α1-Receptor–Independent Activation of Protein Kinase C in Acute Myocardial Ischemia

Mechanisms for Sensitization of the Adenylyl Cyclase System

R.H. Strasser, R. Braun-Dullaeus, H. Walendzik, and R. Marquetant

The activity of the adrenergic system plays an important role in the genesis of malignant arrhythmias and the spreading of the infarcted zone in acute myocardial ischemia. Acute myocardial ischemia induces an increased activity of adenylyl cyclase. This sensitization at the enzyme level as shown in the isolated perfused rat heart occurs rapidly after the onset of ischemia (5–15 minutes) and is rapidly reversible on reperfusion. With prolonged ischemia, it is only transient and is followed by a gradual loss of the adenylyl cyclase activity. The increased activity of adenylyl cyclase is even retained after partial purification, suggesting a covalent modification of the enzyme. Blockade of α1-adrenergic receptors does not prevent this sensitization, demonstrating that it occurs independently of α1-adrenergic receptor activation. Only blockade of protein kinase C by various inhibitors, such as polymyxin B or staurosporine, is able to completely prevent this sensitization process. Moreover, in acute myocardial ischemia an activation of protein kinase C could be identified using its translocation from the cytosol to the particulate fraction as an indicator. Blockade of α1-adrenergic receptors using prazosin fails to prevent the activation of protein kinase C and consequently the sensitization of the adenylyl cyclase system, indicating that the ischemia-induced translocation of protein kinase C occurs independently of α1-adrenergic receptors. These data characterize for the first time an important interaction of two effector enzymes of two distinct signal transduction pathways, i.e., the adenylyl cyclase system and the protein kinase C system in acute myocardial ischemia. Further studies are necessary to identify the mechanisms involved in the activation of protein kinase C in acute ischemia in order to develop therapeutic strategies to prevent such activation of protein kinase C and consequently the sensitization of adenylyl cyclase. (Circulation Research 1992;70:1304–1312)

**KEY WORDS** • acute myocardial ischemia • β-adrenergic receptors • adenylyl cyclase • α1-adrenergic receptors • sensitization • protein kinase C

Coronary heart disease leading to acute myocardial ischemia may lead to a life-threatening event with the occurrence of malignant arrhythmias or a severe reduction of left ventricular function. Both the spreading of the ischemic zone, resulting in an impairment of contractility, and the genesis of malignant arrhythmias are influenced by the sympathetic system.1,2 Acute myocardial ischemia causes several alterations of the sympathetic system: 1) Local and central mechanisms lead to an increased presynaptic release of endogenous catecholamines.3–5 2) In contrast to expectation, these catecholamines do not induce a desensitization of receptors postsynaptically. Instead, acute myocardial ischemia leads to an increased density of functionally coupled β-adrenergic receptors6,7 at the expense of intracellular receptors, leading to a receptor-linked sensitization of the adenylyl cyclase system. Ischemia may also lead to an increase of α1-adrenergic receptors.8 3) The activity of the transducing enzyme of the β-adrenergic system, adenylyl cyclase, is altered in two ways. With prolonged ischemia (≥30 minutes), a decreased activity could be demonstrated.9–12 This late reduction is accompanied by the functional decrease of G proteins, predominantly the stimulatory G protein, Gs, after 1 hour of myocardial ischemia.13 In contrast, short periods of ischemia induce an increased activity of adenylyl cyclase. Two distinct and as yet little understood mechanisms at the postreceptor level contribute to the sensitization of the adenylyl cyclase system in acute myocardial ischemia: a G protein–linked process (B. Rauch, C. Weinbrenner, F. Niroomand, R. Marquetant, T. Beyer, C. Schwenke, W. Kübler, W. Hasselbach, and R. H. Strasser, submitted manuscript) and an enzyme-linked sensitization process.7,12 These sensitization processes potentiate the temporal accumulation of second messenger cAMP in
the ischemic zone, which has been claimed to promote ventricular arrhythmias.

The present study characterizes the mechanisms contributing to the enzyme-linked sensitization of adenylate cyclase in the very early phase of acute myocardial ischemia. This transient sensitization of adenyl cyclase in acute myocardial ischemia develops independently of $\alpha_1$-adrenergic receptors and, as shown previously, independently of $\beta$-adrenergic receptors. Inhibition of protein kinase C can completely prevent sensitization of adenyl cyclase. Additionally, it can be demonstrated that acute ischemia activates protein kinase C by an as yet undefined but $\alpha_1$-adrenergic receptor–independent mechanism. Activation of protein kinase C may promote the sensitization of adenyl cyclase. For the ischemic heart, these findings open a new perspective of interaction of these two enzymes, which are components of two distinct signal transduction pathways, i.e., the adenyl cyclase system and the phosphatidylinositol system.

