In Vitro Effect of Acetylcholine on Function of Sinoatrial Node in Rat Heart

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Electrophysiological parameters of true pacemakers in the sinoatrial node of rat heart were recorded intracellularly using glass microelectrodes. In 11 of 13 experiments acetylcholine in increasing doses did not induce migration of the dominant pacemaker region, while in two cases its minor migration upstream the sinus node artery was observed.

Key Words: *sinoatrial node; pacemaker cells; dominant pacemaker region; acetylcholine*

The sinoatrial node in mammals contains true and latent pacemaker cells differing much by their electrophysiological characteristics [5,9]. Two morphological types of cells are distinguished: typical nodal and transitional [3]. Functioning of the mammalian sinoatrial node is inseparable from the process of migration of the dominant pacemaker region (DPR) in response to various factors, such as injection of neurotransmitters [2,5], stimulation of nervous conductors [1,6,7], electrical stimulation [8], *etc.*

A peculiarity of the sinoatrial node of rat heart is its morphological connection to the sinus node artery [3,4], which allows quantitative evaluation of the DPR movement along the vessel and was used in studies of the DPR migration in response to norepinephrine *in vitro* [2].

We studied migrations of the dominant pacemaker region in response to application of acetylcholine in increasing doses.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (60-90 g). The hearts were removed from animals under nembutal narcosis (40 mg/kg) and placed into cuvettes with Hanks' solution (pH 7.35, 15-20°C). A fragment of the right atrium containing the anterior wall, upper and lower venae cavae, and the auricula was isolated. The sinoatrial node was located between the vena cava superior and the auricula along the sinus node artery [3,4]. The preparation was fixed and placed into a flow chamber with modified Krebs—Ringer solution [9] equilibrated with 5% carbogen to pH 7.4 at 38°C (1.7 ml/min medium flow rate). The DPR was located using glass microelectrodes and its position was fixed using a graduated ruler mounted on the objective (1 interval of the ruler was equal to 0.0235 mm). The shape of action potential of the pacemaker cells (the presence of slow diastolic depolarization phase, smooth transfer from slow diastolic depolarization phase into the phase of initial rapid increase of potential, and low rate of the initial rapid increase of the potential) served as the criterion of the true pacemaker cells [2-5,9]. After location of DPR, acetylcholine iodide solution (Sigma) in ascending concentrations (2.5×10^{-6}, 5.0×10^{-6}, 7.5×10^{-6} g/liter) was successively (after 15-min washing) added to the cuvette and DPR location was redetermined (using the ruler) after each addition. Basal action potentials repetition rate (APRR) and changes in this parameter after addition of acetylcholine in different concentrations were recorded.

RESULTS

As was expected, APRR of pacemaker cells decreased after application of acetylcholine. Successive increase in acetylcholine concentrations caused a nonlinear decrease in APRR with the formation of a plateau (Fig.

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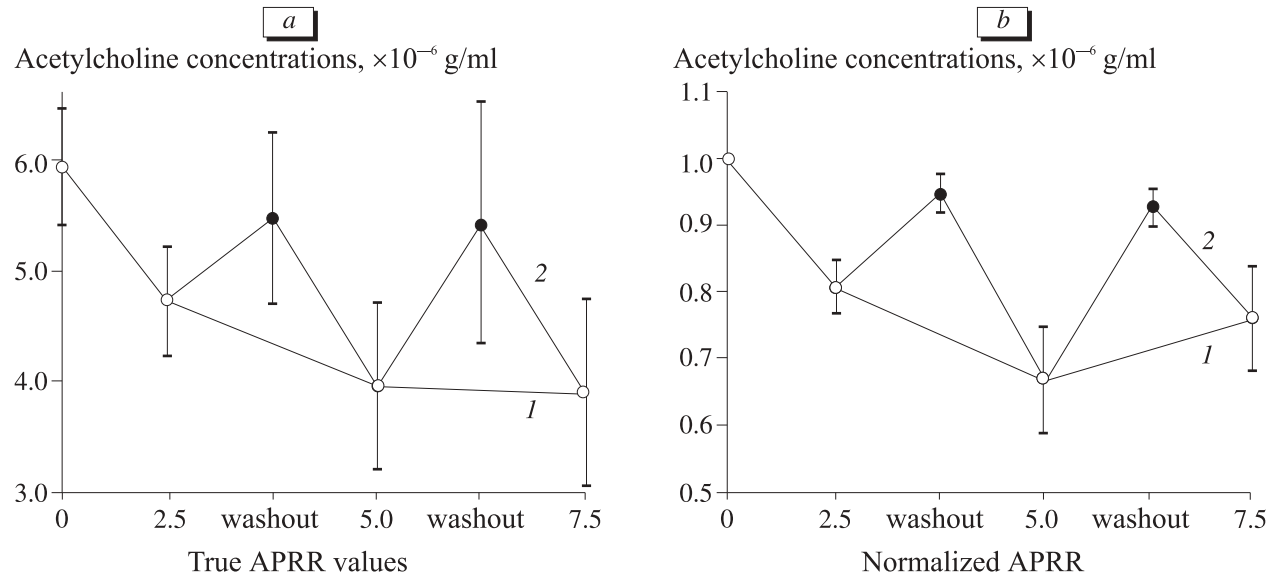

Fig. 1. Changes in action potential repetition rate (APRR) in rat sinoatrial node in response to ascending concentrations of acetylcholine (n=13). *1*) successive addition of acetylcholine in ascending concentrations; *2*) addition of acetylcholine in ascending concentrations with washing between additions.

1, *a*). It is noteworthy that 3-fold concentration of acetylcholine led to arrhythmization of the preparation in 9 of 13 preparations, which made precise measurements of APRR impossible. High scattering of APRR values results from significant differences in its basal levels in individual preparations.

The shape of curves changed when we used standardized frequency $F_a=F_t/F_0$ (Fig. 1, *b*), where F_0 is APRR at the beginning of experiment (Hz) and F_t is the current frequency (Hz). The chronotropic effect of 3-fold concentration of the transmitter was slightly (though insignificantly) lower than that of 2-fold one, which can be explained by instability of the preparation at this stage of the experiment. Incomplete recovery of APRR in comparison with the basal frequency (F_0) was observed in the majority of the experiments.

In similar experiments we previously determined the pattern of DPR migrations after addition of ascending concentrations of norepinephrine to the bathing solution. The migrations in response to the stimulator could be described by a linear curve. Further increase of norepinephrine concentration showed a limit of DPR migration (about 0.3 mm). In all cases DPR moved down along in the sinus node artery [2].

In experiments with acetylcholine DPR migration was not observed in 11 of 13 cases. DPR position remained stable. In 2 of 13 experiments DPR moved up along the sinus node artery by 0.05 and 0.12 mm. Hence, the sinoatrial node in rat is characterized by distant lower functional border and close upper border.

The vectors of DPR migrations in response to acetylcholine and norepinephrine were differently or even oppositely directed [5-7]. Hence, the rat sinoatrial node is a functionally asymmetrical formation with a functional nucleus in the upper part of the sinus node artery and an appreciable functional "tail" extended down along the artery. DPR migration up the sinus node artery in two cases confirms published data and suggests that for some reasons DPR in rats lay outside the functional nucleus at the start of the experiment. Our experiments on identification of the true pacemaker cell structure and function showed that these cells morphologically corresponded to typical nodal cells [3,4]. It seems that the functional nucleus and tail of the sinoatrial node ensuring the effects of DPR migration consist of similar groups (clusters) of typical nodal cells with different sensitivity to cardiac rhythm regulators.

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