

**RELATIONSHIP BETWEEN ATRIAL FIBRILLATION
AND HYPERTENSION IN RATS**

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June, 2005

**A thesis submitted to McGill University in partial fulfilment of the
requirements of the degree of Masters of Science**

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ISBN: 978-0-494-22701-5

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Abstract

The importance of hypertension as a risk factor for the development of AF is well recognized. Despite this leading importance, the role of essential hypertension in providing a substrate for AF is incompletely understood. The present study was undertaken to investigate the possibility of having rats as an adequate animal model for the relationship between hypertension and AF in man and to elucidate the role of hypertension as a risk factor for AF. The results of this study show that structural remodeling especially in the form of increased amount of interstitial fibrosis seems to be the major contributing factor to the AF sustainability. Although it can be concluded that hypertension could accelerate the accumulation of fibrosis which occurs during the normal process of aging, a clear relationship between hypertension and AF in this rat model was not found. Further work in the other animal models of hypertension would be interesting.

Résumé

L'Hypertension est reconnue comme un facteur de risque important dans le développement de la fibrillation auriculaire (FA). Cependant, le rôle exact de l'hypertension essentielle dans la pathogenèse du FA n'a pas été élucidé. La présente expérience a comme but d'investiguer la possibilité d'utiliser des rats comme modèle animal adéquat pour étudier la relation entre l'hypertension et la FA. De plus, nous visons à éclaircir le rôle de l'hypertension comme facteur de risque pour la FA. Les résultats démontrent que le remodelage structurel, se manifestant sous forme de fibrose interstitielle, contribue largement à soutenir la FA. Alors qu'il peut être conclu que l'hypertension peut accélérer l'accumulation de fibrose qui survient normalement avec l'âge, la présente étude n'a pu trouver de relation claire entre l'hypertension et la FA dans le modèle animal employé. D'autres travaux avec différents modèles animaux de l'hypertension seraient intéressants.

Acknowledgements

I would like to express my sincere gratitude to Dr. Stanley Nattel, my supervisor, who has provided me with the great opportunity to become familiar with research and to pursue my graduate studies in his lab. I thank him for his ongoing support, and pertinent guidance. He is a great inspiration and I am grateful for what I learn as a member of his team. I would also like to thank Mr. Robert Clement and Mr. Marc-Antoine Gillis, the technicians in the Montreal Heart Institute, for their patience and their great technical support. In addition, I would like to thank Dr. Yan Fen Shi who performed the echocardiography experiments in this study. Her teamwork spirit and her technical skills are admirable. Also many thanks would go to Ms. Chantal Maltais, Ms. Evelyn Landry, and Ms. Nathalie L'heureux, the technicians in our lab, for making life easier everyday for everyone at work. I thank the other lab colleagues especially Dr. Akiko Takeshita for always being there to answer my questions. And last but not least I would like to thank the department of Pharmacology and Therapeutics for its great support. It was indeed a wonderful privilege to be a part of this department with its diverse and yet unique character.

Contributions of Authors

Experimental design, hemodynamic measurements, in-vivo electrophysiology studies, cholinergic stimulation experiments, thoracic aortic constriction experiments, and data analysis were all performed by Ali Andalib. Collaboration of Dr. Yan Fen Shi and Dr. Jean-Claude Tardif was needed in the Echocardiography measurements. These measurements as well as their analysis were performed by Dr. Shi. Histological preparations were done by Dr. Tack Ki Leung's team and his guidance has been used in performing the histological analysis. Dr. Stanley Nattel is the director of the laboratory whose supervision has the most important role in the project.

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List of Abbreviations

AF	Atrial fibrillation
ECG	Electrocardiogram
AV	Atrioventricular
CHF	Congestive heart failure
ERP	Effective refractory period
EAD	Early afterdepolarization
DAD	Delayed afterdepolarization
APD	Action potential duration
BP	Blood pressure
LVH	Left ventricular hypertrophy
LAE	Left atrial enlargement
SHR	Spontaneously hypertensive rat
SHHF	Spontaneously hypertensive heart failure
RAA	Renin-angiotensin-aldosterone
DOCA	Deoxycorticosterone acetate
ACE	Angiotensin converting enzyme
AGE	Advanced glycation endproducts

1. Introduction

1.1. Atrial Fibrillation

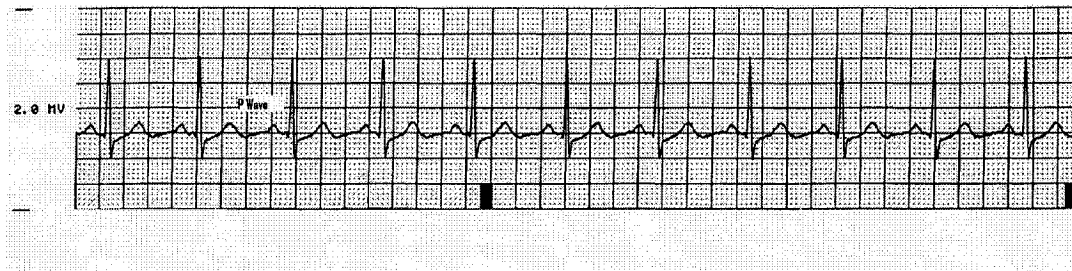
1.1.1. Definition

Atrial fibrillation (AF) is characterized by wavelets propagating in different directions and causing disorganized atrial depolarizations without effective atrial contraction. Electrical activity of the atrium can be detected on electrocardiogram (ECG) as small irregular baseline undulations of variable amplitude and morphology, called f waves, at a rate of 350 to 600 beats/min (Figure 1). Each recorded f wave is not conducted through atrioventricular (AV) junction, so a rapid ventricular response comparable to the atrial rate does not occur. Many atrial impulses are concealed because of a collision of wavefronts, or they are blocked in the AV junction without reaching the ventricles. The ventricular response is grossly irregular and, in an untreated patient with normal AV conduction, is usually between 100 and 160 beats/min¹.

Owing to the loss of effective atrial contraction, and the irregular and excessively rapid ventricular rhythms that can be caused by AF, acute and sometimes life-threatening decompensation of otherwise compensated cardiac disease might occur. In fact, sustained AF with an uncontrolled ventricular response rate can, by itself, cause severe congestive heart failure (CHF) after several weeks to months, but this is reversible with proper rate and/or rhythm control². The loss of atrial contraction also leads to stasis of blood in the atria, which promotes clot formation and the occurrence of thromboemboli. These thromboemboli tend to propagate, particularly to brain but also to other organs (including the kidneys, mesenteric circulation and the heart itself), potentially leading to infarction³. These considerations probably account for the

significant role of AF in the occurrence of stroke: AF is the single most important cause of ischaemic stroke in people older than 75⁴.

A.



B.

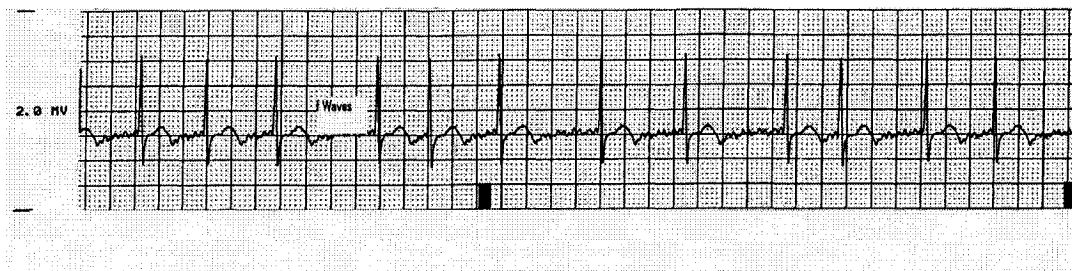


Figure 1: ECG from a normal subject (A) with clear P waves compared to one from a patient with AF (B) with f waves. The irregularity of the ventricular rhythm could be seen in the ECG from the patient with AF. (adapted from Braunwald. Heart Disease: A Textbook of Cardiovascular Medicine. 2001: 833-835)

1.1.2. Epidemiology

1.1.2.1. Incidence of AF

Atrial fibrillation is the most common sustained cardiac arrhythmia encountered by clinicians⁵. Currently, more than 2.2 million people in United States have atrial fibrillation⁶. Although data are difficult to compare, the prevalence of AF seems to be similar in the western world, Europe and U.S.A, but perhaps lower in Asia⁷.

1.1.2.2. Increase in Prevalence of AF

Recent data suggests that AF is a disease that is increasing in prevalence⁸. This could be due to a number of reasons such as an increase in the number of elderly patients in the population, recent improvements in survivals after cardiovascular incidences such as myocardial infarction, or simply a trend toward an increase in the telemetry and routine electrocardiograms. However, data from the Framingham Heart Study support an increase in the prevalence of AF over time in men when the analysis was restricted to the electrocardiograms performed routinely in biennial examinations. In this study between 1968 and 1989, the prevalence of AF in men nearly tripled from 3.2% to 9.1%⁹.

1.1.2.3. AF, Disease of Aging

The incidence of AF increases dramatically with increasing age in a way that it doubles with each decade after 50 years^{10;11}. The prevalence increases from 0.1% for those <50 years of age to between 7.3% and 13.7% for those ≥80 years of age¹². Figure 2 is taken from the Renfrew/Paisley study that was performed in Scotland¹³.

