Inhibition of Ca\(^{2+}\)-dependent PKC isoforms unmasks ERK-dependent hypertrophic growth evoked by phenylephrine in adult ventricular cardiomyocytes

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Abstract

Objective: The duration of extracellular signal-regulated kinase (ERK) activation and the ERK-dependency of hypertrophic growth differ between stimulation of \(\alpha\)-adrenoceptors or angiotensin II receptors. As both receptor systems activate different protein kinase C (PKC) isoforms, we hypothesized that PKC isoforms contribute to the specific effect of \(\alpha\)-adrenoceptor stimulation. Methods: Isolated adult ventricular cardiomyocytes from rats were used. Different PKC isoforms were inhibited either pharmacologically by six different PKC inhibitors or specifically downregulated by antisense oligonucleotides. ERK activation was determined by phosphorylation relative to total ERK. The rate of protein synthesis was determined by \(^{14}\)C-phenylalanine incorporation. Results: The hypertrophic response of phenylephrine was inhibited in a concentration-dependent fashion by three different inhibitors of Ca\(^{2+}\)-independent PKC isoforms (Gö6983, rottlerin, Gö6850), but not by three distinct PKC inhibitors directed preferentially against Ca\(^{2+}\)-dependent PKC isoforms (Ro32-0432, HBDDE, Gö6976). Antisense oligonucleotides directed against PKC-\(\alpha\), -\(\delta\), or -\(\varepsilon\) downregulated their specific isoforms. Their corresponding sense oligonucleotides did not affect PKC isoform expression. The phenylephrine-induced increase in protein synthesis was blocked by antisense oligonucleotides directed against PKC-\(\delta\) or PKC-\(\varepsilon\) but not PKC-\(\alpha\), confirming the pharmacological experiments. Inhibition of Ca\(^{2+}\)-dependent PKC isoforms by HBDDE or Gö6976 converted a transient activation of ERK by phenylephrine into a sustained response. Under these conditions, phenylephrine increased protein synthesis in an ERK-dependent way. Conclusion: Inhibition of Ca\(^{2+}\)-dependent PKC isoforms unmasks the ERK-independent effect of phenylephrine on protein synthesis. We conclude that co-activation of Ca\(^{2+}\)-dependent PKC isoforms by phenylephrine contributes to the specific effect on adult ventricular cardiomyocytes from rat.

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Keywords: Myocardial hypertrophy; Protein synthesis; Protein kinase C inhibitors; Angiotensin II

1. Introduction

The participation of G-protein-coupled receptors in an acceleration of protein synthesis during the induction of myocardial hypertrophy appears to be mandatory for the acute load-induced hypertrophic response. Among these, G-protein-coupled receptors activating phospholipase C and, subsequently, protein kinase C (PKC) have been shown to contribute to this process. Although one might expect that stimulation of different receptors from this family induces a more or less similar response, it has turned out that this is not the case. In a previous study, we focused on two members of the G-protein-coupled receptor family that are known to contribute to the development of myocardial hypertrophy in response to pressure overload. These are the \(\alpha_{1A}\)-adrenoceptors and the angiotensin II type 1 receptors (AT\(_1\)). They trigger the hypertrophic response of either selective \(\alpha\)-adrenoceptor agonists, i.e. phenylephrine, or angiotensin II,
respectively. The intracellular signal transduction pathway by which adult ventricular cardiomyocytes respond to both agonists in regard to protein synthesis is different. While α-adrenoceptor stimulation strongly increases protein synthesis via activation of PKC, PI 3-kinase, and p70S6K but independent of an activation of early response kinases (ERK) [1–4], angiotensin II moderately increases protein synthesis via a PKC- and ERK-dependent pathway [5–8]. This example clearly shows ligand-specific effects although similar, initial second messenger pathways—in this case, PKC activation—are used.

