Morphological Analysis of Myocyte Relationships in the Sinoatrial Node in Rats

P. V. Sutiagin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 11, pp. 589-595, November, 2009
Original article submitted July 14, 2009

The presence of action potentials of working atrial cardiomyocytes among action potentials of true and latent pacemakers was revealed in the part of bidimentional maps of distribution of action potentials of sinoatrial node cells on the frontal surface of the right atrium under conditions of short-term culturing using intracellular glass microelectrodes. Detailed submicroscopical analysis of the node tissue was conducted. Sinoatrial node of rat heart consists of typical nodal cells and transition-type cardiomyocytes. Working atrial cardiomyocytes are absent in the tissue of the node. Action potentials of atrial cardiomyocytes among true and latent pacemakers in the course of electrophysiological study are recorded from cells of working myocardium of the thin subepicardial plate coverig the sinoatrial node.

Key Words: *sinoatrial node; pacemaker cells; intracellular lead; action potential; submicroscopical analysis*

According to classical concepts [4,5], the sinoatrial node (SAN) in mammals consists of true and latent pacemakers (PM). Basic processes of spontaneous generation of action potential and regulation of heart chronotropy take place within the central part of SAN, while latent PM of the peripheral node region protect the central part from the hyperpolarizing action of working atrial cardiomyocytes surrounding SAN [4]. However, recent complex studies of the structure and function of enzymatically dissociated cardiomyocytes from rabbit SAN [7] showed that atrial cardiomyocytes are present in all studied SAN regions, their proportion in the dominant pacemaker region (DPR) being about 22%. Interdigitations between bundles of nodal and working atrial cardiomyocytes in mouse sinoatrial node were demonstrated by immunohistochemical methods [6].

The aim of the study was to investigate the relationship between muscular elements within topographical boundaries of SAN in rat heart.

Department of Morphology, Medico-Biological Faculty, N. I. Pirogov Russian State Medical University, Moscow, Russia. ***Address for correspondence:*** nilan-2008.56@mail.ru. P. V. Sutyagin

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n=19) weighing 80-140 g. The animals were sacrificed by intraperitoneal injection of phenobarbital in a dose of 60 mg/kg. Then the chest was opened and 10 ml 0.01% trypan blue in Hanks solution, pH 7.35 (t=37-38°C), was slowly injected into the left ventricle. The heart was removed and placed in a dish with Hanks solution (15-20°C), a fragment of the right atrium containing the frontal wall, right cranial and caudal venae cava, and the auricle were isolated. SAN is located at the boundary of upper vena cava and auricle along the SAN artery detected by trypan blue staining. The preparation was fixed and placed into a thermostated flow chamber filled with modified Krebs–Ringer solution adjusted to pH 7.4 at 38°C with 5% carbogen. The rate of medium replacement in the chamber was 1.7 ml/min. The shape of action potential of true and latent PM was determined using glass microelectrodes. Bidimentional coordinates of the location of true and latent PM as well as working atrial cardiomyocytes were plotted on a previously drawn (on scale paper) scheme of the preparation of the right atrium by in-

0007-4888/09/1485-0834

troducing microelectrodes under an MBI-3 light microscope.

The material for electron microscopy was collected and processed using conventional methods. Five animals were used in the analysis. Specimens were embedded in Epon-812. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined under a Hitachi HU-12 electron microscope. The results were analyzed using Student *t* test.

RESULTS

According to electrophysiological data, rat SAN consists of true (Fig. 1, *a*) and latent PM. Latent PM include two subpopulations. One population forms a functional tail of SAN [1] and is characterized by shape action potential similar to that of true PM, but differs from that by a sharp transition from the slow diastolic depolarization phase (phase 4) to the phase of initial rapid increase of potential (phase 0) and higher rate of potential growth in phase 0. Another subpopulation of latent PM is characterized by high polymorphism of action potential curves, which represent various intermediate forms between true PM to perinodal atrial cardiomyocytes located nearby (Fig. 1, *b*). This subpopulation of latent PM builds the peripheral part of SAN [2]. Morphological identification of PM conducted using ionophoretic intracellular administration of La^{3+} ions showed that true PM and PM constituting functional tail of the central SAN part represent a consolidated population morphologically identical to typical nodal cells described previously. Peripheral part of SAN consists of latent PM, which were morphologically identified as cell of transient type [3].

Bidimentional analysis of distribution of cells with rhythmical electric activity on the frontal surface of right atrium confirmed previously obtained data on morphological and functional organization of rat SAN [2]. However, in some cases it also revealed cardiomyocytes with action potential shape typical for atrial working cardiomyocytes among true and latent PM (Fig. 1) [1]. The presence of action potential typical for atrial cardiomyocytes among SAN-building cells was the basis for a more detailed submicroscopic analysis of SAN structure in order to reveal possible mosaicism of its structure [7].

