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Regional Choline Acetyltransferase Activity in the Guinea Pig Heart

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SUMMARY Choline acetyltransferase is the enzyme that catalyzes the biosynthesis of acetylcholine, the neurotransmitter of the pre- and postganglionic parasympathetic system. To assess the extent of parasympathetic innervation, enzyme activity was measured in specialized and contractile regions throughout the guinea pig heart. Enzyme activity in the right atrial appendage was 137 nmol g\(^{-1}\) hr\(^{-1}\). Activity was greatest in the region of the sinus node (187 nmol g\(^{-1}\) hr\(^{-1}\)). Also, enzyme activity was high in the regions of the atrioventricular node (153 nmol g\(^{-1}\) hr\(^{-1}\)), the proximal conduction bundles (133 nmol g\(^{-1}\) hr\(^{-1}\)), and the base of the anterior papillary muscle of the right ventricle (179 nmol g\(^{-1}\) hr\(^{-1}\)), which contains the moderator band and Purkinje fibers. In contrast, the enzyme activity in the inferior interventricular septum and the free walls of the right and left ventricles, which are more predominantly contractile tissue, was 67 ± 6, 108 ± 14, and 56 ± 11 nmol g\(^{-1}\) hr\(^{-1}\), respectively. This activity is significantly lower than in the right atrial appendage. These results suggest that the density of parasympathetic innervation is similar in all the components of the conduction system, from the sinus node to Purkinje tissues. Furthermore, the parasympathetic innervation of regions specialized for conduction is up to four times more dense than that of contractile regions.

ACETYLCHOLINESTERASE activity\(^{1,2}\) and the effects of efferent vagal nerve stimulation\(^{3-6}\) vary considerably throughout the heart. Therefore, it is inferred that the parasympathetic innervation of the heart is non-uniform.\(^{8}\) However, these variations are difficult to quantitate. For example, acetylcholinesterase activity is identified histochemically and variations cannot be readily quantitated; furthermore, acetylcholinesterase activity may be non-specific, since it is found in red blood cells and other non-neural tissues.\(^{9,10}\) In a similar context, the magnitude of chronotropic, dromotropic, and inotropic effects of efferent vagal nerve stimulation may not be representative solely of the density of parasympathetic innervation.

These responses also could be affected by regional variability in cholinergic receptors and sympathetic neural influences.\(^{8}\) In view of these considerations, it seemed appropriate to examine another index of the parasympathetic innervation that might be more specific and also quantifiable.

Accordingly, in guinea pig heart, we have investigated the activity of choline acetyltransferase, the enzyme that catalyzes the biosynthesis of acetylcholine in neural tissues.\(^{5,11,12}\) The in vitro determination of choline acetyltransferase activity minimizes the possible influence of modulating factors and uses optimal concentrations of substrates.\(^{11}\) Thus, the enzyme activity quantitatively represents the parasympathetic innervation in discrete specimens of the heart.\(^{11,12}\)

In previous studies of the guinea pig heart, we determined the choline acetyltransferase activity in contractile tissues, the atria, and ventricles.\(^{11,12}\) The present study was designed to assess choline acetyltransferase activity in specialized regions containing pacemaking and conducting tissues and to compare the activity in specialized and contractile regions.
Methods

Tissues were obtained from eight male guinea pigs weighing 650–800 g. The animals were killed with a blow to the base of the neck and the hearts were excised and dissected. Tissue specimens were blotted, weighed, and stored in liquid nitrogen. This was accomplished within 30 minutes; we find that the enzyme activity in heart tissue under these conditions is stable over intervals of at least 45 minutes.

The specialized regions were dissected using the landmarks specified by Anderson.15 The sinoatrial node (SA node) was contained in a wedge of tissue bounded by the superior vena cava-atrial junction, the midportion of the atrial and inferior vena cava, and the midportion between the superior vena cava and the interatrial septum. The wedge excluded the atrial appendage. The atrioventricular node (AV node) was contained in a rectangular block of tissue bounded by the coronary sinus ostium, the posterior membranous septum, and the points just inferior to the attachments of the septal leaflet of the tricuspid valve. The proximal conduction bundles were contained in the anterior superior interventricular septum. The base of the anterior papillary muscle of the right ventricle and a portion of the right ventricular specialized conducting system were taken as a sample with Purkinje tissue. In three separate guinea pigs, smaller samples were taken from the middle of these regions, as close as possible to the structures of interest. The intent was to exclude contractile tissues and obtain more discrete samples of the specialized regions.

