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ACETYLCHOLINE, CHOLINE ACETYLTRANSFERASE AND CHOLINESTERASES IN THE RAT HEART

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Abstract—The distribution of acetylcholine (ACh), choline acetyltransferase (CAT), acetylcholinesterase (AChE) and cholinesterase (ChE) in the rat heart was investigated. The hearts were dissected into various regions and homogenized. Substrate specificity and inhibition studies indicated the presence of two cholinester hydrolyzing enzymes. Both hydrolyze ACh and either acetyl-β-methylcholine or propionylcholine and butyrylcholine respectively. Enzyme activity was found to be higher in the atria than in the ventricles. In both the atria and the ventricles, a higher enzyme activity was found in the right than in the left portions. The enzyme hydrolyzing propionylcholine was the prevailing enzyme. The distribution of ACh was determined by pyrolysis-gas chromatography. ACh concentrations were found to be higher in the atria than in the ventricles with the right side having the higher ACh content in both portions: right atrium, 30.4 nmoles/g, compared to left atrium, 13.1 nmoles/g; and right ventricle, 4.2 nmoles/g, compared to left ventricle, 2.5 nmoles/g. CAT activity was greatest in the right atrium, 12.4 nmoles/g/min, and right ventricle, 11.0 nmoles/g/min, followed by the left ventricle, 8.1 nmoles/g/min and left atrium, 7.6 nmoles/g/min. The distribution of ACh and CAT is in good agreement with the histochemical localization of AChE and ChE and the parasympathetic fibers in these areas of the heart.

While most investigators agree that there is vagal innervation of the atria, ventricular parasympathetic innervation has been subject to considerable debate. Although the presence of parasympathetic fibers throughout the ventricles is now established, their density is considerably lower than in the atria [1].

The distribution of acetylcholine (ACh) and the activity of its synthetic enzyme, choline acetyltransferase (CAT), and its hydrolytic enzymes, acetylcholinesterase (AChE) and butyryl cholinesterase (BuChE), have been studied previously for rat heart preparations; the resulting data [2–13], however, are controversial. The total ACh values for whole heart (3.08 μg/g [2] to 6.34 μg/g [5] of some of these earlier studies [2, 4, 5, 8–10] are truly in conflict, with others [3, 6, 7, 11] that report values of 5.9 μg/g for atria only. These discrepancies led us to reinvestigate the presence of the cholinergic system in rat heart. Few systematic studies have been made on the distribution of ACh, CAT, AChE and BuChE in left and right atria, left and right ventricles, and the septum. Therefore, the present study was undertaken in an effort to examine these separate regions with respect to the distribution of ACh and its enzymes, AChE, BuChE and CAT, utilizing improved analytical techniques for the latter two enzymes and specific substrates for the ACh hydrolyzing enzymes.

MATERIALS AND METHODS

Male Sprague–Dawley rats (200–300 g) were maintained on a 12 hr light/dark cycle with food and water available *ad lib.* for at least 1 week prior to experiments. Animals were killed by decapitation between 9.30 and 11.30 a.m. The hearts were excised and divided into five distinct portions, viz. right atrium, left atrium, septum, right ventricle and left ventricle. Tissues were rinsed in 0.9% saline and utilized as described in the following sections.

Enzyme assays. Tissues were kept on ice, blotted dry, weighed, and homogenized in Ringer solution with a Polytron ST-10 homogenizer (Brinkmann Instruments).

AChE and cholinesterase (ChE) activity was measured by the colorimetric method of Hestrin [14] which determines the residual substrate after periods of incubation. The substrates used were 5 mM acetylcholine chloride (ACh), 10 mM butyrylcholine iodide (BuCh), 10 mM acetyl-β-methylcholine bromide (MeCh), or 5 mM propionylcholine iodide (PrCh) as substrates at pH 7.7 and approximately 23°. The lower limit of measurements was about 0.04 μM of ACh/ml. When iso-OMPA (50 μM, tetramonoisopropylpyrophosphortetramide), a specific inhibitor for butyrylcholinesterase (BuChE), was used, the tissue was preincubated for 30 min with the inhibitor. The remaining enzyme activity was then determined in the presence of inhibitor and substrate.

CAT activity was measured in tissue homogenates preincubated for 30 min at approximately 0° with 0.4 mM physostigmine sulfate and incubated for 2 hr at pH 7.4 and 37° with 0.5 mM [^{14}C]acetyl coenzyme A, 30 mM choline iodide and 0.2 mM physostigmine sulfate. The rate of ACh synthesis was determined using the liquid cation extraction method of Fonnum [15].

ACh content. Portions of the dissected hearts were blotted, weighed, and homogenized in acetonitrile with propionylcholine iodide as internal standard. Tissue to acetonitrile ratio was less than 1g/10 ml, sample size was always 2 or 5 ml, and the period from time of death to homogenization was less than 2 min for

Table 1. Distribution of cholinester hydrolysis in rat heart*

	Right atrium	N	Left atrium	N	Right ventricle	N	Left ventricle	N	Septum	N
Tissue wt	21.8±1.1	32	16.7±1.1	32	111.7±3.1	48	302.5±8.8	42	117.0±4.0	56
ACh	302.7±16.3	6	243.3±12.2	6	158.5±14.3	12	114.7±6.5	12	119.0±4.0	12
PrCh	697.1±53.5	8	653.5±40.3	8	357.3±7.1	6	312.6±22.3	6	281.0±12.0	12
BuCh	525.2±37.3	6	462.2±27.5	6	268.4±8.5	12	222.1±10.2	6	221.0±11.0	12
MeCh	33.4±4.7	4	26.3±3.1	4	10.2±1.4	10	5.3±1.1	10	7.0±1.0	10
ACh†	107.4±8.6	8	62.2±12.4	8	28.7±1.6	8	15.8±0.6	8	18.0±3.0	8
	35.5%		25.6%		18.1%		13.8%		15.1%	

* Enzyme activity is given as μmoles of substrate hydrolyzed/g wet wt/hr (mean±S.E.).

