Hypertrophy of isolated adult feline cardiac muscle cells may be induced in culture by either α- or β-adrenergic agonists. However, it has been shown previously that each of these agonists activate different subsets of immediate-early response genes and have different effects on expression of "fetal" protein isoforms and stimulation of protein synthesis. Moreover, in adult feline heart cells, β-adrenergic agonists, such as isoproterenol, activate sustained synchronous beating and sarcomeric reorganization while α-adrenergic agonists, such as phenylephrine, do not. The objective of the present study was to determine whether these differences in proximal signaling events converged in a common signal pathway during activation of contractile protein synthesis. By direct comparisons of actin and myosin heavy chain (HC) synthesis and accumulation following isoproterenol and phenylephrine, it was determined that both agonists stimulate a coordinated accumulation of these proteins during cardiomyocyte growth. However, each agonist stimulated a very different program of contractile protein synthesis. During phenylephrine-induced hypertrophy, actin and myosin HC synthesis were rapidly and coordinately activated and continuously maintained at rates 10-25% greater than untreated cultures. The pattern of myosin HC synthesis following isoproterenol was very much more complex with periods during which it was as much as 40% greater or 25% less than in control cultures. Furthermore, there was no correlation between rates of actin and myosin HC synthesis following isoproterenol. It was concluded that actin and myosin HC syntheses and accumulation were regulated independently and in a very different manner following isoproterenol or phenylephrine. Since protein accumulation was not correlated with synthesis rates during development of hypertrophy, it was also concluded that post-translational mechanisms played a significant role in the maintenance of contractile protein stoichiometry during β-adrenergic/β-agonist-induced hypertrophy. Myosin HC synthesis also appeared to be independently regulated during cardiomyocyte atrophy induced by the calcium channel blocker nifedipine. Unlike the case in hypertrophy, however, protein balance was not maintained in nifedipine, and the depression of myosin HC synthesis and loss of myosin HC content were much greater than in the case of other contractile proteins.

Cardiac muscle hypertrophy has been characterized as a complex set of genetic, biochemical, physiological, and morphological changes which ultimately result in an increase in cardiac muscle size and mass. Understanding the signal pathways involved in the generation and regulation of hypertrophy has been complicated by several factors. 1) There are a number of diverse stimuli which produce a similar set of phenotypic responses. For example, α-adrenergic stimulation (1-7), endothelin (8-10), angiotensin II (11-13), passive stretch (14-17), pressure overload (6, 18, 19), phorbol esters (20, 21), and microinjection of activated Ras (22) evoke similar patterns of gene expression, fetal isoform re-expression, up-regulation of protein synthesis, and/or increased cell size and mass. 2) However, there are also stimuli which induce cardiac hypertrophy with a somewhat different pattern of phenotypic responses. These include: thyroid hormone (23-25), β-adrenergic agonists (4, 7, 26, 27), and various forms of active loading (exercise 28, 29), pressure overload (6, 30-32), and pacing (33-36). 3) A specific stimulus may also activate multiple signal pathways (36-38). While some pathways represent essential components of the hypertrophic response, others may only represent epiphenomena. 4) Finally, both the signal pathways and hypertrophic responses may vary between species (e.g. rodents versus large mammals) and during different developmental stages (36, 39, 40).

While it is recognized that both α- and β-adrenergic agonists produce cardiomyocyte hypertrophy, the proximal signaling events following each agonist are quite different (3, 4, 41). The present study investigates whether early differences in signal mechanisms converge during later stages of genetic signaling. Specifically, does the activation of contractile protein synthesis occur in the same time frame following each agonist? Is synthesis of different contractile proteins fully coordinated following each agonist? Does each agonist promote a coordinate accumulation of different contractile proteins during hypertrophy? And, finally, is myofibrillar protein synthesis and content also coordinated during myocyte atrophy induced by calcium channel blockade?

