主論文の要旨

Accumbal D2R-medium spiny neurons regulate aversive behaviors through PKA-Rap1 pathway

（側坐核ドーパミン D2 受容体−中型有棘神経細胞が PKA-Rap1 シグナル経路を介して忌避行動を制御する）

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【Introduction】

It is well established that accumbal D2R-MSN, one of the main components of nucleus accumbens (NAc), controls aversive learning. Previous reports have shown that aversive stimulus, i.e. electric footshock, activates only D2R-MSN through protein kinase A (PKA). However, the molecular mechanism of how accumbal D2R-MSN controls aversive learning remains unclear. Electric shock is known to decrease dopamine release in NAc core, resulting in activated D2R-MSN due to the attenuated effect of D2R, a Gi-coupled receptor that inhibits PKA signaling. When dopamine level is less dominant in NAc, D2R-MSN is activated by various neuromodulators and neurotransmitters, including adenosine. D2R-MSN expresses adenosine A2A receptor (A2AR), a Gs-coupled receptor that activates PKA. Based on our lab's previous report that D2R antagonist activates A2AR-PKA signaling through Rap1GAP phosphorylation in D2R-MSN in vivo, we aim to clarify adenosine-A2AR signaling in D2R-MSN that controls aversive learning. In this study, we monitored the phosphorylation level of Rap1gap S563 and the effect of A2aR antagonist on Rap1gap phosphorylation after electric foot shock aversive stimulus. We also examined the impact of AAV-mediated manipulation of PKA-Rap1 pathway in accumbal D2R-MSN on aversive behaviors in passive avoidance tests and real-time place aversion tests. Our findings suggested that accumbal D2R-MSNs regulate aversive behaviors through the A2aR-PKA-Rap1-MEK pathway.

【Materials and Result】

1. A2aR mediating PKA-Rap1 signaling is involved in aversive learning.

To determine whether Rap1gap phosphorylation in accumbal D2R-MSNs responds to aversive stimulus, we investigated the anatomical profile of phosphorylated Rap1gap-positive cells in the NAc of Drd2-YFP transgenic mice by immunohistochemistry. The phosphorylated Rap1gap S563-positive cells in the NAc significantly increased in vehicle-treated group 60 min after exposure to electric foot shock (0.4 mA, 60 Hz, 2 sec) compared with that in vehicle-treated control mice without shock. Pretreatment with KW6002 decreased the number of phosphorylated Rap1gap S563-positive cells evoked by the electric foot shock (Fig. 1A).

To further investigate the role of PKA-Rap1 signaling in D2R-MSN, we performed passive avoidance tests, which are fear-motivated tests classically used to assess memory on laboratory animals. We used Cre-Flex system to express dominant negative mutant PKA (PKAdn) and SPA1 (SPA1ca) in the accumbal D2R-MSNs. The PKAdn is a cAMP-binding domain that leads to the

inhibition of PKA activity. And SPA1ca was used to inhibit the Rap1 activity in D2R-MSNs. Three weeks after the AAV injection, Adora2a-Cre transgenic mice were subjected to the passive avoidance test. Compared with an mCherry-expressed control group, Adora2a-Cre mice expressing PKAdn and SPA1ca showed significantly decreased step-through latency ($p<0.01$, Fig. 1B and C). On the other hand, adora2a-Cre transgenic mice were injected with AAV encoding wild-type PKA (PKAwt), constitutively active mutant PKA (PKAca), wild-type Rap1 (Rap1wt), or constitutively active mutant Rap1 (Rap1ca) into the NAc. PKAca is a PKA catalytic subunit that is not regulated by cAMP whereas Rap1ca is a fast-cycling variant of Rap1a. As expected, the step-through latency of PKAca or Rap1ca-expressed Adora2a-Cre transgenic mice significantly increased compared with mCherry-expressed control mice ($p<0.05$ Fig. 1D and E). These results indicated that PKA-Rap1 signaling in accumbal D2R-MSN plays a critical role in aversive learning.

