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Short communication

Manifestation of automaticity in the pulmonary-vein myocardium of rats with abdominal aorto-venocaval shunt

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ABSTRACT

Effect of abdominal aorto-venocaval shunt (AVS) on the automaticity of the pulmonary-vein myocardium was studied in the rat. Spontaneous electrical activity was observed in one third of the isolated pulmonary-vein preparations from the AVS rats, but scarcely in those from sham-operated rats; the activity was induced by tertiapin and suppressed by carbachol or chelation of intracellular Ca^{2+}. The evoked action potentials in AVS rats had less negative resting membrane potential and longer action potential duration than those in sham-operated rats. These results suggest that the automaticity of the rat pulmonary-vein myocardium is manifested under chronic volume overload.

1. Introduction

The pulmonary vein contains a myocardial layer which is connected to the left atrial myocardium and is capable of generating spontaneous or triggered action potentials (1). At the end of the 20th century, it was reported that paroxysmal atrial fibrillation is initiated by trains of rapid discharges from the pulmonary veins (2). Since then, the electrical activity of the pulmonary-vein myocardium has received great attention as a key player in the generation and maintenance of atrial fibrillation (3, 4). Microelectrode recordings from the myocardial layer have been performed in isolated pulmonary-vein preparations from several experimental animal species including the dog, rabbit, guinea-pig, rat, and mouse. Information on the firing pattern of action electrophysiological properties of the pulmonary-vein myocardium was also reported to be affected by various endogenous factors such as neuronal activity, hormones and atrial dilatation (5, 6).

In case of the isolated rat pulmonary-vein preparations, action potentials could be evoked by electrical stimulation, but spontaneous firing was not observed under normal condition (7–9). Although the reason for the lack of spontaneous activity is unknown at present, this led us to the notion that certain pathological conditions might manifest the intrinsic automaticity of the rat pulmonary-vein myocardium. In the present study, we examined the effect of an abdominal aorto-venocaval shunt (AVS) which has been used as a simple method to induce chronic volume overload to the heart, leading to cardiac hypertrophy (10). We found that the pulmonary-vein myocardium of the AVS rat shows spontaneous action potential firing, and thus examined its electrophysiological properties.

2. Materials and methods

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. An AVS was surgically created in 8-week-old male Wistar rat (150–200 g) by fistulation between the abdominal aorta and inferior vena cava with an 18 gauge needle, as described

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previously (10). More than 3 months after the surgery, the hearts with lungs were quickly removed from AVS and sham-operated rats and preparations were made from the three major pulmonary-vein trunks. The experimental procedures for microelectrode experiments were basically the same as those in our previous studies (8, 11, 12). Carbachol (Sigma–Aldrich; St. Louis, MO, USA) and tertiapin (Peptide Institute; Osaka) were dissolved in distilled water and O,O′-Bis (2-aminophenyl) ethyleneglycol-N,N,N′,N′-tetraacetic acid, tetraacetoxymethyl ester (BAPTA-AM; Tokyo Chemical Industry; Tokyo) in dimethylsulfoxide. Small aliquots were added to the organ bath to obtain the desired final concentration. All other chemicals were commercial products of the highest available quality. All data are expressed as means ± SEM. Data were analyzed by the Student's *t* test, the paired *t*-test or the Welch's *t*-test. A *P* value less than 0.05 was considered significant.

3. Results

In the AVS rats, the heart weight, the ratio of heart weight to body weight and the weight of pulmonary vein were higher than those in sham-operated rat (heart weights: sham 1.10 ± 0.04 g, AVS 2.40 ± 0.10 g, $p < 0.05$, $n = 23$; ratio of heart weight to body weight: sham 0.29 ± 0.01%, AVS 0.51 ± 0.02%, $p < 0.05$, $n = 23$; weight of pulmonary vein: sham 86.0 ± 3.8 mg, AVS 120.0 ± 5.8 mg, $p < 0.05$, $n = 23$).

