Aging-associated changes in L-type calcium channels in the left atria of dogs

TIAN-YI GAN1, WEIWEI QIAO2,3*, GUO-JUN XU1, XIAN-HUI ZHOU1, BAO-PENG TANG1, JIAN-GUO SONG1, YAO-DONG LI1, JIAN ZHANG1, FA-PENG LI1, TING MAO1 and TAO JIANG1

1Department of Cardiology, The First Affiliated Hospital, Xinjiang Medical University, Urumqi, Xinjiang 830011; 2The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Public Health, Shandong University, Qilu Hospital, Jinan, Shandong 250012; 3Department of Cardiology, Yantaishan Hospital, Yantai, Shandong 264001, P.R. China

Received March 29, 2013; Accepted July 1, 2013

DOI: 10.3892/etm.2013.1266

Abstract. Action potential (AP) contours vary considerably between the fibers of normal adult and aged left atria. The underlying ionic and molecular mechanisms that mediate these differences remain unknown. The aim of the present study was to investigate whether the L-type calcium current (I\textsubscript{Ca-L}) and the L-type Ca\textsuperscript{2+} channel of the left atria may be altered with age to contribute to atrial fibrillation (AF). Two groups of mongrel dogs (normal adults, 2-2.5 years old and older dogs, >8 years old) were used in this study. The inductibility of AF was quantitated using the cumulative window of vulnerability (WOV). A whole-cell patch-clamp was used to record APs and I\textsubscript{Ca-L} in left atrial (LA) cells obtained from the two groups of dogs. Protein and mRNA expression levels of the α1C (Cav1.2) subunit of the L-type calcium channel were assessed using western blotting and quantitative PCR (qPCR), respectively. Although the resting potential, AP amplitude and did not differ with age, the plateau potential was more negative and the APD\textsubscript{90} was longer in the aged cells compared with that in normal adult cells. Aged LA cells exhibited lower peak I\textsubscript{Ca-L} current densities than normal adult LA cells (P<0.05). In addition, the Cav1.2 mRNA and protein expression levels in LA cells were decreased in the aged group compared with those in the normal adult group. The lower AP plateau potential and the decreased I\textsubscript{Ca-L} of LA cells in aged dogs may contribute to the slow and discontinuous conduction of the left atria. Furthermore, the reduction of the expression levels of Cav1.2 with age may be the molecular mechanism that mediates the decline in I\textsubscript{Ca-L} with increasing age.

Introduction

Aging is known to increase the propensity for the occurrence of atrial arrhythmias, particularly atrial fibrillation (AF) (1,2). AF usually occurs in conjunction with other cardiovascular diseases; however, not all patients with AF have an underlying disease (3). This suggests that age-associated changes in the atrium may be important in the development of AF (4). However, the electrophysiological changes that cause the atria of elderly individuals to be more susceptible to AF than those of younger adults remain poorly understood.

The role of the left atrium in the development of AF has previously been identified (5), and previous studies have shown that in left atrial (LA) tissue, the action potential (AP) duration (APD) is prolonged and the AP plateau becomes increasingly negative with age (6,7). These alterations in the AP may provide a substrate for reentry, which facilitates the occurrence and maintenance of reentrant arrhythmias, including AF (8). The L-type Ca\textsuperscript{2+} current (I\textsubscript{Ca-L}) is the major current determining the plateau level of the AP; thus, variations in I\textsubscript{Ca-L} may lead to changes in the AP plateau level. Previous studies have reported that I\textsubscript{Ca-L} is reduced in the right atrial (RA) cells of aged canines compared with that in cells from younger adult canines (9,10). However, to the best of our knowledge, there are few published data on the effects of age on LA I\textsubscript{Ca-L} (7).

The I\textsubscript{Ca-L} is mediated by the L-type Ca\textsuperscript{2+} channel. The α1C (Cav1.2) subunit is considered to be the most important polypeptide of the Ca\textsuperscript{2+} channel-forming proteins, since it forms the channel pore for ion flow. However, published data concerning the effects of age on LA Cav1.2 expression levels are lacking.