1.1.2.4. Gender Differences in AF

Although the etiology of gender difference was not clear, in a study which was performed 10 years ago¹⁰, men developed AF at 1.5 times the rate of women even after adjustment for potential confounders. While the incidence and prevalence of AF was greater in men, because of the greater longevity in women, women made up the majority of AF cases. It is estimated that 53% of all people affected by AF are female⁶. The result from the Renfrew/Paisley study shows higher incidences of AF in males than in females up to the age of 64 (figure 2).

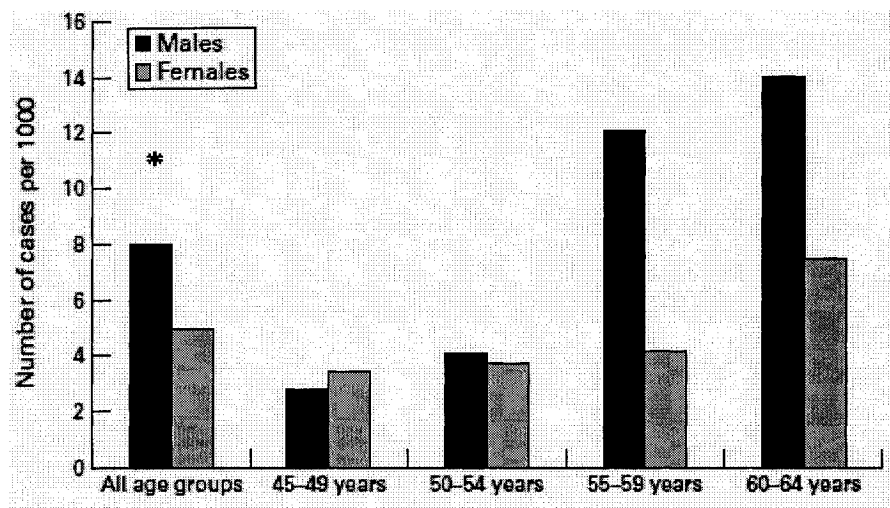


Figure 2: Age and sex specific prevalence of AF in a population cohort from Scotland. A higher prevalence of AF is noted among men up to the age of 64 compared to women. (adapted from Stewart S, Hart CL, Hole DJ et al. Population prevalence, incidence, and predictors of atrial fibrillation in the Renfrew/Paisley study. *Heart*. 2001;86:516-521)

1.1.3. Mechanisms

In Order to better describe each of the mechanisms of AF, a review of the atrial action potential followed by a brief definition of re-entry and its conceptual models is presented.

1.1.3.1. Atrial Action Potential

The atria are composed largely of fast-channel tissue, with atrial-cell activation depending on the large phase 0 inward Na^+ -current (I_{Na}) that depolarizes atrial myocytes from their normal resting potential of between -70 mV and -80 mV to an overshoot potential of about +30 mV (figure 3). The large size of I_{Na} explains the rapid atrial conduction velocity of around 1 meter per second in the direction parallel to fiber orientation. Following depolarization, I_{Na} is inactivated and atrial cells remain in their refractory phase (the refractory period) until inactivation starts to be removed, at about -60 mV. Repolarization, is the process by which atrial cells return from their depolarized state to their resting state. It depends on a series of time-dependent outward K^+ currents. These currents include the short-lasting transient outward current I_{to} (phase 1 of the action potential), the ultra-rapid delayed rectifier current I_{Kur} (phases 1 and 2), and the rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier currents. The time from initial depolarization until the time at which excitability is restored during late phase 3 of the action potential defines the refractory period. The refractory period is often defined experimentally by the longest coupling interval of a twice-diastolic threshold current pulse that fails to capture the tissue, known as the effective refractory period (ERP).

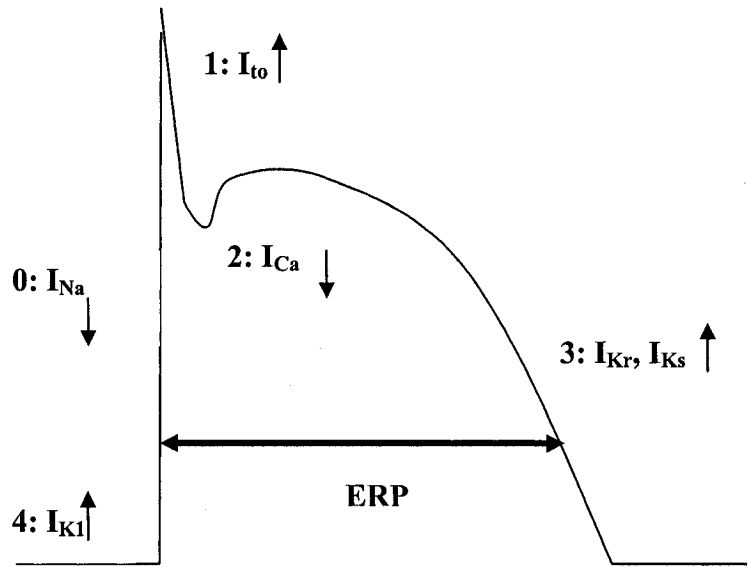


Figure 3: A schematic representation of the atrial action potential with inward current (downward arrows) and outward currents (upward arrows). Numbers indicate the phases of the action potential. I_{Na} : Sodium current responsible for depolarization of atrial myocytes; I_{to} : Short-lasting transient outward current; I_{Ca} : Inward calcium current during phase 2 of the action potential; I_{Kr} : Rapid potassium current responsible for repolarization; I_{Ks} : Slow potassium current responsible for repolarization; I_{K1} : Inward rectifier potassium current responsible for maintaining the cell membrane potential during phase 4 of the action potential.

1.1.3.2. Re-entry

In the normally-activated heart, the propagating impulse initiated by the sinus node stops after sequential activation of the atria and ventricles because it is surrounded by refractory tissue that has been just excited and because it meets the inexcitable fibrous annulus. Under special conditions, the impulse may not die out after complete activation of the heart and may persist to re-excite the atria or the ventricles after the end of the refractory period. This phenomenon is called re-entrant excitation.

Re-entry arises from abnormal impulse propagation between different zones of tissue (figure 4, I and II). After initial depolarization of an action potential, Na^+ channels are inactivated and the cell cannot be re-fired until the cell repolarizes to a potential (about -60mV) at which Na^+ channels recover from inactivation, refractory period. An ectopic complex (figure 4, 2) arising in zone II during the refractory period of action potential 1 in zone I will initially fail to activate zone I, but may propagate through an alternative pathway to return to zone I when its refractory period is over, causing reactivation at this site (figure 4, 3). The impulse will now leave zone I and move towards zone II and, if the time to return to zone II is sufficiently long, then zone II will be reactivated (figure 4, 4). If there is an appropriate substrate for re-entry, zones I and II can repeatedly reactivate each other once re-activation has been initiated, resulting in persistent re-entrant activity³.

Re-entrant excitation as a cause of cardiac arrhythmia has been studied since the second decade of this century. The characteristics of re-entrant excitation and the conditions necessary for its initiation and maintenance were well defined by Mines^{14;15}, and Garrey¹⁶.

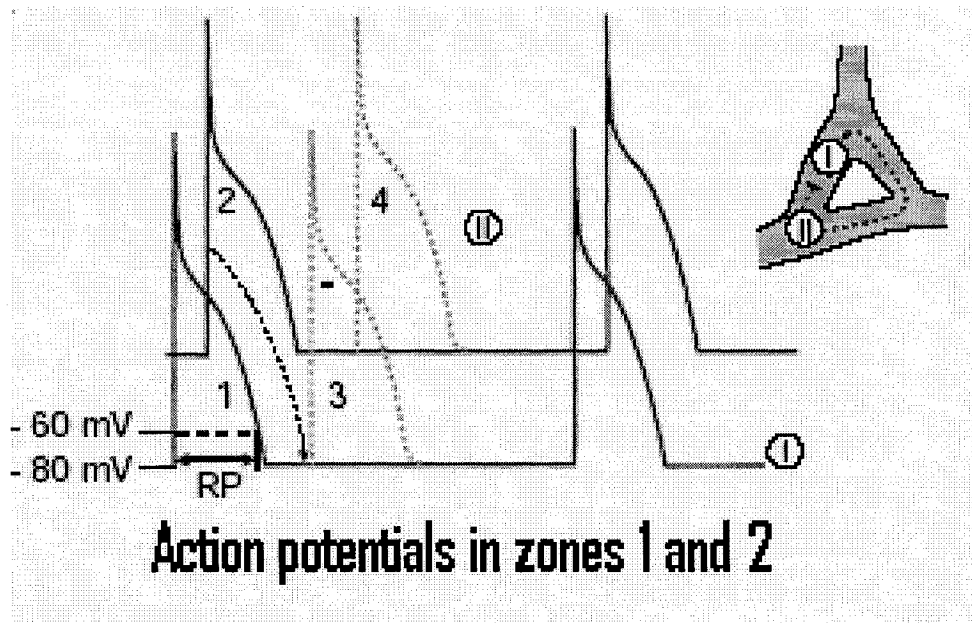


Figure 4: Re-entry occurring between two tissue zones, I and II, which are connected as shown on the right. A premature activation (2) in zone II, which fails to initiate firing in zone I because zone I is still in refractory period, may conduct back to zone I (dashed line) at a time when it can respond with an action potential (3). This action potential may propagate to initiate (4) in zone II, and the process can continue indefinitely. RP, refractory period.

