Role of phosphatidylinositol 3-kinase activation in the hypertrophic growth of adult ventricular cardiomyocytes

Klaus-Dieter Schlüter*, Yaron Goldberg, Gerhild Taimor, Matthias Schäfer, Hans Michael Piper
Physiologisches Institut, Universität Giessen, Aulweg 129, D-35392 Giessen, Germany
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Abstract
Objective: The present study investigated whether activation of phosphatidylinositol 3-kinase (PI3-kinase) is involved in the stimulation of hypertrophic growth of adult ventricular cardiomyocytes under α- or β-adrenoceptor stimulation. Methods: Adult ventricular rat cardiomyocytes were used either directly after isolation (day 1 culture) or after cultivation for 6 days in presence of 20% fetal calf serum (day 7 culture). PI3-kinase activity was determined in extracts of cardiomyocytes after immunoprecipitation with an antibody against the p85 subunit of PI3-kinase. The influence of PI3-kinase inhibition on myocardial growth was determined using the specific PI3-kinase inhibitors wortmannin and LY294002. Results: In day 1 cultures α-adrenoceptor stimulation, but not β-adrenoceptor stimulation caused activation of PI3-kinase. In response to α-adrenoceptor stimulation but not β-adrenoceptor stimulation an acceleration of protein synthesis (incorporation of C-phenylalanine) and an increase in the total masses of cellular protein and RNA was observed. In these cultures inhibition of PI3-kinase attenuated the acceleration of protein synthesis and the increase in cellular masses of protein or RNA in response to α-adrenoceptor stimulation. In day 7 cultures α- and β-adrenoceptor stimulation caused activation of PI3-kinase and increased protein synthesis. In these cultures inhibition of PI3-kinase attenuated the growth response to α- and β-adrenoceptor stimulation. Conclusions: PI3-kinase activation via protein kinase C-dependent or cAMP-dependent pathways is required for hypertrophic growth of adult cardiomyocytes.

Keywords: Hypertrophy; Myocytes; Protein kinases; Second messengers; Signal transduction

1. Introduction
Cardiac hypertrophy is an important compensatory response of the heart to altered work load [1]. Although this process is initially compensatory, hypertrophied myocardium may eventually become dysfunctional. Enlargement of cardiac mass is a well established predictor of subsequent heart failure [2]. Myocardial hypertrophy is characterized by an increased rate of protein synthesis, an enlargement of translational capacity (increase in total RNA) and often a re-expression of fetal type proteins, i.e. myosin heavy chain-β, atrial natriuretic peptide, and creatine kinase B. The different intracellular signals involved in the induction of these cellular changes are as yet only partly understood.

Various growth factors have been identified which are able to induce hypertrophic growth of isolated adult rat ventricular cardiomyocytes [3]. Among these, catecholamines accelerate myocardial growth by stimulation of either α-adrenoceptors [4–7] or β-adrenoceptors [7–11]. The latter effect of catecholamines, however, requires cultivation of cardiomyocytes with 20% (v/v) fetal calf serum (FCS) or activated TGF-β prior to stimulation of β-adrenoceptors [7,10]. It has been concluded that exposure of cardiomyocytes to TGF-β alters their intracellular signal transduction in response to β-adrenoceptor stimulation. This effect seems important because TGF-β, is expressed in hypertrophying hearts before these hearts develop a non-adapted hypertrophy [12]. In cultures demonstrating a hypertrophic response to both α- and β-adrenoceptor stimulation, signal transduction pathways

*Corresponding author. Tel.: +49-641-994-7243; Fax: +49-641-994-7239; E-mail: klaus-dieter.schluter@physiologie.med.uni-giessen.de

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must converge at some level. This level has not yet been identified. We investigated the hypothesis that PI3-kinase is situated at or below the level of convergence.

