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*Short Communication*

# Electrical Activity of the Mouse Pulmonary Vein Myocardium

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**Abstract.** We recorded the electrical activity from the myocardial layer of isolated mouse pulmonary veins with the glass microelectrode technique. Spontaneous electrical activity was observed in about half of the preparations, which appeared either as constant firing or as repetitive bursts. Noradrenaline enhanced, while acetylcholine reduced, automatic activity. The action potentials evoked in quiescent preparations showed a resting membrane potential less negative than the atria and an extremely rapid early repolarization followed by a late plateau. The present study revealed that the mouse pulmonary vein myocardium shows diverse electrical activity, which is influenced by autonomic neurotransmitters.

***Keywords***: pulmonary vein myocardium, automaticity, autonomic neurotransmitter

The mouse heart has a high beating rate and a unique action potential configuration with an extremely rapid repolarization phase due to the presence of the transient outward potassium current ($I_{to}$) as the major repolarizing current. The myocardium of the mouse has characteristic excitation–contraction mechanisms and autonomic regulation to support its rapid contraction (1, 2). Mice are used as genetically-modified animals in which specific genes related to various cardiac diseases are overexpressed or knocked out. Thus, investigation of the mouse heart is of value for basic cardiac physiology and for the interpretation of experimental results from genetically-modified mice.

Atrial fibrillation is a common cardiac arrhythmia in adults which increases the risk of cardiac failure and stroke. The initiation and maintenance of atrial fibrillation are closely related to factors such as autonomic nerve activity and distension of the atrial region (3). The pulmonary vein is receiving attention as the major source of ectopic beats to initiate atrial fibrillation (4, 5). It contains a myocardial layer, which is a continuation from the left atrial myocardium and is capable of generating spontaneous electrical activity (6 – 11). Although studies on the mechanism of generation and the pharmacological properties of the pulmonary vein automaticity are now in progress in many animal species, the electrical activity of the mouse pulmonary vein myocardium has not yet been reported. In the present study, we recorded the electrical activity of the mouse pulmonary vein myocardium with the glass microelectrode technique and examined the effect of autonomic neurotransmitters.

All experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. Hearts with lungs were quickly removed from 6 – 8-week-old male ddY mice and preparations were made from the three major pulmonary vein trunks (Fig. 1Aa) and the left atria. The experimental procedures for histochemical and microelectrode experiments were basically the same as those in our previous studies (8 – 11). All values were expressed as means ± S.E.M. The statistical significance of differences between means was evaluated by the paired *t*-test, unpaired *t*-test, or one-way analysis of variance followed by Dunnett's test for multiple comparisons.

Masson-trichrome staining showed that about half of the mouse pulmonary vein wall was muscular tissue (Fig. 1Ab). Staining with anti-$\alpha$-smooth muscle actin revealed that the smooth muscle was confined to a thin layer on the luminal side (Fig. 1Ac). This indicates that the myocardial layer formed a major portion of the pulmonary vein wall. The myocardial layer of the mouse pulmonary vein was only 30 $\mu$m in thickness with 3 to 4 cell layers, which was thinner than that of other experimental animal species (9 – 12).