In the present study, we tested the hypothesis that I\textsubscript{Ca-L} and Cav1.2 expression levels in the left atrium change with age, creating a substrate that favors the initiation of AF. This was achieved by investigating the differences in I\textsubscript{Ca-L} and Cav1.2 expression in LA myocardia between adult and aged dogs.

Materials and methods

Ethics. All experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (11). All animal studies were approved by

Correspondence to: Professor Bao-Peng Tang, Department of Cardiology, The First Affiliated Hospital, Xinjiang Medical University, 137 Liyushan South Road, Urumqi 830011, P.R. China E-mail: tangbpgan@163.com

*Contributed equally

Key words: atria, calcium channel, cellular electrophysiology, aging
the Animal Use and Care Committee of the First Teaching Hospital, Xinjiang Medical University (Urumqi, China).

**Animal preparation.** Seven adult (2-2.5 years old) and ten aged (>8 years old) mongrels of either gender, weighing 18-26 kg, were used in this study. The ages of the dogs were estimated by a veterinarian based on standard measures for age, including dentition, coat, eyes and musculoskeletal and conformational descriptors. All dogs were anesthetized with sodium pentobarbital (30 mg/kg) and ventilated with atmospheric air using a positive pressure respirator. Core body temperature was maintained at 36.5±1.5°C. The dogs were subjected to twelve-lead electrocardiograms (ECGs) to confirm sinus rhythm and echocardiograms were performed to exclude structural heart disease. Subsequently, continuous recording of standard ECG leads was carried out to determine the heart rate and rhythm. Blood pressure (BP) was continuously monitored via a pressure transducer positioned in the right femoral artery.

The chest was entered via a left thoracotomy at the 4th intercostal space. Multi-electrode catheters ( Biosense-Webster, Diamond Bar, CA, USA) were secured to allow recording at the LA appendage (LAA), left superior pulmonary vein (LSPV) and left inferior pulmonary vein (LIPV). Similar electrode catheters were attached to the RA appendage (RAA), right superior pulmonary vein (RSPV) and right inferior pulmonary vein (RIPV) via a right thoracotomy at the 4th intercostal space. All traces from the electrode catheters were amplified and digitally recorded using a computer-based Lab System (GE 2000; General Electric Company, Fairfield, CT, USA). Bipolar electrograms were filtered at 30-500 Hz. ECG filter settings were 0.1-250 Hz.

**Induction of AF.** Rapid atrial pacing was delivered (1,000 bpm; 2X threshold; duration, 1 msec) at the RAA. After 30 min, rapid atrial pacing was terminated in order to measure AF inducibility. Programmed stimulation at atrial myocardial sites or pulmonary vein (PV) sleeves was performed using a programmable stimulator (DF-4A; Suzhou Dongfang Electronic Instrument Factory, Suzhou, China). Programmed pacing consisted of eight consecutive stimuli (S1-S1, cycle length=330 msec) followed by a premature stimulus (S1-S2) that was progressively decremented until refractoriness. Pacing was performed at 2X diastolic threshold (TH) and at 4X TH. AF was defined as irregular atrial rates (>500 bpm) associated with irregular atrioventricular conduction (lasting >5 sec). The window of vulnerability (WOV) was used as a quantitative measure of AF inducibility. AF inducibility was quantitated as the longest S1-S2 minus the shortest S1-S2 that induced AF at each pacing TH. The cumulative WOV was the sum of the individual WOVs.

