*Current Perspective***Regulatory Mechanisms and Pathophysiological Significance of IP₃ Receptors and Ryanodine Receptors in Drug Dependence**Koji Mizuno¹, Kazuhiro Kurokawa¹, and Seitaro Ohkuma^{1,*}¹Department of Pharmacology, Kawasaki Medical School, Matsushima 577, Kurashiki 701-0192, Japan

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Abstract. Calcium is a ubiquitous intracellular signaling molecule required for initiating and regulating neuronal functions. Ca²⁺ release from intracellular stores in the endoplasmic reticulum into intracellular spaces via intracellular Ca²⁺-releasing channels, inositol 1,4,5-trisphosphate receptors (IP₃Rs) and ryanodine receptors (RyRs), is one mechanism altering the intracellular Ca²⁺ concentration. Functional abnormalities in endoplasmic calcium channels can disturb cellular calcium homeostasis and, in turn, produce pathological conditions. Indeed, our recent studies have indicated the involvement of these upregulated calcium channels in development of the rewarding effect of a drug of abuse and the suppression of its rewarding effect by calcium-channel inhibitors, which suggests a possible functional relationship between intracellular dynamics and the development of the rewarding effects induced by an abused drug. Although previous reports showed that the most important regulators of both RyR and IP₃R channel functions are changes in the intracellular Ca²⁺ concentration and in phosphorylation of these channels by numerous kinases and calcium modulators, little information is available to clarify how the expression of intracellular calcium channels is regulated. In this review, we therefore introduce the roles and regulatory mechanisms of intracellular calcium channels in drug dependence, especially in the rewarding effect induced by the abused drug.

Keywords: drug dependence, type 1 inositol 1,4,5-trisphosphate receptors, AP-1, nuclear factor of activated T-cells c4 (NFATc4), calcium channel

1. Introduction

Two main intracellular calcium channels, inositol 1,4,5-trisphosphate receptors (IP₃Rs) and ryanodine receptors (RyRs), present in endoplasmic reticulum (ER) of mammalian cells are responsible for Ca²⁺ release from intracellular stores to the intracellular cytoplasm. Even though the amino acid sequences of IP₃Rs and RyRs molecules have little homology, both channels have very similar domain interfaces in the N-terminal region (1) that regulate the cytosolic Ca²⁺ concentration. In addition, the function of both families of channels is also regulated by intracellular Ca²⁺ and several types of kinases (2, 3). However, the triggers that initiate Ca²⁺ release in these

two types of receptors are different. Calcium release through IP₃Rs is dominantly mediated by IP₃ produced after G protein-coupled receptor activation, whereas that through RyRs is mediated by cyclic ADP ribose or direct interaction of RyRs and an elevated intracellular Ca²⁺ concentration in association with the opening of L-type voltage-dependent calcium channels (VDCCs), the latter called calcium-induced calcium release (4). The processes that modify physiological functions mediated via changes of the free intracellular Ca²⁺ concentration are complex and multifaceted. In neurons, Ca²⁺ regulation by ER plays various roles in neuronal activities, e.g., modification of synaptic vesicle release (5) or regulation of nerve growth (6) in the axon. Thus, it is supposed that changes in intracellular Ca²⁺ concentration induce biological and physiological actions in the cells and body. Considering the importance of calcium signals for cellular functions, functional abnormalities in endoplasmic calcium channels could result in disturbance in

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cellular calcium homeostasis and, in turn, produce pathological conditions. For example, IP₃Rs in the brain have been hypothesized to contribute to opisthotonus in mice (7). In addition, IP₃R-knockdown mice showed longer immobility in the forced swimming test, and type 1 IP₃R (IP₃R-1)-deficient mice exhibited ataxia and epileptic seizures (8, 9). Further, functional disturbances of IP₃Rs and RyRs appear to be involved in neurodegenerative diseases such as Alzheimer's disease and Huntington's disease (10–12).