(adapted from Nattel S. New ideas about atrial fibrillation 50 years on. *Nature*. 2002; 415:219-226)

1.1.3.3. Conceptual Models of Re-entry¹⁷

1.1.3.3.1. Closed-Circuit Re-entry

In the beginning of this century, Mines considered that re-entry occurs in “closed circuits in myocardium”¹⁵. The determinants of this form of closed-circuit re-entry are illustrated in figure 5. The inexcitability of the core that underlies the substrate may be caused by the anatomical arrangement of potential conducting pathways, by anatomical obstacles like the venae cavae or pulmonary veins, or by a region of inexcitability caused by heart disease. Because the size of the circuit is fixed, re-entry depends critically on a circuit time that is greater than the refractory period, and the circuit time equals the path length divided by conduction velocity. The refractory period determines whether re-entry can be maintained (refractory period must be less than circuit time), but does not directly affect circuit time or tachycardia rate.

1.1.3.3.2. Leading-Circle Re-entry

A great limitation of the closed-circuit re-entry concept is that it does not account for the dynamic nature of re-entry in arrhythmias like AF, in which the re-entry substrate appears functional rather than fixed, as pointed out by Garrey in early twentieth century¹⁶. Allesie et al presented the first detailed conceptual model of functional re-entry in 1977¹⁸. Re-entry is maintained in a leading circle, which establishes itself in the smallest circuit that can maintain continuous activity (figure 5). This minimum circuit size for re-entry is given by the wavelength, a concept first presented by Mines¹⁵ and later quantified by Wiener & Rosenbluth¹⁹ as the product of conduction velocity and refractory period. The core of the re-entry circuit is continuously invaded by centripetal

impulses from the circulating re-entrant wave and is thus continuously excited. A change in conduction velocity causes the re-entrant impulse to move concentrically based on the path length traveled in one refractory period; increased conduction velocity moves the wave outwards, to travel in a longer orbit, whereas decreased conduction velocity allows the wave to move inwards to a smaller path. Because the circuit time equals the refractory period (by definition in a path equal to wavelength), refractory period is the sole determinant of circuit time and tachycardia rate.

1.1.3.3.3. Spiral-Wave Re-entry

More recently, Pertsov et al suggested that the concept of spiral wave activity, a generalized form of continuous activity in excitable media, may be applicable to cardiac re-entry²⁰. As indicated in figure 5, spiral re-entry differs from the other models in that the core is fully excitable. Maintenance of spiral-wave re-entry depends on the curvature of wavefronts at the tip of the spiral²¹. A present limitation on the applicability of the spiral-wave concept is the difficulty of formulating predictions regarding the stability and rate of re-entry based on simple electrophysiological properties like conduction velocity and refractory period.

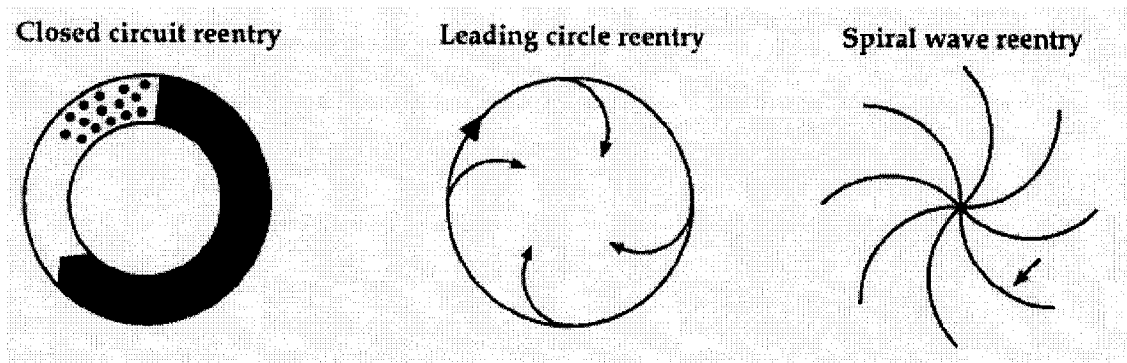


Figure 5: Schematic diagrams of closed-circuit re-entry (*left*) as suggested by Mines in the beginning of the century with a core that is not excitable and a fixed circuit size; leading-circuit re-entry (*middle*), a model that was proposed by Allesie et al. in 1977 to account for the dynamic nature of the re-entry. The re-entry wave establishes itself in the smallest circuit determined by wavelength (product of refractory period and conduction velocity) that can maintain continuous activity with a core that is continuously activated by centripetal impulses from the leading circle; and spiral-wave re-entry (*right*), a more recent model suggested by Pertsov et al. which differs from the other models in that the core is fully excitable and that maintenance of re-entry wave depends on the curvature of wavefronts at the tip of the spiral.

(adapted from Nattel S, Li D, Yue L. Basic mechanisms of atrial fibrillation--very new insights into very old ideas. *Annu Rev Physiol.* 2000;62:51-77)

1.1.3.4. AF Mechanisms

The conceptual framework for understanding the mechanisms of AF has been grounded in ideas developed in the early twentieth century²². The predominant competing theories at the time were that AF is caused by:

- Rapidly discharging, spontaneously active, atrial ectopic foci
- Multiple-circuit reentry
- Single-circuit reentry with fibrillatory conduction

Over the past 50 years, the dominant concept of AF mechanism has been based on the multiple-wavelet hypothesis of Gordon Moe²³. However, observations obtained over the last few years have resulted in a better understanding of the mechanisms of AF. This, recently evolving evidence have thrown us back to the debates of the early twentieth century suggesting that the mechanisms mentioned above may all be involved in atrial fibrillation²⁴.

1.1.3.4.1. Rapid Ectopic Activity

Focal ectopic activity can arise from enhanced normal automaticity or from triggered activity related to early and delayed afterdepolarization.

1.1.3.4.1.1. Automaticity

One of the main differences between cardiac muscle and most other kinds of excitable cells is the spontaneous, intrinsic rhythm generated by some specialised pacemaker cells, which is called automaticity. Pacemaker activity is normally found only in nodal and conducting tissue. These cells share a common characteristic: during

diastole the membrane potential slowly goes up to less negative values. This slow change in membrane potential at phase 4 (figure 3) is called diastolic depolarization which has different slope in different pacemaker tissues. The fastest rate of diastolic depolarization is found in the sinus node cells which act as the normal pacemaker for the heart²⁵. The functional determinants of spontaneous automaticity are the maximum diastolic potential, the rate of phase 4 depolarization, the threshold potential, and the action potential duration. Enhanced automaticity normally results when phase 4 depolarization is accelerated, reaching threshold earlier and increasing automatic rate.

1.1.3.4.1.2. Triggered Activity

Triggered activity is the term used to describe impulse initiation in cardiac fibers that is dependent on afterdepolarization²⁶. Afterdepolarization is an oscillation of the membrane potential that occurs near the time that the cell is repolarizing. The oscillations occurring during phase 2 or 3 of the cardiac action potential are known as “early afterdepolarizations” (EADs)²⁷ and those occurring just after repolarization or during phase 4 of the cardiac action potential are known as “delayed afterdepolarizations” (DADs)²⁸ (figure 6). When afterdepolarizations are large enough to reach the threshold potential, the resultant action potentials are referred to as “triggered”.

EAD-induced triggered activity is generally cycle-length dependent²⁹. Any factors which cause excessive lengthening of action potential duration (APD) and promote inward Na^+ and/or Ca^{2+} current may induce EADs, particularly at slow activation rates³⁰.

DADs usually occur under a variety of conditions in which there appears to be a large increase in Ca^{2+} in the cytoplasm and the sarcoplasmic reticulum of the myocytes. This explains the tendency of drugs such as digitalis to cause DADs³¹. The ionic

mechanism responsible for DAD is the result of a net inward current known as the transient inward current which could occur when the intracellular concentration of $[Ca^{2+}]_{in}$ increases beyond its normal range³². A rise in $[Ca^{2+}]_{in}$ could cause a transient inward current by activating the Na^+/Ca^{2+} exchanger across the cell membrane³³. This counter-transport system acts to transfer one Ca^{2+} ion out of the cell in exchange for three Na^+ ions, resulting in a net influx of one positive charge which acts to depolarize the cell membrane.

A single rapidly firing atrial focus would be expected to produce a regular tachycardia, but if the firing rate is so rapid that areas of the atria are unable to respond in a 1:1 fashion, a fibrillatory atrial response will result. In a study by Haissaguerre and colleagues, it has been shown that AF is frequently initiated by ectopic activity arising from the pulmonary vein region³⁴.

