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In Vitro Acetylcholine Biosynthesis in Normal and Failing Guinea Pig Hearts

By Robert Roskoski, Jr., Philip G. Schmid, Howard E. Mayer, and Francois M. Abboud

ABSTRACT

Choline acetyltransferase activity, which is rate limiting in acetylcholine biosynthesis, was measured in the four heart chambers of guinea pigs subjected to (1) sham surgery, (2) constriction of the ascending aorta, (3) constriction of the descending thoracic aorta, and (4) constriction of the pulmonary artery. After 30 days when hypertrophy and heart failure were fully established, choline acetyltransferase was quantified in vitro by a radiochemical assay. In the sham-operated group, enzyme activity expressed in terms of unit weight of cardiac tissue was greatest in the right atrium and the right ventricle and lower in the left atrium and the left ventricle (3.62 ± 0.30, 2.96 ± 0.52, 1.64 ± 0.15, and 1.67 ± 0.22 nmoles/min g⁻¹, respectively). Enzyme activity was reduced (P < 0.05) in the right atria and the right ventricles of guinea pigs with constriction of the pulmonary artery (1.68 ± 0.37 and 1.31 ± 0.29 nmoles/min g⁻¹, respectively). Enzyme activity also tended to be reduced in the left atria and the left ventricles of guinea pigs with constriction of the aorta. These changes represented a relative dilution of enzyme activity per unit weight but not an absolute depletion, since choline acetyltransferase activity per ventricle was not reduced. The absence of significant changes in the total amount of the neuronal enzyme, choline acetyltransferase, per ventricle contrasted with the observed increases in the myocardial enzyme, carnitine acetyltransferase. These results confirm the presence of significant parasympathetic innervation of the ventricles as well as the atria but do not demonstrate alterations in parasympathetic neurotransmitter biosynthesis in hypertrophied and failing myocardium. The absence of absolute reductions in choline acetyltransferase activity in hypertrophied and failing ventricle contrasts strikingly with the previously reported reductions in tyrosine hydroxylase, which is rate limiting in sympathetic neurotransmitter biosynthesis.

KEY WORDS parasympathetic neurotransmitter cardiac chambers carnitine acetyltransferase cardiac hypertrophy parasympathetic innervation of the heart choline acetyltransferase

In hypertrophied and failing hearts, defects in neurogenic control involve both sympathetic and parasympathetic abnormalities. These sympathetic defects are characterized by decreases in catecholamine biosynthesis and depletion of the sympathetic neurotransmitter (1-4), but the nature of the parasympathetic defects is uncertain (5). Depletion of the parasympathetic neurotransmitter, acetylcholine, has been postulated but not confirmed (6). Technical difficulties in the bioassay of acetylcholine emphasize the need for alternative assessments of cardiac parasympathetic innervation (7). Measuring the activity of choline acetyltransferase, the enzyme that catalyzes the rate-limiting step in the biosynthesis of acetylcholine, represents one approach. This enzyme is found almost exclusively in nerves (8); thus, it represents a reliable marker of postganglionic and intrinsic parasympathetic innervation to the heart. In the present report, choline acetyltransferase activity was determined in vitro in cardiac tissue from normal guinea pigs and guinea pigs with left or right ventricular hypertrophy and heart failure.

Methods

Four groups of seven guinea pigs were studied. Guinea pigs in one group had a sham operation consisting of a left thoracotomy and a blunt dissection between the proximal aorta and the main pulmonary artery. In the second group, a band 1.8 mm in diameter was placed on the proximal ascending aorta. A band 1.3 mm in diameter was placed on the descending thoracic aorta in the third group, and constriction of the main pulmonary artery was produced with a band 1.8 mm in diameter in the fourth group. Banding reduced vessel lumens to an average of 15% of normal. Thirty days after surgery when cardiac hypertrophy and heart failure were fully established, the guinea pigs were killed by dislocation of cervical vertebrae; their hearts were rapidly

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Excised, drained of blood, blotted, and weighed. Samples were taken from the right atrial appendage (excluding the area of the sinoatrial node), the left atrial appendage, and the free walls of the right and left ventricles between the apex and the base (excluding papillary muscles). These samples (50–200 mg) were blotted, weighed, and stored in liquid nitrogen.

