Desflurane Preconditioning Induces Time-dependent Activation of Protein Kinase C Epsilon and Extracellular Signal-regulated Kinase 1 and 2 in the Rat Heart In Vivo

Octavian Toma, M.D.,* Nina C. Weber, Ph.D.,† Jessica I. Wolter, M.D.,‡ Detlef Obal, M.D.,§ Benedikt Preckel, M.D.,|| Wolfgang Schlack, M.D.#

Background: Activation of protein kinase C epsilon (PKC-ε) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) are important for cardioprotection by preconditioning. The present study investigated the time dependency of PKC-ε and ERK1/2 activation during desflurane-induced preconditioning in the rat heart.

Methods: Anesthetized rats were subjected to regional myocardial ischemia and reperfusion, and infarct size was measured by triphenyltetrazoliumchloride staining (percentage of area at risk). In three groups, desflurane-induced preconditioning was induced by two 5-min periods of desflurane inhalation (1 minimal alveolar concentration), interspersed with two 10-min periods of washout. Three groups did not undergo desflurane-induced preconditioning. The rats received 0.9% saline, the PKC blocker calphostin C, or the ERK1/2 inhibitor PD98059 with or without desflurane preconditioning (each group, n = 7). Additional hearts were excised at four different time points with or without PKC or ERK1/2 blockade: without further treatment, after the first or the second period of desflurane-induced preconditioning, or at the end of the last washout phase (each time point, n = 4). Phosphorylated cytosolic PKC-ε and ERK1/2, and membrane translocation of PKC-ε were determined by Western blot analysis (average light intensity).

Results: Desflurane significantly reduced infarct size from 57.2 ± 4.7% in controls to 35.2 ± 16.7% (desflurane-induced preconditioning, mean ± SD, P < 0.05). Both calphostin C and PD98059 abolished this effect (58.8 ± 13.2% and 64.2 ± 15.4% respectively, both P < 0.05 versus desflurane-induced preconditioning). Cytosolic phosphorylated PKC-ε reached its maximum after the second desflurane-induced preconditioning and returned to baseline after the last washout period. Both calphostin C and PD98059 inhibited PKC-ε activation. ERK1/2 phosphorylation reached its maximum after the first desflurane-induced preconditioning and returned to baseline after the last washout period. Calphostin C had no effect on ERK1/2 phosphorylation.

Conclusions: Both, PKC and ERK1/2 mediate desflurane-induced preconditioning. PKC-ε and ERK1/2 are both activated in a time dependent manner during desflurane-induced preconditioning, but ERK1/2 activation during desflurane-induced preconditioning is not PKC dependent. Moreover, ERK1/2 blockade abolished PKC-ε activation, suggesting ERK-dependent activation of PKC-ε during desflurane-induced preconditioning.

Volatile anesthetics can induce pharmacological preconditioning,¹ ² where protein kinase C (PKC) isoforms are involved as important steps of the signal transduction cascade.³ ⁴ Activation of PKC affects several downstream targets like the mitogen activated protein kinases.⁸ Among the mitogen activated protein kinases, the extracellular signal-regulated kinase 1/2 (ERK1/2) was found to be involved in preconditioning.⁹ ¹⁰ The activation of ERK1/2 is regulated by phosphorylation, and this process was shown to be PKC-dependent.¹¹ It has been suggested that ERK1/2 is a downstream effector of PKC. Moreover, it was shown that ERK1/2 is a potential mediator and downstream target of PKC in anesthetic induced preconditioning.¹²

Otani et al.¹³ showed that despite the fact that administration of a mitochondrial K ATP channel opener or a nitric oxide donor preconditioned the rat heart, PKC-ε activation was no more detectable 20 min after washout of the preconditioning drugs. It was recently shown that ERK1/2 was not activated shortly after anesthetic preconditioning but that it was significantly activated later after ischemia and reperfusion in anesthetic preconditioned hearts.¹² These data suggest a time-dependent activation of PKC and ERK1/2 during the preconditioning phase.

The aim of the present study was to investigate, in desflurane-induced preconditioning, the role of PKC and ERK1/2, the interaction of both enzymes, and the time course of their activation.

