Increased Cyclic AMP Content Accelerates Protein Synthesis in Rat Heart

Xenophon P. Xenophontos, Peter A. Watson, Balvin H.L. Chua, Takashi Haneda, and Howard E. Morgan

Elevation of cyclic AMP (cAMP) content in perfused rat hearts by exposure to glucagon, forskolin, and 1-methyl-3-isobutylxanthine (IBMX) increased rates of protein synthesis during the second hour of perfusion with buffer that contained glucose in the absence of added insulin. When tetrodotoxin was added to arrest contractile activity, glucagon, forskolin, and IBMX still elevated cAMP content and rates of protein synthesis. Perfusion of beating rat hearts at elevated aortic pressure (120 mm Hg vs. 60 mm Hg) also accelerated rates of protein synthesis and raised cAMP content and cAMP-dependent protein kinase activity during the second hour of perfusion. Insulin accelerated rates of protein synthesis in beating hearts during the first and second hour of perfusion but did not increase cAMP content. Elevation of aortic pressure in insulin-treated hearts raised cAMP content but had no further effect on rates of protein synthesis. Perfusion of arrested hearts for as little as 2 minutes at 120 mm Hg resulted in a rapid and sustained increase in cAMP content, cAMP-dependent protein kinase activity, and rate of protein synthesis after 60–120 minutes of additional perfusion at 60 mm Hg. Exposure of arrested hearts to 0.2 mM methacholine, a muscarinic-cholinergic agonist, for 5 minutes before elevation of perfusion pressure blocked the pressure-induced increases in cAMP content, cAMP-dependent protein kinase activity, and rates of protein synthesis. When hearts were removed from pertussis toxin–treated animals, methacholine did not block the effects of forskolin on these same three parameters. These studies indicated that elevation of tissue cAMP by hormone binding, direct activation of adenylate cyclase, or inhibition of phosphodiesterase resulted in acceleration of protein synthesis. Furthermore, the effects of increased aortic pressure to accelerate synthesis appeared to involve a cAMP-dependent mechanism that was independent of changes in contractile activity but could be blocked with a muscarinic-cholinergic agonist. Acceleration of protein synthesis by insulin was not associated with an elevation of cAMP. (Circulation Research 1989;65:647–656)
perfusion phenylalanine when the perfusate concentration was increased to 0.4 mM. As a result, increased incorporation of radioactivity into protein in response to agents that increase cAMP, as found in the present experiments, could not be due to higher specific radioactivity of the immediate precursor pool.

Another important determinant of heart size is the pressure load placed upon the heart. Kira et al demonstrated that elevation of aortic pressure in rat hearts perfused as Langendorff preparations in the absence of insulin resulted in restoration of protein synthesis rates in beating, drained, or arrested drained hearts to values observed in vivo or in vitro in the presence of insulin. When insulin was present, elevation of aortic pressure increased the rate of protein synthesis by 19% during the second hour of perfusion. This effect of elevated aortic pressure in insulin-treated hearts could not be confirmed in later experiments from this laboratory that involved a much larger sample size (n=5 vs. n=20). Stretch of the ventricular wall secondary to elevated perfusion pressure was concluded to be the mechanical parameter that linked increased aortic pressure to faster protein synthesis. This conclusion was supported by subsequent experiments with arrested hearts that contained a ventricular drain that allowed for alteration of intraventricular pressure independent of aortic perfusion pressure. Elevation of intraventricular pressure from 0 to 25 mm Hg in hearts perfused at an aortic pressure of 60 mm Hg accelerated protein synthesis to rates observed in vivo or in vitro in hearts perfused at 120 mm Hg aortic pressure. Elevation of aortic pressure to 120 mm Hg or intraventricular pressure to 25 mm Hg restored the balance between aortic pressure and intraventricular pressure.

