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IDENTIFICATION OF SINUS NODE PACEMAKER CELLS OF THE RAT HEART BY INTRACELLULAR INJECTION OF LANTHANUM IONS

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The study of the structural basis for activity of different types of cardiomyocytes requires the use of methods that enable morphological and functional parameters of the same cell to be investigated. The localization of cells with a particular type of electrical activity is usually based on injection of solutions of dyes [3, 4], or cobalt [6] or lanthanum [7] ions through the recording electrode. The demands for a combination of electrophysiological and electron-microscopic methods of analysis of a single cell are best satisfied by the method suggested by Taylor et al. [7].

The aim of the present investigation was to study qualitative and quantitative characteristics of action potentials (AP) of sinus node pacemaker cells of the rat heart and also the suitability of the lanthanum labeling method for morphological identification of these cells.

EXPERIMENTAL METHOD

Male Wistar rats weighing 130-180 g were used. Under pentobarbital anesthesia (40 mg/kg) thoracotomy was performed and the heart was perfused with 10 ml of 0.01% trypan blue solution in Hanks' solution (pH 7.4) at 38°C. The right atrium was placed in a continuous-flow constant-temperature cuvette with modified Krebs–Ringer solution, equilibrated with a mixture of 5% CO_2 and 95% O_2 to pH 7.4 [8]. The rate of change of the medium was 5-10 ml/min. A glass microelectrode was filled with a mixture of 3 M KCl and 2% $LaCl_3$ and introduced into the sinus node by means of a KM-1 micromanipulator, under control of a stereoscopic microscope. The location of the sinus node was determined from the nodal artery, stained with trypan blue. The recording system consisted of a UPT-2 dc amplifier and dual-beam S8-2 storage oscilloscope. La^{+++} ions were injected after recording AP of the pacemaker cell by applying positive square pulses of current with a strength of 2-6 μA, frequency 5 Hz, and duration 100 msec to the microelectrode for 2 min. In this way from six to 15 pacemaker cells were labeled in each of the five animals studied. Parameters of AP of pacemaker cells obtained in this series were checked against traces of potentials recorded by microelectrodes filled with 3 M KCl only. The preparations were then fixed with 4% glutaraldehyde solution in Millonig's buffer at 4°C for 2 h, rinsed in buffer, the region of the sinus node was then isolated and post-fixed in 2% OsO_4 in Millonig's buffer, dehydrated, and embedded in Epon. Ultrathin sections, both stained with uranyl acetate and lead citrate, and also unstained, were examined in the Hitachi 11-E-2 electron microscope. The structure of the $LaCl_3$ tags was analyzed after drying the lanthanum chloride solution on a formvar support.

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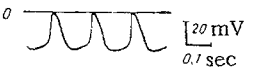

Fig. 1. Shape of AP of sinus node pacemaker cell recorded in rat heart by microelectrode filled with 3 M KCl and 2% $LaCl_3$.

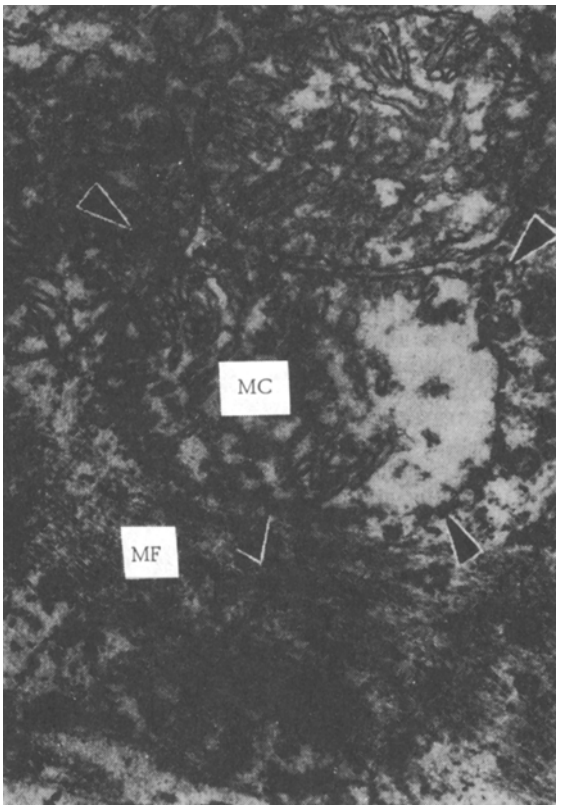

Fig. 2. Localization of lanthanum agglomerations (arrows) in cytoplasmic matrix, on outer membrane, and in mitochondrial matrix (40,000 ×). MC) Mitochondria, MF) myofibrils.