In a previous study, we found two major differences between these two systems with regard to the activation of second messenger pathways: First, α-adrenoceptor stimulation strongly activates Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent isoforms of PKC, namely PKC-α, PKC-β, PKC-δ, and PKC-ε, but angiotensin II has a clear preference for PKC-δ and PKC-ε [5]. Second, angiotensin II causes a long-lasting activation of ERK, while α-adrenoceptor stimulation activates ERK transiently [5]. These findings, together with the different outcome in regard to protein synthesis in terms of absolute values and ERK-dependency, prompted us to investigate in more detail the relationship between PKC isoform activation, ERK activation, and acceleration of protein synthesis. We hypothesized that co-activation of Ca\(^{2+}\)-dependent PKC isoforms by phenylephrine compared to angiotensin II contributes to the ligand-specific effects of the former agent. To confirm our hypothesis, we inhibited Ca\(^{2+}\)-dependent PKC isoforms. In a first set of experiments, six PKC inhibitors with different isoform specificity were used. Their impact on the ability of phenylephrine to stimulate protein synthesis was investigated. Concentration–response curves were determined for each of them. In the second set of experiments, these pharmacological studies were confirmed by down-regulation of PKC isoforms α or δ using antisense oligonucleotides. In a third set of experiments, we investigated the impact of pharmacological inhibition of Ca\(^{2+}\)-dependent PKC isoforms on the duration of ERK activation caused by phenylephrine. Finally, using these antagonists, we converted the specific α-adrenoceptor and ERK-independent effect on protein synthesis into an ERK-dependent effect. In summary, our study expands our present knowledge about how similar signals are converted into ligand-specific responses in adult ventricular cardiomyocytes.

2. Materials and methods

2.1. Cell culture

Ventricular heart muscle cells were isolated from 200- to 250-g male Wistar rats and plated in basic culture medium on 35-mm culture dishes (Falcon 3001) that had been pre-incubated overnight with 4% (vol/vol) fetal calf serum as described previously [1]. Animal handling conforms with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23). The basic culture medium consisted of a modified glucose-free medium 199 with Earle’s salts, 5 mmol/l creatine, 2 mmol/l L-carnitine, 5 mmol/l taurine, 100 IU/ml penicillin and 100 μg/ml streptomycin. To prevent growth of non-myocytes, media were also supplemented with 10 μmol/l cytosine-beta-arabinofuranoside. All incubations were carried out at 37 °C. Four hours after completing plating, the dishes were washed twice with basic culture medium to remove round and non-attached cells and were supplied with serum-free experimental medium (basic culture medium plus ascorbic acid (100 μmol/l)) in which the cells were incubated for the different time periods according to the treatment protocols at 37 °C.

2.2. Treatment protocols

Cells were stimulated with phenylephrine at a concentration of 10 μmol/l. The concentration was chosen on the basis of previously published concentration–response curves [4]. For each of the PKC inhibitors, we determined a concentration–response curve and calculated the IC\(_{50}\) values. In each case, inhibitors were added 15 min before adding the agonist. In some of the experiments, a MAP kinase kinase inhibitor (PD98059) was used at a concentration of 10 μmol/l, based on a concentration–response curve obtained for this inhibitor on the same cell culture system [3]. To downregulate the expression of PKC isoforms in adult ventricular cardiomyocytes, cells were incubated overnight with 10 μg/ml phosphorothioated antisense or sense oligonucleotides that corresponded to the region of the translation initiation site at the mRNA. The sequences are given in Table 1. In experiments in which we investigated the growth response of the cells under these conditions, cells were stimulated with phenylephrine 24 h after initiating oligonucleotide treatment.

