Extracellular Signal-regulated Kinase Plays an Essential Role in Hypertrophic Agonists, Endothelin-1 and Phenylephrine-induced Cardiomyocyte Hypertrophy*

Received for publication, August 3, 2000, and in revised form, September 8, 2000 Published, JBC Papers in Press, September 12, 2000, DOI 10.1074/jbc.M007037200

Tian-Li Yue‡, Juan-Li Gu, Chuanlin Wang, Alastair D. Reith‡, John C. Leef‡, Rosanna C. Mirabile‡, Reinhold Kreutz**, Yibin Wang‡‡, Beverly Maleeff§, Andrew A. Parsons, and Eliot H. Ohlstein

From the Departments of Cardiovascular Pharmacology, Neuroscience, Bone and Cartilage Biology, and Safety Assessment, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406, the Department of Physiology, University of Maryland, Baltimore, Maryland 21201, and the Department of Clinical Pharmacology and Toxicology, Freie Universitaet Berlin, 12200 Berlin, Germany

The extracellular signal-regulated kinase (ERK) pathway is activated by hypertrophic stimuli in cardiomyocytes. However, whether ERK plays an essential role or is implicated in all major components of cardiac hypertrophy remains controversial. Using a selective MEK inhibitor, U0126, and a selective Raf inhibitor, SB-386023, to block the ERK signaling pathway at two different levels and adenovirus-mediated transduction of dominant-negative Raf, we studied the role of ERK signaling in response of cultured rat cardiomyocytes to hypertrophic agonists, endothelin-1 (ET-1), and phenylephrine (PE). U0126 and SB-386023 blocked ET-1 and PE-induced ERK but not p38 and JNK activation in cardiomyocytes. Both compounds inhibited ET-1 and PE-induced protein synthesis and increased cell size, sarcomeric reorganization, and expression of β-myosin heavy chain in myocytes with IC50 values of 1–2 μM. Furthermore, both inhibitors significantly reduced ET-1- and PE-induced expression of atrial natriuretic factor. In cardiomyocytes transfected with a dominant-negative Raf, ET-1- and PE-induced increase in cell size, sarcomeric reorganization, and atrial natriuretic factor production were remarkably attenuated compared with the cells infected with an adenovirus-expressing green fluorescence protein. Taken together, our data strongly support the notion that the ERK signal pathway plays an essential role in ET-1- and PE-induced cardiomyocyte hypertrophy.

Cardiac hypertrophy is an adaptive response of the heart to virtually all forms of cardiac diseases (1). Although the hypertrophic response is initially a compensatory mechanism that augments cardiac output, sustained hypertrophy can lead to dilated cardiomyopathy, heart failure, and sudden death. Despite the diverse stimuli that lead to cardiac hypertrophy, there is a prototypical final molecular response of cardiomyocytes to hypertrophic signals that involves an increase in cell size and protein synthesis, enhanced sarcomere organization, and re-expression of embryonic cardiac genes, β-mycine heavy chain (β-MHC), and atrial natriuretic factor (ANF) (2–4). The cause and effect of cardiac hypertrophy have been extensively documented, but the underlying molecular mechanisms that couple hypertrophic signals initiated at the cell membranes to the reprogramming of cardiomyocyte gene expression remain poorly understood.

Numerous studies have proposed that extracellular signal-regulated kinase (ERK) cascade is involved in the hypertrophic response (for review see Ref. 5). The initial finding was that G-protein-coupled receptor (GPCR) hypertrophic agonists stimulate ERK activation. Subsequent studies focusing on the role of the Ras/Raf/ERK pathway have provided considerable support for this hypothesis (6–9). However, there is still much debate and controversy concerning the role of the ERK pathway in cardiac hypertrophy. The controversy stems from various reports suggesting that blockade of ERK signaling pathway effects some, but not all, components of hypertrophic responses (7, 9–13). In some instances, opposite effects have been reported (10, 12, 13). Therefore, whether the ERK pathway plays an essential role in all or only some components of cardiac hypertrophic responses remains unclear (10–16). The controversies may stem from the different techniques used in the studies.