To prepare stored tissues for assay of choline acetyltransferase, samples were disrupted in a tight-fitting stainless steel pulverizer cooled with liquid nitrogen. The powdered samples were further disrupted with a Potter-Elvehjem (Teflon to glass) motor-driven homogenizer in a minimum amount (less than 3 ml) of buffer (5 mm potassium phosphate, 0.1 mm ethylenediaminetetraacetic acid [EDTA], pH 7.4) containing 1% n-butanol. The assay of acetylcholine biosynthesis in myocardial tissue was carried out as described previously (9) by quantifying the formation of [14C]acetylcholine from [14C]acetyl coenzyme A. The final incubation mixture contained 50 μM [14C]acetyl coenzyme A (50 μc/μ mole), 2 mm choline chloride, enzyme, 50 mm potassium phosphate, 0.1 mm EDTA, 0.1 mm eserine sulfate, 1% n-butanol, and 100 mm KCl, all adjusted to pH 7.4 in a final volume of 20 μliters. Incubations were at 37°C for 15 minutes. The reaction was stopped by adding 10 μliters of a 100 mm acetylcholine chloride-0.5N formic acid solution. To resolve [14C]acetylcholine from labeled precursor, samples were subjected to low-voltage (35 V/cm) paper electrophoresis (Whatman no. 1) in a 1M formic acid–1M acetic acid solution for 15 minutes in a Gelman deluxe electrophoresis apparatus. After 15 minutes of drying at 100°C to remove volatile acid, acetylcholine was developed in an I2 chamber, and the marker zones were cut out for determination of radioactivity as described previously (9). The activity of choline acetyltransferase, a neuronal enzyme that catalyzes the preceding reaction, was distinguished from that of carnitine acetyltransferase, a myocardial enzyme, as previously described (9). At the time of analysis, tissues from guinea pigs representing each of the four groups were processed simultaneously so that any possible variations in methodology would affect results from all groups similarly.

Statistical analyses were carried out using analysis of variance and Tukey’s test to assess all of the possible differences among group means (10).

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Final body weight (kg)</th>
<th>Ventricular weight (g/kg)</th>
<th>Atrial weight (combined left and right atria) (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>7</td>
<td>0.78 ± 0.044</td>
<td>1.81 ± 0.10</td>
<td>0.62 ± 0.06</td>
</tr>
<tr>
<td>Constriction of ascending aorta</td>
<td>7</td>
<td>0.81 ± 0.047</td>
<td>3.04 ± 0.17</td>
<td>0.97 ± 0.08*</td>
</tr>
<tr>
<td>Constriction of descending aorta</td>
<td>7</td>
<td>0.66 ± 0.046</td>
<td>2.71 ± 0.15</td>
<td>0.76 ± 0.06</td>
</tr>
<tr>
<td>Constriction of pulmonary artery</td>
<td>7</td>
<td>0.75 ± 0.033</td>
<td>1.60 ± 0.09</td>
<td>1.24 ± 0.11*</td>
</tr>
</tbody>
</table>

Values are means ± SE.

* P < 0.05 compared with the mean for the sham-operated guinea pigs using analysis of variance and Tukey’s test (10).
In vitro choline acetyltransferase activity in cardiac tissue obtained from four groups of guinea pigs: (1) a sham-operated group (S), (2) a group with pulmonary artery constriction (PA), (3) a group with constriction of the descending thoracic aorta (D), and (4) a group with constriction of the ascending aorta (A). Values for individual guinea pigs are represented by points, and group means are represented by bars. Constriction of vessels reduced luminal cross-sectional areas to 15% of normal and resulted in hypertrophy and heart failure. Note that activity is expressed in terms of unit weight of cardiac tissue. Groups were compared using analysis of variance and Tukey's test (10).