2.3. Analytical procedures

2.3.1. Protein synthesis

Protein synthesis was determined by the incorporation of phenylalanine into cells as described previously [9]. Briefly, cells were exposed to L-[\(^{14}\)C]phenylalanine (3 × 10\(^{3}\) Bq/ml) for 24 h, and incorporation of radioactivity into the acid-insoluble cell mass was measured. Non-radioactive phenylalanine (0.3 mmol/l) was added to the medium to minimize variations in the specific activity of the precursor pool responsible for protein synthesis. In incorporation studies,
experiments were terminated by removing the supernatant medium from the cultures and washing three times with ice-cold phosphate-buffered saline (PBS; composition in mmol/l: 1.5 KH$_2$PO$_4$, 137 NaCl, 2.7 KCl, and 1.0 Na$_2$HPO$_4$, pH 7.4). The nucleic acids were digested with benzonase (Merck, Darmstadt, Germany). Proteins were separated by SDS-polyacrylamide gel electrophoresis under high-resolution conditions (acrylamide/bisacrylamide 100:1). After SDS-gel electrophoresis, proteins were transferred onto enhanced chemiluminescence nitrocellulose by semi-dry blotting. The sheets were saturated with 2% (wt/vol) bovine serum albumin (BSA) and incubated for 2 h with rabbit polyclonal anti-rat p42 mitogen-activated protein kinase (= ERK2, 0.2 µg/ml; Santa Cruz Biotechnology). After the sheets were washed, alkaline phosphatase-labeled sheep anti-rabbit IgG (50 mU/50 ml) was added for 2 h. Bands were visualized by alkaline phosphatase activity using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. For quantification, the two bands of the activated, phosphorylated form with retarded gel mobility and of the non-activated, non-phosphorylated form were scanned densitometrically. Box plots were put on the two bands and the arbitrary units (AU) were assessed. The results were expressed as the ratio of the arbitrary units of the activated (phosphorylated) ERK to those of total ERK (phosphorylated and non-phosphorylated): ERK activation = AU$_{ERK-P}$/AU$_{ERK-P}$ + AU$_{ERK}$. Immunoblots were performed to determine the protein expression of PKC isoforms after transfection of the cardiomyocytes with antisense oligonucleotides directed against some of the isoforms. The following changes to the above-mentioned methods for detection of ERK activation were used: the acrylamide:bisacrylamide ratio was 30:1 instead of 100:1 and the polyclonal antibodies used were anti-PKC-$\gamma$ (lot K1962 SA-148), anti-PKC-$\varepsilon$ (lot K2087 SA-149), and anti PKC-$\alpha$ (lot K2085 SA-144; all from Biomol).

2.4. Statistics

Data are given as mean ± S.E. from $n$ different culture dishes. Statistical comparisons were performed by one-way

2.3.2. Determination of ERK2 and immunoblotting

ERK2 activation was determined as described elsewhere in detail [3]. Briefly, after stimulation, cells were lysed in lysis buffer (composition: 50 mmol/l Tris–HCl, pH 6.7, 2% (wt/vol) SDS, 2% (vol/vol) mercaptoethanol, and 1 mmol/l orthovanadate). The nucleic acids were digested with benzonase (Merck, Darmstadt, Germany). Proteins were separated by SDS-polyacrylamide gel electrophoresis under high-resolution conditions (acrylamide/bisacrylamide 100:1). After SDS-gel electrophoresis, proteins were transferred onto enhanced chemiluminescence nitrocellulose by semi-dry blotting. The sheets were saturated with 2% (wt/vol) bovine serum albumin (BSA) and incubated for 2 h with rabbit polyclonal anti-rat p42 mitogen-activated protein kinase (= ERK2, 0.2 µg/ml; Santa Cruz Biotechnology). After the sheets were washed, alkaline phosphatase-labeled sheep anti-rabbit IgG (50 mU/50 ml) was added for 2 h. Bands were visualized by alkaline phosphatase activity using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. For quantification, the two bands of the activated, phosphorylated form with retarded gel mobility and of the non-activated, non-phosphorylated form were scanned densitometrically. Box plots were put on the two bands and the arbitrary units (AU) were assessed. The results were expressed as the ratio of the arbitrary units of the activated (phosphorylated) ERK to those of total ERK (phosphorylated and non-phosphorylated): ERK activation = AU$_{ERK-P}$/AU$_{ERK-P}$ + AU$_{ERK}$. Immunoblots were performed to determine the protein expression of PKC isoforms after transfection of the cardiomyocytes with antisense oligonucleotides directed against some of the isoforms. The following changes to the above-mentioned methods for detection of ERK activation were used: the acrylamide:bisacrylamide ratio was 30:1 instead of 100:1 and the polyclonal antibodies used were anti-PKC-$\gamma$ (lot K1962 SA-148), anti-PKC-$\varepsilon$ (lot K2087 SA-149), and anti PKC-$\alpha$ (lot K2085 SA-144; all from Biomol).

2.4. Statistics

Data are given as mean ± S.E. from $n$ different culture dishes. Statistical comparisons were performed by one-way

### Table 2

<table>
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<th>Inhibitor</th>
<th>IC$_{50}$</th>
<th>Calculated</th>
<th>Alpha</th>
<th>Beta</th>
<th>Delta</th>
<th>Epsilon</th>
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<td>8</td>
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<td>210</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Go6893</td>
<td>11 ± 6</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Rottlerin</td>
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<td>30,000</td>
<td>42,000</td>
<td>6000</td>
<td>80,000</td>
<td></td>
</tr>
<tr>
<td>Ro32-0432</td>
<td>–</td>
<td>9</td>
<td>28</td>
<td></td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>HBDE</td>
<td>–</td>
<td>43,000</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Go6976</td>
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<td>2</td>
<td>6</td>
<td></td>
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</tr>
</tbody>
</table>

Data are given as IC$_{50}$ values calculated from the concentration–response curves shown in Figs. 1 and 2. IC$_{50}$ values for the different PKC isoforms are given as citations from the literature as indicated by the references.
analysis of variance and with the use of Student–Newman–Keuls test for post hoc analysis. Differences with $p < 0.05$ were regarded as statistically significant. The IC$_{50}$ values were calculated from the derived values of four separate experiments and analyzed by the GraphPad Prism 3.0 program.

