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## Aged atria: electrical remodeling conducive to atrial fibrillation

**Wen Dun**

Department of Pharmacology, Center for Molecular Therapeutics, Columbia University, New York, NY, USA

**Penelope A. Boyden**

Department of Pharmacology, Center for Molecular Therapeutics, Columbia University, New York, NY, USA

Department of Pharmacology, Columbia College of Physicians and Surgeons, 630 West 168th ST., New York, NY 10032, USA

### Abstract

The incidence of atrial fibrillation (AF) increases with age. Alterations in structure and function of atrial ion channels associated with aging provide the substrate for AF. In this review we provide an overview of current knowledge regarding these age-related changes in atria, focusing on intrinsic ion channel function, impulse initiation and conduction. Studies on the action potentials (APs) of atria have shown that the AP contour is altered with age and the dispersion of AP parameters is increased with age. However, studies using human tissues are not completely consistent with experimental animal studies, since specimens from humans have been obtained from hearts with concomitant cardiovascular diseases and/or that are under the influence of pharmacologic agents. Ionic current studies show that while there are no age-related changes in sodium currents in atrial tissue, the calcium current is reduced and the transient outward and sustained potassium currents are increased in aged cells. While sinoatrial node firing is reduced with age, enhanced impulse initiation may occur in aged atrial cells, for example in the pulmonary veins and coronary sinus. Fibrous tissue is increased in aged atria, which is associated with an increased likelihood of abnormal electrical conduction. Thus, age-related AF involves alterations in the substrate as well as in the passive properties of aged atria.

### Keywords

Age; Atria; Ion channels; Calcium; Atrial fibrillation

### 1 Introduction

The objective of this article is to provide an overview of current knowledge regarding age-related changes in atria, focusing on intrinsic ion channel function, impulse initiation and conduction. It is well established that the incidence and prevalence of atrial fibrillation (AF)

increase with age. This observation raises the question: are there changes in intrinsic ion channel function, impulse initiation, and impulse conduction that predispose aged atria to AF?

## 2 Substrate changes

### 2.1 Action potential (AP)

In laboratory animals, action potential durations (APDs) in atrial tissue are prolonged with age, thus providing a substrate for reentry. However, clear-cut evidence for age-related changes in action potentials (APs) in normal human atria is lacking, since most studies have been done in patients with significant heart disease, or who are taking various cardiovascular medications. In addition, although some studies have included patients up to 70 years of age, few have included patients over age 70, and none have involved patients 80 years or older. For example, Brorson et al. [1] used four age groups to study right atrial (RA) effective refractory periods (ERP) and monophasic action potentials (MAP) in healthy male humans *in vivo*. Group one was 25–34 years, group two 35–44 years, group three 45–54 years and group four 55–64 years. They found that group three (45–54 years) showed a significantly longer RA ERP than the others. However, there was no progressive trend in APD with age. Interestingly, the ERP of the oldest group (55–64 years) was comparable to that of the younger age groups. Similarly, the duration of MAP was significantly longer in group three during sinus rhythm and there was no progressive trend with age. Thus, these results do not support the view that APDs are prolonged with age. Spach et al observed the shape of RA APs at different ages (2, 10, 43 and 62 year old subjects) [2]. A similar “spike and hump” AP configuration was demonstrated at all ages (for example see Fig. 1). Although a quantitative analysis of the size of the notch was not performed, the authors suggested that the qualitative similarities in AP shape strongly suggested that there were no significant differences in the basic ionic mechanisms that control the AP shape in atrial pectinate muscle bundles from subjects varying between 1 and 70 years of age [2]. These results imply that passive properties determine propagation and the arrhythmogenic behavior of aged human atria. Notably, left atrial (LA) APs were not studied.

Toda [3] reported that  $APD_{90}$  (i.e. APD at 90% repolarization) at day 360 (aged) was significantly longer than that at day 90 (adult) in isolated rabbit RA (Table 1) without concomitant changes in  $APD_{10}$ , resting potential, or AP overshoot. No data were reported for animals over 360 days old. Su et al. [4] determined the maximum diastolic potential (MDP),  $APD_{50}$  and  $APD_{95}$  in adult (6–8 months) and aged (26–28 months) rat RA tissues. The MDP was significantly depolarized in atria of aged ( $-66.8 \pm 1.5$  mV) versus adult ( $-76.4 \pm 1.8$  mV) rats (Table 1). Notably,  $APD_{50}$  and  $APD_{95}$  repolarization did not vary significantly with age. Interestingly, carbachol-induced hyperpolarization was significantly greater in aged compared to adult rats. This age-associated enhancement in carbachol-induced hyperpolarization may be due to a greater increase in  $I_{KACH}$  (acetylcholine-induced potassium conductance) in aged atria, since the key mechanism for hyperpolarization of MDP is activation of  $I_{KACH}$  through stimulation of muscarinic cholinergic receptors in the atria. More recently, Huang et al. [5] used Langendorff-perfused rat hearts to record MAPs and ERPs of RA and LA epicardium from rats in 3 age groups: adult (7–8 months), middle-

aged (13–15 months) and aged (over 2 years). Both MAPD<sub>90</sub> and ERPs were prolonged with age in RA (S1S1 = 400 ms) (Table 1), but in LA they were shorter in the aged group than in the other 2 groups. Furthermore, there were no differences in MAPD<sub>90</sub> or ERP between the RA and LA in the adult and middle-aged groups, but in the aged group the MAPD<sub>90</sub> and ERP of RA were significantly longer than in the LA. Importantly, in the aged group, the MAPD<sub>90</sub> of the RA shortened by 38% as the stimulation frequency increased, while LA MAPDs only shortened 11%. Thus with age it appears that the RA MAPD is more sensitive to rate dependent shortening than the LA MAPD.