PI3-kinase is activated in various mammalian cell types by growth factors like insulin, PDGF and others. The enzyme catalyses the formation of a family of phosphoinositides with phosphate at the D-3 position of the inositol ring [13,14]. Activation of PI3-kinase correlates well with the ability of several growth factors to induce mitogenesis, suggesting an important role in the transduction of trophic signals [15–17]. Whether PI3-kinase activation is part of an intracellular signaling inducing hypertrophic growth of adult ventricular cardiomyocytes has not yet been investigated. PI3-kinase has been identified as a potential upstream activator of p70 ribosomal S6-kinase (p70S6b) in HepG2 cells [18] and it has already been reported that hypertrophic growth of neonatal cardiomyocytes depends on the activation of p70S6 [19]. It was therefore of interest to investigate whether hypertrophic stimuli activate PI3-kinase in adult cardiomyocytes. The availability of two chemically distinct inhibitors of PI3-kinase, wortmannin and LY 294002, was an useful adjunct to clarify the role of PI3-kinase in the induction of hypertrophic growth of cardiomyocytes.

Our studies were performed in a well characterized culture model of ventricular cardiomyocytes isolated from adult rats. In this model the role of agonists on intracellular signaling and protein metabolism can be studied in media devoid of other stimuli. Since cardiomyocytes are mechanically quiescent in these cultures, effects of agonists mediated through a modulation of the mechanical behavior of the cells are also excluded. Two different culture models were used in our study: (1) Freshly isolated cardiomyocytes which exhibit a hypertrophic response to the α-adrenoceptor agonist phenylephrine but not the β-adrenoceptor agonist isoprenaline (day 1 culture) and (2) cardiomyocytes, precultured for 6 days in presence of 20% fetal calf serum and studied on day 7 (day 7 culture), which exhibit a hypertrophic response to phenylephrine and isoprenaline. The reason for the use of both culture models in this study is that the hypertrophic responsiveness to β-adrenoceptor stimulation is induced only in day 7 cultures [7,10].

2. Methods

2.1. Cell culture

Ventricular heart muscle cells were isolated from 200- to 250-g male Wistar rats as previously described [20,21]. Isolated cells were suspended in FCS-free culture medium and plated at a density of 1.4×10^5 elongated cells/35 mm culture dish (Falcon type 3001). The culture dishes had been preincubated overnight with 4% FCS in medium 199. The basic culture medium consisted of medium 199 with Earle’s salts, 5 mM creatine, 2 mM L-carnitine, 5 mM taurine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. To prevent growth of nonmyocytes, media were also supplemented with 10 μM cytosine-β-D-arabinofuranoside.

Four hours after plating, cultures were washed twice with culture medium to remove round and nonattached cells. Experiments were either performed on cells on the first or the seventh day in culture. In day 1 cultures, FCS-free experimental media were added and cells were incubated in these media up to 24 h. To obtain day 7 cultures, incubation with FCS supplemented (20% v/v) cell culture media was continued for 6 days. On day 7, culture media were exchanged for FCS-free experimental media and cells were incubated in these media up to 24 h. Experimental media consisted of basic culture media (see above) with additions of phenylephrine, isoprenaline, dibutyryl-cAMP or phorbolmyristate acetate, at concentrations indicated. Ascorbic acid (100 μM) was added to all cultures as an antioxidant.