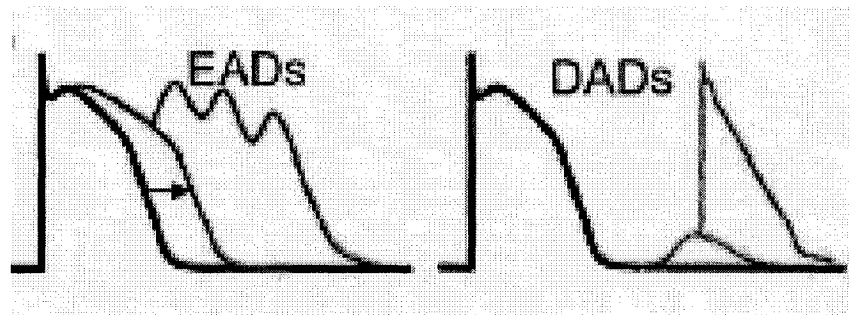


Figure 6: Schematic diagram of early after depolarizations (EADs) and delayed after depolarizations (DADs).

(adapted from Pogwizd S, Bers D. *Trends Cardiovasc Med.* 2004; 14:61-66)

1.1.3.4.2. Multiple-Circuit Re-entry

On the basis of earlier studies, in late 1950s and early 1960s, Moe formulated the so-called “multiple-wavelet” hypothesis to explain the mechanisms of true fibrillation^{23;35}. Since, at that time, simultaneous recording from a sufficient number of atrial sites to document the complex excitation pattern was impossible, Moe et al³⁶ developed a computer model of AF which was based on multiple re-entrant wavelets in a two-dimensional sheet with realistic properties. The key feature of the model was a non-homogenous distribution of refractory periods in otherwise identical elements.

According to Moe’s finding based on his computer model, fibrillation is maintained by the presence of a number of independent wavelets that wander randomly through the myocardium around islets or strands of refractory tissue. Each of the wavelets may accelerate or decelerate as it encounters tissue in a more or less advanced state of recovery of excitability. They may extinguish, divide, or combine with a neighbour wavelet, and they may continuously fluctuate in size and change direction of propagation. In Moe’s theory the number of wavelets present at one time could vary, and could be changed by the changing of electrophysiologic properties of the atrial tissue. The likelihood of spontaneous termination of fibrillation depends on the average number of wavelets present. If many wavelets exist (fine fibrillation), the chance that all wavelets extinguish simultaneously is small. On the other hand, with only a small number of wavelets present (coarse fibrillation), at a certain moment the waves may die out or fuse into a broad single wave front. This may result in transition into atrial flutter or resumption of normal sinus rhythm.

A direct test of the multiple-circuit hypothesis was performed by Allesie et al.³⁷. They inserted two egg-shaped multipolar electrodes, containing 480 recording terminals

in each, into the atrial cavities of isolated, Langendorff-perfused dog hearts. Atrial fibrillation was induced by a single premature stimulus while acetylcholine was administered continuously to the perfusion fluid. During maintained fibrillation, excitations of right and left atria were mapped consecutively. As Moe's theory mentioned, the presence of multiple independent wavelets was demonstrated.

The persistence of multiple-circuit re-entry depends on the continuous presence of excitable tissue in front of the head of propagating wave fronts. The leading circuit re-entry theory suggests that functional re-entrant circuits spontaneously establish themselves in the minimum path length for re-entry, the wavelength, given by the product of refractory period and conduction velocity. Thus, short ERPs and small conduction velocities favour multiple-circuit re-entry by reducing minimum circuit size and allowing the atria to accommodate more circuits. Correspondingly, increased atrial size should favour multiple-circuit re-entry by allowing for more wavelets to coexist. Refractoriness heterogeneity should also promote multiple-circuit re-entry, because of spatial variability of conduction around variably refractory tissue. Premature impulses are generally required to initiate re-entrant activity of any form.

1.1.3.4.3. Single-Circuit Re-entry

As a re-entrant mechanism, the maintenance of single circuit-re-entry depends on the wavelength being shorter than the circuit size. Because a single functional re-entrant circuit tends to be less stable than multiple circuits, particular conditions are needed to stabilize single-circuit re-entry in the atria. Atrial flutter, the classic example of atrial single-circuit re-entry, is usually maintained by a macroreentry circuit, depending on a combination of anatomic factors and a slowly conducting atrial region. AF results if the

impulses coming from a single, rapidly firing circuit encounter conduction barriers in the atria, resulting in zones that do not respond in a 1:1 fashion (fibrillatory conduction). Fibrillatory conduction can result from spatially varying ERPs or from source-sink mismatches related to anatomic properties of atrial tissue³⁸.

Mapping studies have suggested the importance of single-circuit re-entry in underlying AF of isolated canine right atria exposed to high acetylcholine concentrations³⁹. A mathematical modeling approach points to single spiral wave rotors underlying AF in simulated canine atrial tissue with heterogeneous acetylcholine distribution⁴⁰. Similar evidence for the ability of single macroreentry circuits to form the basis of AF is shown in experimental congestive heart failure⁴¹. The relevance of this mechanism is supported by clinical data as well^{42,43}.

1.1.4. Risk Factors for Atrial Fibrillation

Although underlying cardiovascular disease and age are the best predictors of increased risk of AF⁴⁴, non-cardiac causes of AF have been reported. The non-cardiac predisposing conditions include excessive alcohol intake, hyperthyroidism, severe infections, and pulmonary disorders⁴⁵. Obstructive sleep apnea may also be related, in which case the provision of continuous positive airway pressure reduces the risk of the recurrence of AF⁴⁶. Both vagal and sympathetic mechanisms of paroxysmal atrial fibrillation have been described (neurogenic atrial fibrillation)⁴⁷, as have familial forms of the condition⁴⁸. “Lone” AF (i.e., that occurring in the absence of a cardiac or other

explanation) is common, particularly in patients with paroxysmal atrial fibrillation-up to 45% of such patients have no underlying cardiac disease⁴⁹.

Aside from age and male gender, two of the most important risk factors of atrial fibrillation¹², the recognized cardiac conditions associated with AF are various types of valvular heart disease, acute myocardial infarction, myocarditis, pericarditis, hypertrophic cardiomyopathy, congenital heart disease, congestive heart failure, and hypertensive cardiovascular disease^{10;12}. In fact, currently, hypertension is recognized as the most prevalent, independent, and potentially modifiable risk for atrial fibrillation^{11;12}.

1.2. Hypertension

1.2.1. Definition and Epidemiology

Blood pressure (BP) is distributed in a typical bell-shaped curve within the overall population (figure 7). The long-term risks for cardiovascular mortality associated with various levels of pressure rise progressively over the entire range of blood pressure, with no threshold that clearly identifies potential danger. Therefore, the definition of hypertension is somewhat arbitrary. Perhaps the best operational definition is “the level at which the benefits (minus the risks and costs) of action exceed the risks and costs (minus the benefits) of inaction.”¹

Essential or primary hypertension, a term that is used to indicate hypertension of unknown cause, refers to a lasting increase in blood pressure with heterogeneous genetic and environmental causes. Its prevalence rises with age, irrespective of the type of blood pressure measurement. In developed and developing countries alike, essential hypertension affects 25-35% of the adult population, and up to 60-70% of those beyond the seventh decade of life. Hypertension aggregates with other cardiovascular risk factors, such as abdominal obesity, dyslipidaemia, glucose intolerance, hyperinsulinaemia, and hyperuricaemia, possibly because of a common underlying cause⁵⁰.

Originally, kidney was identified as the source of elevated intravascular pressure, but later studies in the 19th century refuted that interpretation. This left open the possibility of other causes. That possibility was bolstered by laboratory investigations of the 1930s and 1940s that clearly established the multifactorial nature of blood pressure control and, therefore, the likelihood of several origins of hypertension⁵¹.

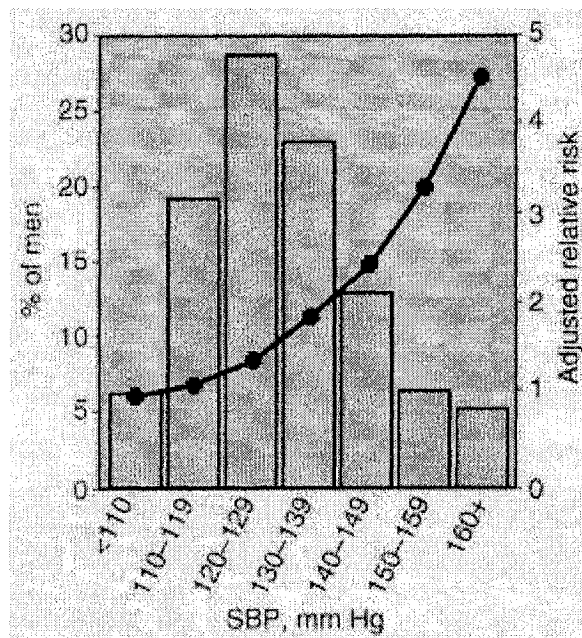


Figure 7: Percent distribution of systolic blood pressure (SBP) for men screened for the Multiple Risk Factor Interventional Trial who were 35 to 57 years old and had no history of myocardial infarction ($n = 347,978$) (bars) and corresponding 12-year rates of cardiovascular mortality by SBP level adjusted for age, race, total serum cholesterol level, cigarettes smoked per day, reported use of medications for diabetes mellitus, and estimated household income (using census tract of residence).