Materials and Methods

The study was performed in accordance with the regulations of the German Animal Protection Law and was approved by the Bioethics Committee of the District of Düsseldorf, Nordrhein-Westfalen, Germany.

Materials

Desflurane was purchased from Baxter Deutschland GmbH (Unterschleissheim, Germany). Calphostin C and PD98059 were purchased from Sigma (Taufkirchen, Germany). The enhanced chemoluminescence protein detection kit was from Santa Cruz (Heidelberg, Germany). Phospho-PKC-ε and total PKC-ε polyclonal antibodies were from Upstate (Lake Placid, NY, USA). Pho-
ERK1/2 and total ERK1/2 polyclonal antibodies were from Cell Signaling (Frankfurt/M, Germany). Monoclonal anti-α-tubulin and antiactin antibodies were from Sigma (Taufkirchen, Germany). Peroxidase-conjugated donkey antirabbit and peroxidase-conjugated goat antimouse antibodies were from Jackson Immunolab (Dianova, Hamburg, Germany). All other materials were either purchased from Sigma or Merck-Eurolab (Munich, Germany).

**Surgical Preparation**

Male Wistar rats (250–300 g) were anesthetized by intraperitoneal Sc(+)-ketamine injection (150 mg/kg). Further preparation and infarct size measurement by triphenyltertrazoliumchlorid staining were performed as described previously. In summary, after tracheal intubation, the lungs were ventilated with oxygen-enriched air and a positive end-expiratory pressure of 2–3 cm H2O. Respiratory rate was adjusted to maintain partial pressure of carbon dioxide within physiologic limits. Body temperature was maintained at 38°C by the use of a heating pad. The right jugular vein was cannulated for saline and drug infusion, and the left carotid artery was cannulated for measurement of aortic pressure. Anesthesia was maintained by continuous α-chloralose infusion. A lateral left sided thoracotomy followed by pericardiotomy was performed and a ligature (5–0 prolene) was passed below the main branch of the left coronary artery. The ends of the suture were threaded through a propylene tube to form a snare, and the coronary artery was occluded by tightening the snare. Successful coronary artery occlusion was verified by epicardial cyanosis. The area of risk and the infarcted area were determined by planimetry using SigmaScan Pro 5® computer software (SPSS Science Software, Chicago, IL) and corrected for dry weight.

**Experimental Protocol**

The study protocol is shown in figure 1. For infarct size measurement, 42 rats were randomly assigned to one of the six groups and all animals were subjected to 25 min of left coronary artery occlusion followed by 120 min of reperfusion. After surgical preparation, the rats were allowed to recover for 30 min and then received either vehicle (0.9% saline), the highly selective PKC inhibitor calphostin C (0.1 mg/kg) in dimethyl sulfoxide 1% aqueous solution, or the ERK1/2 mitogen-activated protein kinase kinase (MEK) inhibitor PD98059 (1 mg/kg in dimethyl sulfoxide 1% aqueous solution), or the ERK1/2 mitogen-activated protein kinase kinase (MEK) inhibitor PD98059 (1 mg/kg in dimethyl sulfoxide 1% aqueous solution). The excised hearts were frozen in liquid nitrogen. The membrane-, cytosolic-fraction and ERK1/2 phosphorylation. The aortic pressure signal was digitized using an analog-to-digital converter (PowerLab/8SP, ADInstruments Pty Ltd, Castle Hill, Australia) at a sampling rate of 500 Hz and continuously recorded on a personal computer using Chart for Windows v5.0 (ADInstruments Pty Ltd).

**Separation of Membrane and Cytosolic Fraction**

Tissue specimens were prepared for protein analysis to investigate PKC-ε and ERK1/2, twelve additional groups received either vehicle, calphostin C or PD98059, and the hearts were excised at four different time points (fig. 1B): without further treatment (baseline), after the first period of 1 MAC desflurane (Des I), after the second period of 1 MAC desflurane (Des II), and after the last washout phase before infarct inducing ischemia (Wash II).

