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Distribution of ^{3}H-Dopamine and ^{3}H-DAGO Binding Sites in the Central Part of Rat Sinoatrial Node

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

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Distribution of ^{3}H-dopamine and ^{3}H-DAGO binding sites was studied by autoradiography on semithin sections of total preparations of rat sinoatrial node. The relative density of ^{3}H-dopamine and ^{3}H-DAGO binding sites in the functional nucleus of the sinoatrial node was minimum and increased in the cranial and caudal directions. The level of ^{3}H-dopamine binding in the perinodal atrial myocardium was appreciably lower (22±6%), while binding of ^{3}H-DAGO was similar (76±16%) to that in the periarterial zone of the sinoatrial node.

Key Words: *sinoatrial node; dominant pacemaker region; autoradiography; ^{3}H-dopamine; ^{3}H-DAGO*

The sinoatrial node (SAN) of rat heart is located along the sinoatrial artery and consists of a 0.3-mm long central part (functional nucleus and tail) containing true pacemaker cells (PMC) and peripheral part occupied by latent PMC [4]. The SAN region is densely innervated by cholinergic and adrenergic terminals [10]. Stimulation of these conductors *in vivo* and direct *in vitro* treatment (*e.g.* with norepinephrine) leads to the effect of transposition of the dominant pacemaker region (DPR) [1,3]. A possible cause of this effect is uneven distribution of cholinoceptor and adrenoceptor structures in the central part of SAN [5]. Apart from basic transmitters of the autonomic nervous system, myocardial tissue, specifically SAN, contains a spectrum of neurotransmitters; of these, enkephalins [12,13] and dopamine [2,11] are particularly interesting, as the former are responsible for acetylcholine and norepinephrine release from nerve terminals and the latter is a catecholamine precursor in sympathetic terminals, on the one hand, and an independent neurotransmitter with peripheral receptor formations and specific physiological effects, including the chronotropic effect [2,11], on the other.

Department of Morphology, Russian State Medical University, Moscow

The aim of this study was autoradiographic evaluation of the distribution of relative density of ^{3}H-dopamine and ^{3}H-DAGO binding sites along the artery in the periarterial space of the central part of rat SAN.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (60-90 g). Hearts were removed under Nembutal narcosis (40 mg/kg) and transferred into Hanks' solution (pH 7.35, 15-20°C), a fragment of the right atrium containing the anterior wall, right cranial and caudal venae cavae, and the auricle was isolated. SAN was located at the boundary of the right cranial vena cava and the auricle along the artery [4]. The preparation was fixed in a frame and placed into a flow chamber with modified Krebs—Ringer solution equilibrated with 5% carbogen to pH 7.4 at 38°C. The rate of medium replacement in the chamber was 1.7 ml/min.

The location of DPR was detected by glass microelectrodes. The shape of PMC action potential (presence of slow diastolic depolarization phase, smooth transfer from this phase into the phase of initial rapid increase of potential, and low rate of initial rapid elevation of potential) served as the criterion of the true

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PMC [4,6]. After detection of DPR a glass microelectrode was left in the tissue, and culture medium in the cuvette was replaced with washing medium (50 mM Tris-HCl (pH 7.4) with 0.1% ascorbic acid, 0.1 nM pyrocatechine, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ for ^{3}H-dopamine; 50 mM Tris-HCl (pH 7.4) with 0.2% BSA and 0.04% bacitracin for ^{3}H-DAGO) for 15 min. The ligand binding was carried out as described previously [5] by replacing washing medium with medium with ^{3}H-dopamine (Amersham, 49.7 Ci/mmol) and ^{3}H-DAGO (Amersham, 48 Ci/mmol) to a final concentration of labeled ligand 2 nM. The binding was carried out for 1 h at ambient temperature (20-25°C). Three animals were used in each experiment. After the end of incubation the medium in the cuvette was replaced with washing medium (2×3 min) and the preparation was fixed for 2 h with 4% glutaraldehyde in Millonig buffer. At the end of fixation a sketch of the SAN artery and the position of the tip of the glass microelectrode was made for subsequent topographic orientation of the preparation. The microelectrode was then removed from the 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 preparation was washed in buffer, postfixed in 1% OsO_4 in Millonig buffer, dehydrated, and embedded in epon-812.

