Journal of Pharmacological Sciences xxx (2018) 1–10

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Propofol induced diverse and subtype-specific translocation of PKC families

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ARTICLE INFO

Article history:
Received 11 September 2017
Received in revised form
20 February 2018
Accepted 23 March 2018
Available online xxx

Keywords:
Propofol
Protein kinase C
PKC translocation

ABSTRACT

Propofol is the most commonly used anesthetic. Immunohistochemical studies have reported that propofol translocated protein kinase Cs (PKCs) in cardiomyocyte in a subtype-specific manner; however detailed features of the propofol-induced translocation of PKCs remain unknown. In this study, we performed real-time observation of propofol-induced PKC translocation in SH-SY5Y cells expressing PKCs fused with a fluorescent protein. Propofol unidirectionally translocated γPKC-GFP, a conventional PKC, and ζPKC-GFP, an atypical PKC, to the plasma membrane and nucleus, respectively, whereas the propofol-induced translocation of novel PKCs was diverse and subtype-specific among δPKC, εPKC and ηPKC. The propofol-induced translocation of εPKC-GFP was especially complicated and diverse, that is, 200 μM propofol first translocated εPKC-GFP to the perinuclear region. Thereafter, εPKC was translocated to the nucleus, followed by translocation to the plasma membrane. Analysis using a mutant εPKC in which the C1 domain was deleted demonstrated that the C1b domain of εPKC was indispensable for its translocation to the perinuclear region and plasma membrane, but not for its nuclear translocation. An in vitro kinase assay revealed that propofol increased the activities of the PKCs activities at the concentration that triggered the translocation. These results suggest that propofol could translocate PKCs to their appropriate target sites in a subtype-specific manner and concomitantly activated PKCs at these sites, contributing to its beneficial or adverse effects.

1. Introduction

Protein kinase C (PKC), a family of serine/threonine kinase, is involved in diverse cellular functions.[1,2] In the central nervous system, PKC plays an important role in neurotransmission by regulating neurotransmitter release and modifying functions of neurotransmitter receptors and ion channels.[2,3]

Among the PKC family, there are more than ten subtypes, which are calcified into three groups, conventional PKCs (cPKCs), novel PKCs (nPKCs) and atypical PKCs (aPKCs), based on the structure of their regulatory domain (Fig. 1).[1,4]

PKC consists of two common domains, a kinase domain and a regulatory domain. The kinase domain functions as a kinase itself, and the regulatory domains accept various stimulants. As a regulatory domain, the C1 domain is known to respond to phospholipids, and the C2 domain binds to calcium ions. The C1 domain is subdivided into two regions, the C1a and C1b domains.

cPKCs have both a C1 and C2 domain; therefore they are activated by membrane-derived phospholipids and Ca^{2+}, which is mobilized into the cytosol from the endoplasmic reticulum (ER).

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Peer review under responsibility of Japanese Pharmacological Society.
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https://doi.org/10.1016/j.jphs.2018.03.008

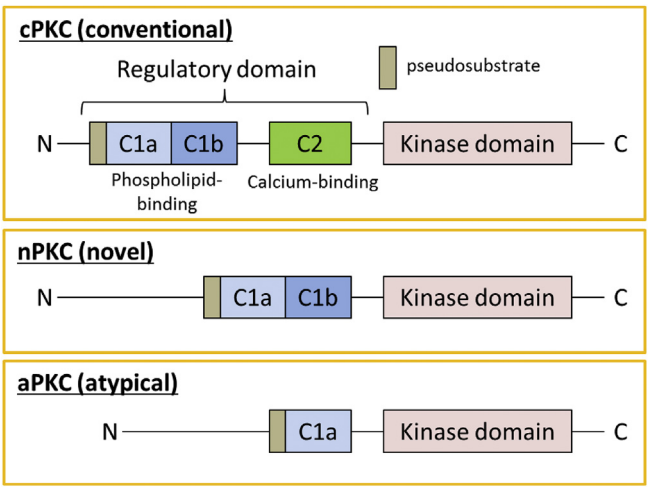

Fig. 1. Structure of PKC families.

nPKCs do not require Ca^{2+} for their activation because nPKCs do not contain a C2 domian.[5] aPKCs only have one C1 subdomain, and Ca^{2+} is not necessary for their activation.[6,7]

The cellular localization of PKCs is altered upon its activation, the phenomenon of which is called "PKC translocation".[8–10] For instance, cPKCs are transiently translocated from the cytosol to the plasma membrane upon the stimulation of G-protein-coupled receptors (GPCRs).[9,10]

In addition to GPCR stimulation, various lipids mediators can trigger PKC translocation, the target sites of which depend on the lipid mediators and the PKC subtypes.[11]{ For example, arachidonic acids, one of the lipid mediators, translocate γPKC to the plasma membrane, εPKC to the Golgi apparatus and ζPKC to the nucleus.[10–12] Ceramide, another lipid mediator produced by sphingomyelinase activation, also translocates δPKC to the Golgi apparatus.[13] These findings lead to the hypothesis that the function of PKC depends on the location of its translocation and what types of substrates the PKC phosphorylates at the translocated sites in case of its activation.[9,10] For this reason, PKC translocation is a very important phenomenon when considering the role of PKC.

