Progress in Neuro-Psychopharmacology & Biological Psychiatry 63 (2015) 47–53

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

PKC in rat dorsal raphe nucleus plays a key role in sleep–wake regulation

Sheng-Jie Li [1], Su-Ying Cui [1], Xue-Qiong Zhang, Bin Yu, Zhao-Fu Sheng, Yuan-Li Huang, Qing Cao, Ya-Ping Xu, Zhi-Ge Lin, Guang Yang, Xiang-Yu Cui, Yong-He Zhang *

Department of Pharmacology, Peking University, School of Basic Medical Science, Beijing 100191, China

ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form 28 April 2015
Accepted 6 May 2015
Available online 10 May 2015

Keywords:
Dorsal raphe nucleus
Protein kinase C
Serotonin
Sleep
Slow wave sleep

ABSTRACT

Studies suggest a tight relationship between protein kinase C (PKC) and circadian clock. However, the role of PKC in sleep–wake regulation remains unclear. The present study was conducted to investigate the role of PKC signaling in sleep–wake regulation in the rat. Our results showed that the phosphorylation level of PKC in dorsal raphe nucleus (DRN) was decreased after 6 h sleep deprivation, while no alterations were found in ventrolateral preoptic nucleus (VLPO) or locus coeruleus (LC). Microinjection of a pan-PKC inhibitor, chelerythrine chloride (CHEL, 5 or 10 nmol), into DRN of freely moving rats promoted non rapid eye movement sleep (NREMS) without influences on rapid eye movement sleep (REMS). Especially, CHEL application at 5 nmol increased light sleep (LS) time while CHEL application at 10 nmol increased slow wave sleep (SWS) time and percentage. On the other hand, microinjection of $CaCl_2$ into DRN not only increased the phosphorylation level of PKC, but also reduced NREMS time, especially SWS time and percentage. While CHEL abolished the inhibitory effect of $CaCl_2$ on NREMS and SWS. These data provide the first direct evidence that inhibition of intracellular PKC signaling in DRN could increase NREMS time including SWS time and percentage, while activation of PKC could suppress NREMS and reduce SWS time and percentage. These novel findings further our understanding of the basic cellular and molecular mechanisms of sleep–wake regulation.

1. Introduction

Protein kinase C (PKC) is highly expressed within the brain, where it plays important roles in regulating neurotransmission, coordinating intracellular signaling in response to external stimuli, and modulating changes in gene expression and neuronal plasticity (Calabrese and Halpain, 2005; Ramakers et al., 1997; Zarate and Manji, 2009). Intensive studies on circadian clock have provided an insight into the role of PKC in it. Conventional Ca^{2+}-sensitive PKC signaling pathway is not limited to relaying external stimuli but is rhythmically activated by internal processes, forming an integral part of the circadian feedback loop (Robles et al., 2010). The alternative between sleep and arousal is one of the most typical behaviors that have circadian rhythm. The tight relativity between PKC and circadian clock implies the potential role of PKC signaling in sleep–wake regulation.

The PKC family is composed of twelve isozymes that are classified as either conventional, novel, or atypical according to the nature of their regulatory domains. The conventional PKCs (cPKCs) possess a Ca^{2+}-regulated domain that binds Ca^{2+} to be activated. cPKCs are designed to exactly decode two second messengers, Ca^{2+} and signaling lipids. cPKCs reside in the cytosol during resting periods waiting to be triggered by rises in intracellular Ca^{2+} concentration (Lipp and Reither, 2011). Circadian oscillations of free Ca^{2+} have been widely observed (Harrisingh et al., 2007; Imaizumi et al., 2007), which may be involved in the rhythmical activation of PKC. Several reports have revealed that Ca^{2+} oscillations drive oscillations of the activity of cPKCs (Bartlett et al., 2005; Violin et al., 2003). In addition, our recent study has demonstrated that the elevation of calcium function by BAY-K-8644, the L-type calcium channel agonist, and $CaCl_2$ reduces NREMS and REMS, whereas down-regulation of Ca^{2+} function by diltiazem, the L-type calcium channel antagonist, and EGTA, the Ca^{2+} chelating agent in dorsal raphe nucleus (DRN) may promote NREMS especially the SWS percentage in pentobarbital treated rats (Cui et al., 2011a). And pharmacological evidences strongly suggest a correlation between L-type Ca^{2+} channel and serotonergic (5-HT) system in signaling transduction involved in sleep regulation (Cui et al., 2011b).

