Japanese Journal of Physiology, **54**, 23–29, 2004

Identification of Caspase-Independent PKCε-JNK/p38 MAPK Signaling Module in Response to Metabolic Inhibition in H9c2 Cells

Yong-Sam JUNG, Yi-Sook JUNG*, Mi-Young KIM*, and Eunhee KIM

Research Center for Biomedicinal Resources and Division of Life Science, PaiChai University, Daejeon 302–735, Korea;
* Department of Physiology, School of Medicine, Ajou University, Suwon 442–749, Korea

Abstract: To understand the molecular mechanism of ischemia-induced cardiac myocyte cell death, H9c2 cells were studied by chemical hypoxia (CH), using metabolic inhibition buffer. CH suppressed the activities of caspase-3, -8, and -9. c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) were activated, whereas extracellular regulated kinase (ERK) was inactivated. Only protein kinase Cε (PKCε) among PKC isotypes was translocated to the membrane fraction implying its activation. Moreover, the administration of PKCε inhibitor suppressed the phosphorylations of JNK/p38 MAPK and reduced CH-induced cell death. An administration of JNK/p38 MAPK inhibitors also decreased CH-induced cell deaths, implying JNK/p38 MAPK's causative roles in the deaths. Collectively, this study identified a novel caspase-independent PKCε-JNK/p38 MAPK signaling module induced by CH in cardiac myocytes. Our data show that the PKCε-JNK/p38 MAPK signaling module contributes to CH-induced H9c2 cell death. This contrasts with previous notions, i.e., PKCε's protective effect against ischemic death. Thus our data suggest that PKCε can mediate alternative signals, i.e., beneficiary or deleterious signals, depending on the cell type, intensity, and/or type of injury. [The Japanese Journal of Physiology 54: 23–29, 2004]

Key words: H9c2, Ischemia, JNK, p38 MAPK, PKCε.

Myocardial cell death following ischemia/reperfusion in the heart is attributed to necrosis and apoptosis caused by ATP depletion, acidosis, and oxidative stress [1, 2]. Necrosis is characterized by cell swelling, rupture, and a rapid release of cytosolic constituents as a result of damage to plasma membrane [3]. Apoptosis is an energy-dependent cell death resulting in caspase activation, chromatin condensation, cell shrinkage, and DNA fragmentation [4]. Regarding myocardial cell death, it is difficult to define the death type because myocardial cells are in different regions and phases of ischemic damage.

The mitochondrial caspase cascade is initiated by cytochrome C release from the mitochondria. The cytochrome C forms apoptosome together with Apaf1 and caspase-9, and subsequently an ATP-dependent activation of caspase-9 occurs [5]. There are several upstream signaling molecules. The ativation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinases (MAPK) contributes to the nucleosomal DNA fragmentation in the heart and the kidney by ischemic damage, whereas the activation of extracellular signal-regulated kinase (ERK1/2) results in protection against cell injury [6, 7]. The intracellular MAPK signaling pathway participates in diverse cellular processes based on cell types and stress conditions.

Ischemic preconditioning (PC) is the phenomenon in which a brief ischemic episode can protect myocardial cells against subsequent lethal ischemia [8, 9]. One effector in this protection has been reported to be the mitochondrial ATP-sensitive K (mitoK_{ATP}) channel [10]. The PC signaling pathway is G-protein coupled receptors → phosphatidylinositol 3′-kinase → pro-

Received on October 28, 2003; accepted on December 11, 2003
Correspondence should be addressed to: Eunhee Kim, Division of Life Science, PaiChai University, 439–6 Doma-2-dong, Seo-gu, Daejeon, 302–735, Korea. Tel and Fax: +82–42–520–5463, E-mail: ehkim@mail.pcu.ac.kr

tein kinase C (PKC)→mitoK_{ATP} [11, 12]. PKCε, a PKC isotype characterized as a calcium-independent and diacylglycerol (DAG)-sensitive serine/threonine kinase, mediates delayed cardioprotection [13]. PC results in the activation of two PKC isoforms, PKCδ and PKCε, and modulates the MAPK pathway [14–18]. However, specific details, such as which MAPK component interplays with PKCs for cardioprotection, are unknown.

In the present study, we present a novel signaling pathway, i.e., a caspase-independent PKCε-JNK/p38 MAPK signaling module in a CH model using metabolic inhibition buffer in heart-derived H9c2 cells. Moreover, this study demonstrates a detrimental effect of PKCε in CH-induced myocardial damage.