Materials and Methods

Materials

$[\alpha\text{-}{}^3\text{P}]$ATP and $[\gamma\text{-}{}^3\text{P}]$ATP were purchased from New England Nuclear, Boston. Alprenolol was a generous gift from Haessler, Sweden. Reagents for the protein assay according to Bradford were from Bio Rad, Munich, FRG. All other reagents were from Sigma, Munich, FRG. Male Wistar rats (200–250 g) were from Ivanovas, Kiesslegg.

Perfusion of Isolated Hearts

Isolated rat hearts were perfused according to the method of Langendorff with a modified Krebs–Henseleit solution consisting of (mM) NaCl 125, MgCl$_2$ 1, CaCl$_2$ 1.85, KCl 4, sodium EDTA 0.027, glucose 11, NaHCO$_3$ 17, and Na$_2$HPO$_4$ 0.2 (pH 7.4 at 37°C). The perfusion medium was continuously gassed with 95% O$_2$–5% CO$_2$. After preperfusion for 10 minutes for equilibration, the hearts were perfused with drugs or kinase inhibitors as indicated. Ischemia was induced by the termination of perfusion (0–50 minutes as indicated) with simultaneous gassing of the incubation chamber (37°C) with nitrogen to prevent oxygen uptake at the surface. The control hearts were continuously perfused under normoxic conditions. At the end of the experiments, the hearts were immediately frozen in liquid nitrogen and stored at −80°C until further use.

Preparation of Cardiac Plasma Membranes

Cardiac plasma membranes were prepared as described previously. Briefly, the hearts were homogenized using a polytron (three times for 10 seconds each, 10,000 rpm, Brinkmann Instruments, Inc., Westbury, N.Y.) in 40 ml buffer A containing (mM) Tris-HCl 50, sodium EDTA 5, sodium EGTA 2, and dithi oretiol (DTT) 1 (pH 7.2 at 4°C). The homogenate was passed through two layers of cheesecloth and centrifuged (360g for 10 minutes at 4°C). The supernatant was sedimented (45,000g for 10 minutes at 4°C) and washed twice in 40 ml buffer A. The resulting pellet was washed once in 40 ml of 50 mM Tris-HCl (pH 7.4 at 4°C) and resuspended in the same buffer to give a final concentration of 2–3 mg protein/ml.

Solubilization and Partial Purification of Adenylate Cyclase

Partial purification of the enzyme was performed according to a method by Pfeuffer et al. Briefly, hearts were homogenized using a Brinkmann polytron (three times for 10 seconds each, 10,000 units/min) in 40 ml buffer A containing (mM) Tris-HCl 50, sodium EDTA 5, and sodium EGTA 2 (pH 7.2 at 4°C). The homogenate was filtered through two layers of cheesecloth and centrifuged (360g for 10 minutes at 4°C). The supernatant containing 60–65% of total cyclase activity was sedimented (45,000g for 15 minutes at 4°C). The resulting pellet was resuspended in 10 ml solubilization buffer containing 50 mM Tris-HCl (pH 7.2), 1 mM DTT, 1 mM sodium EDTA, 500 mM sucrose, 50 μM GTP, 0.5% lubrol, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mM benzamidine. Solubilization (continuous stirring for 45 minutes at 4°C) was stopped by centrifugation (100,000g for 45 minutes at 4°C). The resulting supernatant was incubated with 0.3 ml of a 50% suspension of wheat germ sepharose for 30 minutes (4°C). The sepharose was washed three times in 20 ml buffer containing 100 mM NaCl, 25 mM Tris-HCl (pH 7.4 at 4°C), 1 mM DTT, 1 mM sodium EDTA, and 0.5% lubrol by centrifugation (200g for 3 minutes). Adenyl cyclase was eluted in 500 μl of the same buffer containing 300 mM N-acetylglucosamine. The eluate was used directly in the adenyl cyclase assay (1.98±0.12 μg protein per assay tube). The yield of adenyl cyclase was 3,023±34 pmol per heart in the purified plasma membranes, 1,513±12 pmol per heart in the soluble fraction, and 525±23 pmol per heart in the partially purified adenyl cyclase. The yields were identical in control and ischemic hearts (~17% of the membrane fraction and ~34% of the soluble fraction), and they were in good agreement with previously published data on purification of adenyl cyclase. The specific activity reached approximately 1,500 pmol cAMP·min$^{-1}$·mg protein$^{-1}$.

