Journal of Pharmacological Sciences xxx (2018) 1–6

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Influence of chronic volume overload-induced atrial remodeling on electrophysiological responses to cholinergic receptor stimulation in the isolated rat atria

Q4 Xin Cao [a, c], Megumi Aimoto [a, c], Marie Fukumoto [b], Yoshinobu Nagasawa [a], Hikaru Tanaka [b], Akira Takahara [a, *]

[a] Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba, 274-8510, Japan
[b] Department of Pharmacology, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba, 274-8510, Japan

ARTICLE INFO

Article history:
Received 31 August 2017
Received in revised form
8 December 2017
Accepted 26 December 2017
Available online xxx

Keywords:
Aorto-venocaval shunt
Carbachol
Atrial fibrillation
Effective refractory period
Conduction velocity

ABSTRACT

Whereas molecular mechanisms of atrial fibrillation (AF) have been widely investigated, there is limited information regarding interrelation between chronic volume overload and parasympathetic nervous system in the pathophysiology of AF. In this study, we investigated the influence of abdominal aorto-venocaval shunt (AVS) -induced atrial remodeling on electrophysiological responses to cholinergic receptor stimulation in the isolated rat atria. Interstitial fibrosis, cardiomyocyte hypertrophy and atrial enlargement, known as structural arrhythmogenic substrates for AF, took place after one month of AVS operation. Carbachol at 0.1 and 1 μM shortened the effective refractory period, acting as functional arrhythmogenic substrates, but increased the conduction velocity both in the atria of the sham-operated and AVS rats. The extents of the electrophysiological responses to carbachol in the atria of the AVS rat were greater than those in the sham-operated ones. Also, the higher inducibility and longer duration of carbachol-mediated AF were detected in the AVS atria than those in the sham-operated ones. These results showed that chronic volume overload-induced atrial remodeling promoted electrophysiological responses to cholinergic receptor stimulation in the isolated atria of rats, suggesting possible synergistic actions between structural arrhythmogenic substrate in the remodeled atria and functional arrhythmogenic substrates modulated by parasympathetic nerve activity.

© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of Japanese Pharmacological Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Atrial fibrillation (AF) is one of the most common cardiac arrhythmias in clinical practice, which impacts on both life expectancy and quality of life.[1] Autonomic nervous system is well recognized as an important contributor in triggering and maintaining AF, of which activation of parasympathetic nerves shortens the atrial action potential duration and the effective refractory period, and increases dispersion of atrial repolarization,[2–4] thus creating functional arrhythmogenic substrates for AF.

Congestive heart failure and mitral valve diseases are also regarded as important risk factors for AF,[5] which can generate structural substrates through volume overload to the atria. The role of the parasympathetic nervous system or volume overload in the pathogenesis of AF has a long and rich history independently.[5,6] Whereas molecular control mechanisms of AF have been investigated widely, linked to gene expression, cell Ca^{2+} handling, and conduction disturbance,[7] interrelation between volume overload and parasympathetic nervous system in triggering and maintaining AF remains elusive. Recently, we established a rat model to deliver long-term volume overload to the heart by aorto-venocaval shunt (AVS) operation, where initiating trigger of AF, namely, increased automaticity in the pulmonary-vein myocardium could be found.[8] In that study, carbachol suppressed the spontaneous electrical activity in pulmonary-vein myocardium of AVS via a significant hyperpolarizing effect on the resting membrane potential, exerting an antiarrhythmic effect.[8] To clarify cholinergic modulation of

* Corresponding author.
E-mail address: akirat@phar.toho-u.ac.jp (A. Takahara).
Peer review under responsibility of Japanese Pharmacological Society.
[c] Equally contributed.

https://doi.org/10.1016/j.jphs.2017.12.009
1347-8613/© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of Japanese Pharmacological Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: Cao X, et al., Influence of chronic volume overload-induced atrial remodeling on electrophysiological responses to cholinergic receptor stimulation in the isolated rat atria, Journal of Pharmacological Sciences (2018), https://doi.org/10.1016/j.jphs.2017.12.009

UNCORRECTED PROOF

arrhythmogenic properties in the atrium itself, we assessed the influence of chronic volume overload-induced atrial remodeling on electrophysiological responses to cholinergic receptor stimulation in the atria. This study may provide new insights into pathophysiology of interrelation between functional and structural substrates in AF.

2. Materials and methods

Experiments were performed by using 8-week-old male Wistar rats ($n = 37$), weighing approximately 150–200 g (Sankyo Labo Service, Tokyo, Japan). Animals were kept at 23 ± 1 °C under a 12-h light–dark cycle, where food and water were available *ad libitum*. All experiments were approved by the Animal Research Committee for Animal Experimentation at Faculty of Pharmaceutical Sciences of Toho University (No. 17-51-359) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animal of Toho University.

Rats were randomly divided into two groups: sham group ($n = 19$) and AVS group ($n = 18$). Briefly, animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Then, rats in the AVS group received fistulation between the abdominal aorta and inferior vena cava by an 18-gauge needle, as described previously.[9] Rats in the sham group underwent similar operation procedure, but did not receive a fistulation. Four weeks later, histological and morphometric analyses in addition to electrophysiological testing were performed.

