

REGIONAL DISTRIBUTION OF TYROSINE HYDROXYLASE AND DOPAMINE β -HYDROXYLASE ACTIVITIES IN GUINEA PIG HEART

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ABSTRACT

The sympathetic and parasympathetic autonomic nervous systems regulate heart rate and myocardial contractility. Using sensitive radioisotopic assays, we examined the regional distribution of tyrosine hydroxylase and dopamine β -hydroxylase activity, which are markers for sympathetic autonomic innervation, in specialized pacemaker and conduction tissue and in contractile heart tissue. Of the 20 regions of guinea pig heart examined, we find that the highest activities occur in the sinoatrial node and the right atrial appendage. Intermediate activity occurs in the left atrium, interatrial septum and right ventricular papillary muscle. Activities in the remainder of the heart are lower and rather uniform. Comparing the enzyme markers for the parasympathetic [17] and sympathetic system, we find that these systems have different distributions. The former, for example, is closely associated with the specialized pacemaker and conduction system including the sinoatrial and atrioventricular nodes and regions rich in Purkinje fibers. The sympathetic system, on the other hand, is associated with both contractile and conducting tissue.

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INTRODUCTION

The sympathetic and parasympathetic divisions of the autonomic nervous system play a pivotal role in the regulation of heart rate, myocardial contractility and cardiac conduction. In general, activity of the sympathetic system increases heart rate and myocardial contractility and the parasympathetic system mediates the opposite responses [10]. There is, furthermore, a functional link between the parasympathetic and sympathetic system which allows modulation of one system by the other through interactions involving both presynaptic and postsynaptic mechanisms [10]. The activity of choline acetyltransferase, which catalyzes acetylcholine synthesis and is a neurochemical marker of the parasympathetic system has been reported [16,17].

The neurotransmitter of the postganglionic sympathetic neuron which innervates the heart is norepinephrine. Tyrosine serves as a precursor for its synthesis in a 3-step reaction pathway. The rate-limiting enzyme for norepinephrine biosynthesis in guinea pig heart is tyrosine hydroxylase which catalyzes the conversion of tyrosine to dihydroxyphenylalanine (DOPA) [9]. After the enzymatic conversion of DOPA to dopamine, the final step in biosynthesis is catalyzed by dopamine β -hydroxylase activity [14]. The activity of aromatic amino acid decarboxylase, which is also required for norepinephrine synthesis, remains unchanged. We determined the activities of tyrosine hydroxylase and dopamine β -hydroxylase in the specialized pacemaker and conduction tissue and in the contractile regions analogous to the previous study [17]. These enzymes serve as specific markers for the postganglionic adrenergic neurons [14]. Our experiments indicate that the regional distribution of the sympathetic systems in guinea pig heart differs from that of the parasympathetic system.

MATERIALS AND METHODS

Tissue preparation

Male guinea pigs (600–800 g) were killed by a blow to the base of the neck and the hearts were excised and dissected according to the format shown in Fig. 1. The samples were blotted, weighed, frozen and stored in liquid nitrogen within 10 min after sacrifice. Pilot studies showed that the activity of each enzyme is stable in liquid nitrogen for at least 10 months. Subsequent manipulations were performed at 0–4°C unless otherwise noted. Tissues were homogenized (20 vols. of 5 mM potassium phosphate–0.1 mM EDTA (pH 7.4) per g wet weight) with four 10 s bursts with a Tekmar tissue-mixer at a speed of 70% maximum. After aliquots of 10% (v/v) Triton X-100 were added to the homogenate to give a final concentration of 0.2%, the samples were centrifuged at 13,000 g (4 min) in an Eppendorf microfuge and portions of the supernatant were taken for tyrosine hydroxylase and dopamine β -hydroxylase determinations.

Enzyme determinations

Tyrosine hydroxylase activity was measured in duplicate 50 μ l portions of the extract during a 15 min incubation at 37°C using the procedure of Coyle [5]. The final concentration of [3 H]L-tyrosine and 2-amino-4 hydroxy-6,7-dimethyltetrahydropteridine (Cal Biochem) were 0.2 mM and 0.9 mM, respectively. The rate of the reaction was linear for 20 min and was proportional to protein concentrations up to 5 mg/ml under these conditions. Dopamine β -hydroxylase activity was measured in 50 μ l portions by the procedure of Coyle and Axelrod [6]. Incubations were performed for 30 min (37°C) and activity was proportional to protein concentrations to 5 mg/ml. The rates were linear for at least 60 min.

