MORPHOFUNCTIONAL CHARACTERISTICS OF PACEMAKER FORMATIONS IN THE ATRIOVENTRICULAR VALVES OF THE HEART

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Multicellular formations responsible for the generation of spontaneous pacemaker activity were found in the cusps of the atrioventricular valves of the rabbit heart by means of a microelectrode technique and electron microscopy. Cells of different morphological structure were found: P cells, cells of the Purkinje type, and transitional cells. These formations were found to have a rich innervation, mainly cholinergic in nature. Definite correlation was found between the morphological structure and electrophysiological parameters of these cells.

KEY WORDS: atrioventricular valves; automatic activity; membrane potentials; P cells.

Work on pacemaker activity in the atrioventricular valves of the dog [12] and monkey [14] heart has recently been published. The present writers found lasting pacemaker activity in the atrioventricular valves of the rabbit heart [2]. This activity is characterized by the presence of spontaneous action potentials (AP) with slow diastolic depolarization (SDD), and it is due mainly to the functioning of the slow sodium-calcium channel.

The pacemaker formations of the mammalian heart are characterized by a definite morphological structure: by the presence of P cells, cells of the Purkinje type, and transitory or intermediate cells [7, 8, 10]. Formations of this type in the atrioventricular valves had not been found previously, although there is morphological evidence that they contain nervous and myocardial elements [3, 4, 6].

In the investigation described below a multicellular morphofunctional complex responsible for the generation of spontaneous pacemaker activity in the atrioventricular valves of the rabbit's heart was found and is described for the first time.

EXPERIMENTAL METHOD

The heart was removed from 72 rabbits under urethane anesthesia, the left and right ventricles were opened, and the cusps of the atrioventricular valves excised. The spontaneously contracting preparations were perfused with Tyrode's solution of the following composition (in mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 12, NaH_2PO_4 0.4, glucose 5.5. The solution was oxygenated with a gas mixture containing 95% O_2 and 5% CO_2; the pH of the solution was 7.4 and its temperature 36-37°C. The membrane potentials of the pacemaker cells were derived by glass microelectrodes by the method used previously [2]. The electrophysiological characteristics of the pacemaker cells were studied in 100 preparations of the atrioventricular valves: the frequency of spontaneous AP, their shape, the magnitude of the overshoot, and the levels of the critical and maximal diastolic potentials.

Regions of the valves (30 preparations) in which pacemaker activity was detected electrophysiologically were investigated in the electron microscope. Material was fixed in buffered osmic acid solution, dehydrated,

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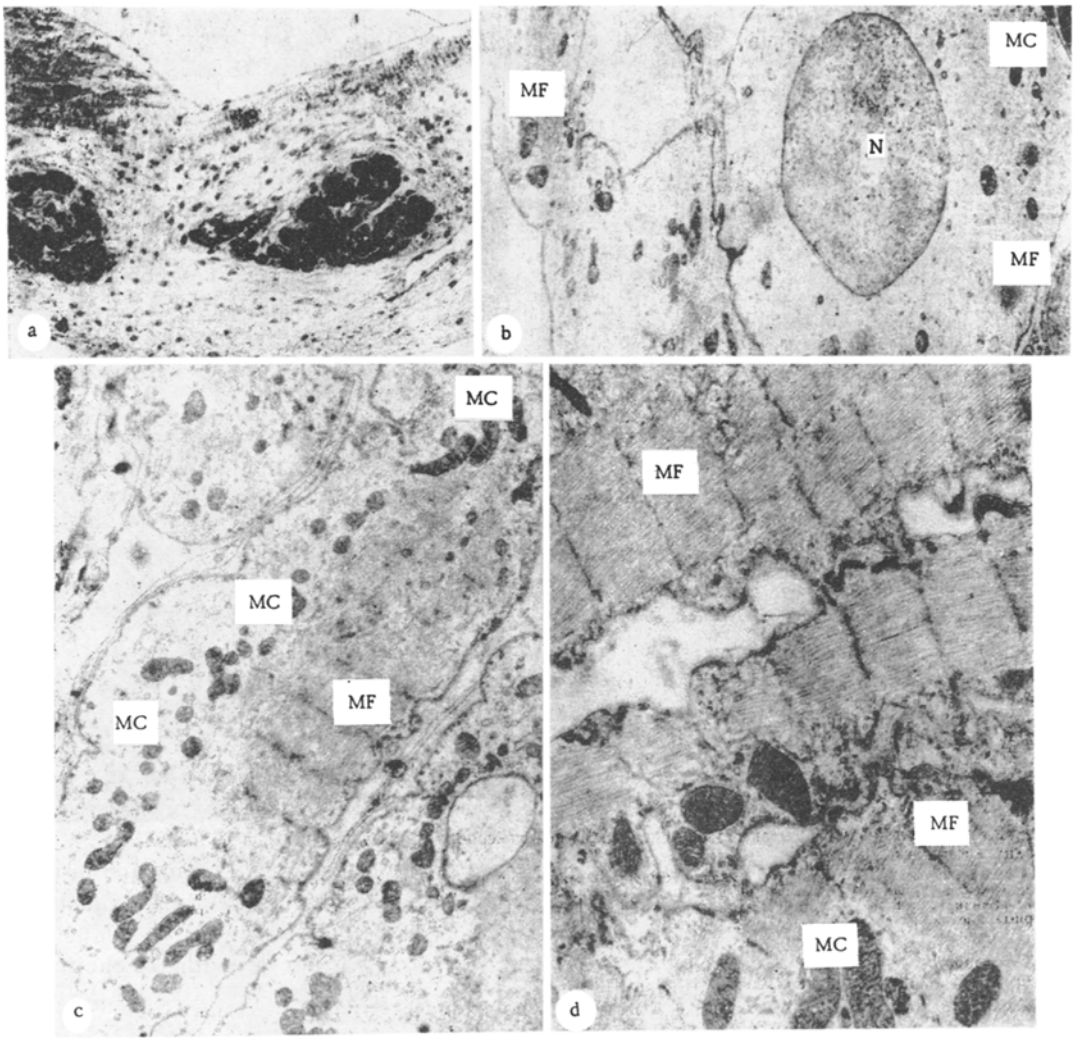

