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Full paper

Involvement of alpha- and beta-adrenoceptors in the automaticity of the isolated guinea pig pulmonary vein myocardium

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ABSTRACT

We examined the involvement of adrenoceptors in the automaticity of the pulmonary vein myocardium, which probably plays a crucial role in the generation of atrial fibrillation. The automatic activity of the myocardium in guinea pig pulmonary vein tissue preparations were monitored by contractile force or membrane potential measurement. In quiescent preparations, application of noradrenaline induced an automatic activity. The firing frequency was reduced by prazosin or atenolol. Methoxamine induced an automatic activity of low frequency, which was accelerated by further application of isoproterenol. In preparations driven at a constant frequency, noradrenaline, in the presence of atenolol, caused a depolarizing shift of the resting membrane potential and an increase in the slope of the diastolic depolarization. In contrast, in the presence of prazosin, noradrenaline had no effect on the slope, but caused acceleration of the late repolarization and a hyperpolarizing shift of the maximum diastolic potential. At clinically relevant concentrations, carvedilol significantly inhibited the noradrenaline-induced activity but bisoprolol did not. It was concluded that α_1- and β_1-adrenoceptor stimulation enhance automaticity through different mechanisms in the guinea pig pulmonary vein myocardium. Dual blockade of these adrenoceptors appears to be effective for suppressing noradrenaline-induced pulmonary vein automaticity and probably atrial fibrillation.

1. Introduction

The pulmonary vein contains a myocardial layer connected to the left atrial myocardium which is capable of generating spontaneous or triggered action potentials. The ectopic automatic activity of the pulmonary vein is overdriven by the normotopic pacemaking activity of the sinus node in the normal heart, but may be manifested under certain pathological conditions. Earlier studies has highlighted the role of repetitive focal activity of the pulmonary vein myocardium in the triggering and maintenance of atrial fibrillation,[1,2] the most common arrhythmia in clinical practice. Atrial fibrillation increases the risk of heart related complications such as heart failure and stroke due to thrombus, and its therapeutic need is high. Although presently available antiarrhythmic agents are moderately effective for the treatment of atrial fibrillation, these agents have broad electrophysiological effects on the atrial and ventricular working myocardium and are not free from arrhythmogenic risk.[3] Thus, antiarrhythmic agents with high efficacy and safety are desired, and it is anticipated that agents which inhibits the automatic activity of the pulmonary vein myocardium would achieve the desired profile.

The major risk factors of atrial fibrillation include high-blood pressure, heart failure and valvular dysfunction: conditions which are accompanied by elevated sympathetic drive of the heart.[4] Reduction of pulmonary vein automaticity through ablation of the ganglionated plexi has received increasing attention as an efficient therapeutic strategy for atrial fibrillation.[5] The effectiveness of this strategy implies that pharmacological blockade of the

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autonomic nerve influence to the pulmonary vein myocardium would reduce its automaticity and lead to prevention or termination of atrial fibrillation. Experimental manipulation of pulmonary vein automaticity with agents related to autonomic nerves and receptors has been reported in several animal species such as the dog,[6] rabbit,[7] rat[8–10] and mouse.[11]

The guinea pig heart has been widely used in various physiological and pharmacological studies including the development of atria-specific antiarrhythmic agents for the treatment of atrial fibrillation.[12–15] Microelectrode recording of the action potential of the isolated guinea pig pulmonary vein myocardium was conducted by several researchers including us.[16–18] We have reported the basic firing mechanisms of the guinea pig pulmonary vein myocardium,[19–21] the effect of acute and chronic mechanical stretch[22,23] and the effects of antiarrhythmic agents.[24] In the present study, we intended to clarify the effect of adrenoceptor stimulation on the automaticity of the guinea pig pulmonary vein myocardium. The results indicated that α_1- and β_1-adrenoceptors are involved in the enhancement of pulmonary vein automaticity through different mechanisms.

2. Materials and methods

2.1. General

All experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and the Guide for the Care and Use of Laboratory Animals at Faculty of Pharmaceutical Sciences, Toho University.

2.2. Preparation of guinea pig pulmonary vein

The hearts with lungs were quickly removed from Hartley strain male guinea pigs weighing 300–450 g and preparations were made from the four major pulmonary vein trunks. The region of the pulmonary vein close to the orifice was cut open and suspended in a 20 ml organ bath containing the physiological salt solution following composition (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.9 and glucose 11.1 (pH 7.4), gassed with 95% O_2–5% CO_2 and maintained at 36 ± 0.5 °C.