About a half (65 in 144) of the pulmonary vein prepa-

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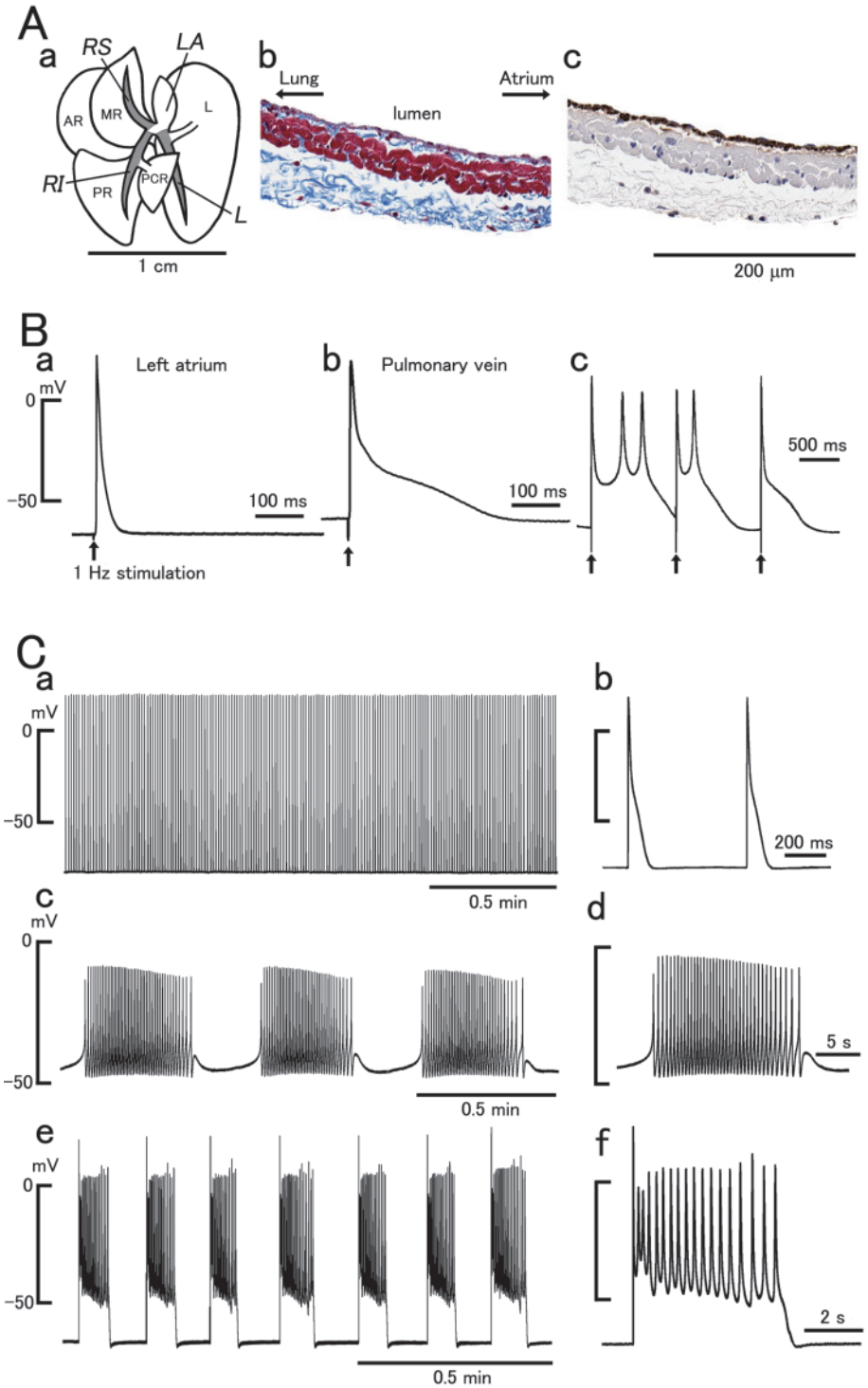


**Fig. 1.** Anatomy and electrical properties of the mouse pulmonary vein. Aa: Illustration of the left atrium and lung with pulmonary veins. Pulmonary lobes are indicated as L: left, PCR: postcaval right, PR: posterior right, MR: media right, AR: anterior right. Shadowed area and oblique type shows the areas of dissection of the preparations. L: left pulmonary vein, RS: right superior pulmonary vein, RI: right inferior pulmonary vein, LA: left atrium. Ab and Ac: Masson-trichrome staining (b) and staining with anti-smooth muscle $\alpha$-actin (c) of serial longitudinal sections. B: Typical traces of action potentials evoked at 1 Hz. a: left atrium, b: pulmonary vein, c: pulmonary vein with an EAD. Arrows indicate electrical stimulation. C: Typical traces of spontaneous electrical activity in pulmonary vein myocardium in normal (a, c, e) and expanded (b, d, f) time scale. The type 1 automaticity was a constant firing (a, b); the type 2 automaticity was a repetitive burst (c, d); and the type 3 automaticity was a repetitive burst with a more negative maximum diastolic potential (> 5 mV) between the bursts than during the bursts (e, f).

rations showed spontaneous electrical activity, and the other half was quiescent. Action potentials evoked in quiescent pulmonary vein and left atrial preparations by electrical stimulation at 1 Hz (Fig. 1B) showed extremely rapid early repolarization, which ceased at about 20 ms when the membrane potential reached −30 to −40 mV. Unlike the atrial myocardium, which showed further repolarization (Fig. 1Ba), the pulmonary vein myocardium showed a late plateau lasting longer than 100 ms (Fig. 1Bb). Early after-depolarizations (EADs) were observed in 4 out of 14 pulmonary vein preparations (Fig. 1Bc). The resting potential (RP) was significantly less negative and action potential duration at 90% repolarization ($APD_{90}$) was significantly longer in the pulmonary vein than in the atria (Fig. 1B). The action potential parameters of the pulmonary vein preparations were as follows: RP: −67.5 ± 0.9 mV, peak membrane potential: 18.2 ± 1.2 mV, action potential duration at 20% repolarization ($APD_{20}$): 5.5 ± 0.6 ms, that at 50% repolarization ($APD_{50}$): 14.6 ± 1.6 ms, $APD_{90}$: 142.4 ± 24.5 ms (n = 14). The action potential parameters of left atria were as follows: RP: −76.7 ± 0.3 mV, peak membrane potential: 18.8 ± 2.3 mV, $APD_{20}$: 4.8 ± 1.3 ms, $APD_{50}$: 9.2 ± 1.1 ms, $APD_{90}$: 40.8 ± 3.0 ms (n = 6). 4-Aminopyridine (1 mM), a blocker of $I_{to}$, prolonged the $APD_{20}$ of the pulmonary vein from 4.4 ± 0.3 to 11.6 ± 1.3 ms (n = 7, $P < 0.001$).