2.2. Incorporation of [14C]phenylalanine, [14C]-juridine, and changes in cellular protein and RNA mass

Incorporation of phenylalanine into cells was determined by exposing cultures to L-[14C]phenylalanine (0.1 μCi/ml) for 24 h and determining the incorporation of radioactivity into acid-insoluble cell mass as reported earlier [7]. Nonradioactive phenylalanine (0.3 mM) was added to the medium to minimize variations in the specific activity of the precursor pool responsible for protein synthesis. These experiments were terminated after 24 hrs. Within this time period the incorporation of radioactivity from [14C]-phenylalanine into acid-insoluble mass of the cell was linear and the portion of cellular precursor radioactivity, not incorporated into the acid-insoluble cell mass, was small and identical under all investigated conditions. Experiments were terminated by removal of the supernatant medium from the cultures. Culture dishes were washed three times with ice-cold phosphate-buffered saline (PBS; composition in mM: 1.5 KH PO , 137 NaCl, 2.7 KCl, and 1.0 NaHPO , pH 7.4). Subsequently, ice-cold 10% (wt/vol) trichloroacetic acid was added. After storage overnight at 4°C, the acid was removed from the dishes. Radioactivity contained in this acid fraction was taken to present the intracellular precursor pool as described in [7]. The dishes were then washed twice with ice-cold PBS. The remaining precipitate on the culture dishes was dissolved in 1 N NaOH–0.01% (wt/vol) sodium dodecyl sulfate (SDS) by an incubation for 2 h at 37°C. In these samples protein contents [22] and DNA contents [23] were determined, and the radioactivity was counted. RNA was determined from an aliquot of these samples after precipitation with an equal volume of 10% (wt/vol) perchloric acid in the remaining supernatant [24]. The RNA content was also expressed relative to the DNA content of the
samples. Incorporation of \(^{14}C\text{-uridine}\) into cells was determined by exposing cultures to [U-\(^{14}C\text{-uridine}\) (0.1 \(\mu\text{Ci/ml}\)) for 6 h and determining the incorporation of radioactivity into RNA as described earlier [7]. The rate of incorporation of \(^{14}C\text{-uridine}\) into the acid-insoluble material was constant under all investigated conditions. For these studies the experiments were terminated by removal of the supernatant medium from the cultures and washed three times with ice-cold phosphate-buffered saline (PBS). Subsequently cells were scraped off with a rubber policeman, collected, centrifuged and resuspended in 300 \(\mu\text{l}\) RNA clean (AGS, Heidelberg, Germany). RNA was extracted by addition of chloroform and subsequently precipitated with isopropanol. The remaining RNA pellet was washed twice with ethanol (70% (v/v)), dried and resuspended in sterile \(\text{H}_2\text{O}\). The amount of RNA was determined photometrically and the incorporated radioactivity was counted. RNA synthesis is expressed as the amount of \(^{14}C\text{-uridine}\) per mg RNA.

2.3. Determination of PI3-kinase activation

PI3-kinase activity was determined in immunoprecipitates as described by Whitman et al. [25]. Briefly, cardiomyocytes were washed twice with PBS and the cells were lysed in lysis buffer (composition: 10% (v/v) glycerol, 1% (v/v) nonidet P40, 1 mM PMSF). After centrifugation (10 min at 10,000 \(\times\) g) the supernatant was diluted to equal amounts of protein and used for immunoprecipitation with an antibody against the \(\alpha\)-subunit of bovine PI3-kinase and the immunoprecipitates were sedimented with Protein A-Sepharose. The pellets were washed with PBS, twice with buffer A (composition: 0.5 M LiCl, 0.1 M Tris, pH 7.4) and once with buffer B (composition: 10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA) and resuspended in 25 \(\mu\text{l}\) of buffer B. 1 mg/ml phosphatidylinositol (Sigma, Deisenhofen, Germany) was dispersed by sonification in 5 mM HEPES-buffer, pH 7.4 and 20 \(\mu\text{l}\) of this solution was added to the resuspended immunoprecipitates. After preincubation for 30 min at room temperature, the phosphorylation reaction was started by addition of 20 \(\mu\text{Ci}\) \(\gamma\text{-}^{32}\text{P-ATP}\) in starting buffer containing 50 \(\mu\text{M}\) ATP and 5 mM MgCl\(_2\). The total volume in the reaction tubes was 50 \(\mu\text{l}\). The reaction mixture was incubated for 20 minutes at 25\(^{\circ}\)C and terminated by addition of 100 \(\mu\text{l}\) of 1 M HCl. Phospholipids were then extracted with 200 \(\mu\text{l}\) of CHCl\(_3\)/MeOH (1:1). The organic phase was spotted onto a silica gel thin layer chromatography (TLC) plate pre-treated with 1% (w/v) potassium oxalate. Phosphorylated products were separated by TLC in a CHCl\(_3\)/MeOH/4 M \(\text{NaOH}\) (9:7:2) developing solvent and visualized on a phosphorimagery plate which varied between the reaction tubes (origin). The organic phase was spotted onto a silica gel thin layer chromatography plate pre-treated with 1% (w/v) potassium iodine vapor. An autoradiogram of the thin layer chromatography plate generated on a phosphorimagery plate is shown. The position of spots corresponding to \(^{32}\text{P-phosphatidylinositol (PIP)}\) and origin (Ori) is indicated.

