Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1040 (2005) 129–136
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Role of protein kinase Cβ in phorbol ester-induced c-*fos* gene expression in neurons of normotensive and spontaneously hypertensive rat brains

Takahiro Amemiya, Toshie Kambe, Ryuji Fukumori, Takao Kubo*

Department of Pharmacology, Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan

Accepted 24 January 2005
Available online 11 March 2005

Abstract

We have previously demonstrated that pressure application of the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA) onto some neurons in the anterior hypothalamic area of rats increases neural activity in vivo and that this PKC activation-induced increase of neural activity is enhanced in spontaneously hypertensive rats (SHR), an animal model for genetic hypertension. Activation of PKC increases expression of the c-*fos* gene, an important transcription factor and proto-oncogene thought to be a marker of neural activity. To evaluate PKC isoforms responsible for neural activation, we examined which isoforms of PKC are involved in the PKC activation-induced c-*fos* gene expression in neuronal cultures of Wistar rat and spontaneously hypertensive rat (SHR) brains. PMA increased c-*fos* gene expression in neuronal cultures of Wistar rat brain and the PMA-induced c-*fos* gene expression was inhibited by the PKC inhibitor 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7). The PKCα,β,γ activator thymeleatoxin also increased c-*fos* gene expression, while the PKCδ,ε activator ingenol did not affect it. In addition, the PMA-induced c-*fos* gene expression was inhibited by PKCβantisense oligonucleotides (AON) but not by PKCα and PKCγAONs. In SHR brain neuronal cultures, the PMA-induced c-*fos* gene expression was enhanced as compared with that of Wistar Kyoto rats (WKY), while basal c-*fos* gene expression was almost the same in both neuronal cultures. The enhancement of PMA-induced c-*fos* gene expression in SHR brain cultures was abolished by PKCβAON. These findings suggest that in rat brain neuronal cultures, PMA increases c-*fos* gene expression via activation of PKC and that PKCβisoforms are partly involved in the PMA-induced c-*fos* gene expression. In neuronal cultures of SHR brain, it appears that the PMA-induced c-*fos* gene expression is also enhanced via PKCβ.
© 2005 Elsevier B.V. All rights reserved.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Second messengers and phosphorylation

Keywords: Protein kinase C; c-*fos*; Phorbol 12-myristate 13-acetate; Neuronal culture; PKCβ; Spontaneously hypertensive rat

1. Introduction

The role of protein kinase C (PKC) in the regulation of many biological function is widely accepted [1,5,11,24]. PKC is also thought to be involved in the pathogenesis of various diseases including hypertension [6,12,31,35]. Molecular cloning and biochemical analysis have revealed that PKC exists as a family of at least eleven isoforms that can be further divided into three groups [25,30]. The first group comprises the α, β1, β2, and γ-isoforms, the second group consists of the δ, ε, η, θ, and μ isoforms, and the third group consists of the ζ and ι isoforms. The second group differs from the first group in that it lacks the Ca^{2+}-binding domain in the amino acid sequences. The first and second groups but not the third group are sensitive to phorbol esters.

Many agonists via their receptors stimulate hydrolysis of inositol phospholipids in neurons [8]. The receptor-mediated hydrolysis of inositol phospholipids leads to the generation of inositol phosphates and diacylglycerol (DAG) which activates PKC. In neurons, PKC is thought

* Corresponding author. Fax: +81 42 721 1588.
E-mail address: kubo@ac.shoyaku.ac.jp (T. Kubo).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.01.073

to be involved in the expression of various genes including the angiotensin II receptor gene [2,5,22]. Indeed, it has been demonstrated that activation of PKC stimulates the expression of the c-*fos* gene, an immediate early gene and a proto-oncogene thought to be a marker of neural activity, and that c-*fos*/c-*Jun* complexes interact with AP-1 site on the promoter to regulate the expression of various genes [29].

We have previously reported that some neurons in the anterior hypothalamic area (AHA) are tonically activated by endogenous angiotensins in rats [10] and that activities of these angiotensin II-sensitive neurons in the AHA are enhanced in spontaneously hypertensive rats (SHR) [14]. Pressure application of the PKC activator phorbol 12-myristate 13-acetate (PMA) onto angiotensin II-sensitive neurons in the AHA of Wistar rats increased their firing rate and the increase of unit activity by PMA was inhibited by the PKC inhibitor 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7) [15]. The increase of unit firing by PMA was enhanced in SHR as compared with Wistar Kyoto rats (WKY), a normotensive control. Pressure application of H-7 alone decreased the basal firing activity of angiotensin II-sensitive neurons in SHR but not in WKY. These findings indicate that activation of PKC increases neural activity in angiotensin II-sensitive neurons in the AHA and that this PKC activation-induced increase of neural activity is enhanced in the AHA of SHR. In this study, we have examined (1) the effect of the PKC activator, PMA, on c-*fos* gene expression and (2) PKC isoforms responsible for the PMA-induced expression of the c-*fos* gene in neuronal cultures of normotensive and spontaneously hypertensive rat (SHR) brains.