Propofol was discovered and clinically applied to anesthesia in 1977 by Kay and Rolly.[14] At present, this anesthetic agent is most commonly used because of its rapid anesthetic induction and reversal. Propofol has been considered to exert its anesthetic effects by binding to the $GABA_A$ receptor, thereby, enhancing the inhibitory effects of GABA on the central nervous system.[15] Regarding the involvement of PKC in the action of propofol, this anesthetic is known to dose-dependently enhance the activity of purified PKC in vitro.[16] Additionally, propofol has been reported to exert its protective effects on ischemia and reperfusion damage via the activation of PKC in the heart.[17,18] Immunoblotting and immunocytochemistry have revealed that propofol translocates various types of PKC to the subcellular region in a subtype-specific manner in cardiomyocytes.[19]

Thus, PKC is hypothesized to be implicated in the exertion of various effects of propofol; however, how propofol induces PKC translocation and activation remains unclear. In this study, we aimed to elucidate the effects of propofol on the translocation of PKC and enzymatic activity. For this purpose, we performed the following studies: 1) an analysis of propofol-induced PKC translocation using PKC-GFP, 2) an examination of the significance of the C1 domain in propofol-induced PKC translocation, and 3) an investigation of the mechanism by which propofol affects PKC activity as a kinase in vitro.

2. Materials and methods

2.1. Materials

Propofol was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). 12-O-Tetradecanoylphorbol 13-acetate (TPA) was purchased from Sigma (St. Louis, MO, USA). [^{32}P]γATP and Protein A Sepharose 4-fast flow were purchased from GE Health Care (Little Chalfont, UK). All of the expression plasmids for PKC-GFPs or mutant PKC-GFP, which have been described throughout the literature,[8,11–13,20] and the polyclonal anti-GFP antibody for immunoprecipitation were purchased from Dr. Saito (Biosignal Research Center, Kobe University). Glass-bottom culture dishes were purchased from MatTek Corporation (Ashland, OR, USA). All of the other chemicals used were of analytical grade.

2.2. Construction of adenovirus vectors for the expression of PKC-GFPs or δPKC-DsRed

To construct adenovirus vectors that can express PKC-GFPs or δPKC-DsRed, we used the pAdEasy system (Agilent Technology, Santa Clara, CA, USA). Briefly, cDNAs for PKCs except δPKC were first inserted into the pEGFP-N1 (Clontech Takara, Japan). Thereafter, these PKC cDNAs combined with EGFP were inserted into the pShuttle-CMV vector. δPKC cDNA was inserted into the pDsRed monomer-N1 (Clontech Takara, Japan), and a δPKC-DsRed fragment was inserted into the pShuttle-CMV. These shuttle vectors were recombined with pAdEasy-1, an adenoviral backbone cosmid vector, in the BJ5183 *E. coli* strain. The recombinant adenoviral genome was digested from the cosmid vector by PacI and transfected into HEK293 cells, which stably expressed the E1 gene and produced the E1 gene-deleted adenoviral vector. Proliferated adenoviral vectors were extracted from HEK293 cells and concentrated by cesium chloride ultracentrifugation. The αPKC, γPKC and δPKC cDNAs originated from human, and the εPKC, ηPKC and ζPKC cDNAs were from rat.

2.3. Construction of plasmids encoding the deletion mutants of εPKC-GFP or γPKC-GFP

The plasmids encoding the deletion mutants of εPKC-GFP were generated as previously described.[12] The constructs encoding GFP-fused rat εPKC were previously described.[11] Briefly, the cDNA encoding deletion mutants of εPKC were generated by PCR using BS 495 (rat εPKC in pCRTM2.1)[11] as the template. The cDNAs encoding domain-deleted εPKCs were produced using the ExSiteTM PCR-based Site-directed Mutagenesis kit (Stratagene, La Jolla, CA) with BS495 as a template. The PCR products for deletion mutants of εPKC were subcloned into the EGFP expression vector (BS340). The plasmids encoding mutant γPKC, the lipid-binding sites in the C1a and C1b domains of which were lost (non-lipid-biding γPKC) were produced as previously described.[4,8]

2.4. Cell culture and transfection

COS-7 and SH-SY5Y cells were purchased from the Riken Cell Bank (Tsukuba, Japan). Human Umbilical Vein Endothelial Cells (HUVECs) were purchased from Sumitomo Dainippon Pharma Co., Ltd. (Osaka, Japan). COS-7 cells were cultured in Dulbecco's modified Eagle's medium (Wako, Osaka, Japan), and SH-SY5Y cells were maintained in Dulbecco's modified Eagle's medium/Ham's F-12 medium (Wako, Osaka, Japan). These media for COS-7 and SH-SY5Y cells contained 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). HUVECs were cultured in EBM-2 medium (CAMBREX, East Rutherford, USA) containing Clonetics

Please cite this article in press as: Miyahara T, et al., Propofol induced diverse and subtype-specific translocation of PKC families, Journal of Pharmacological Sciences (2018), https://doi.org/10.1016/j.jphs.2018.03.008