Effects of Basic Nuclear Proteins on Cardiac Muscle Function

By Arnold Schwartz, Ph.D., Arline H. Laseter, B.Sc., and Gary G. Ferguson, B.Sc., M.Sc.

Recent data obtained in this laboratory suggest that basic nuclear proteins may exert some controlling extranuclear influence on cellular activity. For example, very low concentrations of purified histones obtained from thymus, liver, kidney and brain, as well as semipurified histones derived from heart and skeletal muscle, inhibit a membrane-ATPase system associated with active cation transport, stimulate a Mg++-dependent mitochondrial ATPase and inhibit ATPases and syneresis of actomyosin and myofibrillar suspensions. These effects were particularly evident when histones prepared from cardiac muscle nuclei were reacted with the subcellular components from the same muscle.

The role of the nucleus or nuclear proteins in cardiac muscle function has received very little attention. In fact, it is only recently that some studies have been directed towards RNA and DNA metabolism in cardiac hypertrophy. Other than a brief reference to the presence of histones or basic nuclear proteins in cardiac muscle, no data on heart histones have been reported. The basic nuclear proteins are thought to be associated with gene expression. According to this theory a histone-DNA binding results in "selective" inhibition of DNA-directed RNA synthesis. If this hypothesis is correct, the biochemistry of heart muscle nuclear histone should receive serious consideration in cardiac muscle hypertrophy. Furthermore, extranuclear functions for the histones have not been considered in spite of evidence which implicates them in various membrane activities.

In view of the marked effects of histones on a variety of energy utilizing and liberating systems, both in heart and other organs, as cited above, the present paper describes a study of the effects of histones on actomyosin of cardiac and skeletal muscle. Some observations on in situ effects of histones on heart muscle contractions are also presented.

Methods

HISTONE PREPARATION FROM HEART AND SKELETAL MUSCLE

Freshly obtained calf hearts and hearts and skeletal muscle from albino rats were used for isolation of histone fractions. All manipulative procedures were carried out in the cold room. The muscles were carefully trimmed of all fat; the atria and great vessels were removed from the hearts; and the ventricles were cut into small pieces and blended in a Waring macro blender at top speed for one minute with 0.14 M NaCl containing 0.01 M sodium citrate at pH 7 (5 ml/g tissue). Diisopropyl fluorophosphate was added (0.1 mM) and the suspension was passed through cheesecloth. Histones were isolated according to the procedure of Hnilica and Busch slightly modified as indicated in the flow diagram (table 1).

Other histones (from thymus, liver, kidney, brain, and skeletal muscle) were prepared by the procedure of Hnilica and Busch or were generously furnished by Drs. H. Busch, W. C. Starbuck, and C. W. Taylor of this department.

ISOLATION OF A "CRUDE HISTONE FRACTION" FROM SKELETAL OR CARDIAC MUSCLE

Tissue extracts which possessed all of the properties of the "purified histones" could be prepared rapidly by suspending thrice-washed,
TABLE 1
Flow Diagram for Preparation of "Cardiac Histones"

Citrate-NaCl suspension

\[ \downarrow \]

Centrifuged (1000 \times g for 50 min)

Supernate discarded

Precipitate

Washed by blending in medium and centrifuging until supernate is clear and precipitate is white.

Precipitate

Supernate discarded

Washed with 4:1 95% ethanol: H\(_2\)O and centrifuged (1000 \times g for 20 min)

Precipitate

Supernate discarded

Extracted with 4:1 (95% ethanol: 1.25 N HCl)

Precipitate

Combined supernates

Extracted with 0.25 N HCl

Combined supernates

Precipitate discarded

Dialyzed vs 6 vol 100% ethanol and centrifuged (1000 \times g for 20 min)

Supernate

Precipitate

Made 20% with TCA* and centrifuged (1000 \times g for 20 min)

Precipitate washed twice with acid-acetone and twice with ether

Crude

F\(_1\) + F\(_2b\)

Supernate discarded

Precipitate

Washed twice with acetone and twice with ether

Crude

F\(_2a\)

Supernate discarded

Add 2 to 3 vol acetone

Washed twice with ether

Crude

F\(_3\)

*Trichloroacetic acid.
HISTONES AND CARDIAC FUNCTION

600 X g centrifuged pellets of 10% tissue homogenates made in 0.32 M sucrose + 0.5 mM EDTA, in 2 ml of 0.25 N HCl per g original tissue, with the aid of a small glass homogenizer and Teflon pestle. The suspension was kept at 0°C with occasional stirring for two to four hours, and then centrifuged at 600 X g for 15 minutes. The supernatant fluid was neutralized with 1 M Tris and stored at 5°C.