These questions are addressed by assessing rates of protein synthesis and accumulation in cultured adult cardiomyocytes following α- and β-adrenergic agonists. Protein synthesis rates provide a view of the hypertrophic response during the final stage at which transcriptional regulation might exert direct influence on product accumulation. Several studies have shown a correlation between mRNA levels and protein synthesis rates in the heart (42-44) and skeletal muscle (45). Thus, the coordination of specific mRNA levels may be indirectly indicated from coordination of specific protein synthesis rates. This provides some view into the role of transcriptional regulation in the coordination of specific protein accumulation. However, subsequent post-translational processing may also play a significant role in the regulation and coordination of cardiomyocyte growth. The significance of regulatory mechanisms at this level would be most readily revealed from a mismatch between rates of protein synthesis and protein accumulation.

The model of cultured adult feline cardiomyocytes employed...
in this study provides a number of features which facilitate analysis of the cardiac hypertrophic response. First, these cells may be maintained in a stable, postmitotic, highly differentiated state for many weeks in culture. During this period, myocytes remain fully responsive to both α- and β-adrenergic agonists and develop many characteristics of myocyte hypertrophy following exposure to either agonist (27, 46–48). Second, protein turnover in adult heart cultures corresponds more closely to that in the intact adult heart than does turnover in neonatal heart cultures (27, 49). Third, a stable culture environment greatly facilitates paired, serial time studies of specific protein synthesis and accumulation while also providing unique access to sensitive protocols for assessment of protein synthesis which would not be possible in the intact heart. And, fourth, the response of adult feline cardiomyocytes also provides a unique opportunity to compare hypertrophy induced through two distinct signal pathways representing both hormonal activation and cell loading in the form of active beating.

The results of this study show that myosin HC synthesis and accumulation is regulated by distinctly different mechanisms following α- and β-adrenergic agonist-induced hypertrophy in adult cardiac muscle cells. Furthermore, myosin HC synthesis is also regulated in a manner which is independent from or other cardiac muscle proteins.

**EXPERIMENTAL PROCEDURES**

**Culture Procedures**—Adult feline cardiomyocytes were enzymatically dissociated and plated in culture according to procedures described in Clark et al. (47). All experiments were conducted on cardiomyocytes maintained for 14 days on laminin-coated culture dishes in Dulbecco’s modified Eagle’s medium containing 5% fetal bovine serum (Whittaker Bioproducts), 5% Nu-Serum (Collaborative Research), and 2.5 μg/ml insulin, 2.5 μg/ml transferrin, and 2.5 ng/ml selenous acid (ITS supplement, Collaborative Research). Cytosine arabinoside, 10 μmol/liter (Sigma), was maintained in the culture medium to prevent fibroblast overgrowth. Cultures were maintained for at least 7 days prior to administration of agonists and antagonists. Variable treatment periods were obtained by adding test reagents at staggered intervals so that all cultures were the same age (14 days) at the time of analysis.

**Radioisotope Labeling and Determination of Synthesis Rates**—Fractional protein synthesis rates and relative changes in synthetic rates of specific SDS-PAGE fractionated proteins were determined using a dual isotope labeling protocol which has been described previously (50). Briefly, protein synthesis rates were derived from the ratios of isotopically labeled leucine incorporated into protein over two intervals, t1 and t2. Long-term labeling, t2, was conducted by labeling for 168 h in 0.1 μCi/ml [3H]-leucine from day 7 to 14 in vitro. Short-term labeling, t1, was 4 h in 2.5 μCi/ml [3,4-3H]-leucine administered immediately before harvesting cultures on day 14. All isotopes were purchased from Amersham.

Fractional rates of protein synthesis were obtained from the following relationship:

\[
R = \frac{\left(\frac{[3H]}{[14C]}\right)_{t2} - 1}{\left(\frac{[3H]}{[14C]}\right)_{t1} - 1} = e^{k_{t2} - k_{t1}}
\]

where \( R \) equals the \(^{3}H/^{14}C \) ratio in protein, \( P' \), divided by the \(^{3}H/^{14}C \) ratio in free leucine in the culture medium, \( F' \). This function was derived from a relationship described by Zilversmit (Equation 10 in Ref. 51) by substituting different isotopes for each labeling period in the original derivation. This method differs from “pulse-equilibrium” ratio protocols (33, 54) in that the denominator in the exponential term is not assumed to be 1.0 as would be the case if the \( t_1 \) labeling period was sufficiently long to achieve equilibrium between the precursor and product specific radioactivity. That is, \( P' = F' \).