Detailed morphological analysis of lateral region of central SAN part showed that it consists exceptionally of tightly packed typical nodal cells (Fig. 2, *a*). True and latent PM from the lateral region detected electrophysiologically in close proximity to SAN arteria were in fact located closely to artery *tunica media* and formed junctions of special type with its smooth muscle cells. At the junction points, typical nodal cells formed short outgrowths towards smooth muscle elements where basal membranes of contacting cells merge (Fig. 2, *b*). While moving from SAN artery, transition-type cells appeared at the boundary of central and peripheral parts of the node among typical nodal cells (latent PM, Fig. 2, *c*). Farther from SAN artery, an opposite picture was observed: individual typical nodal cells surrounded by cells of transient type (Fig. 2, *d*). Thus, an interdigitations of typical nodal cells and cells of transient type were in fact observed in rat SAN. Working atrial cardiomyocytes were seen on the preparation much more laterally, they contact to transition-type cells only. The presence of action potential of working cardiomyocytes on the

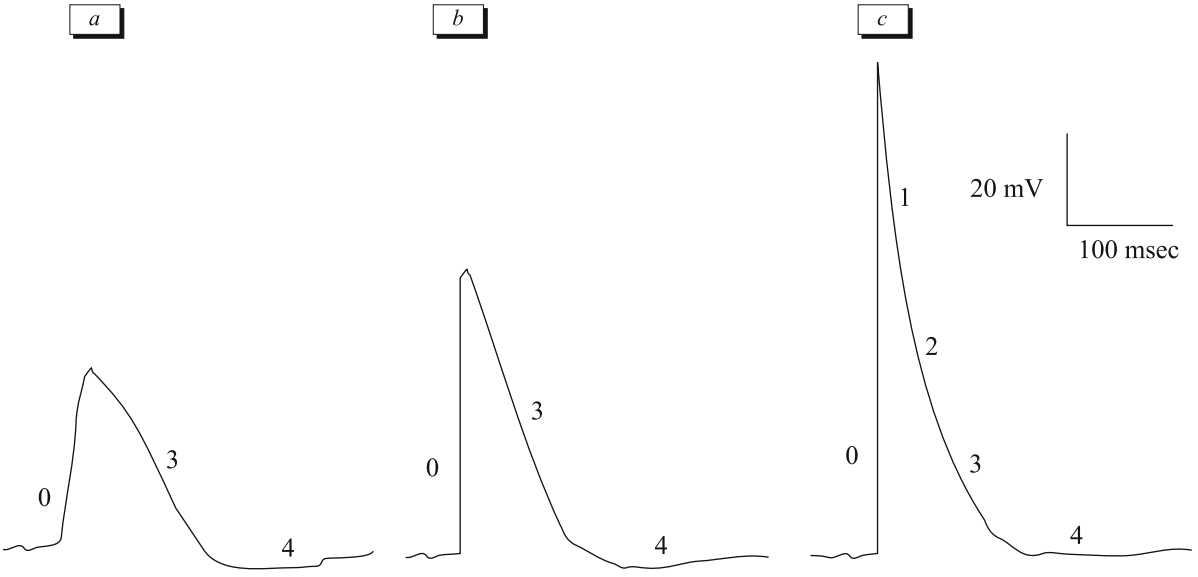

Fig. 1. Action potentials of true (*a*), latent PM (*b*) and working atrial cardiomyocytes (*c*). 0 – phase of initial rapid increase of potential; 1, 2, 3 – repolarization phases; 4 – diastolic phases (for true and latent PM – phase of low diastolic depolarization).