The present study used an experimental design that does not require overexpression of the proteins involved but rather inhibition of the endogenous kinases by the two types of inhibitors, U0126, a new potent and selective inhibitor of MEK1/2 (17), and SB-386023, a selective Raf inhibitor (18), to block the ERK signaling pathway at two different levels and to determine changes in GPCR agonist-induced hypertrophic responses in rat neonatal cardiomyocytes. In addition, we used adenovirus-mediated gene transfer to express Raf−, a dominant inhibitory mutant of Raf (19, 20) in cardiomyocytes to specifically inhibit ERK activation, enabling us to further determine the role of the ERK pathway. Cardiomyocyte hypertrophy was monitored by the following characteristic phenotypic changes: 1) an increase in protein synthesis; 2) an increase in myocyte size; 3) increased organization of contractile protein actin into sarcomeric units; 4) enhanced expression

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† To whom correspondence should be addressed: Dept. of Cardiovascular Pharmacology, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., P. O. Box 1539, UW2510, King of Prussia, PA 19406. Tel.: 610-270-5313; Fax: 610-270-5080; E-mail: tian-li_yue@sbphrd.com.

This paper is available on line at http://www.jbc.org

© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
of embryonic genes β-MHC and ANF. Our data demonstrate that the ERK pathway is essential to the hypertrophic responses of myocytes to GPCR agonists, ET-1 and PE.

**EXPERIMENTAL PROCEDURES**

Primary Neonatal Rat Cardiac Myocyte Cultures—Primary cultures of neonatal rat cardiomyocytes from 1- to 2-day-old Harlan Sprague-Dawley rats were prepared by the method reported previously (21). The myocytes were purified on a discontinuous Percoll gradient, suspended in a plating media containing 10% serum, and plated in Laminin Cellware 24-well plates (Becton Dickson) or Permanox® Lab-Tek chamber slides coated with laminin for 24 h. The media was then replaced with a serum-free maintenance media and incubated for 24 h before being used for study. The purity of cardiomyocytes assessed by immunohistochemical analysis with an antibody against sarcomeric α-actinin (Sigma) was above 95%.

**Protein Synthesis Measurement ([3H]Leucine Incorporation)—**Cardiomyocytes cultured in 24-well plates were treated with vehicle or U0126 (Promega, Madison, WI) or SB-386013 (SmithKline Beecham, King of Prussia, PA) for 30 min, stimulated with ET-1 or PE for 24 h in serum-free medium, and then incubated in the same medium with 1.0 μCi/ml [3H]leucine for an additional 6 h. The radioactivity incorporated into the trichloroacetic acid-precipitable material was determined. The [3H]leucine incorporation in the cells treated with ET-1 or PE alone was normalized 100% (14).

MAPK Activity Assay—MAPK activities were assayed as described previously (22). The cell lysates were immunoprecipitated with antibodies specific for ERK1/2 (sc-154-G; Santa Cruz Biotechnology), p38 (sc-535-G; Santa Cruz Biotechnology), and JNK1/2 (sc-474-G; Santa Cruz Biotechnology) and were assayed by using myelin basic protein for ERK, glutathione S-transferase-activating transcription factor-2 for p38, and glutathione S-transferase-e-Jun(J1–85) for JNK as the substrate, respectively.

Measurement of Cell Surface Area—Cardiac myocyte cultured on chamber slides (2.5 × 10⁵ cells/well) were fixed and immunostained with a monoclonal antibody against sarcomeric α-actinin (Sigma) (23) as described below. Planimetry was performed using Image-Pro software. At least 300 cells per slide were scored for size measurement. The cell surface area in control cells was normalized 100% (basal), and results were expressed as the fold-change in cell size compared with the basal level (24).

Determination of Sarcomere Organization—for staining of filamentous actin, the myocytes were fixed in 3.7% formaldehyde in PBS, washed with PBS, treated with 0.1% Triton X-100 for 3–5 min, washed again, and incubated with 1% bovine serum albumin in PBS for 30 min, and then stained with Oregon Green 514 phalloidin (Molecular Probes) at 5 units/ml for 20 min at room temperature (25, 26). The cells were visualized by fluorescence microscopy.