2.3. Contractile force measurement

Contractile force was recorded isometrically with a force-displacement transducer (TB-611T, Nihon Kohden, Tokyo) and a carrier amplifier (AP-621G, Nihon Kohden, Tokyo). One end of the pulmonary vein preparation was pinned down on a silicon block at the bottom of the organ bath and the other end was attached to a needle connected to the transducer. The output of the carrier amplifier was digitized by an A/D converting interface (Power Lab, AD instruments, Dunedin, New Zealand).

2.4. Action potential measurement

The experimental procedures for microelectrode experiments were basically the same as those in our previous studies.[19,21–23] The quiescent preparations were driven by rectangular current pulses (1 Hz, 3 msec, 1.5 × threshold voltage) through a pair of platinum plate electrodes generated from an electronic stimulator (SEN-3301, Nihon Kohden). The action potential parameters: resting membrane potential (RMP); maximum diastolic potential (MDP); overshoot (OS); maximum rate of rise (dV_{max}/dt); action potential duration at 20%, 50% and 90% repolarization ($APD_{20, 50, 90}$) and the slope of the diastolic depolarization phase were measured.

2.5. Chemicals

Noradrenaline-bitartrate, prazosin, atenolol, ICI118551, methoxamine and isoproterenol were purchased from Sigma–Aldrich (St. Louis, MO, USA), SR59230A from Wako Pure Chemical Industries (Osaka, Japan) and 1,2-Bis (2-aminophenoxy) ethane-N, N, N′, N′-tetraacetic acid acetoxymethyl ester (BAPTA-AM) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo). Prazosin, atenolol, SR59230A and BAPTA-AM were dissolved in dimethyl sulfoxide and small aliquots were added to the organ bath to obtain the desired final concentration. The other chemicals were dissolved in distilled water. All other chemicals were commercial products of the highest available quality.

2.6. Data analysis and statistics

Data were expressed as means ± standard error of the mean (S.E.M.). Contractile force and action potential parameters were analyzed by Chart7 (AD instruments, Dunedin, New Zealand). Statistical significance between means was evaluated by the paired *t*-test, the Student's *t*-test or Welch's *t*-test, using the GraphPad PRISM 6.07 software (San Diego, USA). A *P* value less than 0.05 was considered significant.

3. Results

3.1. Induction of automatic activity by noradrenaline

In quiescent isolated pulmonary vein preparations, noradrenaline (0.1–10 μM) induced automatic contractile activity (Fig. 1A). The incidence as well as frequency of contractile activity was concentration dependent; contractions were induced at 0.1 μM or 0.3 μM, and the frequency of contractions in each preparation increased with the increase in noradrenaline concentration (Fig. 1B). The automatic activity induced by 10 μM noradrenaline was partly inhibited by chelation of intracellular Ca^{2+} by BAPTA (300 μM BAPTA-AM). The firing frequency before and after the application of BAPTA-AM was 2.4 ± 0.2 Hz and 0.6 ± 0.2 Hz, respectively (n = 6).

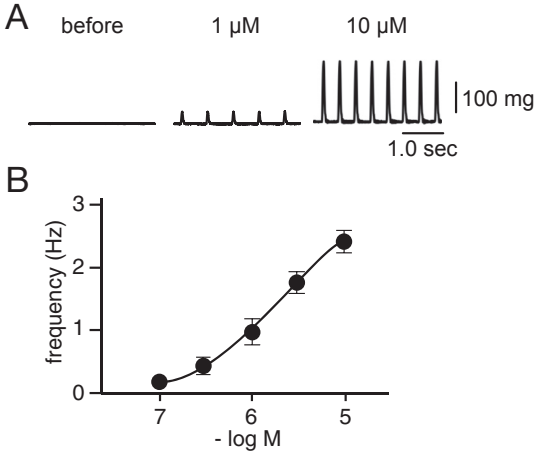

Fig. 1. Noradrenaline-induced automatic activity in the quiescent pulmonary vein preparations. Typical traces of contractile force before and after application of 1 μM and 10 μM noradrenaline (A). Summarized concentration-frequency relationship (n = 11) (B). Symbols and vertical bars indicate mean ± S.E.M.