Effects of metabolic inhibition on phosphorylation levels of PKC isoforms in the guinea pig taenia caeci

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Abstract

We investigated which isoform of protein kinase C (PKC) is responsible to metabolic inhibition in the guinea pig taenia caeci with respect to their phosphorylation levels. By Western blot analysis using isoform-specific antibodies, at least four isoforms of PKC, α, β2, ε and ζ were identified in the taenia. Prolonged metabolic inhibition of hypoxia, hypoxia+glucose depletion, and addition of cyanide (all in the presence of high K+) for more than 60 min, but not glucose-depletion only, elicited dephosphorylation of PKCs, α, β2 and ε, except ζ. Ca2+ depletion from the medium prevented the dephosphorylation of PKCs induced by hypoxia, and apparently inhibited the dephosphorylation induced by hypoxia+glucose depletion. Acute treatment with hypoxia for 10–30 min elicited a gradual dephosphorylation of PKCβ2, but not of other tested PKC isoforms. Considering the ATP level under various metabolic conditions reported previously, PKCβ2 is suggested to be primarily responsible to hypoxia, and its dephosphorylation is closely associated with the alteration of adenylate compounds in the cell. Re-oxygenation after prolonged hypoxia did not restore the phosphorylation level of any tested PKCs, suggesting that the dephosphorylation of PKCs is associated with the irreversible damage of the cell under hypoxia. Presumably, the dephosphorylation of PKCs, particularly PKCβ2, plays a role in the signal transduction pathway under metabolic inhibition of the taenia, as reported in proliferative and pathophysiological processes in many other cells.

Key words: PKC isoform, PKCβ2, hypoxia, glucose-depletion, taenia caeci

Introduction

Smooth muscle responds to hypoxia mostly with relaxation, except the pulmonary artery with hypoxic constriction and some arteries with endothelium-dependent vasoconstriction. The relaxing effect of hypoxia on contractile responses differs among different smooth muscle types (Paul et al., 1987). The imposition of hypoxia in the presence of high concentration (more than
40 mM) of K+ by bubbling nitrogen instead of oxygen rapidly abolishes the isometric force of the guinea pig taenia caeci (Ishida et al., 1984; Ishida and Paul, 1990), but slowly in the rate and marginal in the magnitude of relaxation of conduit arteries of hog coronary artery (Paul et al., 1987) and rabbit aorta (Coburn et al., 1979). Therefore, the contractile response of the taenia caeci is highly sensitive to the presence of oxygen compared with those of other smooth muscle types. Since the relaxation response of the taenia to hypoxia occurred without a significant reduction in the phosphorylation level of myosin regulatory light chain (MRLC), the energy limitation for the contractile apparatus has been proposed for the hypoxia-induced relaxation of the taenia (Obara et al., 1997).

Recently, the role of second messengers in smooth muscle function has been more precisely understood. Among messengers, protein kinase C (PKC) has been reported to be capable of regulating smooth muscle contractility at the contractile apparatus through 1) the regulation via interaction with thin filament-associated proteins of caldesmon and calponin, 2) the activation of myosin light chain kinase activity via the interaction with ERK1/2-ILK pathway, p38/HSP27-ZIP kinase pathway, 3) the direct regulation of phosphorylation MRLC, and 4) the inhibition of myosin phosphatase activity via the interaction with CPI-17 (Morgan and Gangopadhyay, 2001; Bitar, 2003; Harnett and Biancani, 2003). Also, in the taenia caeci, PKC has been reported to regulate its contraction, for example, muscarinic M2 receptor-mediated contraction (Shen and Mitchelson, 1998), inhibition of high K+ (45 mM KCl)-induced contraction due to addition of 100 µM carbachol (Mitsui-Saito and Karaki, 1996). Since many isoforms are identified in PKC, individual isoform has been implicated to take a specific role in many cellular responses. Further, phosphorylation state and cellular localization are now thought to be key determinants of isoform activity and specificity (Dempsey et al., 2000; Parekh et al., 2000). Thus, we here investigated which isoform of PKC is responsible for hypoxic conditions with respect to the phosphorylation level of PKC and attempted to understand the relation between levels of PKC phosphorylation and relaxation responses to hypoxia in the taenia caeci. Present experiments firstly showed that dephosphorylation of PKCβ2 is most sensitive to hypoxia, while the onset of dephosphorylation lagged behind the relaxation response in the taenia caeci.

