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Relative Distribution Densities of Cholinergic and Adrenoceptor Structures in the Central Part of the Sinoatrial Node in Rat Heart

P. V. Sutyagin, E. E. Kalinina, and A. S. Pylaev

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Characteristics of distribution of cholinergic and adrenoceptor structures along the sinoatrial node artery in rat heart were evaluated by autoradiography on semithin sections by determining the density of ^{3}H-dihydroalprenolol and ^{3}H-quinuclidinyl benzilate binding sites. The relative density of binding sites for ^{3}H-dihydroalprenolol and ^{3}H-quinuclidinyl benzilate was minimum in the functional nucleus of the sinoatrial node and asymmetrically increased to maximum values to cranial (sharply) and caudal (smoothly) directions. The relative level of binding for ^{3}H-dihydroalprenolol in the perinodal atrial myocardium tissue was markedly lower than in the periarterial zone of the central part of the sinoatrial node and comparable to that for ^{3}H-quinuclidinyl benzilate.

Key Words: *sinoatrial node; dominant pacemaker region; autoradiography; ^{3}H-dihydroalprenolol; ^{3}H-quinuclidinyl benzilate*

The effects of classical transmitters on the sinoatrial node (SAN) of mammals, specifically rats, are paralleled by the effect of migration of the dominant pacemaker region (DPR) [1,2]. Mapping of the SAN area in rat heart showed that this formation of the conduction system is situated along the artery and has a central part (functional nucleus and tail) consisting of true pacemaker cells (total length of about 0.3 mm) and peripheral part occupied by latent pacemaker cells [3]. Migration of DPR is realized within the central part; it is considered to be due to heterogeneity of SAN cells, which differ by their sensitivity to classical transmitters and by the number and presence of various types of ionic channels providing pacemaker electrogenia [8,10]; another reason is possible uneven density of the cholinergic and adrenergic innervation in the SAN and adjacent cardiac tissue [6].

Department of Morphology, Russian State Medical University, Moscow

We carried out autoradiographic evaluation of the distribution of relative density of binding sites for ^{3}H-dihydroalprenolol (^{3}H-DHA) and ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) along the artery in the periarterial space of the central part of SAN in rat heart.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (60-90 g). Hearts were removed under Nembutal narcosis (40 mg/kg) and put into a cuvette with Hanks' solution (pH 7.35, 15-20°C). The right atrial region including the anterior wall, right cranial and caudal venae cavae, and the auricle was isolated. SAN was situated at the interface between the right cranial vena cava and auricle along the right cranial caudal artery [3]. The preparation was then fixed in a frame and placed into a flow incubation chamber with modified Krebs—Ringer solution saturated with 5% carbogen to pH 7.4 at

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38°C. The medium was replaced at a rate of 1.7 ml/min. DPR was located using glass microelectrodes. The form of action potential of pacemaker cell (presence of slow diastolic depolarization phase, smooth transition from slow diastolic depolarization into the initial rapid increase of potential phase and low rate of the initial rapid increase of the potential) served as the criterion [3,4]. After detection of DPR the glass microelectrode was left in the tissue and the medium in the cuvette was replaced with washout medium (10 mM $MgCl_2$ in 0.17 M Tris-HCl (pH 7.7) for ^{3}H-DHA and 0.9% NaCl in 15 mM phosphate buffer (pH 7.0) for ^{3}H-QNB) for 15 min. Ligand binding was carried out as described previously [5] by replacing washout medium with a medium with ^{3}H-DNA (Amersham, 38 Ci/mmol) and ^{3}H-QNB (Amersham, 30 Ci/mmol) to final concentrations of 2 and 1 nM, respectively. Binding reaction was carried out for 1 h for ^{3}H-DHA and 2 h for ^{3}H-QNB (in the darkness) at 20-25°C. Three rats were used in each experiment. After binding the preparation was washed (2×3 min), 4% glutaraldehyde solution in Millonig's buffer was added to the cuvette, and the preparation was fixed for 2 h. At the end of fixation sketches of the SAN artery pattern and position of the tip of the glass microelectrode were made for subsequent topographic orientation of the preparation. The microelectrode was then removed from tissue, the preparation was removed from the frame, and the anterior wall of the right atrium containing the SAN region was cut out under a stereomicroscope. The cut-out fragment was washed in buffer, postfixed in 1% OsO_4 in Millonig's buffer, dehydrated, and embedded in epon-812. Radioautographic evaluation of the relative density of tritium label distribution was carried out on successive semithin sections (3 μ). Blocks containing the total preparation of the right atrial anterior wall were oriented so that the preparation plane maximally coincided with the plane of the resultant semithin sections, which were then mounted on slides, stained with 1% methylene blue, coated by photographic Amersham LM-1 emulsion in darkness, dried, and exposed at 4°C. After one year the preparations were routinely developed in D-19 developer (Kodak). The images were analyzed on a Lumam-I-3 measuring microscope at ×40. The image was projected from the microscope objective to the videocamera with visual field of 26.3×32.5 μ^2 (Fig. 1, *a*). Densitometry was carried out in the immediate vicinity of the SAN artery wall. The position of the microelectrode tip (DPR; Fig. 1, *a*; 2) was taken for the zero point of measurement. Using an ocular micrometer, the preparation was shifted (at a 0.05 mm step) down along the artery (along the bloodflow — positive measuring) and at the same step shifted up along the artery (opposite to the bloodflow — negative measuring). The measurements were carried out only in cases when SAN artery wall was in the visual field. This circumstance mainly determined the number of analyzed visual fields. Densitometrical measurements in the preparations were maximally 0.5 mm down the SAN artery and 0.25 mm up the artery. For comparison the density of autoradiographic label was evaluated in the tissue of the adjacent perinodal atrial working myocardium. Images shown by the videocamera with the AverMedia EZCapture 2.5 device (Fig. 1, *b*) were computer processed (Adobe Photoshop 5.9; Fig. 1, *c*). The result of processing was the percentage of the visual field area occupied by autoradiographic label grains. Relative density of autoradiographic label was estimated as the ratio of the percentage of area occupied by the label in the current visual field to that maximum for the given semithin section. Distribution curves of the relative density of autoradiographic label along the SAN artery were plotted for each semithin section (Fig. 3). The results were statistically processed using Student's *t* test.