(adapted from National High Blood Pressure Education Program Working Group: National High Blood Pressure Education Program Working Group report on primary prevention of hypertension. *Arch Intern Med* 1993; 153: 186)

A number of so-called secondary hypertension have been recognized. Most of these “secondary hypertension” have been discovered through the study of hypertensive patients with signs or symptoms inappropriate for essential hypertension. Some of the major secondary causes include: pheochromocytoma, Cushing’s syndrome, aortic coarctation, renal arterial disease, and primary aldosteronism⁵¹.

1.2.2. Pathophysiology

Sodium and fluid balance and vasomotor tone are cornerstones in BP regulation. Both mechanisms are affected by numerous genetic and environmental factors, and are controlled by hormonal, nervous system, paracrine, and intracellular feedback loops. The interactions between these factors change with age. These interactions account for the heterogeneous pattern of the hemodynamic alterations that lead to and sustain high blood pressure throughout life.

1.2.3. Genetics of Hypertension

Blood pressure is a quantitative trait with continuous variation from low to high values in outbred populations of humans or animals. As with most quantitative traits, differences in BP result from the contributions of many genes (i.e. BP is a polygenic trait) interacting with each other and the environment.

The vast majority (98-99%) of patients with essential hypertension, in whom the cause is not due to obvious lesions in a single gene, are in a highly complex situation.

The many genetic alterations responsible for high BP would be expected to be subtle, such as upregulated or downregulated expression of an active gene or point mutations that may alter but not abolish the activity of a protein. Because human essential hypertension is a polygenic disorder that is intrinsically genetically heterogeneous, different patients carry different subsets of genes that lead to elevated blood pressure. These genes that affect BP can be described as having alternate variant forms associated with either increased or decreased BP. The genetic component of essential hypertension are thought to be largely additive in nature, such that BP observed in a particular patient is dependent on the interaction of the environment with the balance between the number and the relative strength of the low and high BP alleles present. Understanding of the molecular basis of BP regulation is further complicated by the existence of genes that have no direct effect on BP but that influence BP in a specific environmental context, such as high dietary salt intake⁵².

1.2.4. Experimental Models of Hypertension

Model organisms have been utilized for over a century to understand biological processes. The difficulty in studying a disease process such as hypertension begins with the fact that the etiology of this disease is heterogeneous. Due to the fact that hypertension can be primary or secondary and that the pathophysiology of essential (primary) hypertension is heterogeneous, a spectrum of experimental animal models of hypertension has been developed. These experimental models of hypertension could be placed in the following categories:

1.2.4.1. Renovascular Model

More than 150 years ago, Richard Bright first linked the kidney to high blood pressure, but it was another 100 years before Goldblatt firmly established the potential of the kidney to cause hypertension. Goldblatt et al.⁵³ produced the first reliable form of experimental hypertension with remarkably simple intervention of narrowing the main renal artery to perturb renal hemodynamics. Since then, interest in the kidney in the development of essential hypertension has waxed and waned. Chronic renovascular hypertension has been produced in different large and small experimental animals by constriction of the renal artery such as in non-human primates⁵⁴, dogs^{55;56}, and rodents^{57;58}. The advantage of these models is that one can start the hypertension process at investigator's convenience and that the main pathophysiologic mechanism which causes hypertension is known.

The chief pathophysiologic mechanism underlying renovascular hypertension involves activation of both limbs of the renin-angiotensin-aldosterone (RAA) system. The reduction of renal blood flow due to renal artery stenosis initiates hypersecretion of renin, which accelerates conversion of angiotensin I to angiotensin II and enhances adrenal release of aldosterone. The result is profound angiotensin-mediated vasoconstriction and aldosterone-induced sodium and water retention leading to hypertension⁵⁹.

1.2.4.2. Page Kidney Model

In 1939 Page induced hypertension in dogs by wrapping a kidney with cellophane and described an intense inflammatory reaction to this foreign material producing constrictive perinephritis, compression of the kidney parenchyma, and hypertension⁶⁰.

Page proved that extirpating the affected kidney could cure this high blood pressure. Since then other animals such as rabbits⁶¹ have been used as an experimental model of hypertension induced by renal wrapping. Page's observations were experimental until 1955 when he and a colleague reported a case of a football player who suffered a blunt injury to the kidney producing renal hematoma and renin-mediated hypertension⁶². From then on cases of hypertension secondary to kidney compression are referred to in literature as "Page" kidney.

Any significant external compression of the kidney causes renal hypoperfusion and ischemia which activates the RAA axis ultimately leading to hypertension⁶³⁻⁶⁵. Despite the clinical evidence from Page kidney cases showing the importance of RAA system in development of hypertension, Hart et al. have found no systemic or local activation of the RAA pathway in the experimental canine model of hypertension due to renal wrapping⁶⁶. Therefore, the pathophysiologic mechanism of hypertension due to renal wrapping is rather ambiguous.

1.2.4.3. Undernutrition, Glucocorticoids Models

There is now strong epidemiological evidence that maternal malnutrition and reduced growth in utero is linked with higher incidence of known cardiovascular disease, including hypertension^{67;68}. Therefore, animal experiments have been created in which cardiovascular disease including hypertension have been programmed by maternal malnutrition^{69;70}. In addition, there is growing interest in the role of glucocorticoids as mediators of the effects of intra-uterine deprivation. In one experiment maternal protein restriction during pregnancy in the rat results in high blood pressure in the offspring and this effect is prevented by the inhibition of maternal

corticosteroids biosynthesis during pregnancy⁷¹. In fact, animal models of hypertension exist by treatment of pregnant rats⁷² and ewes⁷³ with the synthetic glucocorticoid dexamethasone which results in induction of hypertension in the offspring.

The mechanisms underlying the programming of hypertension by maternal undernutrition are likely to be multifactorial and complex. However, a role for the kidney and the endothelium in the maintenance of raised blood pressure has been reported. Nephrogenesis has been compromised in kidneys submitted to intrauterine undernutrition leading to a reduction in total nephron number based on clinical and experimental studies⁷⁴⁻⁷⁸. This is likely to contribute to the development of hypertension in adult life. Moreover, a broad spectrum of evidences suggests that intrauterine undernutrition promotes alterations in NO, a well-known vasodilator, pathways⁷⁹⁻⁸¹. Decrease in NO synthesis play an important role in endothelium dysfunction observed in experimental models of undernourished-induced hypertension.

1.2.4.4. Environmentally-Induced Hypertension Models

In recent years, the important role of environmental factors in cardiovascular disorders, particularly hypertension has received great attention from scientists and physicians. To better understand the ways in which the environmental factors affect the cardiovascular functions, researchers have tried to produce experimental hypertension in animals by exposing them to different environmental challenges.

Stress-induced hypertension by exposing rats to two weeks of irregular foot electric shocks combined with buzzing noise⁸², diet-induced hypertension by chronic fructose treatment of rats associated with insulin resistance and hyperinsulinemia⁸³, and cold-induced hypertension including cardiac hypertrophy by chronic exposure of rats to

mild cold (5 °C)⁸⁴ are important examples for models of environmentally-induced hypertension. Activation of sympathetic nervous system and the RAA system are accepted to be at the core of the pathophysiological mechanisms underlying environmental-induced hypertension^{83;85;86}.

1.2.4.5. Pharmacologically-Induced Hypertension Model

The DOCA-salt-induced model of hypertension is a typical representative of pharmacologically-induced hypertension. A very high dose of deoxycorticosterone acetate (DOCA) in the presence of isotonic saline in drinking water as a co-factor is required to induce hypertension in rats. This model often needs surgical reduction of renal mass or unilateral nephrectomy. Therefore, the combination of DOCA-salt and unilateral nephrectomy results in hypertension, cardiac and renal hypertrophy, and nephrosclerosis⁸⁷.

There is evidence for the involvement of arginine vasopressin⁸⁸, sympathetic nervous system⁸⁹, the endothelin system⁹⁰, and oxidative stress⁹¹ in the pathogenesis of DOCA-salt hypertension. The major limitations of this model are: 1) large doses of drug are required; 2) surgical reduction of renal mass is required; and 3) ingestion of large amount of salt is required. This model would be potentially useful to investigate the role of sodium in the developmental stages of hypertension.

1.2.4.6. Genetically-Induced Hypertension Rodent Models

Over the past decade, transgenic and gene-targeting technology has been utilized to study the cardiovascular effects of over-expression or ablation of genes which have been considered candidates in the genetic basis of hypertension. These types of genetic

models usually have relatively good normotensive controls, the same strain of animals without genetic alteration. These are excellent models to study the role of a specific gene in the pathogenesis of hypertension and its consequence, especially with the new advances in the field of molecular biology which allows scientists to target genes in specific tissues. Several experimental rodent models of hypertension exist in which known genes responsible for controlling the blood pressure have been modified. Several examples could be found in the literature such as introduction and overexpression of mouse renin-2 gene in Sprague-Dawley rats⁹².

Other types of genetically-induced hypertension models are produced by selectively breeding rats with high blood pressure. Many hypertensive rat strains have been produced ranging from spontaneously hypertensive rats (SHRs) to spontaneously hypertensive heart failure (SHHF) rats which are the strains used in the experiments presented in this thesis. These models exhibit end-organ damage phenotypes similar to those seen in human essential hypertension, including LVH, stroke and renal failure.

