Electrophysiological response to acehytisine was modulated by aldosterone in rats with aorto-venocaval shunts

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ABSTRACT

Aldosterone induces cardiac electrical and structural remodeling, which leads to the development of heart failure and/or atrial fibrillation (AF). However, it remains unknown whether aldosterone-induced remodeling may modulate the efficacy of anti-AF drugs. In this study, we aimed to jeopardize the structural and functional remodeling by aldosterone in rats with aorto-venocaval shunts (AVS rats) and evaluate the effect of acehytisine in this model. An AVS operation was performed on rats (n=6, male) and it was accompanied by the intraperitoneal infusion of aldosterone (AVS+Ald) at 2.0 μg/hr for 28 days. The cardiopathy was characterized by echocardiography, electrophysiologic and hemodynamic testing, and morphometric examination in comparison with sham-operated rats (n=3), sham+Ald (n=6), and AVS (n=5).

Aldosterone accelerated the progression from asymptomatic heart failure to overt heart failure and induced sustained AF resistant to electrical fibrillation in one out of six rats. In addition, it prolonged PR, QT interval and Wenckebach cycle length. Acehytisine failed to suppress AF in the AVS+Ald rats. In conclusion, aldosterone jeopardized electrical remodeling and blunted the electrophysiological response to acehytisine on AF.

Key words: Acehytisine; Aorto-venocaval shunt; Aldosterone; Atrial fibrillation
INTRODUCTION

Atrial fibrillation (AF) and congestive heart failure (CHF) frequently coexist (1). In a setting where CHF and AF coexist, the renin-angiotensin-aldosterone system (RAAS) is always activated (2). Aldosterone is the terminal effector of the RAAS. Patients with high aldosterone display a 12-fold higher prevalence of AF in comparison to those with essential hypertension (3). Long-term exposure to aldosterone induced cardiac hypertrophy, fibrosis, and conduction disturbances lead to the development of heart failure and/or atrial fibrillation (3-6). In our previous study, rats only presented asymptomatic heart failure after one month of aorto-venocaval shunts (AVS) operation (7). On the other hand, chronic treatment with aldosterone in rats via an osmotic minipump induced structural and electrical remodeling despite normal blood pressure, including left ventricular hypertrophy, prolonged QT-intervals, a higher rate of ventricular premature beats and non-sustained ventricular tachycardia, accompanying with downregulation of mRNA expressions of Kv4.2 and Kv4.3 (I_{o}), Kv1.5 (I_{Kur}), Kir2.1 and Kir2.3 (I_{K1}) and of Cav1.2 (L-type Ca^{2+} channel) (8). To display both the overt CHF and AF phenotypes, aldosterone was selected to jeopardize the cardiac remodeling in AVS rats. A previous study indicated that the severity of remodeling may modify the effectiveness of anti-AF drugs (9); however, it is unclear whether
aldosterone-induced remodeling may modulate the effectiveness of anti-AF drugs.

Our team recently showed that acehytisine—a multi-ion channel inhibitor that blocks Na⁺, Ca²⁺, and K⁺ channels—exhibited a favorable anti-AF effect and low proarrhythmic risk in AVS rats (7, 10-13). To test its efficacy in aldosterone-remodeled rats, additional aldosterone was chronically intraperitoneally infused into the AVS rats to jeopardize the structural and functional remodeling, following which the efficacy of acehytisine in doses of 4 and 10 mg/kg was further evaluated in the AVS+Ald model.
MATERIALS AND METHODS

Experiments were conducted in 8-week-old male Wistar rats (n=20, weighing 180-220 g). The rats were purchased from Sankyo Labo Service and housed at a constant temperature (23 ± 1°C) under a 12/12-h light-dark cycle, with free access to food and water. All animal experimental procedures were approved by the Animal Research Committee for Animal Experimentation of Toho University (No. 17-51-359) and Chengdu University of Traditional Chinese Medicine (No. 2018-10).

