The Effect of the Lathyrogen β-Amino Propionitrile (BAPN) on the Mechanical Properties of Experimentally Hypertrophied Rat Cardiac Muscle

O.H.L. Bing, B.L. Fanburg, W.W. Brooks, and S. Matsushita

SUMMARY β-Amino propionitrile (BAPN) at dietary concentrations of 1, 5, and 10 g BAPN/kg rat chow was administered to rats for 14–21 days following surgical constriction of the ascending aorta. Five and 10 g BAPN/kg rat chow prevented the increase in left ventricular collagen content which occurred with cardiac hypertrophy in rats following aortic constriction. In spite of this block in the increase in collagen in the ventricles, isolated trabecular muscles from hypertrophied hearts showed a decrease in maximum velocity of shortening at a preload of 0.5 g/mm² (max V) and an increase in time to peak tension as compared with values for sham-operated animals. Max V for rats with aortic constriction receiving 10 g BAPN/kg rat chow, max V was decreased 0.66 muscle length/sec (P < 0.05), and time to peak tension was prolonged by 21 msec (P < 0.001). Resting tension was increased to 1.70 ± 0.18 (mean ± SEM) g/mm² as compared with shams, 1.15 ± 0.09 g/mm² (not significant). We conclude that the decrease in maximum velocity of shortening and prolongation of time to peak tension in experimental cardiac hypertrophy occur independently of elevated collagen content, whereas elevations in resting tension appear to depend upon an increase in collagen content of these hearts.

IN THE past decade, numerous studies have attempted to characterize the mechanical performance of hypertrophied cardiac muscle. Several investigators have reported a decrease in maximum shortening velocity of hypertrophied cardiac muscle when the hypertrophy was induced by a pressure overload. Whether the decrease in shortening velocity reflects a depression of cardiac "contractility" is a matter of controversy. It seems clear, nonetheless, that an abnormality of cardiac performance follows acute experimental pressure overload. Additional mechanical changes have been described to accompany cardiac hypertrophy induced by pressure overload. These include a prolongation of contraction and a change in passive compliance. Additional changes have been described to accompany cardiac hypertrophy induced by pressure overload. These include a prolongation of contraction and a change in passive compliance.

In addition to the increase in RNA and protein synthesis, which accompanies experimental cardiac hypertrophy, enhanced thymidine incorporation into DNA has been demonstrated primarily in fibroblasts. Also, increased quantities of collagen are found in hypertrophied heart muscle. β-Amino propionitrile (BAPN) is a lathyrogen which has been shown in vivo and in tissue culture to inhibit the cross-linking of collagen probably by blocking the initial step in the enzymatic conversion of lysyl residues in peptide linkages to their aldehyde derivatives. In the present study, the rat model of left ventricular hypertrophy induced by aortic constriction was used to elucidate further the physiological role of the increased collagen seen in cardiac hypertrophy by inhibiting the connective tissue response to cardiac hypertrophy by BAPN administration.

Cardiac hypertrophy following surgical constriction of the aorta in the rat develops relatively rapidly. An increase in the left ventricular-body weight ratio has been noted 3 days after operation and reaches a peak at approximately 7 days. Since no further hypertrophy, as indicated by the left ventricular/body weight ratio, occurred, and most parameters of mechanical performance were stable between 7 and 28 days, 14–21 days was selected as the time to study the effect of BAPN on muscle performance in the present groups of animals.

Methods

Male Charles River CD rats, weighing 180–220 g, were used. Ninety-nine rats were subjected to con-
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striction of the aortic arch and 88 were sham-operated controls. Operations were carried out as previously described, except that the rats were anesthetized with 60–70 mg of chloral hydrate intraperitoneally. The rats were started on BAPN diets immediately after surgery. The BAPN was mixed with ground rat chow and rats were fed ad libitum until they were killed. One, 5, or 10 g of BAPN were added to each kilogram of rat chow. The rats were weighed and decapitated 14–21 days after operation. The chest was opened, and the heart was removed rapidly and placed in oxygenated Krebs-Henseleit solution at 30°C.