**Data Analysis**

The aortic pressure signal was digitized using an analog-to-digital converter (PowerLab/8SP, ADInstruments Pty Ltd, Castle Hill, Australia) at a sampling rate of 500 Hz and continuously recorded on a personal computer using Chart for Windows v5.0 (ADInstruments Pty Ltd).
fraction. The remaining pellet was resuspended in lysis buffer containing 1% Triton X-100, incubated for 60 min on ice and vortexed. The solution was centrifuged at 16000×g, 4°C, for 15 min and the supernatant was collected as membrane fraction.

**Western Blot Analysis**

After protein determination by the Lowry method, equal amounts of protein were mixed with loading buffer containing Tris-HCl, glycerol, and bromphenol blue. Samples were vortexed and boiled at 95°C before being subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. The proteins were separated by electrophoresis and then transferred to a polyvinylidene difluoride membrane by tank blotting. Unspecific binding of the antibody was blocked by incubation with 5% fat dry milk powder solution in Tris buffered saline containing Tween for 2 h. Subsequently, the membrane was incubated overnight at 4°C with the respective first antibody (either phospho specific or total in the case of PKC-ε and ERK1/2, anti-α-tubulin, and antiaction as internal standard) at indicated concentrations. After washing in fresh, cold, Tween-containing Tris buffered saline, the blot was subjected to the appropriate horseradish peroxidase conjugated secondary antibody for 2 h at room temperature. Immunoreactive bands were visualized by chemiluminescence detected on autoradiography film (Hyperfilm ECL; Amersham, Freiburg, Germany) using the enhanced chemiluminescence system Santa Cruz. The blots were quantified by SigmaScan Pro 5® computer software (SPSS Science Software, Chicago, IL). The results were calculated as ratio of phosphorylated protein to total protein (to detect phosphorylation) or as ratio of total protein to α-tubulin (to detect translocation) and they were presented as absolute values of average light intensity.

**Statistical Analysis**

Data are expressed as means ± SD. Statistical analysis of infarct size and PKC-ε or ERK1/2 measurements was performed by Student’s t test with Bonferroni’s correction for multiple comparisons. Statistical analysis of the hemodynamic variables was performed by two-way repeated-measures analysis of variance for time and treatment effects. If an overall significance was found, comparisons between groups were done for each time point using one-way analysis of variance followed by Tukey post hoc testing. Time effects within each group were analyzed by repeated-measures analysis of variance followed by Dunnett post hoc test with the baseline value as the reference time point. P < 0.05 was considered statistically significant.

**Results**

**Hemodynamics**

Table 1 shows the time course of heart rate and mean aortic pressure during the experiments. No significant differences in heart rate and aortic pressure were observed between the experimental groups during baseline. Calphostin C or PD98059 administration did not influence heart rate or aortic pressure. Desflurane transiently reduced mean aortic pressure, and the values recovered to baseline during the washout periods. At the end of experiment, mean aortic pressure was significantly decreased compared with baseline in calphostin C treated groups.
Infarct Size Measurement

Desflurane preconditioning reduced infarct size from 57.2 ± 4.7% in controls to 35.2 ± 16.7% (P < 0.05; fig. 2). Both calphostin C and PD98059 preinfusion abolished this cardioprotection (Calphostin + desflurane, 58.8 ± 13.2%; PD98059 + desflurane, 64.2 ± 15.4%; both P < 0.05 versus desflurane-induced preconditioning). Calphostin C or PD98059 infused without desflurane-induced preconditioning had no effect on infarct size (58.8 ± 13.2% and 48.8 ± 11.6%, respectively; P = 0.27).

Time Course of PKC-ε Phosphorylation

PKC-ε phosphorylation increased to the highest values after the second desflurane administration (1.54 ± 0.96 versus 1.10 ± 0.41; P < 0.05 versus baseline; fig. 3, white columns) and returned to baseline after the last washout period (1.17 ± 0.82; P = 0.7 versus baseline).

The PKC blocker calphostin C abolished the increased phosphorylation at the respective time points. As evidenced by equal actin identifications, protein content in the different probes was similar, excluding the fact that the observed effect was attributable to different protein amounts.