2.1. Histological examination

More than 4 weeks after the AVS surgery, the animals in the sham ($n = 2$) and AVS ($n = 2$) groups were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). To prevent blood clotting, 1 mL of heparinized saline (100 IU/mL) was intravenously administered. Immediately after euthanization, beginning of the aorta was cannulated for retrograde perfusion with saline and vena cava was incised. The perfusion fixation with a 10% formalin neutral buffer solution was conducted through the cannula, and the segments were processed into paraffin blocks. Longitudinal sections ($n = 1$ for the sham group and $n = 1$ for the AVS group) in 4-μm-thick were cut that all four chambers of the heart were seen from the paraffinized tissue blocks. In addition, cross sections ($n = 1$ for the sham group and $n = 1$ for the AVS group) in 4-μm-thick were also cut from four chambers and mounted on charged slides. One slide each was stained with Masson trichrome to accentuate muscle and connective tissues. Microphotographs in the upper panel were taken by Aperio scanscope (Leica Microsystems K.K., Tokyo) at 4× (objective), and in the lower at 20× (objective).

2.2. Morphometric examination

For morphometric examination, the hearts were immediately dissected and blotted dry in the sham ($n = 8$) and AVS groups ($n = 8$). Right and left ventricles, interventricular septum, and atrium were separated and weighed. Body weight was also measured and used for normalization of heart masses (Table 1). The wall thickness was measured at the thickest part where was identified with naked eye.

2.3. Electrophysiological testing

The isolated atrial preparation consisted of the entire right and left atrium from sham ($n = 9$) and AVS ($n = 8$) groups, which was incubated with the Krebs–Henseleit solution of the following composition (in mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2,

Table 1
Morphometric parameters.

Parameters	Sham ($n = 8$)	AVS ($n = 8$)
Body weight (g)	274 ± 6	282 ± 6
Heart weight (mg)	761 ± 29	1234 ± 48****
Heart weight (mg)/body weight (g)	2.8 ± 0.1	4.4 ± 0.2****
Atrial weight (mg)	78 ± 5	145 ± 11****
Ventricular weight (mg)	684 ± 26	1089 ± 43****
RV weight (mg)	131 ± 4	212 ± 13****
LV weight (mg)	553 ± 27	877 ± 33****
RV wall thickness (mm)	1.2 ± 0.0	1.7 ± 0.1***
Septal thickness (mm)	2.6 ± 0.2	3.4 ± 0.1****
LV wall thickness (mm)	3.8 ± 0.1	5.1 ± 0.2****

Data are presented as mean ± S.E.M. ($n = 8$ for each group). ***$P < 0.001$, ****$P < 0.0001$ vs. Sham. RV: right ventricle; LV: left ventricle; AVS: aorto-venocaval shunt.

KH_2PO_4 1.2, $NaHCO_3$ 24.9, glucose 11.1, gassed with 95% O_2/5% CO_2 (pH 7.4 at 37 °C). Electrophysiological parameters were recorded as described previously.[10,11] Carbachol at 0.1 and 1 μM was added to isolated atria of the sham and AVS groups. After obtaining electrophysiological data of 1 μM carbachol, atropine at 10 μM was additionally applied to the atria ($n = 3$ for each group) to confirm association of muscarinic receptors. Briefly, the stimulating and recording electrodes for measuring intra-atrial conduction time were attached on the atrial epicardium close to the sinus nodal region and on the left atrial appendage, respectively. On the other hand, the stimulating electrode for burst pacing was attached on the septum of atria. Electrograms were amplified with a bioelectric amplifier (AB-621G; Nihon Kohden, Tokyo, Japan) and fed into a computer-based data acquisition system (PowerLab, ADInstruments, Castle Hill, Australia). The preparation was electrically driven at cycle lengths of 200, 150, or 100 ms using an electrical stimulator (SEN-7203, Nihon Kohden) and an isolator (SS-104J, Nihon Kohden) with rectangular pulses (about 1.5 times of the diastolic threshold voltage and 3-ms width). The effective refractory period of right atria was assessed by a pacing protocol consisting of ten beats of basal stimuli (S_1) in cycle lengths of 200, 150, or 100 ms followed by an extra stimulus (S_2) of various coupling intervals. The intra-atrial conduction time was measured as the difference between right and left atrial electrograms to calculate the intra-atrial conduction velocity. The pacing cycle lengths were set shorter than those during spontaneous sino–atrial activity, which were around 220–250 ms. Atrial tachyarrhythmia was induced by pacing at the septum of atrium with burst pacing (5 V output; 3-ms pulse width; 10-ms cycle length for 30 s) for 10 times. An episode of more than 3 continuous premature atrial contractions and/or atrial tachyarrhythmia such as AF and atrial flutter (AFL) lasted for 30 s were regarded as an incidence of AF/AFL. AF/AFL duration was expressed as an average of the 10 times of burst pacing. All experiments were performed at 36.5 ± 0.5 °C.

2.4. Drugs

Carbachol and atropine sulfate were purchased from Sigma–Aldrich (catalog # 074K0777, St. Louis, MO, USA) and Wako Pure Chemical (catalog # 015-4853, Osaka), respectively, which were dissolved in distilled water. Small aliquots were added to the organ bath to obtain the desired final concentration. All the other chemicals were commercial products of the highest available quality. The concentration of carbachol was determined by previous studies, in which the low concentration (0.1 μM) was reported to produce physiological responses mimicking parasympathetic nerve activity at night or rest time.[8,12]

Please cite this article in press as: Cao X, et al., Influence of chronic volume overload-induced atrial remodeling on electrophysiological responses to cholinergic receptor stimulation in the isolated rat atria, Journal of Pharmacological Sciences (2018), https://doi.org/10.1016/j.jphs.2017.12.009

Download English Version:

https://daneshyari.com/en/article/8532918

Download Persian Version:

https://daneshyari.com/article/8532918

Daneshyari.com