Anyukhovskiy et al. [6] used microelectrode techniques to record APs from the endocardium of the RA free wall of dogs 1–5 years (adult) and >8 years (aged). They found that plateau potential was more negative and that APD<sub>90</sub> was longer in the aged versus the adult tissue (Table 1). The differences in APD<sub>90</sub> and plateau voltage between the two groups were rate-dependent. APD<sub>30</sub> was shorter and rate-dependent in aged atria. The calcium channel blocker nisoldipine markedly lowered the plateau in adults but had no effect on plateau height in aged atria, whereas the I<sub>Ca,L</sub> agonist Bay K 8644 elevated the plateau more prominently in aged tissue. These data suggest that a decrease in the calcium current may be a mechanism for the low plateau voltages in aged RA tissue. Anyukhovskiy et al. [7] also recorded APs from four regions of the RA (crista terminalis, appendage, atrioventricular ring and pectinate muscles) and three regions of the LA (appendage, atrioventricular ring and free wall near the pulmonary veins) in dogs of different ages (Fig. 1 (a)). APD<sub>90</sub> averaged across all regions was significantly longer in aged compared to adult tissues (Fig. 1 (b)). Notably, the range of APD<sub>90</sub> values was wider in the aged group versus adults as a result of an increase in duration occurring mainly in the RA fibers. Statistically, both the standard deviation and the coefficient of variation of APD heterogeneity were higher in aged dogs compared to adults [7]. This increased heterogeneity of atrial repolarization may create a substrate for initiation of AF in aged canines. Interestingly, MDP is significantly more depolarized, V<sub>max</sub> is slower and total AP amplitude is lower in aged than in adult tissues (Table 1)[7] perhaps contributing to slowed conduction.

From the studies described above in different species, we conclude that APD<sub>90</sub> and ERP are prolonged and continue to show rate-dependence with age in the RA. In the LA, APs shorten with age and are less rate-dependent, although confirmation by other groups and extension to other species is needed.

## 2.2 Ion currents

**2.2.1 Sodium current (I<sub>Na</sub>)**—Our laboratory was the first to study age-related changes in I<sub>Na</sub> in canine atrial cells [8]. We found that I<sub>Na</sub> density in the LA was greater than in the RA in both adult and aged atrial cells (Fig. 2 (a)), and that there were no age-related changes in I<sub>Na</sub> density in either the RA or LA. Thus, the difference in I<sub>Na</sub> density between atrial chambers is unaffected by age (Table 1). I<sub>Na</sub> kinetic differences between aged and adult cells include a significant acceleration into the inactivated state and an enhanced use-dependent decrease in peak-current in aged RA cells. However, in the LA there is no significant difference in the rate of development of inactivation of I<sub>Na</sub> between aged and adult cells. Other kinetic parameters (activation, steady-state inactivation, and recovery from

inactivation) of  $I_{Na}$  are not altered by age in either RA or LA cells [8]. Immunocytochemistry data show that  $Na_v1.5$  (the protein underlying the  $\alpha$  subunit of the major cardiac sodium channel) staining in RA and LA cells from adult and aged atria is uniform, with staining all along the sarcolemma as well as in the gap junction region [8]. Therefore, there is no structural remodeling of the cardiac  $Na^+$  channel protein  $Na_v1.5$  in aged canine atrial cells. Currently there are no data in humans examining the effect of age on atrial  $Na^+$  channel remodeling.

### 2.3 Calcium currents ( $I_{Ca}$ )

The effect of age on basal calcium ( $Ca^{2+}$ ) currents differs. Our laboratory has reported that L-type  $Ca^{2+}$  currents with either  $Ca^{2+}$  ( $I_{CaL}$ ) or  $Ba^{2+}$  ( $I_{BaL}$ ) as the charge carrier are significantly reduced by 47% and 43% respectively in aged canine RA cells compared to adults (Fig. 2 (b), Table 1)[9]. The current reduction in aged cells was unaccompanied by a significant change in calcium channel availability or recovery from inactivation. Isoproterenol abolished the differences in  $I_{BaL}$  between aged and adult cells, suggesting that adrenergic stimulation can restore  $Ba^{2+}$  currents in aged cells [9]. Using the chelator EGTA with  $Ca^{2+}$  as the charge carrier, Tipparaju et al. [10] also reported that basal  $I_{Ca}$  and maximal  $I_{Ca}$  produced by isoproterenol were similar in RA cells from young adult ( $16 \pm 1$  year old) and aged patients ( $66 \pm 3$  year old). These findings suggest that there may be a pronounced  $Ca^{2+}$ -dependent inactivation of  $I_{CaL}$  in atrial cells under these recording conditions. There are no published data on the effects of age on LA  $I_{CaL}$ .