The SHR was introduced by Okamoto and Aoki in 1963⁹³ as a model of genetic hypertension which is thought to be similar in many respects to hypertension in man⁹⁴. The pathogenesis of hypertension in the SHR appears to be heterogeneous; cellular, central nervous system, neurohormonal, and renal abnormalities have been proposed. The SHR is a “normal-renin” model, and its blood pressure is relatively sodium-independent.

The SHHF rat represents a congenital model of dilated cardiomyopathy with hypertension progressing to decompensated heart failure which exhibits several hallmark signs of human disease state. While progression to decompensated heart failure occurs in

100% of these rats at old age, such transformation does not necessarily occur in all SHRs^{95;96}.

1.2.5. SHR, A Model for Essential Hypertension

Human genetic (essential) hypertension is recognized as a multifactorial disease involving many genes, but the causative genes have not yet been fully identified. Therefore, it seems a logical approach to study the pathophysiological alterations of essential hypertension in a laboratory animal with a short life span in which hypertension is produced naturally through genetic inbreeding that shares similarities with the human disease. The SHR is such an experimental model in which the disease is very similar to essential hypertension in man⁹⁴. Both have their apparent onsets early in life. Their elevated arterial pressure is mediated through a slow and progressively increased total peripheral resistance which demands cardiac and vascular adaptation. Eventually, cardiac failure, strokes, and renal lesions result in a shortened life span by about one-third in both forms of hypertension. In both genetic diseases, neural mechanisms seem to predominate in the early stages, whereas in the later and more complicated phases structural, renal, endocrine, humoral, and metabolic mechanisms also may participate. In both forms of hypertension there seems to be susceptibility for aggravation of the disease by excess dietary sodium, stress, and other environmental factors. Therefore, it is justifiable to use SHRs in this study as a model for essential hypertension in man. However, a major drawback of this model is the lack of an appropriate control which has been genetically altered yet is free from the disease and the complexity of the genetic mutations which have affected not only blood pressure but

many other regulatory systems as well. Regardless of this issue, Wistar rats are commonly accepted as the best normotensive control for SHR.

1.3. Relationship between Atrial Fibrillation and Hypertension

In a review done by Healey et al in 2003⁹⁷, hypertension is associated with cardiac structural changes that are linked with atrial fibrillation. These changes include left ventricular hypertrophy (LVH), left atrial enlargement (LAE), changes in left atrial mechanical function, altered left atrial electrophysiology, and increased atrial ectopic activity. Moreover, in a study done by Verdecchia et al. in 2003¹⁰⁰, on untreated hypertensive patients in sinus rhythm and free of overt cardiovascular disease or hyperthyroidism, age and LV mass were determined as the sole independent predictors of AF.

1.3.1. Left Ventricular Hypertrophy

One of the most important effects of hypertension on the heart that is very well studied is hypertrophy of the left ventricle. LVH by itself, detected by electrocardiography or echocardiography, has been identified as an important risk factor for AF¹¹. In the Framingham study, patients with an electrocardiographically determined diagnosis of LVH had a 3 to 3.8 fold increased risk of developing AF. The risk of developing AF also increased by 28% for each 4-mm increase in echocardiographically measured left ventricular thickness¹². Moreover, recently Verdecchia et al.¹⁰⁰ have substantiated the role of LVH as an independent predictor of AF in apparently uncomplicated subjects with essential hypertension.

1.3.2. Left Atrial Enlargement

Support for LAE as a causal risk factor for AF comes from cohort studies that use enlargement of the left atrium as a prospective predictor of the occurrence of AF and show that the risk of atrial fibrillation is correlated with the severity of increase in left atrial diameter. For example, in the Framingham study, among 4,731 patients who underwent baseline echocardiography, the risk of developing AF increased by 39% for each 5 mm increment increase in left atrial diameter¹². In other prospective studies, it was confirmed that LAE predicts the development of AF¹⁰³. Moreover, newer, more precise measurements of left atrial size by monitoring the left atrial volume echocardiographically have been used recently by Tsang et al.¹⁰⁴. In this cohort, a 30% increase in left atrial volume was correlated with a 43% increased risk of AF, and left atrial volume was a more sensitive marker for AF risk than conventional left atrial diameter.

LAE may develop early in patients with hypertension, before any evidence of LVH or atrial arrhythmia¹⁰⁵. Most studies have reported that the magnitude of atrial enlargement correlates with the degree of hypertension^{106;107}. LAE in hypertensive patients may be the result of elevated left ventricular filling pressure¹⁰⁸ and impairment of left ventricular diastolic function^{109;110}. LAE is an important step in the progression from hypertension to AF. Cross-sectional studies have demonstrated that hypertensive patients with a history of paroxysmal AF have larger left atria than do patients without history of paroxysmal AF¹¹¹. Also, in the study by Verdecchia et al.¹⁰⁰ left atrial size was determined as an independent predictor of chronicization of AF in untreated hypertensive patients.

1.3.3. Left Atrial Mechanical Function

In hypertensive patients who go on to develop AF, methods of assessing left atrial function, such as measuring left atrial fractional shortening, have documented impaired left atrial function compared with hypertensive patients who remain in sinus rhythm¹¹¹. Impaired contractile function of the left atrium has also been shown to predict the development of AF in patients with heart failure¹¹². Moreover, Doppler echocardiographic studies have confirmed the importance of left atrial function as a measure for susceptibility to AF¹⁰⁹. However, in the study which was done by Verdecchia et al., Doppler measurements (E- and A-wave velocities on the transmitral inflow pattern, as well as their ratio) did not predict occurrence of AF¹⁰⁰.

1.3.4. Changes in Atrial Electrophysiology

Changes in atrial electrophysiology has been noted to occur early in hypertensive heart disease, preceding the appearance of LVH and LAE¹¹³. Two distinct changes in atrial electrophysiology, both of which are associated with development and maintenance of AF, have been studied. These two changes are the prolongation of atrial conduction velocity, and the decrease in atrial refractoriness¹¹⁴⁻¹¹⁶. Slowing of atrial conduction velocity was assessed by measuring the p-wave duration on the electrocardiograms. However, the only evidence for increased dispersion of atrial refractoriness and shortened ERP were obtained from animal studies in which the left atrial pressure of the animals were elevated by balloon dilation or in the case of isolated rabbit hearts the atrial pressure was increased by 15 cm H₂O^{117;118}. Therefore, it is reasonable to postulate that changes in atrial electrophysiology could be a mechanism by which hypertension predisposes individuals to AF.

1.3.5. Atrial Ectopic Activity

Atrial fibrillation is now known to be frequently initiated by atrial premature beats arising from within the pulmonary veins³⁴. Hypertension is associated with an increased frequency of premature atrial contractions. However, the site of origin for these contractions is not determined. In a study of 85 patients with essential hypertension, patients with LVH had an increase in the number of atrial premature beats compared with hypertensive patients without LVH, while both groups had more atrial premature beats than an age-matched control group¹¹⁹.

1.4. Hypertension as a Risk Factor for AF

Currently, hypertension is the most prevalent, independent, and potentially modifiable risk factor for atrial fibrillation^{11;12}. The relative risk of developing AF in patients with hypertension is modest (relative risk, 1.4 to 2.1) compared with other conditions such as heart failure (relative risk, 6.1 to 17.5) and valve disease (relative risk, 2.2 to 8.3)⁹⁷. However, because as mentioned before, almost 25-35% of the adult population and up to 60-70% of those beyond the seventh decade of life has high blood pressure, hypertension is the primary risk factor for atrial fibrillation in North America.

Cohort studies suggested that hypertension was present in 50% to 53% of North American patients with AF^{98;99} and was causative in 15%¹². The incidence of AF among hypertensive patients is approximately 94 of 1,000 patient-years. In a cohort of hypertensive patients, those who subsequently developed AF had a higher mean ambulatory systolic pressure¹⁰⁰.

2. Goals of the Study

The importance of hypertension as a risk factor for the development of atrial fibrillation is well recognized. However, despite its leading importance as a highly prevalent risk factor, only a few clinical data are available regarding predictors and outcome of AF in large populations of subjects with essential hypertension free of other coexisting and predisposing cardiovascular conditions. So that, the role of essential hypertension as a risk factor for AF is still incompletely known.

Recent data indicates that in hypertensive patients with sinus rhythm and no other major predisposing conditions, risk of AF increases with age, LV mass, and LA size (which predisposes to chronicization of AF)¹⁰⁰. However, it is important to note that LV mass and LA size increase during the process of aging independently of presence of hypertension and could increase the risk of AF^{101;102}. Therefore, the role of hypertension per se in providing a substrate for AF needs to be elucidated further.

This present study was undertaken to understand the conditions that will provide a substrate for AF in patients with essential hypertension. Understanding these underlying conditions will be useful to design new treatments. The study was undertaken in rats as low-cost experimental animals with short life span. Using an animal model allowed us to study the effects of hypertension alone without the presence of other coexisting cardiovascular diseases and without the confounding factor of drug therapies that are seen in patients. Moreover, the rather short life span of rats allowed us to study the effects of hypertension from the beginning of its course. SHRs have been used in this study as a model of genetic hypertension in man for the reasons explained before.