Although direct solution of \( k_s \) from Equation 1 is complex, a simple empirical cell was derived by fitting predicted ratios with \( k_s \). Over the range 0.015 < \( k_s < 0.50 \) day\(^{-1} \), the function:

\[
k_s = 0.65887 + 3.493732R + 0.19856(\ln R) + 7.51653R^2
\]

provides the fractional synthesis rate per day with a correlation \((r^2\) > 0.9999. In the text and figures, the fractional rate is expressed as percent per day which is defined as \( k_x \times 100 \). It should be noted that this function predicts absolute fractional synthetic rates only under steady-state conditions. However, if there was a change in the ratio, \( R \), following hypertrophic or atrophic stimulation, it can be demonstrated that this change would be equal or less than the underlying change in fractional synthetic rates by the following reasoning. If we let \( x \) represent the term \( t_2 \), and \( y = f(x) = 1 - e^{-x} \), then it can be shown both that \( dy/dx \) and \( y \) are positive for any positive value of \( x \). Thus, it follows that a relative change in \( y \) as defined by \( (y - \text{dy})/y \) must also be less than or equal to the change in \( x \). Since the labeling times would be the same in treated and control cultures, the change in \( R \) provides a minimum estimate for the underlying change in the fractional rate. This holds regardless of whether the test rate was increased or decreased relative to the control rate. Thus, any change in \( R \) could be interpreted as indicating a change in protein synthetic rates at least as great as the change in \( R \).

**Determination of Labeling Ratios, \( R \)**—In order to estimate \( R \), the \(^{3}H/^{14}C \) ratios in both the protein and free leucine in the medium were determined. Immediately following pulse-labeling, the medium was collected and the cultures were harvested. The culture medium was deproteinized with 5% trichloroacetic acid, and amino acids were recovered on a Dowex 50W column (Sigma). Following derivatization with dansyl chloride (9N-dimethylaminonaphthalene-sulfonic acid), it can be demonstrated that dy and \( \text{dy}/y \) change in \( R \) must be less than or equal to the change in \( R \). Since the labeling times would be the same in treated and control cultures, the change in \( R \) provides a minimum estimate for the underlying change in the fractional rate. This holds regardless of whether the test rate was increased or decreased relative to the control rate. Thus, any change in \( R \) could be interpreted as indicating a change in protein synthetic rates at least as great as the change in \( R \).

The cultures were harvested for SDS-PAGE by rapidly rinsing five times and scraping the cells into ice-cold phosphate-buffered saline. After centrifugation, the supernatant was discarded and the cell pellet was resuspended and sonicated in 300 μl of low salt buffer (40 mmol/liter NaCl, 1 mmol/liter dithioretilte, 0.1 mmol/liter EGTA, and 5 mmol/liter sodium phosphate, pH 6.8) containing 1 Triton X-100 (Sigma). A 5× concentration of SDS-PAGE loading buffer was added to the sonicated cell fraction, and proteins were solubilized by heating at 100 °C for 10 min. Total protein content was determined by the Lowry method (47, 55). Equal amounts of protein were applied in each set of gels. Different types of SDS-PAGE separation were achieved by varying acrylamide and bisacylamide concentrations along with different running buffer combinations. The specific method used in each case is given in the figure legends. Coomassie Blue-stained gels were scanned using a Bio-Rad Model 620 CCD densitometer.

Specific proteins were identified both from their mobility relative to prestained molecular weight standards co-migrated in each analysis and by Western blotting with specific antibodies. Radioisotope incorporation was determined by cutting out specific bands from Coomassie stained gels. Gel bands were excised, minced and counted and the 3H/14C ratio in free leucine in the medium (49).

