Extracellular ATP Induces Immediate-Early Gene Expression but Not Cellular Hypertrophy in Neonatal Cardiac Myocytes

Jing-Sheng Zheng, Marvin O. Boluyt, Lydia O'Neill, Michael T. Crow, Edward G. Lakatta

Abstract It is well-documented that norepinephrine (NE) induces the expression of immediate-early genes (IEGs) such as c-fos, c-jun, and jun-B, in cultured neonatal heart cells and leads to cell growth without cell division (ie, hypertrophy). Although purinergic receptors activated by ATP are present on cardiac myocytes and ATP is coreleased with NE from sympathetic nerve endings within the heart, the potential role of the purinergic system in the cascade of events that leads to cardiac hypertrophy is unknown. We report in the present study that stimulation of purinergic receptors by micromolar concentrations of extracellular ATP increased the levels of c-fos and jun-B mRNA as well as FOS and JUN-B proteins in neonatal cardiac myocytes. The magnitude of response to micromolar ATP was comparable to that elicited by NE. The increase in IEG expression induced by ATP was preceded by a rapid transient increase in cytosolic Ca$^{2+}$. Pretreatment of myocytes with the intracellular Ca$^{2+}$ chelator BAPTA-AM prevented the ATP-stimulated increase in cytosolic Ca$^{2+}$ and attenuated the ATP-stimulated increase in c-fos expression. In contrast, NE did not increase cytosolic Ca$^{2+}$ in quiescent myocytes, and pretreatment with BAPTA-AM did not inhibit the NE-stimulated increase in c-fos gene expression. Furthermore, although NE markedly increased $[\text{14C}]$phenylalanine incorporation into protein and myocyte hypertrophy measured by cell size, ATP did not. These results demonstrate that stimulation of purinergic receptors by ATP activates IEGs via a Ca$^{2+}$-dependent pathway in cardiac myocytes that differs from the NE-stimulated activation of these genes. Since ATP activated IEG expression but did not increase the rate of protein synthesis nor augment cell size, we conclude that the activation of c-fos and jun-B expression is not sufficient to induce cellular hypertrophy in neonatal cardiac myocytes. (Circ Res. 1994;74:1034-1041.)

Key Words • purinergic receptors • c-fos • jun-B • cardiac myocytes

On nerve stimulation, ATP is coreleased with norepinephrine (NE) from the endings of the sympathetic nerves that innervate the heart. Corelease probably accounts for the majority of extracellular ATP found in the heart. Extracellular ATP, however, can also be released by platelet degranulation and from anoxic myocytes or damaged cells. When ATP is released, it activates purinergic receptors in the cardiac cell membrane and leads to a number of biochemical changes in the heart. Stimulation of P$_2$-purinergic receptors by ATP increases [Ca$^{2+}$] in freshly isolated adult rat ventricular myocytes. Extracellular ATP also increases phosphoinositide turnover in adult ventricular myocytes and has been shown to stimulate phosphoinositide hydrolysis and inhibit accumulation of cAMP in cultured ventricular myocytes isolated from fetal mice.

There is a growing body of evidence that NE released from sympathetic nerve endings may be an important nonhemodynamic stimulus for cardiac growth. In neonatal cardiac myocytes, NE induces cardiac hypertrophy primarily via activation of a$_1$-adrenergic receptors.

The responses to a$_1$-adrenergic receptor stimulation include induction of the immediate-early genes (IEGs) c-fos, c-jun, jun-B, and Egr-1, followed by selective upregulation of early developmental isogenes (eg, cardiac β-myosin heavy chain and skeletal α-actin), increased synthesis and release of atrial natriuretic factor (ANF) from ventricular myocytes, and an increase in protein content and cell size of individual ventricular myocytes. These descriptive studies have led to the speculation that the induction of IEGs has a critical role in the activation of the ANF gene and the increase in cell size.