Adenyl Cyclase Activity

Adenyl cyclase activity was determined according to the method of Salomon et al. 10 mM Tris-HCl (pH 7.5), 12.5 mM MgCl$_2$, 1 mM sodium EDTA, 100 μM GTP, 1 mM DTT, 100 mM CAMP, 1 mM isobutylmethylxanthine, 10 units creatine kinase, and 20 mM phosphocreatine. For stimulation (–), isoproterenol (10$^{-3}$ M), NaF (10$^{-2}$ M), or forskolin (10$^{-4}$ M) was used (as indicated). The incubation (37°C) was started by the addition of cardiac membranes (~100 μg per tube) or the partially purified cyclase preparation (~2 μg per tube) and stopped after 10 minutes by the addition of 500 μl cold (4°C) 120 mM NaHCO$_3$ and 500 μl zinc acetate (125 mM). The radio labeled cAMP was isolated according to the method by Jakobs et al. using aluminum oxide columns. For quantitation, Cerenkov radiation was determined.

Protein Kinase C Activity

Protein kinase C activity was determined in the cytosol and in the particulate fraction according to the method of Takai et al. Briefly, hearts were homogenized using a Brinkmann polytron (two times for 6
seconds each, 10,000 rpm) in solution containing (mM) Tris-HCl 20, sucrose 250, sodium EDTA 1, sodium EGTA 1, vanadate 1, PMSF 1, and \( \beta \)-mercaptoethanol 10 (pH 7.4 at 4°C). Crude particles and nuclei were sedimented (360g for 10 minutes at 4°C). The resulting supernatant was centrifuged (100,000g for 45 minutes at 4°C) to give the soluble fraction (cytosol). To this fraction, Triton X-100 was added to give a final concentration of 0.3%. The pellet was resuspended in 10 ml buffer containing (mM) Tris-HCl 20, sucrose 250, sodium EDTA 1, sodium EGTA 1, vanadate 1, PMSF 1, and \( \beta \)-mercaptoethanol 10 (pH 7.4 at 4°C) with the inclusion of 0.3% Triton X-100. Solubilization was performed by continuous stirring on ice for 45 minutes. Insoluble particles were sedimented (100,000g for 45 minutes at 4°C). The resulting supernatant (solubilized membranes) and the cytosol (see above) were subjected to ion exchange chromatography using DEAE columns (bed volume, 2 ml). After application of the samples to the columns by gravity, the columns were washed with 10 ml of the above buffer, and protein kinase C activity was eluted (2 ml) using the identical buffer with the inclusion of 400 mM NaCl. The eluate was used directly for the determination of protein kinase C activity.

The incubation containing 20 mM Tris-HCl (pH 7.5), 1 mM MgCl\(_2\), 20 \( \mu \)M [\( \gamma ^{32} \)]P]ATP (200,000 cpm), and 1,000 \( \mu \)g/ml histone III was started by the addition of cytosol or solubilized particulate fraction after DEAE chromatography and continued for 5 minutes at 30°C. Basal kinase activity was determined in the presence of 1 mM sodium EDTA and 1 mM sodium EGTA. Stimulated protein kinase C activity was determined in the presence of CaCl\(_2\) (1 mM) alone or in the presence of CaCl\(_2\) (1 mM), phosphatidyserine (200 \( \mu \)g/ml), and diacylglycerol (200 \( \mu \)g/ml). Before incubation, phosphatidyserine and diacylglycerol were solubilized under nitrogen in 20 mM Tris-HCl (pH 7.5 at 4°C) using sonication (model B 15 sonifier, Branson Ultrasonics Corp., Danbury, Conn.). Basal kinase activity was not significantly altered in the presence of calcium alone. The incubation was stopped by adding excessively cold ATP (25 mM, 10 \( \mu \)l) on ice. Equal aliquots (50 \( \mu \)l) were spotted on phosphocellulose P81 (Whatman, Inc., Clifton, N.J.). The filters were washed twice for 15 minutes each at 4°C, and radioactivity was counted using Cerenkov counts. Protein kinase C activity was calculated as the difference between basal kinase activity (no calcium or lipids) and the stimulated protein kinase C activity per milligram protein or as protein kinase C activity per heart in each fraction as indicated.