Time Course of PKC-ε Translocation

PKC-ε in the membrane fraction increased during desflurane preconditioning and a maximum increase was observed after the second washout period (0.79 ± 0.27 versus 0.63 ± 0.32; P = 0.1 versus baseline; fig. 4, white hatched columns). Conversely, PKC-ε in the cytosolic fraction decreased (after the second...
washout period 0.50 ± 0.27 versus 0.62 ± 0.28 baseline; P = 0.2; fig. 4, white columns). The PKC blocker calphostin C abolished the respective changes in PKC content.

**Time Course of ERK1/2 Phosphorylation**

ERK1 phosphorylation (fig. 5, white columns) reached its maximum after the first desflurane-induced preconditioning (1.83 ± 1.20 versus 0.95 ± 0.53 in baseline; P < 0.01), slightly decreased after the second desflurane-induced preconditioning (1.56 ± 0.93; P < 0.01 versus baseline), and further decreased toward baseline after the last washout period (1.23 ± 0.58, P < 0.05 versus baseline).

As shown in figure 6, ERK2 phosphorylation (white columns) followed a similar time course pattern as ERK1 phosphorylation: 2.02 ± 1.22 after the first desflurane-induced preconditioning (P < 0.01 versus baseline), 1.57 ± 1.06 after the second desflurane-induced preconditioning (P < 0.01 versus baseline), and 0.77 ± 0.49 after the last washout (P < 0.05 versus baseline) versus 0.50 ± 0.32 during baseline.

**Causal Relationship Between PKC-ε and ERK1/2**

Phosphorylation of PKC-ε was blocked by PD98059 (fig. 3, black columns): 1.02 ± 0.75 after the first desflurane-induced preconditioning, 0.94 ± 0.58 after the second desflurane-induced preconditioning, and 0.80 ± 0.50 after the last washout versus 1.03 ± 0.59 during baseline. PD98059 also blocked the increase of PKC-ε in the membrane fraction (fig. 4, black hatched columns): 0.64 ± 0.33 after the first desflurane-induced preconditioning, 0.63 ± 0.26 after the second desflurane-induced preconditioning, and 0.68 ± 0.17 after the last washout versus 0.69 ± 0.28 during baseline. Similarly, PD98059 blocked the decrease of PKC-ε in the cytosolic fraction (fig. 4, black columns): 0.57 ± 0.48 after the first desflurane-induced preconditioning, 0.55 ± 0.31 after the second desflurane-induced preconditioning, and 0.54 ± 0.28 after the last washout versus 0.59 ± 0.16 during baseline.

In contrast, phosphorylation of ERK1 was not affected by the PKC blocker calphostin C (fig. 5, black columns): 1.91 ± 1.57 after the first desflurane-induced preconditioning (P < 0.05), 1.89 ± 1.38 after the second desflurane-induced preconditioning, and 1.78 ± 1.24 after the last washout.}

---

**Fig. 5.** One representative Western blot experiment of cytosolic fraction showing time-course of ERK1 phosphorylation during desflurane-induced preconditioning. (Top) phosphorylated form of ERK1; (bottom) total ERK1. The histogram presents densitometric quantification of four experiments as x-fold average light intensity. Data are presented as mean ± SD; **P < 0.01 versus vehicle baseline; *P < 0.05 versus vehicle baseline; †P < 0.05 versus Calph C baseline.

**Fig. 6.** One representative Western blot experiment of cytosolic fraction showing time-course of ERK2 phosphorylation during desflurane-induced preconditioning. (Top) phosphorylated form of ERK2; (bottom) total ERK2. The histogram presents densitometric quantification of four experiments as x-fold average light intensity (AVI). Data are presented as mean ± SD; **P < 0.01 versus vehicle baseline; *P < 0.05 versus vehicle baseline; †P < 0.05 versus Calph C baseline.
rane-induced preconditioning \( (P < 0.05) \), and \( 1.12 \pm 0.72 \) after the last washout period versus \( 1.10 \pm 0.90 \) during baseline. Phosphorylation of ERK2 (fig. 6, black columns) was also not blocked by calphostin C treatment: \( 1.10 \pm 1.13 \) after the first desflurane-induced preconditioning \( (P < 0.05) \), \( 1.35 \pm 1.50 \) after the second desflurane-induced preconditioning \( (P < 0.05) \), and \( 0.65 \pm 0.52 \) after the last washout \( (P = 0.2) \) versus \( 0.40 \pm 0.32 \) during baseline in calphostin C treated hearts. The failure of PKC blockade to attenuate ERK1/2 phosphorylation suggests that ERK1/2 phosphorylation during desflurane-induced preconditioning is not PKC dependent.