The hypotheses that were tested in the present experiments were 1) that elevation of cAMP content whether induced by hormone binding, adenylate cyclase activation, or cAMP phosphodiesterase inhibition would accelerate protein synthesis in hearts that were supplied glucose in the absence of insulin, 2) that stretch of the ventricular wall by acute elevation of aortic pressure in Langendorff preparations would result in restoration of in vivo rates of protein synthesis through a cAMP-dependent mechanism, and 3) that elevation of aortic pressure in insulin-treated hearts would elevate cAMP content and cAMP-dependent protein kinase (cAMP-PK) activity even though stretch did not increase the rate of protein synthesis. Rates of protein synthesis, cAMP content, and the activity of the cAMP-PK were determined in beating and tetrodotoxin-arrested hearts that were exposed to glucagon, forskolin, or 1-methyl-3-isobutylxanthine (IBMX) or to acute elevation of aortic pressure from 60 mm Hg to 120 mm Hg in control and insulin-treated hearts. Arrested hearts were exposed to methacholine, a muscarinic-cholinergic agonist, during the period of elevated aortic pressure to determine whether inhibition of adenylate cyclase by this mechanism would uncouple the effect of elevated aortic pressure to accelerate protein synthesis.

Materials and Methods

Animals

Male rats (Sprague-Dawley, Charles River Breeding Laboratory, Wilmington, Massachusetts) weighing approximately 300 g were either provided food and water ad libitum or fasted overnight, as indicated, and maintained on a light-dark cycle (LD 12:12) until killed. Animals used for the experiments that examined the ability of pertussis toxin to prevent muscarinic-cholinergic inhibition of cAMP metabolism and protein synthesis were administered pertussis toxin (1 μg/100 g body wt i.p.) in saline; Sigma, St. Louis, Missouri) daily for 3 days before death. All animals were injected with heparin (30 units/100 g body wt i.p.) 30 minutes before being anesthetized with pentobarbital sodium (8 mg/100 g body wt i.p.). Once the rats were anesthetized, hearts were excised from the animals.

Heart Perfusion

After excision, hearts were quickly placed in cold 0.9% NaCl (2° C). Hearts were perfused as Langendorff preparations, as described previously.
ing the first 15 minutes of perfusion, Krebs-Henseleit bicarbonate buffer containing plasma levels of 19 amino acids,18 0.4 mM phenylalanine, and 15 mM glucose passed through the heart a single time. Perfusion pressure during this period was 40 mm Hg, and perfusate was maintained at 37°C and gassed with 95% O₂, 5% CO₂. After preliminary perfusion, 15 ml of the same buffer containing 0.1% bovine serum albumin (fraction V, diagnostic grade, American Research Products, Solon, Ohio) was recirculated through the heart for the times indicated. Glucagon, forskolin, IBMX, or methacholine were added to the perfusate as listed in Tables 1, 2, and 6. In experiments involving elevated aortic pressure, hearts were exposed to an aortic pressure of 120 mm Hg for the time period indicated in Tables 3-6 and Figures 1 and 2. In experiments involving arrested hearts, tetrodotoxin (9 μg/ml) was added to the buffer recirculated through the heart. Hearts were exposed to tetrodotoxin at an aortic pressure of 40 mm Hg until arrest occurred; after arrest, aortic pressure was elevated in the experimental groups. At the conclusion of perfusion, hearts were rapidly frozen between blocks of aluminum cooled to the temperature of liquid nitrogen.

**Estimation of Protein Synthesis Rates**

Rates of protein synthesis were estimated in the final 30–120 minutes of perfusion by the incorporation of [14C]phenylalanine (0.1 μCi/ml) into total heart protein. Frozen ventricles were weighed and powdered using porcelain mortars and pestles cooled to the temperature of liquid nitrogen. Dry-to-wet weight ratios were determined by drying approximately 100 mg powdered tissue at 105°C for 18 hours. Percollaric acid extracts were prepared, and ventricular proteins were purified as described previously.19,20 Small aliquots of purified protein were weighed into scintillation vials, solubilized in 1 ml NCS tissue solubilizer (Amersham, Arlington Heights, Illinois), and radioactivity was determined by liquid scintillation spectroscopy. Because the concentration of phenylalanine (0.4 mM) used in these experiments was shown to result in the equilibration of the specific radioactivities of perfusate and tRNA-bound phenylalanine, the specific radioactivity of phenylalanine in the perfusate was used to calculate rates of protein synthesis.7 Rates of protein synthesis were expressed as nanomoles of phenylalanine incorporated into protein per gram dry heart per hour. The protein content of dry heart was 750 mg/g.7 Tissue contents of ATP, ADP, AMP, creatine phosphate, and creatine were determined enzymatically in perchloric acid extracts, as described previously.19