PREPARATION OF ACTOMYOSIN

The procedure described recently by Inchiosa was found to be most suitable for fresh preparations of actomyosin from cardiac muscle (guinea pig and rabbit). For the glycerinated actomyosin preparations, the procedures outlined by Endo and by Ebashi were used.

PREPARATION OF MYOFIBRILS

A slight modification of the procedures of Stam and Honig and Gergely et al. was employed as follows: Guinea pig and rabbit hearts and skeletal muscle were removed, rinsed, minced with scissors (3 to 4 mm pieces), and made up to five times the volume with 65 mM succinate buffer, pH 7.5. The mixture was homogenized in a Waring blender at maximum speed for two minutes and centrifuged at 600 X g for three minutes. The supernatant fluid was then centrifuged at 600 X g for 10 minutes and the upper portion of the pellet, which was relatively free of connective tissue, was removed and suspended in five volumes of succinate buffer with the aid of a hand-operated glass homogenizer fitted with a Teflon pestle. The suspension was centrifuged at 600 X g for 10 minutes. The upper portion of the pellet was again removed, resuspended and recentrifuged at 600 X g for 15 minutes. The resulting pellet was washed two or three times with buffer and suspended in several volumes of 50% glycerol-succinate buffer and stored in polyethylene containers at -20°C.

ASSAY OF ACTOMYOSIN ATPASE ACTIVITY

The procedure outlined by Maruyama and Ishikawa was used. Essentially, this consists of incubating the actomyosin at 37°C in 7 mM Tris, pH 8, 1 mM ATP, 1 mM MgCl2 or 1 mM CaCl2 and KCl for adjustment to the desired ionic strength, in a total volume of 1 ml. After 10 minutes the reaction was stopped by the addition of 0.1 ml of 50% trichloroacetic acid (TCA).

MEASUREMENTS OF ACTOMYOSIN SUPERPRECIPITATION

1) Superprecipitation (Syneresis) Method

The actomyosin in protein concentrations of from 50 to 200 μg/ml was added to the assay mixture consisting of 5 mM Tris pH 7.0, 3 mM MgCl2, 3 mM ATP and 1 mM MgCl2 and ATP were added last. The increase in optical density at 600 μm is a measure of syneresis. The procedure was the same as for actomyosin except that the reaction mixture consisted of 30 mM Tris, pH 7.0, 3 mM MgCl2 and 3 mM ATP in a volume of 1.0 ml.

PREPARATION AND ASSAY OF MITOCHONDRIAL AND "MICROSOMAL" ATPASES

The methods used have been previously described. All ATPase activities reported in this paper are expressed as amounts of inorganic phosphate released.

WHOLE HEART EXPERIMENTS (IN SITU AND ISOLATED)

Histones were injected into guinea pigs, anesthetized by an intraperitoneal injection of 35 mg/kg of pentobarbital sodium, and changes in blood pressure and "contractility" of the left ventricle were measured by a previously described procedure. This involves recording the peak "isometric" pressure after aortic occlusion. In some experiments, contractile force was determined by direct myographic recordings from the left ventricle and atrium.

Hearts of rabbits were perfused with Ringer-Locke's solution in retrograde direction through the aorta (Langendorff perfusion). Histones were introduced via the perfusion medium and contractility of the left or right atria and ventricles recorded. Contractility of the in situ or isolated preparations was recorded by an E and M Physiograph (E and M Instruments, Houston, Texas) using a medium-force (no. B-409) transducer.

CHEMICALS

All chemicals and reagents were of "Reagent Grade" quality.
Results

EFFECTS OF HISTONES ON MYOFIBRILLAR AND ACTOMYOSIN ATPASE ACTIVITY

All of the histones examined affected the ATPases associated with either actomyosin or myofibrillar suspensions in a qualitatively similar manner, i.e., inhibited the activity in the presence of Mg ions and in very low ionic strength media (table 2 and fig. 1). However, as the ionic strength of the incubating medium was increased, presumably during the clearing phase, the inhibition appeared to be converted to stimulation (fig. 1). Thus a biphasic effect was generally observed. In the "inhibitory phase" as little

TABLE 2

<table>
<thead>
<tr>
<th>Histone</th>
<th>ATPase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ag/ml</td>
<td>μmoles of Pi/mg of protein/hr</td>
</tr>
<tr>
<td>None</td>
<td>10.7 ± 0.9†</td>
</tr>
<tr>
<td>10</td>
<td>9.8</td>
</tr>
<tr>
<td>50</td>
<td>6.9</td>
</tr>
<tr>
<td>100</td>
<td>5.5</td>
</tr>
<tr>
<td>200</td>
<td>5.2</td>
</tr>
<tr>
<td>300</td>
<td>4.8</td>
</tr>
</tbody>
</table>

*Glycerinated myofibrils were prepared from rat heart as described in the test and stored at −20°C for 131 days. Assay was done in a total vol of 1 ml which included: 30 mM Tris, pH 7.0, 3 mM MgCl₂ and 3 mM ATP. Protein = 0.46 mg. The histone was derived from heart muscle (Fᵢ Fᵢ b F₂ b F₃) and was preincubated at 37°C with the assay mixture for 10 min before the addition of the enzyme.