On the other hand, L-type VDCCs on the cell surface are a large family of integral membrane proteins that control the selective flow of Ca²⁺ down their electrochemical gradient in response to changes in membrane potential. They are classified into distinct subtypes (L, N, P/Q, R, and T) based on their pharmacological and biophysical properties. As described above, calcium signals are important for cellular functions and participate in several diseases. In drug dependence, our previous study suggests that upregulation of VDCCs, especially $\alpha 1c$ and $\alpha 2/\delta$ subunits, is involved in the development of the rewarding effect induced by abused drugs. Further, inhibitors for VDCCs, such as nifedipine and gabapentin, suppressed the abused drugs-induced rewarding effect (13, 14). These experimental data suggest that calcium influx into the cytoplasm from extracellular sources, that is, an increase in calcium concentration in the cytoplasm, is core to developing drug dependence. This review introduces the roles and regulatory mechanisms of intracellular calcium channels, especially IP₃R-1, in drug dependence.

2. Inositol 1,4,5-trisphosphate receptors

Cell activation by extracellular agonists such as hormones, growth factors, and neurotransmitters often leads to phospholipase-C (PLC) activation subsequent to intracellular IP₃ production. IP₃ diffuses through the cytoplasm until it binds and activates its receptor. IP₃Rs are one of the intracellular Ca²⁺ release channels predominantly located on the ER and responsible for a controlled release of Ca²⁺ ions into the cytoplasm, which is important to initiate complex spatio-temporal Ca²⁺ signals (15, 16). The functional IP₃R/Ca²⁺ release channel is a tetramer, and the 4 subunits composing the channel have similar general structures. The diversity of IP₃Rs is due to the presence of 3 genes (*ITPR1*, *ITPR2*, and *ITPR3*) encoding for IP₃R-1, -2, and -3 respectively, the occurrence of splicing events, and the possible formation of homo- and heterotetramers (2). Their affinities for IP₃ display the rank order IP₃R-2 > IP₃R-1 > IP₃R-3 (17, 18), and a difference in sensitivity to regulatory factors including Ca²⁺, ATP, and protein kinases was observed (2).

The physiological relevance of the existence of multiple IP₃R isoforms is also supported by the fact that their expression pattern in the subcellular localization varies in different types of cells and organs (19) and that it changes during cellular differentiation and development as well as under pathophysiological situations (2).

3. Type I inositol 1,4,5-trisphosphate receptors in drug dependence

Among the 3 subtypes of IP₃Rs with distinct physiological properties (2), IP₃R-1 is the major neuronal member of the IP₃R family in the central nervous system, predominantly enriched in cerebellar Purkinje cells and concentrated in cerebral cortical neurons (20). The most important regulators of IP₃R channel functions are considered to be changes in the intracellular Ca²⁺ concentration and phosphorylation of IP₃R protein by numerous kinases and calcium modulators (2). However, it is not yet clear how the expression of IP₃Rs is regulated.

Dopaminergic neurons in the mesolimbic dopamine system, which originate from the ventral tegmental area and project their axons predominantly to the nucleus accumbens (NAcc) and prefrontal cortex, have been considered to play a crucial role in the rewarding effect and development of drug dependence (15, 21). In addition, a previous study demonstrated that enhancement of activity of dopaminergic neurons in the rat ventral tegmental area by IP₃R-1 stimulation is an essential step for subsequent behavioral changes and that this step probably is involved in amphetamine-induced rewarding effect (22). Similarly, our recent studies show that both methamphetamine and cocaine, well known psychostimulants, upregulate IP₃Rs-1 in association with strengthening of their rewarding effect and that the increases of the rewarding effect in response to both agents were significantly suppressed by the IP₃Rs antagonists 2-aminoethoxydiphenyl borate (2-APB) and xestospongin C. In addition, both events, IP₃R-1 upregulation and the increase of the rewarding effect, were significantly suppressed by antagonists selective to dopamine D₁- and D₂-like receptors (23, 24). Taken together these data indicates that IP₃R-1 upregulation in NAcc and cortex via dopamine D₁- and D₂-like receptors is likely to be one of the mechanisms responsible for the rewarding effect.