**Determination of cAMP Content in Perfused Heart**

The concentration of cAMP was determined by radioimmunoassay, which was performed with the materials and methods acquired from DuPont (New England Nuclear and Rainin Divisions, North Billerica, Massachusetts). Briefly, acid extracts were prepared from 120 mg frozen tissue by the addition of 1.0 ml cold 6% trichloroacetic acid and subsequent homogenization with a loose-fitting Teflon pestle. Homogenates were centrifuged for 30 minutes at 1,500g at 4°C, and supernatants were decanted and stored on ice. Supernatants were extracted four times in 5 vol water-saturated ether. The supernatants were frozen in liquid nitrogen and lyophilized overnight. Lyophilized samples were resuspended in 3 ml acetate buffer (supplied in the Rainin radioimmunoassay kit) and a ninefold dilution prepared in the same buffer to bring cAMP concentrations into the range of the standard curve. Radioimmunoaassay of cyclic nucleotide content was performed using 100 μl extract/assay, as described in the methods supplied by the manufacturer. Content of cAMP was expressed as picomoles per milligram protein. The pellet from the initial centrifugation was dissolved in 0.1N NaOH, and protein was estimated by the method of Lowry et al.21

**cAMP-Dependent Protein Kinase Activity Ratio**

The relative activity of cAMP-PK in tissue from perfused hearts was determined as described by Roskoski22 with minor modifications. Briefly, 110 mg powdered heart tissue was homogenized in 3.0 ml buffer containing (mM) potassium phosphate 10 (pH 7.0), EDTA 10, IBMX 0.5, and sodium chloride 0.25 M with a polytron (5–10 seconds at a setting of 5). Homogenates were centrifuged at 27,000g for 20 minutes at 4°C. The supernatant (10 μl) was added to 45 μl buffer (50 mM MOPS [pH 7.0], 10 mM MgCl₂, 0.25 mg/ml bovine serum albumin, 100 μM ATP, and 0.1 mg Kemptide; Sigma). Reactions were performed in the presence and absence of 7 μM cAMP, with approximately 0.1 μCi of [32P]α-ATP in all reactions. After incubation for 10 minutes at 30°C, 50 μl of reaction mixtures were absorbed into phosphocellulose filters (1 cm×2 cm). The filters were air dried and washed three times for 2 minutes each in 75 mM phosphoric acid. Filters were blotted and dried at 100°C for 5 minutes. Filter-bound radioactivity was assessed in 10 ml ACS scintillation fluid (Amersham), and the data were expressed as the ratio of radioactivity incorporated into Kemptide in the absence and presence of cAMP.

**Statistical Analysis**

For comparison among sample means, Student's t test and two-way analysis of variance and Student-Newman-Keuls multiple-range test were used. Values of p<0.05 were considered to be statistically significant.

**Results**

**Effect of Elevation of cAMP Content on Protein Synthesis**

Agents that elevate cAMP content increase the rate and force of cardiac contractions.1 In the
TABLE 1. Effects of Glucagon and Tetrodotoxin on Cyclic AMP Content and Protein Synthesis

<table>
<thead>
<tr>
<th>Glucagon (1 × 10^{-6} M)</th>
<th>Protein synthesis (nmol phenylalanine/g dry heart/hr)</th>
<th>cAMP (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>1,100±60</td>
<td>4.67±0.40</td>
</tr>
<tr>
<td>+</td>
<td>1351±16*</td>
<td>7.60±0.53*</td>
</tr>
<tr>
<td>-</td>
<td>1,133±50</td>
<td>4.13±0.53</td>
</tr>
<tr>
<td>+</td>
<td>1,412±35**</td>
<td>8.00±0.27**</td>
</tr>
</tbody>
</table>

Values represent the mean±SEM of four hearts.