### 2.4 Potassium currents

The transient outward ( $I_{to}$ ) and sustained potassium current ( $I_{sus}$ ) densities are significantly increased in aged canine RA cells compared to adult cells (Fig. 3, Table 1)[9]. Kinetic studies of  $I_{to}$  show that  $I_{to}$  decay is slowed and that the steady-state inactivation curve is shifted positively in aged cells. Further, TEA (tetra-ethylammonium)-sensitive current densities of  $I_{to}$  and  $I_{sus}$  are greater in aged versus adult cells. However, KN-93, a specific CaMKII inhibitor, produces increases in  $I_{to}$  in both adult and aged RA cells, while dramatically inhibiting  $I_{sus}$  in aged compared to adult RA cells. Thus, increased  $I_{sus}$  in aged RA cells is most likely due to CaMKII upregulation. Interestingly, upregulated  $I_{to}$  and  $I_{sus}$  are not observed in aged LA cells [11]. To our knowledge, no studies have examined the function and molecular basis of other potassium currents ( $I_{Kr}$ ,  $I_{Ks}$ ,  $I_{K1}$ ,  $I_{K(ATP)}$ ) from adult and aged atrial cells.

## 3 Abnormal impulse initiation

Increasing age is associated with abnormal impulse initiation. For example, it is well known that sinoatrial node (SAN) dysfunction increases with age [12]. If the sinus node fails to fire, then there is an increased likelihood that atrial cells with abnormal impulse initiation will become manifest.

To our knowledge, there are no studies available that illustrate the effects of age on the function of  $I_f$  in the atrium. However, recent studies on guinea pigs suggest that the fundamental reason for a change in SAN function with age is that expression of the  $Ca_v1.2$

protein (protein underlying the pore-forming unit of the cardiac calcium channel) within the SAN declines during aging (Fig. 4) [13]. This change is accompanied by a reduction in SAN electrical activity. These findings are consistent with observations that the L-type  $\text{Ca}^{2+}$  current provides  $\text{Ca}^{2+}$  influx into the SAN cell and that  $\text{Ca}^{2+}$  release may control SAN pacemaker function [14]. The status of the other cardiac isoform of this protein,  $\text{Ca}_v1.3$ , was not assessed in this study. Importantly, areas of atrial tissue staining for a major gap junctional protein (Cx43) also decreased with age in this model [15]. Thus, age-associated sinus brady-cardia may be due to declines in  $\text{Ca}^{2+}$  channel protein and Cx43 expression. The mechanisms underlying these changes are unknown.

If the SAN has a reduced rate of firing, then a population of atrial cells that exhibit abnormal pacemaker function may be revealed. Canine atrial fibers, while showing a phenotypic change with age (see section above on substrate changes), apparently show no increased or *de novo* pacemaker activity with age [16]. On the other hand, several cell regions within the atria show triggered activity both *in vivo* and *in vitro*, prompting the question: does the capability for triggered activity increase with age? Over 25 years ago, studies on Purkinje fibers from aged canines demonstrated an increased susceptibility to the Na-K pump inhibitor ouabain [17], manifested by delayed after-depolarizations (DADs) and thus increased potential for triggered activity. Since then it has become clear that abnormal intracellular  $\text{Ca}^{2+}$  handling underlies the  $\text{Ca}^{2+}$  waves that give rise to DADs [18]. In specialized atriovenous junctions, groups of pulmonary vein (PV) cells, coronary sinus cells or mitral valve cells also show triggered activity [19], and recent data suggest that these types of cells can show enhanced DADs in tissues from aged animals (Fig. 5) [20,21]. Interestingly, in both of these studies, a pharmacological agent was needed to provoke the DADs and thus abnormal impulse initiation (glycolytic inhibition or ouabain). In fact, in one study both early after-depolarizations (EADs) and DADs were induced [21]. From our own work, we have reported that there is a change in AP phenotype that occurs with age in the canine (see Fig. 1(a)) such that APs of left PV cells (within circles) show an increased plateau and APD with age. Given the strict relation between APD and the ability of cells to show DADs [19], it follows that the mechanism of enhanced DADs in PV cells from aged animals may be primarily due to changes in APD that occur with age.

While it is clear that abnormal intracellular  $\text{Ca}^{2+}$  dynamics may underlie enhanced DADs in specialized aged atrial cells, as yet there has been no systematic study of intracellular  $\text{Ca}^{2+}$  handling in atrial cells from aged human atria. In old rats, one report [21] suggested that the paced  $\text{Ca}^{2+}$  transient is delayed in aged animals versus the young, but as noted above, this may be just a reflection of an age-related change in APD. In experimental animal models, there has been no systematic study of the effects of age alone on the components of *atrial* excitation-contraction (EC) coupling. Perhaps this is a challenge since unlike ventricular cells, normal EC coupling in adult atrial cells involves a coordinated release from two subcellular compartments [18]. However, a recent study in older patients (average 66 years) with AF [22] reported increased  $\text{Ca}^{2+}$  spark frequency (RA cells), increased  $\text{Ca}^{2+}$  wave frequency, spontaneous inward Na-Ca exchange current ( $I_{\text{NCX}}$ ), and DADs (Fig. 6) relative to patients in the same age range who were in sinus rhythm (but with coronary artery bypass surgery, aortic valve replacement, and/or mitral valve replacement). Comparisons between

atrial  $\text{Ca}^{2+}$  spark characteristics in younger and older patients with sinus rhythm have not been done for atrial cells, but data are available for aged ventricular cells [23].