Radioautographic evaluation of the density of tritium label distribution was carried out on a series of semithin (3 μ) sections. Blocks containing the total right atrial anterior wall preparation were oriented so that the preparation plane maximally coincided with the plane of the resultant semithin sections. The sections were mounted on slides, stained with 1% methylene blue, coated with Amersham LM-1 photographic emulsion in the darkness, dried, and exposed at 4°C for 1 year. After exposure the preparations were developed in D-19 developer (Kodak). The resultant images were analyzed under a Lumam-I-3 measuring microscope at ×40. The image from the objective was projected to a videocamera (visual field 26.5×32.5 μ^2). Densitometry was carried out in the immediate vicinity of the SAN arterial wall. The position of the microelectrode tip was taken for the zero point (Figs. 1, 2). Then the preparation was shifted (0.05-mm steps) down and up the SAN artery (along and against to the bloodflow : positive and negative measurings, respectively). The measurements were carried out if the SAN arterial wall was in the visual field. This circumstance determined the number of analyzed visual fields. Densitometry in the preparations could be made maximally 0.5 mm down the SAN artery and 0.25 mm up the artery. For comparison the density of autoradiographic label in the adjacent perinodal atrial working myocardium was evaluated. The images shown by the videocam were computer-processed [5]. The results were expressed as the percentage of the visual field area occupied by autoradiographic label grains. The relative density of autoradiographic label was estimated as the ratio of the percentage of the area occupied by the label in the current visual field to that maximum for the section. Curves reflecting the distribution of the relative density of autoradiographic label along the SAN artery were plotted for each section (Fig. 3). The results were processed using Student's *t* test.

RESULTS

Topographic identification of the position of the glass microelectrode inserted into the DPR region after binding, washing, fixation, and embedding in epon showed that, similarly to the previous series of experiments [5], the electrode was located in one of atypical myocytes (Figs. 1, 2) forming a fine (2-3 layers of cells) muff around the SAN artery. The morphology of these myocytes was studied previously using intracellular La^{3+} treatment and corresponded to the morphology of typical nodal cells [6]. The microelectrode tip in all cases was situated only on one side of the SAN artery in a region, denoted as the lateral SAN area [5]. For evaluation of possible relationships between migration processes the DPR along the SAN artery in response to autonomic neurotransmitters and peculiarities in the distribution densities of receptor structures to these modulators, we carried out densitometry in the narrow area in the immediate vicinity of the SAN artery *t. media* (only in the lateral area). This approach already proved to be effective in the study of the distribution of cholinoceptor and adrenoceptor structures in this region [5].

The distribution of silver grains in the SAN lateral area along the artery is heterogeneous (Fig. 3, *a*). The least number of receptors is characteristic of the SAN functional nucleus. The label density gradually increased below this site down the SAN artery (parallel to the bloodflow) in the functional tail, while up the SAN artery the number of binding sites sharply increased to virtually maximum values. The relative number of ^{3}H-dopamine binding sites in the periarterial region notably surpasses that in the perinodal atrial myocardial tissue (22±6% of the maximum). The curve of relative binding sites for ^{3}H-dopamine virtually completely repeats the curve for ^{3}H-dihydroalprenolol (^{3}H-DHA) [5]. Since dopamine with increase of its concentration in cardiac tissue reacts first with dopamine receptors (D1, D2, D4) [2,8] and then with β- and α-adrenoreceptors [11], we cannot exclude cross-binding of ^{3}H-dopamine to β-adrenergic receptors of the SAN central part. However, the pattern of β-ad-