For mechanical measurements the columnar carnea muscle of the left ventricle was dissected free, mounted vertically between two spring clips, and placed in a chamber containing Krebs-Henseleit solution containing 5.5 mM glucose. The solution was gassed with 95% O₂ + 5% CO₂ (pH = 7.4) and maintained at a temperature of 28°C with a Lauda K-2 constant-temperature circulating pump. The lower spring clip was attached to a No. 30-gauge length of stainless steel tubing which passed through a mercury seal at the bottom of the chamber to a Statham model G7B-0.75-350 force transducer. The upper spring clip was connected to a thin gold chain which was attached to an isotonic lever arm above which a micromanometer stop was mounted to adjust resting muscle length. The lever arm was made from magnesium with a ball-bearing fulcrum and the lever arm ratio was 8:1. A Sanborn DC DT-050 displacement transducer was mounted above the short end of the lever arm. The total equivalent mass was calculated for each of the four lever arm systems used in the present experiment and averaged 300 mg.

Muscles placed in the chambers were stimulated 12 times per minute through parallel platinum electrodes by rectangular pulses lasting 7 msec at voltages which were 10% greater than the minimum required to produce a maximum mechanical response. After an equilibration period of 1 hour, mechanical performance was recorded for each muscle which had been carefully stretched to the apex of its length-tension curve. The following measurements were made from the collected data: active tension, time-to-peak tension, maximum rate of tension development, and maximum shortening velocity at a load of 0.5 g/mm². The velocity index will be referred to in this report as maximum muscle shortening velocity (max V). Measurements of maximum rate of tension development and maximum velocity of shortening were determined from tangents drawn to the rapidly rising phase of the relevant curves. The accuracy of these measurements was verified subsequently by comparing this technique to results obtained using an electronic differentiator with a high frequency cut-off at 600 Hz. Resting tension at the apex of the length tension curve was recorded after 5 minutes in each study. Over a subsequent 5-minute period, there was a further change in tension of less than 5% due to stress relaxation.

Muscle length was determined at the apex of the length-tension curve with a Gaertner cathometer and telescope. At the end of each experiment, the muscle between the spring clips was weighed and muscle cross-sectional area was calculated assuming cylindrical uniformity and a specific gravity of 1.050. Values for tension were normalized for muscle cross-sectional area, and shortening was corrected for length. Each experiment was carried out with four muscles contracting simultaneously in four chambers with common temperature regulation and oxygenation. Most experiments were organized so that each set of four muscles included those from two sham-operated rats and two with aortic constriction.

After removal of the trabecular muscles, the left ventricle, including the septum, was dissected from the remainder of the heart, blotted, and weighed. A portion of the left ventricle also was weighed after drying to a constant weight at 60°C. Hydroxyproline was measured in samples of approximately 10 mg of tissue (dry weight) from the free wall of the left ventricle according to the method of Prockop and Udenfriend. In addition, saline-soluble collagen was measured from pooled left ventricles according to the method of Nimni. For this measurement, four or five left ventricles were homogenized in 4°C in 20 volumes of 0.5 m NaCl in a Virtis homogenizer at half the maximum speed setting for 5 minutes. The homogenate was shaken overnight and centrifuged at 20,000 g for 1 hour. The supernatant extract was dialyzed overnight against water and samples of the dialysates were analyzed for hydroxyproline after HCl hydrolysis (6 N HCl, 120°C, 2 hours). For expression as collagen, the hydroxyproline values were multiplied by 7.46.

Results

Body and Left Ventricular Weights

The body weight of sham-operated rats receiving 1 g BAPN/kg rat chow was not significantly different from that of rats not receiving BAPN (Table 1). When BAPN, 5 and 10 g/kg chow, was added to the diet, there was a dose-related progressive decrease in body weight. Rats with aortic constriction generally showed a slight but consistent decrease in body weight in comparison to sham-operated controls, although statistical significance (at P < 0.05) at any amount of BAPN in the diet was not present.

In sham-operated rats receiving 5 and 10 g of BAPN/kg rat chow, there was a decrease in left ventricular weight which paralleled the fall in body weight. A similar decrease in left ventricular weight was seen in rats with aortic constriction at the 5- and 10-g concentrations of BAPN. At all dietary amounts of BAPN, the left ventricular weights of
TABLE 1  Effect of Dietary BAPN on Rat and Left Ventricular (LV) Weight