**RESULTS**

**Contractile Protein Balance following Adrenergic Agonists**—While previous studies have shown that quiescent adult cardiomyocytes disassemble their myofibrillar structure during prolonged culture (47, 48), they nevertheless appear to retain contractile protein content, albeit in a somewhat less ordered
state. This was shown by quantitative densitometry of SDS-PAGE fractionated proteins from adult feline cardiomyocytes maintained for up to 25 days in culture (Fig. 1). Over this period, the relative proportions of different proteins remained fairly constant in gels loaded with equal amounts of protein. In quiescent 14-day cultures, quantitative scanning indicated that myosin HC constituted approximately 9.8% of the total SDS-soluble protein fraction. Actin represented approximately 12% of total protein. Activation of beating with the β-adrenergic agonist isoproterenol results in a 60% increase in total protein over the period from 7 to 21 days in vitro (27). However, since gel scans of beating and nonbeating cultures are virtually identical (Fig. 1), the accumulation of different proteins must be fully coordinated during isoproterenol-induced cell growth. No splitting of the myosin HC band was observed following any treatment which would be indicative of a myosin HC isoform shift. The β-adrenergic agonist, phenylephrine, also stimulates growth of adult feline cardiomyocytes (27). However, as was the case in isoproterenol, 7 days in phenylephrine also did not result in any significant changes in the relative amounts of SDS-PAGE separated proteins (data not shown). These data, therefore, imply a coordinate increase in proteins in both α- and β-adrenergic agonist-induced hypertrophy. To determine whether this coordination results from regulation at pre- or post-translational levels, the synthesis rates of individual proteins were compared following each of these agonists.

Fractional Protein Synthesis Rates in Quiescent Steady State—Determination of the ratio of labeled amino acids incorporated into specific proteins following short- and long-term labeling provides a sensitive means for estimating and comparing fractional synthesis rates of different myocardial proteins. Since this approach could also provide a measure of change in protein synthesis rates following agonists, it also provided insight into the coordination of growth at the level of mRNA translation. Application of this approach is shown in Fig. 2 which relates Coomassie Blue densitometry, long-term [14C]leucine incorporation, and the labeling ratio, R, in SDS-PAGE-separated proteins from quiescent 14-day cultures. A correspondence between Coomassie Blue staining density and [14C]leucine incorporation is expected following prolonged labeling since this label becomes directly proportional to leucine content as incorporation approaches equilibrium with the precursor (53). After 7 days of labeling, total protein is slightly more than 30% equilibrated with the precursor in adult cardiomyocytes (49, 50). The top graph in Fig. 2 shows the mean 4- to 168-h ratio, R, in each gel section. These data indicate that between 4 and 6% of total protein-bound leucine was incorporated during the 4-h pulse. The right ordinate scale in the top graph of Fig. 2 also shows the corresponding fractional protein synthesis rates computed from the ratio data (by Equation 2) and indicates that the fractional synthesis rates of most proteins in quiescent cultures are in the range of 20 to 33% day⁻¹.

Data in the first row of Table I shows the mean fractional synthesis rates (computed according to Equation 2) of four major protein bands indicated in Fig. 2, i.e. myosin HC, α-actinin, desmin, and actin. Since the direct relationship between fractional synthetic rates and the labeling ratio R only applies under steady-state conditions, fractional synthetic rates in Ta-
the presence of phenylephrine, myosin HC synthesis appeared and isoproterenol was in the effect on myosin HC synthesis. In highly coordinated with that of desmin and actin (Fig. 4 and Table I). In contrast, no such coordination was apparent following isoproterenol (Fig. 3). At no time was the depression of synthesis of myosin HC observed in phenylephrine as was the case in isoproterenol. Apart from the activation of beating in isoproterenol, the most notable difference between the effect of phenylephrine and isoproterenol was in the effect on myosin HC synthesis. In the presence of phenylephrine, myosin HC synthesis appeared highly coordinated with that of desmin and actin (Fig. 4 and Table I). In contrast, such coordination was equal to or less than a change in the fractional synthesis rate, %, Table I also provides the observed labeling ratio, R, for each protein following isoproterenol and phenylephrine.

