主論文の要旨

Balance between dopamine and adenosine signals regulates the PKA/Rap1 pathway in striatal medium spiny neurons

（ドーパミンシグナルとアデノシンシグナルとのバランスは、線条体中型有棘神経細胞における PKA / Rap1 経路を制御する）

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[Background]

In the brain, dopamine functions as a neuromodulator and is associated with motor function, motivation, learning and reward. Two major dopaminergic pathways project dopamine neurons from the substantia nigra and the ventral tegmental area to the striatum including the nucleus accumbens (NAc). Medium spiny neurons (MSNs) are major components of the striatum including MSNs expressing the dopamine D1 receptor (D1R-MSNs) or dopamine D2 receptor (D2R-MSNs). In the NAc, D1R-MSNs are involved in rewarding behavior, while D2R-MSNs are involved in aversive behavior. D1R is coupled to G_{olf}, whose stimulation activates protein kinase A (PKA) through adenylate cyclase. D2R is coupled to G_i, whose activation results in an inhibition of the cAMP/PKA signaling pathway. We have recently carried out a comprehensive phosphoproteomic analysis of PKA substrates downstream of D1R and identified novel PKA substrates including Rasgrp2. Rasgrp2 is a guanine nucleotide exchange factor (GEF) for Rap1 that can activate Rap1 signaling to regulate neuronal excitability and cocaine-induced reward responses by acting through the MAPKK/MAPK pathway. In addition to Rasgrp2, we have also found that Rap1gap is phosphorylated by D1R agonist. Rap1gap is a GTPase activating protein (GAP) that can downregulate Rap1 activity. These findings suggest that D1R/PKA/Rap1gap is another signaling pathway for dopamine action to control Rap1 activity *in vivo*. However, whether dopamine D1R stimulation promotes phosphorylation of Rap1gap *in vivo* remains unclear.

Dopamine suppresses neuronal activity through D2R, whereas adenosine appears to increase the activity of D2R-MSNs. In the striatum, D2R-MSNs express adenosine A2A receptor (A2AR), which couples to the G_{olf} protein, whereas D1R-MSNs express adenosine A1 receptor (A1R), which couples to the G_i protein. Thus, PKA activity in D2R-MSNs is speculated to be positively controlled by adenosine/A2AR and negatively by dopamine/D2R. However, how these two pathways co-operatively act in D2R-MSNs remains largely unknown.

[Methods and results]

We first examined whether SKF81297 and forskolin, an inducer of cAMP, enhanced Rap1gap phosphorylation in striatal slices using the phospho-specific antibody. The phosphorylation level of Rap1gap at S563 was significantly increased after the treatment of the striatal slice with SKF81297 or forskolin, indicating that D1R and PKA stimulation promotes Rap1gap phosphorylation (Fig. 1).

To monitor Rap1gap phosphorylation in D2R-MSNs, striatal slices were treated with the A2AR agonist CGS21680 and D2R agonist quinpirole. Treatment of striatal slices with CGS21680 significantly increased the phosphorylation level of Rap1gap at S563. The CGS21680-stimulated phosphorylation of Rap1gap at S563 was completely blocked by

pretreatment with quinpirole (Fig. 2).

To investigate D1R's effect on Rap1gap phosphorylation *in vivo*, we examined Rap1gap phosphorylation level in the NAc of C57BL/6 mice by immunoblotting. The phosphorylation level of Rap1gap at S563 was significantly increased in the SKF81297-treated mice (Fig. 3a). We also immunohistochemically investigated if SKF81297 stimulates Rap1gap phosphorylation in accumbal D1R-MSNs by treating Drd1-mVenus transgenic mice, which express mVenus in the D1R-MSNs, with SKF81297. SKF81297 treatment increased the number of phosphorylated Rap1gap-positive cells, and most of the signals were colocalized with mVenus-positive cells in the NAc of Drd1-mVenus transgenic mice (Fig. 3b). These results indicate that D1R stimulation increased PKA activity in the accumbal D1R-MSNs *in vivo*.

We further investigated the effect of the A2AR agonist on Rap1gap phosphorylation *in vivo*. Treatment with CGS21680 slightly but not significantly increased Rap1gap phosphorylation level in the NAc of C57BL/6 mice (Fig. 4a). This may be because that the basal levels of extracellular dopamine interfere with the effect of A2AR activation towards the Rap1gap phosphorylation by acting through D2R. Therefore, we measured Rap1gap phosphorylation level after treatment with the D2R antagonist eticlopride in mice. Treatment with eticlopride significantly increased Rap1gap phosphorylation level in a dose-dependent manner (Fig. 4b). Furthermore, the eticlopride-induced Rap1gap phosphorylation was detected in accumbal D2R-MSNs of Drd2-mVenus transgenic mice, in which D2R-MSNs express mVenus (Fig. 4c), suggesting that the basal levels of extracellular dopamine inhibit Rap1gap phosphorylation *in vivo*.

To examine the interaction between A2AR and D2R in the NAc of mice, we further investigated the effect of the A2AR antagonist SCH58261 on eticlopride-induced Rap1gap phosphorylation. Pretreatment with SCH58261 significantly suppressed the Rap1gap phosphorylation induced by eticlopride treatment (Fig. 5). Thus, A2AR stimulation is required for the D2R antagonist-induced Rap1gap phosphorylation. Taken together, these results suggest that adenosine promotes Rap1gap phosphorylation through A2AR, but dopamine counteracts this effect through D2R in MSNs in basal conditions.

[Discussion]

We have recently demonstrated that dopamine phosphorylates and activates Rasgrp2 through D1R/PKA in D1R-MSNs. Consequently, Rasgrp2-mediated Rap1 activation stimulates MAPK, which increases the excitability of D1R-MSNs to enhance reward-related behaviors. In this study, we found that Rap1gap is phosphorylated through D1R/PKA in D1R-MSNs or through A2AR/PKA in D2R-MSN. Phosphorylation of Rap1gap has been shown to decrease its GAP activity on Rap1 according to previous report. Therefore, Rap1 is efficiently activated through these two signaling pathways in D1R-

MSNs or D2R-MSNs, thereby activating the Rap1/MAPKK/MAPK signaling pathway (Fig. 6).

The extracellular dopamine concentration is changed dynamically and rapidly in the striatum released from dopaminergic neurons in response to stimuli. Since the Kd values of high affinity site of D1R and D2R are around 200 nM and 10 nM, respectively, extracellular dopamine seems to bind to D2R rather than D1R under basal condition. According to previous report, basal adenosine concentrations are estimated to be in the range of 25–250 nM. The affinity of A1R and A2AR is around 100 nM. Therefore, basal extracellular adenosine concentrations are sufficient to tonically activate A1R and A2AR. Taking these matters into account, we assumed that PKA was activated at low concentration of dopamine and inactivated at high concentration of dopamine in D2R-MSNs when basal adenosine was present. In D1R-MSNS, PKA activity may be controlled in the opposite way, which means that PKA is inactivated at low concentration of dopamine and activated at high concentration. Thus, when basal adenosine is present, the switch between the D1R-MSN and D2R-MSN activation states occurs efficiently depending on the concentration of extracellular dopamine (Fig. 6).