There is no current evidence that ATP invokes changes that could positively or negatively influence the hypertrophic process stimulated by NE. Our purpose was to determine whether extracellular ATP constitutes a stimulus sufficient to induce changes in the pattern of IEG expression associated with cardiac myocyte hypertrophy and, if so, whether ATP, like NE, stimulates cardiac hypertrophy. We used neonatal ventricular myocytes to study the effects of extracellular ATP on expression of the IEGs c-fos and jun-B and on the rate of $[\text{14C}]$phenylalanine incorporation into myocyte proteins. Our results indicate that both ATP and NE increase the expression of IEGs in neonatal myocytes and suggest that the effect of ATP is mediated via a Ca$^{2+}$-dependent pathway, distinct from that stimulated by NE. Additionally, NE increased protein synthesis and cell size in cardiac myocytes, whereas ATP did not. This discordance between the induction of IEGs and

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cardiac hypertrophy by ATP suggests that activation of c-fos or jun-B is not sufficient to stimulate myocyte hypertrophy.

Materials and Methods

Cell Culture

Neonatal ventricular myocytes were cultured as previously described by Iwaki et al. with some modifications. Hearts from 1- to 3-day-old Wistar rats were removed, and the ventricles were trisected and then digested with collagenase type II (0.5 mg/mL, Worthington) and pancreatin (0.6 mg/mL, Sigma Chemical Co.) for 20 minutes at 37°C. The cell supernatant was collected by centrifugation, and the pellet was resuspended in 100% horse serum. The above steps were repeated 7 to 10 times until the hearts were completely digested. The cells were combined, washed, and centrifuged through a discontinuous Percoll gradient of 0.5, 1.050, and 1.082 g/mL, respectively. The band at the 1.062/1.082 interface was used for myocyte culture. Forty-eight hours after the initial plating, the purity of such cultures was >95% myocytes as assessed by immunohistochemistry with antibodies to sarcomeric myosin (MAB E5939) or sarcomeric actin (Sigma). The myocytes were resuspended in culture medium containing 4:1 Dulbecco’s modified Eagle’s medium (DMEM)/medium 199 (M199, GIBCO Laboratories) supplemented with 10% horse serum, 5% fetal bovine serum, and penicillin (100 U/mL)/streptomycin (100 μg/mL, GIBCO). Myocytes were then plated on gelatin-coated 100-mm plastic tissue culture plates (Costar) at a density of 1.08 × 10^5 cells per square centimeter for RNA analyses and on gelatin-coated glass or plastic eight-chamber culture slides for immunocytochemistry studies. Plating efficiency was sufficient that at the time of study, cell-cell contact was minimal. Addition of ATP did not increase the percentage of cultured neonatal myocytes exhibiting contraction either in the short term (<5 minutes) or in the longer term (>24 hours). NE did not increase the number of cells contracting in short term, but it did increase the proportion of myocytes contracting from <2% at 5 minutes to >80% after 24 hours of incubation. Myocyte cultures were maintained in serum-containing medium at 37°C and 5% CO2 in humidified air at pH 7.3. The medium was then changed to serum-free DMEM/M199. All experiments were initiated 24 hours after the change to serum-free culture medium.

Immunocytochemistry Studies

After incubation with experimental reagents (such as ATP and NE) for the designated periods of time, chamber slides were rinsed twice with phosphate-buffered saline (PBS) and fixed with 3.7% formaldehyde-PBS for 5 minutes at room temperature. Cells were then washed three times with PBS, permeabilized with 1% Triton X-100 in PBS for 15 minutes, washed three additional times in PBS, and dehydrated with 100% ethanol for 5 minutes. After three PBS washes, cells were incubated with 2% bovine serum albumin (BSA) in PBS for 30 minutes. Incubation with the primary antibody (5 μg/mL anti-rabbit FOS IgG, Oncogene Science) in 2% BSA was carried out overnight at 4°C. The next day, the slide chambers were washed three times with PBS for 5 minutes each. Cells were then incubated with a secondary antibody (1 μg/mL anti-rabbit IgG conjugated with alkaline phosphatase, Promega) for 4 hours at room temperature and then washed three times with PBS. Cells were incubated with alkaline phosphatase buffer (mmol/L: NaCl 100, MgCl2 5, and Tris-HCl 100, pH 9.5) containing substrates, nitro blue tetrazolium (330 μg/mL) and 5-bromo-4-chloro-3-indolyl phosphate (165 μg/mL) for 15 minutes. Photomicrographs were made at an original magnification of ×400.