**Adrenergic Stimulation of Myofibrillar Protein Synthesis**— Although cardiomyocyte hypertrophy and increased rates of total protein synthesis are induced by both α- and β-adrenergic agonists (27), the data in Table I suggest that these agonists may have significantly different effects on the metabolism of different cardiac proteins. The effects of isoproterenol and phenylephrine on the synthesis of myosin HC, desmin, and actin are directly compared in Figs. 3 and 4, respectively. During the first 4 h following activation of beating with isoproterenol, protein synthesis is uniformly depressed in each protein (Fig. 3). However, by 24 h, the pattern of synthesis is very different. While synthesis of desmin and actin increased from 17 to 22% above basal levels, that of myosin HC was depressed by more than 25%. By 48 h in isoproterenol, the rate of myosin HC synthesis began to increase and eventually reached a level more than 40% above basal levels on the 4th day. Thereafter, the rate of myosin HC synthesis declined in parallel with that of desmin and actin.

In contrast to the effect in isoproterenol, phenylephrine induced a fairly homogeneous elevation in the synthesis of myosin HC, desmin, and actin. The synthesis rates of each of these proteins rose between 10 and 20% by 24 h in phenylephrine and thereafter remained elevated and constant for at least 7 days (Fig. 4). At no time was the depression of synthesis of myosin HC observed in phenylephrine as was the case in isoproterenol. Apart from the activation of beating in isoproterenol, the most notable difference between the effect of phenylephrine and isoproterenol was in the effect on myosin HC synthesis. In the presence of phenylephrine, myosin HC synthesis appeared highly coordinated with that of desmin and actin (Fig. 4 and Table I). In contrast, no such coordination was apparent following isoproterenol (Fig. 3). Relative differences in synthesis of each protein are directly compared in Figs. 5 and 6. These data show the relation between myosin HC versus actin synthesis as well as desmin versus actin synthesis in proteins from the same gels. A fairly constant relationship in the desmin to actin synthesis ratio following isoproterenol suggests that the synthesis of these proteins is well coordinated during the development of hypertrophy. However, a very different pattern was observed in the relationship between myosin HC and actin synthesis. Differences in activation of synthesis of these proteins were evident as early as 4 h following isoproterenol. By 24 h, the ratio of

### Table I

**Myocardial protein synthesis in control, isoproterenol-, and phenylephrine-treated adult cardiomyocytes**

<table>
<thead>
<tr>
<th>Treatment (time)</th>
<th>Myosin HC</th>
<th>α-Actinin</th>
<th>Desmin</th>
<th>Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24±1.5 ± 4.9 ± 0.18</td>
<td>5.26 ± 0.19 ± 4.41 ± 0.13</td>
<td>20.3 ± 1.1 ± 5.14 ± 0.13</td>
<td>23.0 ± 1.1 ± 4.72 ± 0.14</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>4 h</td>
<td>5.30 ± 2.3 ± 5.30 ± 0.38</td>
<td>5.03 ± 2.3 ± 5.03 ± 0.38</td>
<td>5.38 ± 0.27 ± 5.38 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>2 days</td>
<td>5.06 ± 2.3 ± 5.06 ± 0.38</td>
<td>5.03 ± 2.3 ± 5.03 ± 0.38</td>
<td>5.38 ± 0.27 ± 5.38 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>5.34 ± 2.3 ± 5.34 ± 0.38</td>
<td>5.03 ± 2.3 ± 5.03 ± 0.38</td>
<td>5.38 ± 0.27 ± 5.38 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>5.14 ± 2.3 ± 5.14 ± 0.38</td>
<td>5.03 ± 2.3 ± 5.03 ± 0.38</td>
<td>5.38 ± 0.27 ± 5.38 ± 0.27</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>4 h</td>
<td>5.82 ± 2.3 ± 5.82 ± 0.38</td>
<td>5.03 ± 2.3 ± 5.03 ± 0.38</td>
<td>5.38 ± 0.27 ± 5.38 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>6.00 ± 2.3 ± 6.00 ± 0.38</td>
<td>5.03 ± 2.3 ± 5.03 ± 0.38</td>
<td>5.38 ± 0.27 ± 5.38 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>5.39 ± 2.3 ± 5.39 ± 0.38</td>
<td>5.03 ± 2.3 ± 5.03 ± 0.38</td>
<td>5.38 ± 0.27 ± 5.38 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>6.03 ± 2.3 ± 6.03 ± 0.38</td>
<td>5.03 ± 2.3 ± 5.03 ± 0.38</td>
<td>5.38 ± 0.27 ± 5.38 ± 0.27</td>
</tr>
</tbody>
</table>