**Discussion**

We investigated the role of PKC-ɛ and ERK1/2 mitogen activated protein kinase in desflurane-induced preconditioning in the rat heart *in vivo*. The new findings of the current study are, first, that both PKC and ERK1/2 are involved in desflurane-induced pharmacological preconditioning as proved by the loss of cardioprotection after infusion of specific PKC and MEK/ERK1/2 inhibitors calphostin C and PD98059, respectively. Second, during desflurane administration, cytosolic PKC-ɛ and ERK1/2 were phosphorylated in a time-dependent manner and returned toward the baseline level at the end of the preconditioning protocol when PKC-ɛ translocation from cytosol to membrane reached the highest level. Third, desflurane administration induced ERK1/2 phosphorylation even after PKC blockade, indicating that ERK1/2 activation during desflurane-induced preconditioning is not PKC-dependent. Taken together, we showed that both enzymes in active form are necessary for cardioprotection elicited by desflurane.

Ischemic and anesthetic-induced preconditioning share several steps in the signal transduction cascade. Besides opening of mitochondrial \( K_{\text{ATP}} \) channels, activation of PKC plays a central role in anesthetic-induced preconditioning. Zaug et al.\(^5\) showed that PKC represents an important signaling pathway upstream to the mitochondrial \( K_{\text{ATP}} \) channels: volatile anesthetics seem to prime rather than directly increase the open state probability of mitochondrial \( K_{\text{ATP}} \) channels, and this process is accomplished through multiple PKC-dependent pathways.\(^5,20\) Using an *in vivo* animal model, we previously demonstrated that PKC-ɛ phosphorylation was increased after three 5-min periods of isoflurane inhalation and that the PKC-ɛ inhibitor calphostin C abolished both the isoflurane-induced infarct size reduction and the increased phosphorylation of PKC-ɛ.\(^21\) In the current study, we demonstrate that PKC-ɛ also plays an important role in desflurane-induced cardioprotection.

Interestingly, after the last washout period, we detected a translocation of PKC-ɛ to the membrane without an increase of cytosolic phosphorylation. This finding seemed to disagree with the existing data about structural and spatial regulation of PKC: to accomplish its biologic function, PKC must be processed by phosphorylation, have its pseudosubstrate exposed, and be localized at the correct intracellular location for signaling. On ligand binding at the membrane, phosphorylation at the activation loop triggers autophosphorylation of the two carboxyl-terminal sites and the fully phosphorylated form is then released into the cytosol where generation of diacylglycerol provides the allosteric switch of PKC.\(^22,23\)

Therefore, we investigated the time course of intracellular PKC-ɛ changes and found a strong time-dependent pattern of PKC-ɛ activation during desflurane-induced preconditioning, with a maximum increase of cytosolic phosphorylated PKC-ɛ after the second desflurane administration and a complete return to baseline before the infarct-inducing ischemia (fig. 3). During desflurane-induced preconditioning, PKC-ɛ progressively translocated from cytosol to the membrane fraction and we detected the maximum before the infarct-inducing ischemia (fig. 3). The specific PKC blocker calphostin C abolished the increased phosphorylation/translocation at the respective time points. These findings are in agreement with the mechanisms of PKC regulation mentioned above\(^22,23\) and with the fact that prolonged PKC activation by phorbol ester results in complete dephosphorylation coupled to translocation to a detergent-insoluble cell fraction.\(^22\) Anesthetic preconditioning was found to be dose-dependent,\(^24,25\) and at least two cycles of short exposure to a volatile anesthetic followed by its washout enhance cardioprotection afforded by anesthetic preconditioning.\(^26\) However, it is difficult to compare the strength of the stimulus we used (two 5-min periods of 1 MAC desflurane) to induce anesthetic preconditioning with the PKC activation by phorbol esters.