In the sham-operated rat, isolated pulmonary vein rarely showed spontaneous electrical activity (3/69). However, 36.2% (25/69) of the isolated AVS rat pulmonary vein showed spontaneous electrical activity (Fig. 1A), and the others were quiescent. The spontaneous electrical activity of the AVS rat pulmonary-vein preparations appeared in two different waveform types. The majority (77%) was a constant firing (type 1; Fig. 1A: a, b; Table 1) and the rest was a repetitive burst (type 2; Fig. 1A: c, d; Table 1). The action potential parameters of quiescent pulmonary-vein preparations of AVS rat when driven by electrical stimulation at 1 Hz were compared with that of sham-operated rat (Fig. 1C; Table 2). The resting membrane potential (RP) was less negative in AVS rat than in sham-operated rat. The action potential duration at 90% repolarization (APD_{90}) was longer in AVS rat than in sham-operated rat. The maximum rate of rise of the diastolic depolarization phase (slope) was observed only in AVS rat.

Carbachol at 0.3 μM suppressed the spontaneous electrical activity of pulmonary vein of AVS rat within 1 min (5/7, 71.4%) and caused a negative shift of the maximal diastolic potential (Fig. 1B). The same concentration of carbachol shortened the action potential duration of the pulmonary vein of AVS rat electrically driven at 1 Hz with a significant hyperpolarizing effect on the resting membrane potential (Fig. 1C; Table 2). The hyperpolarizing effect was larger in AVS rat than in sham-operated rat. Tertiapin, a blocker of the acetylcholine-activated potassium current, induced spontaneous activity in the quiescent pulmonary-vein preparations from the AVS rat, but not from those of the sham-operated rat. The tertiapin-induced activity was completely abolished by the addition of 300 μM BAPTA-AM ($n = 3$).

4. Discussion

The main finding of the present study is that, in the AVS rat pulmonary-vein myocardium, spontaneous action potentials were observed in about one third of the preparations. This is in contrast to the normal or sham-operated rat pulmonary vein myocardium in which spontaneous action potentials were not or only scarcely observed; spontaneous firing of action potentials were observed only after treatment with activating agents such as noradrenaline (7–9). This suggests that the intrinsic automaticity of the

