Effects of Acetylcholine on Electrical Remodeling of Human Atrial Fibers

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Summary: Autonomic nervous system activation can result in significant changes of atrial electrophysiology and facilitate induction of atrial fibrillation. By recording influence of different concentrations of acetylcholine (ACh) on atrial fibers (AF), we investigated the role of the increased vagal tone in electrical remodeling in atrial fibrillation. Parameters of action potentials and force contraction (Fc) in atrial fibers were recorded by using standard intracellular microelectrode technique and force transducer. It was found that: (1) ACh at 0.1 µmol/L had no significant influence on spontaneous action potentials (SAPs) and Fc (n=6, P>0.05); ACh at both 1.0 and 10.0 µmol/L shortened action potential duration (APD) and Fc of human AF from right atrium (n=6, P<0.05); there was no significant difference in shortening APD between 10.0 and 1.0 µmol/L of ACh; (2) ACh at 0.1 µmol/L had no significant desensitization (n=6, P>0.05), but ACh at 1.0 and 10.0 µmol/L had desensitization (n=6, P<0.05) to SAPs and Fc. The desensitization of ACh on APD in AF was concentration- and time-dependent. It was shown that APD was longer than the control along with extending time of continuous Tyrode’s solution perfusion after desensitization. It is concluded that ACh changes the electrophysiological characteristics of human AF, indicating that increased vagal tone plays a role in the development of a vulnerable substrate for atrial electrical remodeling in atrial fibrillation.

Key words: electrical remodeling; vagal nervous system; acetylcholine; desensitization

Chiou et al[1] and many investigators have demonstrated that atrial fibrillation can be initiated by focal electrical activity of autonomic nervous system, particularly the parasympathetic nervous system in the thoracic veins (pulmonary vein, vena cava, coronary sinus and ligament of Marshall)[2-4]. Once present, atrial fibrillation changes the electrophysiological and structural properties of the atrial myocardium (atrial remodeling) in a way that induces its self-perpetuation and self-recurrence[5]. However, there are few data evaluating the relationship between parasympathetic tone and recovery from electrical remodeling. Previous studies have suggested that high vagal tone was associated with a short atrial effective refractory period after rapid pacing and that there was a prolonged recovery from remodeling in goats[6]. Moreover, it is widely accepted that vagal stimulation changes the electrophysiological and structural properties of the atrial myocardium (atrial remodeling) in a way that induces its self-perpetuation and self-recurrence. Therefore, the present study was undertaken to investigate effects of different concentrations of ACh (neurotransmitter of vagus) on electric activity of human atrial fibers (AF) to define the role of increased vagal tone in atrial electrical remodeling in atrial fibrillation.

1 MATERIALS AND METHODS

1.1 Specimens

In accordance with the institutional guidelines for human subject research, approximately 1 cm² isolated specimen from anterior free wall of the right atrial appendage was removed from each heart of 18 patients undergoing corrective open-heart surgery requiring the routine cannulation procedure for cardiopulmonary bypass. All patients were under 40 years old and suffered from congenital heart diseases including ventricular septal defects (n=9) or atrial septal defects (n=9). The specimens of atrial tissues were considered to be physiologically normal if they met the following clinical criteria: (1) According to the New York Heart Association (NYHA) Cardiac Functional Classification, all preoperative patients were fallen into class I; (2) Preoperative ultrasound and chest X-ray in all patients only revealed expansion of the atrium; (3) No patients had been in congestive heart failure and none had received any cardiotonic, antiarrhythmic, or diuretic medication; (4) No patients had a history or electrocardiographic evidence of cardiac arrhythmia or pulmonary hypertension. The ex-
peripheral program was done in accordance with the criteria of the Declaration of Helsinki and accepted by the Scientific and Ethics Committee of Union Hospital, Wuhan. Prior to cardiac surgery, informed consent was obtained from each patient.

The tissue was immersed in cold oxygenated Tyrode’s solution immediately after excision from the atrium and brought rapidly to the laboratory. The specimens were then transferred to a conventional perfusion chamber and fixed with fine pins to the silica gel of the Lucite chamber perfused with Tyrode’s solution maintained between 36°C and 37°C for 60 min. The specimens and Tyrode’s solution were equilibrated with 100% O₂ and improved Tyrode’s solution was composed of (mmol/L): 140 NaCl, 5 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), 0.33 NaH₂PO₄, 1 MgCl₂, 1.8 CaCl₂, 5 KCl, 10 glucose, with a pH of 7.35±0.05. The perfusion rate was 4 mL/min.

1.2 Electrophysiological Measurements

Parameters of spontaneous action potentials (SAPS) and force contraction (FC) in human AF were recorded by using standard intracellular microelectrode technique and force transducer. One end of the preparation was fixed with fine pins to the silica gel on the base of a perfusion chamber; other end of the preparation was set on stainless steel wire with 0.3 mm in diameter which was directly connected to the muscle transducer (Chengdu Instrument Factory, China). The tissue was impaled with 3 mol/L KCl-filled glass capillary microelectrodes having tip diameters about 0.5 µm and resistances of 10–30 MΩ. The electrodes were coupled by a 3 mol/L KCl interface to an Ag–AgCl bar which led to an amplifier (Axopatch 200, Axon) having a high input impedance. The amplified signals were fed to the A/D convertor and processed by a microcomputer. Measurements included maximal diastolic potential (MDP), rate of pacemaker firing (RPF), and duration of 90% and 80% repolarization of action potential (APD₉₀, APD₈₀), Fe with the program designed by Chengdu Instrument Factory, China. Measurement parameters were memorized into the microcomputer for analysis.