The spontaneous electrical activity of the pulmonary vein myocardium appeared in three different waveform types (Fig. 1C, Table 1). The first was a constant firing (type 1; Fig.1C: a, b) and the second was a repetitive burst with a similar maximum diastolic potential (MDP) during and between bursts (type 2; Fig. 1C: c, d). The third was also a repetitive burst but with a more negative MDP (> 5 mV) between the bursts than during the bursts and thus an EAD-like waveform (type 3; Fig. 1C: e, f). The incidence of type 1, 2, and 3 was 27.7% (18/65), 30.8% (20/65), and 41.5% (27/65), respectively, and spontaneous interconversion of waveform types was occasionally observed. A pacemaker-like waveform, which is characterized by a diastolic depolarization and its smooth transition into the action potential upstroke, was observed in all types. The incidence of the pacemaker-like waveform was 27.8% (5/18), 65.0% (13/20), and 37.0% (10/27) for type 1, 2, and 3 activity, respectively. Automatic electrical activity was not observed in the left atrium (n = 14). Noradrenaline (1 $\mu$M) increased the frequency of type 1 activity from 0.7 ± 0.3 to 3.9 ± 2.0 Hz (3/3) and changed type 2 activity to type 1 (2/3) or type 3 (1/3) and type 3 activity to type 1 (4/9) or type 2 (5/9). Acetylcholine (0.3 $\mu$M, 5/5), as well as adenosine (10 $\mu$M, 5/5), inhibited all types of activity.

In quiescent pulmonary vein preparations, noradrenaline (1 $\mu$M) induced automatic electrical activity (59/79), which appeared in the three waveform types mentioned above (Fig. 2A: a – f); the incidence of type 1, 2, and 3 were 44.1% (26/59), 42.4% (25/59), and 13.6% (8/59), respectively. The generation of automatic electrical activity was preceded by depolarization (22/59, 3.2 ± 0.3 mV), hyperpolarization (16/59, −5.9 ± 1.2 mV), or no change (< 2 mV, 21/59) of the RP. Noradrenaline-induced activity was inhibited by 1 $\mu$M propranolol (12/16, Fig. 2Ag). Noradrenaline did not induce automatic electrical activity in the left atrium (n = 8). Acetylcholine (0.3 $\mu$M) induced a negative shift of RP in the pulmonary vein preparations (6/6), which was reversed by 0.1 $\mu$M atropine (6/6); the RP before acetylcholine was −49.8 ± 7.9 mV, that after acetylcholine was −67.5 ± 3.6 mV ($P < 0.01$), and that after further application of atropine was −49.6 ± 6.9 mV (Fig. 2Ba). Such a shift was not observed in the left atrium (n = 6, Fig. 2Bb). In the pulmonary vein myocardium, acetylcholine (0.3 $\mu$M) inhibited noradrenaline-induced activity (5/11), decreased the frequency of type 1 activity from 4.7 ± 0.8 to 1.3 ± 0.9 Hz (4/11), or changed the firing pattern from type 1 to type 2 (1/11) or type 3 (1/11). The inhibitory effect of acetylcholine was reversed by atropine (5/5, Fig. 2Bc). Adenosine (10 $\mu$M) also inhibited the noradrenaline-

**Table 1.** Electrical parameters of the spontaneous activity

| Waveform type | Type 1 (n = 7) | | Type 2 (n = 7) | Type 3 (n = 17) |
|---|---|---|---|---|
| Maximum diastolic potential (mV) | −73.0 ± 1.8 | during bursts | −45.8 ± 2.2 | −42.3 ± 0.9 |
| | | between bursts | −45.5 ± 2.0 | −67.0 ± 1.5 |
| Peak membrane potential (mV) | 16.3 ± 4.3 | | −10.5 ± 4.4 | −5.1 ± 2.0 |
| Frequency (Hz) | 3.1 ± 0.8 | during bursts | 2.2 ± 0.2 | 4.7 ± 0.2 |
| | | burst initiation | 0.03 ± 0.004 | 0.05 ± 0.01 |
| Duration of bursts (s) | | | 16.0 ± 1.7 | 10.9 ± 1.8 |

Values are reported as the mean ± S.E.M. The peak membrane potential was measured at the midpoint of the burst.