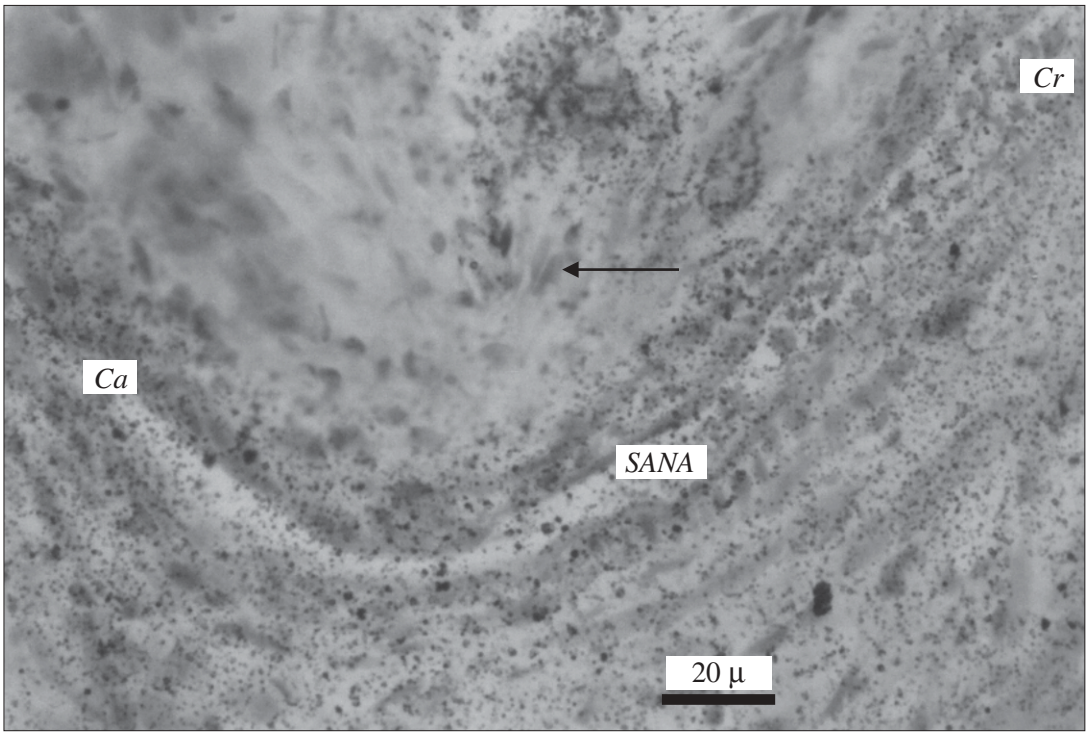

Fig. 1. Distribution of autoradiographic label of ^{3}H-dopamine binding sites in the immediate vicinity to the dominant pacemaker region (DPR). Arrow shows the trace from the glass microelectrode tip. Here and in Fig. 2: SANA: sinoatrial node artery; Ca: caudal direction; Cr: cranial direction.

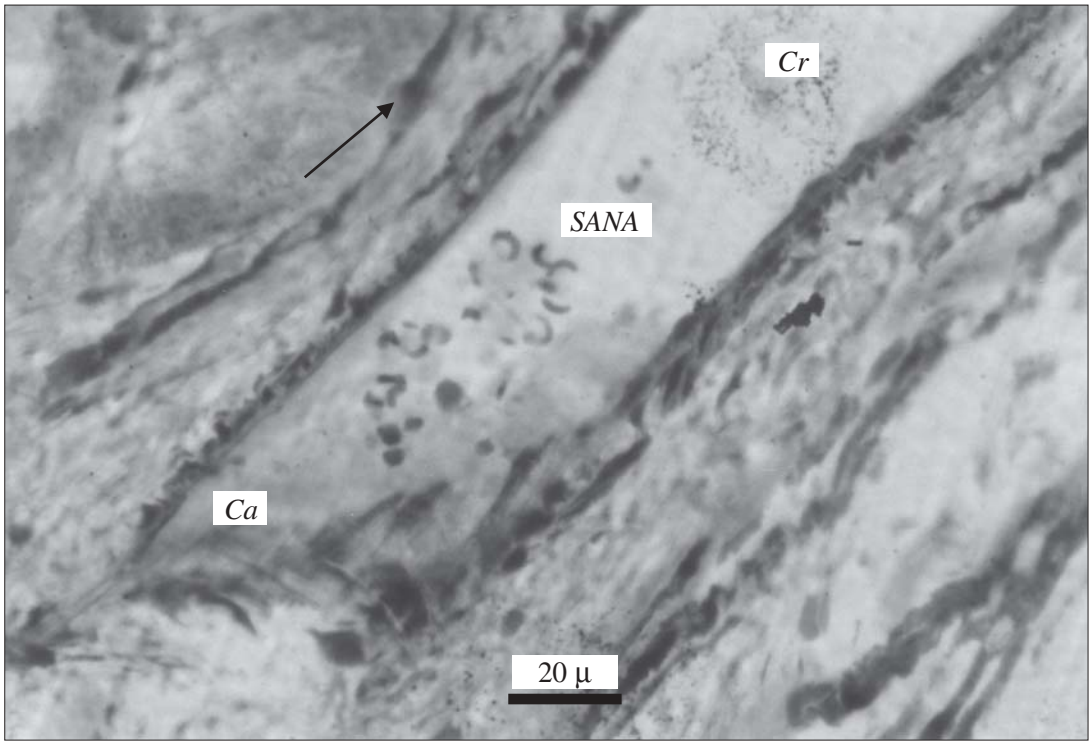

Fig. 2. Distribution of autoradiographic label of ^{3}H-DAGO binding sites in the immediate vicinity of DPR. Arrow shows the position of the trace from the glass microelectrode tip.

renoreceptor and dopamine receptor distributions in the central part of SAN most probably coincide, and ^{3}H-dopamine label is just additive [11]. Greater difference between binding values in the periarterial zone in comparison with the values in the adjacent working myocardium (22±6%) for ^{3}H-dopamine in comparison