Hears were removed from overnight-fasted rats and perfused as Langendorff preparations at 40 mm Hg aortic pressure with buffer containing 15 mM glucose, 0.1% bovine serum albumin, 0.1 mM EDTA, 50 μM bathocuproine disulfonate, and normal plasma concentrations of amino acids except for 0.4 mM phenylalanine. Aortic pressure was then adjusted to 60 mm Hg, and the same buffer containing 0.1 μCi [14C]phenylalanine/ml was recirculated for 2 hours. Glucagon and tetrodotoxin were added as indicated for the entire 2-hour period. Glucagon had no effects on the contents of ATP, ADP, AMP, total adenine nucleotides, creatine-P, creatine, or the creatine-P/creatine ratio in either control or tetrodotoxin-arrested hearts when measured at 130 minutes of perfusion (data not shown).

*p<0.05 vs. no addition.

**p<0.05 vs. tetrodotoxin.

isolated perfused rat heart exposed to epinephrine or isoproterenol, the increase in contractile activity was accompanied by a fall in ATP and total adenine nucleotide content. These changes interfered with an assessment of the effects of β-adrenergic agonists on protein synthesis. When cAMP was elevated approximately twofold by exposure of beating or arrested hearts in 1 × 10^{-6} glucagon, high energy phosphates were maintained (Table 1). Protein synthesis increased 22–25% over 2 hours of perfusion in glucagon-treated hearts. The stimulatory effect of glucagon was of a similar magnitude in beating and tetrodotoxin-arrested hearts and indicated that the acceleration of protein synthesis by glucagon was not dependent on increased mechanical activity. A 2-minute exposure of arrested hearts to forskolin, an activator of adenylate cyclase, resulted in elevated cAMP content and cAMP-PK activity 1 hour later (Table 2). Protein synthesis was increased 19% in forskolin-treated hearts when measured between 70 and 100 minutes of perfusion. When forskolin (1 × 10^{-6} M) exposure was continued for 90 minutes in arrested hearts, cAMP content was 8.69±0.16 pmol/mg protein (n=3), and the cAMP-PK activity ratio was 0.45±0.04 (n=3). Despite the further elevations of these parameters, the rate of protein synthesis when measured between 70 and 100 minutes was 797±23 nmol phenylalanine/g/hr (n=3), a value that was not significantly higher than that in controls.

TABLE 2. Effects of Exposure to Forskolin and 1-Methyl-3-Isobutylxanthine on Protein Synthesis, Cyclic AMP Content, and Cyclic AMP-Dependent Protein Kinase Activity in Arrested Rat Hearts

<table>
<thead>
<tr>
<th>Treatment and time of measurement (min)</th>
<th>Protein synthesis (nmol phenylalanine/g dry heart/hr)</th>
<th>cAMP (pmol/mg protein)</th>
<th>(PK-cAMP)/(PK+cAMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70–100</td>
<td>682±10 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>. . .</td>
<td>5.0±0.20 (5)</td>
<td>0.225±0.009 (5)</td>
</tr>
<tr>
<td>100</td>
<td>. . .</td>
<td>4.74±0.13 (9)</td>
<td>0.233±0.012 (9)</td>
</tr>
<tr>
<td>Forskolin (10^{-6} M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70–100</td>
<td>809±26 (5)*</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>. . .</td>
<td>7.1±0.6 (5)*</td>
<td>0.313±0.011 (5)*</td>
</tr>
<tr>
<td>100</td>
<td>. . .</td>
<td>5.01±0.20 (5)</td>
<td>0.334±0.014 (5)*</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70–130</td>
<td>673±33 (8)</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>. . .</td>
<td>6.15±0.45 (4)</td>
<td>ND</td>
</tr>
<tr>
<td>IBMX (10^{-5} M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70–130</td>
<td>815±13 (7)*</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>. . .</td>
<td>7.96±0.27 (4)*</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values represent mean±SEM of the number of observations shown in parentheses. cAMP, cyclic AMP; PK, protein kinase activity; IBMX, 1-methyl-3-isobutylxanthine; ND, not determined.