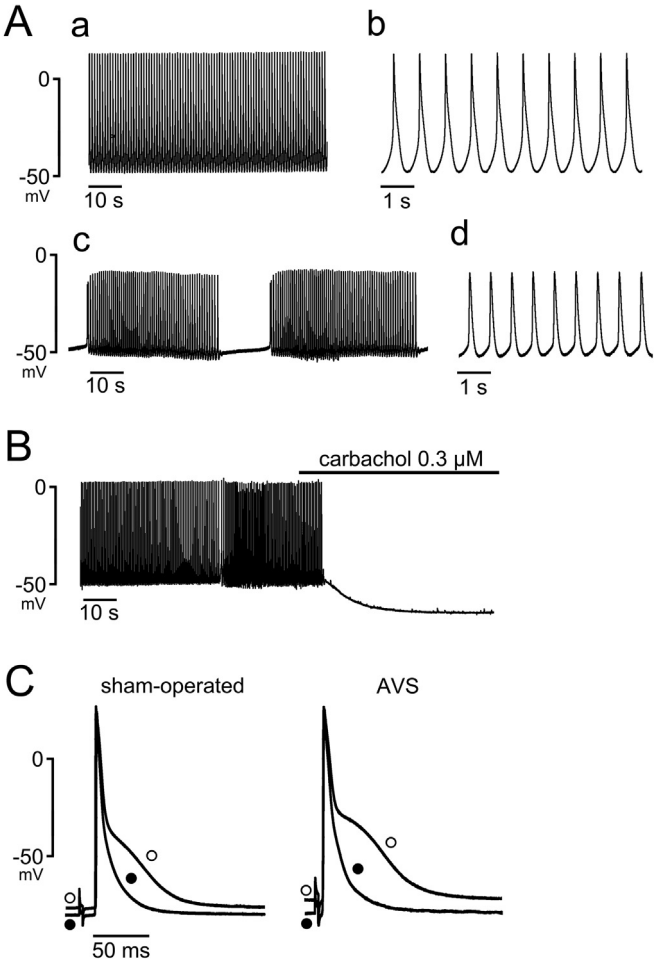

Fig. 1. Electrical properties of the pulmonary-vein myocardia from sham-operated and AVS rat. A: Typical traces of spontaneous electrical activity in AVS rat pulmonary-vein myocardium in normal (a, c) and expanded (b, d) time scale. The type 1 automaticity was a constant firing (a, b); the type 2 automaticity was a repetitive burst (c, d). B: Effect of 0.3 μM carbachol on spontaneous electrical activity in AVS rat pulmonary-vein myocardium. C: Typical traces of action potentials driven by 1 Hz electrical stimulation in pulmonary-vein myocardia from sham-operated and AVS rat in the absence (open circle) and presence (closed circle) of 0.3 μM carbachol.

pulmonary-vein myocardium is manifested under pathological conditions. Thus, the AVS rat pulmonary-vein myocardium provides a useful model to study the mechanisms for the manifestation of myocardial automaticity.

The AVS model is characterized by volume overload to the heart which markedly affects cardio-pulmonary circulation and induces histological remodeling of the heart such as eccentric hypertrophy and atrial enlargement (13, 14). Our present finding that the action potential properties and automaticity are altered in AVS myocardium indicates that electrophysiological remodeling is also taking place. Our previous study with another model of volume overload, the chronic atrio-ventricular block dog, also indicated that both histological and electrophysiological remodeling of the myocardium are taking place, and that the pulmonary-vein myocardium is more sensitive to volume overload than the atrial (15). The AVS rat and the chronic atrio-ventricular block dog, the two models of volume overload, appear to have different characteristics. The AVS rat has a pathophysiological condition of high-output heart failure (16), whose preload to the left ventricle (15 ± 5 mmHg) is higher than that in the chronic atrio-ventricular block dog (10 ± 2 mmHg) complicating low-output heart failure (17). This may imply greater

Table 1
Electrical parameters of spontaneous activity of the pulmonary vein from AVS rat.

Waveform type	Type 1 (constant firing)		Type 2 (repetitive burst)
	13/17 (76.5%)		4/17 (23.5%)
Maximum diastolic potential (mV)	−59.0 ± 3.1	During bursts Between bursts	−53.0 ± 0.6 −50.3 ± 1.5
Peak membrane potential (mV)	4.2 ± 3.3		−0.5 ± 3.0
Frequency (Hz)	2.3 ± 0.4	During bursts Burst initiation	1.7 ± 0.1 0.021 ± 0.007
Duration of bursts (s)			13.0 ± 3.2

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

influence of mechanical stretch to the pulmonary vein in the AVS rat model. Concerning the endogenous factors enhancing the automaticity of the pulmonary vein, humoral factors in addition to mechanical effects on the pulmonary vein are probably involved in both models; compensatory neuro-humoral control of the circulation such as increased sympathetic nerve activity and the angiotensin-aldosterone system has been reported both in atrio-ventricular block- (17) and AVS-induced heart failure (18).

The spontaneous electrical activity of the pulmonary-vein myocardium in various animal species was reported to be sensitive to interventions which affect intracellular Ca^{2+} mobilization such as chelation of intracellular Ca^{2+} or repetitive electrical stimulation, and application of agents such as ouabain, ryanodine and 2-aminoxydiphenyl borate. Sarcolemmal transporters and ion channels such as the forward-mode Na^{+}–Ca^{2+} exchanger and the Ca^{2+}-activated chloride Ca^{2+} channels were reported to be responsible for the intracellular Ca^{2+}-dependent depolarization triggering the action potential (5, 8, 9, 11, 12). Our present result that the tertiapin-induced electrical activity of the AVS rat pulmonary vein was inhibited by chelation of intracellular Ca^{2+} by BAPTA indicates the involvement of intracellular Ca^{2+}-dependent mechanisms. The source of intracellular Ca^{2+} and sarcolemmal transporters and/or channels responsible for the electrical activity of the AVS rat pulmonary-vein myocardium remain to be investigated.