<table>
<thead>
<tr>
<th>BAPN added to rat chow (g/kg)</th>
<th>0</th>
<th>1 g</th>
<th>5 g</th>
<th>10 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>272 ± 8</td>
<td>271 ± 6</td>
<td>215 ± 3</td>
<td>202 ± 5</td>
</tr>
<tr>
<td>Constricted</td>
<td>285 ± 5</td>
<td>262 ± 6</td>
<td>212 ± 4</td>
<td>193 ± 4</td>
</tr>
<tr>
<td>LV wt (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.614 ± 0.015</td>
<td>0.531 ± 0.015</td>
<td>0.396 ± 0.008</td>
<td>0.377 ± 0.009</td>
</tr>
<tr>
<td>Constricted</td>
<td>0.764 ± 0.022*</td>
<td>0.728 ± 0.027*</td>
<td>0.540 ± 0.022*</td>
<td>0.476 ± 0.012*</td>
</tr>
<tr>
<td>LV wt/100 g body wt (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>190 ± 4</td>
<td>196 ± 3</td>
<td>184 ± 1</td>
<td>187 ± 3</td>
</tr>
<tr>
<td>Constricted</td>
<td>297 ± 6*</td>
<td>278 ± 9*</td>
<td>254 ± 9*</td>
<td>248 ± 7*</td>
</tr>
<tr>
<td>Number of rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>23</td>
<td>15</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Constricted</td>
<td>22</td>
<td>16</td>
<td>27</td>
<td>34</td>
</tr>
</tbody>
</table>

* P < .001 compared to sham.

The left ventricle-body weight ratio was relatively constant for sham-operated rats for all amounts of dietary BAPN. The constancy of this ratio was maintained despite loss of body weight at the higher dietary concentrations of BAPN. The left ventricle-to-body weight ratios for rats with aortic constriction were increased by 56, 43, 38, and 33% above values for sham-operated controls at 0, 1, 5, and 10 g BAPN/kg rat chow.

Left Ventricular Hydroxyproline

Total left ventricular hydroxyproline content as determined by multiplying ventricular weight by hydroxyproline concentration in the ventricles was elevated in rats with aortic constriction (0.56 ± 0.06 mg/left ventricle) as compared to the value for sham-operated rats (0.32 ± 0.01 mg/left ventricle) when BAPN was not added to the diet (P < 0.001) (Fig. 1). However, differences in hydroxyproline content between sham-operated rats and those with aortic constriction were less marked at increasing concentrations of BAPN in the diet, and at 10 g BAPN/kg rat chow there was no significant difference in content between rats with aortic constriction and those which had undergone a sham operation. Hydroxyproline content of left ventricles of sham-operated rats was not influenced significantly by BAPN.

The concentration of hydroxyproline in the left ventricle of rats with aortic constriction did not differ significantly from that of sham-operated rats when BAPN was not added to the diet or at 1 g BAPN/kg chow (Fig. 2). At 5 and 10 g BAPN/kg rat chow, sham-operated rats showed a significant increase in left ventricular hydroxyproline concentration in comparison with rats with aortic constriction. The concentration of hydroxyproline also increased significantly in sham-operated rats at 5 and 10 g BAPN/kg rat chow as compared to that in sham-operated rats not receiving BAPN. A significant increase in saline-soluble collagen in the left ventricles of rats with aortic constriction was associated with an increase in hydroxyproline content.

**Figure 1** The effect of BAPN and aortic constriction on left ventricular hydroxyproline content (hydroxyproline concentration × left ventricular weight). In rats administered 10 g BAPN/kg rat chow, there was no significant difference in hydroxyproline content in sham-operated rats compared to those with aortic constriction.

**Figure 2** The effect of BAPN and aortic constriction on left ventricular hydroxyproline concentration. At 0 and 1 g BAPN/kg rat chow, there was no significant difference between rats with sham operation or aortic constriction. However, at 5 and 10 g BAPN/kg rat chow, the hydroxyproline concentration of the sham-operated rats increased significantly above values for rats with aortic constriction.
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TABLE 2  
Saline-Soluble Collagen

<table>
<thead>
<tr>
<th>g BAPN/kg rat chow</th>
<th>Sham (mg collagen per g wet ventricle)</th>
<th>Constricted (mg collagen per g wet ventricle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.19 ± 0.06</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>1</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>0.37 ± 0.06*</td>
<td>0.38 ± 0.01†</td>
</tr>
<tr>
<td>10</td>
<td>0.35 ± 0.09*</td>
<td>0.40 ± 0.12*</td>
</tr>
</tbody>
</table>

Mean ± SD of three or four groups of left ventricles (each containing 4-5 left ventricles) are shown. Only single groups were measured at 1 g BAPN/kg rat chow.