Fig. 1. Activation of PI3-kinase by phenylephrine. Cardiomyocyte PI3-kinase was immobilized on protein G-Sepharose beads through anti-PI3-kinase \(\alpha\)-p85 antibodies. Immunoprecipitates were generated from cardiomyocytes cultured under control conditions (C) or in presence of phenylephrine (PE, 10 \(\mu\text{M}\)) for 15 min. PI3-kinase activation in immunoprecipitates was assayed as described in Section 2. The lipid products were extracted and separated by thin layer chromatography as described. An autoradiogram of the thin layer chromatography plate generated on a phosphorimagery plate is shown. The position of spots corresponding to \(^{32}\text{P-phosphatidylinositol (PIP)}\) and origin (Ori) is indicated.

2.4. Statistics

Data are given as means±SD or SEM as indicated from n different culture preparations. Statistical comparisons were performed by one-way analysis of variance and use of the Student–Newman–Keuls test for post hoc analysis [26]. Differences with \(p<0.05\) were regarded as statistical significant.

2.5. Materials

Falcon tissue culture dishes were obtained from Becton-Dickinson (Heidelberg, Germany). Boehringer Mannheim (Mannheim, Germany) was the source for glutamine-free medium 199 and fetal calf serum. Cytosine-\(\beta\)-\(\alpha\)-arabinofuranoside, \(\text{l}\)-carnitine, creatine, taurine, \(\text{l}\)-
phenylephrine hydrochloride, D,L-isoproterenol hydrochloride, phorbol 12-myristate 13-acetate and bisindolylmaleimide were obtained from Sigma (Deisenhofen, Germany). Wortmannin and LY 294002 were obtained from Calbiochem-Novabiochem, Bad Soden, Germany. All other chemicals were of analytical grade. Radiochemicals were purchased from Amersham-Buchler (Braunschweig, Germany).

3. Results

3.1. PI3-kinase activation in day 1 cultures

It was shown previously that in day 1 cultures the α-adrenoceptor agonist phenylephrine but not the β-adrenoceptor agonist isoprenaline accelerates protein synthesis. We therefore investigated whether PI3-kinase is activated under the same experimental conditions. Activation of PI3-kinase was evaluated in anti-PI3-kinase p85 immunoprecipitates. In day-1-cultures incubation with phenylephrine but not isoprenaline activated PI3-kinase (Fig. 2). This activation was completely abolished when cardiomyocytes had been preincubated with 100 nM wortmannin (from 192.527±42.521 dpm to 12,748±9,448 dpm; p<0.01, n=3). Addition of wortmannin had no influence on PI3-kinase activity of isoprenaline treated cultures (Fig. 2).

![Fig. 2. PI3-kinase activation in cardiomyocytes treated for 15 min with phenylephrine (10 μM) or isoprenaline (1 μM) with or without wortmannin (WORT, 100 nM). Data show the phosphorylation of phosphatidylinositol by immunoprecipitates of the p85 α-subunit of PI3-kinase as described in Section 2. Data are expressed as the amount of phosphorylated phosphatidylinositol relative to the amount of 32P in the lipid extracts of the reaction mixture. Phosphorylation products were separated by thin layer chromatography and the radioactivity counted with a phosphorimaging process. Data are means ± SEM from three experiments. *, p <0.05 vs. control (C).](image)