RNA Analysis

Total RNA was isolated from cultured ventricular myocytes as described by Chirgwin et al. After denaturation with formamide and formaldehyde, equal amounts of RNA (10 μg per lane) were size-fractionated by electrophoresis through a 1% agarose gel containing 3% formaldehyde. RNA was electrophoretically transferred to a nylon (Duralon) membrane at 5 V/cm, cross-linked by ultraviolet irradiation (120 mJ), and then hybridized at 62°C with either a 32P-radiolabeled ribo-probe for rat c-fos or a CDNA probe for mouse jun-B or at 60°C with an end-labeled oligonucleotide to ribosomal 18S as described by Church and Gilbert. Hybridization intensity was quantified in disintegrations per minute directly from blots with a Betascope 603 (Betagen Corp). The signal from each sample was normalized to the signal obtained with a synthetic oligonucleotide probe specific for the 18S ribosomal RNA.

Probes

The probe used to detect c-fos mRNA was an ≈500-bp [32P]CTP-labeled riboprobe synthesized from the vector pCRfos(500) by in vitro RNA transcrip. pCRfos(500) encodes ≈500 bp of the conserved coding region of the rat c-fos cDNA and was generated by a polymerase chain reaction (PCR) by use of primers complementary to the published sequence and cloned into the vector PCR1 according to the manufacture’s instructions (In Vitrogen Corp). The complete sequence of the cDNA insert derived by PCR was verified by the dyeoxy-mediated chain-termination method. The probe used to detect jun-B was a 1.917-kb EcoRI fragment cDNA from clone 465.2 (American Type Culture Collections). The probe for 18S RNA was a synthetic end-labeled oligonucleotide as previously described.

Measurement of \[^{40}Ca^{2+}\]i

Neonatal cardiac myocytes were cultured on coverslips coated with gelatin. Before loading with indo 1-AM, the incubation solution was changed from DMEM/M199 to a HEPES buffer solution containing (mmol/L) NaCl 137, KCl 5.4, CaCl2 1.5, MgSO4 1.2, and HEPES 20 at pH 7.4. Myocytes were then loaded with indo 1-AM (25 μmol/L) for 20 to 30 minutes at 37°C. Indo 1 fluorescence was excited at 350 nm. Paired photomultipliers collected indo 1 emission by simultaneous measuring spectral windows of 391 to 434 and 457 to 507 nm selected by bandpass interference filters. Since indo 1-AM may be partially hydrolyzed and the \[^{40}Ca^{2+}\]i indicator may be compartmentalized in mitochondria or other organelles, calibration of \[^{40}Ca^{2+}\]i, in neonatal myocytes remains uncertain. The 410/490-nm ratio of emitted fluorescence was taken as an index of \[^{40}Ca^{2+}\]i, as described previously. \[^{40}Ca^{2+}\]i measurements were performed at room temperature.