†Standard error of the mean.

FIGURE 1

Effect of histones on actomyosin ATPase from cardiac muscle at various ionic strengths. Histone was obtained from calf thymus (Fᵢ) and used in a concentration of 100 μg/ml in the assay procedure described in the text. Protein concentration of actomyosin = 0.22 mg/ml. Ionic strength was adjusted with KCl. Values represent a compilation of five experiments, and are statistically significant.

Effect of Mg²⁺ concentration on histone-induced inhibition of actomyosin ATPase from cardiac muscle. Conditions were the same as described in figure 1. Protein concentrations = 1.4 mg/ml. Ionic strength was less than 0.02.

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TABLE 3

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Ion</th>
<th>Histone</th>
<th>ATPase activity µmoles of Pi/mg of protein/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh liver mitochondria</td>
<td>Mg</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>+</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>+</td>
<td>19</td>
</tr>
<tr>
<td>Aged liver mitochondria</td>
<td>Mg</td>
<td>-</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>+</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>Heart &quot;microsomes&quot;</td>
<td>Mg</td>
<td>-</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>+</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>-</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>+</td>
<td>54</td>
</tr>
<tr>
<td>Cardiac actomyosin</td>
<td>Mg</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>+</td>
<td>21</td>
</tr>
<tr>
<td>Skeletal actomyosin</td>
<td>Mg</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>+</td>
<td>24</td>
</tr>
</tbody>
</table>

Tissue fractions were obtained from either guinea pig or rat by methods indicated in the text or as previously described. Histones were obtained from rat or beef heart or from calf thymus and were used in a concentration of 50 µg/ml, in assays described in the text or references.

Figure 2 shows that, in low ionic strength medium (less than 0.02), the histone (100 µg) markedly inhibited actomyosin ATPase in the presence of Mg ions, over a wide concentration range.

The effects of the basic proteins on Ca++-activated actomyosin ATPase differed considerably from those on the Mg++-activated actomyosin ATPase. At all ionic strengths studied, the histones markedly stimulated ATPase activity (fig. 3) in the presence of 1 mM CaCl₂.
It seemed possible that the "contractile" preparations might have been contaminated with mitochondrial or "microsomal" ATPases but this was unlikely because these enzymes reacted differently towards the histones than did the actomyosin or myofibrillar ATPases (table 3). The values obtained in the presence of Ca++ are noteworthy. Possible "nuclear ATPase" contamination has not been ruled out. The histones themselves exhibited no ATPase activity (unpublished observations). The inhibition of ATPase was overcome or "reversed" by increasing the Ca++ concentrations (fig. 4). It is interesting that the stimulation of ATPase, induced by the histone in the presence of Ca++, was also reversed by high concentrations of Mg++ (fig. 4).

**EFFECT OF HISTONES ON SUPERPRECIPITATION**

The results in table 4 represent a typical experiment in which various histones markedly reversed or prevented superprecipitation (syneresis) of actomyosin or myofibrillar suspensions. The rate of syneresis was also markedly depressed as measured by the turbidity procedure.15

**TABLE 4**

<table>
<thead>
<tr>
<th>Histone</th>
<th>Mg++ ATP</th>
<th>Precipitate height % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>F₁, b (thymus)</td>
<td>+</td>
<td>31</td>
</tr>
<tr>
<td>F₁ (thymus)</td>
<td>+</td>
<td>21</td>
</tr>
<tr>
<td>F₁, F₁, b (heart)</td>
<td>+</td>
<td>68</td>
</tr>
</tbody>
</table>

*Actomyosin was prepared from guinea pig heart and used in a concentration of 0.85 mg protein/ml in the assay described in the test. Histones were obtained from rat heart or calf thymus as indicated; 400 µg were preincubated with the actomyosin at 37°C for 10 minutes in the presence of Mg++. This is a typical experiment.

**EFFECT OF HISTONES ON CONTRACTILITY OF MUSCLE IN SITU**

Recordings taken from the left ventricle of an anesthetized, open-chest guinea pig are shown in figure 5. The histone, F₁, which is a lysine-rich histone obtained in this case from calf thymus, markedly depressed contractility. A dose of 1.5 mg/kg almost completely stopped the heart. Although the tracings are not shown, in five experiments, isolated perfused rabbit hearts responded...
Effect of histone (F₁) on guinea pig heart contractility. Left ventricular contractions were recorded from an anesthetized open-chest animal (wt = 480 g) as described in the text. Histone was injected at indicated points in the trace, into the right ventricle.