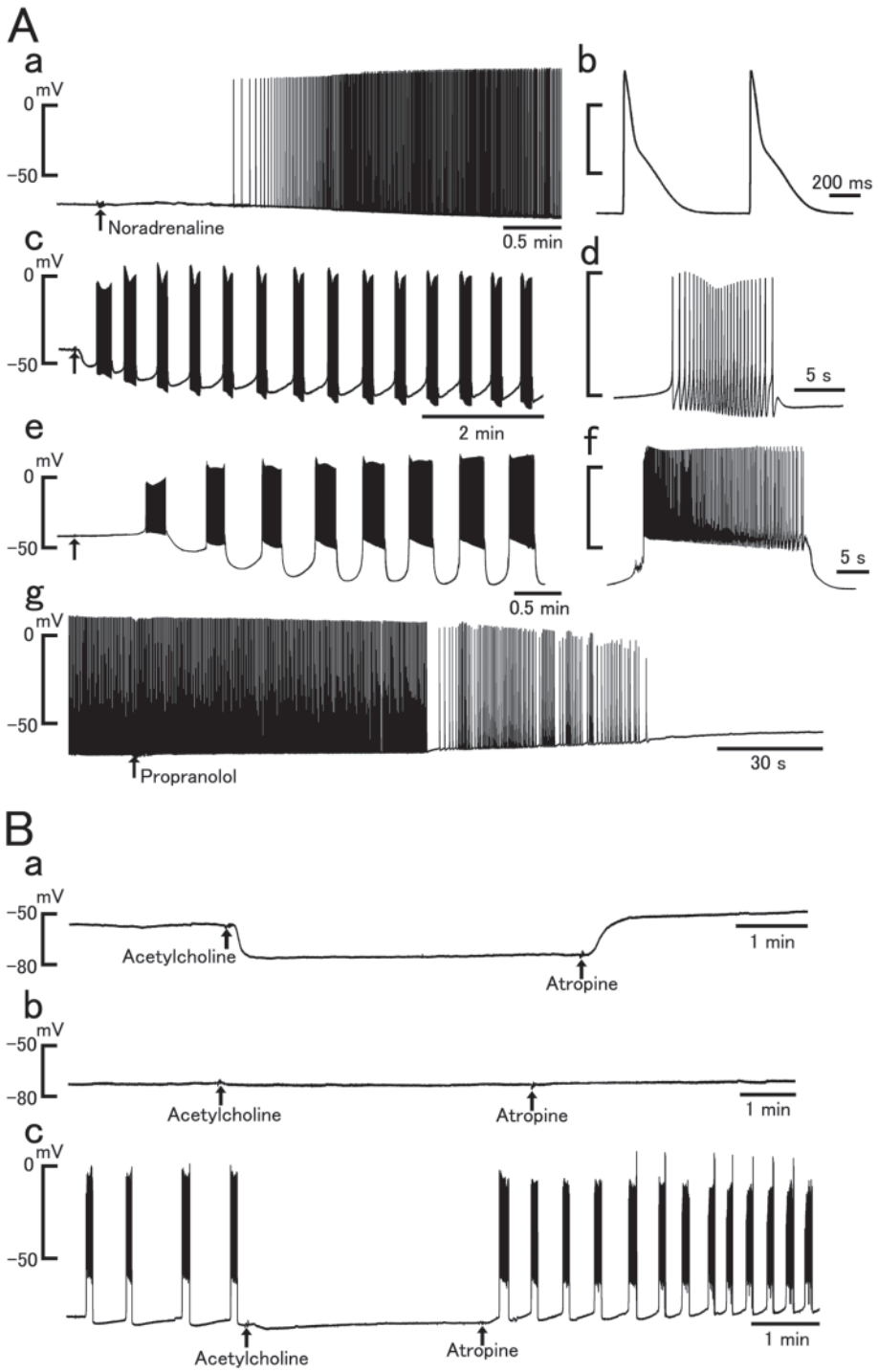


**Fig. 2.** Autonomic control of pulmonary vein automaticity. A: Typical traces of noradrenaline-induced automaticity of the pulmonary vein myocardium in normal (a, c, e) and expanded (b, d, f) time scale, and its inhibition by 1 $\mu$M propranolol (g). Type 1 automaticity was a constant firing (a, b), type 2 automaticity was a repetitive burst (c, d), and type 3 automaticity was a repetitive burst with a more negative maximum diastolic potential (> 5 mV) between the bursts than during the bursts (e, f). B: Effects of acetylcholine (0.3 $\mu$M). a: shift of the resting potential by acetylcholine and reversal by atropine (0.1 $\mu$M) in a quiescent pulmonary vein, b: lack of acetylcholine effects in the left atrium, c: inhibition of noradrenaline-induced automaticity by acetylcholine (0.3 $\mu$M) and recovery by atropine (0.1 $\mu$M). Note that the frequency of the burst initiation after atropine application is higher than that before acetylcholine treatment.

induced activity (6/7).

The extremely rapid early repolarization of the pulmonary vein myocardium (Fig. 1Bb) could be attributed to $I_{to}$ being the major repolarizing current; prolongation of the action potential by 4-aminopyridine supports this view. Cessation of the rapid repolarization could be explained by the time-dependent inactivation and the voltage-dependent deactivation of $I_{to}$ (13). The presence of a late plateau in the pulmonary vein myocardium is probably a reflection of the low density of the inwardly rectifier potassium current responsible for the final phase of repolarization. Low density of the inwardly rectifier potassium current in the pulmonary vein myocardia has been reported in many experimental animal species (12, 14, 15). The finding that the resting membrane potential of the pulmonary vein myocardium was less negative than the atrial also supports this view.

The automatic electrical activity of the mouse pulmonary vein myocardium appeared in three different waveform types (Fig. 1C); in all types the frequency of the repetitive firing was higher than those reported in other experimental animal species such as the guinea pig, rabbit, and canine in which the delayed rectifier potassium current serves as the major repolarizing current (8, 14, 16). As the activation–inactivation kinetics of the $Na^+$ and $Ca^{2+}$ currents are similar among these animal species (1, 2, 12, 14, 15), the high-frequency firing in the mouse could be attributed to the rapid kinetics of $I_{to}$. There seemed to be two different levels of MDP regardless of the waveform