RESULTS

After glutaraldehyde fixation in the cuvette after binding and washing a stable trace formed at the site of the glass microelectrode tip; this trace was detected at 2-4 successive semithin sections (Fig. 1, 2). The tip of the glass microelectrode was in all cases situated in the immediate vicinity of *t. media*, this electrophysiologically confirming the presence of only 2-3 layers of atypical cells forming a specific muff around the artery in the central part of rat SAN [11]. Analysis of morphological and electrophysiological parameters of these cells was carried out in our previous study [4]. Here we studied the distribution of autoradiographic label in the immediate vicinity of *t. media*, *i.e.* in the area occupied by typical nodal cells. According to our estimations, the length of the central part of rat SAN is about 0.3 mm [3]. The microelectrode tip trace was fixed only on one side from the SAN artery in all the cases, this suggesting morphofunctional asymmetry of SAN. A part of SAN between the SAN artery and right auricle we arbitrarily denoted as the median area, the contralateral area (SAN part on the other side of the SAN artery) as the lateral area. The microelectrode tip was always seen in the lateral SAN area. Based on our previous findings [1-3], we hypothesized that the main processes of DPR shifting also took place in the lateral area of the SAN central part, and hence, densitometric measurements were carried out along the artery only on the lateral side.

According to the curve of relative density of ^{3}H-DHA binding (Fig. 3, *a*), the distribution of catecholamine receptors along the artery in the SAN lateral