† Determined in the presence of 50 μM iso-OMPA. The per cent values are per cent remaining activity when compared to hydrolysis in the absence of iso-OMPA.

atria and less than 5 min for septum and ventricles. Further, it should be noted that tissue was kept on ice until homogenized. Samples were prepared for pyrolysis-gas chromatography according to the method of Schmidt *et al.* [16]. This technique was used to minimize interference from endogenous choline. Pyrolysis-gas chromatography conditions were as described previously [17], except that nitrogen was used as the carrier gas with a flow rate of 60 ml/min, with a column temperature of 122°. For comparisons of means, Student's '*t*' test was used.

RESULTS

Distribution of ChE. ChE activities of different portions of the heart are shown in Table 1. All heart regions hydrolyze PrCh and BuCh more rapidly than ACh and MeCh. Hydrolysis rates are highest in the right atrium, followed by left atrium, right ventricle, left ventricle and septum. The latter two show very little difference in hydrolytic activity. The hydrolysis pattern of the four substrates is consistent with the presence of at least two different enzymes, both of which can hydrolyze ACh at different rates and hydrolyze either MeCh or PrCh and BuCh respectively. To investigate this possibility, the effect of iso-OMPA on the hydrolysis of these substrates was examined (Table 1). The inhibitor-treated tissue showed virtually no activity for hydrolyzing BuCh and a significantly reduced hydrolytic activity for ACh (<0.001).

Distribution of CAT activity and ACh in the heart. CAT activity and ACh distribution are shown in Table 2. The enzyme activity of the right atrium and right ventricle is significantly greater than that of the other three portions ($P < 0.01$) with the septum having the lowest activity. The atria show the highest amount of ACh and both the right atrium and the right ventricle contain significantly higher amounts of ACh than the corresponding left components ($P < 0.01$). There is about a 12-fold difference between the highest amount of ACh found in the right atrium (30.4 nmoles/g), and the lowest value, found in the left ventricle (2.5 nmoles/g). The heart portions with higher rates of MeCh and ACh hydrolysis also show higher CAT activity and ACh content. Neither PrCh nor BuCh was found in any of the tissues tested.

Table 2. Distribution of ACh and CAT in rat heart

	ACh (nmoles/g wet wt)	N	CAT*	N
Right atrium	30.4±2.1	4	12.40±0.56	5
Left atrium	13.1±1.6	4	7.62±0.18	5
Right ventricle	4.2±0.2	4	11.06±0.53	5
Left ventricle	2.5±0.4	4	8.18±0.80	5
Septum	2.7±0.3	4	6.28±0.48	4

* Enzyme activity is given as nmoles ACh synthesized/g wet wt/min.

DISCUSSION

The data presented in Table 1 indicate the presence of at least two forms of ChE in all of the heart regions tested, viz. BuChE and AChE. The former, as indicated by the high rate of hydrolysis of BuCh and PrCh, appears to be the prevailing enzyme. Also, it should be noted that the atria, relative to the other heart sections, and the right side, as compared to the left side of the heart, contain a higher percentage of AChE as evidenced by the experiment utilizing iso-OMPA, a specific inhibitor of BuChE. The distribution of the ACh hydrolyzing enzymes, as presented in Table 1, is in reasonable agreement with and amplifies earlier investigations [12, 13, 18], in which no distinction was made between left and right portions of the heart and the specific choline esters BuCh and PrCh were not used as substrates.

While the ACh values contained in this report are in the same range as some earlier reports [3, 6, 7, 11], they are considerably lower than those of several other reports [2, 4, 5, 8–10]. Reports of ACh levels for whole heart, such as 3.91 μg/g [10], 3.08 μg/g [2], or even 6.34 μg/g [5], are in the same range as values reported for whole brain or specific brain regions [19, 20]. That this could be the case seems highly unlikely. A possible explanation for the high levels found in the only earlier report [10] which utilized gas chromatography is interference from endogenous acetylcarnitine. In the original use [21] of this technique, the authors reported that large quantities of acetylcarnitine could lead to erroneously high ACh determinations. While acetylcarnitine levels in the brain have been reported as below 10 nmoles/g, reported heart levels are 377 nmoles/g [22]. The earlier reports [2, 4, 5, 8, 9] with higher ACh levels were all determined by bioassay and do not agree with other bioassay findings [3, 6, 7, 11].

Further, it should be noted that these earlier reports, as is the case for the ChE determinations, suffer from a limited scope of investigation because either ACh content was determined for whole heart [2, 4, 5, 8, 10], or pooled atria [3, 6, 7], with no distinction between right and left, or were limited to the left and right auricles only [11].

The distribution of ACh, CAT, AChE and BuChE is in good agreement with the histochemical localization of AChE and BuChE [23]. Activity for both enzymes was demonstrated in the atrioventricular node, bundle of His, its two main branches, the neurons of the cardiac ganglia, their postganglionic fibers, and in atrial and ventricular muscle fibers. The activity was greater in the atria than in the ventricle.

Whether ACh and CAT are limited to nerve elements or are perhaps found in the myocardial cells as well remains to be seen.

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