**Atrial myocyte preparation.** Following intravenous (i.v.) administration of pentobarbital (30 mg/kg) and thoracotomy, the heart was rinsed in oxygenated Ca²⁺-free Tyrode's solution [137 mmol/l NaCl, 5.4 mmol/l KCl, 1.0 mmol/l MgCl₂, 1.8 mmol/l CaCl₂, 0.33 mmol/l NaH₂PO₄, 10 mmol/l HEPES and 10 mmol/l glucose (adjusted to pH 7.4 with NaOH)]. The aorta was cannulated and the heart was retrogradely perfused on a Langendorff apparatus (ADInstruments, Inc., New South Wales, Australia) at 37°C. A perfusion with Ca²⁺-free Tyrode's solution for 5 min was followed by perfusion with Ca²⁺-free Tyrode's solution containing 0.03% collagenase-II (Worthington Biochemical, Lakewood, NJ, USA) and 1% BSA for 35 min. The left atria were dissected, minced and gently triturated with a pipette in the low-Ca²⁺ Tyrode's solution containing 1% BSA at 37°C for 10 min. The cells were filtered through 200-μm nylon mesh and resuspended in the Tyrode's solution, in which the Ca²⁺ concentration was gradually increased to 1.0 mmol/l. Only cells with a rod-shaped morphology and clear cross-striation were used for subsequent experiments.

**Cellular electrophysiology.** LA cells were continuously superfused (2-3 ml/min) in a 1-ml bath with normal Tyrode's solution [137 mmol/l NaCl, 5.4 mmol/l KCl, 1.0 mmol/l MgCl₂, 1.8 mmol/l CaCl₂, 0.33 mmol/l NaH₂PO₄, 10 mmol/l HEPES and 10 mmol/l glucose (adjusted to pH 7.4 with NaOH)]. The solution was bubbled with 100% O2. Membrane currents and APs were recorded using whole-cell patch-clamp techniques with an EPC 10 Double amplifier and Patchmaster software (HEKA Elektronik Dr. Schulze GmbH, Lambrecht/ Pfalz, Germany). Patch pipette resistances ranged from 2.0-3.0 MΩ when filled with an internal solution. APs were recorded in current-clamp mode. The solution for AP recordings contained 137 mmol/l NaCl, 5.4 mmol/l KCl, 1.0 mmol/l MgCl₂, 1.8 mmol/l CaCl₂, 10 mmol/l HEPES and 20 mmol/l glucose (adjusted to pH 7.4 with KOH). The internal electrode solution for AP recordings contained 140 mmol/l KCl, 2.0 mmol/l MgCl₂, 2.0 mmol/l egtazic acid, 5.0 mmol/l HEPES, 5 mmol/l EGTA and 4.0 mmol/l Na₂ ATP (adjusted to pH 7.4 with KOH). The Ca²⁺ currents were recorded in voltage-clamp mode. The external solution for Iₑ₅₋₄ recording contained 137 mmol/l choline-Cl, 2.0 mmol/l CaCl₂, 1.0 mmol/l MgCl₂, 5.0 mmol/l HEPES, 10 mmol/l glucose, 4.6 mmol/l CsCl, 10 mmol/l TEA-Cl and 5 mmol/l 4-aminopyridine (4-AP) (adjusted to pH 7.30 with CsOH). The internal solution for Iₑ₅₋₄ recording contained 120 mmol/l CsCl, 1.0 mmol/l MgCl₂, 5.0 mmol/l MgATP, 10 mmol/l BAPTA, 10 mmol/l HEPES and 10 mmol/l TEA-Cl (adjusted to pH 7.3 with CsOH). Data acquisition was initiated 10 min after membrane rupture. Iₑ₅₋₄ magnitudes were normalized by the membrane capacitance (βP) of each cell and expressed as current density (pA/ρF). Recordings were filtered using low-pass (2 Hz) and high-pass (30 Hz) filters.

**Detection of Cav1.2 gene expression.** Total RNA was extracted from LAA samples using TRIzol (Gibco-BRL, Carlsbad, CA, USA). Total RNA was reverse transcribed using ReverTra Ace (Toyobo Biotech Co., Ltd., Osaka, Japan). The expression levels of target genes were measured by quantitative PCR (qPCR) using Sybr-Green qPCR Master Mix (Bio-Rad, Hercules, CA, USA). In each assay, β-actin (used as an endogenous control) and Cav1.2 genes from the same samples were amplified in triplicate in separate tubes. The mRNA levels of Cav1.2 were determined using the relative standard curve method, normalized against the corresponding β-actin mRNA levels and then expressed as the relative change from the control ± SD. The expected sizes of amplicons were confirmed by gel electrophoreses. The sequences of the genes studied were obtained
from GenBank and the primers were designed using PRIMER 5.0 software (Applied Biosystems, Carlsbad, CA, USA). The amplicon size and primer sequences for the genes are shown in Table I.