3.2. Effect of wortmannin and LY 294002 on myocyte growth induced by phenylephrine on day 1 cultures

The influence of the PI3-kinase inhibitors wortmannin and LY294002 on protein synthesis under α-adrenoceptor stimulation was investigated in the same culture model. For this purpose cardiomyocytes were incubated for 24 h in presence of phenylephrine and protein synthesis was evaluated by determination of the incorporation of 14C-phenylalanine into cell mass. Without wortmannin phenylephrine increased incorporation of 14C-phenylalanine by 57±6% (Fig. 3). Wortmannin, a specific inhibitor of PI3-kinase, reduced the phenylephrine mediated increase in protein synthesis in a dose dependent way. At the highest concentration tested (100 nM) the phenylephrine induced increase of 14C-phenylalanine incorporation was reduced to 12±5% (Fig. 3). The same concentration of wortmannin did not significantly influence the basal incorporation of 14C-phenylalanine (2.4±0.6×10^2 dpm/μg DNA basal vs. 2.2±0.5×10^2 dpm/μg DNA in presence of wortmannin, n=4). Similar results were also obtained with another PI3-kinase inhibitor, LY 294002. At 100 μM, the phenylephrine induced increase of 14C-phenylalanine incorporation was reduced to 6±5% (Fig. 3). In presence of phenylephrine alone a 25±11% increase in total cellular protein was observed in a 24 h period. When wortmannin or LY 294002 were applied, this increase remained absent (Table 1). The growth response of cardiomyocytes to α-adrenoceptor stimulation was accompanied by an increase in the total cellular RNA mass by 17±8%. This
Table 1

Influence of wortmannin and LY 294002 on protein and RNA mass of cardiomyocytes (Day 1 culture)

<table>
<thead>
<tr>
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<th>Protein/DNA (%)</th>
<th>RNA/DNA (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>100±7</td>
<td>100±6</td>
</tr>
<tr>
<td>Wortmannin</td>
<td>97±5</td>
<td>98±4</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>125±11*</td>
<td>117±8*</td>
</tr>
<tr>
<td>Phenylephrine + Wortmannin</td>
<td>109±12*,7</td>
<td>102±10*</td>
</tr>
<tr>
<td>Phenylephrine + LY 294002</td>
<td>106±9*,+</td>
<td>106±9*</td>
</tr>
<tr>
<td>PMA</td>
<td>110±4*</td>
<td>111±2*</td>
</tr>
<tr>
<td>PMA + Wortmannin</td>
<td>105±1**</td>
<td>99±5**</td>
</tr>
</tbody>
</table>

Cardiomyocytes were cultured for 24 h under control conditions (control), in presence of wortmannin (100 nM), LY 294002 (100 µM), phenylephrine (10 µM), phorbolmyristate acetate (PMA, 100 nM), or in combinations as indicated. Data are presented relative to the control values as means ± SD from n cultures. Under control conditions protein/DNA ratio amounted to 31.13 ± 2.18 µg/µg and the RNA/DNA ratio amounted to 2.24 ± 0.13 µg/µg.

* p<0.05 vs. control; †, p<0.05 vs. phenylephrine; ‡, p<0.05 vs. PMA.

increase in RNA content was abolished when wortmannin or LY 294002 were present (Table 1).

3.3. Effect of wortmannin on myocyte hypertrophy induced by phorbolmyristate acetate

Similar results as seen with phenylephrine were observed when, instead of α-adrenoceptor stimulation, the phorbol ester phorbolmyristate acetate (PMA), which activates directly protein kinase C, was used. PMA increased 14C-phenylalanine incorporation by 69±11% but only by 10±9% when wortmannin was also applied (p<0.05, n=16). Wortmannin reduced also the PMA mediated increase in total cellular protein (Table 1) and RNA (Table 1).

3.4. PI3-kinase activation in day 7 cultures

As mentioned in the introduction α- and β-adrenoceptor stimulation accelerate protein synthesis in day 7 cultures. We now investigated whether PI3-kinase is activated by phenylephrine and isoprenaline in these cultures. In day 7 cultures, both phenylephrine and isoprenaline caused PI3-kinase activation (Fig. 4).

3.5. Effect of wortmannin on myocyte growth in day 7 cultures

The hypertrophic response under α-adrenoceptor stimulation was also studied in day 7 cultures. As in day 1 cultures it was found that in phenylephrine treated cardiomyocytes wortmannin and LY 294002 inhibited the increase in of 14C-phenylalanine incorporation, the increase in cellular protein and RNA mass (Fig. 5, Table 2). Phenylephrine, but not isoprenaline, increased the rate of RNA synthesis, as determined by incorporation of 14C-uridine into RNA (Fig. 6). This increase was also attenuated by wortmannin. It was further investigated whether wortmannin also attenuates the β-adrenoceptor mediated hypertrophy on day 7 cultures. Isoprenaline increased 14C-phenylalanine incorporation in day 7 cultures by 21±11% and this hypertrophic effect of isoprenaline was blocked completely by wortmannin and LY 294002 (Fig. 5). In day 7 cultures wortmannin and LY 294002 blocked also the accumulation of total cellular protein by isoprenaline (Table 2) but not that of total RNA (Table 2).