**Methods**

**Tissues preparation**

Male guinea pigs weighing 300–400 g were stunned and exsanguinated. The smooth muscle of the taenia caeci was dissected from the caecum and cut into strips about 30 mm long. Strips were incubated in a physiological salt solution: NaCl 120, KCl 5.4, CaCl₂ 2.0, MgCl₂ 1.0, Na₂HPO₄ 1.3, NaHCO₃ 24 and glucose 5.6 (mM). The solution was maintained at 37°C and bubbled with 95% O₂: 5% CO₂ for aerobic conditions or 95% N₂: 5% CO₂ for hypoxic conditions (pH 7.4).

For preparing the muscle extracts to detect PKC isoforms, the muscles were frozen in liquid nitrogen promptly after the incubation in desired conditions and stored at –80°C until use. Frozen muscles were pulverized with a pulverizer (Mikro-Dismembrator, B. Braun Biotech,
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Melsungen, Germany), and homogenized using the Dawns glass homogenizer with 5 volumes of RIPA buffer: 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% nonidet P40, 0.5% deoxycholic acid, 0.1% SDS, 10 µg/ml protease inhibitors (leupeptin, antipain, pepstatin A, chemostatin and phosphoramidon), 250 µM p-amidinophenylmethanesulfonyl fluoride, 10 nM calyculin A, 0.32 µM okadaic acid, 1 mM sodium orthovanadate, 50 µM bisperoxovanadium, 1 mM dithiothreitol and 10 µM calpain inhibitor I. Homogenized samples were further sonicated for 5 min, then centrifuged at 15,000 g for 30 min at 4°C, and supernatants were served as a cellular fraction to be subjected to SDS-PAGE. Protein concentration was determined by bicinchoninic acid using a kit (Pierce, Rockford, IL, USA). The animal treatment and the experimental protocol were approved by the Animal Welfare Committee of Mitsubishi Kagaku Institute of Life Sciences.

Western blotting

Protein extracts of the taenia caeci were incubated with SDS-PAGE sample buffer (2% SDS, 10% glycerol and 62.5 mM Tris-HCl, pH 6.8), then separated by 7.5% SDS-PAGE for Western blotting. Proteins on the SDS-PAGE gel were transferred to nitrocellulose membranes (400 mA, 45 min). Membranes were blocked in 5% BSA/TBST (TBS containing 0.1% Tween-20) for 2 h at room temperature. Membranes were incubated with rabbit anti-PKCα antibody (Sigma, St. Louis, MO, USA), monoclonal anti-PKCβ1 antibody (Sigma), monoclonal anti-PKCβ2 antibody (Sigma), rabbit anti-PKCδ antibody (Sigma), rabbit anti-PKCε antibody (Sigma), and rabbit anti-PKCζ antibody (Sigma) for 2 h at room temperature or overnight at 4°C. For internal standard of the immunosignal, monoclonal mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Research Diagnostic, Flanders, NJ, USA) or rabbit anti-14-3-3β antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used. Then, membranes were incubated with anti-rabbit IgG horse radish peroxidase (HRP)-linked antibody (Cell Signaling Technology, Beverly, MA, USA) or anti-mouse IgG (Fab specific) HRP-linked antibody (Sigma) in 5% BSA/TBST for 1 h at room temperature. Signals were visualized with the enhanced chemiluminescence detection reagent.

Results

Effects of metabolic inhibition on the electrophoretic mobility of PKC isoforms

To identify PKC isoforms expressed in the guinea pig taenia caeci, homogenates prepared from the muscle were subjected to SDS-PAGE followed by Western blotting using antibodies against PKC isoforms of α, β1, β2, δ, ε and ζ. The specific immunoreactivities against PKC isoforms of α, β2, ε and ζ were detected in the extracts of the taenia exposed to aerobie conditions, and their molecular masses were around 80 kDa, being consistent with the molecular mass of PKCs (Fig. 1). However, the significant immunosigals for PKCs of β1 and δ were not detected in the extracts of the taenia with used antibodies at the present experimentaldata not shown). These results indicate that at least four PKC isoforms of α, β2, ε, and ζ are expressed in the taenia caeci.