METHODS

Cell culture and reagents. The embryonic rat-heart–derived myogenic H9c2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotics (penicillin/streptomycin) at 37°C in humidified 95% air, and 5% CO_2 atmosphere. Go6976, rottlerin, Myr-εV1-2, and Myr-PKCζ were purchased from Biomol Research Laboratories (Plymouth Meeting, PA, USA). SP600125 was obtained from TOCRIS (Ellisville, MO, USA). SB203580 was from Calbiochem (La Jolla, CA, USA).

Metabolic inhibition model. The cells were washed once with phosphate-buffered saline (PBS) before the addition of metabolic inhibition buffer (106 mM NaCl, 4.4 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 38 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 20 mM 2-deoxyglucose, 1 mM NaCN, pH 6.6) [19] and placed in the metabolic inhibition buffer at the indicated time.

Measurement of cell viability and cytotoxicity. Cell viability was quantified by using XTT (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis[4-methoxy-6-nitro] benzene sulfonic acid) assay (Roche, Mannheim, Germany) according to manufacturer instructions. The XTT labeling mixture was measured by using an ELISA reader at 460 nm. The cytotoxicity was assessed by monitoring LDH activity in the extracellular medium. LDH activity was measured colorimetrically according to manufacturer instructions (Promega, Madison, WI, USA). The results were expressed as the percentage (%) of the total LDH activity, assuming the absorbance of control cells to be 100%.

Caspase activity assay. Caspase assay was performed by a modification of the manufacturer's protocols (Peptron, Daejeon, Korea). Briefly, 1×10^5 cells were harvested and resuspended in 50 μl lysis buffer. Enzymatic reactions were carried out at 37°C for 30 min in reaction buffer (200 mM HEPES, pH 7.5, 20% sucrose, 0.2% CHAPS, 10 mM DTT) containing 50 μM Ac-DEVD-AMC and 50 μM Ac-IETD-AMC for caspase-3 and caspase-8, respectively. The release of fluorescent AMC was measured with a Perkin-Elmer LS-50B luminescence spectrometer (Perkin Elmer Life and Analytical Sciences, Inc., Boston, MA, USA) at excitation and emission wavelength, 360 and 460 nm, respectively. The caspase-9 activation assay was performed in the same reaction buffer as the above, containing 50 μM Ac-LEHD-AFC, and the release of fluorescent AFC was measured at excitation and emission wavelength, 400 and 505 nm, respectively.

Translocation of PKC isoforms. The subcellular localization of PKC isoforms was assessed by immunofluorescence by using PKC isoform-specific antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). A Western blot analysis of cytosolic and particulate fractions of cells was carried out as described [20]. Briefly, the cells were washed with PBS and harvested in ice-cold homogenization buffer (20 mM Tris-HCl, 2 mM EDTA, 5 mM EGTA, 5 mM DTT, 6 mM β-mercaptoethanol, 1 mM PMSF, 20 μM leupeptin, and 10 mg/ml aprotinin). The cells were sonicated and centrifuged at 100,000×*g* for 1 h at 4°C. Supernatant was recovered as a cytosolic (soluble) fraction. The pellet was resuspended in 1% Triton X-100 containing homogenization buffer and centrifuged 10,000×*g* for 10 min at 4°C. The supernatant was recovered as a membrane (particulate) fraction. To normalize the differences in protein loading, the blots were reprobed with a monoclonal antibody to actin (Sigma, St. Louis, MO, USA) and cytochrome C oxidase subunit IV (Cox IV, Molecular Probes, Eugene, OR, USA).

MAPK phosphorylation assay. To measure MAPK activity, the cells were washed twice in 1 X PBS and immediately lysed in the mammalian lysis buffer (50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.4 mM phenylmethylsulfonyl fluoride) and sonicated. After sonication, they were centrifuged at 13,000×*g*, and cell debris was removed. Cell extract proteins were incubated at 30°C for 30 min in kinase buffer (20 mM MOPS, 2 mM EGTA, 10 mM $MgCl_2$, 1 mM DTT, 0.1% Triton X-100, 0.1 mM Na_3VO_4, 15 μM ATP, 10 μCi [γ-^{32}P]ATP), the substrate (GST-c-Jun for JNK, GST-ATF-2 for p38 MAPK). SDS sample buffer was added, and proteins were eluted from the beads by boiling for 5 min. After centrifugation, the supernatants were separated on

SDS-PAGE. The phosphorylations of substrates were detected by autoradiography. The activation of ERK1/2 was determined by immunoblot, using anti-phospho-ERK1/2 antibody (Cell Signaling Technology, Beverly, MA, USA).