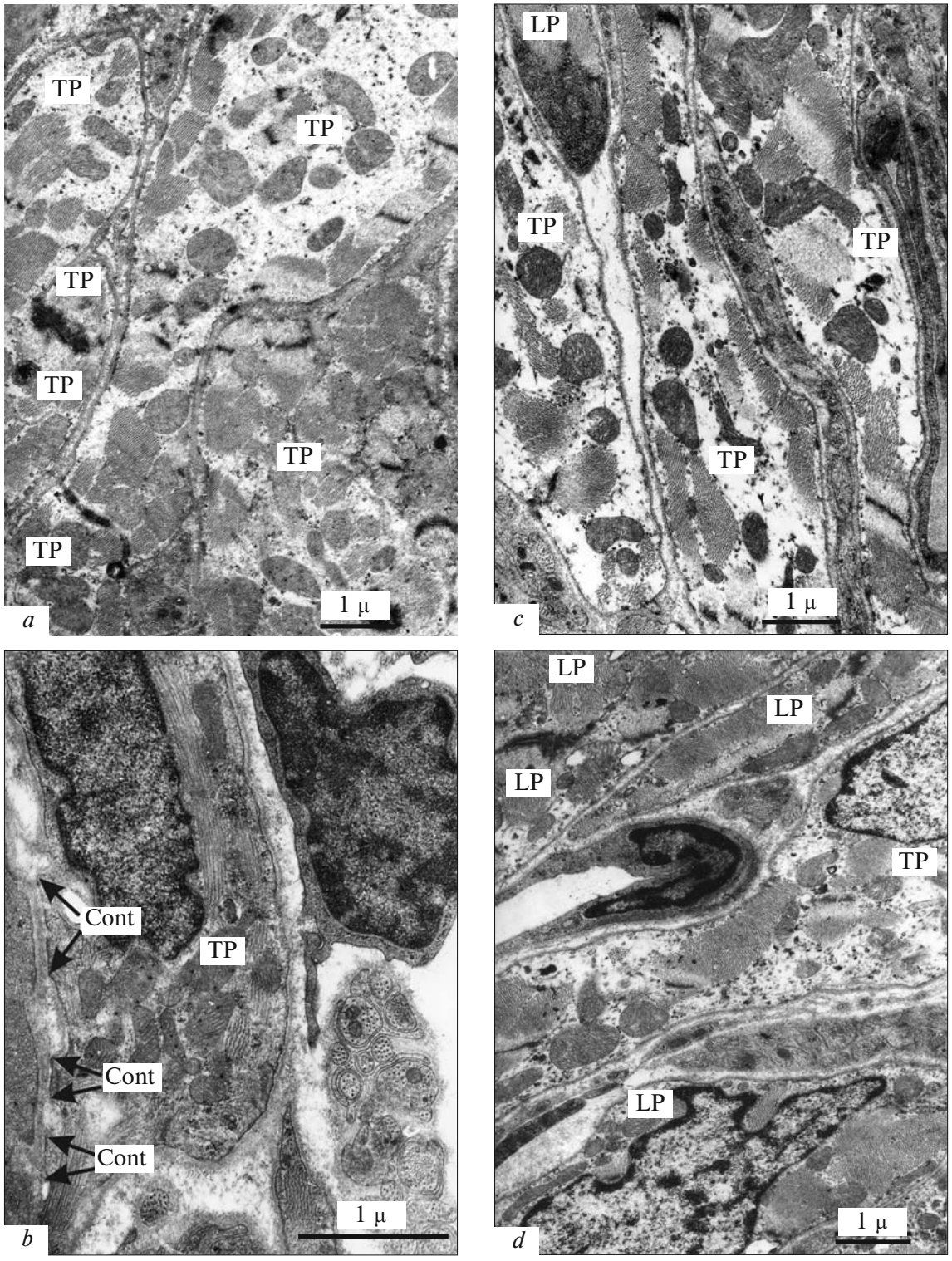

Fig. 2. Relative position of true (typical nodal cells) and latent PM (cells of transient type) in various regions of rat SAN. *a*) true PM of lateral part of the central SAN region; *b*) junction of true PM with a smooth muscle cell of *t. media* of the SAN artery; *c*) interdigitation of latent and true PM at the boundary of central and peripheral SAN regions; *d*) boundary of central and peripheral parts of SAN. Interdigitation of true and latent PM. TP: true PM, LP: latent PM, Cont: zone of junction of true PM and smooth muscle cell of *t. media* of SAN artery.

bidimentional SAN map [1] can be explained by the existence of a thin subepicardial layer of working atrial myocardium, which covers SAN in rats. Presence of working atrial cardiomyocytes from subepicardial and subendocardial layers in tissue samples for enzymatic dissociation of SAN myocardium can introduce

certain error during estimation of cellular composition of the node.

REFERENCES

1. P. V. Sutiagin, A. G. Kamkin, and O. Yu. Gurina, *Bull. Exp. Biol. Med.*, **148**, No. 9, 343-346 (2009).
2. P. V. Sutiagin, E. E. Kalinina, and A. S. Pylaev, *Ibid.*, **139**, No. 2, 227-230 (2005).
3. P. V. Sutiagin, I. A. Chervova, and A. S. Pylaev, *Kardiologiya*, **28**, No. 2, 84-87 (1988).
4. M. R. Boyett, H. Honjo, and I. Kodama, *Cardiovasc. Res.*, **47**, No. 4, 658-687 (2000).
5. T. N. James, Prog. *Cardiovasc. Dis.*, **45**, No. 3, 235-267 (2002).
6. J. Liu, H. Dobrzynski, J. Yanni, *et al.*, *Cardiovasc. Res.*, **73**, No. 4, 729-738 (2007).
7. E. E. Vercheijck, A. Wessels, A.C. van Ginneken, *et al.*, *Circulation*, **97**, No. 16, 1623-1631 (1998).