**Protein Assay**

Protein concentrations were determined according to the method of Bradford\(^6\) using bovine albumin as a standard.

**Statistical Analysis**

Statistical analysis was performed using Student’s \( t \) test.\(^{22}\)

**Results**

**Sensitization of Adenylyl Cyclase in Acute Myocardial Ischemia**

To investigate the total adenylyl cyclase activity after various periods of global ischemia, forskolin-stimulated adenylyl cyclase activity was determined in cardiac plasma membranes after 5–50 minutes of global ischemia. As shown in Figure 1, even very brief periods of ischemia induce an increased enzyme activity with a maximal augmentation of \(-30\%\) after 10 minutes of global ischemia (306±32 vs. 392±40 pmol cAMP·min\(^{-1}\)·mg protein\(^{-1}\)). This sensitization of forskolin-stimulated adenylyl cyclase activity is also observed in the whole homogenate. It is, however, only transient. After 20 minutes of global ischemia, the enzyme activity has returned to control values (Figure 1), with a further decrease after 30 and 60 minutes of ischemia (data not shown). Moreover, on reperfusion the early sensitization of the adenylyl cyclase activity is rapidly reversible within 5 minutes (Figure 2).

To determine whether other membrane components and specifically G proteins may be responsible for this early ischemia-induced sensitization of adenylyl cyclase, the enzyme was solubilized and partially purified using wheat germ chromatography. As shown in Figure 3, direct stimulation by forskolin or manganese reveals that the ischemia-induced sensitization of adenylyl cyclase is retained even after solubilization and partial purification of the enzyme. Stimulation by activation of \( \beta \)-adrenergic receptors using isoproterenol (data not shown) or stimulation via the stimulatory G protein, \( G_s \), using NaF (Figure 3) is lost in this preparation, indicating that the enzyme was effectively separated from receptors and G proteins yet still retained its increased activity after ischemia.

**Effect of Prazosin on the Ischemia-Induced Sensitization of Adenylyl Cyclase**

To test whether activation of \( \alpha \)-adrenergic receptors may be involved in this early sensitization process, isolated hearts were perfused with prazosin before the ischemic insult. However, ischemia-induced sensitiza-
and purification of the enzyme was suggestive of a covalent regulatory modification of the enzyme. To investigate whether protein kinase C might be involved in this process, we tested different inhibitors of protein kinase C to determine whether they might be able to prevent the ischemia-induced sensitization.

Preperfusion with the specific protein kinase C inhibitor polyoxymyxin B \(^{22}\) (Figure 5) or the isoquinolinesulfonamide H-7 \(^{24}\) (data not shown) abolished the increase of activity that was observed after 10 minutes of global ischemia. As shown in Figure 5, perfusion with polyoxymyxin B alone had no effect on forskolin-stimulated adenylyl cyclase. Additionally, preperfusion with the highly potent inhibitor of protein kinase C, staurosporine, \(^{25}\) even at a concentration of \(5 \times 10^{-6} \text{M}\), was able to completely prevent sensitization of adenylyl cyclase after short periods of global ischemia (Figure 6). In separate experiments, it could be demonstrated that staurosporine at this concentration completely inhibits protein kinase C in vitro (data not shown).

These data indicate that an activation of protein kinase C in acute myocardial ischemia may be involved in the increased adenylyl cyclase activity. In fact, direct activation of protein kinase C in the isolated perfused rat hearts using perfusion with phorbol esters such as phorbol 12-myristate 13-acetate (PMA) for 5 or 10 minutes was able to induce a sensitization of the adenylyl cyclase comparable to the one observed after global ischemia (Figure 7). Preperfusion with staurosporine \(^{25}\) was able to prevent the phorbol-induced sensitization of adenylyl cyclase (data not shown). The metabolically inactive phorbol ester 4\(\alpha\)-phorbol 12,13-didecanoate, which does not stimulate protein kinase C, also failed to sensitize adenylyl cyclase (Figure 7).