Fig. 1. Structure of pacemaker formation in atrioventricular valve of rabbit heart: a) local pacemaker formations in cusp of valve: endothelium above, followed by fibrous layer and two clusters of muscular type in stroma of valve, consisting of loose connective tissue (70×); b) P cells (6000×); c) transitional cells (9000×); d) cells of Purkinje type (12,000×). Here and in Fig. 2: MF) myofibrils, MC) mitochondria; N) nucleus.

and embedded in Araldite. The required area was visualized through the whole surface of the block by the direct vision ultramicrotomy method in semithin sections as clusters of cells of muscular type, in the region of which the block was then trimmed to a pyramid in order to obtain ultrathin sections. The sections were stained and examined in the ŒM-100-B electron microscope with a magnification of 5000-40,000 times.

EXPERIMENTAL RESULTS

Under the light microscope, in semithin sections, individual clusters of cells of muscular type were revealed in the stroma of the valves, which consisted of loose connective tissue, in those areas in which pacemaker activity was found electrophysiologically (Fig. 1a). Examination in the electron microscope revealed cells of varied morphological structure in these formations.

Some cells were large (5-10 μ in diameter), with a large, central nucleus and pale cytoplasm, on account of the small number of intracellular organelles (Fig. 1b). Solitary mitochondria with a pale matrix and a few cristae were observed in the cytoplasm of these cells; the individual contractile elements or myofilaments were arranged haphazardly or as small bundles without any characteristic cross striation. The sarcoplasmic reticulum was poorly developed. No transverse T system was present. Contact between the cells was established through simple approximation of their plasma membranes, which were separated by a distance of

