, Nakauchi S, Nishioka T, Takano T, Zhang X, Tsuboi D, Funahashi Y, Nakano T, Yoshimoto J, Kobayashi K, Uchigashima M, Watanabe M, Miura M, Nishi A, Kobayashi K, Yamada K, Amano M, Kaibuchi K. Phospho-proteomics of the dopamine pathway enables discovery of Rap1 activation as a reward signal *in vivo*. Neuron, Jan.21,2016.

Japanese ver.

http://www.med.nagoya-u.ac.jp/medical/dbps_data/_material_/nu_medical/_res/topix/2015/Rap1_20160122jp.pdf

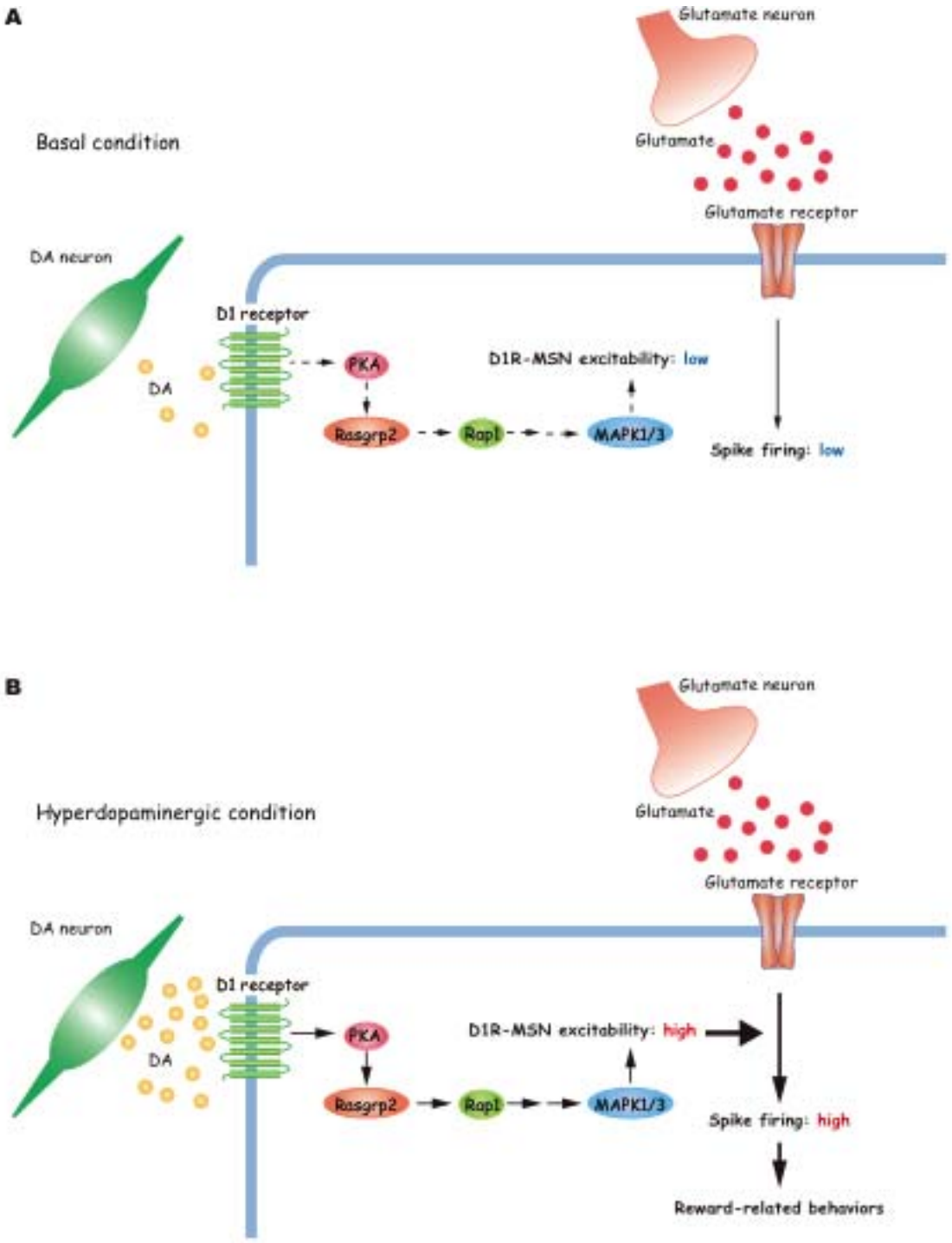

Figure 1. Working model of D1R-dependent Rap1 signaling

(A) Basal condition. When DA levels are relatively low at rest, D1R-MSNs appear to be less excitable. (B) Hyperdopaminergic condition. The binding of DA to D1Rs activates PKA to phosphorylate Rasgrp2. The phosphorylation of Rasgrp2 leads to Rap1 activation, followed by recruitment of the MAPK pathway, which stimulates the excitability of accumbal D1R-MSNs. The enhancement of D1R-MSN excitability increases spike firing in response to excitatory glutamatergic input from the cortex and/or thalamus. The D1R-MSN pathway is subsequently activated, which eventually results in reward-related behaviors.