6. S. K. Sudakov, Byull. Éksp. Biol. Med., No. 6, 662 (1980).
7. H. Hyden, Nature, 184, 433 (1959).
8. G. M. Prisco, M. Baney-Schwartz, and H. D. Strecker, in: Pyridine Nucleotide-Dependent Dehydrogenases (H. Sund, ed.), Berlin (1970), p. 305.

MORPHOMETRIC DETECTION OF SPECIALIZED INTERNODAL CONDUCTING PATHWAYS OF THE HEART

E. R. Pavlovich and I. A. Chervova

UDC 611.127-018.86

KEY WORDS: specialized internodal pathways of the heart; morphometry.

Three basic hypotheses are constantly discussed in the literature on conduction of the impulse from the cardiac pacemaker — the sinus node (SN) to the atrioventricular node (AVN): 1) There are no specific conduction pathways in the atria and the impulse spreads over the whole working myocardium [1, 8]; 2) selective conduction pathways exist, i.e., pathways spreading the impulse more rapidly than the surrounding myocardium but without any specialized morphological substrate [7, 11-13]; 3) specialized pathways exist, i.e., pathways differing in structure from the working myocardium and conducting the impulse more rapidly than the surrounding myocardium of the right atrium (RA) [4-6, 9, 10]. It is difficult to give an unequivocal answer to this question because of the absence of a common nomenclature in investigations at different structural levels of organization (anatomy, histology, cytology) [5, 9, 10], and also the absence of morphofunctional correlations [4, 7, 13]. The technical difficulties encountered in previous investigations resulted from the study of hearts of relatively large animals: rabbit, monkey, dog, man [6, 9, 12], in which it is difficult to detect components of the intracardiac conduction system because of constraints imposed by the methods of light and electron microscopy and microelectrode techniques for such material. Furthermore, accurate quantitative methods have not been adequately used in the search for specialized conduction pathways [6] and most workers have contented themselves with qualitative, descriptive and, consequently, subjective criteria of identification.

The object of this investigation was to attempt to obtain quantitative morphological evidence in support of the existence of specialized conduction pathways in RA of the rat heart, using morphometric methods.

EXPERIMENTAL METHOD

Mature noninbred male rats weighing 250-300 g were used. The animals were anesthetized by intraperitoneal injection of pentobarbital (0.05 mg/g body weight) after which the heart was perfused with 2.5% glutaraldehyde in phosphate buffer (pH 7.4). Samples were taken from the region of the superior vena cava with the adjacent myocardium of RA and, after standard processing for electron microscopy, they were embedded in one block. Tissues of SN and AVN were identified among the working myocardium in semithin sections [3]. Quantitative data on the tissue composition of the regions chosen for study were obtained by the dot method from negatives [2], with a final magnification of 7500 (the area of one field of vision was 640 μ^2). The material was subjected to statistical analysis and differences were evaluated by Student's t test.

EXPERIMENTAL RESULTS

Quantitative analysis of regions of SN and the adjacent working myocardium of RA revealed a significant difference ($P < 0.001$) in the content of muscle, connective-tissue, and nerve cells in these regions, but differences in the relative proportions of vascular struc-

Department of Morphology and Cytology, Medicobiological Faculty, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 10, pp. 496-498, October, 1981. Original article submitted February 19, 1981.