There was a tendency for enzyme activity to be reduced in the left heart chambers of these guinea pigs, but activity was reduced significantly only in the left atrial appendages of guinea pigs subjected to constriction of the ascending aorta. Constriction of the aorta did not seem to affect enzyme activity in the right heart chambers with one notable exception; constriction of the ascending aorta was associated with significant decreases in enzyme activity in the right atrial appendages.

Choline acetyltransferase activity per ventricle is shown in Figure 2. This activity tended to be higher in the left ventricle than it was in the right ventricle, but the total enzyme activity of either ventricle appeared to be unaffected by constriction of the pulmonary artery or the aorta.

Activation or inhibition of choline acetyltransferase might occur in the failing heart and obliterate significant changes in enzyme activity. This possibility was tested by reconstituting homogenates of right atria from sham-operated guinea pigs with corresponding homogenates from guinea pigs with heart failure. The sum of choline acetyltransferase activity in individual homogenates from the two groups equaled the activity in combined homogenates (Table 2), indicating the absence of adventitious effectors of choline acetyltransferase activity. If a cofactor (activator) were present in the homogenates, the sum of activity in the reconstituted homogenates would have been less than the sum of activity in the individual homogenates (9). Values are means ± SE for determinations on extracts from three guinea pigs in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental (pmoles/min)</th>
<th>Sham-operated (pmoles/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>17.4 ± 0.31</td>
<td>23.6 ± 0.25</td>
</tr>
<tr>
<td>Constriction of pulmonary artery</td>
<td>6.2 ± 0.59</td>
<td>28.0 ± 0.24</td>
</tr>
<tr>
<td>Constriction of ascending aorta</td>
<td>9.6 ± 0.34</td>
<td>23.2 ± 0.29</td>
</tr>
<tr>
<td>Constriction of descending aorta</td>
<td>6.2 ± 0.50</td>
<td>23.6 ± 0.25</td>
</tr>
</tbody>
</table>

Right atrial extracts (50 μg of protein) from the specified experimental guinea pigs were dialyzed for 4 hours against 5 mM potassium phosphate and 0.1 mM EDTA (pH 7.4) at 3°C. Choline acetyltransferase activity was measured in the samples alone (experimental) and after recombination (experimental + sham-operated) (9). Values are means ± SE for determinations on extracts from three guinea pigs in each group.

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enates from sham-operated guinea pigs but not in the homogenates from guinea pigs with heart failure, the 15-minute incubation period in the reconstitution experiments, in which homogenates from guinea pigs subjected to sham-operation and those from guinea pigs with heart failure were combined, should have been sufficient for any allosteric or enzyme-catalyzed modifications of the reaction to occur. Since no modifications of the reaction were detected, we conclude that all of the necessary cofactors were in the homogenates from guinea pigs with heart failure.

To test for functional changes in the choline acetyltransferase from sham-operated and experimental guinea pigs, the apparent $K_m$ for acetyl coenzyme A was measured (Table 3). The $K_m$ was similar in all groups, indicating that the enzyme in sham-operated guinea pigs and guinea pigs with heart failure had similar functional characteristics and, therefore, presumably similar structural characteristics. The apparent $K_m$ for choline could not be measured reliably, because the carnitine acetyltransferase that is also present in the homogenates catalyzes choline acetylation when choline is present in high concentrations.

$V_{max}$ was determined using acetyl coenzyme A as the substrate. $V_{max}$ varied in the different groups of guinea pigs (Table 3). However, the values for $V_{max}$ were similar to the corresponding values for choline acetyltransferase activity per unit weight in each group (Fig. 1 and Table 3). Since the reconstitution experiments excluded the possibility of inhibitors of choline acetyltransferase, we conclude that the decreases in $V_{max}$ in cardiac tissue from guinea pigs with heart failure represent decreases in the quantity of the enzyme per unit weight.

During the dialysis to remove endogenous carnitine, which required 3 hours, the lability of the choline acetyltransferase from guinea pigs with heart failure might have been greater than that of the enzyme from sham-operated guinea pigs. Such an effect could explain the decreases in the quantity of choline acetyltransferase per unit weight of cardiac tissue in guinea pigs with heart failure. This explanation seems unlikely, however, since the apparent $K_m$ for acetyl coenzyme A indicates similar functional characteristics of the enzyme for all of the groups of guinea pigs. Similar conditions for the extraction and assay of choline acetyltransferase existed for tissue from all of the guinea pigs. Therefore, any lability of the enzyme should affect determinations of choline acetyltransferase activity similarly in guinea pigs with heart failure and those subjected to sham-operations.