Choline acetyltransferase activity was measured by determining the amount of [14C]acetylcholine formed from labeled acetylcoenzyme A.11 To prepare stored tissues for this purpose, samples were disrupted further with a Teflon-to-glass, motor-driven homogenizer in a minimum amount (less than 3 ml) of buffer [potassium phosphate (5 ITIM), ethylenediaminetetraacetic acid (EDTA) (0.1 mM), pH 7.4]. The assay of acetylcholine biosynthesis in myocardial tissues was carried out as described previously.12 The final incubation mixture contained [14C]-acetylcoenzyme A (50 μM) (50 μCi/μmol), choline chloride (2 mM), extract, potassium phosphate (50 mM), EDTA (0.1 mM), eserine sulfate (0.1 mM), Triton X-100 (0.1%), and KCl (100 mM), all adjusted to pH 7.4 in a final volume of 20 μl. Incubations were at 37°C for 15 minutes. The enzyme activity was proportional to the amount of protein in the assay. The reaction was stopped by adding 10 μl of acetylcholine chloride (100 mM)-acetylcarnitine (100 mM)-formic acid (0.5 N) solution. To resolve [14C]-acetylcholine from labeled precursor, samples were subjected to low-voltage (35 V/cm) paper electrophoresis (Whatman no. 1) in a 2% formalin-acetic acid-8% acetic acid solution for 20 minutes in a Gelman deluxe electrophoresis apparatus. After 15 minutes of drying at 100°C to remove volatile acid, acetylcholine and acetylcarnitine were developed in an I2 chamber, and the marker zones of acetylcholine were cut out for determination of radioactivity as described previously.11,16 The activity of choline acetyltransferase, a neuronal enzyme, is distinct from that of carnitine acetyltransferase, a mitochondrial enzyme.11 Kinetic characterization of choline acetyltransferase activity in the various tissues was performed as described previously.17

Comparisons were made using analysis of variance and Tukey's test for multiple group means.14

Results

Choline acetyltransferase activity was greatest in the regions of the SA node, the AV node, the proximal conduction bundles, and the base of the anterior papillary muscle and the moderator band of the right ventricle (Fig. 1). Elsewhere, in the contractile regions, enzyme activity

![Figure 1: Choline acetyltransferase activity in the guinea pig heart. Samples with pacemaker and conducting tissues are indicated by stippling. The bars represent the values as percentages of the levels in the right atrial appendage. The absolute values (mean ± SE) are above the bars; these are expressed as nmol hr⁻¹ g⁻¹. Analysis of variance of this data indicated differences as noted.14 RA Ap = right atrial appendage, SA node = sinoatrial node, IA septum = interatrial septum, LA Ap = left atrial appendage, AV node = atrioventricular node, Prox (Cond) Bundles = proximal conduction bundles, Pap Mus and Mod Band = anterior papillary muscle and moderator band of the right ventricle, Sup = superior, Inf = inferior, Post = posterior, and Ant = anterior.](image-url)
was significantly lower except in the right atrial appendage. The $K_m$, using acetylcoenzyme A as substrate was similar for all the tissues and varied from 16 to 20 $\mu$M so that the differences in choline acetyltransferase activity among tissues were related to the quantity of enzyme and not to qualitative differences in enzyme function.

To test the possibility that excessive contractile tissue contaminated the samples of specialized tissues and artifically reduced the values of choline acetyltransferase activity, we excised tissues from more circumscribed areas of the specialized regions. The ratios of the weights of the larger to the circumscribed tissue samples (in mg) were 42/12 for the SA node, 114/23 for the AV node, 127/40 for the proximal bundles, and 27/6 for the moderator band. Choline acetyltransferase activity in the circumscribed samples averaged $160 \pm 50$ nmol hr$^{-1}$ g$^{-1}$ ($\pm$SE) for the sinus node, $117 \pm 7$ for the atrioventricular node, $92 \pm 10$ for the proximal bundles, and $93 \pm 29$ for the moderator band. These values were not higher than the corresponding values for larger tissue samples. In fact, localizing the sample more specifically to the areas identified by Anderson as specialized tissues had no significant effect on the values.

Despite considerable variability in choline acetyltransferase activity among (1) the specialized regions, (2) the right ventricular free wall, and (3) the left ventricular free wall, there was relatively little variability among the SA node, the AV node, the proximal conduction bundles and the moderator band, or within the right or left ventricle.

**Discussion**

The justification for using the activity of choline acetyltransferase as a quantitative index of the parasympathetic innervation is based on several considerations. First, the enzyme is found almost exclusively in neurons. Enzyme activity in rat heart cells in culture, for example, is undetectable (unpublished observations). The only documented non-neuronal source is primate placenta. Second, enzyme activity is reduced by more than 95% in the atria of rat hearts denervated by transplantation (unpublished observations). Third, the in vitro conditions for the radiochemical assay of choline acetyltransferase are standardized so that optimal tissue enzyme activity is determined. Under these conditions, the values provide an index of the relative density of the parasympathetic innervation in various regions of the heart.