Radioisotopes were purchased from New England Nuclear. Chemicals and drugs, unless otherwise specified, were purchased from Sigma Chemical. Significance of differences between means of multiple samples was assessed by analysis of variance and Tukey's test [18]. A $P \leq 0.05$ was used as the criterion of significance. All assays were performed in duplicate on the same homogenate and the duplicate determinations were averaged. The means from 6 different animals are reported.

RESULTS

To resolve specialized pacemaker and conducting tissue of the heart from contractile tissue, the dissections were performed using the landmarks specified by Anderson [2]. The primary pacemaker of the heart, the sinoatrial node (SA) node, is found in guinea pig at the junction of the superior vena cava and right atrium as depicted in Fig. 1 and fully described by Anderson [2]. The impulse generated in the SA node travels through the atrium to the

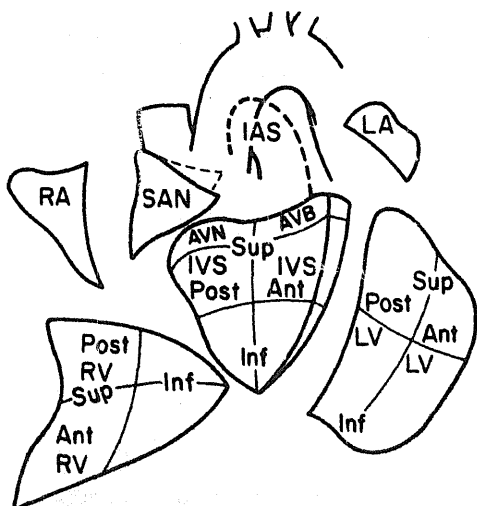


Fig. 1. Dissection of the guinea pig heart into specialized pacemaker and contractile regions for enzyme and receptor determinations. RA, right atrium; SAN, sinoatrial node; LA, left atrium; AVN, atrioventricular node; AVB, atrioventricular bundle; Sup, superior; Inf, inferior; Ant, anterior; Post, posterior; RV, right ventricle; LV, left ventricle.

TABLE I

REGIONAL DENSITY OF TYROSINE HYDROXYLASE AND DOPAMINE β -HYDROXYLASE ACTIVITY IN HEART

The values given represent the means \pm S.E. determined from 6 animals. Data for choline acetyltransferase from ref. 17. N.D., data not determined.

Region	Tyrosine hydroxylase (nmol/h/g)	Dopamine β -hydroxylase (nmol/h/g)	Choline acetyltransferase (nmol/min/g)	mg wet weight
Sinoatrial node	207 \pm 15	1 600 \pm 170	187 \pm 37	17.4 \pm 1.9
Atrioventricular node	45 \pm 16	620 \pm 90	153 \pm 26	21.7 \pm 2.1
Atrioventricular bundle	47 \pm 9	600 \pm 74	133 \pm 15	27.5 \pm 4.5
Right ventricular moderator band	105 \pm 10	890 \pm 110	179 \pm 19	13.2 \pm 1.6
Right atrial appendage	234 \pm 33	1 800 \pm 200	137 \pm 14	27.3 \pm 1.0
Left atrial appendage	130 \pm 32	1 100 \pm 200	64 \pm 7	58.9 \pm 3.7
Interatrial septum	129 \pm 9	950 \pm 92	82 \pm 11	13.3 \pm 1.7
Interventricular septum				
Superior anterior	33 \pm 7	530 \pm 67	N.D.	58.3 \pm 7.3
Inferior anterior	27 \pm 4	590 \pm 120	N.D.	46.1 \pm 7.3
Superior posterior	28 \pm 8	560 \pm 110	N.D.	48.9 \pm 4.9
Inferior posterior	27 \pm 3	560 \pm 100	N.D.	40.2 \pm 5.3
Right ventricle				
Superior anterior	74 \pm 8	880 \pm 130	108 \pm 14	97.5 \pm 4.6
Inferior anterior	64 \pm 11	910 \pm 140	N.D.	61.5 \pm 4.0
Superior posterior	67 \pm 7	760 \pm 160	94 \pm 11	61.0 \pm 6.4
Inferior posterior	62 \pm 5	920 \pm 140	N.D.	54.7 \pm 2.4
Left ventricle				
Superior anterior	39 \pm 5	670 \pm 120	55 \pm 12	152 \pm 19.9
Inferior anterior	27 \pm 5	590 \pm 140	47 \pm 9	133 \pm 8.0
Superior posterior	33 \pm 4	640 \pm 140	49 \pm 6	151 \pm 6.2
Inferior posterior	20 \pm 3	560 \pm 130	56 \pm 11	144 \pm 5.8
Papillary muscle	33 \pm 8	540 \pm 81	85 \pm 20	42.4 \pm 2.5