All rats were initially anesthetized in an induction chamber with 4% isoflurane (Mylan Seiyaku, Osaka, Japan) for a brief period. Then, they were quickly taken to a face mask under 2% isoflurane. After exposing abdominal viscera, isoflurane was maintained with 1.5%, and fistula between the abdominal aorta and inferior vena cava was created with an 18-gauge needle in the AVS group (n=5) according to a previous study (14). In addition, osmotic mini pumps (ALZET pump 2ML4, Durect Cor., Cupertino, CA, USA) were steriley implanted into the AVS+Ald group (n=6) after fistulation to enable the subcutaneous infusion of 2.0 μg/hr for 28 days. In the sham group, rats received a similar operation procedure without abdominal aortocaval fistulation (n=3). Simultaneously, in the sham+Ald group (n=6), osmotic mini pumps were added to the sham-operated rats.
Echocardiography

Four weeks later, cardiac structure and function were evaluated. All rats were subjected to cardiac echocardiography (Noblus; Hitachi, Ltd., Tokyo, Japan) with 1.5% isoflurane. M-mode cardiac images of the left ventricle (LV) from the parasternal long axis view were measured to analyze LV end-diastolic diameter (LVEDD), LV ejection fraction (EF) and LV fractional shortening (FS). Pulse wave Doppler images of mitral inflow from the apical 4-chamber view was obtained to detect the early diastolic filling wave (E), atrial contraction wave (A) and transmitral E/A ratio. In addition, the left atrium major axis (LAMA) was also calculated via the apical four-chamber image.

Electrophysiological and Hemodynamic Testing

Following echocardiography, rats were inserted with a tracheal cannula and mechanically ventilated with a tidal volume of 10 mL/kg and respiratory rate of 12 breaths/min (SN-480-7; Shinano Manufacturing Co., Ltd., Tokyo, Japan). The lead II electrocardiogram (ECG) was recorded with needles punctured to limb. The right femoral vein was cannulated for saline and drug infusion and the right femoral artery was punctured to monitor the blood pressure. The value of ECG and blood pressure was averaged by twelve recordings of consecutive waves. The right jugular vein was cannulated to position an electrode catheter (3 F, inter-electrode distance of 4 mm,
SMC-304; Physio-Tech) into the septum of the right atrium. The stimulator (SEN-8203; Nihon Kohden, Tokyo, Japan) and an isolator with rectangular pulses (2 times of the diastolic threshold voltage and 3-ms width) (SS-104J, Nihon Kohden) were used for programmed stimulation. The atrial effective refractory period (ERP) was measured with a train of eleven beats ($S_1$) of basic cycle lengths at 120 (ERP ($CL_{120\text{ms}}$)) and 100 ms (ERP ($CL_{100\text{ms}}$)) followed by an extra stimulus ($S_2$). The ERP was defined as the shortest $S_1S_2$ interval that captured the atria. The Wenckebach cycle length (WCL) was measured by decreasing the stimulation cycle length in 1-ms steps every 4 s stimuli until atrioventricular block occurred. AF was induced by atrial burst pacing for 20 s (15-ms cycle length; 3-ms pulse width; 5 V output) at the site of RA where two biphasic P wave was detected. An episode of AF was defined as more than three continuous premature atrial contractions. AF duration was averaged after ten times atrial burst pacing application. If the duration of AF was more than 10 min, defibrillation was initiated with programmed stimulation coupling. Then, the episode of the AF was regarded as 10 min.

Following the basal control assessment, acetylethysine was infused in a dose of 4 mg/kg (in 10 min bolus) with an infusion pump (KD Scientific Inc, MA, USA), and the same assessment was performed as in basal control. Finally, an identical procedure was
repeated with acehytisine in doses of 10 mg/kg. Acehytisine (Angene International, Ltd.,
Hong Kong) was initially dissolved in saline in a concentration of 5 mg/mL, then
diluted to 2 mg/mL with saline. It was infused at the rate of 2 mL/kg per 10 min via
right femoral vein.

**Morphometric Examination**

After electrophysiological and hemodynamic testing, the hearts were quickly
removed. Then, the atria, left ventricle plus septum and right ventricle were dissected
and weighed (Table 3). Heart weight was normalized to body weight to quantify the
cardiac hypertrophy. Furthermore, the thickness of the free LV, interventricular septum,
and RV were measured at the thickest part.

**Statistical Analysis**

All data are expressed as mean ± standard error of mean (S.E.M.). One-way
repeated-measures ANOVA followed by Dunnett (GraphPad Prism 6.07) was used for
assessing the efficacy of acehytisine, whereas One-way repeated-measures ANOVA
followed by Turkey was applied for evaluating the difference among sham, sham+AVS,
or AVS and AVS+Ald rats. A p value<0.05 was considered significant.
RESULTS

After one month of operation, one out of six rats developed overt heart failure, which included signs of dyspnea (Supplementary video 1), varicose veins in the chest, barrel chest, and pleural fluid (Supplementary video 2). The other five rats showed asymptomatic heart failure, which included atrial dilation, depression of hemodynamic function, and hypertrophy. Furthermore, one out of six rats showed sustained AF refractory to electrical defibrillation in AVS+Ald group, but no overt heart failure or sustained AF resistant to electrical defibrillation was found in the other three groups.