3.6. Effect of wortmannin on protein synthesis induced by PMA and dibutyryl cAMP in day 7 cultures

In day 7 cultures PMA increased of C-phenylalanine incorporation by 60±20% but only by 22±14% when wortmannin was also applied (p<0.05, n=16). In the same culture dibutyril-cAMP increased of 14C-phenylalanine incorporation by 61±22% but only by 21±9% when wortmannin was also applied (p<0.05, n=16).

![Fig. 4. PI3-kinase activation in cardiomyocytes treated for 15 min with phenylephrine (10 µM) or isoprenaline (1 µM). Experimental conditions are the same as shown for Fig. 2 but cardiomyocytes were cultured for 6 days in serum supplemented medium before the experiments were performed. The experiments were then performed on the seventh day. Data are means ± SEM from three experiments. *, p <0.05 vs. control (C).](image-url)
4. Discussion

It was the aim of the present study to identify an involvement of PI3-kinase activation in the intracellular signaling leading to hypertrophic growth of adult ventricular cardiomyocytes. For this purpose adult ventricular cardiomyocytes were stimulated by the α-adrenoceptor agonist phenylephrine or the β-adrenoceptor agonist isoprenaline. The main finding of the present investigation is that PI3-kinase activation is a common step in the hypertrophic response to either agonist investigated. This conclusion is based on two findings: First, activation of PI3-kinase was observed in cardiomyocytes under conditions where catecholamines induced hypertrophic growth, i.e., in day 1 cultures in presence of phenylephrine but not isoprenaline and, in day 7 cultures in presence of phenylephrine as well as isoprenaline. Second, wortmannin and LY 294002, two specific inhibitors of PI3-kinase [27], blocked the hypertrophic response of cardiomyocytes to phenylephrine or isoprenaline.

We have previously studied the hypertrophic response of adult ventricular cardiomyocytes to α-adrenoceptor stimulation. This hypertrophic response is characterized by an increase in the rate of protein synthesis, as determined by the incorporation of [14C]-phenylalanine into cellular proteins, and increases in total protein and total RNA mass [5,7]. An increase in total RNA mass indicates enlargement of the translational capacity of the cardiomyocytes, because total RNA reflects mainly ribosomal RNA [24].

Table 2

<table>
<thead>
<tr>
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<th>Protein/DNA</th>
<th>n</th>
<th>RNA/DNA</th>
<th>n</th>
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<td>Control</td>
<td>100±17</td>
<td>24</td>
<td>100±7</td>
<td>24</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>118±11*</td>
<td>16</td>
<td>121±12</td>
<td>16</td>
</tr>
<tr>
<td>Phenylephrine + Wortmannin</td>
<td>105±3*</td>
<td>16</td>
<td>108±6*</td>
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</tr>
<tr>
<td>Phenylephrine + LY 294002</td>
<td>106±5*</td>
<td>16</td>
<td>104±16</td>
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</tr>
<tr>
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<td>113±3*</td>
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<td>Isoprenaline + Wortmannin</td>
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<td>115±8*</td>
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<tr>
<td>Isoprenaline + LY 294002</td>
<td>102±18</td>
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<td>114±24*</td>
<td>16</td>
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</tbody>
</table>

Cardiomyocytes were cultured for 6 days in presence of 20% (v/v) fetal calf serum. Thereafter serum free incubations started on the seventh day for 24 h under control conditions (control), in presence of wortmannin (100 nM), LY 294002 (100 μM), phenylephrine (10 μM), isoprenaline (1 μM), or in combinations as indicated. Data are presented relative to the control values as means ± SD from n cultures. Under control conditions protein/DNA ratio amounted to 25.14±4.27 μg/μg and the RNA/DNA ratio amounted to 1.64±0.11 μg/μg.