Statistical analysis. The data were expressed as mean±SEM. Comparisons between groups of data were analyzed by Student's *t*-test. Data analysis and graph generation were performed with Sigmaplot (Chicago, IL, USA). A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Chemical hypoxia (CH)-mediated H9c2 cell injury and death

The incubation of H9c2 cells for increasing time with metabolic inhibition buffer produced a significant decrease in mitochondrial respiration in a time-dependent manner, as demonstrated by the use of XTT assay (Fig. 1). After 10 min of incubation with metabolic inhibition buffer, mitochondrial respiration decreased from 100 (untreated control) to 83.9±1.1% of control, and after 3 h to 13.1±9.3% of control. CH also produced a significant release of LDH into the medium (Fig. 1). LDH release was increased 0.5-fold after 10 min of CH and further elevated to 11-fold after 3 h of CH when compared to the untreated cells.

Caspases were not activated by CH

Caspase assay and Western blot analysis were performed to learn the effect of CH on caspases. The activities of all three caspases decreased as incubation prolonged (Fig. 2A). The characteristic cleavage fragments of caspase-3, -8, and -9 were not detected in the lysates from H9c2 cells during CH (data not shown), and caspase expressions were not changed (Fig. 2B).

Time course of MAPK activations by CH

Time course experiments for CH-induced cell death were performed to determine an appropriate time to evaluate the effect of the MAPKs. H9c2 cells were subjected to an increasing period of CH. JNK, p38 MAPK, and ERK1/2 activities in ischemic H9c2 cells are shown in Fig. 3A. JNK and p38 MAPK were transiently activated in response to CH, but ERK1/2 phosphorylations were decreased in a time-dependent manner. JNK activation was detectable within 10 min of CH and continued to increase, reaching maximal

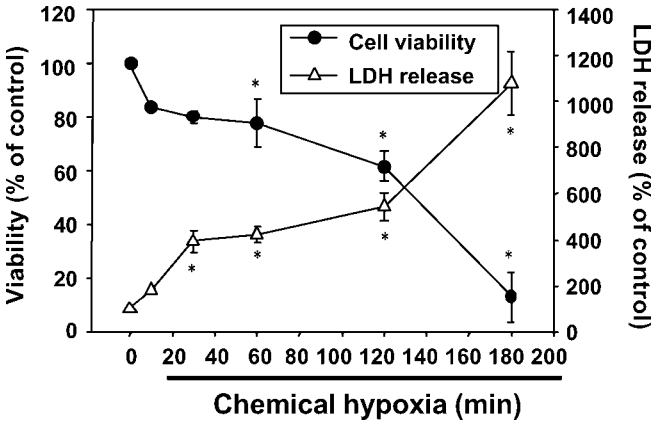


Fig. 1. Time course of cell death induced by chemical hypoxia in heart-derived H9c2 cells. H9c2 cells were plated and grown for 24 h in culture medium. After the desired incubation time (10, 30, 60, 120, 180 min), an XTT assay was performed for cell viability. Data, calculated as % of control values, are the arithmetic mean±SEM of quadruplicate determinations of three independent experiments. * $p<0.05$ vs. time zero.