Echocardiography

Table 1 summarizes the parameters of cardiac structure and function by echocardiography among sham, sham+Ald, AVS and AVS+Ald rats. AVS plus aldosterone enlarged the chamber size of the left atrium and ventricle without altering systolic and diastolic function when compared with sham rats. The increased LAMA of an AVS+Ald rat is shown in supplementary video 2. These results indicated that dilation took place in the AVS+Ald rats. No difference was detected between AVS and AVS+Ald rats.

Electrophysiological and Hemodynamic Testing

Figure 1 shows the typical traces of the electrophysiological and hemodynamic
parameters in a sham and an AVS+Ald rat before acehytisine administration. Table 2
summarized the electrophysiological and hemodynamic parameters in sham, sham+Ald,
AVS and AVS+Ald rats. The P-wave duration and QT interval in the AVS+Ald group
were prolonged than those in the sham group. The mean blood pressure (MBP), P-wave
duration, PR interval, WCL, ERP\textsubscript{(CL120ms)} and ERP\textsubscript{(CL100ms)} in the AVS+Ald rats were
significantly altered in comparison with sham+Ald rats. Burst pacing-induced AF
duration tended to be longer in AVS+Ald rats ($p=0.0512$) as compared to the sham
ones. Sustained AF that is resistant to electrical defibrillation occurred in an AVS+Ald
rat, but none was found in the other three groups. Significant difference was detected in
heart rate, PR interval, QT interval, WCL between AVS and AVS+Ald rats. These
results indicated that aldosterone accelerated electrical remodeling.

**Effect of Acehytisine on AVS+Ald Atria**

Figure 2 shows the effects of acehytisine on electrophysiological and
hemodynamic parameter in AVS+Ald rats. The baseline parameters of AF duration,
WCL, ERP\textsubscript{(CL120 ms)}, ERP\textsubscript{(CL100 ms)}, heart rate, MBP, P-wave duration, PR interval, QRS
width, and QT interval were 93.4 ± 39.0 s, 126.5 ± 5.6 ms, 53.8 ± 3.7 ms, 51.5 ± 2.5 ms,
250 ± 18 beats/min, 75 ± 6 mmHg, 25.0 ± 2.4 ms, 64.4 ± 4.3 ms, 17.6 ± 0.5 ms, and
108.7 ± 9.1 ms, respectively. Acehytisine was unable to suppress the AF duration, but
altered the heart rate, MBP, ERP (CL100 ms), and QRS width at 10 mg/kg.

Morphometric Examination

After four weeks’ operation, similar body weights were detected among sham, sham+Ald, AVS and AVS+Ald rats (Table 3), whereas the heart-weight/body-weight was higher in the AVS and AVS+Ald group when compared to sham group. In addition, the wall thickness of the right ventricle, left ventricle, and septum were higher in the AVS+Ald rats in comparison to the sham-operated ones (Table 3). No significant difference was detected between AVS and AVS+Ald rats.

DISCUSSION

In this study, one out of six rats in the AVS+Ald group developed overt heart failure and sustained AF. The other five rats exhibited marked atrial structural and electrophysiological changes (dilation and hypertrophy, conduction delay) and enhanced AF vulnerability compared to the sham controls. Acehytisine failed to suppressed the burst pacing-induced AF in the AVS+Ald rats.

Aldosterone jeopardized electrical remodeling in the AVS+Ald rats.

In this study, one out of six rats developed overt heart failure and burst-pacing induced sustained AF. However, the coexistence of CHF with sustained AF was not
found in sham, sham+Ald and AVS rats. In addition, PR interval, QT interval and WCL were much longer than AVS rats and sustained AF that is resistant to electrical defibrillation took place, indicating that aldosterone jeopardized electrical remodeling and accelerated the progression from asymptomatic heart failure to overt heart failure. This finding is in accordance with a previous study showing that aldosterone contributed to heart failure (6).

The structural remodeling induced by dilation and hypertrophy paralleled AVS model changes. Atrial dilation and hypertrophy are well-known proarrhythmia substrates (15, 16). Chronic mechanical stretch by dilation decreased activity of Ca\(^{2+}\) channels and prolonged the action potential duration (17, 18), which increased the risk of AF and thus supported our results that the mean AF duration was 93.4 s in the AVS+Ald rats in comparison to the sham rats, who had an AF duration 7.9 s. The prolongation of P-wave-duration—namely delayed intra-atrial conduction—also occurred in the AVS+Ald rats, but not in AVS rats, which is in good accordance with our previous study (19). As we know, intercellular ion channels, fibrosis, or connexins at gap junctions are major determinants of conduction velocity (20). A significant reduction in the phosphorylation of connexin 43 was observed in hearts with arteriovenous fistula (21). In addition, the contribution of aldosterone to fibrosis in the
cardiovascular system has been reported (22, 23). The synergic effect of the reduced
phosphorylation of connexin 43 and fibrosis by aldosterone may imply this different
response in conduction delay between the AVS and AVS+Ald rats when compared to the
control rats (23).