3. Results

3.1. The increase in protein synthesis evoked by $\alpha$-adrenoceptor stimulation depends on the activation of $\text{Ca}^{2+}$-independent PKC isoforms

In a first set of experiments we evaluated the influence of six PKC inhibitors with either no PKC isoform specificity or with different specificity on $\alpha$-adrenoceptor-dependent increases in protein synthesis. Fig. 1 summarizes the results for three PKC inhibitors that inhibited the increase of protein synthesis evoked by phenylephrine in a concentration-dependent way. Gö6983 inhibited the increase in protein synthesis caused by phenylephrine with an IC$_{50}$ of approximately 11 nmol/l, and rottlerin and Gö6850 had IC$_{50}$ values of 6 nmol/l and 145 nmol/l, respectively. The observed IC$_{50}$ values suggest that $\text{Ca}^{2+}$-independent PKC isoforms are mainly involved in the $\alpha$-adrenoceptor-dependent effect on protein synthesis (Table 2). In a subsequent set of experiments, we used three PKC inhibitors that are mostly specific for $\text{Ca}^{2+}$-dependent PKC isoforms (Ro32-0432, HBDDE, Gö6976). None of them, however, inhibited the hypertrophic response of the cells to phenylephrine (Fig. 2).

These pharmacological experiments were confirmed by transfection of adult ventricular cardiomyocytes with antisense oligonucleotides directed against either PKC-α, PKC-δ, or PKC-ε. As shown in Fig. 3, transfection with antisense oligonucleotides significantly reduced the expression of the corresponding PKC isoforms. On average, transfection with antisense oligonucleotides directed against PKC-δ reduced the expression of PKC-δ to 40 ± 5% of control ($n = 3$, $p < 0.05$ vs. control) but not that of PKC-α (94 ± 9%, $n = 3$, n.s. vs. control). Transfection with antisense oligonucleotides directed against PKC-α reduced the expression of PKC-α to 29 ± 11% of control ($n = 3$, $p < 0.05$) and that of PKC-δ to

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Fig. 2. Concentration–response curves for the three different PKC inhibitors Ro32-0432, HBDDE, and Gö6983 and their impact on phenylephrine-dependent increases in protein synthesis. The maximal response to phenylephrine (10 µmol/l) in the absence of inhibitors was set at 100%. This was 29% above control in experiments using Ro32-0432, 40% above control in experiments using HBDDE, and 28% above control in experiments using Gö6983. Closed symbols represent data in the presence of phenylephrine and open symbols represent data in the absence of phenylephrine. Data are means ± S.E. from $n = 4$ preparations (each run in quadruplicate).

Fig. 3. (A) Representative immunoblots indicating PKC isoform expression 24 h after incubation with either sense or antisense oligonucleotides directed against PKC-α, PKC-δ, or PKC-ε. (B) Representative immunoblots indicating the specificity of isoform-selective downregulation by transfection with antisense oligonucleotides directed against either PKC-α or PKC-δ as indicated. The top panel shows the PKC-α isoform (effects of antisense for PKC-δ and PKC-α) and the bottom panel PKC-δ.
67 ± 15% of control (n = 3, p < 0.05 vs. control). Transfection with antisense oligonucleotides against PKC-ε reduced the expression of PKC-ε to 53 ± 4% of control (n = 3, p < 0.05 vs. control). After having confirmed specificity of the antisense oligonucleotides used in the experiments, the effect of phenylephrine on protein synthesis was determined in cells in which one of these three PKC isoforms had been downregulated. Selective down-regulation of either PKC-δ or PKC-ε significantly impaired the increase in protein synthesis of adult ventricular cardiomyocytes in response to phenylephrine (Fig. 4). However, downregulation of PKC-α did not influence the response of the cells. Although both PKC-δ and PKC-ε significantly reduced the phenylephrine-induced increase in protein synthesis to a similar extent, neither of the two treatment strategies was sufficient to attenuate the response completely (Fig. 4B).