The taenia caeci develop the maximum tension in the presence of high concentration (45.4 mM) of K+ (high K+) (Ishida et al., 1984; Ishida and Paul., 1990; Urakawa and Holland, 1964)
indicating that the muscle consumes energy nearly maximally. To clearly observe the effects of metabolic inhibiting conditions, the taenia caeci were exposed to hypoxic conditions with bubbling N₂ instead of O₂ for 120 min (N₂ 120 min), glucose-depleted medium under normoxia for 120 min (Glc depl 120 min), conditions of hypoxia and glucose depletion for 120 min (N₂ Glc depl 120 min), 5 mM NaCN for 60 and 120 min (CN 60 min and CN 120 min, respectively), all in the presence of high concentration (45.4 mM) of K⁺ (high K⁺). Ca²⁺-free solution was made by a removal of Ca²⁺ from the medium and an addition of 5 mM EGTA to the medium. For Western blotting, muscle extracts were loaded onto 7.5% SDS-PAGE. PKC isoforms were detected using antibodies of rabbit anti-PKCα antibody, monoclonal anti-PKCβ² antibody, rabbit anti-PKCε antibody, and rabbit anti-PKCs antibody. Control electrophoretic pattern of proteins were shown as O₂ 120 min, that represents the exposure of the muscle to high K⁺ under normoxia for 120 min. For internal standard, monoclonal mouse anti-GAPDH antibody were used.

Fig. 1. Using Western blotting, effects of metabolic inhibiting conditions in the presence of 2.5 mM Ca²⁺ (A) and the absence of Ca²⁺ (B) on the electrophoretic mobility of PKC isoforms of α, β², ε and ζ in taenia caeci isolated from the guinea pig. For treatments with metabolic inhibition, the taenia caeci were exposed to hypoxic conditions with bubbling N₂ instead of O₂ for 120 min (N₂ 120 min), glucose-depleted medium under normoxia for 120 min (Glc depl 120 min), conditions of both hypoxia and glucose depletion for 120 min (N₂ Glc depl 120 min), 5 mM NaCN for 60 and 120 min (CN 60 min and CN 120 min, respectively), all in the presence of high concentration (45.4 mM) of K⁺ (high K⁺). Ca²⁺-free solution was made by a removal of Ca²⁺ from the medium and an addition of 5 mM EGTA to the medium. For Western blotting, muscle extracts were loaded onto 7.5% SDS-PAGE. PKC isoforms were detected using antibodies of rabbit anti-PKCα antibody, monoclonal anti-PKCβ² antibody, rabbit anti-PKCε antibody, and rabbit anti-PKCs antibody. Control electrophoretic pattern of proteins were shown as O₂ 120 min, that represents the exposure of the muscle to high K⁺ under normoxia for 120 min. For internal standard, monoclonal mouse anti-GAPDH antibody were used.

Effects of metabolic inhibiting conditions on the phosphorylation level of PKCs were analyzed using SDS-PAGE (7.5% of acrylamide in the separation gel) followed by Western blotting (Fig. 1), since the non-phosphorylated form has a lower molecular mass and moves faster than the phosphorylated form on the gel (Keranen et al., 1995; Dutil et al., 1994; Borner et al., 1989; Zhang et al., 1994). When the taenia caeci were kept in normal conditions, PKC isoforms were detected as a single band with a relatively high molecular mass of 80 kDa. Exposure of the taenia to hypoxia for 120 min elicited an increase in the antibody signal for the lower molecular mass (76 kDa) of PKCs, α, β² and ε, but not ζ, indicating that hypoxia induces dephosphorylation of these PKC isoforms (Fig. 1A). The extents of dephosphorylation were approximately 60% of total signal (phosphorylated form + non-phosphorylated form) in PKCβ², 10% in α and 30% in ε (number of experiments 2–4). Thus, PKCβ² appeared to be mostly
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Similarly to hypoxia, treatment of the taenia with cyanide (5 mM) elicited dephosphorylation of PKC isoforms of \( \alpha, \beta_2 \) and \( \varepsilon \) faintly at 60 min and clearly at 120 min after hypoxia (Fig. 1A). Also, the extent of dephosphorylation was greatest in PKC\( \beta_2 \), medium in PKC\( \varepsilon \) and least in PKC\( \alpha \). The electrophoretic mobility of PKC\( \zeta \) was not affected by the cyanide-treatment.

When the taenia caeci were exposed to the strong metabolic inhibiting condition of hypoxia+glucose depletion for 120 min, detected PKC isoforms, except \( \zeta \), were more prominently dephosphorylated in comparison with the effects of hypoxia only (Fig. 1A). PKCs of \( \alpha \) and \( \varepsilon \) were dephosphorylated by more than 60%. In the immunosignal for PKC\( \beta_2 \), the signal for high molecular mass seemed to disappear, instead the one for low molecular mass appeared in this strong metabolic inhibiting condition. Therefore, PKC\( \beta_2 \) seems to be entirely shifted down to the dephosphorylated form in hypoxia+glucose depletion. These results also suggest that the PKC isoform of \( \beta_2 \) is more sensitive to the hypoxia-related metabolic inhibition than those of \( \alpha, \varepsilon \) and \( \zeta \).