Activation of PKC affects mitogen activated protein kinases like the extracellular signal-regulated kinase (ERK1/2), an enzyme that has been described to be involved in preconditioning.\(^9,10\) The existing data regarding the role of ERK1/2 in acute cardioprotection by ischemic preconditioning are controversial. Increased phosphorylation of mitochondrial ERK seems to mediate cardioprotection, in part by phosphorylation and inactivation of proapoptotic proteins.\(^8,10\) MEK overexpression in isolated cardiomyocytes up-regulates ERK1/2 activation and induces cardiomyocyte hypertrophy (an equivalent for cardioprotection).\(^27\) Isolated hearts from rats treated with the MEK/ERK1/2 inhibitor PD98059 failed to show complete functional recovery after ischemia/reperfusion.\(^28\) Fryer et al.\(^29\) found that ERK1 and ERK2 are differentially regulated in the rat heart *in vivo* and that cytosolic activation of ERK1 could be an important part in the signal transduction mediating acute cardioprotection by ischemic preconditioning or by opioid agonists.\(^29\) Su et al.\(^30\) and Zhong et al.\(^31\) found that
effects of isoflurane and halothane in vascular tissues in vitro were reversed by inhibitors of PKC or ERK1/2. Using the specific MEK/ERK1/2 inhibitor PD98059, we clearly demonstrated that the cardioprotection after desflurane preconditioning was abolished (fig. 2), showing an important role of ERK1/2 in desflurane-induced preconditioning. In addition, we demonstrated an early increase of ERK1/2 phosphorylation after the first administration of the anesthetic that rapidly decreased after the second desflurane administration and after the second washout period (fig. 4). Taken together, our data clearly show an involvement of ERK1/2 in desflurane-induced preconditioning and a time-dependent activation of ERK1/2.

The activation of ERK1/2 is regulated by phosphorylation and this process was shown to be PKC-dependent and it has been suggested that ERK1/2 is a downstream effector of PKC mediating effects of volatile anesthetics. To investigate a possible cross-link between PKC and ERK1/2, phosphorylation of each enzyme was determined after blockade of the other enzyme. Our results show that desflurane increased ERK1/2 phosphorylation even after PKC blockade (figs. 5 and 6). In addition, phosphorylation of ERK1/2 occurred earlier (after the first desflurane period) compared with phosphorylation of PKC (after the second desflurane period). These data suggest that desflurane activates ERK1/2 independent of PKC and that in desflurane-induced preconditioning, ERK1/2 is not downstream of PKC. In contrast, blockade of ERK1/2 by PD98059 abolished PKC activation by desflurane (figs. 3 and 4), suggesting that ERK1/2 might be upstream of PKC. Alternative, PKC-independent activation pathways of ERK1/2 are already known from other settings such as α-adrenergic stimulation, when ERK1/2 activation occurs by a tyrosine kinase-dependent pathway. In contrast to our results, ERK1/2 activation after isoflurane was found to be PKC-ε-dependent using either in vitro vascular cell preparates or an isolated rat model. Better functional recovery after anesthetic preconditioning was not abolished by PD98059, but based on late activation during ischemia/reperfusion, ERK1/2 was supposed to play a role as a mediator, and not as a trigger, in anesthetic preconditioning. We used an in vivo model and the catecholamine release by desflurane could produce early activation of ERK1/2, providing evidence for ERK1/2 acting as a trigger in desflurane-induced preconditioning. Adrenergic stimulation triggers pharmacological preconditioning, and the cardioprotective effects of desflurane in isolated human cardiac tissue involve α and β adrenoceptor stimulation. In our setting, catecholamine release by desflurane might have resulted in early ERK1/2 activation via adrenergic receptors and tyrosine kinases, and cardioprotection might have occurred after subsequent PKC-ε phosphorylation and translocation.

