Hypophyseal Influence on Function and Composition of Cardiac Muscle

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With the technical assistance of Ryo Kubota

Relationships between cardiac and hypophyseal functions have been recognized since early clinical observations of cardiac enlargement and failure in acromegaly. Experimental attention has been directed at hypophyseal influences on cardiac size, cardiac output, and the ability of the heart to hypertrophy. The relationship of cardiac changes to peripheral circulatory events following hypophysectomy has been investigated.

Although hypophyseal hormones are known to have widespread effects on growth and metabolism, the functional characteristics of the myocardium following modification of hypophyseal activity have been barely studied. Beznae found that the response of the intact heart to an increased input or resistance load was not abnormal in hypophysectomized rats. Direct studies on cardiac muscle are lacking. Such studies would seem to be highly desirable, since the investigations of Plattener and Reed, Gray and Young, and Bigland and Johring have raised questions regarding the functional significance of hypophyseal hormone effects on skeletal muscle. Moreover, since Scow reported effects of hypophyseal growth hormone on various protein fractions of skeletal muscle, it would be of interest to have comparable data, particularly with regard to contractile protein concentrations, for cardiac muscle.

It was the object of this study to determine the mechanical properties of isolated cardiac muscle of hypophysectomized and growth hormone-treated rats and to relate these to the concentration and nature of ventricular contractile protein under these conditions.

Methods

Two groups of experiments were performed:

GROUP 1: MECHANICAL PROPERTIES OF SURVIVING CARDIAC MUSCLE

Hypophysectomized female albino rats, of the Sprague-Dawley strain, and unoperated littermates were obtained from Hormone Assay Laboratories, Chicago. All animals were maintained for 21 to 24 days in a temperature- and humidity-controlled environment and given a special diet containing milk, meat, fresh fruit, and vegetables. At the end of this time, they were sacrificed by decapitation. Body weight, adrenal weight, and uterine weight were determined and the completeness of the hypophysectomy verified. The heart was removed, the atria and great vessels trimmed off, and the weight of the ventricles determined. A left ventricular muscle bundle was removed and placed in a muscle bath, according to the technique of Ullrick and Whitehorn. Resting and developed length-tension relationships were obtained. The muscle was then removed, weighed wet, and dried to constant weight. Complete data were obtained from 8 control and 10 hypophysectomized animals.

GROUP 2: COMPOSITION OF CARDIAC MUSCLE

Hypophysectomized and unoperated animals were obtained and maintained as described. Animals were placed in four groups as follows: (1) saline-injected controls; (2) unoperated controls injected intraperitoneally with 150 ng of growth hormone (GH) (NIH-GH-B-2, Bovine) per day in saline; (3) saline-injected hypophysectomized animals; and (4) hypophysectomized animals injected intraperitoneally with 50 ng of growth hormone (GH) per day. The amounts of hormone used were calculated to produce a normal rate of growth in the hypophysectomized animals and to ensure an increased growth rate in unoperated animals. After 21 to 24 days, the animals were sacrificed and body and organ weights determined as in group 1. The heart was removed, the ventricles trimmed, and a sample taken for determination of water content. Actomyosin was ex-
tracted from the remainder of the ventricles by the method of Benson, Hallaway, and Freier and redissolved in 0.6 M KCl. Total ventricular protein concentration and actomyosin concentration were determined by the micro-Kjeldahl technique. Relative viscosity before and after the addition of ATP was determined on samples of the redissolved protein with Ostwald viscometers at a temperature of 24 C. and a pH of 7.0. The protein concentration of the solution approximated 1 mg./ml. Relative viscosity ($\eta_{rel}$) was determined as the ratio of outflow time for the protein solution to that of 0.6 M KCl. The viscosity number ($Z_n$) was obtained from the expression:

$$Z_n = \frac{2.303 \log (\eta_{rel})}{C},$$

where $C$ is the concentration of the solution in Gm. per L. The “ATP sensitivity,” in per cent, is equal to:

$$\frac{Z_n - Z_n \text{ ATP}}{Z_n \text{ ATP}} \times 100,$$

where $Z_n \text{ ATP}$ is the viscosity number after the addition of ATP.

Data were obtained from a total of 49 animals. In 23 of these, length-tension relationships of a surviving left ventricular muscle bundle were determined as in group 1.

**Results**

**BODY AND HEART WEIGHS**

Growth curves of the animals of group 2 are presented in figure 1. In the dosage given, growth hormone (GH) restored the rate of growth to normal in hypophysectomized rats.

Data on ventricular and body weights of all animals are given in table 1. As expected, hypophysectomy was associated with reduction of ventricular weight, both absolutely and relative to body weight. Of more significance is the observation that ventricular weights and ventricle-body weight ratios of the hypophysectomized animals are significantly less than those predicted for controls of similar weight from regression equations based on observations in this laboratory. GH, in sufficient dose to produce an essentially normal rate of growth, did not bring these values to normal. Similarly, administration of GH to intact animals did not result in significant modification of ventricle-body weight ratios. A specific “cardiotrophic action” of the hormone is therefore not apparent.