**Activation of Protein Kinase C in Acute Myocardial Ischemia**

These data suggest that acute myocardial ischemia may promote an activation of protein kinase C that is responsible for the sensitization of adenylyl cyclase. Previous studies have reported that protein kinase C, when activated, becomes translocated from the cytosol

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**Figure 2.** Bar graph showing reversibility of the ischemia-induced sensitization of adenylyl cyclase with reperfusion. Isolated hearts were preperfused normoxically for 10 minutes. Ischemia was induced by termination of perfusion for 10 minutes (ischemia). Reperfusion was started after 10 minutes of ischemia for 5 to 25 minutes. In the control hearts, normoxic perfusion was continued for 35 minutes. Forskolin-stimulated adenylyl cyclase activity was determined in the cardiac plasma membranes. Shown are mean ± SEM of six hearts at each point with triplicate determinations. **p < 0.01 and *p < 0.05** compared with the control value.

**Effect of Protein Kinase Inhibitors**

The rapid and rapidly reversible sensitization of adenylyl cyclase that was retained after solubilization

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**Figure 3.** Bar graph showing sensitization of the partially purified adenylyl cyclase after myocardial ischemia. Adenylyl cyclase was partially purified from normoxically perfused hearts (control) or from ischemic hearts (15 minutes of global ischemia). The activity of adenylyl cyclase was determined in the absence of any stimulators (basal) and in the presence of forskolin (\(10^{-4} \text{M}\)), manganese (\(20 \text{mM}\)), or \(\text{NaF} (10 \text{mM}\)). Shown are mean ± SEM of four hearts at each point with triplicate determinations. *p < 0.02 compared with normoxic control hearts.
to the particulate fraction in isolated cells and in the heart.

To determine whether protein kinase C becomes activated in ischemia, we investigated its subcellular distribution after various periods of global ischemia as a parameter for its activation. As shown in Figure 8, in normoxically perfused hearts (controls), approximately 80–90% of the protein kinase C activity resides in the cytosol, whereas in the particulate fraction, only a small portion of the enzyme activity can be demonstrated (4,408±157 versus 462±68 pmol/min, Figure 8). Indicated are the total enzyme activities recovered in each fraction from individual hearts. The specific activities amounted to 89±13 versus 10±5 pmol·min⁻¹·mg protein⁻¹. A similar distribution of protein kinase C was found in nonperfused rat hearts (data not shown). In contrast, in acute myocardial ischemia, protein kinase C activity is significantly reduced in the cytosol (4,408±157 versus 3,620±108 pmol/min or 89±13 ver-

**Figure 4.** Bar graphs showing that blockade of the α₁-adrenergic receptor fails to influence the ischemia-induced sensitization of adenylyl cyclase. Isolated hearts were preperfused with the α₁-adrenergic blocker prazosin (10⁻⁴ M). Normoxic perfusion was continued for 10 minutes (control); ischemia was induced by termination of perfusion for 10 minutes. In isolated cardiac plasma membranes, adenylyl cyclase was determined in the absence of any stimulators (basal), in response to the β-agonist isoproterenol (10⁻⁵ M), or in response to forskolin (10⁻⁴ M). Shown are mean±SEM of three hearts at each point with triplicate determinations. *p<0.01 compared with normoxic control value.

**Figure 5.** Bar graph showing that inhibition of protein kinase C by polymyxin B completely prevents the ischemia-induced sensitization of adenylyl cyclase. Isolated hearts were preperfused with or without polymyxine B (10⁻⁶ M) in modified Krebs-Henseleit (Tyrode's) solution for 10 minutes. Ischemia was induced by termination of perfusion for 10 minutes. For control hearts, normoxic perfusion was continued. In isolated cardiac plasma membranes, adenylyl cyclase activity in response to direct stimulation by forskolin (10⁻⁴ M) was determined (see “Materials and Methods”). Shown are mean±SEM of three hearts at each point with triplicate determinations. *p<0.01 compared with corresponding value with polymyxin B.