Assessment of Cav1.2 protein expression. For western blotting, 50 µg protein was solubilized for 5 min at 95°C in one volume of loading buffer, loaded onto 10% SDS-PAGE gels and then transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% nonfat dry milk in PBST (containing 0.05% Tween 20), incubated overnight at 4°C with the primary antibody (Cav1.2, 1:2,000; goat IgG, polyclone; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), washed in PBST, incubated with horseradish peroxidase-conjugated secondary antibody and revealed using Immun-Star HRP Substrate (Bio-Rad). For normalization of gel loading, the same western blots were reprobed with anti-β-actin (dilution, 1:200; Santa Cruz Biotechnology Inc.). The densities of the bands on the western blots were quantified using an automatic gel imaging and analysis system (Bio-Rad).

Table I. Amplicon size and primer sequences of genes.

| Gene   | Primer sequence | Amplicon size (bp) |
|--------|-----------------|--------------------|
| β-actin| F: 5’-AAGGACCTGTATGCCAAACCA-3’<br>R: 5’-ATCCACAGAAATCTTGCCTT-3’ | 152 |
| Cav1.2 | F: 5’-GACGCTATGGGCTATGAGTTAC-3’<br>R: 5’-AGTCCAGGTAGCCCTTTAGGT-3’ | 199 |

Statistical analysis. Statistical analysis was performed using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). All values are expressed as the mean ± SD. Comparisons between the two groups were made using the Student's t test. P<0.05 was considered to indicate a statistically significant difference.

Results

ECG data. ECG data concerning the sinus rhythm for the adult and aged groups are shown in Table II. The ECGs of the aged group exhibited prolonged P wave durations and increased P wave dispersion (PWD) compared with those of the adult

Table II. ECG data of adult and aged dogs (mean ± SD).

| Group | P wave (msec) | PWD interval (msec) | PR interval (msec) | QRS interval (msec) | QT interval (msec) |
|-------|--------------|---------------------|-------------------|-------------------|-------------------|
| Adult | 66.1±6.4     | 19.1±4.1            | 123.9±7.2         | 63.1±4.3          | 248.9±11.7        |
| Aged  | 75.9±5.3     | 26.7±3.1            | 130.0±7.7         | 64.7±5.4          | 246.5±17.3        |

P<0.05 vs. adult canines. ECG, electrocardiogram; PR interval, from starting point of P wave to the starting point of QRS wave in electrocardiogram; QRS interval, from starting point of QRS wave to the ending point of QRS wave in electrocardiogram; QT interval, from starting point of QRS wave to the ending point of T wave in electrocardiogram.

Table III. Action potential characteristics recorded from adult and aged canine atria at a cycle length of 2000 msec (mean ± SD).

| Group | MDP (mv)  | APA (mv)  | Plateau (mv) | APD₉₀ (msec) |
|-------|-----------|-----------|--------------|--------------|
| Adult | -78.8±0.8 | 109.8±1.4 | -6.4±1.1     | 320.0±7.9    |
| Aged  | -79.2±1.4 | 110.5±4.9 | -9.5±1.7     | 340.5±10.1   |

P<0.05 vs. adult canines. MDP, maximum diastolic potential; APA, action potential amplitude; APD₉₀, action potential duration to 90% repolarization.

Figure 1. Determination of the window of vulnerability (WOV) for atrial fibrillation (AF) when programmed stimulation was performed at the RAA in adult and aged dogs. *P<0.05 vs. adult dogs. RAA, right atrial appendage; TH, threshold.
Other variables were not observed to differ between the two groups.

