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Original article

Modulation of rate by autonomic agonists in SAN cells involves changes in diastolic depolarization and the pacemaker current

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Abstract

Two distinct intracellular mechanisms have been proposed to affect the firing rate of cardiac pacemaker cells: one involves modulation of the I_f current by the second messenger cAMP, and one relies upon disruption or alteration of SR Ca^{2+} transients during activity. Although both mechanisms are necessary for proper automaticity and autonomic rate control, the specific contribution of each to pacemaking is still debated. We investigated if the two processes can be separated based on potentially different effects on action potential characteristics during rate modulation. To identify specific I_f-mediated effects, we used the selective I_f blocker ivabradine and found that ivabradine (3 μM) slows rate (−16.2%) by selectively reducing (−31.9%) the steepness of early diastolic depolarization (EDD). On the other hand ryanodine (3 μM), used to evaluate the effects of abolishment of SR Ca^{2+} transients, slowed rate (−31.3%) by depolarizing the take-off potential (TOP, 18.1%) without affecting EDD. We therefore used these two parameters to identify I_f-based or SR Ca^{2+} transients-based processes and analyzed the effects on action potential's characteristics of Rp-cAMPs (50 μM), a membrane permeable cAMP analogue directly activating f-channels; we found that Rp-cAMPs accelerates rate by increasing EDD (+42.3%) without modifying TOP. Finally, rate modulation was achieved by muscarinic (ACh 0.01 μM) or β-adrenergic (Iso 1 μM) stimulation; in both cases, rate changes were associated with modifications of EDD (ACh, −29.3% and Iso, +47.6%) and not of TOP. We conclude that rate-related changes in the EDD induced by autonomic agonists are mediated by I_f and not by processes involving SR Ca^{2+} transients.

Keywords: I_f current; Pacemaker rate; SR Ca transients; Ivabradine; Ryanodine; Diastolic depolarization; SA node; Autonomic modulation

1. Introduction

A contribution of the funny (I_f) pacemaker current of sino-atrial node (SAN) cells to the generation and autonomic modulation of spontaneous activity in the heart has been proposed since the early description of this current [1,2] and is now well established [3–5], although debate continues on the extent of this contribution and participation of other mechanisms. Several reports have proposed a role for Ca^{2+} transients in rate regulation based on observations of Ca^{2+} release from the sarcoplasmic reticulum during activity [6–11]. These reports have highlighted a correlation between the amplitude of the Ca^{2+} transients and rate. For example, β-adrenoreceptor (βAR) stimulation causes parallel increases in Ca^{2+} transients and rate in SAN cells, and reduction or abolishment of Ca^{2+} transients leads to proportional changes in the frequency of spontaneous activity of pacemaker cells, as well as reduced responsiveness to βAR stimulation [11].

While these studies clearly illustrate the importance of normal Ca^{2+} homeostasis to the persistence of stable automaticity, the data have led to the further proposal that Ca^{2+} transients represent a main mechanism responsible for both normal SAN rate maintenance and β-adrenergic-induced positive chronotropism. However, experiments investigating the mode of action of Ca^{2+} transients inhibition on rate modulation showed that while abolishment of Ca^{2+} transients by ryanodine does inhibit βAR-induced rate acceleration, it does not abolish the rate acceleration caused by increasing intracellular cAMP levels [12]. This suggests that although normal Ca^{2+} homeostasis is required for maintenance of a proper activation cascade leading from βAR stimulation to effectors, including f-channel ac-

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tivation, rate regulation also involves the contribution of a cAMP-mediated, I_f-dependent mechanism.

To further investigate the mechanisms controlling rate regulation, and specifically the control of rate by autonomic agonists, we asked the question whether different processes affecting rate can be classified based on their effects on action potential configuration. What is the "signature", for example, of changes of rate induced by I_f changes? Is this different from the signature of rate changes attributable to modifications of Ca^{2+} transients? Can any of these signatures be recognized in the way autonomic neurotransmitters affect rate? These questions were prompted by the preliminary observations that ryanodine slows rate both by altering the late diastolic depolarization and by shifting the action potential threshold in SAN cells to more positive values [12], while direct modulation of I_f by Rp-cAMPs increases rate by altering the early diastolic depolarization without an apparent effect on the action potential threshold (see for example [12], Fig. 4). We therefore proceeded to identify parameters describing action potential features such as action potential threshold and early diastolic depolarization rate and to study how different rate-modifying stimuli, and specifically neurotransmitters, affect these parameters.

We found that rate changes due to I_f modification are associated with specific changes of EDD, while those due to SR Ca^{2+} modifications are associated with specific changes of TOP. We further found that rate changes elicited by autonomic agonists involve changes of EDD, but not of TOP. These data confirm that I_f modulation is a major mechanism responsible for rate control.