In contrast, treatment of the taenia with another metabolic inhibition, glucose depletion under aerobic conditions in the presence of high-K\(^+\) for 120 min, did not elicit any apparent effect on the electrophoretic mobility of tested PKC isoforms of the taenia (Fig. 1A). PKCs of \( \alpha, \beta_2 \) and \( \varepsilon \) stayed in the phosphorylated level. Either, the mobility of PKC\( \zeta \) was not affected by the glucose depletion.

Figure 1B shows the effects of Ca\(^{2+}\)-depletion from the external medium on phosphorylation levels of PKC isoforms of the taenia exposed to various metabolically inhibited conditions. For Ca\(^{2+}\)-depletion, Ca\(^{2+}\) was removed from the medium and 5 mM EGTA was added to the medium containing high K\(^+\). The contractile activity of the taenia was lost in this Ca\(^{2+}\)-depleted medium (data not shown). In the absence of Ca\(^{2+}\), exposure of the taenia to hypoxia for 120 min did not change the electrophoretic mobility of all tested PKC isoforms, and their immunosignals stayed at the phosphorylated level as that under normoxia. Treatment with cyanide for 60 and 120 min only elicited a marginal increase in dephosphorylation of PKC \( \alpha, \beta_2 \) and \( \varepsilon \), but the extent of dephosphorylation is much smaller in the absence of Ca\(^{2+}\) than in the presence of Ca\(^{2+}\). Exposure of the taenia to glucose depletion for 120 min did not elicit any shift in electrophoretic mobility of PKC isoforms either in the absence or presence of external Ca\(^{2+}\). The exposure to the strong metabolic inhibition, hypoxia+glucose-depletion, for 120 min in the absence of Ca\(^{2+}\) elicited an appreciable shift towards the dephosphorylated form in electrophoretic mobility of PKCs \( \alpha, \beta_2 \) and \( \varepsilon \), but the extent of dephosphorylation was apparently smaller when compared with that in the presence of external Ca\(^{2+}\). Therefore, Ca\(^{2+}\)-depletion from the external medium generally attenuated the metabolic inhibition-induced dephosphorylation of PKC isoforms in the taenia. In addition, hypoxia for 120 min did not elicit the shift of any tested PKC isoforms in the normal medium (2 mM Ca\(^{2+}\) without high-K\(^+\) (data not shown), suggesting that PKCs in the relaxed taenia are insensitive to hypoxia.

Acute effects of hypoxia on dephosphorylation of PKC isoforms of the taenia

Since preliminary experiments showed the dephosphorylation of PKC\( \beta_2 \) of the taenia
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exposed to hypoxia for 30 min, muscles were exposed to hypoxia with high K' relatively short term for 3, 5, 10, 15, and 30 min, and the electrophoretic mobility of PKC isoforms was analyzed by Western blotting (Fig. 2). Exposure of the muscle to hypoxia for 3 and 5 min had no effect on the electrophoretic mobility of tested PKC isoforms. Further, during 30 min after hypoxia, PKCs of α, ε and ζ were detected as a single band at the equivalent place of those for aerobic conditions. On the other hand, the immunosignal for fast migrated form of PKCβ2 was slightly observed at 10 min, 20% of total isoform at 15 min, and 50% at 30 min after imposition of hypoxia. These results clearly shows that PKCβ2, but not other tested isoforms, of the taenia is gradually dephosphorylated in the acute phase after hypoxia + high-K'. Also, among tested isoforms, PKCβ2 appears to be most sensitive to hypoxia in dephosphorylation, being corroborated with the above results of the most extensive dephosphorylation in PKCβ2 under prolonged hypoxia for 120 min.