**Induced AF.** As shown in Fig. 1, programmed electrical stimulation at the RAA in the aged group induced a larger WOV (15±7.5 ms) compared with that of the adult group (5±2.5 msec) during 2X TH. A similar result was observed during 4X TH (aged group, 22±12 msec vs. adult group, 9±4.5 msec).

**AP characteristics.** LA cells from aged atria exhibited longer APDs and lower plateau potentials compared with those from adult atria. Representative AP recordings from the adult and aged groups are shown in Fig. 2. AP characteristics, at a cycle length of 2,000 msec, are shown in Table III. While there were no significant differences in the maximum diastolic potential (MDP) or action potential amplitude (APA), action potential duration to 90\% repolarization (APD_{90}) was longer in the aged dogs, indicating that the slope of phase 3 repolarization was more gradual in the aged than in the adult cells.

**I\textsubscript{Ca.L}.** Typical I\textsubscript{Ca.L} recordings from LA cells of the adult and aged dogs are shown in Fig. 3. Aged LA cells had lower peak I\textsubscript{Ca.L} densities than adult LA cells (-8.1±0.5 vs. -14.1±0.8, respectively, P<0.05; measured at +10 mV). Activation voltage dependence was assessed from depolarization-induced currents and the driving force was corrected with driving force corrected by membrane potential-reversal potential, where reversal potential is the voltage axis intercept of the ascending limb of the current-voltage relation. There were no significant differences in half-activation voltage or slope factor between the two groups (Table IV). Inactivation was assessed with 1-sec prepulses of -60, -50, -40, -30, -20, -10, 0, 10, 20, 30 and 40 mV, followed by 250-msec test pulses to +10 mV. Furthermore, the current reduction in the aged cells was not accompanied by a significant change in the refractory period (Table IV).

**Cav1.2 gene expression in LA cells.** To study Cav1.2 expression in LA cells, Cav1.2 mRNA levels were analyzed using qPCR. The specificity of the amplified PCR product was verified using agarose gel electrophoresis and non-specific DNA fragments were not detected (Fig. 4A). Cav1.2 mRNA levels were decreased in the aged group compared with those in the adult group (P<0.05, Fig. 4B).

**Cav1.2 protein expression in LA cells.** To confirm the qPCR results, western blotting was performed with Cav1.2 antibodies. Fig. 5A presents the bands from a gel on which the Cav1.2 protein levels from LA cells were studied. The β-actin bands were used to confirm that the loading was equal. Densitometric data demonstrated that the Cav1.2 protein levels were significantly lower in the aged group compared with those in the adult group (P<0.05, Fig. 5B).

### Table IV. Electrophysiological characteristics of the L-type calcium current (I\textsubscript{Ca.L}) in adult and aged canine LA cells (mean ± SD).

| Group | n  | I\textsubscript{Ca.L} density (pA/pF) | Steady-state activation | Steady-state inactivation | Monoexponential recovery time constants (msec) |
|-------|----|-------------------------------------|--------------------------|---------------------------|-----------------------------------------------|
|       |    |                                     | V\textsubscript{0.5} (mV) | K (mV)                    |                                               |
| Adult | 14 | -8.1±0.5                            | -7.1±1.5                 | 5.7±0.4                   | -23.1±2.1                                    |
| Aged  | 16 | -14.1±0.8\*                         | -6.7±2.8                 | 5.5±0.5                   | -22.9±3.3                                    |

*P<0.05 vs. adult. n, cell number; V\textsubscript{0.5}, half-activation or -inactivation voltage; K, slope factor; pA/pF, current density.

Figure 2. Action potential (AP) recording from the left atrial (LA) cells of (A) aged and (B) adult dogs.