*, p <0.05 vs. control; **, p <0.05 vs. phenylephrine; ***; p <0.05 vs. isoprenaline.
We found that the hypertrophic response of cardiomyocytes to α-adrenoceptor stimulation can be abolished by protein kinase C inhibitors, namely staurosporine and bisindolylmaleimide [5,28]. In the present study we found that the hypertrophic response of cardiomyocytes to the presence of a direct activator of calcium dependent and calcium independent protein kinase C isoforms, phorbolmyristate acetate, is sensitive to wortmannin. This suggests that in cardiomyocytes PI3-kinase activation is downstream to protein kinase C in signal transduction towards a hypertrophic growth. This is an important finding because we and others have identified several agonists that are potential hypertrophic stimuli for cardiomyocytes and have in common that they all activate protein kinase C. Apart from α-adrenoceptor agonists these include parathyroid hormone [5,29], calcitonin-gene-related peptide [28] and endothelin-1 [30,31]. These data do not indicate, however, that protein kinase C directly interacts with PI3-kinase. Whether other intracellular steps are involved in the activation of PI3-kinase downstream protein kinase C activation was not in the scope of this study. In fetal rat brown adipocytes the protein kinase C zeta isoform represents a downstream target of PI3-kinase [32]. If this is the case also in cardiomyocytes is unclear.

Wortmannin and LY 294002 blocked also the increase in the rate of protein synthesis and cellular protein accumulation in presence of β-adrenoceptor stimulation, in day 7 cultures. This indicates that PI3-kinase activation is involved in the hypertrophic response to β-adrenoceptor stimulation. In contrast to the inhibitory effect of wortmannin and LY 294002 on the RNA increase in presence of phenylephrine, wortmannin did not attenuate the increase in RNA mass in cardiomyocytes treated with isoprenaline. This difference is not related to differences in the culture types used in the experiments (day 1 and day 7 cultures) because wortmannin and LY 294002 blocked the phenylephrine effect on RNA mass in either culture type. It indicates, however, that the increase in total RNA mass occurs for different reasons in α- and β-adrenoceptor stimulated cardiomyocytes. Under α-adrenoceptor stimulation [14C]-uridine incorporation, indicating RNA synthesis, is increased as shown in [7] and the present study and this process as well as the resulting increase in total RNA mass is wortmannin-sensitive. In contrast, β-adrenoceptor stimulation leads to an increase in RNA mass by a mechanism not sensitive to wortmannin. It is not related to an increase in [14C]-uridine incorporation and, therefore, likely due to deceleration of RNA degradation. The exact mechanism by which β-adrenoceptor stimulation leads to an deceleration of RNA degradation has not yet been investigated. It has been shown before, however, that β-adrenoceptor mediated hypertrophy in vivo is linked to an acceleration of the polyamine synthesis, which is known to stabilize RNA and DNA.

It has been suggested by previous work that marked elevations of total protein synthesis in cardiomyocytes are only possible if the translational capacity becomes also enlarged [24]. Since the translational capacity is determined by the number of functional active ribosomes and ribosomal RNA make up about 90% of total RNA, distinct elevations of total protein synthesis should go along with distinct elevations of total RNA. This is indeed the case in all experiments here performed. The converse is not the case as the increase in protein synthesis observed under β-adrenoceptor stimulation is reduced in presence of wortmannin while total RNA mass stays elevated. Thus, an increase in translational capacity is not sufficient to increase protein synthesis but requires an acquisition of existing ribosomes as well.

In conclusion, our study demonstrates for the first time an activation of PI3 kinase in adult ventricular cardiomyocytes and its involvement in the stimulation of protein synthesis of adult cardiomyocytes. Activation of PI3-kinase by α- and β-adrenoceptor stimulation indicates that different second messenger pathways converge at the level of PI3-kinase. The differences in RNA metabolism under α- and β-adrenoceptor stimulation also show, however, that signaling events independent of PI3-kinase are additionally involved in the control of myocardial growth and that these events differ between α- and β-adrenoceptor stimulation.

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