Effects of reoxygenation on the electrophoretic mobility of PKC isoforms

The taenia caeci were exposed to hypoxia with high K' for 120 min and again oxygenated for 15 min (reoxygenation), and the electrophoretic mobility of PKC isoforms was analyzed by Western blotting (Fig. 3). Hypoxia for 120 min elicited the dephosphorylation of PKCs, α, β2 and ε, being consistent with the results shown in Fig. 1. The following reoxygenation for 15 min did not significantly change the electrophoretic pattern of tested PKC isoforms: the extent of dephosphorylated isoform after reoxygenation was remained nearly at the same level as that in hypoxia right before reoxygenation. During treatments with hypoxia followed by reoxygenation, PKCζ always appeared as a single band at the constant place without change in mobility (data not shown). These results indicate that PKC isoforms of α, β2 and ε, but not ζ, in the taenia contracted with high K' are dephosphorylated by the prolonged hypoxia in a relatively irreversible manner.
Discussion

In the present experiments using the Western blot method, PKC isoforms of α, β2, ε and ζ were identified in the taenia caeci of the guinea pig. The presence of multiple isoforms in the muscle suggests that the signal transduction in the taenia can be regulated in a PKC isoform-dependent manner, since the activation of PKC in the taenia was diversely reported to be inhibitory in spontaneous activities of membrane potential and contraction (Baskakov et al., 1987), both inhibitory in response of [Ca^{2+}], and stimulatory in sensitization of contractile protein (Mitsui and Karaki, 1993) and stimulatory in carbachol-induced activation of diacylglycerol kinase (Nobe et al., 1997). Also in other smooth muscles, Obara et al. (1999) reported the association of PKCδ with endothelin-1-induced contraction of the porcine coronary artery, and the association of PKCα with the inhibition of K_{Ca} channel leading to contraction of the canine basilar artery (Obara et al., 2002).

The presently detected PKC isoforms of the taenia were also differently responded to metabolic stress conditions in increased electrophoretic mobility on SDS-PAGE. PKCβ2 was the most sensitive isoform to hypoxia and hypoxia+glucose depletion, PKCε was moderately, PKCα was least sensitive, and PKCζ was totally insensitive. Therefore, PKCβ2 of the taenia is shown to be the primarily responsible isoform to the metabolic stress, particularly to hypoxia.

The increased electrophoretic mobility of PKCs under hypoxia and hypoxia+glucose depletion in the presence of high K^{+} may be caused by dephosphorylation or facilitated dephosphorylation of PKCs, since hypoxia was reported to increase in phosphatase 1 activity to lead the dephosphorylation of PKCs (Harada et al., 1999). Furthermore, the reported fact that phosphatase successively changes substrates one another (Prevostel et al., 2000; Hansra et al., 1999) may represent the difference in time initiating the dephosphorylation of different PKC isoforms.
isoforms observed in the present experiments, as phosphatase 1 may initially dephosphorylates PKCβ2 thereafter PKCs of ε and α after hypoxia in the taenia. However, the level of phosphorylated MRLC (MRLC-P) under hypoxia was reported to be high at nearly the same level as that of the contracted taenia under aerobic conditions (Obara et al., 1997). Since MRLC-P level is also regulated by protein phosphatase, the activation of phosphatase may not simply be responsible for the PKC dephosphorylation. The different compartmentation of phosphatase from myofibrillar site in the cell is considerable, though not warrant at the present moment.

The change in phosphorylation potential under metabolically stressed conditions could be alternative or cooperative mechanism for the metabolic stress-induced dephosphorylation of PKCs in the taenia, as the phosphorylation level of enzymes was reported to be associated with cellular ATP levels (Yung and Tolkovsky, 2003; Bhat et al., 1996; Bonz et al., 1998). Hypoxia in the presence of high K+ was reported to reduce ATP to 1/2 of control and phosphocreatine (PCr) to 1/10 in the taenia (Ishida and Paul, 1990). While, Nakayama et al. (1988) reported that glucose depletion in the presence of high K+ reduced ATP and PCr to 1/2 and 1/4, respectively. Many years ago, Urakawa et al. (1969) reported that the oxygen consumption of the taenia is not totally suppressed but remained appreciably for more than 1 h after glucose depletion. Further, hypoxia in a Ca2+-removed medium did not elicit a significant reduction of ATP in the taenia (Ishida and Shibata, 1982), indicating that the relaxing muscle does not consume ATP extensively. Considering those reported change in phosophagen levels, exposure of the fully contracted taenia to hypoxia may elicit the dephosphorylation of PKC isoforms due to the significant reduction in phosphagens. Whereas, glucose depletion inhibits the energy metabolism, but the reduction of phosphagen may be relatively small not enough to induce the PKC dephosphorylation. In addition, the PKC dephosphorylation induced by cyanide suggests that the suppression of mitochondrial oxidative phosphorylation reduces more phosphagen than the inhibition of glycolytic pathway in the taenia. Also, the strong metabolic stress of hypoxia+glucose depletion was reported to nearly deplete ATP of the taenia (Ishida and Shibata, 1982). This ATP depletion may be responsible for the hypoxia+glucose depletion-induced dephosphorylation of PKCs in either stimulated or non-stimulated taenia.