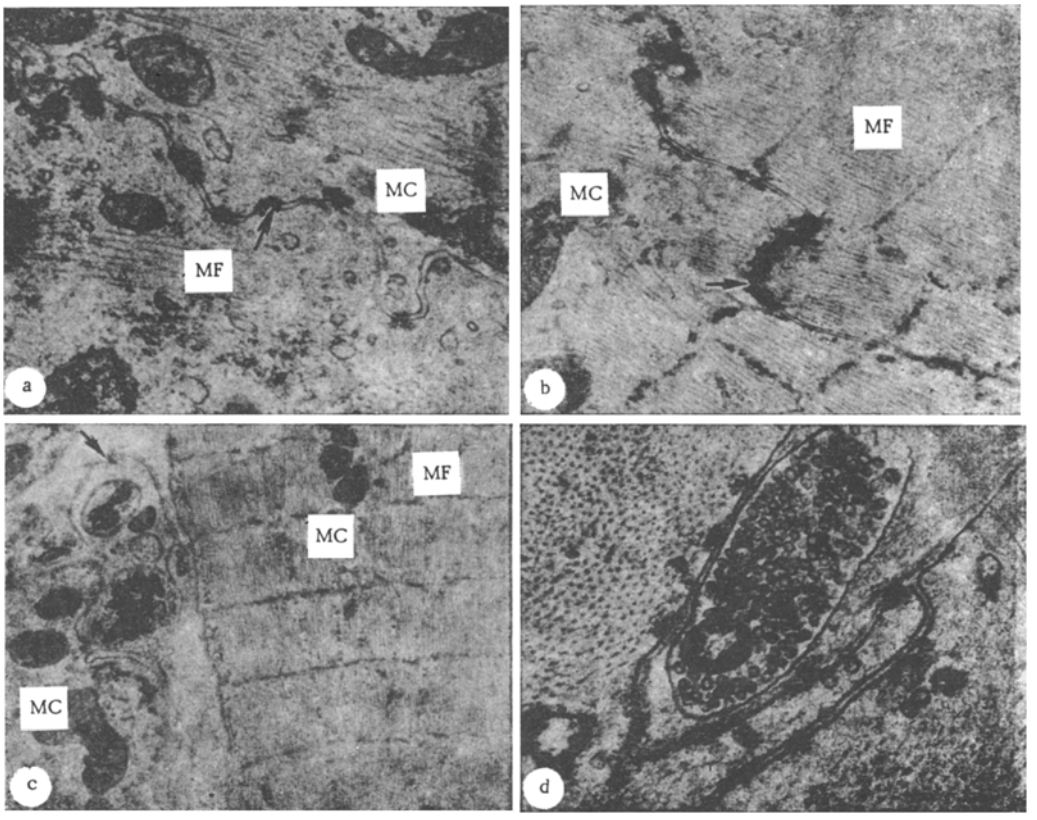

Fig. 2. Appearance of intercellular contacts and innervation of pacemaker formation in atrioventricular valve of rabbit heart: a) desmosomal type of contact (arrow) between two transitional cells (35,000×); b) intercalated disk (arrow) separating two cells of Purkinje type (20,000×); c) nerve fiber (arrow) close to cells of Purkinje type (10,000×); d) nerve ending of cholinergic nature close to pacemaker cells (45,000×).

70-200 Å. Specialized intercellular junctions in the form of desmosomes were less frequent. Cells of this type were relatively infrequent, only occasionally were two to five cells observed together, and as a rule they were situated in the depth of the formation. The characteristics of the intracellular and intercellular organization of the cells of this type suggest that they can be identified with the pale cells that were first found in the sinoatrial node of the mammalian heart and called P cells [7].

Cells of a different type, found mainly in the surface parts of the cluster of muscle cells, also were few in number and were characterized by a different morphological structure (Fig. 1e, d). These cells were large (over 15 μ in diameter) and had a central nucleus. Nearly all their cytoplasm was filled with numerous bundles of myofibrils with characteristic cross striation. Mitochondria with numerous cristae were arranged in chains between the myofibrils. The longitudinal L system of the sarcoplasmic reticulum was well developed in the cells, whereas the transverse T system as a rule could not be seen. Numerous granules of glycogen and a well-developed Golgi complex were found in the sarcoplasm. The cells of this type were joined together end-to-end by well-developed intercalated disks (Fig. 2b). In their morphological characteristics the cells of this type resembled cells of the Purkinje type [8, 9].

The most numerous cells were those which, by their morphological characteristics, could be identified as transitional cells between the two types of cells described above (Figs. 1c and 2a). They were characterized by variability of size, shape, and content of intracellular organelles, and they had intercellular contacts which ranged from simple approximation of the plasma membranes of adjacent cells to specialized junctions in the form of desmosomes and intercalated disks.

The cells of transitional type and also those of the Purkinje type were richly innervated. Numerous nerve endings, most frequently cholinergic, less frequently adrenergic in nature, were found close to the surface of these pacemaker cells (Fig. 2c, d).

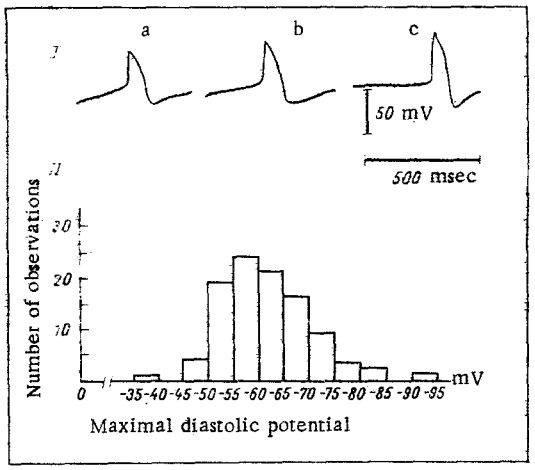

Fig. 3. Electrophysiological characteristics of pacemaker cells of atrioventricular valves of rabbit heart. I) Action potentials recorded from three types of pacemaker cells: P cells (a), transitional cells (b), cells of Purkinje type (c). II) Histogram of distribution of maximal diastolic potential based on 100 observations.