* a Myosin HC versus α-actinin.  
  b Myosin HC versus desmin.  
  c α-Actinin versus desmin.  
  d Desmin versus actin.  
  e Myosin versus actin.
Analysis of the correlation between synthetic rates of these proteins over all time periods is given in Table II. The Pearson linear correlation coefficients relating synthesis of myosin HC, desmin, and actin suggests that there was a significant correlation ($p < 0.01$) between the synthesis rates of these proteins in both control and phenylephrine-treated cultures. However, there was no significant correlation between myosin HC and actin synthesis rates during development of $\beta$-adrenergic-induced hypertrophy. Since there was no evidence of discordant accumulation of actin and myosin HC following isoproterenol (Fig. 1), it would appear that regulation of accumulation of these proteins must occur during post-translational processing.

Accelerated Myosin HC Loss during Atrophy—While adult cardiomyocyte hypertrophy may be induced by both $\alpha$- and $\beta$-adrenergic agonists, treatment with calcium channel antagonists has been shown to induce atrophy in isolated cardiomyocytes (27, 47). Contractile proteins such as myosin HC (27, 55) and actin (56) appear particularly sensitive to calcium channel antagonists and are rapidly depleted following these agents. The coordination of contractile protein synthesis and amounts during adult cardiomyocyte atrophy was also evaluated during contraction blockade induced by the L-type calcium channel.
antagonist, nifedipine. To determine whether the hypertrophic effect of isoproterenol could be produced independently of beating, relative protein content and synthesis were determined in the presence of both isoproterenol and nifedipine.

The requirement for L-type calcium channel activity for the maintenance of myosin HC content is shown by quantitative densitometry of SDS-PAGE fractionated proteins from cultures maintained in isoproterenol and nifedipine (Fig. 7). Although the proportions of desmin and actin appear to remain constant, myosin HC declined nearly 70% over 5 to 7 days in nifedipine. The specific loss of myosin HC was also indicated from the decline in [14C]leucine recovered in myosin HC, desmin, and actin SDS-PAGE bands following isoproterenol plus nifedipine, Table III. Whereas the level of 14C in the myosin HC band declined more than 60%, the effect on desmin and actin was negligible. Analysis of protein synthesis rates following 24 h in nifedipine also showed markedly greater inhibition of myosin HC synthesis than that for desmin or actin. After 24 h in nifedipine, myosin HC synthesis rates were depressed to 52% (±8, n = 4) of basal rates, while desmin and actin were maintained at 95% (±13, n = 4) and 83% (±7, n = 4) of controls, respectively.

**DISCUSSION**

The results of this study provide support for three main conclusions. 1) Myosin HC accumulation is fully coordinated with other muscle proteins during myocyte growth induced through α- or β-adrenergic signal pathways. 2) Myosin HC depletion is accelerated and not coordinated with other muscle proteins during myocyte atrophy induced by contractile blockade. 3) The synthesis of myosin HC is coordinated with other muscle proteins following α-adrenergic agonists, but there is no significant correlation between myosin HC and actin synthesis during β-adrenergic-stimulated hypertrophy. The apparent mismatch between contractile protein synthesis and accumulation following β-adrenergic/beat-induced hypertrophy lead to the conclusion that post-translational processing is an essential factor for ensuring coordinated protein accumulation during this type of hypertrophy. The independent and uncoordinated relationships evident in the activation of contractile protein synthesis following isoproterenol also suggests different modes of transcriptional regulation for different contractile proteins.  