FIGURE 5

Discussion

Since the discovery of the basic nuclear proteins in 1870, much research has been done in order to determine the function or functions of these compounds. The hypothesis of the Stedmans in 1943 developed the concept that basic nuclear proteins, particularly the histones, could serve as gene-repressing agents presumably by combining with certain areas or sites on the DNA molecule. Subsequent studies have not given complete support to this concept and much doubt remains with regard to the function or functions of the histones.

In recent years evidence has been presented which suggests that histones may "shuttle" into and out of the nucleus and may perhaps be involved in coordination of nuclear and cytoplasmic activities during some phase of cellular development. McIlwain's studies have shown clearly that, under certain conditions, histones "diffuse" from the nucleus of brain cells to acidic sites in the endoplasmic reticulum, resulting in a loss of respiratory response of tissue slices to electrical stimulation. Displacing the histones with acidic substances, such as gangliosides, reverses this phenomenon. On the basis of this and other investigations, McIlwain has formulated a hypothesis of active cation transport in neural tissues involving acidic and basic proteins present in membranes. The importance of polyelectrolytes in membrane structure and function has been stressed by Katchalsky. Since macromolecules do pass through pores in the nuclear membrane, there is no reason to believe that histones cannot transverse membranes, particularly the nuclear membrane. On the contrary, there is some suggestion that histones may be synthesized in the cytoplasm and later migrate to the nucleus. The present observations indicate that very low concentrations of basic proteins derived from nuclei of a variety of cells markedly affect an extranuclear structure, the "contractile apparatus." The inhibitory effects of histones on actomyosin ATPase activity of heart or skeletal muscle appear to be accompanied by inhibition of syneresis of suspensions of actomyosin or myofibrils although this association may not be directly related. The interesting aspect is that the inhibition of ATPase can be converted to stimulation by increasing the ionic strength or changing the Mg⁺⁺ to Ca⁺⁺ ratio. It is thought that at high ionic strengths, actomyosin dissociates into actin and myosin, resulting in the so-called "clearing phase," although there has been some question concerning this. The finding that the histones appear similarly to the histones. While all of the histone fractions examined (F₁, F₃, F₂a; F₂b) acted in a qualitatively similar manner, the most active was F₂a, a slightly lysine-rich histone.
to inhibit ATPase when actomyosin is associated, and to stimulate when actomyosin is dissociated, points out the complexities of this reaction. The importance of ionic strength on effects of thiol inhibitors on actomyosin has recently been emphasized. The Ca**+-activated ATPase activity of actomyosin, surprisingly enough was markedly increased by histones at all ionic strengths and Ca**+ concentrations studied. It is not yet known whether this stimulation is accompanied by changes in superprecipitation. While the significance of these observations is not clear, in view of current evidence which supports the view that Ca**+ is necessary to activate the Mg**+-dependent ATPase of actomyosin, the histone-calcium "synergism" is interesting.

On the more organized level, inhibition of cardiac muscle contractility in situ by the histones, appears to be consistent with the observed data on the actomyosin systems, although other factors are undoubtedly involved in the intact systems.

A few reports have dealt with studies on the effects of basic proteins and nonprotein compounds on muscle. Of these the recent report by Wollenberger is of particular interest. He observed an excitatory effect of protamine or histones on the rhythmic beat of isolated cultured cardiac cells, but only in the presence of a serum protein factor, presumably sialic acid (ganglioside). In addition, a stimulatory effect of basic compounds on a brain microsomal ATPase system was noted. Wollenberger has suggested the interesting possibility that the basic polyelectrolytes may function as carriers of acidic substances in the serum into cardiac cells.

It is apparent, then, from these and other investigations that basic compounds can cause marked changes in muscle activity. Intracellular redistribution of proteins may be an important consideration in cardiac function. It must be emphasized that this is a working hypothesis, the value of which can only be assessed after suitable testing.

**Summary**

A method for the preparation of semi-purified basic proteins from cardiac muscle was presented. At low ionic strengths (below 0.05) and in the presence of Mg**+, these compounds, as well as purified histones from a variety of tissues, inhibited the Mg**+-ATPase activity of cardiac and skeletal muscle actomyosin or myofibrillar suspensions. Under similar conditions, a retardation of superprecipitation occurred. At high ionic strengths (above 0.06) the histones stimulated the Mg**+-ATPase. In the presence of Ca**+, and at all ionic strengths studied, the histones elicited a marked stimulation of actomyosin ATPase.

Administration of histones to anesthetized, open-chest guinea pigs, caused a significant depression of the force of contraction of the hearts. These data suggest a possible involvement of basic nuclear proteins in cardiac activity.

**Acknowledgment**

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**References**


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