2. Inhibition of PKA-Rap1 signaling in accumbal D2R-MSN attenuates the aversive response caused by optogenetic inactivation of mesolimbic DAergic neurons

We applied an optogenetic technique to inhibit mesolimbic DAergic neurons. ArchT-mCherry was expressed in the VTA/SNc (Fig. 2A). To manipulate D2R-MSNs, we injected AAV-PKAdn or SPA1ca, and AAV-tetO-ArchT-mCherry into the NAc and VTA/SNc of Adora2a-Cre::DAT-PF-tTA double transgenic mice, respectively. Real-time place aversion test was carried out 3 weeks after the AAV injection (Fig. 2B and C). Optogenetic inhibition of the mesolimbic DAergic pathway reduced the time spent in a chamber with yellow light stimulation ($p<0.05$, Fig. 2C). These results indicate that inhibiting mesolimbic DAergic neuron causes aversive behavior. This aversive response was suppressed by PKAdn and SPA1ca expression in accumbal D2R-MSNs ($p<0.05$, Fig. 2C). These data clearly indicated that PKA-Rap1 signaling in accumbal D2R-MSNs controls aversive response.

【Discussion and Conclusion】

In this study, we proposed a new perspective on the balance between activation of A2aR and inactivation of D2R in accumbal D2R-MSN using a combination of neurochemical, histochemical, AAV expression system, optogenetics, and behavioral techniques. In basal condition, adenosine tonically stimulates PKA-Rap1 through A2aR, but the activation of PKA-Rap1 is inhibited by D2R activation under basal DA concentration. The decrease of dopamine release and the subsequent inactivation of D2R in response to aversive stimuli activates PKA-Rap1

signaling due to disinhibition. Thus, the NAc may mediate aversive behavior through the A2aR-PKA-Rap1 signaling pathway following the reduction of DA release from mesolimbic DAergic neurons in the NAc after aversive stimuli.

We demonstrated that electric foot shock-evoked phosphorylation of Rap1gap S563 was evident in D2R-MSNs of the NAc and that this phosphorylation was inhibited by A2aR antagonist KW6002. This suggests that A2aR in accumbal D2R-MSN is the upstream receptor involved in aversive responses. In fact, A2aR antagonist attenuated aversive memory and reduced PKA-Rap1 signaling caused by electric footshock in accumbal D2R-MSNs. These findings indicate that A2aR is a trigger that promotes PKA-Rap1 signaling after aversive stimuli. To investigate how PKA-Rap1 signaling contributes to aversive behavior, we used AAV-mediated cell-type specific PKA and Rap1 manipulation in accumbal D2R-MSNs. Inhibition of PKA-Rap1 signaling suppressed the step-through latency in passive avoidance tests, whereas the activation of PKA-Rap1 signaling potentiated the latency.

According to the results of real-time place aversion, the inhibition of PKA-Rap1 signaling in accumbal D2R-MSNs can attenuate aversive response induced by photostimulation. These results suggested that A2aR-PKA-Rap1-MAPK signaling mainly plays a role in aversive response; thus, memory formation is impaired.

We have recently proposed that the balance between DA and adenosine signals regulates the PKA-Rap1 pathway in D1R-MSNs and D2R-MSNs (Zhang et al. 2019). Basal DA concentration cannot activate D1R but activates D2R to suppress D2R-MSN activity. A high DA state (e.g., reward acquirement and abused drugs intake) activates D1R-MSN and inactivates D2R-MSN, whereas a low DA state (e.g., aversive experience) activates D2R-MSN due to the inability of D2R to suppress the A2aR-PKA-Rap1-MAPK pathway. The activation of accumbal D2R-MSNs regulates aversive response and memory formation. Our findings provide a novel negative reinforcement regulating mechanism managed by the activation of PKA-Rap1 in D2R-MSNs.