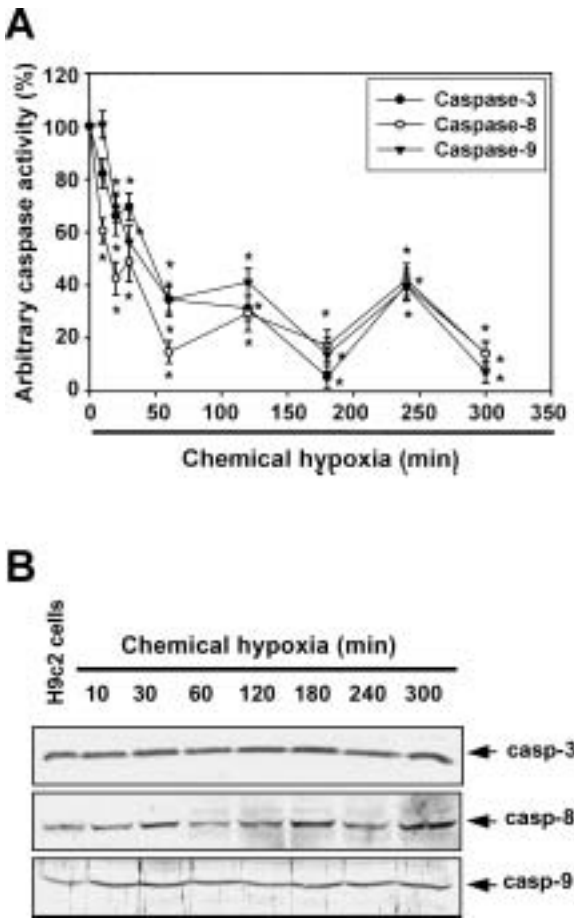


Fig. 2. Caspase activities during chemical hypoxia in H9c2 cells. A: The time course of caspase-3, -8, and -9 activities during chemical hypoxia. The cells were subjected to chemical hypoxia for various periods, as indicated (10, 30, 60, 120, 180, 240, 300 min). The data represent mean±SEM ($n=4$). * $p<0.05$ vs. time zero for each. **B**: H9c2 cells were incubated with metabolic inhibition buffer. After the indicated time, the cells were harvested and analyzed by Western blot for caspase-3, -8, and -9.

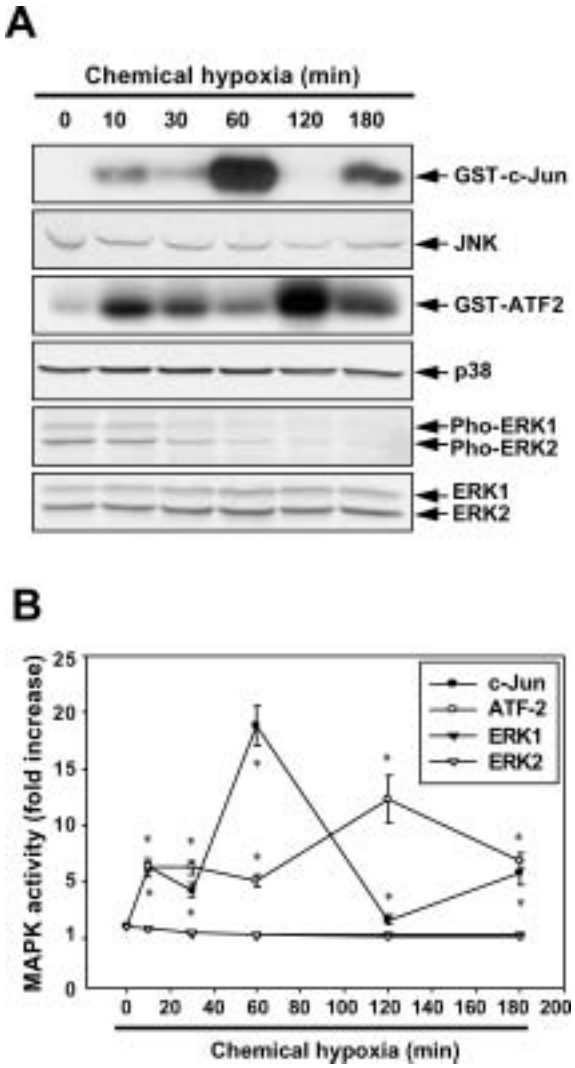


Fig. 3. MAPK activities during chemical hypoxia in H9c2 cells. A: The cells were exposed to chemical hypoxia for various times ranging from 0 to 3 h, as indicated. The activations of JNK and p38 MAPK were examined by *in vitro* kinase assays as described in Methods. The activation of ERK1/2 was determined by Western blot analysis, using anti–phospho-ERK1/2 antibody. **B**: The relative levels of phosphorylated–c-Jun, –ATF-2, and –ERK1/2 were analyzed by densitometry (BAS-1800, Fujiphotofilm, Tokyo, Japan). The data were normalized by setting the control values to 1. The data are means±SEM from two determinations. * $p<0.05$ vs. time zero for each.