4. Mechanisms of upregulation of inositol 1,4,5-trisphosphate receptors via dopamine receptors

As described above, although several reports have demonstrated that the most important regulators of IP₃R channel functions are likely to be the changes in intra-

Fig. 1. Schema of regulatory mechanisms of IP₃R-1 and RyR expression. The figure shows regulatory mechanisms of IP₃R-1 and RyR expression via dopamine D₁-like receptors and D₂-like receptors, especially mediated via the calcium signal transduction pathways. Inhibitors of calcium signal transduction pathways, such as U73122, 2-APB, BAPTA-AM, W7, KN-93, and FK506, suppressed the expression of IP₃Rs-1. Furthermore, the CaMKs inhibitor KN-93 and the PKA inhibitor KT5720 also suppressed RyR expression. D₁R, dopamine D₁-like receptors; D₂R, dopamine D₂-like receptors; PKA, protein kinase A; CREB, cyclic-AMP-responsive element-binding protein; PLC, phospholipase C; DAG, diacylglycerol; IP₃R, inositol 1,4,5-trisphosphate receptors; 2-APB, 2-aminoethoxydiphenyl borate; CaMKIV, calmodulin-dependent protein kinase IV; NFAT, nuclear factor of activated T cells; AP-1, activator protein-1.

cellular Ca²⁺ concentration and phosphorylation of IP₃R protein by numerous kinases and calcium modulators (2), it is considered that the rewarding effect would be affected by psychostimulant-induced IP₃R-1 upregulation in the NAcc and cortex via dopamine D₁-like and D₂-like receptors. However, there has been little information on the regulatory mechanisms for IP₃R expression by dopamine receptors. Previous reports have already shown that IP₃R-1 mRNA expression is regulated by the calcium signal transduction pathway (25, 26) and that the expression is dominantly regulated by nuclear factor of activated T-cells c4 (NFATc4) (26). Recently, we also obtained similar findings that NFATc4, which is translocated from the cytoplasm to the nucleus by activated calcineurin, binds to the IP₃R-1 promoter region via activation of Gαq-linked D₁-like receptors in mouse cortical neurons (27). In addition to these known regulatory mechanisms of IP₃R-1 mRNA expression, we revealed that CaMKIV promotes the binding of AP-1, a heterodimer composed of cFos and cJun, to the IP₃R-1

promoter region to accelerate IP₃R-1 mRNA transcription via dopamine Gαq-linked D₁- and D₂-like receptors in mouse cerebral cortical neurons (27, 28). Dopamine promotes Gαs protein activation, and the PKA cascade, downstream of Gαs protein, regulates numerous protein expressions via D₁-like receptors. On the other hand, our study showed that a PKA inhibitor had no effect on IP₃R-1 expression via D₁-like receptor activation and that IP₃Rs-1 are upregulated without an increase in cAMP production (27). Fig. 1 shows regulatory mechanisms of IP₃R-1 and RyR expression by dopamine stimulation. Thus, dopamine receptors coupled to Gαq play an important role in not only behavioral changes associated with drug dependence but also regulating the expression of intracellular calcium channels to modify intracellular Ca²⁺ dynamics. Furthermore, it is considered that IP₃R-1 expression is regulated by positive feedback mechanisms via the calcium signal transduction pathway. That is, such neurochemical events are likely to be involved in reinforcement by psychostimulants.

5. RyRs in drug dependence

RyRs, one of the intracellular calcium channels, are classified to a multigene family of channel proteins that mediate calcium release from intracellular calcium stores such as the ER into the cytoplasm, as IP₃Rs do. Three different genes coding for isoforms of RyRs also have been identified and cloned (*RyR-1*, *RyR-2*, and *RyR-3*). All 3 RyR isoforms and their mRNA were detected in the brain, and RyR-2 mRNA is recognized to be the most abundant (29). In addition, enhancement of calcium release from intracellular calcium stores by stimulation of RyRs as well as of IP₃Rs also gives rise to calcium signals recognized as the calcium-induced calcium release that is considered to propagate small and localized calcium signals to mitochondria and nuclei with signal amplification (30).