**Tension Production in Surviving Myocardium**

No significant differences were found in static length-tension relationships of muscles of group 1. Developed tension-length curves are presented in figure 2. The curves are generally similar to those previously reported for rat cardiac muscle. The mean maximum tension developed by the hypophysectomized group was 0.333 ± 0.027 Gm. per mg. wet weight of muscle, as compared with 0.273 ± 0.024 Gm. per mg. wet weight for the controls. Differences between groups are not significant. Mean maximum developed tensions were also determined on a total of 23 animals of group 2. Data are presented in table 2. The results are comparable to those of group 1 and further indicate that GH exerted no significant effect on contractility in either intact or hypophysectomized rats.

**Total Protein and Actomyosin Concentration**

Values for total ventricular protein and actomyosin concentration are given in table 3. When corrections are made for slight differences in water content, total protein concentration does not differ from group to group. Ventricular actomyosin concentration, however, is elevated in both hypophysectomized groups, and this difference becomes more ap-
parent when expressed as a percentage of ventricular total protein, thus eliminating effects of differences in water content. Analysis of variance indicates this difference to be highly significant between control and hypophysectomized groups. Administration of GH, either to intact or hypophysectomized animals, did not produce significant changes.

Significant viscosimetric data for the extracted actomyosin are given in table 4. There were no significant differences in viscosity per unit of protein. Response to administration of ATP was, however, modified. Reduction in viscosity, upon the addition of ATP, expressed as ATP sensitivity, was consistently less in the hypophysectomized groups. Moreover, analysis of variance applied to these data indicates a significant increase in ATP sensitivity between GH-treated and untreated hypophysectomized animals. A similar tendency is apparent if comparison is made between GH-treated and untreated intact animals, but the difference is not statistically significant.

**Discussion**

The effects of hypophysectomy on heart size noted here are confirmatory of previous reports. It is significant that the size of the heart in the hypophysectomized animals is less than that expected on the basis of body weight, indicating a true cardiac atrophy. In the dosage used, GH did not correct this disproportion in such animals, although it induced a normal rate of growth. Neither did it increase the heart size in intact animals. These results are in agreement with those of Beznak, but are in some contradiction to the conclusions of de Grandpre and Raab, who

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**TABLE 1**

<table>
<thead>
<tr>
<th>Body and Ventricle Weights*</th>
<th>Body weight Gm.</th>
<th>Ventricle weight mg.</th>
<th>Ventricle/body weight mg./Gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Terminal</td>
<td>Actual</td>
</tr>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>——</td>
<td>191.5 ± 5.3</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>10</td>
<td>——</td>
<td>122.6 ± 1.2</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>181 ± 2.2</td>
<td>213.3 ± 5.1</td>
</tr>
<tr>
<td>Control + GH</td>
<td>8</td>
<td>184 ± 2.9</td>
<td>225.4 ± 2.6</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>16</td>
<td>153 ± 2.1</td>
<td>157.8 ± 8.2</td>
</tr>
<tr>
<td>Hypophysectomy + GH</td>
<td>15</td>
<td>155 ± 2.1</td>
<td>181.5 ± 2.1</td>
</tr>
</tbody>
</table>

*Means ± S.E. [Predicted ventricle weight = 197.2 ± 1.87 (body weight)].

**TABLE 2**

| Mean Maximum Developed Tension ± S.E. Gm./mg. Wet Weight of Tissue (Group 2) |
|-----------------------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Number | Control | Control + GH | Hypophysectomy | Hypophysectomy + GH |
| Tension|     6   |       5       |          6       |                  6       |

*Circulation Research, Volume X, June 1962*
TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Total protein % wet ventricle</th>
<th>Actomyosin % wet ventricle</th>
<th>Actomyosin % total protein</th>
<th>Dry heart weight % wet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9 20.2 ± 0.27</td>
<td>8 2.16 ± 0.11</td>
<td>7 10.8 ± 0.61</td>
<td>9 23.7 ± 0.36</td>
</tr>
<tr>
<td>Control + GH</td>
<td>8 20.2 ± 0.26</td>
<td>8 2.06 ± 0.09</td>
<td>8 10.2 ± 0.44</td>
<td>8 23.8 ± 0.19</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>15 18.9 ± 0.13</td>
<td>15 2.48 ± 0.09</td>
<td>15 13.1 ± 0.46</td>
<td>16 22.2 ± 0.25</td>
</tr>
<tr>
<td>Hypophysectomy + GH</td>
<td>11 18.7 ± 0.28</td>
<td>9 2.31 ± 0.14</td>
<td>9 12.4 ± 0.91</td>
<td>14 21.9 ± 0.21</td>
</tr>
</tbody>
</table>

*Mean Values ± S.E.

reported marked increase in myocardial mass upon administration of STH to hypophysectomized rats. These authors expressed terminal organ weights in terms of initial body weights, and the validity of conclusions based on this procedure would seem doubtful. Differences in dosage and purity of the preparations used may also be of significance. Our data would seem to support the conclusion that GH, in an amount sufficient to promote a normal rate of bodily growth, does not reverse the processes responsible for microcardia in hypophysectomized animals.