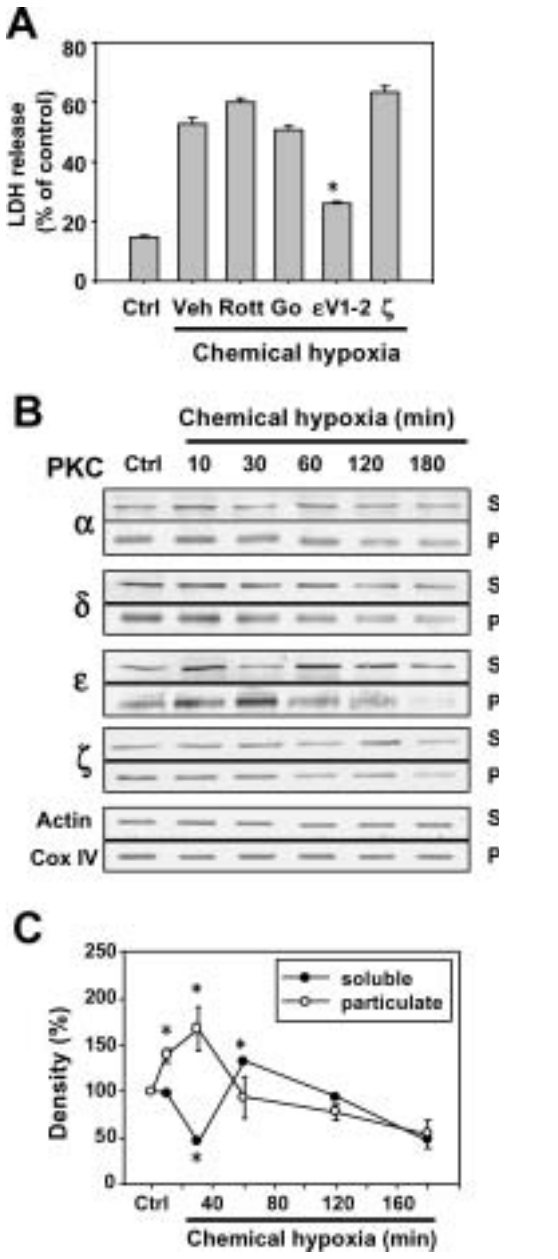
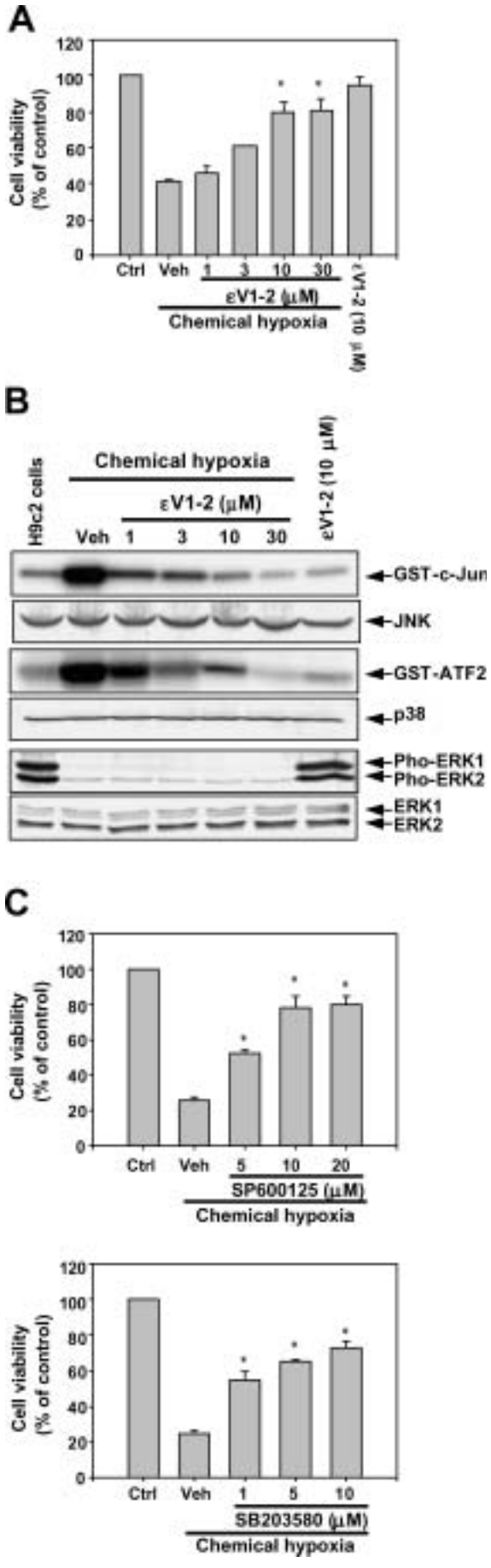