Aikawa et al. showed that the tyrosine kinase inhibitor genistein suppressed the ERK1/2 activation induced by hydroxyl radicals, whereas inhibitors of PKC did not influence the ERK1/2 activation. Treatment of cardiac myocytes either with a PKC activator or inhibitor did not affect H2O2-induced ERK activation, suggesting PKC-independent activation of ERK1/2. Reactive oxygen species are also involved in anesthetic preconditioning and a direct generation of reactive oxygen species by volatile anesthetics could also explain a PKC-independent ERK1/2 activation.

For the induction of anesthesia, rats were anesthetized with S(+)-ketamine because it has been shown that S-enantiomer of ketamine does not interfere with the mechanisms of preconditioning. Racemic ketamine and R(-)-ketamine, but not S(+)-ketamine, block the protective effects of preconditioning. The absolute specificity and potential toxicity of PD98059 are difficult to evaluate. PD98059 prevents the activation and phosphorylation of MEK1 in vitro and in vivo and does not interfere with the mechanisms of PKC activation. PD98059 has a high level of specificity for MEK/ERK1/2 pathway and we can conclude that an effect of PD98059 on PKC is very unlikely. The pharmacokinetic profile of pharmacologic inhibitors affecting signaling pathways, such as PD98059, is normally not known and the in vivo protocol has to be adapted with short periods between drug infusion and measurement points. Giving the dose we infused in the current study it is less probable that the concentrations of PD98059 before the last measurement point (40 min after infusion) are lower than the effective inhibitory concentration of the targeted kinase. The reason for choosing calphostin C instead of other widely used PKC inhibitors (such as chelerythrine) was the higher specificity in the used concentration. The 50% inhibitory concentration of chelerythrine chloride for PKC is approximately 150-fold and 260-fold less than for protein tyrosine kinase and protein kinase A, respectively, whereas the 50% inhibitory concentration of calphostin C for PKC (0.05 μM) is more than 1000-fold less than for protein tyrosine kinase and protein kinase A. Calphostin C acts on the regulatory domain of PKC, which is distinct from other protein kinases, and induces a more specific inhibition of PKC than other PKC inhibitors. An inhibitor whose specificity for PKC is higher in the used concentration decreases the chance to inhibit other kinases probably also involved in preconditioning. There exist very few data about the pharmacokinetic features of calphostin C in the rat in vivo. In mice, calphostin C has a half-life time of 91.3 min after intraperitoneal injection. In the current study, the last measurement point is 40 min after calphostin C infusion; therefore, a bolus injection should result in a sufficient concentration of the inhibitor during the preconditioning period to affect the targeted enzyme which is up-regulated in this period of time. This was confirmed by the western blot data showing a clear
inhibition of the enzyme phosphorylation after inhibitor treatment.

The translocation of PKC-ε to the membrane was not statistically significant. This finding could be the result of an already existing membrane-bound fraction of PKC-ε; i.e., the changes induced by desflurane are probably relative small when compared to the total membrane-bound PKC-ε, including constitutively translocated PKC-ε existing in the virgin myocardium. The first step in the physiologic posttranslational changes of PKC is represented by membrane binding in a conformation that favors the action of PKC kinase. We did not present the absolute values of phosphorylated PKC-ε into the cytosolic fraction and we do not have a measure of this phenomenon in our settings. We detected an increase in the phosphorylated form of PKC-ε compared with the control conditions, and it might have been that this rather nonabrupt increase was also attributable to constitutively existent cytosolic phosphorylated PKC-ε.

In summary, the current study provides, for the first time evidence, that both PKC and ERK1/2 are involved in desflurane-induced pharmacological preconditioning and that both enzymes are necessary for cardioprotection. Phosphorylation of PKC-ε and ERK1/2 is time dependent and rapidly reversible, returning to baseline in the physiologic posttranslational changes of PKC isoenzymes. ANESTHESIOLOGY 2004; 100:59–69

References


2. Cason BA, Gamperl AK, Slocum RE, Hickey RF. Anesthetic-induced preconditioning: Previous administration of isoflurane decreases myocardial infarct size in rats. ANESTHESIOLOGY 1997; 87:1182–90.


Downloaded From: http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931184/ on 06/19/2017