Northern Analysis of ANF Expression in Cardiomyocytes—The probe for ANF spanned a 216-base pair fragment of the rat ANF cDNA (accession number M27498). It was generated by reverse transcriptase polymerase chain reaction from total RNA obtained from rat heart (Penninsula Laboratory), mouse anti-rat skeletal β-MHC, and mouse anti-rat sarcomeric α-actinin monoclonal antibody (Sigma). As a negative control, the cell samples were incubated with nonimmune IgG instead of the primary antibody. An anti-rabbit IgG biotinylated secondary antibody was used for ANF staining, and an anti-mouse IgG biotinylated secondary antibody was used for β-MHC.
dominant-negative mutants of Raf (K375R), and GFP (pEGFP; CLONTECH) were subcloned into a shuttle vector containing the cytomegalovirus promoter/enhancer. Recombinant adenovirus was generated as described previously (19). The recombinant adenovirus was tested for transgene expression in cardiomyocytes by reverse transcriptase polymerase chain reaction, Western blot, or kinase assays as described (20). Cardiomyocytes were infected with adenovirus-expressing dominant-negative Raf (AdRaf−) or adenovirus-expressing GFP (AdGFP) as control for 24 h. The cells were exposed to ET-1 (100 nM) or PE (50 μM) in serum-free medium for an additional 24–48 h before being fixed and stained with anti-actinin antibody (cell size measurement) or Oregon Green 514 phalloidin to visualize actin fibers. Culture supernatants were collected for ANF assay as described above.

Statistical Analysis—All values in the text and figures are presented as mean ± S.E. of n independent experiments. Statistical evaluation was performed by using one-way analysis of variance with subsequent post hoc paired comparisons. Differences with a value of p < 0.05 were considered significant.
performed in quadruplicate).

0.05; **, p < 0.01 versus cells treated with ET-1 or PE alone (n = 4, performed in quadruplicate).

FIG. 5. Effects of U0126 on ET-1- or PE-induced ANF expression in cardiomyocytes. Lower panel, immunohistochemical analysis. Cardiomyocytes cultured on chamber slides and in serum-free media for 24 h were treated with vehicle (A), ET-1 (100 nM) (B), ET-1 + U0126 (10 μM) (C), or PE (50 μM) (D), or PE + U0126 (10 μM) (E) for 24 h. The cells were processed for immunohistochemical analysis using a rabbit anti-rat ANF antiserum (Peninsula Laboratory) following the manufacturer’s instruction. Upper panel, ELISA. Myocytes cultured in 24-well plates and serum-free medium for 24 h were treated with vehicle or U0126 (10 μM) for 20 min prior to the addition of ET-1 (100 nM) or PE (50 μM), and incubation was continued for 24 h. The supernatants were collected for ANF assay using a ELISA kit (Phoenix Pharmaceuticals) following the manufacturer’s instruction. The level of ANF in vehicle-treated cells (basal) was 18.7 ± 3.2 ng/ml (n = 4). *, p < 0.05; **, p < 0.01 versus cells treated with ET-1 or PE alone (n = 4, performed in quadruplicate).

U0126 Inhibits ET-1- or PE-induced Sarcomeric Reorganization in Cardiomyocytes—Rat neonatal cardiomyocytes cultured in serum-free condition displayed thin and rudimentary sarcomeric structure (Fig. 4A), and treatment of myocytes with 100 nM ET-1 or 50 μM PE caused a reorganization of sarcomere with highly organized actin fibers as shown by the brightly stained straight, thick bundles when the cells were stained for polymerized actin by phalloidin. (Fig. 4, B and C). However, pre-treatment of myocytes with U0126 (10 μM) abolished ET-1- or PE-induced sarcomere reorganization (Fig. 4, D and E).