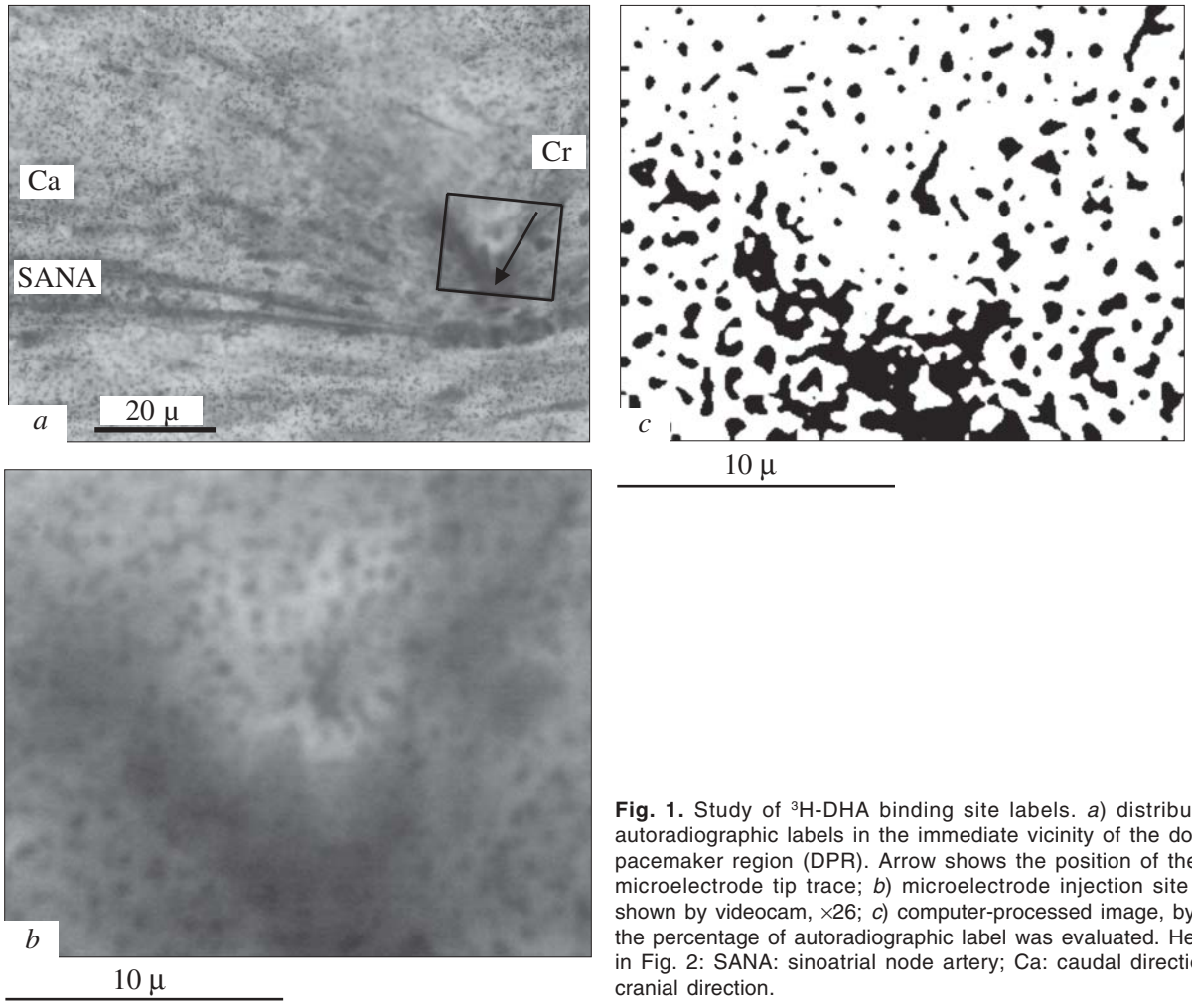

Fig. 1. Study of ^{3}H-DHA binding site labels. *a*) distribution of autoradiographic labels in the immediate vicinity of the dominant pacemaker region (DPR). Arrow shows the position of the glass microelectrode tip trace; *b*) microelectrode injection site image shown by videocam, ×26; *c*) computer-processed image, by which the percentage of autoradiographic label was evaluated. Here and in Fig. 2: SANA: sinoatrial node artery; Ca: caudal direction; Cr: cranial direction.

area is uneven. The least number of receptors is characteristic of the SAN functional nucleus. The density of catecholamine receptor label gradually increases down the SAN artery (parallel to bloodflow) to the maximum values and then somewhat decreases, while up along the SAN artery the number of receptors sharply increases to virtually maximum values. The relative number of ^{3}H-DHA binding sites in the periarterial region is appreciably higher than in the perinodal atrial myocardial tissue (30±13% of the maximum). This is in line with previously reported data [9] indicating appreciable increase in the density adrenergic receptors in the periarterial area of the SAN region at the length of more than 1 mm.

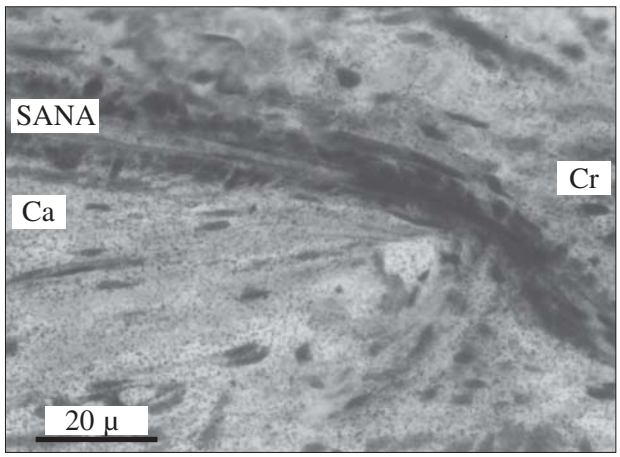

Fig. 2. Distribution of autoradiographic labels of ^{3}H-QNB binding sites in the immediate vicinity of DPR.