Early dephosphorylation of PKCβ2 after hypoxia in the taenia seems to be concomitantly accompanied with the reported alteration of adenylate compounds of the muscle. The PKCβ2 dephosphorylation was initiated around 10 min after hypoxia in the presence of high K+, then gradually proceeded to 50% of total PKCβ2 30 min after hypoxia. Ishida and Paul (1990) reported that the change in adenylate compounds of the taenia, loss of ATP and increase in AMP, was also initiated around 10 min after hypoxia to reach the steady level in 30 min. On the other hand, dephosphorylation of PKCs of α and ε delayed with the lag time more than 30 min after hypoxia, suggesting that these isoforms are dephosphorylated via other signal transduction process as discussed above, yet uncertain. These results again suggest that the PKC isoform of the taenia primarily responsible to the alteration of adenylate compounds is PKCβ2.

Although PKCβ2 responded well to change in the phosphorylation potential after imposition of hypoxia, reoxygenation of the taenia did not produce the re-phosphorylation of PKCs of α, β2 and ε, while ATP level was reported to restore to 75% of normal (Ishida and Paul, 1990). Ishida
and Paul (1990) also reported that the contractility of the taenia partially, but not completely, recovered after reoxygenation. Thus, the lack of rephosphorylation of PKCs may represent the irreversible damage in the function of the taenia. Further, the phosphorylation of once dephosphorylated PKC was reported not to be achieved merely by the presence of ATP (Dutil et al., 1994; Borner et al., 1989), but to require the activation of kinase (Dempsey et al., 2000; Parekh et al., 2000). Therefore, the prolonged hypoxia may suppress the responsible kinase activity of the taenia.

Present experiments clearly show that hypoxia-related metabolic inhibition in the contracted taenia elicits the dephosphorylation of PKCs of α, β2 and ε, but not ζ. These results further suggest that PKCs of the taenia are present mostly in a phosphorylated form, so called as a matured form, in the aerobic conditions. This is consistent to the reported fact that PKCs in the cells are present mostly in a matured form, which is phosphorylated on activation loop and fully phosphorylated on autophosphorylation site and priming site on carboxyl terminus (Karinen et al., 1995; Zhang et al., 2000). While, the phosphorylation level of PKCζ of the taenia could not be determined in the present experiments, since any tested metabolic stress conditions did not change the electrophoretic mobility.

With respect to the cellular function, hypoxia-induced dephosphorylation of PKCβ2 may not be directly associated to the relaxation response of the taenia, but may be related to other cellular functions, since the dephosphorylation occurred after the muscle relaxed under hypoxia: the relaxation response attained the steady level in 5 min after hypoxia (Ishida and Paul, 1990). Recently, in other cells, PKCβ2 has been reported to regulate many pathophysiological functions such as cell proliferation (Murray et al., 1993; Murray et al., 1999), immune response (Leitges et al., 1996), cardiomyophaty response (Wakasaki et al., 1997; Takeishi et al., 1998), and diabetic conditions (Kaneto et al., 2002; Finton et al., 2002). Also, in the hypoxic vasculature, PKCβ2 is a trigger for events leading to induction of Egr-1, being distinct from HIF-1 pathway (Yan et al., 2000). Further, the ischemia induced the increase in retinal neovascularization in PKCβ transgenic mice, but not much in PKCβ knock-out mice (Suzuma et al., 2002). Thus, the alteration of PKC phosphorylation level upon the metabolic inhibition may be a new perspective for understanding the ischemic responses, especially at the impaired response. Future investigation on this process will shed the light on the mechanism of ischemia and to develop the way for effective treatments of ischemia in various organs.

Acknowledgments

Authors express their gratitude to Dr. Akira Omori and Ms Yukie Kakiyama at Mitsubishi Kagaku Institute of Life Sciences for valuable advice how to detect the phosphorylation level of PKC using SDS-PAGE and their valuable comments on this work. They also acknowledge Prof. Richard J. Paul at University of Cincinnati College of Medicine for encouragement during description of the manuscript.
References


(Received May 10, 2004; Accepted June 15, 2004)