U0126 Inhibits ET-1- or PE-stimulated ANF Expression in Cardiomyocytes—The effect of U0126 on ERK pathway in modulating the induction of ANF in response to ET-1 or PE in myocardies was examined by the following three different assays: 1) Detection of ANF mRNA expression by Northern blot analysis. The basal level of ANF mRNA was detected in myocytes as reported previously (24). In myocytes treated with ET-1 (100 nM) or PE (50 μM) for 24 h, the expression of ANF mRNA was markedly enhanced. In the presence of U0126 (10 μM), however, ET-1- or PE-induced ANF mRNA expression was remarkably attenuated (data not shown). 2) Detection of ANF protein expression by immunohistochemical analysis (Fig. 5, lower panel). Expression of ANF protein in myocytes was markedly enhanced by the treatment of cells with ET-1 (100 nM) or PE (50 μM) for 24 h. ANF-positive cells showed a characteristic perinuclear staining pattern, consistent with that reported previously (13, 30). In the presence of U0126 (10 μM), ET-1- or PE-induced ANF expression was markedly reduced. 3) Measurement of ANF levels in the medium by ELISA (Fig. 5, upper panel). A detectable level of ANF (18.7 ± 3.2 ng/ml, n = 4) was found in the medium of untreated myocytes and was increased approximately 3-fold in the presence of 100 nM ET-1 or 50 μM PE for 24 h. (51.4 ± 4.9 and 64.4 ± 7.1 ng/ml, respectively, p < 0.01 versus basal, n = 4), indicating an enhanced production of ANF from the cells. U0126 reduced, in a dose-dependent manner, ET-1- and PE-induced elevation of ANF levels in the culture medium with IC50 values of 1.0 and 1.3 μM, respectively.

U0126 Inhibits ET-1- or PE-induced β-MHC Expression in Cardiomyocytes—As shown in Fig. 6, expression of β-MHC in cardiomyocytes was significantly enhanced when cells were treated with ET-1 (100 nM) or PE (50 μM) for 24 h. However, in the presence of U0126 (10 μM), ET-1- or PE-stimulated expression of β-MHC in myocytes was markedly reduced.

Effects of SB-386023 on ET-1- or PE-induced Hypertrophic Responses in Cardiac Myocytes—The similar inhibitory effects of SB-386023 on ET-1- or PE-induced hypertrophic responses
in myocytes were demonstrated, and the data with ET-1 are mainly presented. Treatment of cardiomyocytes with 10 \( \mu M \) SB-386023 for 20 min prior to the addition of ET-1 (100 nM) abrogated ET-1-induced activation of ERK without affecting activation of p38 or JNK (Fig. 7A). In the presence of SB-386023, ET-1-induced increase in \( ^{[3H]} \)leucine incorporation was reduced (IC \(_{50} = 1.4 \mu M \)) (Fig. 7B). SB-386023 inhibited ET-1 (100 nM)- or PE (50 \( \mu M \))-induced cell size increase in IC \(_{50} \) values of 1.9 and 2.0 \( \mu M \), respectively (Fig. 7C). A representative photograph with ET-1 stimulation is presented in Fig. 7D. Moreover, ET-1-induced increased organization of contractile protein actin into sarcomeric units (Fig. 7E) and expression of \( \beta \)-MHC (Fig. 7F) and ANF protein (Fig. 7G) in cardiomyocytes were all attenuated in the presence of SB-386023.

Effects of Dominant-Negative Raf Transfection on ET-1- or PE-induced Hypertrophic Responses in Cardiac Myocytes—More than 95% of cardiomyocytes were positive for GFP expression after incubation of cells with AdGFP for 24 h under the experimental condition. Transfection of cells with AdGFP had no effect on PE- or ET-1-induced cell size increase. However, in cardiomyocytes infected with recombinant adenovirus encoding the dominant inhibitory mutant of Raf (AdRaf\(^{-}\)), PE- or ET-1-induced increase in cell size was inhibited (data not shown). As shown in Fig. 8, upper panel, ET-1-induced increase in sarcomeric reorganization was inhibited in myocytes transfected with AdRaf\(^{-}\) but not in cells infected with AdGFP. Moreover, ET-1- or PE-induced ANF production was markedly inhibited in myocytes infected with AdRaf\(^{-}\) but not with AdGFP (Fig. 8, lower panel).