**Molecular Weight (kDa)**

![Graph showing molecular weight distribution](image)

**Figure 7. Effect of calcium channel blockade on contractile protein balance in adult cardiomyocytes.** Proteins from 14-day cultures treated with 1 × 10⁻³ mol/liter isoproterenol and the same concentration of nifedipine for the times indicated were separated on SDS-PAGE as described in Fig. 2. Gel scans were conducted on Coomassie Blue-stained gels.

<table>
<thead>
<tr>
<th>Treatment* (time)</th>
<th>Myosin</th>
<th>Desmin</th>
<th>Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 8</td>
<td>100 ± 9</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>ISO + Nifed 1-2 days</td>
<td>79 ± 6</td>
<td>106 ± 11</td>
<td>104 ± 9</td>
</tr>
<tr>
<td>ISO + Nifed 3-5 days</td>
<td>79 ± 6</td>
<td>103 ± 20</td>
<td>92 ± 17</td>
</tr>
<tr>
<td>ISO + Nifed 6-7 days</td>
<td>76 ± 5</td>
<td>97 ± 13</td>
<td>85 ± 13</td>
</tr>
</tbody>
</table>

*ISO + Nifed, isoproterenol + nifedipine.

These observations raise the question whether cardiomyocyte hypertrophy represents a transcriptionally coordinated phenotypic response, or alternatively whether the expression of each protein is independently regulated during transcriptional, post-transcriptional, and post-translational processing. If the latter proves to be the case, then rather than be regarded as a single defined state ‘hypertrophy’ would more accurately be characterized as an integration of many distinct adaptations which may exist in many qualitatively different states. In documenting the latter view, it must be demonstrated that different phenotypic elements may exist in different relationships in different forms of hypertrophy.

**The α-Adrenergic Response in Adult Cardiomyocytes**—The most highly coordinated and integrated set of responses to hypertrophic stimuli appears to develop following α-adrenergic agonists, although other stimuli may produce similar effects. The general phenotypic pattern following α-adrenergic agonists includes up-regulation of protein kinase C (10, 20, 21, 57-60), activation of immediate early response genes (3, 22, 61-63), up-regulation of fetal protein isoforms (37, 63-65), with a general up-regulation of protein synthesis (1, 5, 27, 66-68), accumulation (7, 20, 69), and net cell growth (20, 27, 70). A similar response pattern has been observed in both isolated neonatal (1, 3, 41, 69, 70) and adult (5, 7, 27, 71) heart cells, as well as in intact heart preparations (46, 66, 67). The α-adrenergic response appears rapidly (minutes for immediate-early response genes (3, 5, 61), to a few hours for up-regulation of protein synthesis (1, 5, 27, 66)). The entire constellation of responses following α-adrenergic agonists appear highly coordinated and are mediated through stimulation of a single α₁-receptor subtype (72), and, in the case of β-myosin HC, a single core enhancer element (73). The α-adrenergic response appears to be independent of active cell loading since these adaptations have also been observed in non-beating cells (7, 60, 68, 70).

**The β-Adrenergic Response**—There are a number of indications, including this report, which suggest that β-adrenergic-induced hypertrophy of cardiac myocytes represents a different type of response. However, since β-adrenergic agonists stimulate beating in adult feline heart cells (47) and have isotropic effects in the intact heart, there is some ambiguity regarding whether hormonal or mechanical signals represent the primary hypertrophic stimulus (74). However, the concern of the present study is to focus on the type of phenotypic response rather than clarification of the signal pathway. Specifically, is there a convergence at some point following isoproterenol to produce a similar phenotypic response as has been identified following α-adrenergic agonists and are the component elements coordinated in a similar fashion. The response to β-adrenergic agonists and/or beating is considerably more complex and less coordinated than following α-adrenergic agonists. β-Adrenergic agonists activate a smaller subset of immediate response genes (3, 61) and do not acutely stimulate cardiomyocyte contractile protein synthesis (1, 5, 27, 48, 66, 75-78) or ANF (79-81). Over longer intervals, however, β-adrenergic agonists clearly induce
significant hypertrophy, cell growth (3, 27, 47, 82), sarcomere reorganization (3, 27, 47, 83), increased secretion of ANF (27), and up-regulation of protein synthesis (27, 82).