The mechanism of enhanced  $\text{Ca}^{2+}$  wave and spark formation in AF atria from aged patients has been examined, and one study suggests that ryanodine receptor dysfunction (hyperphosphorylation) [24] occurs in human atria from older AF patients (mean 65 years old). However, due to the lack of a young age control group, it is not clear whether the cause of the ryanodine receptor change is due to age, the presence of AF, or another mechanism. The molecular underpinnings for these changes are also unknown. Some have suggested that aging reduces the activity of the sarcoplasmic reticulum calcium (SERCA) pump (a major protein needed to transport  $\text{Ca}^{2+}$  back into the sarcoplasmic reticulum) [25]. Recent data using aged human atrial tissues (mean 68 years) suggest that abnormal  $\text{Ca}^{2+}$  handling is due to impaired phosphorylation-dependent regulation of many  $\text{Ca}^{2+}$  handling proteins, one of which is phospholamban [26]. Again, whether levels of phosphorylation of these proteins change with age alone has not been evaluated.

In summary, data on the effects of age alone on the cell properties of specialized atrial cells suggest that enhanced impulse initiation may occur in such cells, while SAN firing is reduced. However, the specific mechanisms for these changes remain unknown.

#### 4 Abnormal impulse conduction

To understand changes in conduction in the aged atria, it is important to comprehend the nature of both the electrical and structural remodeling that occur with age. In particular, the profound structural remodeling that occurs in aged atria affects the ability of a premature beat that is triggered in the PV cells, for example, to penetrate the substrate and initiate propagation.

The most significant change in aged atrial bundles is the increased content of fibrous tissue interspersed between the atrial cells (Fig. 7) [6] and characterized by accumulation of collagen in the extracellular space. The impact of remodeling of the atrial microstructure on impulse conduction in atrial bundles isolated from patients with no atrial arrhythmias has been studied by Spach et al. [27-31]. Recent data from this laboratory [31] suggest that arrhythmogenic conduction depends on the atrial microarchitecture, the precise location of the premature beat within the atrial bundle, and its precise timing.

Another important effect of increased fibrosis is a change from typical uniform anisotropic conduction to non-uniform conduction [27]. Studies in human atria (17 to 75 years) show an age-related reduction in wavefront velocities in both atria [32], but only septal refractoriness correlated with age. In another study [33], changes in anisotropic conduction properties in atrial tissue were quantified in infant, young and aged canines (Fig. 8). This study showed that age-related fibrosis preferentially affects transverse conduction (Table 2). Furthermore, as discussed above, this abnormal conduction pattern is not due to a change in intrinsic  $\text{Na}^+$  channel function.

The implication of these studies is that as collagen is laid down between atrial bundles, muscle cells and bundles become electrically isolated, forcing impulse propagation along



nontraditional alternative pathways. In guinea-pig hearts, protein quantification by immunofluorescence and Western blot confirmed a significant loss of Cx43 with age in SAN tissue but not in RA tissue [15]. The expression of other cardiac connexins, Cx40 and Cx45, was unaffected by age in either SAN or RA tissues. However, connexin quantity cannot always be equated with altered function of gap junctions. Thus, regional studies on the effects of age on intercellular connections in both the RA and LA are needed.

## 5 Summary

Age related changes in both  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  currents underlie specific AP changes in RA but not LA cells. It is unlikely that conduction changes in aged atrial tissues are due to changes in intrinsic sodium channel function, but rather are due to the complex interplay between age-related fibrosis (whether patchy or diffuse), wavefront velocities, and increased likelihood of block. AF will be initiated if and only if suitable electrophysiologic conditions are present, including a trigger or initiation of an ectopic beat, propagation of the waveform to atrial tissue, and perpetuation of the dysrhythmia through a re-entrant circuit. Age-related changes in atrial tissue increase the likelihood that these conditions will be present in older compared to younger hearts.