Hears were removed from rats fed ad libitum and perfused as Langendorff preparations at 40 mm Hg aortic pressure with the buffer described in Table 1. After 10 minutes of preliminary perfusion, hearts were arrested by the addition of tetrodotoxin (final concentration, 9 μg/ml). In experiment 1, buffer containing 10^{-4} M forskolin was recirculated through the heart for 2 minutes followed by a wash through perfusion with forskolin-free buffer and recirculation of forskolin-free buffer containing tetrodotoxin for 90 minutes. Aortic pressure during the periods when buffer was recirculated through the heart was 60 mm Hg. In experiment 2, arrested hearts were exposed to IBMX for the final 120 minutes of perfusion. Protein synthesis was determined by the incorporation of [14C]phenylalanine into protein during the final 30 minutes of perfusion in experiments using forskolin and during the final hour in IBMX experiments.

*p<0.05 vs. no addition.
Effect of Perfusion at Elevated Aortic Pressure on cAMP Content, cAMP-PK Activity, and Protein Synthesis Rates in Beating Hearts

Elevation of aortic pressure from 60 mm Hg to 120 mm Hg for as little as 15 seconds in beating rat hearts perfused in the absence of insulin resulted in a significant increase in the rates of protein synthesis when measured in the second hour of perfusion (Figure 1). In addition, cAMP content and cAMP-PK activity (Figure 2) were elevated after the increase in aortic pressure. Within 15 seconds of the initiation of perfusion at 120 mm Hg, cAMP content was increased but declined within 2 minutes to values not significantly different from values that were observed in hearts perfused with an aortic pressure of 60 mm Hg (Figure 2). The pressure-induced spike in cAMP content was accompanied by a rapid and sustained increase in cAMP-PK activity for the duration of the 2-hour perfusion (Figure 2). After 60 and 120 minutes of continued perfusion at 120 mm Hg, cAMP levels were again significantly increased as compared with those in hearts perfused at 60 mm Hg. These findings indicated that the aortic pressure--induced acceleration of protein synthesis also could be mediated by cAMP.

Earlier experiments from this laboratory showed that insulin accelerated protein synthesis in the perfused rat heart by preventing development of a block in peptide chain initiation. In the absence of the hormone, rates of protein synthesis declined during the second hour of perfusion at an aortic pressure of 60 mm Hg. The slower rate of protein synthesis was accompanied by greater tissue content of ribosomal subunits; this occurrence indicated that peptide chain initiation was restrained. Stretch of the ventricular wall by elevation of aortic pressure or intraventricular pressure also accelerated protein synthesis and decreased tissue content of ribosomal subunits as compared with control hearts perfused at an aortic pressure of 60 mm Hg. As shown in Table 3, the effects of insulin and elevated aortic pressure on protein synthesis were not additive.

The most likely explanation for the lack of an additive effect was that either elevated aortic pressure or addition of insulin prevented development of the peptide chain initiation block and sustained an optimal rate of protein synthesis.

Although both insulin and elevated aortic pressure prevented development of a block in peptide initiation, insulin did not increase content of cAMP after 1 hour of exposure to the hormone (Table 3). However, elevation of aortic pressure increased cAMP content in hearts perfused in the presence of the hormone. Elevation of aortic pressure increased ribosome formation during the first hour of perfusion in insulin-treated hearts and could be a consequence of elevated cAMP. During the first hour of perfusion, insulin increased rates of total protein synthesis and ribosome formation to a similar extent. These findings suggest that insulin and stretch of the ventricular wall affect total protein synthesis and ribosome formation through different intracellular signaling mechanisms.

Effect of a Brief Exposure to Elevated Aortic Pressure in Tetrodotoxin-Arrested Hearts on cAMP, cAMP-PK, and Rates of Protein Synthesis

When arrested hearts were exposed to elevated aortic pressure (120 mm Hg) for 2 minutes, the rate of protein synthesis was increased when measured in the final 30 minutes of a 2-hour perfusion in the absence of insulin (Table 4). Additionally, cAMP content and cAMP-PK activity were increased at
70, 100, and 130 minutes of continued perfusion at 60 mm Hg. In other experiments, cAMP content and cAMP-PK activity were measured at intervals from 15 seconds to 8 minutes after elevation of aortic pressure in arrested hearts (Table 5). Content of cAMP increased significantly 15 seconds after elevation of aortic pressure to 120 mm Hg, but cAMP content was not higher after 2, 4, and 8 minutes. After 60 minutes of exposure to elevated aortic pressure, cAMP content again was increased. The activity of cAMP-PK was increased after 2, 4, 8, and 60 minutes of exposure to an aortic pressure of 120 mm Hg. These findings are similar to those reported for beating hearts (Figure 2) and indicate that the effect of increased aortic pressure on the increase in cAMP content was not dependent on contractile activity of the heart.