Figure 3. Typical L-type calcium current (I\textsubscript{Ca.L}) recordings obtained from left atrial (LA) myocytes in adult and aged dogs. (A) Adult LA cell; (B) aged LA cell; (C) mean I\textsubscript{Ca.L} density-voltage relationship in adult (cells, n=11; hearts, n=7) and aged (cells, n=13; hearts, n=10) LA cells.
Ca. is important in the maintenance of Ca. The current reduction in aged cells was not accompanied by a significant change in Ca. The most marked alteration was a significant lowering of the plateau potential in aged LA cells. Previous studies have shown that negative plateau potentials have a lower driving force in the conduction of early premature beats. Therefore, our results imply that alterations in the APs of aged atrial cells are likely to lead to a decreased conduction of premature beats in aged atria. The slow conduction of early premature impulses may further facilitate the onset of AF. The slow conduction of early premature beats in aged atria become more susceptible to AF. These findings may constitute a mechanism via which aged atria become more susceptible to AF.

In cardiac myocytes, Ca. currents through L-type Ca. channels are the main mechanism for Ca. influx from the extracellular space into the cytoplasm. Cardiac L-type Ca. channels are composed of four polypeptide subunits (α1, β, α2, and δ). The α1 subunit is the most important polypeptide of the Ca. channel-forming proteins; it forms the channel pore for ion flow and is responsible for voltage-dependent Ca. channel opening and channel selectivity for Ca. ions. To date, at least 10 different α1 subunit genes have been identified, but only the α1C (Cav1.2) isoform is expressed at high levels in cardiac muscle. The current study demonstrated that Cav1.2 mRNA and protein expression levels in LA cells were significantly lower in the aged group compared with those in the adult group. This may be the main cause of the reduction in I. and AP plateau potential in aged LA cells.
Therefore, atria Cav1.2 protein levels may decrease with age. However, research has been limited to studies using Cav1.2 protein from only one region of the left atrium and SA node (one region of RA). The mechanisms underlying these changes are unknown. We hypothesized that aging may result in the progressive deterioration of physiological functions and metabolic processes, which alters the density and distribution of ion channels.

Previous studies have attached particular importance to the left atrium in the initiation and maintenance of AF (26,27). The present study demonstrated that there were age-associated changes in the electrophysiological properties and ion channels of the LA myocardium. A lower AP plateau potential and decreased I_{Ca,L} in the LA cells of aged canines may contribute to the slow and discontinuous conduction in the left atrium. These changes increase the susceptibility of aged atria to AF. Furthermore, the decreased expression of Cav1.2 with age may be the cause of the reduction in I_{Ca,L} with increasing age. However, further studies of the mechanisms of alteration in Cav1.2 expression are required.

Although the present study demonstrated age-associated electrophysiological and molecular changes in the LA cells of aged canines, the extent to which these effects are clinically applicable remains to be determined.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (no. 308660299), the Natural Science Foundation of the Xinjiang Uygur Autonomous Region (no. 200821143) and the Doctoral Fund of the Ministry of Education (no. 200807600004).