1.3 Experimental Protocols

The experiment began after the specimens had been equilibrated in the perfusate for 60 min and stable SAPs and Fe were recorded. The experiments consisted of: (1) Recording the spontaneous electric activity of human AF from right atrium after spontaneous rhythm was stabilized; (2) Recording the effect of ACh on the electrophysiology of human AF from right atrium. After recording SAPs of three stable controls, Tyrode’s solutions containing ACh (0.1, 1, and 10 µmol/L) were applied, accumulatively; (3) Washing for 10 min with Tyrode’s solution when the desensitization of ACh appeared.

1.4 Drugs

ACh synthesized by Sigma Chemical Co, USA was dissolved in deionized water. ACh was prepared as 10 mmol/L stock solution. Before the experiments, the stock solution was diluted with external solution to reach the desired final concentrations.

1.5 Statistical Analysis

All values were expressed as ±xs. The analysis of data was performed using paired t-test. A difference of P<0.05 was considered significant. Sigma Plot 9.0 software (SPSS Incorporation, USA), Clampfit 10.0 software (Molecular Devices, USA) and Originpro 7.5 software (Origin Lab Corporation, Northampton, MA, USA) were used to fit the data and plot graphs.

2 RESULTS

After 60 min of equilibration, the glass capillary microelectrodes were exploited to find human AF with spontaneous electric activity. There were two types of fibers in human atrium: the first showing electrical characteristics typical of atrial contractile cells and the second showing those of atrial specialized fibers. Autonomicity developed only in the latter type of cells[10–12]. In all specimens, intracellular potentials of human AF showed the characteristics of a “pacemaker” cell; there was a smooth transition from slow depolarization of phase 4 to the more rapid depolarization of phase 0. After attainment of stable sustained spontaneous electric activity, the characteristics of the SAPs were recorded.

2.1 Effects of ACh on SAPs of Human AF

No significant effect of ACh on SAPs of human AF from right atrium was seen at the concentration of 0.1 µmol/L used (n=6, P>0.05, compared with control group). Both 1.0 and 10.0 µmol/L of ACh significantly decreased the RPF, APD and increased MDP (n=6, P<0.05). The effect of 1.0 and 10.0 µmol/L of ACh on shortening APD in atrial cells was not concentration-dependent. There was no significant difference in shortening APD between 10.0 and 1.0 µmol/L ACh (table 1). Furthermore, when compared to APD before the exposure to ACh, APD under ACh at 1.0 and 10.0 mol/L was rather prolonged after wasout (table 1).

Table 1 Effect of different concentrations of ACh (µmol/L) on spontaneous action potentials of human AF from right atrium

<table>
<thead>
<tr>
<th>Groups</th>
<th>RPF (beat/min)</th>
<th>MDP (mV)</th>
<th>APD₉₀ (ms)</th>
<th>APD₈₀ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.5±0.3</td>
<td>-49.2±0.3</td>
<td>306±4</td>
<td>356±6</td>
</tr>
<tr>
<td>ACh (0.1)</td>
<td>34.4±0.2c</td>
<td>-49.8±0.5c</td>
<td>305±5c</td>
<td>358±6c</td>
</tr>
<tr>
<td>Control</td>
<td>33.1±0.2</td>
<td>-52.9±0.2</td>
<td>334±8</td>
<td>379±6</td>
</tr>
<tr>
<td>ACh (1.0)</td>
<td>30.1±3.0a</td>
<td>-56.0±1.0b</td>
<td>317±7a</td>
<td>368±7a</td>
</tr>
<tr>
<td>Control</td>
<td>31.9±0.2</td>
<td>-60.9±0.4</td>
<td>338±4</td>
<td>394±9</td>
</tr>
<tr>
<td>ACh (10.0)</td>
<td>26.1±3.6b</td>
<td>-63.2±0.2b</td>
<td>319±3b</td>
<td>376±3c</td>
</tr>
</tbody>
</table>

Data are expressed as ±xs (n=6). aP<0.05, bP<0.01, cP>0.05, compared with the corresponding controls by paired t-test

2.2 Effects of ACh on APD and Fe of Human AF

0.1 µmol/L ACh had no significant effects on APD shortening and desensitization in human AF (n=6, P>0.05). Both 1.0 and 10.0 µmol/L ACh had significant effect on desensitization (n=6, fig. 1 and 2). Moreover, more pronounced desensitization and shortened desensitization time of human AF from right atrium were associated with the increasing concentration of ACh. As shown in fig. 3, different concentrations of ACh had desensitization to human AF from right atrium. SAPs of