Effects of Methacholine on Rates of Protein Synthesis, cAMP Content, and cAMP-PK Activity in Arrested Hearts Exposed for 2 Minutes to Elevated Aortic Perfusion Pressure

As noted above, brief exposure of arrested rat hearts to elevated aortic perfusion pressure in the absence of added insulin resulted in a significantly increased rate of protein synthesis, as well as a rapid and sustained activation of cAMP-PK. Exposure of arrested hearts to 0.2 mM methacholine for 5 minutes before elevation of perfusion pressure blocked the pressure-induced increases in cAMP content, cAMP-PK activity, and rates of protein synthesis that occurred in arrested control hearts after brief exposure to 120 mm Hg aortic pressure (Table 6).

When hearts were removed from animals administered pertussis toxin for 3 days before death, methacholine did not block the effect of elevated aortic pressure on cAMP content, activity of cAMP-PK, or the rate of protein synthesis (Table 6). Pertussis toxin treatment did not block the effect of increased aortic pressure on cAMP metabolism or protein synthesis in arrested control hearts.

In other experiments, $10^{-6}$ M forskolin was added during the final 120 minutes of perfusion to directly activate adenylate cyclase (Table 6). At the end of the experiment, cAMP content and the activity ratio of cAMP-PK were greater than observed in arrested control hearts. The rate of protein synthesis in these hearts was elevated when measured between 105 and 135 minutes of perfusion. Addition of 0.2 mM methacholine to forskolin-treated hearts reduced cAMP content and cAMP-PK ratio, but the values were still higher than in arrested control hearts. Rates of protein synthesis were still elevated in hearts exposed to forskolin and methacholine. Elevation of perfusion pressure to 120 mm Hg had
**TABLE 3. Effect of Insulin and Elevated Aortic Pressure on Cyclic AMP Content and Rates of Protein Synthesis in Beating Hearts**

<table>
<thead>
<tr>
<th>Parameter and time of measurement</th>
<th>Insulin (2.8 nM)</th>
<th>Aortic pressure</th>
<th>Protein synthesis (nmol phenylalanine/g dry heart/hr)</th>
<th>cAMP content at 75 min (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 mm Hg</td>
<td>120 mm Hg</td>
<td>10–70 min</td>
<td>0</td>
<td>5.27±0.27 (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>848±81 (16)*</td>
<td>8979±94 (17)*</td>
</tr>
<tr>
<td>70–130 min</td>
<td></td>
<td>0</td>
<td>447±24 (12)</td>
<td>602±27 (12)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>810±69 (20)*</td>
<td>799±45 (22)*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Protein synthesis values are taken from an earlier study in our laboratory by Chua et al.13 cAMP, cyclic AMP.

Hearts were perfused initially for 10 (protein synthesis experiments) or 15 minutes (cAMP experiments) as Langendorff preparations with an aortic pressure of 40 mm Hg with buffer that contained 15 mM glucose, 0.4 mM phenylalanine, and plasma levels of 19 amino acids. This buffer was discarded after a single pass through hearts. Perfusion was continued by recirculating 30 ml same buffer containing 0.2% bovine serum albumin for the periods indicated. When protein synthesis was measured, [U-14C]phenylalanine (0.1 μCi/ml) was added to the buffer. When second hour rates were measured, radioisotope was added after 70 minutes. Perfusion pressure was adjusted to 60 or 120 mm Hg at beginning of recirculation period.

*p<0.05 vs. no added insulin, same aortic pressure, and same period of perfusion.