2. Materials and methods

One-day-old spontaneously hypertensive rats (SHR/Izm) and Wistar Kyoto rats (WKY/Izm) maintained by Disease Model Cooperate Research Association (Kyoto, Japan) were used in this study. Primary cultures from 1-day-old rat brains were prepared according to the methods of Raizada [26]. Brains were removed from the cranium and placed in isotonic salt solution containing 100 U penicillin G, 100 μg streptomycin, and 0.25 μg of amphotericin B per ml. The brains (brainstem and hypothalamus) were washed and chopped into less than 2-mm^3 chunks. Brain pieces were subjected to trypsin and deoxyribonuclease I treatment. Dissociated cells were suspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U penicillin G, and 100 μg streptomycin per ml, and sedimented at 1000 × *g* for 10 min. Cells (1 × 10^7 cells per dish) suspended in DMEM containing 10% FBS were plated in 100-mm-diam Falcon tissue culture dishes pretreated with poly-L-lysine. Cells were incubated for 3 days at 37 °C in a humidified incubator with 5% CO_2–95% air. Then, the medium was replaced with DMEM containing 10% horse serum, 5% FBS, and 10 μM cytosine arabinoside to inhibit the multiplication of all cells. After 2 days, the medium was replaced with DMEM containing 10% horse serum and 5% FBS, and cells were allowed to grow for further 5 days before being used for the experiments. Histological examination showed that neuronal culture contains 80% neurons and 20% glia-like cells (data not shown).

Phorbol esters were added into DMEM and incubation was started at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. PMA, thymeleatoxin, and ingenol were dissolved in dimethylsulfoxide (DMSO) and added into the medium in a volume of 1 μl/ml. H-7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride, and other drugs were dissolved in physiological saline (0.9% NaCl) and added into the medium in a volume of 1 μl/ml.

2.1. Construction of oligonucleotides

Oligonucleotides were synthesized by Japan Gene Research Laboratory (Tokyo, Japan). They were synthesized, purified by high-performance liquid chromatography, washed with 70% ethanol, and dried. The following sequences were used; PKCα sense (+6/+20): 5′-TCGGGGGGACCATG-3′, PKCα antisense (+20/+6): 5′-CATGGTCCCCCCCGA-3′, PKCβ sense (+4/+18): 5′-GCTGACCCGGTGCG-3′, PKCβ antisense (+18/+4): 5′-CGCAGCCGGTCAGC-3′, PKCγ sense (+5/+19): 5′-CGGGTCTGGGTCCTG-3′, PKCγ antisense (+19/+5): 5′-CAGGACCCAGACCCG-3′. These oligonucleotides were modified by backbone phosphorothioation. Antisense and sense oligonucleotides were incorporated into liposome (DAC-30, Nippon Gene, Tokyo) by shaking.

2.2. Western blot analysis

Western blot analysis was performed as described [20]. Briefly, cells were lysed, homogenized in ice-cold buffer, and equal amounts of proteins (5 μg) were loaded on 10% sodium dodecyl sulphate (SDS)–polyacrylamide gel and transferred to a polyvinylidene defluoride (PVDF) membrane (Millipore, Bedford, MA, USA) for 45 min at 15 V. Membranes were blocked with blocking buffer and incubated for 24 h at 4 °C. Membranes were incubated for 2 h at 37 °C with a rabbit anti-PKCβ1 antibody (Sigma). A biotinylated anti-rabbit IgG was then applied, followed by a streptavidin-biotin-labeled horseradish peroxidase complex. Membranes were then incubated with chemiluminescent reaction solution (ImmunoStar Kit, Wako, Osaka, Japan) exposed to X-ray film.

2.3. RNA analysis

Total RNA was extracted according to the guanidinium thiocyanate/cesium chloride centrifugation method [3], as

Download English Version:

https://daneshyari.com/en/article/9416464

Download Persian Version:

https://daneshyari.com/article/9416464

Daneshyari.com