It was not feasible to assess the stability of choline acetyltransferase before the 3 hours required for the removal of carnitine. This substrate is present in appreciable amounts in cardiac tissue, and, if it is not removed, it will react adventitiously with $[^{14}C]$acetyl coenzyme A (9). Therefore, the dialysis step prior to the measurement of choline acetyltransferase was necessary. Rapid dialysis techniques that would allow assessment of enzyme stability in less than 3 hours have proven unfeasible thus far. At this time, little can be said about enzyme stability except that measurements of choline acetyltransferase activity after 3 and 6 hours of dialysis are similar.

The measurements of enzyme activity reported in the present paper represent the total cellular content; 1% n-butanol was used to disrupt subcellular elements and release the enzyme from all cell constituents.

**CARNITINE ACETYLTRANSFERASE**

In the sham-operated group, carnitine acetyltransferase activity, expressed per unit weight, tended to be greater in the left chambers than it was in the corresponding right chambers (Table 4). Similar results have been observed previously in normal guinea pigs (9). Constriction of the ascending aorta was associated with significant increases in enzyme activity per left ventricle (Table 4). Activity per unit weight, however, was unchanged. The activity of carnitine acetyltransferase was unchanged under the other experimental conditions. Of greater consequence, the specific changes

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**TABLE 3**

Kinetic Characterization of Choline Acetyltransferase Activity in Right Atrium from Sham-Operated Guinea Pigs and Guinea Pigs with Heart Failure with Acetyl Coenzyme A as the Variable Substrate

<table>
<thead>
<tr>
<th>Group</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (nmol/min g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>18</td>
<td>4.3</td>
</tr>
<tr>
<td>Constriction of ascending aorta</td>
<td>19</td>
<td>2.1</td>
</tr>
<tr>
<td>Constriction of descending aorta</td>
<td>21</td>
<td>3.4</td>
</tr>
<tr>
<td>Constriction of pulmonary artery</td>
<td>18</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Choline acetyltransferase activity was measured as described in Methods using 2 mM choline and acetyl coenzyme A from 15 μM to 100 μM. The experiment was performed on tissues from three guinea pigs in each group, and the variability was less than 6%.
TABLE 4

<table>
<thead>
<tr>
<th>Carnitine Acetyltransferase</th>
<th>Activity per unit weight (µmoles/min g⁻¹)</th>
<th>Activity per ventricle (µmoles/min ventricle⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RA</td>
<td>RV</td>
</tr>
<tr>
<td>Sham-operated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.54 ± 0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constriction of ascending aorta</td>
<td>3.54 ± 0.39</td>
<td>4.88 ± 0.68</td>
</tr>
<tr>
<td>Constriction of descending aorta</td>
<td>3.72 ± 0.38</td>
<td>6.00 ± 0.51</td>
</tr>
<tr>
<td>Constriction of pulmonary artery</td>
<td>3.79 ± 0.23</td>
<td>4.01 ± 0.92</td>
</tr>
</tbody>
</table>

Values are means ± SE for determinations on tissue samples from seven guinea pigs. Comparisons between groups were made using analysis of variance and Tukey’s test (10). RA = right atrium, RV = right ventricle, LA = left atrium, and LV = left ventricle.

* Group mean differs from the mean for the sham-operated group (P < 0.05).
† Group mean differs from the mean for activity per left ventricle in guinea pigs with a banded ascending aorta (P < 0.05).