MBP tended to be reduced in the AVS+Ald rats. The reduced blood pressure
may be ascribed to the loss of blood circulation due to aldosterone-induced water
retention and lower terminal peripheral resistance due to the decrease of the flow to the
small artery by the AVS (14).

* **Aldosterone modulated the electrophysiological response to acehytisine in the AVS+Ald rats.**

Pretreatment with acehytisine failed to decrease the AF duration. In our
previous AVS rat model, acehytisine shortened the AF duration from 4 mg/kg and
significantly prolonged the P-wave duration, PR interval, and WCL, possibly via the
blockade of Na⁺ and Ca²⁺ channels (7). However, such effects were blunted by
aldosterone in the AVS+Ald model, which indicates that additional aldosterone to AVS
rats may remodel Na⁺ and Ca²⁺ channels (24, 25). The significant alternation of PR
interval and the prolongation tendency of P-wave duration in AVS+Ald rats may partly
support this hypothesis. Acehytisine prolonged ERP in the AVS+Ald rats, but not in the
AVS rats. The different response to acehytisine may be ascribed to the reduced repolarization reserve in myocyte that occurs due to the downregulation of the $I_{K}$ channel by aldosterone, which may modulate the model to be more sensitive to the $K^+$ channel–inhibition and thus prolong the atrial ERP significantly (26).

In conclusion, aldosterone jeopardized electrical remodeling and modulated the electrophysiological response to acehytisine on AF.

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**Conflict of interest:** The authors declare no conflict of interest.

**Supplementary Materials**

The online version of this article contains supplementary materials.
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Fig. 1  Typical traces showing the electrophysiological and hemodynamic differences between a sham (left) and AVS+Ald (right) rat. The upper panel showed a bradycardia and prolonged P-wave duration in the AVS+Ald (right) rat. The middle panel indicated that blood pressure was much lower in the AVS+Ald (right) rat. The lower panel showed that burst pacing-induced atrial fibrillation (AF) was 167 s in the AVS+Ald (right) rat and 10 s in the sham rat (left). Sinus rhythm (SR).
Fig. 2  Acehytisine at 4 (light grey) and 10 mg/kg (dark grey) failed to suppress the
burst pacing-induced atrial fibrillation (AF) duration when compared with pre-drug
control (white). WCL: Wenckebach cycle length; ERP\(_{(CL120\,\text{ms})}\): the effective
refractory period (ERP) at 120 ms; ERP\(_{(CL100\,\text{ms})}\): the effective refractory period (ERP)
at 100 ms; MBP: mean blood pressure. AVS+Ald: aorto-venocaval shunt plus
aldosterone.  * \(p < 0.05\) vs. pre-drug control.
Table 1  Cardiac structure and function

| Parameters                  | Sham (n=3)       | Sham+Ald (n=6) | AVS (n=5)      | AVS+Ald (n=6)  |
|-----------------------------|-------------------|----------------|----------------|----------------|
| **Structure**               |                   |                |                |                |
| LAMA (mm)                   | 4.07±0.31         | 4.13±0.21      | 5.34±0.17      | 5.69±0.38*$$   |
| LVEdD (mm)                  | 5.87±0.37         | 6.78±0.11      | 7.70±0.14*     | 7.82±0.52*     |
| **Systolic function**       |                   |                |                |                |
| EF (Teich) (%)              | 87.88±1.17        | 90.47±0.89     | 85.32±1.59     | 86.74±3.95     |
| %FS (%)                     | 52.43±1.64        | 56.52±1.41     | 51.72±3.38     | 51.99±4.46     |
| **Diastolic function**      |                   |                |                |                |
| E                           | 0.83±0.12         | 0.94±0.02      | 1.07±0.05*     | 1.04±0.04      |
| A                           | 0.69±0.12         | 0.58±0.03      | 0.82±0.02      | 0.71±0.11      |
| E/A                         | 1.23±0.06         | 1.64±0.07      | 1.30±0.08      | 1.64±0.28      |