***Relationship between Atrial Fibrillation and Hypertension in
Rats***

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Introduction:

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia encountered by clinicians and is responsible for substantial morbidity and mortality in general population¹. The most important risk factors for AF are age, male gender, hypertension, thyrotoxicosis, smoking, diabetes, left ventricular (LV) hypertrophy, left atrial (LA) enlargement, valvular and coronary heart disease, congestive heart failure, and stroke².

Because of its high prevalence in the population (53% according to the Manitoba Follow-up study³), hypertension independently accounts for more AF cases than any other risk factors⁴. However, despite its leading importance as a highly prevalent risk factor, only a few clinical data are available regarding predictors and outcome of AF in large populations of subjects with essential hypertension free of other coexisting and predisposing cardiovascular conditions. So that, the role of hypertension as a risk factor for AF is still incompletely known.

Recent data indicates that in hypertensive patients with sinus rhythm and no other major predisposing conditions, risk of AF increases with age, LV mass, and LA size (which predisposes to chronicization of AF)⁴. However, it is important to note that LV mass and LA size increase during the process of aging independently of presence of hypertension and could increase the risk of AF⁵⁻⁷. Therefore, the role of hypertension per se in providing a substrate for AF needs to be elucidated further.

The present study was undertaken to investigate the possibility of having rats as an adequate animal model for the relationship between hypertension and AF in man and to understand the role of hypertension as a risk factor for AF.

Methods:

Animal Groups and Experimental Protocols

The research protocol was approved by the Institutional Animal Care and Use committee of Montreal Heart Institute. Rats (obtained from Charles River laboratories) were studied at three different age groups of young (6 months), middle-aged (13 months), and old (19 months). In all the age groups, studies were performed on Wistar rats (control), and spontaneously hypertensive rats (SHRs). In the old age group, studies were also performed on spontaneously hypertensive heart failure (SHHF) rats. Moreover, 10 three-month old Wistar rats were used for the cholinergic stimulation and the thoracic aortic constriction experiments. In the experiments requiring a thoracotomy, animals were anesthetized with 3% isoflurane. Endotracheal intubation was performed as described before⁸. The cannula was connected to a volume-cycled rodent ventilator with a tidal volume of 2.5 ml (oxygen supplemented) and respiratory rate of 75 breaths/min. Body temperature was kept at 37°C throughout the experiments with a heating pad.

Hemodynamic and Echocardiography Measurements

Noninvasive blood pressure (BP) was measured by the tail-cuff plethysmography method in unanesthetized rats prewarmed for 10 minutes. For the echocardiography measurements, animals were sedated with midazolam (2 mg/Kg, IP) and lightly anesthetized with 1% isoflurane. Two-dimensional echocardiography in the rat was performed using an echocardiogram (HP 5500) equipped with a 5-7.5 MHz transducer. All measurements were done from leading edge to leading edge according to the American Society of Echocardiography guidelines.

In-Vivo Electrophysiology Study

A Lead I electrocardiogram was recorded from the skin. Right atrium (RA) was exposed by a right thoracotomy between the third and the fourth rib. Two Teflon-coated (except at their tips) bipolar electrodes were hooked on the RA. The effective refractory period (ERP) of the RA was measured by the S₂ extrastimulus method using 10 regularly paced S₁ beats at three different cycle lengths (CL), 100, 120, and 150-msec with twice threshold current. Burst RA pacing (CLs of 15 to 5-msec above ERP, in 10-msec increments) for various durations with 10 times threshold current was used to induce AF. The induced arrhythmia was considered to be AF when the atrial bipolar electrogram morphology was nonuniform, the activation intervals were irregular, and had a cycle length < 35 msec. AF was said to be sustained if it lasted for > 30 seconds.

Cholinergic Stimulation

Experimental protocol was followed as explained before. Baseline (before injection) electrophysiology study was performed. Carbamylcholine chloride (carbachol) was injected (2 mg/Kg, IP). Five minutes post-injection the electrophysiology study was repeated.

Thoracic Aortic Constriction

The chest cavity was entered in the second intercostals space at the left upper sternal border through a small incision. The thymus was then gently deflected out of the field of view to expose the aortic arch. After the aorta was isolated distal to the carotid arteries, it was constricted by a 2-0 silk suture ligature tied firmly against a 25-gauge needle. The needle was promptly removed after ligature. The change in blood pressure proximal to the point of constriction was verified with a fluid-filled catheter inserted into the right carotid artery and connected to a pressure transducer. Electrophysiology studies

were carried out at before, and 10, 30, 60 minutes after aortic constriction. It should be noted that atropine (0.05 mg/Kg, IM) was injected every 25 minutes starting before the first electrophysiology study.

Histological Analysis

The hearts of the rats were weighed after conclusion of the *in-vivo* studies. The hearts were then fixed in 4% buffered formalin for >24 hours and then placed in 70% alcohol. Trimming was done in all the paraffin-embedded tissue blocks for 400 μ m. Five sections were made in each RA and LA at every 200 μ m of intervals. On each level, transmural sections of 5 μ m were cut perpendicularly to the epicardium and mounted onto slides. The sections were stained with either trichrome Masson or HPS. The slides were examined by light microscopy at 400X magnification. The ratio of the area occupied by interstitial fibrosis to the total area was determined in each trichrome-stained sample using a computer-assisted image analysis software (SigmaScan). Results were given as percentage fibrosis of total atrial surface area.

Statistical Analysis

All statistical analyses were performed using Systat. Data are expressed as mean $\pm$ SEM. When appropriate, statistical analyses were performed using multiple-way analysis of variance (ANOVA), multiple-way repeated measures of analysis of variance, Chi-square test.

Results:

Cholinergic Stimulation and Thoracic Aortic Constriction

Cholinergic stimulation experiment was performed on five rats in order to investigate the feasibility of inducing sustained AF in rats. The mean ERP at cycle length of 120 msec before injection of carbachol was 37 ± 2.6 msec. After injection of carbachol, ERP decreased to values below 20 msec. Sustained atrial fibrillation was initiated in all the animals while trying to determine the ERP. AF would last for durations longer than 20 minutes. Attempts to stop the fibrillation by burst pacing were not successful.

Thoracic aortic constriction experiment was performed on five rats in order to investigate the effect(s) of an acute increase in atrial pressure and the resulting atrial stretch on development of AF. Atropine was injected in three animals before constriction of the aorta to block the parasympathetic effect that might be activated as a result of aortic constriction. The electrophysiology study was performed on the other two rats without the injection of Atropine. Sustained AF was not induced in any of the rats at any time. ERP increased in all the animals from this group after constriction of the aorta. Figure 1 show the change in ERP of the right atrium from the three rats with atropine injection at different times following constriction as well as the change in blood pressure measured from the right carotid artery after constriction of the aorta. The change in blood pressure is comparable to the difference in blood pressure between normal Wistar rats and SHRs.

Hemodynamic and Echocardiography Measurements

The hemodynamic and echocardiography measurements are summarized in table 1. As expected blood pressure is significantly higher in the SHRs compared to their age-

matched controls. Also, the blood pressure of SHHF rats is comparable to that of SHR. The echocardiography measurements show that EF% and PWSV are lower in the SHR compared to their age-matched controls. Although at old age EF% seems to be lower in the SHR compared to controls, a statistical significance is not present. Left ventricular hypertrophy exists in the middle-aged and old SHR when their LV mass is compared to that of age matched control rats. Left ventricular mass increases significantly in both Wistar and SHR as they age up to 13 months, but it remains unchanged to the old age. Left atrial dimension (LAD) is lower in the young and middle-aged SHR compared to the Wistar rats. But the difference between the LADs of the two groups is disappeared at old age. However, LAD increases significantly in both strains of rats as a result of aging up to middle age.

Electrophysiology Study

ERP was longer in the SHR only at young age in all the basic cycle lengths when compared to young Wistar rats (table 2). At the other age groups this difference was not present. When comparison was made at different ages within the same strain, no difference was found in the ERP except at the basic cycle lengths of 100 msec for the Wistar and 120 msec for the SHR ($P < 0.05$). Figure 2 represents a sample electrocardiogram from Lead I and the associated electrogram from RA while it is fibrillating. There is no difference in the AF cycle length and duration among all the groups. The percentage of rats with sustained AF in every group is shown in table 2. It should be noted that sustained AF was induced in 100 % of the controls, SHR, and SHHF rats at old age.

Histological Analysis

Figure 3 shows representative atrial sections with and without increase amount of interstitial fibrosis and figure 4 shows the percentage amount of interstitial fibrosis in both atria of each group. In the left atrium of the SHR, interstitial fibrosis is significantly more than that of the controls in middle age and old rats. Moreover, while, the amount of interstitial fibrosis is increased only in the left atrium of the middle-aged SHR when compared to young SHR, fibrosis is increased in both atria in controls and SHR as they grow from 13 months to old age. The amount of interstitial fibrosis in the left atrium of the SHHF rats is comparable to that of old SHR (Figure 4).