**DISCUSSION**

U0126 is a recently discovered selective MEK inhibitor with IC \(_{50} \) values that are 350-fold higher for inhibiting JNK and p38 than for ERK. It has no effect on protein kinase C, Cdk2, or Cdk4 (17). Our data (Fig. 1) have confirmed the selective ERK...
inhibition by U0126 in cultured cardiomyocytes. Besides its selectivity for ERK pathway, U0126 has two important features, better potency and solubility compared with PD98059. The compound displays significantly higher affinities than PD98059 for the MEK-ERK complex and the free MEK enzyme; therefore it is at least 10-fold more potent than PD98059 in inhibiting ERK activation (17). As shown in Figs. 2 and 3, U0126 reduced ET-1- or PE-stimulated increase in cell size and protein synthesis with IC50 values between 1 and 2 μM. Our results demonstrate that ET-1- or PE-induced myofibrillar organization in cardiomyocytes was remarkably inhibited by U0126, consistent with previous studies that demonstrated a positive link between the ERK activation and the enhanced myofibrillar reorganization (12) and the inhibitory effect of PD98059 (50 μM) on sarcomeric reorganization (16). Our data are in contrast with another study in which myocytes treated with 2-aminopurine or 6-thioguanine failed to block PE-induced organization of actin in myocytes (10). Interestingly, PE-induced activation of ERK was not completely inhibited by the either pharmacological reagents. In addition, it is unclear whether those two reagents specifically affected the ERK cascade. The present study also demonstrated that blockade of ERK activation by U0126 inhibits ET-1- or PE-induced expression of β-MHC and ANF in myocytes. There have been conflicting conclusions regarding the role of ERK activation in the regulation of ANF expression. To carefully examine the effect of U0126 on ET-1- or PE-stimulated ANF expression in myocytes, three methods for the detection of ANF were used in this study. Rat neonatal cardiomyocytes express basal levels of ANF that were variable from one preparation to another as reported previously (13, 24, 31). However, data from the three different assays clearly demonstrate that inhibition of ERK pathway by U0126 attenuated ANF expression in myocytes exposed to ET-1 or PE. Our data disagree with a previous study in which transient transfection of dominant-negative ERK mutant or treatment of myocytes with PD98059 failed to block PE-stimulated ANF reporter gene expression in cardiomyocytes (13). This may be due to the lack of sensitivity of the ANF reporter gene system to inhibition, which could be a result of a low transfection efficiency associated with transient transfection. Another reason could be due to the fact that a low dose of PD98059 (10 μM) was used in that study that might be insufficient as it has been reported that PD98059 only at 50 μM completely inhibited ET-1-induced ERK activation in cardiomyocytes (14). A recent study showed different effects on ANF expression by PD98059, and ANF activation was only affected at higher concentrations of the inhibitor (15). It is notable that PD98059 tends to be insoluble at or above 50 μM, making the study using a higher concentration of PD98059 difficult. The difference in the potency and solubility between U0126 and PD98059 could be an important consideration to explain the discrepancy regarding the effects of ERK inhibition on myocyte hypertrophy among different laboratories. Our data clearly demonstrate that all observed hypertrophic responses in cardiomyocytes in this study were inhibited or attenuated by U0126.

There are limitations in previous studies using the transient transfection protocol to block Ras and define the role of ERK pathway in cardiac myocyte hypertrophy (8, 9, 12). Interpretation of these results may be hampered by the fact that Ras has at least three major effectors, namely, Raf, PI3K, and Ralguanine nucleotide dissociation stimulator besides ERK activation (32–34), and transfection of the dominant-negative Ras mutant has been found to inhibit PI3K activity (35). Because expression of c-Raf and A-Raf in cardiac myocytes has been demonstrated (36), the present study used a new potent and selective Raf inhibitor, SB-386023, to further define the role of ERK pathway in ET-1- and PE-induced myocyte hypertrophy. This compound has been demonstrated to inhibit both c-Raf and B-Raf at 1–10 μM in a variety of cellular assays, without affecting JNK or p38 (18). As shown in Fig. 7A, SB-386023 inhibited ET-1-stimulated ERK activation in a concentration-dependent manner but had no effect on p38 or JNK, and activation of ERK was almost completely inhibited at 10 μM. All observed hypertrophic responses of myocytes to ET-1 or PE were significantly blocked in a dose-dependent manner by SB-386023 (Fig. 7, B–G). The data are in agreement with that obtained with U0126, indicating that the Raf/MEK/ERK pathway plays a critical role in myocyte hypertrophy.