**Figure 6.** Bar graph showing that inhibition of protein kinase C by staurosporine abolishes the ischemia-induced sensitization of adenylyl cyclase. Isolated hearts were preperfused with or without staurosporine (5×10⁻⁴ M). Ischemia was induced by termination of perfusion for 10 minutes; control hearts were perfused normoxically. In cardiac plasma membranes, forskolin-stimulated adenylyl cyclase activity was determined (compare with Figure 5). Shown are mean±SEM of three hearts at each point with triplicate determinations. *p<0.01 compared with corresponding value with staurosporine.
Sus 53±11 pmol·min⁻¹·mg protein⁻¹ with a concomitant increase of the enzyme activity in the particulate fraction (462±68 versus 1,019±159 pmol/min or 10±5 versus 49±9 pmol·min⁻¹·mg protein⁻¹). The total available cellular activity of protein kinase C was slightly but not significantly reduced after these brief periods of ischemia (Figure 8). Also, the total unstimulated or basal protein kinase activity or protein kinase C activity in the presence of calcium alone remained unaltered (data not shown). These data demonstrate that with acute myocardial ischemia protein kinase C becomes translocated from the cytosol to the particulate fraction, indicating its activation. Since stimulation of α1-adrenergic receptors by the presynaptically released catecholamines could be responsible for the activation and translocation of protein kinase C in ischemia, we investigated the effect of the α1-adrenergic receptor antagonist prazosin. In contrast to expectation, blockade of α1-adrenergic receptors was not able to prevent the ischemia-induced translocation of protein kinase C (Figure 9). After preperfusion with prazosin, the significant decrease of protein kinase C activity in the cytosol after 10 minutes of ischemia was unaltered (4,522±172 versus 3,672±125 pmol/min without prazosin, 4,432±137 versus 3,643±213 pmol/min after prazosin). Similarly, the ischemia-induced increase of protein ki-
nase C activity in the particulate fraction was not influenced by prazosin.

Discussion

The activity of the sympathetic adrenergic system plays a crucial role in the genesis of malignant arrhythmias in acute myocardial ischemia and in the spreading of the ischemic zone. An increased activity of the sympathetic adrenergic system in ischemia may potentiate these effects. The salient findings of the present study are that in acute myocardial ischemia adenyl cyclase is sensitized at the postreceptor level and that this sensitization of adenyl cyclase is retained after solubilization and partial purification of the enzyme, suggesting a covalent modification of the enzyme molecule. This sensitization of adenyl cyclase is completely blocked by polymyxin B and staurosporine, inhibitors of protein kinase C. Moreover, it could be demonstrated that brief periods of ischemia induce a translocation of protein kinase C from the cytosol to the plasma membranes. Both sensitization of adenyl cyclase and translocation of protein kinase C are not prevented by the α1-adrenergic receptor blocker prazosin. These data suggest that acute myocardial ischemia induces an α1-receptor–independent activation of protein kinase C that may mediate the sensitization of adenyl cyclase in the ischemic heart.

The present study demonstrates that in the ischemic heart sensitization of adenyl cyclase occurs very rapidly. On reperfusion, this sensitization is rapidly reversible. However, as shown in this well-controlled model system (isolated perfused hearts), this sensitization is only transient. With prolonged periods of ischemia, the activity of adenyl cyclase gradually decreases even below the normoxic control value after only 20 minutes of ischemia. In different in vivo model systems using varying time points after an infarction, both increased or decreased adenyl cyclase activities have been described. Depending on the species or model system used, these differences may be explained by slightly varying time courses of this transient sensitization process. Additionally, with an occlusion of a coronary artery, sensitization of adenyl cyclase may be locally inhomogeneous in the infarcted hearts, with the border zone having a different time course of sensitization and inactivation than the completely ischemic zone. Thus, electrophysiological changes of the “inhomogeneously” sensitized adrenergic system may add to the occurrence of malignant arrhythmias in acute myocardial infarction.

Sensitization of adenyl cyclase in acute ischemia occurs independently of the other components of the adenyl cyclase systems. Several lines of evidence support such a notion. First, as shown previously, blockade of β-adrenergic receptors before the ischemic insult and blockade of α1-adrenergic receptors, as shown here, are ineffective in preventing sensitization of adenyl cyclase. Second, the sensitization of adenyl cyclase could be documented in the partially purified enzyme preparation, in which other components of the adenyl cyclase system were removed or functionally uncoupled. These data indicate that the sensitization is independent of other membrane components and that it may be due to a covalent modification of the enzyme itself.