3.2. Influence of Ca2+-dependent PKC isoforms on ERK activation

In the next set of experiments, we determined the impact of selective inhibition of Ca2+-dependent PKC isoforms on ERK activation. As depicted in Fig. 5A by a representative immunoblot, inhibition of Ca2+-dependent PKC isoforms by either HBDDE or Gö6976 significantly prolonged the duration of strong ERK activation. These inhibitors did not have any effect on the early activation of the enzyme. Thus, after 5 min, PE caused an increase in the ratio of phosphorylated ERK to non-phosphorylated ERK from 14.3 ± 5.4% to 57.5 ± 3.7% (n = 4, p < 0.05 vs. control), PE in presence of HBDDE to 60.4 ± 2.7% (n = 4, p < 0.05 vs. control), and PE and Gö6976 to 55.3 ± 6.7% (n = 4, p < 0.05 vs. control). Therefore, activation of ERK by phenylephrine after 5 min was similar in the presence or absence of PKC inhibitors directed against Ca2+-dependent PKC isoforms. However, in the presence of HBDDE and Gö6976, phenylephrine augmented ERK activation at 90 min more strongly compared to phenylephrine alone (Fig. 5B).
3.3. Impact of ERK activation on protein synthesis

We finally investigated whether the observed effect of inhibition of Ca\(^{2+}\)-dependent PKC isoforms with regard to long-term ERK activation had an effect on the participation of ERK in the regulation of protein synthesis. In a first set of experiments, we confirmed earlier data [3,4], indicating that phenylephrine increases protein synthesis in an ERK-independent way (Fig. 6A). This was evidenced by the inability of PD98059, a MAP kinase kinase inhibitor, to inhibit the response of the cells to phenylephrine. However, under conditions where the Ca\(^{2+}\)-dependent PKC isoforms were inhibited by either HBDDE or G66976, phenylephrine increased protein synthesis via a pathway involving ERK, as PD98059 significantly reduced the increase in protein synthesis (Fig. 6B,C).

4. Discussion

In this study, we hypothesized that inhibition of the activation of Ca\(^{2+}\)-dependent PKC isoforms in the presence of phenylephrine, an \(\alpha\)-adrenoceptor agonist, unmasks an ERK-dependent, hypertrophic response of adult ventricular cardiomyocytes to phenylephrine. The hypothesis was driven by former experiments comparing the effects of angiotensin II and phenylephrine. In those experiments, a difference between both agonists with regard to PKC isoform specificity and ERK-dependency of hypertrophic growth was found [5]. The new study shows that an inhibition of Ca\(^{2+}\)-dependent PKC isoforms significantly prolonged the duration of strong ERK activation. Under these conditions, phenylephrine increased protein synthesis in an ERK-dependent way. In contrast, phenylephrine alone activated ERK transiently and increased protein synthesis in an ERK-independent way. Therefore, we conclude from our experiments, first, that co-activation of Ca\(^{2+}\)-dependent PKC isoforms causes a transient activation of ERK in adult rat ventricular cardiomyocytes, and, second, that coupling of the ERK pathway to protein synthesis requires sustained activation of ERK.

Participation of the ERK pathway in the increase of protein synthesis of cardiomyocytes is discussed in the literature as being quite controversial. On the one hand, clear evidence comes from experiments mainly performed on neonatal cardiomyocytes that different G-protein-coupled receptor systems activate ERK and increase protein synthesis via this pathway [4,7,8]. Nevertheless, reports in the literature have not been convincing; e.g., phenylephrine was found to increase protein synthesis in neonatal cardiomyocytes in an ERK-dependent or ERK-independent manner [8,10,11]. The reason for these different outcomes was unclear. In adult cardiomyocytes, which are more closely related to the cell type that responds to pressure overload under pathophysiological conditions, phenylephrine increases protein synthesis clearly in an ERK-independent manner [1–3]. From these findings, we postulated initially that coupling of the ERK pathway to the regulation of protein synthesis is lost during the transition from neonatal to adult cardiomyocytes. This goes along with a loss of the ability to respond to trophic stimuli by cell division. In line with these suggestions, fetal cardiomyocytes from sheep respond to angiotensin II in an early phase of differentiation by ERK-dependent increases in cell proliferation but later on by ERK-independent increases in protein synthesis [12]. However, adult cardiomyocytes do not completely lose their ability to respond to agonists by hypertrophic growth in an ERK-
dependent way. Recently, we showed that angiotensin II increases protein synthesis in an ERK-dependent manner [5]. It is noteworthy that these observations were made in the same cell preparation as those employing phenylephrine, which excludes any preparation-specific differences. As outlined above, phenylephrine and angiotensin II not only differ in their ability to increase protein synthesis in an ERK-dependent or ERK-independent way, but also with regard to the duration of ERK activation and their ability to activate specific isoforms of PKC. As shown before, phenylephrine causes translocation of PKC-α, PKC-β, PKC-δ, and PKC-ε, but angiotensin II caused translocation of mainly PKC-δ and PKC-ε [5]. Therefore, we investigated in more detail the contribution of these PKC isoforms to the increase in protein synthesis in the presence of phenylephrine.