[14C]Phenylalanine Incorporation Experiments and Cell-Size Measurement

The rate of protein synthesis was assessed as described by Simpson, with the following modifications: After cell isolation, cells were cultured in serum-containing medium and plated on six-well tissue culture plates at a density of 5 × 10^4 per well (5 × 10^5 cells per square centimeter). Several experiments were done at a density range from 1 × 10^4 to 1 × 10^5 cells per square centimeter. Similar results were obtained. To inhibit fibroblast growth in the culture, 1 × 10^4 mol/L bromodeoxyuridine (BrdU, Boehringer Mannheim) was added to the cell culture the first day of cell isolation and was maintained in the culture media throughout the experiment. Cultures were essentially free of fibroblasts as assessed by cell staining with antibodies that recognize the myocyte-specific sarcomeric myosin (monoclonal antibody F59) and sarcomeric actin (Sigma). Serum-containing medium was removed and replaced with serum-free medium supplemented with 1
μmol/L insulin, 5 μmol/L transferrin, and 10 nmol/L selenium 24 hours after plating. After an additional 24 hours, [1^4]Cphenylalanine (0.1 μCi/mL) and either ATP or NE were added to the culture medium. Cells were analyzed for changes in cell size or protein synthesis 3 days later. We had noticed that ATP may be degraded during long-term incubation in culture medium. High-performance liquid chromatography measurement indicated that ATP was stable for 6 hours of incubation and was partially degraded (<25%) at 8 hours. Therefore, in some experiments, ATP was replenished in the medium at 8-hour intervals, and in yet others, ATP and medium were replaced every 8 hours for 3 days. For each experiment, two to three fields were randomly chosen, photomicrographs of the cells were taken at high power (×400), and 120 individual two-dimensional cell areas were measured by planimetry. Rates of [1^4]Cphenylalanine incorporation into trichloroacetic acid (TCA)-insoluble material were then determined. For this, the cultures were rinsed with PBS three times and then incubated with ice-cold 10% TCA for at least 1 hour. The plates were then rinsed with 10% TCA three times, and the TCA-insoluble material was dissolved in 1 mL of 1% sodium dodecyl sulfate (SDS). The entire volume of SDS was then used for scintillation counting. Determination of unincorporated [1^4]Cphenylalanine in the TCA-soluble fraction indicated that neither NE nor ATP altered transport of the labeled compound into the cell.

Statistics

Data were expressed as mean±SEM. For mRNA and protein synthesis experiments, the control group was arbitrarily assigned a value of 1, and deviation of experimental groups from control was tested with the t statistic. P values were corrected by the Bonferroni method. For the cell-size measurements, the mean values of the control and experimental groups were compared by ANOVA where indicated. Post hoc comparisons between groups were made with Tukey’s procedure. A value of P<.05 was considered to be significant.

Results

Extracellular ATP Increases c-fos and jun-B mRNA Levels

To determine whether extracellular ATP induces expression of IEGs (ie, c-fos and jun-B), Northern blot analysis was performed on total RNA isolated from cultured neonatal cardiac myocytes incubated with different concentrations of ATP for 30 minutes. The addition of 100 μmol/L ATP elevated c-fos mRNA level 4.6-fold over control levels (Fig 1A and 1B). The magnitude of the increase in c-fos expression was comparable to that achieved by 2 μmol/L NE. Extracellular ATP also increased the levels of jun-B mRNA 2.6-fold compared with levels in vehicle-treated control myocytes (Fig 1C and 1D).

To determine the time course of extracellular ATP on the induction of c-fos mRNA gene expression, ATP (100 μmol/L) was added to the serum-starved myocyte cultures and incubated for 15 minutes to 4 hours. ATP increased c-fos mRNA level as early as 15 minutes, peaked at 30 minutes, and returned the basal level after 1 hour of incubation (Fig 2). To evaluate whether extracellular ATP was exerting its effect in inducing c-fos mRNA in a physiologically achievable range, a dose-response experiment was carried out. ATP increased the level of c-fos mRNA in a dose-dependent manner, with an EC$_{50}$ of =10 μmol/L and a maximal effect at 100 μmol/L (Fig 3). To determine whether the effect of ATP is mediated by P$_1$- or P$_2$-purinergic receptors in the cardiomyocyte sarcolemma, ATP (100 μmol/L), ADP (100 μmol/L), and adenosine (100 μmol/L) were added to cardiomyocyte cultures. The efficacy order of ATP and its metabolites for induction of c-fos was as follows: ATP>ADP>adenosine (Fig 4), suggesting that induction of IEGs is mediated by activation of P$_2$-purinergic receptors.
Extracellular ATP Increases FOS Protein Levels

To determine whether the increase in c-fos mRNA was followed by an increase in FOS protein, cultures were stained with an anti-FOS antibody. The addition of ATP (100 μmol/L, Fig 5C) to cultured cardiac myocytes, like NE (Fig 5D), resulted in an increase of FOS protein within the nuclear compartment. Increased nuclear staining was observed as early as 45 minutes after addition of ATP to myocyte cultures and increased up to 2 to 3 hours. The effect of ATP was dose dependent, with the maximum observable increase in nuclear staining at 100 μmol/L (data not shown).