2. Materials and methods

2.1. Cell isolation

Animal protocols conformed to the guidelines of the care and use of laboratory animals established by Italian (DL. 116/1992), European (86/609/CEE) and US (National Institutes of Health publication No 85-23) directives.

Young white albino rabbits (0.8–1.2 kg) were deeply anesthetized by intramuscular injection of xilazine (4.6 mg/kg) and ketamine (60 mg/kg) (Sigma-Aldrich Co) and euthanized by cervical dislocation, exsanguination and cardiectomy. After quick removal of the heart, the SAN tissue was dissected out and strips of nodal tissue were dissociated into single cells or small aggregates of cells by an enzymatic and mechanical procedure previously described [13]. Dissociated SAN myocytes were stored at 4 °C for the day. During experiments, cells were placed in a 30 mm plastic petri dish placed on the stage of an inverted microscope and superfused with a Tyrode solution containing (mM): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES-NaOH, 10 D-glucose, pH 7.4. Patch-clamp analysis was performed in the whole-cell configuration. The pipette solution contained (mM): 130 K-Aspartate, 10 NaCl, 2 $CaCl_2$, (pCa=7), 2 $MgCl_2$, 10 HEPES, 5 EGTA, 2 ATP(Na_2), 0.1 GTP, 5 Creatine Phosphate, pH 7.2. Spontaneous activity was recorded from small aggregates of 2–6 cells beating uniformly. In addition to providing stable seals for longer times, recording from cell clusters, rather than single cells, was preferred because it prevented cell dialysis and interference with normal intracellular Ca^{2+} homeostasis. Test solutions were delivered on top of the cell under study by a fast perfusion device allowing solution changes near the cell in less than 1 s. The temperature was 34±0.5 °C.

Isoproterenol (Iso) and acetylcholine (ACh) were purchased from Sigma-Aldrich Co, and ryanodine and Rp-cAMPs were obtained from Calbiochem. Ivabradine (Iva) was provided by the Institut de Recherches Internationales Servier, France.

2.2. Data analysis

All data were acquired with pClamp software and an Axopatch 200B amplifier (Axon Instruments). Action potentials were recorded from small uniformly beating aggregates as continuous traces for several hundred seconds at a sampling rate of 2 kHz and filtered at 1 kHz with pClamp software. Only recordings where the control frequency was stable over a period of several tens of seconds were used. Raw action potential records were digitally smoothed by a 10-point adjacent averaging smoothing procedure and the time derivative calculated according to a second polynomial, 8-point smoothing differentiating routine (Origin 7, Origin Lab, Northampton, MA).

Data thus obtained were then processed with customized software. For each action potential cycle, we obtained the following parameters (Fig. 1):

(a) maximum diastolic potential (MDP, mV), defined as the most negative potential reached during action potential repolarization;

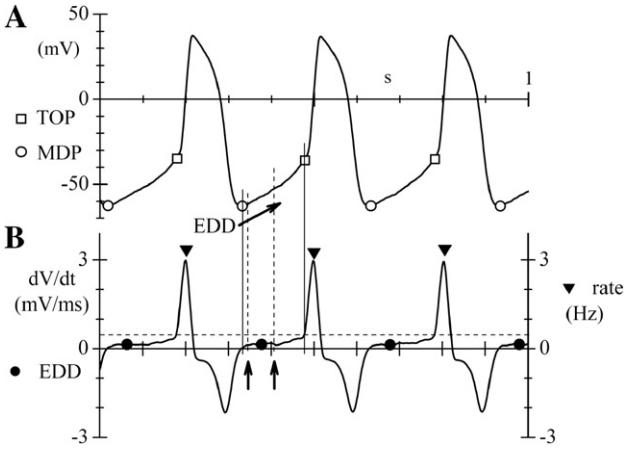

Fig. 1. Measurement of action potential parameters. Sample action potential trace (mV scale in A) and corresponding time derivative dV/dt (mV/ms scale in B) illustrating how action potential parameters were obtained. Both action potential and time derivative traces were digitally filtered as described in the Materials and methods. Time-dependent plots of different variables are indicated by different symbols: MDP, open circles and TOP, open squares (A, mV); EDD, filled circles (B left, mV/ms); rate, filled triangles (B right, Hz). For the sample cycle shown, vertical arrows and corresponding vertical broken lines delimit the time interval used for calculation of the slope of early diastolic depolarization (EDD); this was selected as the interval 0.1 Δt to 0.5 Δt, where Δt is the time interval between MDP and TOP times (vertical full lines). The horizontal broken line is the selected dV/dt threshold (0.5 mV/ms) used to determine the TOP time. A more complete explanation of the algorithms for measurement of all parameters used is given in the text.