atrioventricular (AV) node where conduction is momentarily delayed. The AV node and its extension, the atrioventricular bundle, are found in the posterior and anterior portions, respectively, of the superior interventricular septum (Fig. 1). Conduction continues in the right and left bundles to Purkinje fibers distributed throughout the ventricles. The right ventricular papillary muscle is enriched with the specialized Purkinje fibers of the moderator band. These segments include, in addition to these specialized tissues, surrounding contractile tissue because finer resolution is unobtainable at the macroscopic level. The other segments taken consist largely of contractile tissue. Nerves, blood vessels, and connective tissue, however, occur in all samples to a variable extent.

The density (activity/g wet weight) of tyrosine hydroxylase and dopamine β -hydroxylase activity is greatest in the sinoatrial node and right atrial appendage (Table I). These values, which are not significantly different from each other, are significantly higher ($P < 0.05$) than all other regions of the heart. The values in the right ventricular moderator band, left atrium, and interatrial septum are about 50% and those in the AV node and bundle are about 25% of those in the right atrium. These differences are statistically significant by analysis of variance. Compared with the right atrial appendage, the values in the right ventricle are about 35% and those in the interventricular septum and left ventricle are about 15% as great. The difference between 15 and 35% is not statistically significant. The rank order of density of both tyrosine hydroxylase and dopamine β -hydroxylase activity, the differences of which are significantly different is right atrium $>$ left atrium $>$ right ventricle \approx left ventricle.

In general, the activities of both enzymes parallel one another. In terms of absolute activity, that of tyrosine hydroxylase is about 5–15% that of dopamine β -hydroxylase. This is consistent with the general notion that the former enzyme is rate-limiting in catecholamine biosynthesis [9,14]. There are, however, regional differences in the relative amounts of the two activities. In the atria, for example, the tyrosine hydroxylase activity is about 10–12% that of dopamine β -hydroxylase activity. In the ventricles and interventricular septum, however, corresponding activity is only 6–8%. Since the determinations were performed with crude homogenates, possible alterations in activity by activators or inhibitors are possible. Tyrosine hydroxylase activity, for example, is altered by phosphorylation reactions associated with cAMP-dependent protein kinase [1,12,13,19]. It is also inhibited by catecholamines [9] which are present in the homogenate. Furthermore, dopamine β -hydroxylase activity is inhibited by an endogenous component which is reversed by Cu^{2+} [6]. These components may be altering the observed activities differentially in vitro to produce changes here which are not physiologic in nature. The relationship between the action of these effectors in vitro and nerve activity in vivo remains to be established.

These differences, on the other hand, may reflect conditions in vivo. Angelakos, for example, reported that the guinea pig SA nodal region and atria contain 4- to 8-fold more dopamine than the ventricles [3,4]. One pos-

TABLE II
REGIONAL CONTENT OF TYROSINE HYDROXYLASE AND DOPAMINE
 β -HYDROXYLASE ACTIVITIES IN GUINEA PIG HEART

The values given represent the means \pm S.E. determined from 6 animals

	Tyrosine hydroxylase (nmol/h)	Dopamine β -hydroxylase (nmol/h)
Right atrium	10	84
Left atrium	7.8	64
Interatrial septum	1.8	12
Right ventricle	23.6	252
Left ventricle	18.5	320
Interventricular septum	7.8	138

sible explanation is that chromaffin cells containing dopamine may be present in intracardiac ganglia in this region [7]. These cells, lacking dopamine β -hydroxylase, would account for the higher dopamine content and the lower activity of this enzyme. It might be that the dopamine β -hydroxylase reaction or dopamine uptake into the synaptic vesicles plays a regulatory role in norepinephrine biosynthesis [8]. Alternatively, there may be relatively more tyrosine hydroxylase than dopamine β -hydroxylase activity due to activation of tyrosine hydroxylase in response to relatively greater nerve activity in these regions. Such activation in the guinea pig vas deferens appears to be dependent on protein phosphorylation [19]. More experiments are required to distinguish between these and other possibilities.