†p<0.05 vs. hearts perfused at 60 mm Hg for same test period.

No significant effect on cAMP metabolism or protein synthesis in hearts treated with forskolin and methacholine. These experiments indicated that the inhibitory effect of methacholine on cAMP metabolism and protein synthesis could be prevented by direct activation of adenylate cyclase.

**TABLE 4. Effect of a 2-Minute Exposure to Elevated Aortic Pressure on Cyclic AMP Content, Cyclic AMP–Dependent Protein Kinase Activity, and Protein Synthesis in Arrested Rat Hearts**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period of measurement</th>
<th>Aortic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60 mm Hg (10–130 min)</td>
</tr>
<tr>
<td>Protein synthesis (nmol phenylalanine/g/hr)</td>
<td>100–130</td>
<td>614±15 (5)</td>
</tr>
<tr>
<td>cAMP content (pmol/mg protein)</td>
<td>70</td>
<td>5.42±0.15 (10)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.85±0.07 (8)</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>4.93±0.15 (5)</td>
</tr>
<tr>
<td>(PK–cAMP)/ (PK+cAMP)</td>
<td>70</td>
<td>0.215±0.008 (10)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.233±0.012 (9)</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>0.225±0.005 (5)</td>
</tr>
</tbody>
</table>

Values represent mean±SEM of the number of hearts shown in parentheses. cAMP, cyclic AMP; PK, protein kinase activity.

Hearts were perfused as described in Table 2. After arrest of hearts with tetrodotoxin, aortic pressure was adjusted to either 60 or 120 mm Hg for the duration of the experiment. Hearts were frozen and cAMP content and PK were measured, as described in "Materials and Methods." *p<0.05 vs. 60 mm Hg, 0.25–8 minutes.

†p<0.05 vs. hearts perfused at 60 mm Hg, 60 minutes.

**Discussion**

The major findings of these experiments were 1) that elevation of cAMP content by hormone receptor binding (glucagon), activation of adenylate cyclase (forskolin), or inhibition of cAMP phosphodiesterase (IBMX) accelerated protein synthesis in isolated perfused rat hearts, 2) that increased aortic pressure elevated cAMP content and cAMP-PK activity both during the period of pressure elevation and after it, 3) that only a brief period of exposure to higher aortic pressure was sufficient to increase protein synthesis rate, cAMP content, and cAMP-PK activity in the second hour of perfusion of either beating or arrested hearts, and...
vented or reversed the block of peptide-chain initiation in protein synthesis was observed. In the studies content in insulin-treated hearts although no effect was observed in hearts exposed to 2 minutes of perfusion in the absence of insulin, a block in peptide-chain initiation development of the peptide chain initiation block and its reversal by insulin or by factors that increase cAMP concentrations is present. This possibility deserves further exploration in future experiments. When both the efficiency and capacity of protein synthesis are considered, the ability of increased aortic pressure to accelerate ribosome formation during the first hour of perfusion in insulin-treated hearts indicates that stretch of the ventricular wall is a major factor leading to growth of the heart in response to pressure overload.

Stretch modified protein metabolism in both skeletal and cardiac muscle. Stretch of cardiac muscle in vitro increased protein synthesis rate in isolated papillary muscles. When hearts were arrested by either high perfusate potassium concentrations or addition of tetrodotoxin to the perfusate and perfused in the absence of insulin, elevation of aortic pressure accelerated rates of protein synthesis. These effects were suggested to be due to stretch of the ventricular wall resulting from engorgement of the vascular bed, the so-called erectile properties of the heart. Early attempts to demonstrate that stretch of the ventricular wall by elevation of intraventricular pressure would increase protein synthesis rate were not successful. Howver, recent work from this laboratory used a modified Langendorff preparation that allowed regulation of intraventricular pressure independent of aortic perfusion pressure in arrested hearts and demonstrated that elevated intraventricular pressure accelerated rates of protein synthesis in rat hearts perfused in the absence of insulin.

The identity of the biochemical signal that links myocardial stretch to maintenance of rates of peptide chain initiation and protein synthesis was sought in earlier studies. Increased concentrations of free intracellular calcium and faster rates of prostaglandin synthesis, as well as changes in
Creatine, and glucose-6 phosphate, were considered as potential signals linking stretch and an accelerated rate of protein synthesis in the heart, but a firm relation could not be established.