We further performed the transfection study using adenovirus-mediated transfection of AdRaf–. To avoid adenovirus-
mediated nonspecific changes in cellular morphology or gene expression associated with myocyte hypertrophy, we carefully titrated the adenovirus stock and determined the optimal m.o.i. Infection of myocytes with AdRaf2 but not adenovirus alone blocked ET-1- and PE-induced increase in cell size and sarcomeric reorganization. In addition, ET-1- or PE-induced production of ANF was significantly reduced in myocytes infected with AdRaf2 but not AdGFP. These results are consistent with the data from the study with Raf inhibitor, SB-386023, and MEK inhibitor, U0126.

In conclusion, the present study demonstrates that inhibition of the ERK signaling pathway blocked all observed hypertrophic responses in cardiomyocytes exposed to ET-1 or PE. Although the involvement of other signaling pathways in cardiomyocyte hypertrophy is possible, the present results strongly support the notion that the ERK cascade plays an essential role in the signaling mechanisms that lead to the development of myocardial hypertrophy.

Acknowledgment—We sincerely thank Xiang Li for excellent technical assistance.

REFERENCES

1. Colucci, W. S., Braunwald, E. (1997) in Heart Disease (Braunwald, E., ed) 5th Ed., pp. 394–398, W. B. Saunders Co., Philadelphia, PA
2. Swynghedauw, B. (1999) Physiol. Rev. 79, 215–262
3. Miyata, S., Minobe, W., Bristow, M. R., and Leinwand, L. A. (2000) Circ. Res. 86, 386–390
4. Sugden, P. H. (1999) Circ. Res. 84, 633–646
5. Sugden, P. H., and Clerk, A. (1998) J. Mol. Med. 76, 725–746
6. Bogoyevitch, M. A., Glennon, P. E., Andersson, M. B., Clerk, A., Lazou, A., Marshall, C. J., Parker, P. J., and Sugden, P. H. (1994) J. Biol. Chem. 269, 1110–1119
7. Gillespie-Brown, J., Fuller, S. J., Bogoyevitch, M. A., Cowley, S., and Sugden, P. H. (1995) J. Biol. Chem. 270, 28082–28086
8. Thorburn, A., Thorburn, J., Chen, S. Y., Powers, S., Shubeita, H. E., Feramisco, J. R., and Chien, K. R. (1993) J. Biol. Chem. 268, 2244–2249
9. Hunter, J. J., Tanaka, N., Rockman, H. A., Ross, J., Jr., and Chien, K. R. (1995) J. Biol. Chem. 270, 23173–23178
10. Thorburn, J., Frost, J., and Thorburn, A. (1994) J. Cell Biol. 126, 1565–1572
11. Thorburn, J., McMahon, M., and Thorburn, A. (1994) J. Biol. Chem. 269, 30580–30586
12. Clerk, A., Micheal, A., and Sugden, P. H. (1998) J. Cell Biol. 142, 523–535
13. Post, G. R., Goldstein, D., Thuerer, D. J., Gliemhotski, C. C., and Brown, J. H. (1990) J. Biol. Chem. 271, 8452–8457
14. Choukroun, G., Hajar, R., Kyriakis, J. M., Bonventre, J., Rosenzweig, A., and Force, T. (1998) J. Clin. Invest. 102, 1311–1320
15. Fuller, S. J., Gillespie-Brown, J., and Sugden, P. H. (1998) J. Biol. Chem. 273, 18146–18152
16. Jette, C., and Thorburn, A. (2000) FEBS Lett. 467, 1–6