Left atrium major axis (LAMA), left ventricular end-diastolic diameter (LVEdD), ejection fraction assessed with Teichholz method (EF (Teich)), fractional shortening (FS), peak velocity of E-wave (E), peak velocity of A-wave (A), peak velocity of E-wave/ peak velocity of A-wave (E/A). Data are presented as mean±S.E.M. *P< 0.05 vs. Sham. $$ P< 0.01 vs. Sham+Ald. AVS: aorto-venocaval shunt. Ald: aldosterone.
### Table 2 Electrophysiological and hemodynamic parameters

| Parameters            | Sham (n=3)   | Sham+Ald (n=6) | AVS (n=5)   | AVS+Ald (n=6)          |
|-----------------------|--------------|----------------|-------------|------------------------|
| Heart rate (bpm)      | 309 ± 8      | 361 ± 14       | 343 ± 13    | 250 ± 18<SSS##        |
| MBP (mmHg)            | 103 ± 5      | 107 ± 6        | 83 ± 10     | 75 ± 6$               |
| P-wave duration (ms)  | 16.1 ± 0.8   | 17.9 ± 0.7     | 20.2 ± 1.0  | 25.0 ± 2.4*S          |
| PR interval (ms)      | 53.9 ± 2.0   | 50.2 ± 0.9     | 49.8 ± 2.3  | 64.4 ± 4.3<SS#        |
| QRS width (ms)        | 14.0 ± 1.3   | 16.7 ± 1.2     | 15.8 ± 1.6  | 17.6 ± 0.5            |
| QT interval (ms)      | 75.0 ± 2.6   | 92.7 ± 1.8     | 73.8 ± 2.4  | 108.7 ± 9.1##         |
| AF duration (ms)      | 7.9 ± 0.9    | 67.2 ± 19.7    | 38.5 ± 15.2 | 93.4 ± 39.0           |
| WCL (ms)              | 116.7 ± 5.3  | 99.5 ± 1.0     | 101.4 ± 5.9 | 126.5 ± 5.6<SSS##     |
| ERP (CL120 ms)        | 50.3 ± 2.7   | 36.2 ± 1.4     | 55.2 ± 5.0$S$ | 53.8 ± 3.7$S$        |
| ERP (CL100 ms)        | 49.0 ± 2.5   | 36.0 ± 1.8     | 54.8 ± 4.4$S$ | 51.5 ± 2.5$S$        |

Aorto-venocaval shunt plus aldosterone (AVS+Ald) jeopardized electrical remodeling. Data are means ± S.E.M. *P<0.05 vs. Sham. $P<0.05, ^{SS}P<0.01, ^{SSS}P<0.001$ vs. Sham+Ald. $^P<0.05, ^{##P}<0.01$ vs. AVS. AVS: aorto-venocaval shunt. Ald: aldosterone.
Table 3 Morphometric parameters

| Parameters                                      | Sham (n=3) | Sham+Ald (n=6) | AVS (n=5)  | AVS+Ald (n=6) |
|------------------------------------------------|------------|----------------|------------|---------------|
| Heart weight (mg)                              | 753 ± 32   | 821 ± 16       | 1242 ± 52  | 1150 ± 173    |
| Body weight (g)                                | 309 ± 7    | 290 ± 4        | 290 ± 6    | 320 ± 29      |
| Heart weight (mg)/body weight (g)              | 2.4 ± 0.1  | 2.8 ± 0.0      | 4.3 ± 0.2***$$ | 3.6 ± 0.3*    |
| Atrial weight (mg)                             | 65 ± 4     | 82 ± 5         | 175 ± 18*$$ | 131 ± 31      |
| Ventricular weight (mg)                        | 687 ± 28   | 739 ± 12       | 1067 ± 37  | 1020 ± 141    |
| LV weight (mg)                                 | 560 ± 24   | 596 ± 10       | 830 ± 27   | 796 ± 109     |
| RV weight (mg)                                 | 128 ± 6    | 143 ± 4        | 237 ± 15*$$ | 224 ± 34      |
| LV wall thickness (mm)                         | 2.9 ± 0.1  | 3.5 ± 0.1      | 3.7 ± 0.1* | 3.8 ± 0.2**   |
| Septal thickness (mm)                          | 2.1 ± 0.1  | 2.8 ± 0.1*     | 2.8 ± 0.1* | 2.7 ± 0.1*    |
| RV wall thickness (mm)                         | 1.2 ± 0.0  | 1.3 ± 0.0      | 1.3 ± 0.0  | 1.5 ± 0.1*    |

Data are presented as mean±S.E.M. *P< 0.05, **P< 0.01, ***P<0.001 vs. Sham, $P< 0.05, $$P< 0.01$ vs Sham+Ald.

AVS: aorto-venocaval shunt. Ald: aldosterone.