* P < 0.05
† P < 0.01 compared to 0 BAPN/kg rat chow.

ventricles of both sham-operated rats and those with aortic constriction was seen when rats received 5 and 10 g BAPN/kg chow (Table 2).

Effects of BAPN on Mechanical Performance of Trabecular Muscles of Rats with Aortic Constriction

Variable left ventricular hypertrophy is seen in rats and probably is related to the degree of effective surgical constriction of the aorta. To maintain uniformity for comparisons of physiological parameters, "matched" groups of muscle preparations with similar cross-sectional areas were selected from hearts from both sham-operated rats, serving as controls, and those with aortic constriction. Furthermore, the "matched" muscles from rats with aortic constriction were obtained from those with similar left ventricular-body weight ratios.

Results of studies on isolated muscles are reported in Table 3. Although no significant difference in maximum developed tension or in maximum rate of tension development was found between preparations from rats with aortic constriction and rats after the sham operation, a significant decrease in max V was present in muscles from rats with hypertrophy not receiving BAPN as well as in rats receiving 1, 5, and 10 g BAPN/kg rat chow. Also, significant prolongation of time-to-peak tension occurred in rats with aortic constriction and was not influenced by dietary BAPN. Preparations from hypertrophied hearts reached the same level of peak tension as hearts from sham-operated animals, but tension was maintained at the peak value for a slightly longer period of time. Resting tension at the apex of the length-tension curve was elevated significantly in rats with aortic constriction except for those fed 10 mg BAPN/kg rat chow; in this case the value of 1.23 ± 0.1 g/mm^2 for rats with aortic

TABLE 3  Data for Rats with Matched Trabecular Muscle Cross-Sectional Areas*

<table>
<thead>
<tr>
<th>Body wt (g)</th>
<th>LV wt (g)</th>
<th>LV wt/100 g body wt (mg)</th>
<th>Trabecular muscle cross-sectional area (mm^2)</th>
<th>Developed tension (g/mm^2)</th>
<th>Max. rate of tension development (g/mm^2 per sec)</th>
<th>Max V (lengths/sec)</th>
<th>Time-to-peak tension (msec)</th>
<th>Resting tension (g/mm^2)</th>
<th>Number of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>Constricted</td>
<td>Sham</td>
<td>Constricted</td>
<td>Sham</td>
<td>Constricted</td>
<td>Sham</td>
<td>Constricted</td>
<td>Sham</td>
<td>Constricted</td>
</tr>
<tr>
<td>0</td>
<td>272 ± 8</td>
<td>271 ± 6</td>
<td>190 ± 4</td>
<td>0.94 ± 0.05</td>
<td>8.15 ± 0.46</td>
<td>3.66 ± 0.15</td>
<td>168 ± 2</td>
<td>1.70 ± 0.185</td>
<td>23</td>
</tr>
<tr>
<td>1</td>
<td>250 ± 8†</td>
<td>256 ± 8</td>
<td>295 ± 12§</td>
<td>1.03 ± 0.05</td>
<td>7.07 ± 0.46</td>
<td>3.09 ± 0.19§</td>
<td>180 ± 4</td>
<td>1.70 ± 0.185</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>0.514 ± 0.015</td>
<td>0.531 ± 0.015</td>
<td>273 ± 13§</td>
<td>1.02 ± 0.07</td>
<td>6.02 ± 0.57</td>
<td>2.91 ± 0.22§</td>
<td>189 ± 5§</td>
<td>1.58 ± 0.15§</td>
<td>23</td>
</tr>
<tr>
<td>10</td>
<td>0.736 ± 0.013§</td>
<td>0.691 ± 0.032§</td>
<td>285 ± 13§</td>
<td>1.04 ± 0.03</td>
<td>8.12 ± 0.29</td>
<td>3.04 ± 0.13§</td>
<td>199 ± 5§</td>
<td>1.52 ± 0.15§</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>288 ± 14§</td>
<td>1.07 ± 0.06</td>
<td>7.21 ± 0.25</td>
<td>3.10 ± 0.23§</td>
<td>219 ± 5§</td>
<td>1.15 ± 0.09§</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>288 ± 14§</td>
<td>1.06 ± 0.03</td>
<td>7.39 ± 0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values represent means ± 1 se. P values represent differences between sham-operated rats and rats with aortic constriction for a given BAPN-containing diet. LV = left ventricular.
† Maximum isotonic shortening velocity at a load of 0.5 g/mm^2.
‡ P < 0.05 compared to sham.
§ P < 0.01 compared to sham.
pressed as a percent of developed tension. At increasing from rats with aortic constriction given 5 and 10 g chow, hearts of rats with aortic constriction all were BAPN concentrations plus aortic constriction may
larger sham-operated rats that did not receive BAPN/kg chow was similar to the heart weight of hearts of sham-operated rats and those with aortic constriction. Thus, these groups of rats had relative cardiac hypertrophy without an associated increase in collagen content. Interestingly, the weight of hearts from rats with aortic constriction given 5 and 10 g BAPN/kg chow was similar to the heart weight of larger sham-operated rats that did not receive BAPN. Hence, the opposing effects of high dietary BAPN concentrations plus aortic constriction may result in relatively normal contents of collagen and non-collagen containing structures in the heart, and any change in mechanical properties would have to be explained by alterations in factors other than the absolute quantities of these components.