In the pacemaker and conduction regions of the guinea pig heart, the average activity of choline acetyltransferase varied from 187 to 133 nmol hr$^{-1}$ g$^{-1}$. In the other regions, the average enzyme activity varied from 137 nmol hr$^{-1}$ g$^{-1}$ in the right atrial appendage to 47 nmol hr$^{-1}$ g$^{-1}$ in the anterior inferior left ventricular free wall. Thus in specialized regions, the enzyme activity tended to be relatively uniform. Among the four heart chambers, the enzyme activity varied considerably, but within the right or left ventricle, the activity tended to be uniform.

There was a 2-fold decrease in the activity of choline acetyltransferase between the specialized tissues and the right ventricular free wall. In contrast, there was a 3-fold decrease in the activity of choline acetyltransferase between these tissues and the left ventricular free wall. Thus, there tends to be a greater reduction in the density of parasympathetic nerves in the transition zone between specialized tissues and the left ventricular free wall than there is between these tissues and the right ventricular free wall. Once this transition has occurred, however, the density of innervation in either ventricle is relatively uniform. This is more evident in the left ventricular free wall where four samples of tissue, excluding the papillary muscle, had similar enzyme activity (Fig. 1). The functional significance of these observations remains to be established.

It has not been possible in the guinea pig to quantify the choline acetyltransferase activity in preganglionic and postganglionic parasympathetic neurons. Guinea pigs have not survived bilateral cervical vagotomies or cardiac transplants despite numerous attempts to perform these studies in our laboratory. Therefore, we cannot comment about the regional pre- and postganglionic parasympathetic innervation.

In the present study, the pattern of a higher choline acetyltransferase activity in specialized regions of the guinea pig heart conforms to the predominant histochemical localization of acetylcholinesterase in these same regions in the guinea pig13 and other species.1-5

The regional distribution of choline acetyltransferase activity in the guinea pig heart in the present study and in our previous studies differs somewhat from the regional distribution of acetylcholine in the hearts of cats and muscarinic cholinergic receptors in the hearts of rabbits. In our studies, the rank order of tissues with decreasing enzyme activity is the right atrium > right ventricle > left atrium > left ventricle. In contrast to this, the rank order of decreasing acetylcholine concentration is right atrium > left atrium > right ventricle > left ventricle. The corresponding rank order for muscarinic cholinergic receptors is left atrium > right atrium > right ventricle = ventricular septum = left ventricle. These differences could represent significant variations in the patterns of distribution of parasympathetic neurons, acetylcholinesterase, and muscarinic receptors throughout the heart, or they could be simply the result of species differences. This will have to be investigated.

It is interesting that the activity of choline acetyltransferase is similar in the SA node, the AV node, and the regions with ventricular conduction tissues. This contrasts with our data on norepinephrine turnover in the guinea pig heart (unpublished observations). The turnover rate constant ($K_{NE}$), which is an index of sympathetic neural activity, is high in the SA node and significantly lower in the other specialized regions. This observation and the current data on choline acetyltransferase activity suggest that parasympathetic-sympathetic interactions in the SA node might differ from those in the other specialized regions.

The average choline acetyltransferase activity in the guinea pig heart varied from 49 to 187 nmol hr$^{-1}$ g$^{-1}$. In contrast, acetylcholine turnover has been determined by Haubrich and co-workers to be 12 nmol hr$^{-1}$ g$^{-1}$ in whole guinea pig heart. The difference between enzyme activity in vitro and acetylcholine turnover in vivo may be explained by the differences in choline concentration. A 2
mm choline concentration ($K_m = 0.5 \text{ mm}$) was used in vitro, whereas, the tissue levels in heart are reported to be 0.05 mm.$^{18}$ These considerations are consistent with the concept that acetylcholine biosynthesis is regulated by choline availability and uptake into neurons rather than total enzyme activity.$^{20}$

The present results represent the first attempts to quantify the parasympathetic innervation of specialized cardiac regions containing pacemaking and conducting tissues and to compare systematically the parasympathetic innervation of multiple areas in the right and left ventricular free walls. The innervation appears to be relatively dense and similar in the sinus node, the atrioventricular regions, and the more peripheral Purkinje tissues. In contrast, the innervation of the right and left ventricular free walls is less than in specialized regions. However, within the ventricular free walls, enzyme activity tends to be uniform. These observations and data in the literature$^{16,17}$ raise further questions about the significance of regional variations in the cardiac parasympathetic innervation and about the relative distribution of cholinergic nerves and receptors in specialized and contractile regions of the heart.

References