 0007-4888/81/9210-1446$07.50

TABLE 1. Volume Fractions of Tissue Components of SN, INP, and RA in Rat Heart

Region of heart studied	No. of negatives measured	Muscle components			Connective-tissue components			Vascular components			Nervous components		
		$\bar{X} \pm S_{\bar{x}}$	t	P	$\bar{X} \pm S_{\bar{x}}$	t	P	$\bar{X} \pm S_{\bar{x}}$	t	P	$\bar{X} \pm S_{\bar{x}}$	t	P
SN	54	33,2±2,0	6,78	<0,001	55,9±2,0	5,38	<0,001	7,3±1,6	1,62	>0,1	3,6±0,1	10,73	<0,001
RA	66	54,9±2,5			39,5±2,3			4,4±0,8			1,2±0,2		
IN (content of muscle components ≤35%)	13	25,2±1,7	14,74	<0,001	68,6±4,0	8,41	<0,001	5,0±2,4	0,27	>0,1	1,2±0,6		
RA	53	62,8±1,9			31,7±1,8			4,3±0,9			1,2±0,2		
IN (content of muscle components ≤40%)	16	27,5±1,8	14,06	<0,001	64,9±3,8	8,03	<0,001	6,6±2,2	1,22	>0,1	1,0±0,5	0,17	>0,1
RA	50	64,3±1,9			30,8±1,9			3,7±0,9			1,2±0,2		
IN (content of muscle components ≤45%)	20	30,2±1,9	13,79	<0,001	61,8±3,4	8,47	<0,001	7,0±1,9	1,65	>0,1	1,0±0,4	0,60	>0,1
RA	46	66,3±1,8			28,8±1,9			3,6±0,8			1,3±0,3		
IN (content of muscle components ≤50%)	24	33,1±2,1	16,26	<0,001	57,4±4,1	6,30	<0,001	8,5±2,1	2,78	<0,01	1,0±0,3	0,94	>0,1
RA	42	69,1±0,7			26,9±2,2			2,6±0,3			1,4±0,3		
IN (content of muscle components ≤55%)	30	37,0±2,2	12,39	<0,001	54,7±3,4	7,67	<0,001	7,3±1,7	2,72	<0,01	1,0±0,2	1,39	>0,1
RA	36	70,7±1,6			25,2±1,8			2,6±0,3			1,5±0,3		
IN (content of muscle components ≤60%)	36	40,2±2,3	12,60	<0,001	52,2±3,0	8,65	<0,001	6,6±1,5	2,71	<0,01	1,0±0,1	1,34	>0,1
RA	30	73,5±1,3			22,8±1,6			2,4±0,4			1,3±0,2		

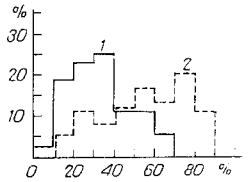

Fig. 1. Histogram of distribution of frequency of discovering muscle components among tissue components of myocardium. 1) SN; 2) RA. Abscissa, proportion of muscular components among all tissue components of myocardium (in %).

tures were not significant (Table 1; $P > 0.1$). The degree of variation of the tissue components was greater in the contractile myocardium than in the conducting system (Fig. 1). This fact could be the result of mechanical amalgamation of the two tissue populations — working and conducting — of the myocardium within the rat RA, i.e., of inability to identify specialized internodal conducting pathways (INP) among the mass of the contractile myocardium. The possibility of detecting these pathways was based on the assumption that tissue components of the main parts of the conducting system of the rat heart have a uniform quantitative composition. This view was confirmed during the present investigation: The tissue composition of SN did not differ significantly ($P > 0.1$) from that in AVN, in the atrioventricular bundle of His (AVB), and in the subendocardial conducting fibers of the ventricular papillary muscles [2]. To detect the conventional boundaries of identification of INP among the working myocardium in RA of the rat heart, the data of variance series of the quantitative composition of tissue components were ranked relative to the actual variable — the number of muscle cells — in order of increasing magnitude. The upper conventional boundaries separating tissue populations into INP and RA were taken to be the following numbers of muscle cells in the myocardium respectively: 35, 40, 45, 50, 55, and 60%. By division in this way, the newly discovered tissue subpopulations of the myocardium were characterized in terms of the number of muscle, connective-tissue, vascular, and nerve components contained in them (Table 1). Comparison of the INP and RA subpopulations in each of these cases of division showed that differences in the numbers of muscle and connective-tissue components were always significant ($P < 0.001$), whereas differences in the number of nervous structures were not significant ($P > 0.1$). Relative differences in the number of vascular components were significant ($P < 0.01$) for divisions in which the proportion of muscle components in INP exceeded 45% but did not exceed 60%; otherwise the differences were not significant ($P > 0.1$). Comparison of conventionally isolated INP with SN with respect to their tissue composition showed that differences were not significant ($P > 0.1$) for muscle, connective tissue, and vascular components in INP subpopulations containing 45, 50, and 55% of muscle components (Table 2). At all levels of identification, INP made a smaller contribution to the content of the nervous component than SN ($P < 0.001$). Having fixed conventional boundaries between INP and RA, it was possible to distinguish the most probable values corresponding to the smallest calculated values of Student's t (Table 2).