Relative density of muscarinic cholinergic receptors (Fig. 3, *b*) was characterized by similar distribution in the lateral area of SAN central part as the adrenergic reception distribution. The minimum values were observed in the SAN functional nucleus with subsequent gradual smooth increase down the SAN artery and a sharp increase up the artery. The relative number of ^{3}H-QNB binding sites in the perinodal working myocardial tissue was 81±10% of the maximum. A similar ratio of the ^{3}H-QNB binding sites density was noted previously [7], the number of binding sites in SAN tissue was lower than in the adjacent atrial myocardium.

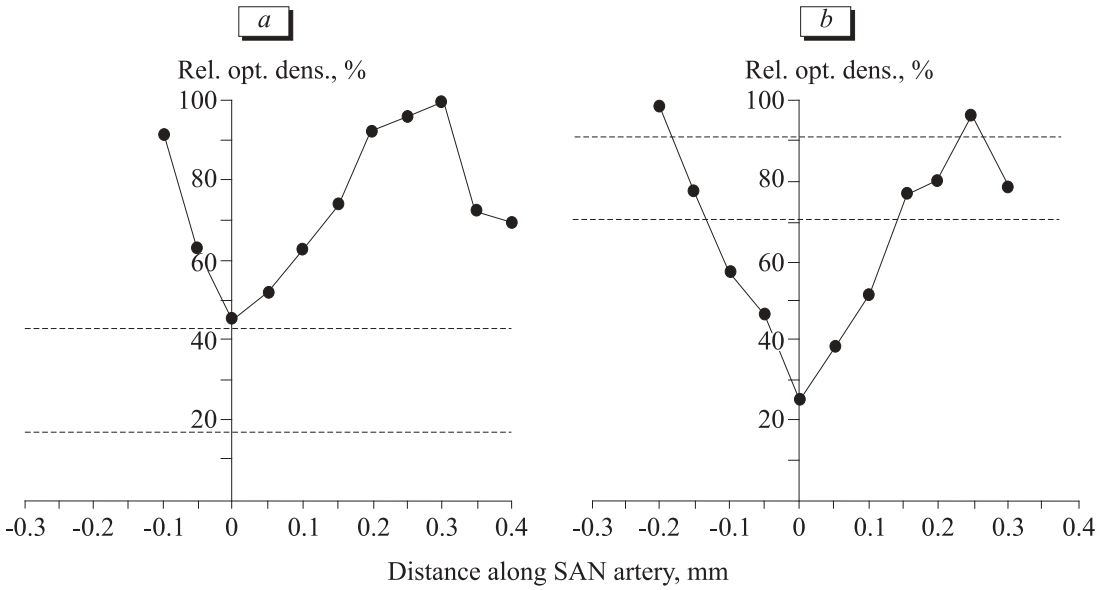

Fig. 3. Distribution of relative optical densities of autoradiographic labels of ^{3}H-DHA (*a*) and ^{3}H-QNB (*b*) binding sites in the structures of the sinoatrial node lateral area along the sinoatrial node artery on a semithin section. Intermittent line: confidence interval of relative optical densities for perinodal atrial myocardium for all the studied sections.

Migration of DPR is capturing of the leading role by one or a group of pacemaker cells possessing the highest successive frequency of action potentials during exposure of SAN to various factors, and therefore our data seem to explain the DPR migration *in vitro*. As the concentration of the added transmitter for all pacemaker cells of SAN center *in vitro* is the same, positive gradient of the adrenoreceptor presentation will promote gradual migration of DPR (with increase of norepinephrine concentration) towards the SAN functional tail [1]. By contrast, distribution of muscarinic cholinergic receptors in the central part of SAN will prevent DPR migration. Cases of DPR migration towards the functional nucleus after acetylcholine treatment [2] are also in line with this concept, as by the moment of acetylcholine addition into the cuvette DPR could be located (due to physiological reasons) in the SAN functional tail.

Uneven distribution of cholinergic and adrenergic innervation densities in the SAN region and right atrium in general will interfere in DPR migration *in vivo* or in experiments with stimulation of sympathetic or parasympathetic conductors. As even distribution of cholinergic and adrenergic innervation is characteristic of the majority of the studied mammalian species (the differences are observed only for the central and peripheral SAN parts) [6], we hypothesize the presence of a mechanism regulating cardiac chronotropy with alteration of DPR position, based on irregular distribution of cholino- and adrenoreceptor structures in the central part of SAN. The studied rat heart SAN is a functionally asymmetric formation. Functional asymmetry is due to the morphological base as well, because the pattern of distribution of relative density of binding sites for both ^{3}H-DHA and ^{3}H-QNB in the lateral and median areas of SAN central part differs appreciably.

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