A major factor which determines the presence or absence of spontaneous activity is the repolarizing power at negative membrane potentials close to the resting membrane potential. In general, spontaneous electrical activity is observed in the pulmonary-vein myocardium but not in the atrial, which can be explained by less repolarizing power in the pulmonary-vein myocardium (3–6). This is supported by the observation that the membrane current density of inwardly-rectifying potassium current, which is responsible for the stabilization of the resting membrane potential, is small in the pulmonary-vein myocardium with spontaneous activity. In the pulmonary-vein myocardium of AVS rats, the resting membrane potential was less negative and the action potential duration was longer than the sham-operated rats, which indicates smaller repolarizing power. This contrasts with the pulmonary-vein myocardium of atrio-ventricular block dogs, in which the action potential duration was rather shortened and the resting membrane potential was not significantly changed (15). The spontaneous electrical activity of the pulmonary-vein myocardium in AVS rats could be inhibited by carbachol, which increases the inwardly rectifying potassium current through the acetylcholine-activated potassium channel. Conversely, tertiapin, an inhibitor of the acetylcholine-activated potassium channel, induced spontaneous electrical activity in the pulmonary-vein myocardium of the AVS rat, but not in the sham-operated rat. Thus, a reduced repolarizing power in the pulmonary-vein myocardium of AVS rats plays a permissive role in the generation of spontaneous electrical activity. Whether the enhanced automaticity of the AVS rat pulmonary-vein myocardium is related to the generation of atrial fibrillation is now under investigation (18, 19).

In conclusion, the pulmonary-vein myocardium of the AVS rat showed spontaneous electrical activity, which appears to be the result of reduced repolarizing power. The pulmonary-vein myocardium of the AVS rat provides a useful model for further studies on the mechanisms of pulmonary-vein automaticity and its relation to atrial fibrillation.

Table 2
Effects of carbachol (0.3 μM) on the action potential parameters of the pulmonary vein from sham-operated and AVS rat.

	Sham-operated		AVS	
	Control	Carbachol	Control	Carbachol
RP (mV)	−76.1±0.5	−79.7±0.9†	−71.7±1.5*	−77.7±1.3†
OS (mV)	21.3±2.6	21.4±2.8	21.2±3.3	27.1±1.7
AMP (mV)	97.4±3.1	101.1±3.6†	92.8±4.7	104.8±2.6†
APD_{20} (ms)	5.9±0.4	4.6±0.4†	6.3±0.7	4.7±0.6†
APD_{50} (ms)	12.7±1.1	8.7±0.7†	26.8±7.6	11.9±1.6
APD_{90} (ms)	64.6±2.9	31.0±4.2†	97.8±11.8*	47.3±8.2†
$\dot{V}_{max}$ (V/s)	159.1±15.1	178.0±20.1	140.3±26.7	203.7±15.1
Slope (mV/s)			2.1±0.4	

Action potential parameters of pulmonary-vein myocardia from sham-operated and AVS rat. RP, OS, AMP, $\dot{V}_{max}$, and slope indicate resting potential, overshoot, amplitude, maximum rate of phase 0 depolarization, and maximum rate of rise of the diastolic depolarization phase, respectively. APD_{20}, APD_{50} and APD_{90} indicate action potential duration at 20%, 50% and 90% repolarization, respectively. Values are the mean ± S.E.M. from 6 to 9 pulmonary-vein preparations. Asterisks indicate a significant difference from corresponding values in sham-operated rat, and daggers indicate a significant difference from corresponding values in the control. Statistical significance was evaluated by paired *t*-test or unpaired *t*-test.

Conflicts of interest

We declare that we have no conflict of interest.

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