In addition to activity per unit weight, activity per region was determined. In this analysis, the activity of the SA node and right atrial appendage was combined and that of the AV node and AV bundle are included with the activity of the interventricular septum. Because of the large mass of the ventricles, the preponderance of total enzyme activity is found here. For example, about 38% of the total heart tyrosine hydroxylase activity is found in the left ventricle and interventricular septum and 34% is found in the free wall of the right ventricle (Table II). Similarly, about 52% of total cardiac dopamine β -hydroxylase activity is found in the left ventricle and interventricular septum and 28% occurs in the right ventricular free wall.

DISCUSSION

The dissection of the guinea pig heart into specialized pacemaker and conduction tissue which is resolved from (although contaminated by) contractile tissue permits an analysis of the potential relative influence of the sympathetic and parasympathetic systems in these regions. From our previous study, we found that the primary pacemaker of the heart — the sinoatrial node — contained the greatest activity of choline acetyltransferase of any heart

region [17]. In conducting elements, enzyme activity was also high in the atrioventricular node, the proximal bundle and the right ventricular papillary muscle (where the Purkinje tissue of the moderator band occurs). Choline acetyltransferase activity was high in the right atrial appendage, which in addition to contractile function, plays an important role in conducting the impulse from the sinoatrial node to the atrioventricular node. Tyrosine hydroxylase and dopamine β -hydroxylase activity, markers for the sympathetic system, also exhibit their highest activity in the sinoatrial node and the right atrial appendage. In contrast to choline acetyltransferase activity and the parasympathetic system, however, tyrosine hydroxylase and dopamine β -hydroxylase activity in the atrioventricular node, proximal bundle and the moderator band are no greater than that of the surrounding contractile tissue. The non-uniform distribution of norepinephrine determined spectrophotofluorometrically parallels that of its biosynthetic enzymes. In guinea pig, the rank order of norepinephrine content is right atrium > left atrium > right ventricle > left ventricle which parallels enzyme activity [3]. Based on the activities of these enzyme markers, the sinoatrial node receives dense innervation from the sympathetic and parasympathetic systems. In contrast to the parasympathetic system, the atrioventricular node, proximal bundle and right ventricular papillary muscle (and the conducting moderator band contained therein) receive proportionately less sympathetic innervation than the sinoatrial node.

In samples consisting mainly of contractile elements, the right atrium contains the greatest density of sympathetic and parasympathetic neuronal enzyme activity. The left atrium and right ventricle contain intermediate levels and the left ventricle contains the lowest density of both the sympathetic and parasympathetic neuronal markers.

The physiologic response to sympathetic and parasympathetic nerve stimulation in dog heart is non-uniform and the response parallels that of the neurotransmitter biosynthetic enzymes: right atrium > left atrium > right ventricle > left ventricle [11,15]. These differences do not parallel the receptor distribution, at least in guinea pig heart.

The distribution of sympathetic neuronal marker enzymes is not greater in the specialized pacemaker and conduction tissue than in the surrounding contractile tissue. This suggests that it functions in the regulation of myocardial contractility as well as heart rate regulation. This correlates with the more profound effects of sympathetic nerve stimulation on ventricular contraction and the weaker response of ventricular contraction seen after parasympathetic nerve stimulation [11,15].

Furthermore, the differences in the relative activity of the sympathetic and parasympathetic system may play a role in the interaction of these two systems. For example, the ratio of choline acetyltransferase to tyrosine hydroxylase is greater in the atrioventricular node and moderator band. In these regions, parasympathetic action might more readily overcome the action of the sympathetic system. Future experiments are required to test this hypothesis.

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