There is evidence that BAPN interferes with collagen synthesis. It appears that this occurs through inhibition of the process by which specific lysyl residues in peptide linkages are converted to their aldehyde derivatives; this interferes with cross-linking. The kinetics of collagen turnover for the sham-operated rats were such that absolute quantities of collagen were not altered by BAPN. An increase in solubilized collagen is consistent with the effect of BAPN and occurred both for rats with sham operation and aortic constriction. The increase in soluble collagen probably results from blockage of inter- and intramolecular cross-linking of collagen to make some collagen soluble in the salt solution. The finding of this effect on sham-operated rats in the absence of a concomitant decrease in the content of insoluble collagen can be explained by the relatively low turnover rates of insoluble collagen in these rats.

BAPN alone at all doses studied did not alter the mechanical performance of heart muscle of sham-operated rats despite the reduction in heart weight at the higher dietary concentrations of BAPN. As previously observed, hypertrophy induced by aortic constriction in rats not receiving BAPN was associated with a depression of maximum shortening velocity, a prolonged contraction time and an increase in resting tension measured at the apex of the length-tension curve, whereas developed tension and maximum rate of tension development were unchanged. Inhibition of collagen cross-link formation and synthesis of insoluble collagen by BAPN did not influence the depression in maximum shortening velocity or the prolonged time to peak tension in the rats with aortic constriction. However BAPN, at a sufficiently high concentration, prevented the increase in resting tension associated with the cardiac hypertrophy which follows acute aortic constriction. Although BAPN prevented the elevation in hydroxyproline content in ventricles of rats with aortic constriction at doses of 5 and 10 g BAPN/kg rat chow, it resulted in increased concentrations of hydroxyproline in ventricles of sham-operated rats relative to those with aortic constriction. This elevation in hydroxyproline concentration occurred as a result of excess loss of non-collagen heart weight relative to collagen content. Resting tension was not elevated for hearts from these groups of sham-operated rats when compared to those with aortic constriction, indicating that resting tension in nonhypertrophied myocardium is not a function of collagen concentration alone.

Thus, these studies indicate that the increase in connective tissue and collagen that occurs with...
experimential cardiac hypertrophy is not the cause of decreased shortening velocity and prolongation of contraction. Other factors unrelated to absolute content of collagen, possibly including organization of collagen, or factors directly related to the contractile proteins, must influence these physiological parameters. However, the elevation in resting tension that occurs with cardiac hypertrophy appears to be due, at least in part, to the increased content of collagen. Increased soluble collagen, as occurs in sham-operated rats fed BAPN, does not have any effect on the contractile properties of cardiac muscle.

It must be emphasized that the left ventricular hypertrophy that follows acute aortic constriction may not necessarily be analogous to that which occurs in man, in whom the hypertrophy is generally more gradual in its development. In naturally occurring disease, not only is the development of hypertrophy much slower, but hypertrophy is progressive and frequently far greater in degree. It is of interest, however, that parallel changes in aspects of myocardial performance and structure have been observed during cardiac hypertrophy both in experimental animals and humans. If the findings of the present study of experimental hypertrophy can be extended to clinical hypertrophy as it occurs in man, the concept of a cause and effect relationship between fibrosis and altered diastolic compliance may be supported.

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