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## Ionic currents

$I_{\text{BaL}}$	Barium, L-type
$I_{\text{CaL}}$	Calcium, L-type
$I_{\text{f}}$	funny current, pacemaker current
$I_{\text{K1}}$	Potassium, inward rectifier current
$I_{\text{KACh}}$	Potassium, acetylcholine-induced
$I_{\text{K(ATP)}}$	Potassium, ATP modulated
$I_{\text{Kr}}$	Potassium, rapid delayed rectifier
$I_{\text{Ks}}$	Potassium, slow delayed rectifier
$I_{\text{Na}}$	Fast Sodium
$I_{\text{NCX}}$	Sodium-calcium exchanger
$I_{\text{sus}}$	Potassium, sustained
$I_{\text{to}}$	Potassium, transient outward

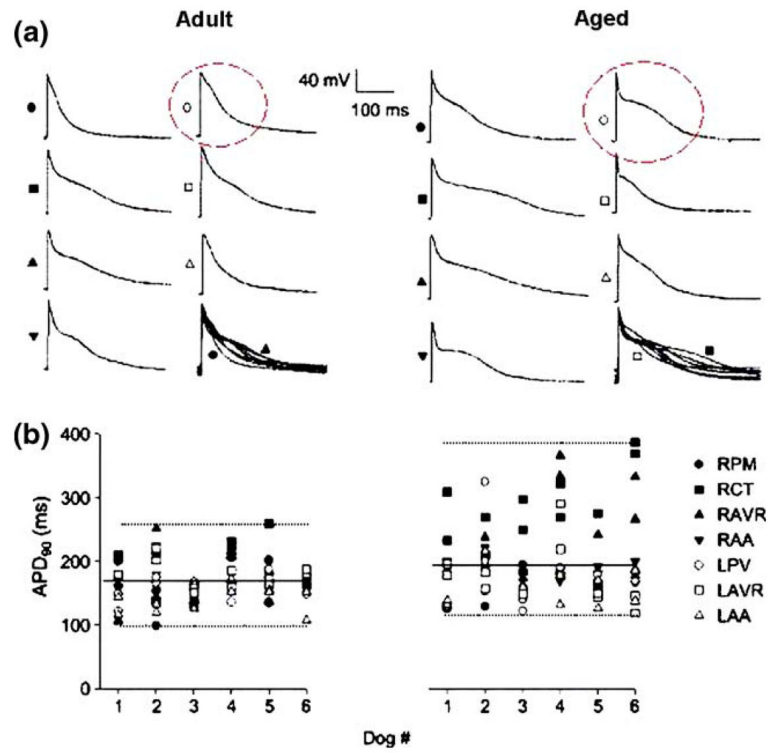
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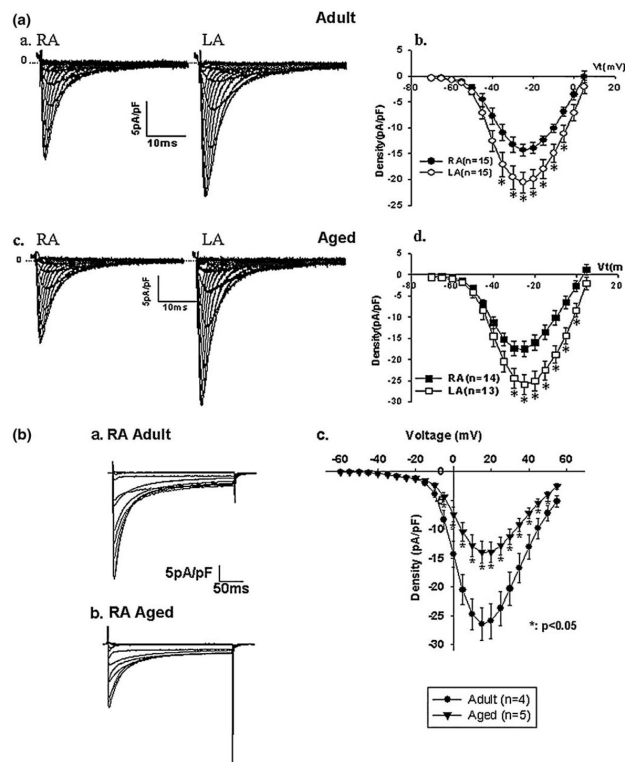


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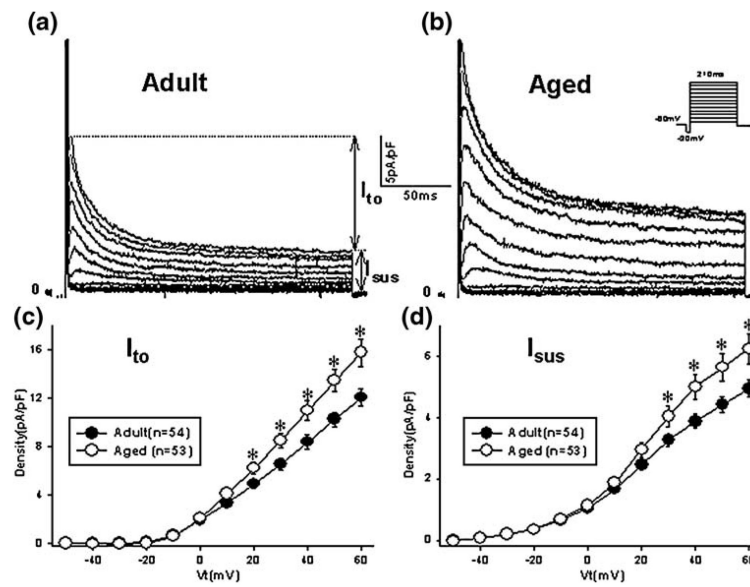
**Fig. 1.**