It appears evident that lack of pituitary hormones does not influence the contractility of cardiac muscle studied in vitro. This normality of the cardiac contractile apparatus in the hypophysectomized animal is in keeping with the observation of Beznač9 that the maximum work capacity of the intact rat heart, per unit weight, is not reduced by hypophysectomy and supports the conclusion that changes in cardiac function are secondary to peripheral circulatory phenomena. Administration of GH did not significantly modify myocardial contractility.

Since pituitary factors are of such prime importance in the regulation of growth processes, the data on myocardial composition are of interest. Scow13 observed a slight reduction in protein content of thigh muscle of hypophysectomized rats, when compared to age controls. Since water content was slightly elevated, the ratio of water to protein was increased. Our data indicate similar changes in the myocardium. Analysis of the contractile protein fraction reveals significant differences between reported data on skeletal muscle and those reported here. Scow found no effect of hypophysectomy on myosin content of thigh muscle if comparison was made with preoperative age or postoperative weight controls. Myosin content was, however, significantly lower than in postoperative age controls. Growth hormone produced an increase in myosin content. On the other hand, our data indicate that ventricular actomyosin concentration was slightly but significantly elevated in the absence of hypophyseal hormones, as compared with age controls, and growth hormone tended, though not significantly, to reduce this value. Although differences in protein extraction procedures, age, and sex of the animals may partially account for this variance, our data suggest that in the heart hypophyseal factors, perhaps GH, may actually operate to reduce the concentration of contractile protein. Such an effect would be compatible with the observations of Gray and Young,11 who noted decreased myofibrillar ATPase activity in quadriceps of GH-treated female rats, and with the observation of Bigland and Jehring12 that tension production per gram of muscle was reduced by GH treatment.

The reduced ATP sensitivity of the extracted protein of the hypophysectomized groups is also of interest. If ATP sensitivity is considered to be an indication of the actin content of the extracted actomyosin, it is clear that the concentration of actin is reduced by hypophysectomy and returned toward normal by GH. Further, it follows that, since the concentration of total extracted actomyosin was increased, this increase must be due to an increase in myosin. The possibility of dif-
HYPOPHYSIS AND CARDIAC MUSCLE

Viscosimetric Data*

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Viscosity number Zn</th>
<th>Viscosity number after ATP Zn ATP</th>
<th>ATP sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.218 ± 0.014</td>
<td>0.119 ± 0.008</td>
<td>85.6 ± 3.8</td>
</tr>
<tr>
<td>Control + GH</td>
<td>6</td>
<td>0.251 ± 0.011</td>
<td>0.127 ± 0.008</td>
<td>97.6 ± 3.2</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>13</td>
<td>0.233 ± 0.006</td>
<td>0.145 ± 0.006</td>
<td>60.7 ± 4.1</td>
</tr>
<tr>
<td>Hypophysectomy + GH</td>
<td>8</td>
<td>0.253 ± 0.010</td>
<td>0.140 ± 0.006</td>
<td>73.6 ± 5.8</td>
</tr>
</tbody>
</table>

*Means ± S.E.

Differential hormonal control of the synthesis of specific contractile protein is thus suggested and invites further study.

Relationship between contractile protein concentration and tension development is best documented for uterine muscle, but has been reported for cardiac muscle. On this basis, it would be expected that the myocardium of the hypophysectomized group would show some increase in maximum tension development, a tendency which does in fact appear in the data of figure 2 and table 2, but is not statistically significant.

Of greater interest, is the lack of significant functional consequences of the marked changes in the viscosimetric response to ATP. Since, in skeletal muscle, actin is present in about half the concentration of myosin, and since each Gm. of actin can combine with 4 or 5 Gm. of myosin, it is clear that an excess of actin obtains. If a similar relationship is present in cardiac muscle, it is understandable that marked changes in actin concentration might exist without functional effects on contraction. In any case, these data should serve to emphasize the uncertainties of functional extrapolations from viscosimetric data.

Summary

The effects of hypophysectomy and of growth hormone (GH) on the properties and composition of rat myocardium were studied. GH, in dosage sufficient to maintain a normal rate of growth, did not correct the microcardia of hypophysectomy. Tension production of surviving left ventricular muscle columns was not modified by hypophysectomy or GH administration. Ventricular actomyosin concentration was slightly but significantly elevated in hypophysectomized animals, with or without GH. However, "ATP sensitivity" of the extracted actomyosin was markedly depressed by hypophysectomy and partially restored by GH. Differential hormonal regulation of cardiac actin and myosin is suggested.

References


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