Effects of ATP on [Ca^{2+}]

Extracellular ATP is known to increase [Ca^{2+}] in adult cardiac myocytes. As a first step to investigate intracellular signal transduction pathways responsible for the effects of extracellular ATP on c-fos and jun-B expression, we determined whether ATP increased the [Ca^{2+}], in cultured neonatal myocytes. On addition of 100 μmol/L ATP, quiescent cultured neonatal cardiac myocytes exhibited a transient increase in [Ca^{2+}], followed by a sustained increase of a lesser magnitude (Fig 6A). The sustained elevation of [Ca^{2+}], varied in duration, but in some cases, it lasted as long as 10 minutes. NE did not elicit an increase in [Ca^{2+}], in quiescent cells, whereas ATP increased [Ca^{2+}], in the same myocytes (Fig 6B). Pretreatment of neonatal myocytes with BAPTA-AM (10 μmol/L) for 30 minutes prevented the ATP-induced increase of [Ca^{2+}], (Fig 6C).

ATP-Induced Elevation in c-fos mRNA Is Mediated by a Ca^{2+}-Dependent Pathway

To determine whether a Ca^{2+}-dependent pathway is required for the ATP-induced increase in the expression of IEGs, we used Northern blot analysis to determine whether the intracellular Ca^{2+} chelator BAPTA-AM modulates the ATP-induced c-fos expression. Preincubation of myocyte cultures with 10 μmol/L BAPTA-AM for 30 minutes inhibited the ATP-induced expression of c-fos but not the NE-induced c-fos expression (Fig 7). These data suggest that NE and ATP induce the expression of IEGs via different intracellular mechanisms and that the increase in [Ca^{2+}], by ATP is required to maximally induce IEG expression in neonatal cardiac myocytes.

Effect of ATP on [14C]Phenylalanine Incorporation Into Myocyte Proteins and on Myocyte Cell Size

Since both ATP and NE increase the expression of IEGs, we investigated whether ATP also increases neonatal myocyte protein synthesis as does NE. Incorporation of [14C]phenylalanine into TCA-precipitable protein was measured after incubation with experimental regimens for 3 days. NE increased [14C]phenylalanine incorporation over the 3-day period by 130% over the control value, whereas extracellular ATP did not increase [14C]phenylalanine incorporation (Fig 8). To eliminate fibroblast proliferation, these experiments were performed in the presence of 100 μmol/L BrdU. The addition of BrdU did not affect the ability of myocytes to activate IEG expression in response to ATP or NE (data not shown). To alleviate concern regarding degradation of ATP or accumulation of ATP-degradation products, additional experiments were conducted in which ATP was replenished or replaced at 8-hour intervals. The three different ATP treatment regimens all decreased [14C]phenylalanine incorporation into TCA-precipitable protein (Table 1). However, neither the replenishment of ATP at 8-hour intervals nor the replacement of ATP and media every 8 hours produced results different from those after treatment with a single dose of ATP.

We also determined whether ATP increased cell size. After treatment with ATP or NE, individual cell areas were measured by planimetry in high-power fields (×400). Although NE increased average myocyte size by ≈50%, extracellular ATP did not significantly increase cell size (Fig 9 and Table 2).