Heterogeneity of action potential duration (APD) in adult and aged dogs in normal sinus rhythm. **(a)** Transmembrane potentials recorded from seven regions of one adult (left) and one aged (right) dog at a cycle length of 1000 ms. In the right lower image at each age, the upstrokes of all action potentials (APs) are superimposed to facilitate comparison of the extent of dispersion. **(b)** Values of APD at 90% repolarization (APD<sub>90</sub>) obtained from each impalement in all regions are shown at a cycle length of 1000 ms for each adult and aged dog. Dotted lines are drawn for maximum and minimum values in each group; solid lines represent APD<sub>90</sub> averaged over all regions. Two impalements were made in each preparation. Preparations from four regions of the right atrium-pectinate muscles (RPM), crista terminalis (RCT), atrioventricular ring (RAVR) and appendage (RAA)—and three regions of the left atrium-free wall near the pulmonary veins (LPV), atrioventricular ring (LAVR) and appendage (LAA)—were dissected in each dog. Reproduced from reference [7]

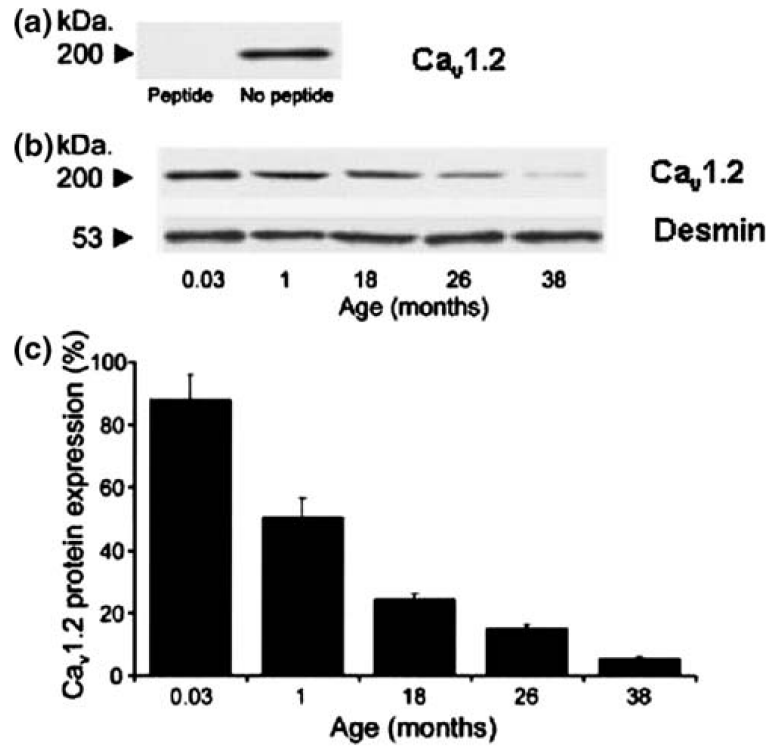


**Fig. 2.**

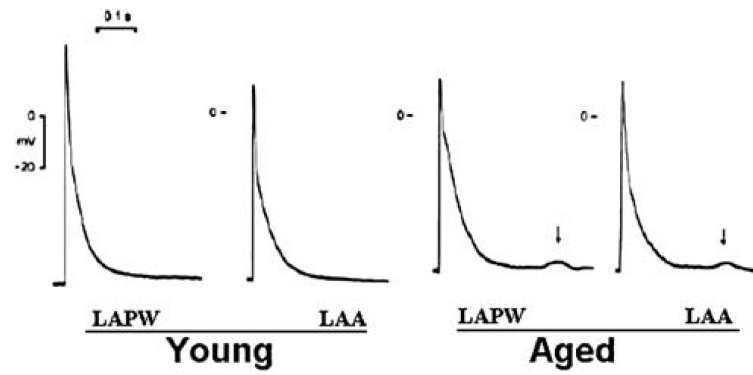
(a), Family of tracings of Na<sup>+</sup> current (I<sub>Na</sub>) in right (RA) and left (LA) atrial cells from adult (Panel a) and aged (Panel c) dogs. I<sub>Na</sub> density-voltage relationship in RA and LA of adult (Panel b) and aged (Panel d) dogs. Reproduced from reference [8]. (b), Current tracings in an adult (Panel a) and aged (Panel b) right atrial cell from dogs under conditions of this experiment: 3 mM Ca<sup>2+</sup>/10 mM BAPTA<sub>i</sub>(internal BAPTA concentration), 2 μM ryanodine, holding voltage of -70 mV to various test voltages. Panel c shows the average peak I<sub>CaL</sub> density in adult and aged cells. Reproduced from reference [9]



**Fig. 3.** K<sup>+</sup> currents in cells dispersed from an adult (Panel **a**) and aged (Panel **b**) dog right atrial cell. (Panel **c**) and (**d**) show average current-voltage relations of I<sub>to</sub> and I<sub>sus</sub> densities in adult and aged right atrial cells. Reproduced from reference 9