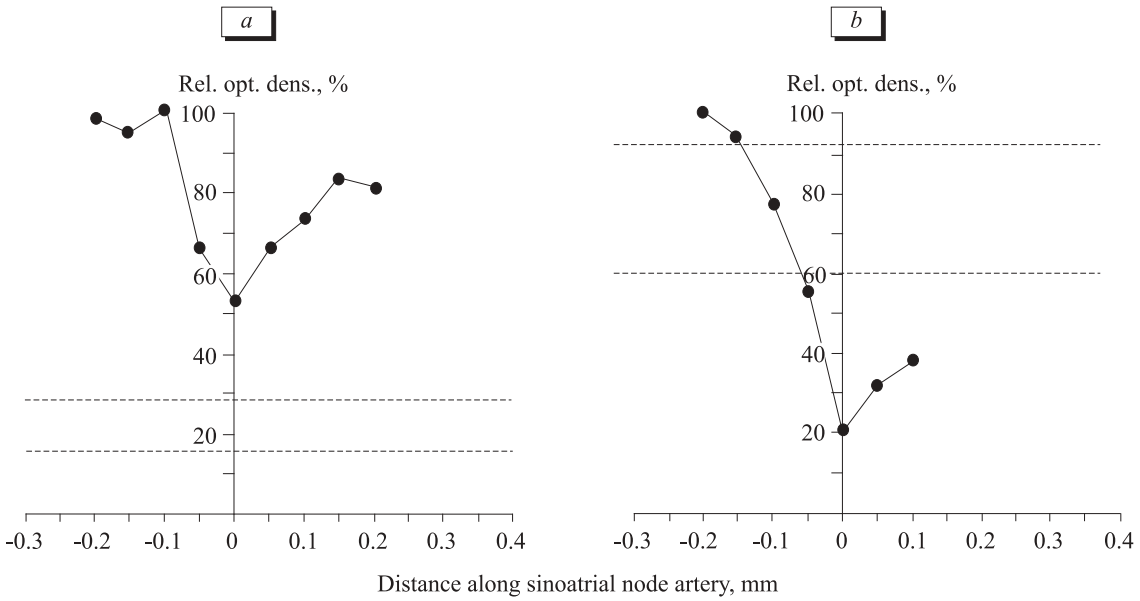

Fig. 3. Distribution of relative optical densities of ^{3}H-dopamine (*a*) and ^{3}H-DAGO (*b*) binding sites for structures of the sinoatrial node lateral area along the SAN artery on one of semithin sections. Interrupted lines show the confidence interval of relative optical densities for the perinodal atrial myocardium in all the studied sections.

with ^{3}H-DHA [5] confirms this hypothesis. In addition to the myocardial tissue, dopamine (D2-like) receptors are present in the coronary arterial walls [9]. The SAN artery is thickly densely with silver grains, indicating the presence of an appreciable number of dopamine receptors in its wall (Fig. 1).

The study of opioid receptors in SAN tissue using a synthetic enkephalin analog ^{3}H-DAGO also showed their uneven distribution in the lateral region of the central part of SAN. Similarly to ^{3}H-quinuclidinyl benzilate (^{3}H-QNB), ^{3}H-DHA, and ^{3}H-dopamine, binding of ^{3}H-DAGO was minimum in the SAN functional nucleus, the relative density of binding increased in the cranial direction. This trend was also observed in the caudal direction, but the SAN artery bent at this site in all three animals, and it was beyond the plane of the studied preparations (Figs. 1, 3). Opioid receptors to enkephalins (κ- and δ-opioid receptors) in SAN tissue are now detected in dogs; they are situated on the membranes of cholinergic and adrenergic terminals and inhibit acetylcholine and catecholamine release from them [12,13]. It is noteworthy that ^{3}H-DAGO labeling was low (in comparison with ^{3}H-QNB, ^{3}H-DHA, and ^{3}H-dopamine labeling) and the relative density of opioid receptors in the periarterial zone was at the level comparable to that in the adjacent perinodal myocardium (76±16%).

Hence, the study of the reception in the lateral region of the central part of SAN in rat heart showed similar distribution of ^{3}H-QNB, ^{3}H-DHA, ^{3}H-dopamine, and ^{3}H-DAGO binding sites. The number of all studied receptors was significantly lower in the functional nucleus (normal location of DPR) in comparison with other parts of SAN. The number of receptors increases in the cranial and caudal directions, the increase in the caudal direction being smoother. This organization of the central SAN part in the presence of rich (compared to other heart compartments) autonomic innervation [10] seems to provide the maximum stability of PMC action potential generation frequency, regulation of PMC cardiac chronotropy in the functional nucleus during exposure to mild factors, and triggering of the DPR translocation under conditions of significant load, sharp alteration of the tone of this or that compartment of the autonomic nervous system or basal hormone level. Disorders in this model of the receptor structures distribution inevitably lead to disregulation of cardiac chronotropy, heart rhythm lability and, presumably, to paroxysmal tachycardia and tachyarrhythmia.

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