Our previous study showed that cocaine, methamphetamine, and alcohol induce the upregulation of RyR-1 and -2 with an increase in the rewarding effect induced by the abused drug mediated via dopamine D₁-like receptors. This abused drug-induced rewarding effect was significantly suppressed by dantrolene, a RyR antagonist (31, 32). It is therefore considered that RyRs are likely to be also one of the important players in development of the rewarding effect induced by an abused drug via upregulation of intracellular Ca²⁺ dynamics as IP₃R-1. In spite of their similar importance in drug dependence, the regulatory mechanism of RyRs is different from that of IP₃R-1. Although IP₃R-1 upregulation is induced by activation of both D₁- and D₂-like receptors, RyR upregulation is regulated by only D₁-like receptors. These conclusions are supported by previous reports showing that RyR-1 and RyR-2 expressions are regulated by the cAMP-PKA cascade and/or CaMKII-CREB pathway (31, 33, 34) and that the expression of IP₃Rs-1 is mediated via pathways that calcineurin and CaMKIV participate in, but not via the cAMP-PKA cascade or CaMKII pathway (27, 28).

6. Conclusion

In this review, we surveyed the role and regulatory mechanism of IP₃R-1 expression in drug dependence. Several *in vivo* studies showed that drugs of abuse upregulate IP₃Rs-1 with showing their rewarding effect. The rewarding effect induced by abused drugs was also suppressed by IP₃R antagonists and antagonists selective to dopamine D₁- and D₂-like receptors functionally linking to Gα_q. *In vitro* studies also showed that IP₃R-1 expression was regulated by the calcium signal transduction pathway following activation of these dopamine receptors. On the other hand, drugs such as methamphet-

amine, cocaine, nicotine, and ethanol induced expression of RyRs. Furthermore, the rewarding effect of these drugs was completely suppressed by the RyR antagonist dantrolene (31, 32). Our recent reports show that RyR-1 and RyR-2 expressions increase via the PKA signaling pathway coupled to dopamine D₁-like receptors, although dopamine D₂-like receptors are not involved in the events (35, 36).

It remains to be elucidated how these differences in regulatory mechanisms of both intracellular receptors by dopamine receptors affect the rewarding effect. We recently found that an inhibitor of RyRs suppressed both development and expression of the abused drug-induced rewarding effect, although inhibitors for IP₃Rs suppressed only the development of the rewarding effect induced by the abused drug (unpublished data). This difference is likely to be a key point in the development of therapeutics to treat and/or prevent psychological dependence on drugs of abuse.

Our recent study also showed that GABA_A-receptor agonists suppressed ethanol-induced IP₃R-1 upregulation (37). Previous investigations reported that ethanol had potential to stimulate dopamine release (38, 39) and increase dopamine D₁-receptor expression in cortical neurons (40). Therefore, inhibition of the ethanol-induced IP₃R-1 upregulation by GABA_A-receptor activation with muscimol is likely to be due to direct inhibitory interaction of GABA_A receptors with dopamine D₁ receptors or suppressive action of GABA_A receptors on ethanol-evoked dopamine release. On the other hand, other reports showed that chronic ethanol treatment induced changes of GABA_A-receptor subunit expression and its function (41–43). In addition, GABA_A-receptor agonists modulated ethanol self-administration *in vivo* (44, 45). Based on these data, furthermore, it is considered that chronic ethanol exposure probably suppresses GABA_A-receptor function and, in turn, reduces the negative regulatory potential of GABA_A receptors on IP₃R-1 expression, and that breaking down the balance between Ca²⁺ and Cl[−] homeostasis is likely to work as a factor in the rewarding effect and development of drug dependence, although the exact mechanisms remain to be elucidated.

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