type, one around −45 mV and the other around −70 mV (Fig. 1C), which may correspond to membrane potentials with lower repolarizing current density. The interconversion of waveform types may be attributed to the thin myocardial layer in the mouse (Fig. 1A); the less averaging effect of cardiomyocytes may result in instability of the depolarizing vs. repolarizing power balance, leading to a shift in the waveform. The burst type waveforms (type 2 and 3) indicate that a periodic change in the depolarizing vs. repolarizing power balance is taking place. Mechanisms such as accumulation of ion channel activation and/or inactivation and periodic change in intracellular ion concentration or phosphorylation status may be involved. The above discussion concerning the mechanisms of electrical activity in the mouse pulmonary vein myocardium is speculative at present and definitive conclusions await further investigation.

Noradrenaline induced depolarization or hyperpolarization of the RP, indicating changes in the depolarizing vs. hyperpolarizing power balance. Adrenoceptor-mediated changes in RP were also reported in the rat pulmonary vein myocardium, and mechanisms such as $\beta$-adrenergic receptor stimulation–induced activation of $Na^+/K^+$ ATPase and L-type $Ca^{2+}$ current, $\alpha$-adrenergic receptor stimulation-induced reduction of $I_{K1}$, and enhancement of $Na^+/Ca^{2+}$-exchanger activity was postulated (17). Induction of automatic activity by noradrenaline in most of the quiescent preparations (Fig. 2A) indicates that the mouse pulmonary vein myocardium has intrinsic automaticity, which is probably related to the induction of atrial fibrillation by the sympathetic nervous system (3). Acetylcholine induced a negative shift in the resting membrane potential and inhibited both spontaneous and noradrenaline-induced automatic activity (Fig. 2B), which was probably through an increase in $I_{K\text{-}ACh}$ (10, 18). However, the enhancement of automaticity observed after further application of atropine (Fig. 2Bc) implies that acetylcholine may have stimulatory influence when its concentration is altered in the presence of adrenergic influence. A similar phenomenon was reported in the canine pulmonary vein (19).

In conclusion, we demonstrated for the first time that the mouse pulmonary vein myocardium shows diverse electrical activity, which is influenced by autonomic neurotransmitters. The mouse pulmonary vein myocardium would be useful for further studies on the molecular mechanisms of pulmonary vein automaticity.

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