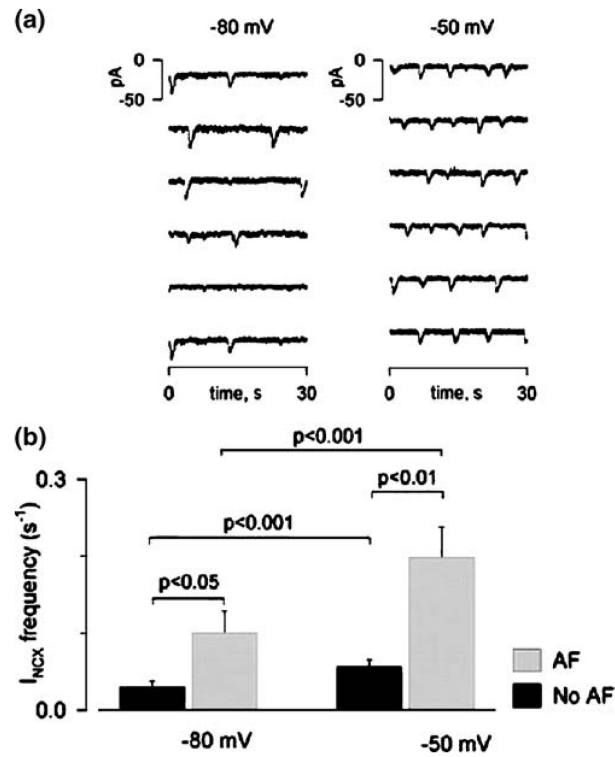


**Fig. 4.** The decline of Ca<sub>v</sub>1.2 protein expression within the sinoatrial (SA) node with increased age in guinea pigs. (a) Specificity of the antibody anti-CNC1 to determine Ca<sub>v</sub>1.2 protein expression was confirmed by the absence of the 200-kDa band in the presence of the supplied control antigen. (b) A typical blot illustrates the expression of desmin (53 kDa) and Ca<sub>v</sub>1.2 (200 kDa) proteins across the examined age range. (c) For every animal from each age group, Ca<sub>v</sub>1.2 protein expression in the SA node region was expressed as a percentage of the paired atrial muscle sample. Data show mean±SEM. Ca<sub>v</sub>1.2 protein expression fell in the SA node region vs age ( $n=5$ ; ANOVA,  $P<0.01$ ;  $y=-1.8363x+67.024$ ;  $R^2=0.81$ ). Reproduced from reference [13]

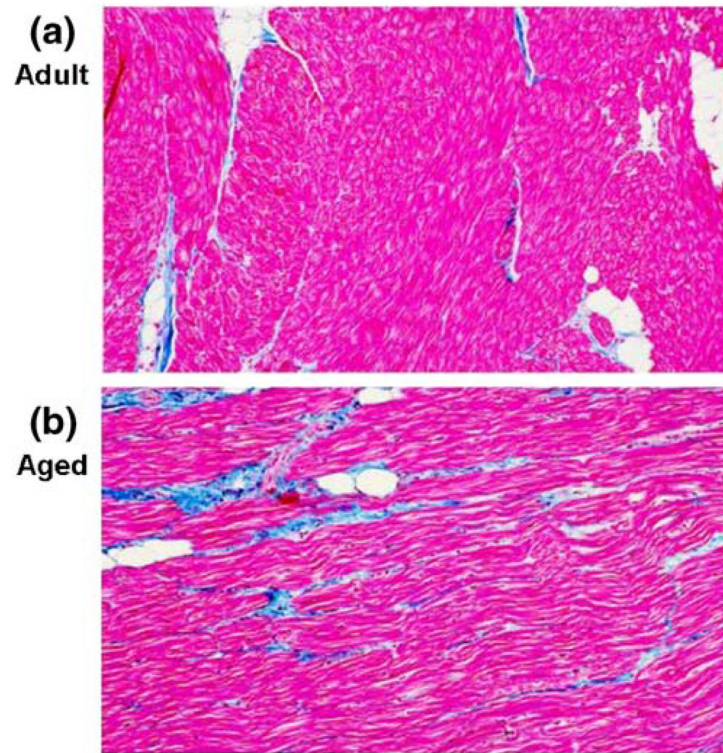


**Fig. 5.** Tracings showing examples of action potentials from young and aged rabbit left atrial posterior walls (LAPWs) and left atrial appendages (LAAs). The delayed after-depolarizations (DADs) (↓) were recorded in the aged LAPW and LAA, but not in young LAPW and LAA, with a 2-Hz electrical stimulus. Reproduced from reference [20]