Fig. 4. Modulation of PKC isoforms during chemical hypoxia. A: H9c2 cells were treated with PKC inhibitors before and during chemical hypoxia. LDH activity was measured 2 h after chemical hypoxia. Ctrl, untreated control; Veh, vehicle-treated; Rott, rottlerin (10 μM); Go, Go6976 (1 nM); εV1-2, myristoylated PKCεV1-2 (10μM); ζ, myristoylated PKCζ peptide inhibitor (10μM). * $p<0.01$ vs. CH (Veh). **B**: Western blots for PKC isozymes detected in the soluble (S) and the particulate (P) fractions after various periods of chemical hypoxia (10, 30, 60, 120, 180 min) in H9c2 cells. For an equivalency of loading, actin and Cox IV were used as controls for the cytosolic fraction (S) and the membrane fraction (P), respectively. **C**: Quantitative Western blot analysis for PKCε isozymes in the soluble and the particulate fraction. The data shown as mean±SEM ($n=4$) represent the percentages (%) of the control. * $p<0.05$ vs. each Ctrl.

level at approximately 1 h of CH. A quantitative analysis of phosphorylated c-Jun by densitometry at 1 h of CH revealed an 18-fold increase compared to that of untreated cells (Fig. 3B). However, JNK activation was discontinued dramatically at 2 h of CH and resurrected at 3 h of CH. This phenomenon has been observed consistently by repeated experiments. The p38 activity reached maximum at 2 h of CH (12-fold increase). A significant level of JNK and p38 MAPK activities was still present at 3 h of CH.



Modulation of PKC isoforms during CH

H9c2 cells were treated with PKC inhibitors 30 min before and during CH. As shown in Fig. 4A, the average of LDH release 2 h after CH was 52.8±1.6%. In the myristoylated PKCε inhibitor (εV1-2, 10 μM) treated cells, 2 h of CH-induced increase in LDH release was significantly inhibited (25.8±0.7%). Inhibitors for other PKC isoforms such as Go6976 (inhibitor for α- and β-isoforms), rottlerin (inhibitor for δ-isoform), and Myr-PKCζ (inhibitor for ζ-isoform) had no effect on CH-induced LDH release. During CH, PKCε was translocated from the soluble to the particulate fraction, whereas PKCα, PKCδ, and PKCζ remained unaffected by CH (Fig. 4B). PKCε was translocated as early as 10 min after CH and persisted up to 30 min of CH (Fig. 4C). Thus the data show that PKCε activation and translocation cause an increase of CH-induced cell death by MAPK activity modulation.

Link between PKCε and JNK/p38 MAPK in ischemic cardiomyocytes

This study examined the presence of a putative link between PKCε and MAPKs. Cell viability was measured by using XTT assay. In the presence of εV1-2 (10 μM), mitochondrial respiration increased to 80±5.7%, from 41.1±0.7% (Fig. 5A). In the presence of εV1-2, a CH-induced increase in JNK and p38 MAPK activities was inhibited significantly, whereas ERK1/2 activity was not affected in ischemic H9c2 cells (Fig. 5B). A Western blot analysis confirmed no change in total protein expression for MAPKs. To examine the effect of inhibition of MAPKs upon CH, we subjected H9c2 cells to a metabolic inhibition buffer with MAPK inhibitors (Fig. 5C). In the presence of the JNK inhibitor SP600125 (10 μM), mitochondrial

Fig. 5. JNK/p38 MAPK activations by PKCε in chemical hypoxia. H9c2 cells were pretreated with the indicated concentration of εV1-2 for 30 min before an onset of chemical hypoxia. **A**: Cell viability of H9c2 cells after chemical hypoxia. Cell injury was evaluated from changes in XTT reduction, as described in Methods. All data shown as mean±SEM (n=4) were calculated as a percentage (%) of the control. **B**: The JNK and p38 MAPK activities were measured by using GST-c-Jun and GST-ATF-2, respectively, as described above. The autoradiographs shown are a representative of at least two separate experiments. **C**: The H9c2 cells were incubated with a JNK specific inhibitor (SP600125) and a p38 MAPK inhibitor (SB203580) for 30 min before and during CH, and they were analyzed by XTT assay. The data shown as mean±SEM (n=4) represent the percentage of viable cells of control. Ctrl, untreated; Veh, vehicle-treated. * p<0.05 vs. each Veh.

respiration increased from 25.6±1.8% to 77.8±6.9%. The administration of SB203580 (p38 MAPK inhibitor, 10 μM) during CH also markedly increased the mitochondrial respiration (to 72.9±3.4%, from 25.1±1.6%). These data suggest that JNK and p38 MAPK play causative roles for the induction of cell death in response to CH. Taken together, these findings indicate that PKCε triggers the activation of downstream MAPKs, JNK, and p38 MAPK during CH in H9c2 cells.