Although this sensitization could be attributed to the enzyme itself, the other components of the adenyl cyclase system may still potentiate the increased activity and responsiveness when adenyl cyclase is active within the domain of the plasma membrane of the ischemic heart. These components may include the ischemia-promoted increased release of endogenous catecholamines, the increase in functionally coupled β-adrenergic receptors, and the rapid functional loss of the inhibitory G protein (B. Rauch, C. Weinbrenner, F. Niroomand, R. Marquetant, T. Beyer, C. Schwenke, W. Kübler, W. Hasselbalch, and R.H. Strasser, submitted manuscript), which precedes the late loss of the stimulatory G protein. The rapid loss of tonic inhibition of adenyl cyclase promoted by the rapid functional impairment of Gs protein may also explain the slight sensitization of basal adenyl cyclase when tested in the plasma membranes. This slight increase in basal adenyl cyclase activity was no longer observed in the partially purified preparation, in which tonic inhibition of adenyl cyclase by the Gs protein was no longer effective even in the control preparations.

The ischemia-induced sensitization of adenyl cyclase could be prevented only by pretreatment with different blockers of protein kinase C, such as polymyxine B, staurosporine, and the isooquinolinesulfonamide H-7. Though none of the inhibitors is totally specific, the complete prevention of sensitization by all these different blockers indicates that protein kinase C might be involved in this sensitization process. In isolated model systems, pharmacological activation of protein kinase C predominantly using phorbol esters has been shown previously to modulate the adenyl cyclase system. In isolated frog erythrocytes, it even has been possible to document phosphorylation and activation of adenyl cyclase by phorbol ester–activated protein kinase C. Similarly, in the present study, in isolated perfused hearts such sensitization of adenyl cyclase by phorbol esters mediated by direct activation of protein kinase C was demonstrated and was comparable to the ischemia-induced sensitization of adenyl cyclase. The molecular mechanism responsible for this sensitization of cardiac adenyl cyclase in ischemia remains to be evaluated. In isolated systems using purified adenyl cyclase, phosphorylation of the amino acid serine 481 of the adenyl cyclase molecule promoted by purified protein kinase C results in a comparable sensitization. These data are also in keeping with the notion that protein kinase C may play a key role in the early, ischemia-induced sensitization of cardiac adenyl cyclase.

These data led us to investigate whether myocardial ischemia promotes an activation of protein kinase C. As shown here, short periods of ischemia led to a rapid translocation of protein kinase C from the cytosol to the plasma membranes or particulate fraction. Both physiological and pharmacological activation of protein kinase C are known to induce such translocation of the enzyme in isolated cells brain, and heart. These data suggest the rapid activation of protein kinase C in the infarcted heart. To our knowledge, this is the first direct demonstration of a translocation of protein kinase C that was induced by acute myocardial ischemia. Only in the fetal brain has severe global ischemia been shown to promote a translocation of protein kinase C.
The mechanisms by which protein kinase C may be activated in acute myocardial ischemia can only be surmised. Despite the previously observed increase of α1-adrenergic receptors during myocardial ischemia in the cat, but not in the rat, α1-adrenergic activation is not involved. At present, it cannot be excluded that activation of other receptor systems which activate the phosphatidylinositol system, receptor-independent stimulation of phospholipase C, or ischemia-induced alterations of intracellular calcium and phospholipid levels may contribute to the activation of protein kinase C. Further studies are necessary to evaluate such possibilities. A proteolytic activation of protein kinase C by calpain, for example, could be excluded since, as shown in the present article, the unstimulated kinase activity in the absence of calcium and phosphatidylinosine was unaltered after ischemia. Also, at present, it has not been evaluated which of the different forms of protein kinase C present in the heart are activated in ischemia.

In the present study, the activation of protein kinase C has been shown to be involved in the sensitization of adenyl cyclase. Other effects of protein kinase C in acute ischemia, such as direct effects on ion channels, G proteins, or the activation of oncogenes or the direct negative inotropic effect on contractility of the heart, may add to the pathophysiological importance of the activation of protein kinase C in the infarcted heart. Further studies are necessary to evaluate such potential roles of activated protein kinase C in acute myocardial ischemia.

In summary, the present study demonstrates that a newly characterized rapid but α1-receptor–independent activation of protein kinase C may induce the early sensitization of adenyl cyclase in acute myocardial ischemia. Further studies are necessary to evaluate the molecular mechanisms responsible for this activation and this newly characterized interaction of these two important signal transduction systems, i.e., the adenyl cyclase system and the phosphatidylinositol system in the acutely infarcted heart. Therapeutic strategies will have to be developed to prevent the deleterious sensitization of adenyl cyclase and the activation of protein kinase C.

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