Increased intracellular content of cAMP has been thought to play a role in regulating cardiac hypertrophy resulting from a variety of physiological and pharmacological stimuli. Schreiber et al observed an increase in adenylyl cyclase activity within 10 minutes of initiation of acute hemodynamic overload in the perfused guinea pig heart. However, the content of cAMP did not change in overloaded ventricles. Singh reported that mechanical stretch overload led Zimmer and Peffer to conclude that tissue concentrations of high energy phosphates, but a firm relation could not be established.

Increased aortic pressure and subsequent stretch of the ventricular wall in vitro elicited a rapid rise in cAMP content. Similarly, recent studies of known stimuli for cardiac hypertrophy including isoproterenol, triiodothyronine, and pressure overload have demonstrated a temporal relation between accelerated rates of protein synthesis and increased myocardial cAMP content in vivo. However, failure of β-adrenergic blockade to attenuate increased rates of protein synthesis as a result of pressure overload led Zimmer and Peffer to conclude that the adenylate cyclase–cAMP pathway did not play a role in pressure-induced cardiac hypertrophy. However, cAMP content was not measured in pressure-overloaded hearts after β-adrenergic blockade. The authors assumed that activation of the adenylate cyclase–cAMP system via the β-receptor was the only stimulatory pathway by which this system might play a role in regulating protein synthesis during pressure-induced cardiac hypertrophy. The present work clearly demonstrated that the adenylate cyclase–cAMP system might play a role in regulating protein synthesis through pharmacological stimuli. Schreibcr et al observed a rise in cAMP content induced by elevated aortic pressure in arrested hearts would be prevented by methacholine and that this effect would prevent the acceleration of protein synthesis rates in these hearts. The results support this hypothesis and further substantiate a role for the stretch-induced activation of cAMP-PK, subsequent to an increase in cAMP content, in the regulation of protein synthesis by aortic perfusion pressure.

A second set of experiments was designed to determine whether the effect of methacholine on protein synthesis was exerted through modification of cAMP metabolism. If methacholine acted in this way, pretreatment of animals with pertussis toxin, which enzymatically ADP-ribosylates G, and effectively prevents its interaction with adenylate cyclase, would block the ability of methacholine to inhibit activation of cAMP-PK and acceleration of protein synthesis induced by stretch. In these experiments, animals were administered pertussis toxin at doses and durations shown to be necessary to ADP-ribosylate the G, protein. Subsequent exposure of the hearts from these animals to methacholine did not block the pressure-induced activation of cAMP-PK and acceleration of protein synthesis. Direct stimulation of adenylate cyclase by inclusion of forskolin in the perfusate overcame the methacholine block of pressure-induced acceleration of protein synthesis.

Mechanical effects on other aspects of heart function such as contractile activity may not be cAMP-dependent. For example, elevation of aortic pressure increased Ca2+ entry; this change rapidly reversed when aortic pressure was lowered from 120 to 60 mm Hg. Increased calcium sensitivity at longer muscle length has been ascribed to greater Ca2+-induced Ca2+ release or to higher sensitivity of cardiac troponin C as the sarcomere is stretched. Alkon and Rasmussen have hypothesized that a transient increase in Ca2+ influx can lead to a persistent enhancement of cell responsiveness through calmodulin activation of adenylate cyclase or translocation of protein kinase C to the plasma membrane. Richter et al have demonstrated that electrical stimulation of the sciatic nerve leads to translocation of protein kinase C from the muscle cytosol to particulate fraction. Further work is needed to identify the intracellular signal that links stretch to peptide chain initiation and ribosome formation. A single signal probably does not mediate all stretch-dependent events.

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32. Key Words • cyclic AMP-dependent protein kinase • aortic pressure • protein synthesis • insulin • muscarinic agonist • pertussis toxin • stretch • forskolin • cyclic AMP • glucagon


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Circ Res. 1989;65:647-656
doi: 10.1161/01.RES.65.3.647

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