DISCUSSION

This study identified a novel caspase-independent PKCε-JNK/p38 MAPK signaling module in response to CH in H9c2 cells. This study disclosed specific interplay between a specific PKC isoform and MAPKs in CH.

The failure of caspase-3 activation is understandable because two major upstream caspases, caspase-8 and -9, were not activated. Caspase-9 inactivation might come from the depletion of ATP, an essential component of apoptosome. However, the reason for caspase-8 inactivation is not clear.

MAPKs are important mediators of intracellular signaling. ERK activation has been implicated in the regulation of cell growth and survival. The activation of JNK and p38 MAPK with a concurrent inhibition of ERK1/2 was found to induce apoptosis in PC12 cells and neonatal cardiomyocytes [21, 22]. However, the elevation of JNK/ p38 MAPK activities and a concurrent decrease of ERK1/2 activities did not result in a typical apoptosis in CH-induced H9c2 cells. Thus other factors seem to be required for the execution of apoptosis.

PKC has been recognized as a key regulatory enzyme in ischemic cardiac physiology. In these studies we show that the inactivation of PKCε renders cells resistant to the CH, which is accompanied with a significant reduction of JNK/p38 MAPK. This suggests that the protective effect of εV1-2 is mediated, at least in part, by the suppression of CH-induced JNK/p38 MAPK activity. To our knowledge, this is the first report demonstrating PKCε involvement in chemical hypoxia-induced myocardial cell death. The results of this study implicated a causative role of PKCε in the CH-induced cell death, which contrasts to literature documenting a beneficial role of PKCε in some conditions [23–25]. However, there is also evidence consistent with our present result that suggests a detrimental role of PKCε in myocytes and NIH3T3 cells [26, 27]. Thus PKCε's pathophysiological role seems to be complicated and might be influenced by other factors, such as switch proteins, which determine the direction that a PKCε signal should be propagated to.

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (02-PJ1-PG10-20905-0007).

REFERENCES

1. Gottlieb RA, Burleson KO, Kloner RA, Babior BM, and Engler RL: Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 94: 1621–1628, 1994
2. Saraste A, Pulkki K, Kallajoki M, Henriksen K, Parvinen M, and Voipio-Pulkki LM: Apoptosis in human acute myocardial infarction. Circulation 95: 320–323, 1997
3. Sperandio S, de Belle I, and Bredesen DE: An alternative, nonapoptotic form of programmed cell death. Proc Natl Acad Sci USA 97: 14376–14381, 2000
4. William GT and Smith CA: Molecular regulation of apoptosis: genetic controls on cell death. Cell 74: 777–779, 1993
5. Adrain C and Martin SJ: The mitochondrial apoptosome: a killer unleashed by the cytochrome seas. Trends Biochem Sci 26: 390–397, 2001
6. Tournier C, Hess P, Yang DD, Xu J, Turner TK, Nimnual A, Bar-Sagi D, Jones SN, Flavell RA, and Davis RJ: Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. Sience 288: 870–874, 2000
7. Yue TL, Wang C, Gu JL, Ma XL, Kumar S, Lee JC, Feuerstein GZ, Thomas H, Maleeff B, and Ohlstein EH: Inhibition of extracellular signal-regulated kinase enhances Ischemia/Reoxygenation-induced apoptosis in cultured cardiac myocytes and exaggerates reperfusion injury in isolated perfused heart. Circ Res 86: 692–699, 2000
8. Murry CE, Jennings RB, and Reimer KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74: 1124–1136, 1986
9. Speechly-Dick ME, Baxter GF, and Yellon DF: Ischaemic preconditioning protects hypertrophied myocardium. Cardiovasc Res 28: 1025–1029, 1994
10. Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, and Grover GJ: Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels. Possible mechanism of cardioprotection. Circ Res 81: 1072–1082, 1997
11. Ping P, Zhang J, Qiu Y, Tang XL, Manchikalapudi S, Cao X, and Bolli R: Ischemic preconditioning induces selective translocation of protein kinase C isoforms ε and η in the heart of conscious rabbits without subcellular redistribution of total protein kinase C activity. Circ Res 81: 404–414, 1997
12. Li Y and Sato T: Dual signaling via protein kinase C and phosphatidylinositol 3′-kinase/Akt contributes to bradykinin B2 receptor–induced cardioprotection in guinea pig hearts. J Mol Cell Cardiol 33: 2047–2053, 2001
13. Ohno S, Akita Y, Konno Y, Imajoh S, and Suzuki K: A novel phorbol ester receptor/protein kinase, nPKC, dis-