Discussion

To allow full recovery from surgery and to avoid, as much as possible, alterations due to the stress of surgery per se, tissues were obtained 30 days after constriction of vessels or the sham-operation. Constriction of the pulmonary artery produced right ventricular hypertrophy and failure associated with hepatic congestion and, in some cases, ascites (11). Constriction of the aorta produced left ventricular hypertrophy and failure associated with pulmonary congestion (11). Our guinea pig preparations with left heart failure resembled those of Gertler (12), Schwartz and Lee (13), and Spann and co-workers (2). In agreement with Spann and co-workers (2), our preparations with left heart failure also exhibited modest right ventricular enlargement.

Except for primate placenta, choline acetyltransferase is found only in nervous tissue (8). The enzyme thus constitutes a marker for cholinergic innervation of the heart. This cholinergic innervation includes the pre- and postganglionic components of the parasympathetic nervous system but excludes the preganglionic component of the sympathetic nervous system, which is entirely extracardiac (14, 15). Therefore, possible changes in the preganglionic sympathetic choline acetyltransferase activity did not contribute to the cardiac enzyme activity measured in this study; only enzyme activity associated with postganglionic and intrinsic parasympathetic innervation was assayed.

Choline acetyltransferase activity, expressed in terms of unit weight of cardiac tissue, was reduced in right atrial appendages and right ventricles of guinea pigs with constriction of the pulmonary artery and tended to be reduced in left atrial appendages and left ventricles of guinea pigs with constriction of the aorta. These reductions appeared to be explained by myocardial hypertrophy and relative dilution of the neuronal enzyme rather than by depletion or destruction of enzyme. Choline acetyltransferase per ventricle was not reduced. The absence of significant changes in the neuronal enzyme, choline acetyltransferase, per ventricle contrasted with the increases in the myocardial enzyme, carnitine acetyltransferase.

We can only speculate about the marked reduction in the activity of choline acetyltransferase per unit weight in right atrial appendages of guinea pigs with constriction of the ascending aorta and left heart failure. Hypertrophy and dilution of the enzyme may have contributed substantially to this reduction, since right ventricular hypertrophy and presumably right atrial hypertrophy accompanied left heart failure. The degree of right heart chamber hypertrophy was more pronounced in the group with ascending aortic constriction than it was in the group with descending aortic constriction (Table 1). In fact, in the two groups of guinea pigs with constriction of the ascending and descending aortas, it is the parallelism between the absolute percent increases in the weights of the right ventricles (+57% and +23%, respectively), the absolute percent increases in the weights of the atria (+78% and +33%, respectively), and the absolute percent reductions in enzyme activity in the right atrial appendages (−71% and −15%, respectively) that most strongly supports the proposal that the reduction in enzyme activity per unit weight is the result of dilution by myocardial hypertrophy. The possible contribution of other factors to the observed alterations must await further investigation.
The values for $V_{\text{max}}$ in right atrial tissues tended to parallel the values for enzyme activity per unit weight (Fig. 1). We believe that the differences in $V_{\text{max}}$ among groups can be explained by different amounts of enzyme per unit weight of tissue rather than by the presence of an enzyme inhibitor, which the reconstitution experiments appeared to exclude.

The results demonstrate that significant choline acetyltransferase activity is present in right and left ventricles, confirming the presence of parasympathetic innervation to these chambers (7, 14, 15). Heart failure does not appear to affect this enzyme activity. Thus, the fate of parasympathetic innervation to the failing heart appears to be quite different from that of sympathetic innervation. Acetylcholine biosynthesis is not impaired, whereas limited investigation suggests that catecholamine biosynthesis is markedly reduced (1-4).

One might postulate that a failure of choline acetyltransferase activity to increase in proportion to myocardial proteins in the failing ventricle contributes to the functional defects of parasympathetic control (5), but this hypothesis requires further investigation. On the other hand, defects in parasympathetic control might involve other components of the system and not necessarily the intrinsic cardiac parasympathetic mechanisms (5); in this case, one would not expect significant alterations in acetylcholine biosynthesis. The functional correlates of the present observations remain to be clarified. However, it is clear that choline acetyltransferase activity per failing ventricle is not reduced in contrast to the reported reduction in activity of tyrosine hydroxylase, the neuronal enzyme that catalyzes the rate-limiting step of norepinephrine biosynthesis (3, 4).

Acknowledgment

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References


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