Adult ventricular cardiomyocytes express three different PKC isoform families. The first one consists of the classical, Ca2+-dependent PKC isoforms, of which PKC-α and PKC-β, but not PKC-γ, are expressed in adult ventricular cardiomyocytes from rats. The second one consists of the Ca2+-independent isoforms, of which PKC-δ and PKC-ε are expressed most prominently. The third family consists of the untypical PKC isoforms that do not respond to phorbol esters. As the increase in protein synthesis can be mimicked by phorbol ester [1,13], members of this latter family do not participate in the hypertrophy response and were not further evaluated in this study. Our present study shows that phenylephrine increases protein synthesis via stimulation of Ca2+-independent PKC isoforms. This finding is based, firstly, on experiments in which we used three different inhibitors specific for either PKC-δ or PKC-ε (see Fig. 1 and Table 2) and, secondly, on experiments in which we downregulated PKC-δ and PKC-ε (see Fig. 4). In contrast, selective inhibition of PKC-α or PKC-β did not result in a clear concentration-dependent inhibition of phenylephrine-stimulated increases in protein synthesis (see Fig. 2 and Table 2). Moreover, downregulation of PKC-α by nearly 80% did not reduce the ability of phenylephrine to increase protein synthesis (Fig. 4). In that aspect, we confirmed work from other groups for our experimental system (e.g., Ref. [14]). A strong prevalence for the involvement of PKC-δ vs. PKC-ε was not demonstrated in this study, although the more robust expression of PKC-δ and the inability of Ro32-0432 to inhibit protein synthesis caused by phenylephrine would appear to favor PKC-δ.

In the second part of the study, we demonstrated that phenylephrine-specific activation of Ca2+-dependent PKC isoforms modifies ERK activation. It was shown in two different studies that phenylephrine activates ERK in a transient manner [3,5]. By blocking Ca2+-dependent PKC isoforms, however, we were able to prolong the duration and efficiency of phenylephrine to activate ERK. Under these conditions, phenylephrine is able to increase protein synthesis in an ERK-dependent way. Thus, we were able to reveal an ERK-dependent component of the regulation of protein synthesis by inhibition of a subset of PKC isoforms in presence of phenylephrine. As speculated previously, coupling of the ERK pathway to protein synthesis occurs under conditions of long-term activation of this pathway. This finding is in line with recent transgenic work in which expression of constitutively active MAP kinase kinase caused myocardial hypertrophy, leading to a constant activation of ERK [15].

In summary, our study identifies a new role for Ca2+-dependent PKC isoforms in adult ventricular cardiomyocytes from rats as a modifier for downstream targets of the other PKC isoforms. Although not investigated in this study, one might speculate that dephosphorylation of ERK is induced by activation of classical, Ca2+-dependent PKC isoforms. This produces a transient ERK activation like that found with phenylephrine. As myocardial hypertrophy can be compensatory or de-compensatory, it is unclear whether in a clinical setting inhibition of myocardial hypertrophy per se is protective or not. However, some of the recent data support the idea that at least PKC-ε is part of a more compensatory, hypertrophic phenotype [15]. The results of this study might indicate that Ca2+-independent PKC isoforms increase protein synthesis via ERK activation. ERK activation has been shown to be anti-apoptotic, for example, further indicating a compensatory rather than mal-adaptive type of hypertrophy. Our study opens new ways to modify myocardial hypertrophy in response to pressure overload instead of complete inhibition of all PKC activity and thus may lead to new concepts for the treatment of myocardial hypertrophy.

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References