tantly related to the protein kinase C family. Cell 53: 731–741, 1988
14. Ping P, Zhang J, Cao X, Li RC, Kong D, Tang XL, Qiu Y, Manchikalapudi S, Auchampach JA, Black RG, and Bolli R: PKC-dependent activation of p44/p42 MAPKs during myocardial ischemia-reperfusion in conscious rabbits. Am J Physiol 276: H1468–H1481, 1999
15. Ping P, Zhang J, Huang S, Cao X, Tang XL, Li RC, Zheng YT, Qiu Y, Clerk A, Sugden P, Han J, and Bolli R: PKC-dependent activation of p46/p54 JNKs during ischemic preconditioning in conscious rabbits. Am J Physiol 277: H1771–H1785, 1999
16. Baines CP, Zhang J, Wang GW, Zheng YT, Xiu JX, Cardwell EM, Bolli R, and Ping P: Mitochondrial PKCε and MAPK form signaling modules in the murine heart: enhanced mitochondrial PKCε-MAPK interactions and differential MAPK activation in PKCε-induced cardioprotection. Circ Res 90: 390–397, 2002
17. Kawamura S, Yoshida K, Miura T, Mizukami Y, and Matsuzaki M: Ischemic preconditioning translocates PKC-δ and -ε, which mediate functional protection in isolated rat heart. Am J Physiol 275: H2266–H2271, 1998
18. Gray MO, Karliner JS, and Mochly-Rosen D: A selective ε-protein kinase C antagonist inhibits protection of cardiac myocytes from hypoxia-induced cell death. J Biol Chem 272: 30945–30951, 1997
19. Gottlieb RA, Gruol DL, Zhu JY, and Engler RL: Preconditioning rabbit cardiomyocytes: role of pH, vacuolar proton ATPase, and apoptosis. J Clin Invest 97: 2391–2398, 1996
20. Johnson JA and Mochly-Rosen D: Inhibition of the spontaneous rate of contraction of neonatal cardiac myocytes by protein kinase C isozymes. A putative role for the ε isozyme. Circ Res 76: 654–663, 1995
21. Xia Z, Dickens M, Raingeaud J, Davis RJ, and Greenberg ME: Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270: 1326–1331, 1995
22. Wang Y, Huang S, Sah VP, Ross J Jr, Brown JH, Han J, and Chien KR: Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. J Biol Chem 273: 2161–2168, 1998
23. Moon CH, Jung YS, Kim MH, Park RM, Lee SH, and Baik EJ: Protein kinase C inhibitors attenuate protective effect of high glucose against hypoxic injury in H9c2 cardiac cells. Jpn J Physiol 50: 645–649, 2000
24. Matsushima H, Shimohama S, Chachin M, Taniguchi T, and Kimura J: Ca^{2+}-dependent and Ca^{2+}-independent protein kinase C changes in the brain of patients with Alzheimer's disease. J Neurochem 67: 317–323, 1996
25. Di-Capua N, Sperling O, and Zoref-Shani E: Protein kinase C-ε is involved in the adenosine-activated signal transduction pathway conferring protection against ischemia-reperfusion injury in primary rat neuronal cultures. J Neurochem 84: 409-412, 2003
26. Shizukuda Y and Buttrick PM: Subtype specific roles of β-adrenergic receptors in apoptosis of adult rat ventricular myocytes. J Mol Cell Cardiol 34: 823–831, 2002
27. Lee YJ, Soh JW, Jeong DI, Cho CK, Jhon GJ, Lee SJ, and Lee YS: PKCε-mediated ERK1/2 activation involved in radiation-induced cell death in NIH3T3 cells. Biochim Biophys Acta 1593: 219–229, 2003