An additional example of uncoordinated hypertrophic responses following different stimuli has also been reported by Buttrick et al. (29). Both renovascular hypertension and swim training in rats produced significant myocardial hypertrophy. However, these treatments induced different levels of mRNA expression for cardiac sarcoplasmic reticulum calcium ATPase (SERCA2), cardiac troponin I (cTnI), and atrial natriuretic factor (ANF), while neither treatment appeared to alter mRNA levels for myosin LC-1 or the fetal isoform of slow skeletal troponin I. It was concluded that distinct mechanisms of gene activation were represented in each of these forms of adaptive hypertrophy.

Independent Regulation of Myosin HC Synthesis and Accumulation—The regulation of myosin HC synthesis and accumulation illustrates an especially striking contrast between α- and β-adrenergic-induced hypertrophy. Myosin HC is the major protein of the thick myofilament and represents approximately 17% of the SDS-soluble protein mass in the adult heart (84), which is not remarkably different than 10% observed in cultured adult cardiomyocytes in this study. Induction of hypertrophy by α-adrenergic agonists resulted in a rapid, coordinated activation of muscle protein synthesis and accumulation in non-beating myocytes. In contrast, regulation of myosin HC synthesis following isoproterenol was clearly more complex and developed in at least four distinct phases. Within 4 h, isoproterenol activates beating (47), but also produces a general depression of protein synthesis (27, 48). It has been suggested that an acute depression in protein synthesis following β-adrenergic agonists may be due to a mismatch between O₂ supply and demand in the face of positive inotropism (74). We cannot rule out this possibility, at least during the initial 4 h following isoproterenol. After 24 h, there was a general elevation of protein synthesis with the notable exception of myosin HC. During this phase, myosin HC synthesis was significantly depressed while that of actin was increased. This type of dichotomy was previously observed that myosin HC accumulation appears to lag behind that of total protein during the first 5 days in isoproterenol and therefore may provide some insight into underlying transcriptional and translational mechanisms due to the slow overall rate of myofibrillar reassembly.

Characterization of Protein Synthesis Rates—This study has addressed the regulation of contractile protein mass during cardiomyocyte hypertrophy and atrophy by focusing on transcription and accumulation of individual proteins. While these processes represent relatively late stages of genetic regulation, they, nevertheless, may provide some insight into underlying transcriptional and translational mechanisms. For example, the steady-state levels of mRNA for myosin HC and LC-2 increased 2–3-fold in cultured neonatal rat cardiomyocytes following phenylephrine (2, 90) while contractile protein synthesis rates increased only 1.3-fold in similar cultures treated with α-adrenergic agonists (1).

The dual isotopic labeling protocol used in this study was employed because it offered sufficient precision to distinguish significant changes in protein synthesis rates at even lower levels than those described above (1, 52, 53). An additional consideration mandating this approach is the slow overall rate of protein synthesis in isolated adult cardiomyocytes (34, 49, 50) as compared to neonatal heart cultures. The sensitivity and precision of dual isotopic labeling is derived from the following features. First, both ³H and ¹⁴C isotopes are introduced in the same amino acid, and thus, no correction is required for differences in amino acid composition in different proteins. Second, errors arising from nonquantitative or differential extraction and separation of proteins on SDS-PAGE are avoided since only the isotope ratio in each fraction (gel band) is required for analysis. And finally, errors related to normalization of incorporated counts with other independent measurements such as cell number, protein, DNA, or amino acid content in the product pool are also avoided.

Conclusions—The results of this study show that myosin HC synthesis and accumulation is regulated by distinctly different
mechanisms following α- and β-adrenergic agonist-induced hyper-pertrophy or during atrophy induced by calcium channel blockage in cultured adult cardiac muscle cells. These mechanisms appear to involve different combinations of transcriptional and post-translational processing following different stimuli. Furthermore, different regulatory mechanisms are operative in the processing of different proteins. Thus, it would appear from these and other data that the regulation of protein mass in adult cardiac myocytes operates at multiple levels of genetic processing with different characteristics for each contractile protein.

REFERENCES