Discussion

The findings presented here demonstrate that extracellular ATP induces the expression of the IEGs c-fos
and jun-B. The time course of c-fos activation by ATP is similar to that observed for \( \alpha_1 \)-adrenergic stimulation of cardiac myocytes\(^{19} \) and is typical of IEG responses.\(^{35,36} \)

The present study also demonstrates that extracellular ATP increases \([\text{Ca}^{2+}]_i\), in quiescent myocytes and that the ATP-induced increase in \([\text{Ca}^{2+}]_i\) precedes the ATP-induced increase in IEG expression (Fig 2 and Fig 6). This suggests that the transient alteration in \([\text{Ca}^{2+}]_i\) induced by ATP may be a stimulus for the induction of IEG expression. Supporting this hypothesis is the finding that chelating intracellular \( \text{Ca}^{2+} \) with BAPTA-AM inhibited the ATP-induced increase in the level of c-fos mRNA. The dependence of ATP on \([\text{Ca}^{2+}]_i\) is consistent with the documented effect of ATP on \( \text{Ca}^{2+} \) entry via L-type \( \text{Ca}^{2+} \) channels and nonselective cation channels.\(^{11} \) It is not clear whether ATP activates other intracellular signaling mechanisms, but the failure of BAPTA-AM to completely inhibit c-fos induction in these experiments suggests that it may.

Fig 5. Photomicrographs showing representative examples of FOS protein levels in neonatal cardiac myocytes. Immunocytochemistry was performed with a primary antibody specific for FOS and a secondary antibody conjugated with alkaline phosphatase as described in "Materials and Methods." A shows vehicle-treated control; B, untreated myocytes incubated without primary antibody; C, ATP-treated myocytes (100 \( \mu \text{mol/L} \)); and D, norepinephrine-treated myocytes (2 \( \mu \text{mol/L} \)). Slides were incubated with various agents for 2 hours before fixation. Arrows point to the nuclear region.

Fig 6. Tracings showing the effects of ATP or norepinephrine and BAPTA-AM+ATP on \([\text{Ca}^{2+}]_i\), in neonatal ventricular myocytes. A, Addition of ATP (100 \( \mu \text{mol/L} \)) to the superfusate entering a chamber containing cultured myocytes increased \([\text{Ca}^{2+}]_i\). \([\text{Ca}^{2+}]_i\), was measured by loading myocytes with indo 1-AM (25 \( \mu \text{mol/L} \)) for 20 to 30 minutes at 37°C. Indo 1 fluorescence was excited at 350 nm, and the 410/490-nm ratio of emitted fluorescence was taken as an index of \([\text{Ca}^{2+}]_i\), as described previously.\(^{28} \) B, Addition of norepinephrine (2 \( \mu \text{mol/L} \)) to quiescent cells did not elicit \( \text{Ca}^{2+} \) response. C, Pretreatment with BAPTA-AM (10 \( \mu \text{mol/L} \)) for 30 minutes and the subsequent addition of ATP (100 \( \mu \text{mol/L} \)) did not increase \([\text{Ca}^{2+}]_i\).

Fig 7. Bar graphs showing the effects of BAPTA-AM on the level of c-fos mRNA stimulated by ATP or norepinephrine (NE). Serum-starved cell cultures were pretreated with the intracellular \( \text{Ca}^{2+} \) chelator BAPTA-AM (10 \( \mu \text{mol/L} \)) for 30 minutes, and then ATP (100 \( \mu \text{mol/L} \)) or NE (2 \( \mu \text{mol/L} \)) was added for an additional 30 minutes. Total RNA was isolated as described in "Materials and Methods." Data are expressed as mean±SEM (n=5) as percent relative to ATP- or NE-treated control.
Fig. 8. Bar graph showing different effects of norepinephrine (NE) and ATP on [14C]phenylalanine incorporation. Serum-starved cell cultures were incubated with ATP (100 μmol/L) or NE (2 μmol/L) and [14C]phenylalanine (0.1 μCi/mL) for 3 days. Cells were washed, treated with 10% trichloroacetic acid for 1 hour, and harvested with 1% sodium dodecyl sulfate. [14C]Phenylalanine incorporation was determined with a beta counter. Data are expressed relative to control (arbitrarily assigned 1) as mean±SEM (n=9).