Based on the important role of PKC on the circadian regulation, the pharmacological actions of calcium in DRN, and the fact that cPKCs can be activated by elevation of intracellular calcium concentration, we hypothesized that the intracellular PKC signaling in the

Abbreviations: CHEL, chelerythrine chloride; cPKCs, conventional PKCs; DRN, dorsal raphe nucleus; EDTA, ethylene diamine tetraacetic acid; EEG, electroencephalogram; EMG, electromyogram; EGTA, ethylene Glycol Tetraacetic Acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LC, locus coeruleus; LS, light sleep; NREMS, non-rapid eye movement sleep; PKC, protein kinase C; REMS, rapid eye movement sleep; SD, sleep deprivation; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SERT, serotonin transporter; SWS, slow wave sleep; TBST, tris buffered saline with tween; TS, total sleep; VLPO, ventrolateral preoptic nucleus.

* Corresponding author. Tel./fax.: +86 10 82801112.
E-mail address: zhyh@hsc.pku.edu.cn (Y.-H. Zhang).

[1] Sheng-Jie Li and Su-Ying Cui contributed equally to the present study.

http://dx.doi.org/10.1016/j.pnpbp.2015.05.005
0278-5846/

DRN may be involved in the regulation of physiological sleep. In the present study, we investigated the role of PKC in DRN on sleep–wake regulation.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (220–240 g, Grade I, purchased from the Animal Center of Peking University, Beijing) were used. All experiments were conducted in accordance with the European Community guidelines for the use of experimental animals and approved by the Peking University Committee on Animal Care and Use. The rats were housed in acrylfiber cages individually and had *ad libitum* access to food and water. They were exposed to a 12:12 h light/dark schedule with lights on at 9:00 A.M. The ambient temperature averaged 23 ± 1 °C and the relative humidity was 50 ± 10%.

2.2. Sleep deprivation

Rats were handled by procedures as reported (Estabrooke et al., 2001). Sleep deprivation was performed by gentle handling while animals were kept in their home cages respectively. The rats were monitored continuously during sleep deprivation. They were aroused by tapping lightly on their cages or touching whenever they appeared to go to sleep. And the rats were never lifted out of the cages. When light tapping did not awaken the animals, crumpled paper was inserted into the cages to stimulate the rats. If needed, louder tapping of the cage was repeated. The deprivation started at light onset (9 A.M.) and lasted for 6 h and the rats were sacrificed at 3 P.M. Control undisturbed rats were sacrificed at the corresponding time point (3 P.M.).

2.3. Surgery

Details of the surgical procedure were described previously (Wang et al., 2014). The animals were implanted chronically with stainless steel screws over the frontal–parietal cortex and a pair of wire electrodes through the nuchal muscles for recording of electroencephalogram (EEG) and electromyogram (EMG), respectively. Additionally, a guide cannula (26 gauge) was implanted 1 mm above the DRN at coordinates, AP = −8.0; L = 0.0 and DV = −5.8 (Paxinos and Watson, 1998). Drug or vehicle was injected into the DRN with an injection cannula (29 gauge), which extended 1 mm beyond the guide, in a 0.2 μl volume over a 2 min period. Histological verification of cannula/injection sites was carried out at the end of the experiments. All the data presented in the sleep recording experiments are derived from animals whose injection site was within the limits of DRN. The location of cannula/injection is shown in Fig. 1B.