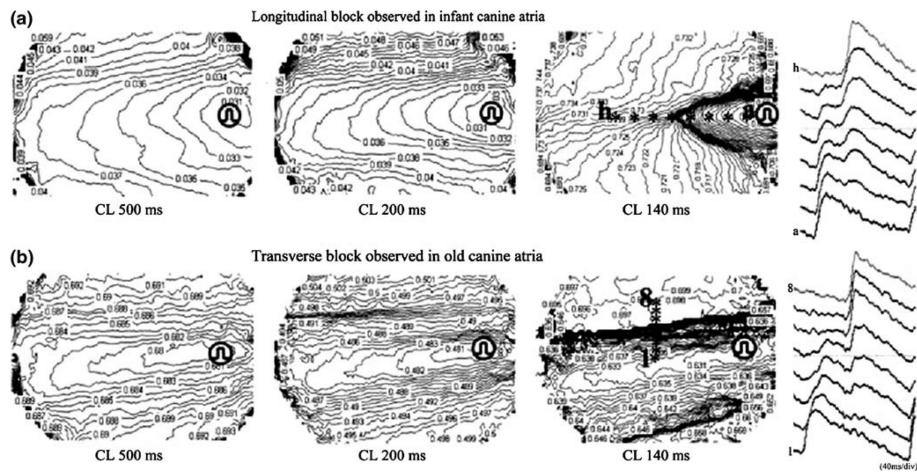




**Fig. 6.** Effect of holding potential on spontaneous INCX frequency in human atrial cells. (a). Switching holding potential from -80 to -50 mV increased the number of spontaneous  $I_{NCX}$ . (b). Average  $I_{NCX}$  frequencies at -80 and -50 mV in the two patient groups. Patients with atrial fibrillation (AF) ( $66.4 \pm 11.2$  years) had more spontaneous  $I_{NCX}$  than those without AF ( $65.5 \pm 13.2$  years). Reproduced from reference [22]



**Fig. 7.** Representative sections of right atrial (RA) pectinate muscle bundles from an adult **(a)** and aged **(b)** dog showing myocytes in red and collagen in blue. Image analysis of these and additional fields showed the amount of fibrous tissue in adult atrium = 4.5% **(a)** and the amount of diffuse interstitial fibrous tissue in old atrium = 17.9% **(b)** of the myocardium. Masson trichrom stain used. Reproduced from reference [6]



**Fig. 8.** Different patterns of conduction block in infant and old canines. The activation sequences of atrial tissue (optical images) of an infant and an old dog under stimulation at cycle length (CL) 500 ms, 200 ms, and 140 ms are presented by 1-ms isochrones. Conduction block at short (140 ms) CL occurred in the longitudinal (L) direction in the infant dog (a), whereas it was observed in the transverse (T) direction in the old dog (b). Eight action potentials recorded across the block line (a to h in the infant tissue, and 1 to 8 in the old tissue) are shown on the right. Reproduced from reference [33]

Table 1

Comparison of electrophysiological characteristics between adult and age right atria

Parameters	Adult	Age	Species	References
AP				
MDP(mV)	-76.4±1.8	-66.8±1.5*	Rat	[4]
	~ -77	~ -72*	Canine	[7]
APD90(ms)	~ 130	~ 150*	Rabbit	[3]
	125±9	146±18*	Rat	[5] [7]
	~ 175	~ 200*	Canine	
ERP(ms)	75±6	86±4*	Rat	[5]
$V_{max}$	~ 180	~ 145*	Canine	[7]
APA(mV)	95±2	84±3*	Canine	[7]
$I_{Na}$				
Density(pA/pF)	14.3±1.2	17.5±1.8	Canine	[8]
$I_{Ca}$				
Density(pA/pF)	19±2.1	12±1.5*	Canine	[9]
$I_{to}$				
Density(pA/pF) at 50 mV	9.9±0.1	12.4±0.1*	Canine	[9]
	8.6±0.9(LA)	11.9±2.1(LA)	Canine	[11]
Decay	$\tau_1$ (ms): 9.9±0.1	12.4±0.1*	Canine	[9]
	$\tau_2$ (ms): 47.1±0.5	58.8±0.7*		
Steady-state inactivation	$V_{0.5}$ (mV): -34.9±1.8	-29.8±1.4*	Canine	[9]
Recovery from inactivation	$\tau_1$ (ms): 45.6±5.6	76.6±6.8*	Canine	[9]
	$\tau_2$ (ms): 313.1±45.6	1083.3±162.4*		
$I_{sus}$				
Density(pA/pF) at 50 mV	4.4±0.3	5.6±0.5*	Canine	[9]
	3.3±0.2(LA)	2.9±0.3(LA)	Canine	[11]

AP=action potential; MDP=maximal diastolic potential; APD90=action potential duration to 90% repolarization; ERP=effective refractory period;  $V_{max}$ = maximum upstroke velocity;  $I_{Na}$ = sodium current;  $I_{Ca}$ = calcium current;  $I_{to}$ = transient outward potassium current;  $I_{sus}$ = sustained potassium current. LA= left atria.

\* P<0.05 Age versus Adult.

**Table 2**

Incidence of longitudinal and transverse block in infant, young and old dogs

	<b>Longitudinal Block</b>	<b>Transverse Block</b>	<b>Whole Refractoriness<sup>a</sup></b>
Infant ( <i>n</i> =10)	40%(4/10) <sup>b</sup>	20%(2/10) <sup>b</sup>	40%(4/10)
Young( <i>n</i> =11)	27%(3/11)	36%(4/11)	36%(4/11)
Old( <i>n</i> =10)	0%(0/10)	50%(5/10)	50%(5/10)

<sup>a</sup> Activation ceased simultaneously in all directions because of refractoriness

<sup>b</sup>  $P < 0.05$  for infant vs old (X2 test). Reproduced from reference [33]