The study of the electrophysiological properties of the formations described above showed that the spontaneous AP of all cells had SDD. The frequency of spontaneous excitation was constant for the same pacemaker formation, whereas considerable variations in it were found in different preparations (from 20 to 190 beats/min).

The shape of the AP, the amount of overshoot, and the levels of the critical and maximal diastolic potentials varied within wide limits in all preparations. The pacemaker formations of the valve contained a very few cells characterized by smooth transition from SDD into phase 0 of the AP (Fig. 3a), a small overshoot (under 8 mV), and low levels of the critical (under −35 mV) and maximal diastolic (under −50 mV) potentials. The electrophysiological characteristics of these cells were similar to those of the leading cells (P cells) of true pacemakers [1, 5, 10, 11]. Cells of the second type, also few in number, were characterized by a more sudden transition from SDD into phase 0 of AP (Fig. 3c), the highest degree of overshoot (20-23 mV), and the greatest value of the maximal diastolic potential (from −80 to −95 mV). With respect to these electrophysiological characteristics, the cells of the second type were similar to cells of the Purkinje type [1, 13]. However, by the level of their critical potential (its maximal values did not exceed −60 mV) the cells of this type differed from the Purkinje cells of the ventricles, for which the critical potential varied from −60 to −80 mV. Most of the pacemaker cells of the valve, as regards the shape of the AP, the size of the overshoot, and the levels of the membrane potentials, could be classed as cells of transitional type (Fig. 3b). These results are thus in good agreement with the morphological data, indicating that P cells and cells of the Purkinje type are not as numerous as cells of the transitional type.

The results of these experiments show that the cusps of the atrioventricular valves of the rabbit heart contain complex multicellular pacemaker formations, the cells of which, by their morphological and electrophysiological characteristics, by the predominant role of the slow sodium–calcium channel in the generation of their pacemaker activity, and by the predominance of their cholinergic innervation over adrenergic, are similar to the supraventricular pacemakers of the heart.

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ELECTRON-AUTORADIOGRAPHIC ANALYSIS OF PROJECTIONS OF SOMATOSENSORY CORTICAL AREAS I AND II IN THE POSTERIOR VENTRAL NUCLEUS OF THE THALAMUS

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Differences in the organization of corticofugal fibers arising from somatosensory cortical areas I (S_I) and II (S_{II}) were detected by electron-microscopic autoradiography in the posterior ventral nucleus (NVP) of the thalamus. The distribution of corticofugal fibers from the corresponding zones of the two somatosensory cortical areas within NVP differs. Endings of both types of fibers form synaptic contacts chiefly with distal dendrites of relay cells of NVP and much less frequently with dendrites of Golgi type II interneurons. No direct convergence of fibers arising from the two somatosensory areas on single cells of NVP was observed.

KEY WORDS: corticofugal fibers; somatosensory cortical areas; posterior ventral nucleus of the thalamus; degeneration; autoradiography; electron microscopy.

Previous electrophysiological investigations showed that somatosensory cortical areas I and II differ in their influence on the relay neurons of the posterior ventral nucleus (NVP) of the thalamus, the axons of which run to somatosensory cortical area I [3-5]. The modulating effect of the somatosensory cortical areas is manifested particularly clearly in relation to the transmission of afferent signals [3] and the formation of trace processes which develop in NVP after the passage of an afferent impulse [4].

The nature of the morphological substrate responsible for differences in the character of cortical control over the mechanism of transmission of signals through the thalamic relay from the two somatosensory areas has not yet been explained. To study these problems the investigation described below was undertaken.

EXPERIMENTAL METHOD

Experiments were carried out on 36 cats weighing 2.5-4 kg. An autoradiographic method was used in the experiments of series I. Leucine-^{3}H, in a concentration of 100 μCi/μl, was injected into the zone of representation of the forelimb in somatosensory area I (S_I) of the cortex (six cats) and somatosensory area II (S_{II}) of

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