17. Favata, M. P., Horiuchi, R. Y., Manos, E. J., Daulerio, A. J., Stradley, D. A., Feeser, W. S., Van Dyk, D. E., Pitts, W. J., Earl, R. A., Hobbs, F., Copeland, R. A., Magolda, R. L., Scherle, P. A., and Trazaos, J. M. (1998) J. Biol. Chem. 273, 18623–18632
18. Hughes, J. P., Staton, P., Wilkinson, M. G., Ken, W. J., Strijbos, P. J. L. M., Skaper, S. D., and Reith, A. D. (2000) Biochem. Mol. Biol. Int. (Abstr. 1568)
19. Hertig, C. M., Kubalak, S. W., Wang, Y., and Chien, K. R. (1999) J. Biol. Chem. 274, 57362–57369
20. Wang, Y., Su, B., Sah, V. P., Brown, J. H., Han, J., and Chien, K. R. (1998) J. Biol. Chem. 273, 5423–5426
21. Yue, T. L., Wang, C., Gu, J. L., Ma, X. L., Kumar, S., Lee, J. C., Feuerstein, G. Z., Thomas, H., Maltez, B., and Ohlstein, E. H. (2000) Circ. Res. 86, 692–699
22. Yue, T. L., Ni, J., Romanic, A. M., Gu, J. L., Keller, P., Wang, C., Kumar, S., Yu, G. L., Hart, T. K., Wang, X., Xia, Z., DeWolf, W. E., and Feuerstein, G. Z. (1999) J. Biol. Chem. 274, 1479–1486
23. Molkentin, J. D., Lu, J. R., Antos, C. L., Markham, B., Richardson, J., Robbins, J., Grant, S. R., and Olsen, E. N. (1999) Cell 93, 215–228
24. Sekiguchi, K., Yokoyama, T., Kurokayashi, M., Okajima, F., and Nagai, R. (1999) Circ. Res. 85, 1000–1008
25. Aoki, H., Sadoshima, J., and Izumo, S. (2000) Nat. Med. 6, 183–188
26. Sells, M. A., Boyd, J. T., and Chernoff, J. (1999) J. Cell Biol. 145, 837–849
27. Kreutz, R., Struk, B., Hubattu, S., Hubner, N., Szpirer, J., Szpirer, C., Ganten, D., and Lindpaintner, K. (1997) Hypertension 29, 131–136
28. Yue, T. L., Wang, X., Sung, C. P., Olsen, B., McKenna, P. J., Gu, J. L., and Feuerstein, J. Z. (1994) Circ. Res. 75, 1–7
29. Bogoyevitch, M. A., Glennon, P. E., and Sugden, P. H. (1993) FEBS Lett. 317, 271–275
30. Hoshijima, M., Sah, V. P., Wang, Y., Chien, K. R., and Brown, J. H. (1998) J. Biol. Chem. 273, 7725–7730
31. Ehle, D. M., Cadre, B. M., Qi, M., Bers, D. M., and Samarel, A. M. (1998) J. Mol. Cell Cardiol. 30, 55–60
32. Joneson, T., and Bar-Sagi, D. (1997) J. Mol. Biol. 275, 587–593
33. Clerk, A., and Sugden, P. H. (2000) Circ. Res. 86, 1019–1023
34. Hagemann, C., and Rapp, U. R. (1999) Exp. Cell Res. 253, 34–46
35. Hu, Q., Klippel, A., Muslin, A. J., Fanti, W. J., and Williams, L. T. (1995) Science 268, 100–102
36. Bogoyevitch, M. A., Marshall, C. J., and Sugden, P. H. (1995) J. Biol. Chem. 270, 26303–26310
Extracellular Signal-regulated Kinase Plays an Essential Role in Hypertrophic Agonists, Endothelin-1 and Phenylephrine-induced Cardiomyocyte Hypertrophy
Tian-Li Yue, Juan-Li Gu, Chuanlin Wang, Alastair D. Reith, John C. Lee, Rosanna C. Mirabile, Reinhold Kreutz, Yibin Wang, Beverly Maleeff, Andrew A. Parsons and Eliot H. Ohlstein

J. Biol. Chem. 2000, 275:37895-37901.
doi: 10.1074/jbc.M007037200 originally published online September 12, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M007037200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 23 of which can be accessed free at http://www.jbc.org/content/275/48/37895.full.html#ref-list-1