EXPERIMENTAL RESULTS

Cardiomyocytes of the right atrium are heterogeneous in their electrophysiological characteristics. Significant differences in the shape of AP could be reduced to variations within its individual phases [1]. For subsequent identification, cells were selected whose AP differed in the abundance of their slow diastolic depolarization, changing smoothly into the phase of rapid rise, which ended with emergence into the repolarization phase at low values of reversal potential (Fig. 1). This type of AP corresponds to sinus node pacemaker cells. Quantitative analysis of AP parameters gave similar results: The following frequency was between 4.5 and 6 Hz, the mean amplitude of AP was 34 ± 5 mV (limits of changes in amplitude 25-50 mV), and the reversal potential varied from −5 to +2 mV, with a mean value of −2 ± 2 mV. Iontophoretic injection of La^{+++} led to a significant decrease in amplitude of AP.

The next step in the investigation revealed some distinguishing features of the intracellular distribution of lanthanum label. Agglomerations of lanthanum in the cytoplasmic matrix had the appearance of electron-dense granules, differing in shape and size (Fig. 2). The lanthanum ions also had increased affinity for the outer membrane of the mitochondria (Fig. 2) and inner surface of the cytolemma (Fig. 3). Most agglomerations of lanthanum were located in the mitochondrial matrix (Fig. 3), in agreement with data according to which the La^{+++} ion, because of its action, is a competitor of the Ca^{++} ion, and interacts with the same membrane formations [2].

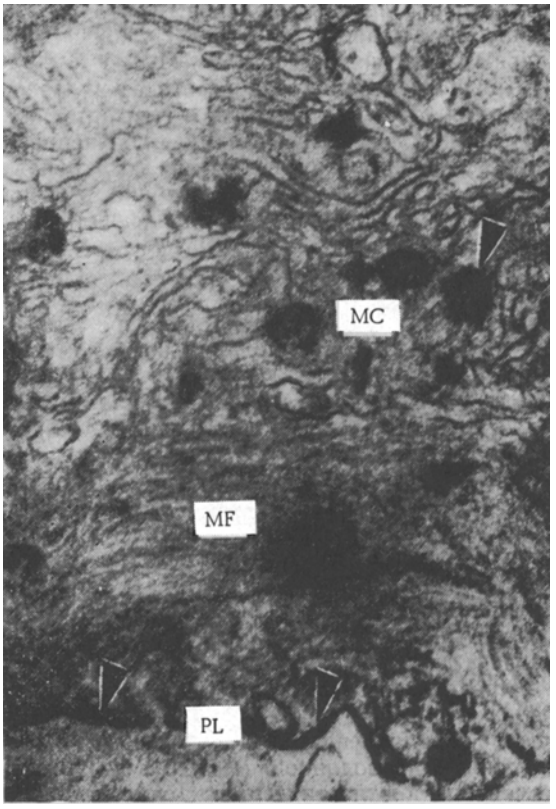

Fig. 3. Structure of lanthanum agglomerations (arrows) in mitochondria and on inner surface of plasma membrane (83,000 ×). MC) Mitochondria, MF) myofibrils, PL) plasmalemma.

Most of the lanthanum label was evidently diffusely distributed within the outlines of the cell section, as shown by the marked increase in electron density of the labeled cell compared with the surrounding cells, with similar morphology.

Investigation of the topography of cells generating AP characteristic of the pacemaker showed that they occupy a definite space around the nodal artery, in agreement with data in the literature [5]. Pacemaker cells of the rat sinus node have morphological parameters similar to those of other animals.

No significant changes in morphology of the lanthanum-labeled cells were observed in this investigation, and this can be taken as evidence that the method is suitable for morphological identification of cells according to their electrophysiological parameters.

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