References

1. Chen LY and Shen WK: Epidemiology of atrial fibrillation: a current perspective. Heart Rhythm 4 (Suppl): S1-S6, 2007.
2. Go AS: The epidemiology of atrial fibrillation in elderly persons: the tip of the iceberg. Am J Geriatr Cardiol 14: 56-61, 2005.
3. Murgatroyd FD and Camm AJ: Atrial arrhythmias. Lancet 341: 1317-1322, 1993.
4. Allessie MA, Boyden PA, Camm AJ, et al: Pathophysiology and prevention of atrial fibrillation. Circulation 103: 769-777, 2001.
5. Corradi D, Callegari S, Maestri R, Benussi S and Alfieri O: Structural remodeling in atrial fibrillation. Nat Clin Pract Cardiovasc Med 5: 782-796, 2008.
6. Anyukhovsky EP, Sosunov EA, Chandra P, et al: Age-associated changes in electrophysiologic remodeling: a potential contributor to initiation of atrial fibrillation. Cardiovasc Res 66: 353-363, 2005.
7. Dun W and Boyden PA: Aged atria: electrical remodeling conducive to atrial fibrillation. J Interv Card Electrophysiol 25: 9-18, 2009.
8. Nattel S: Atrial electrophysiological remodeling caused by rapid atrial activation: underlying mechanisms and clinical relevance to atrial fibrillation. Cardiovasc Res 42: 298-308, 1999.
9. Dun W, Yagi T, Rosen MR and Boyden PA: Calcium and potassium currents in cells from adult and aged canine right atria. Cardiovasc Res 58: 526-534, 2003.
10. Tippuraj SM, Kumar R, Wang Y, Joyner RW and Wagner MB: Developmental differences in L-type calcium current of human atrial myocytes. Am J Physiol Heart Circ Physiol 286: H1963-H1969, 2004.
11. Bayne K: Revised Guide for the Care and Use of Laboratory Animals available. American Physiological Society. Physiologist 39: 199, 208-211, 1996.
12. Sugiu H and Joyner RW: Action potential conduction between guinea pig ventricular cells can be modulated by calcium current. Am J Physiol 263: H1591-H1604, 1992.
13. Yue L, Feng J, Li GR and Nattel S: Transient outward and delayed rectifier currents in canine atrium; properties and role of isolation methods. Am J Physiol 270: H2157-H2168, 1996.
14. Anyukhovsky EP, Sosunov EA, Plotnikov A, et al: Cellular electrophysiologic properties of old canine atria provide a substrate for arrhythmogenesis. Cardiovasc Res 54: 462-469, 2002.
15. Podrid PF: Atrial fibrillation in the elderly. Cardio Clin 17: 173-188, 1999.
16. Spach MS and Dolber PC: Relating extracellular potentials and their derivatives to anisotropic propagation at a microscopic level in human cardiac muscle. Evidence for electrical uncoupling of side-to-side fiber connections with increasing age. Circ Res 58: 536-571, 1986.
17. Spach MS, Miller WT, Dolber PC, Kootsey JM, Sommer JR and Mosher CE Jr: The functional role of structural complexities in the propagation of depolarization in the atrium of the dog. Cardiac conduction disturbances due to discontinuities of effective axial resistivity. Circ Res 50: 175-191, 1982.
18. Shaw RM and Rudy Y: Ionic mechanisms of propagation in cardiac tissue. Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling. Circ Res 81: 727-741, 1997.
19. Rohr S and Kucera JP: Involvement of the calcium inward current in cardiac impulse propagation: induction of unidirectional conduction block by nifedipine and reversal by Bay K 8644. Biophys J 72: 754-766, 1997.
20. Richard S, Perrier E, Fauconnier J, et al: ‘Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} entry’ or how the L-type Ca\textsuperscript{2+} channel remodels its own signalling pathway in cardiac cells. Prog Biophys Mol Biol 90: 118-135, 2006.
21. Yamakage M and Namiki A: Calcium channels - basic aspects of their structure, function and gene encoding; anesthetic action on the channels - a review. Can J Anaesth 49: 151-164, 2002.
22. Wang MC, Dolphin A and Kitimoto A: L-type voltage-gated calcium channels: understanding function through structure. FEBS Lett 564: 245-250, 2004.
23. Bodi I, Mikala G, Koch SE, Akhter SA and Schwartz A: The L-type calcium channel in the heart: the beat goes on. J Clin Invest 115: 3306-3317, 2005.
24. Treiny S and Jurevicius J: Phase 2 L-type Ca\textsuperscript{2+} currents in the heart: structure and regulation. Medicina (Kaunas) 44: 491-499, 2008.
25. Jones SA, Boyett MR and Lancaster MK: Declining into failure: the age-dependent loss of the L-type calcium channel within the sinoatrial node. Circulation 115: 1183-1190, 2007.
26. Tada H, Kurokaki K, Ito S, et al: Left atrial and pulmonary vein ostial ablation as a new treatment for curing persistent atrial fibrillation. Circ J 69: 1057-1063, 2005.
27. Rotheiner FX, Steiner PR, Goseki Y, Sparks PB and Lesh MD: Electrophysiologic effects of selective right versus left atrial linear lesions in a canine model of chronic atrial fibrillation. J Cardiovasc Electrophysiol 10: 1564-1574, 1999.