In contrast to the effects of ATP on [Ca2+]i, NE did not increase [Ca2+]i in quiescent myocytes, although it did activate IEG expression in the present study and in the previous studies of others.19 The NE-induced expression of the c-fos gene was not inhibited by pretreatment with BAPTA-AM. Since BAPTA-AM blocked all detectable Ca2+ activity (ie, Ca2+ transients and beating), an increase in [Ca2+]i is not required for c-fos induction by NE in rat ventricular myocytes and suggests that ATP and NE activate IEG expression by different mechanisms. These data do not distinguish, however, between the activation of cAMP-dependent and diacylglycerol–protein kinase C signaling pathways by NE, both of which are known to activate c-fos gene expression.

A number of studies have suggested that the IEGs play a crucial role in cell growth and proliferation. For example, c-fos has an apparent role in the proliferative response induced by growth factors in BALB/c 3T3 cells;31 high-level expression of c-fos in transgenic mice interferes with normal bone development.38 Although cardiac myocytes do not proliferate when stimulated

Fig. 9. Photomicrographs showing representative examples of the different effects of norepinephrine and ATP on cell size. After 24 hours in serum-free media, cells were treated without (control) or with ATP (100 μmol/L) or with norepinephrine (2 μmol/L) for 3 days. Photomicrographs were prepared using a Nikon phase-contrast inverted microscope in high-power fields (original magnification ×400). Individual cell areas were measured by planimetry and are summarized in Table 2.

with growth factors, hormones, or hemodynamic factors, they do increase in size (ie, hypertrophy) in response to a number of these agents.14,17,39,40 Without apparent

**Table 1. Effects of Different ATP Treatment Regimens on [14C]Phenylalanine Incorporation Into Trichloroacetic Acid–Precipitable Protein**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ATP1</th>
<th>ATP2</th>
<th>ATP3</th>
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</thead>
<tbody>
<tr>
<td>[14C]Phe incorporation (mean±SEM)</td>
<td>1</td>
<td>0.48±0.07</td>
<td>0.48±0.04</td>
<td>0.54±0.08</td>
</tr>
<tr>
<td>Change from control, %</td>
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<td>-52%</td>
<td>-52%</td>
<td>-46%</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>12</td>
<td>16</td>
<td>8</td>
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<tr>
<td>P value vs control</td>
<td>...</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>P value vs ATP1</td>
<td>...</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
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</tbody>
</table>

ATP1 indicates ATP (100 μmol/L) added once to serum-free cell cultures and incubated for 3 days; ATP2, ATP (100 μmol/L) added every 8 hours for 3 days; ATP3, replacement of both ATP and medium every 8 hours to prevent a potential accumulation of ATP degradation products, addition of ATP to the medium, and change of both ATP and medium every 8 hours for 3 days; and Phe, phenylalanine.

[14C]Phe incorporation into trichloroacetic acid–precipitable protein was then measured. For each experiment, a norepinephrine-treated dish was used as a positive control. In these experiments, the norepinephrine-induced [14C]Phe incorporation into trichloroacetic acid–precipitable protein was 1.78±0.2 (mean±SEM, n=8).
exception, the expression of IEGs precedes the hypertrophic response that is observed both in vivo and in vitro.17,39,41 This close association has led to the speculation that IEG may be responsible for induction of cardiac hypertrophy.17,35,42,46 On the other hand, some reports suggest a possible dissociation between IEG expression and hypertrophic growth or markers of hypertrophy.18,47,48 We report in the present study that although ATP increased IEG expression to at least the same extent as did NE, it did not increase the rate of \([3^2P]N\)-phenylalanine incorporation into myocyte protein, nor did it influence myocyte size. This dissociation between the induction of IEGs and cardiac hypertrophy demonstrates that activation of c-fos and jun-B gene expression is not sufficient to induce hypertrophy of cardiac myocytes in the 3-day experiments. Although these data indicate that IEG expression is not sufficient to induce cardiomyocyte hypertrophy, they do not exclude the possibility that IEG activation is necessary for hypertrophic growth.

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References

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