Analysis of muscle components of this most probable of the INP subpopulations isolated by this method of separation showed that they consisted of 1-3 muscle bundles descending from SN, and they formed a distinctive category of specialized myocytes, differing both from the conducting cells of SN and from the working cardiomyocytes of RA. These cells were long, fusiform, and smaller than myocytes of the working myocardium of RA. They contained fewer myofibrils than the working cardiomyocytes of RA, and these organelles were arranged in regular rows parallel to the long axis of the cell. Myocytes of INP resembled in their morphology the specialized type III cells of the conducting system of the rat heart, described by the writers previously [3], found in the lower part of AVN, AVB, and its branches. Unlike some other workers [4, 9, 10, 12, 13], we found morphologically specialized INP that could be distinguished in the surrounding working myocardium of RA both by their quantitative tissue characteristics and by their qualitative cell composition. These morphological data pro-

TABLE 2. Comparison of Volume Fractions of Tissue Components of SN and Conventionally Isolated INP and RA of the Rat Heart

Content of muscle components in conventionally isolated internodel conducting pathways, %	No. of degrees of freedom $K = n_1 + n_2 - 2$	Tissue components of sinus node							
		muscular		connective tissue		vascular		nervous	
		t	P	t	P	t	P	t	P
⩽35	65	3,04	<0,01	2,84	<0,01	0,80	>0,1	3,95	<0,001
⩽40	68	2,12	<0,05	2,10	<0,05	0,26	>0,1	5,10	<0,001
⩽45	72	1,08	>0,1	1,50	>0,1	0,12	>0,1	6,31	<0,001
⩽50	76	0,03	>0,1	0,33	>0,1	0,45	>0,1	8,22	<0,001
⩽55	82	1,28	>0,1	0,30	>0,1	0,00	>0,1	11,63	<0,001
⩽60	88	2,28	<0,01	1,03	>0,1	0,32	>0,1	18,38	<0,001

vide the essential basis for future investigations of morphofunctional correlations, for specialized type III cardiomyocytes may perform the role of structures conducting the depolarization wave rapidly in the conducting system of the heart.

LITERATURE CITED

1. M. G. Udel'nov, Physiology of the Heart [in Russian], Moscow (1975).
2. I. A. Chervova and E. R. Pavlovich, in: Abstracts of Proceedings of the 5th Conference on Tissue–Blood Barriers [in Russian], Moscow (1978), p. 266.
3. I. A. Chervova and E. R. Pavlovich, Arkh. Anat., No. 8, 67 (1979).
4. F. Bojsen-Möller and J. Tranum-Jensen, J. Anat. (London), 112, 367 (1972).
5. B. J. Chuaqi, Arch. Pathol. Anat. Abt. A, 355, 179 (1972).
6. M. J. Davies and A. Pomerance, Br. Heart J., 34, 150 (1972).
7. M. Hiraoka and T. Sano, Am. J. Physiol., 231, 319 (1976).
8. M. J. Janse, R. H. Anderson, F. J. L. van Capelle, et al., Am. Heart J., 91, 556 (1976).
9. L. Sherf and T. N. James, Am. J. Cardiol., 44, 345 (1979).
10. J. Tranum-Jensen, in: The Conduction System of the Heart, Philadelphia (1976), p. 55.
11. M. J. Tynan, in: Embryology and Teratology of the Heart and the Great Arteries, Leiden (1977), p. 34.
12. S. Viragh and C. E. Challice, in: Ultrastructure of the Mammalian Heart, Academic Press, New York (1973), p. 43.
13. S. Viragh and A. Porte, Z. Zellforsch., 145, 191 (1973).