2.4. EEG and EMG recordings and analysis

Seven days after implantation the animals were adapted to the recording and injection procedures. For CHEL experiment, recording was started at 9 P.M. and lasted for 6 h during the night phase. For CHEL and $CaCl_2$ combination experiment, recording was started at 9 A.M. and lasted for 6 h during the light phase. Sleep–wake states were manually classified as wakefulness, light sleep (LS), slow wave sleep (SWS) and rapid eye movement sleep (REMS). Non rapid eye movement sleep (NREMS) time equals LS time plus SWS time. Total sleep (LS) time equals NREMS time plus REMS time. The mean number and duration of TS, NREMS, LS, SWS, and REMS were also quantified, as well as LS, SWS and REMS percentage in TS. Details of the EEG and EMG recordings and analysis were described previously (Wang et al., 2014).

2.5. Drugs and drug administration

The following drugs were used in this study: chelerythrine chloride (CHEL), $CaCl_2$ (both from Sigma-Aldrich, St. Louis, USA). CHEL inhibits PKC activation by blocking the site of DAG/phorbol-ester binding and inhibiting PKC translocation to the membrane for activation (Brennan et al., 2009).

CHEL was dissolved in physiological saline and was microinjected into DRN either alone at 9 P.M. (5 or 10 nmol) or 20 min prior to $CaCl_2$ administration at 5 nmol. $CaCl_2$ was dissolved in saline and the pH of the solution was adjusted to 7.3 with NaOH. $CaCl_2$ was microinjected into the DRN at 9 A.M. (25 or 50 nmol). Each agent was microinjected in a volume of 0.2 μl. The control groups in each experiment were microinjected with saline.

2.6. Tissue sample preparation

The procedure was based on our previous study (Wang et al., 2015). For sleep deprivation experiment, immediately after 6 h sleep deprivation (3 P.M.), rats were decapitated, and the brains were quickly removed to a pre-chilled brain matrix. Ventrolateral preoptic nucleus (VLPO), dorsal raphe nucleus (DRN), and locus coeruleus (LC, with some of their surrounding tissue) were punched (1 mm diameter for VLPO, between 0 mm and 1 mm posterior to the bregma, bilaterally; 2 mm diameter for DRN, between 7 mm and 9 mm posterior to the bregma; 2 mm for LC, between 9 mm and 11 mm posterior to the

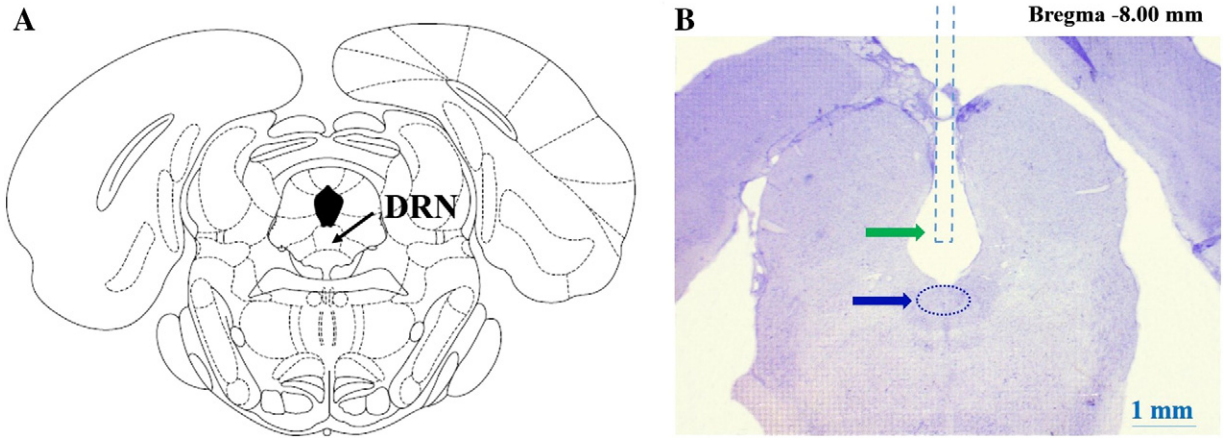

Fig. 1. Photomicrographs of representative cannula placements in DRN. Sections were according to Paxinos and Watson (1998).