Discussion:

Principle Findings

In the present study, we have found no difference in AF sustenance between the normal and hypertensive rats at different age groups. However, despite lack of statistical significance, it is important to note the increase in the percentage of rats with induced sustained AF as the animals from each group age. In Wistar rats, sustained AF is induced in 100% of the old age group while it was only induced in 33% and 29% of middle-aged and young rats. Increased amount of interstitial fibrosis was only noted at old age. In SHR, sustained AF was induced in only 17% of young animals, but increased to 44% and 100% as the interstitial fibrosis accumulated over time. In the SHHF rats, AF was induced in all the animals in the presence of heart failure at old age while severe LV hypertrophy, LA enlargement, and LA interstitial fibrosis was noted in this group.

Moreover, hypertension could accelerate the accumulation of fibrosis which occurs during the process of aging. While fibrosis was increased significantly in the SHR_s at middle age compared to young SHR_s, in Wistar rats such an increase was only observed at old age. Furthermore, the amount of interstitial fibrosis at old age was higher in SHR_s compared to Wistar_s.

Possible Mechanism(s) of AF in this Model

Although epidemiologically linked to an increased incidence of AF, the mechanisms by which hypertension enhances the frequency of AF in man remain incompletely elucidated. In general, AF may result from more than one mechanism, automatic triggers, after depolarizations, and reentrant substrates. AF might have various initiators (autonomic, hormonal, stimulants due to pathological conditions) or it might occur as an isolated disorder-a condition known as 'lone AF'^{9;10}.

Arrhythmogenic remodeling, that promote the occurrence of cardiac arrhythmias could be in the form of alterations in atrial electrophysiology or structure that provide a reentrant substrate or could lead to automatic trigger activities. The multiple wavelet theory has been widely accepted as an explanation for the mechanism of AF sustenance¹¹. According to this theory, fibrillation requires several reentrant wavelets to coexist.

In the electrophysiology studies that we performed in these experiments, ERP does not seem to have a role in arrhythmogenic remodeling. It is conceivable that hypertension, aging, and heart failure cause changes in cellular electrophysiology of atrial myocytes. We do not have enough evidence to be able to exclude the presence of alterations in cellular electrophysiology, which could contribute to AF vulnerability. However, the lack of pro-arrhythmic alteration in ERP should be noted. Moreover, an

acute increase in the afterload and the immediate effect of elevated LV pressure on atrial pressure and stretch did not lead to pro-arrhythmic changes in the ERP and development of AF as evident from the thoracic aortic constriction experiments.

Structural Remodeling and AF

Theoretical models have associated atrial interstitial fibrosis as a substrate for AF^{12;13}. Increased AF vulnerability has been associated with increased atrial interstitial fibrosis in animal models with induced heart failure¹⁴, mitral regurgitation¹⁵, and aging^{16;17}. Also in humans, it has been observed that atrial fibrosis increases with age in patients with AF^{18;19}. Moreover, it has been shown recently that the alterations in atrial conduction produced by atrial interstitial fibrosis alone are sufficient to produce a substrate for AF²⁰.

Studies have shown that LA enlargement is a risk factor for AF and stroke, especially in men^{21;22}. Also, it has been shown that echocardiographic LA enlargement is commonly found in hypertensive patients with LV hypertrophy, and that in such patients, LA enlargement is particularly prevalent in older and more obese patients independent of the degree of LV hypertrophy²³.

In this rat model, structural remodeling especially increased amount of interstitial fibrosis seems to be the major contributor to the AF sustainability. Although the role of LA enlargement is not clear in the Wistar rats and SHRs, its enlargement by approximately 40% in the SHHFs at old age compared to old controls could contribute to the fact that all the animals from this group developed sustained AF.

Furthermore, although, heart failure was present in the SHHF rats, a new study from our lab suggests that structural, not ionic, remodeling is the primary contributor to AF maintenance in experimental heart failure²⁴.

Clinical Implication

In this model, aging and hypertension predispose the heart to AF mainly due to the same mechanism (increased amount of interstitial fibrosis). The signalling pathways that will lead to increase amount of interstitial fibrosis in each condition should be investigated further.

Due to the limited efficacy and the nontrivial adverse effects of the presently available drug therapies for AF, the prevention of arrhythmogenic remodeling is emerging as a potential new treatment strategy^{25;26}. Since the structural remodeling is a process that occurs over time, drug therapies that start at young age and prevent these changes might be found useful.

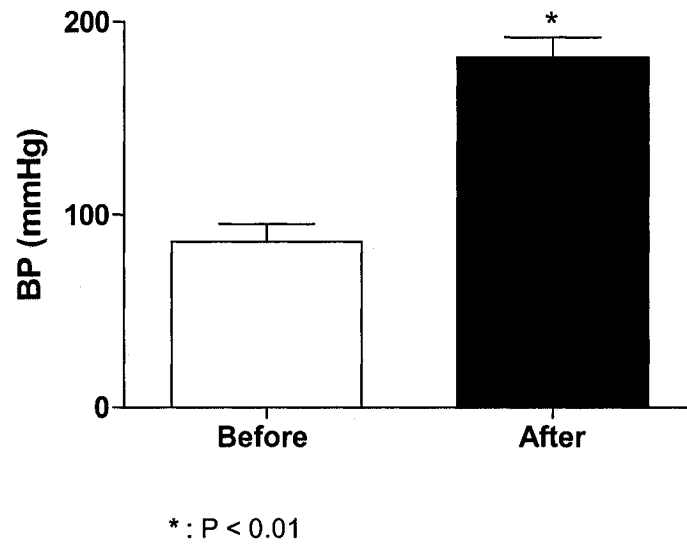
Limitation of the Study

It is important to note that it was feasible for us to only use three animals per group at 19 months due to the unavailability of the strains at older ages. Also, the results observed in this rat model may not mimic the events seen in human. Therefore, one should be careful when extrapolating the results of this study to clinical settings.

In general, our inability to find a clear relationship between hypertension and AF in this rat model was disappointing. Further work in other species and other models of hypertension would be interesting in order to define an adequate animal model for the relationship between hypertension and AF in man. This would allow for a study of the relevant basic mechanisms and potentially for the development of improved preventive approaches.

Figure 1:

A.



B.

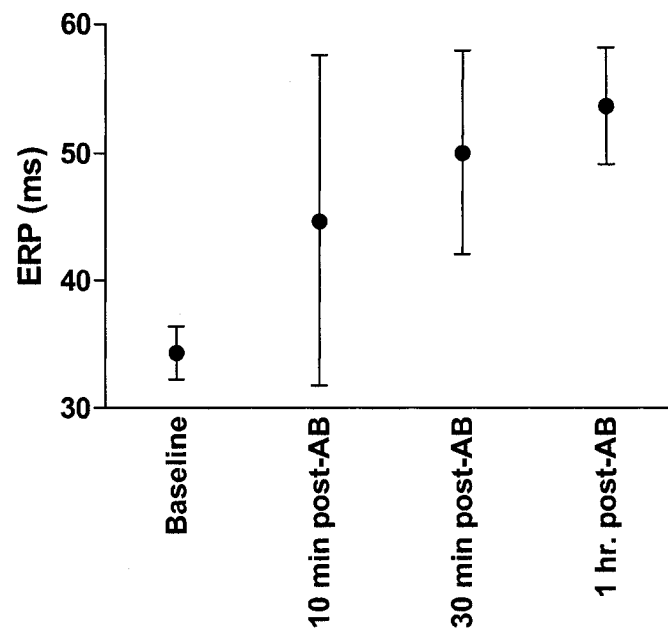


Table 1
Hemodynamic and Echocardiography Measurements

	Young Wistar (n = 7)	Young SHR (n = 6)	Middle- aged Wistar (n = 9)	Middle- aged SHR (n = 9)	Old Wistar (n = 3)	Old SHR (n = 3)	SHHF (n = 3)
BP (mmHg)	130 ± 6.9	192 ± 2.2*	132 ± 3.3	189 ± 6.4*	133 ± 4.1	183 ± 3.9*	185 ± 8.5§§
EF (%)	58 ± 0.8	52 ± 2.2*	57 ± 0.8	49 ± 2.1*	53 ± 1.8	45 ± 3.3	31 ± 2.0§
LV Mass (g)	1.4 ± 0.03	1.3 ± 0.04	1.6 ± 0.04†	1.8 ± 0.06†*	1.6 ± 0.1	1.9 ± 0.1**	2.5 ± 0.1§
PWSV (cm/s)	3.95 ± 0.2	2.92 ± 0.2*	3.4 ± 0.1	2.77 ± 0.2*	3.4 ± 0.3	2.2 ± 0.2**	1.39 ± 0.3§
LAD (mm)	4.8 ± 0.2	3.7 ± 0.2*	5.5 ± 0.1†	4.7 ± 0.3†*	5.6 ± 0.1	5.6 ± 0.9	9.1 ± 0.7§§
AFS (%)	33 ± 0.9	34 ± 1.1	29 ± 1.3	32 ± 2.2	28 ± 0.7	25 ± 6.2	11 ± 0.5§

Values are given as mean ± SEM.

* : P < 0.01, **: P < 0.05 (Wistar Vs. Same age SHR); † : P < 0.01 (age effect)

§ : P < 0.01, §§ : P < 0.05 (Old Wistar Vs. SHHF)

BP: blood pressure; EF: ejection fraction; LV: left ventricular; PWSV: posterior wall shortening velocity; LAD: left atrial dimension; AFS: atrial fractional shortening.

Figure 2:

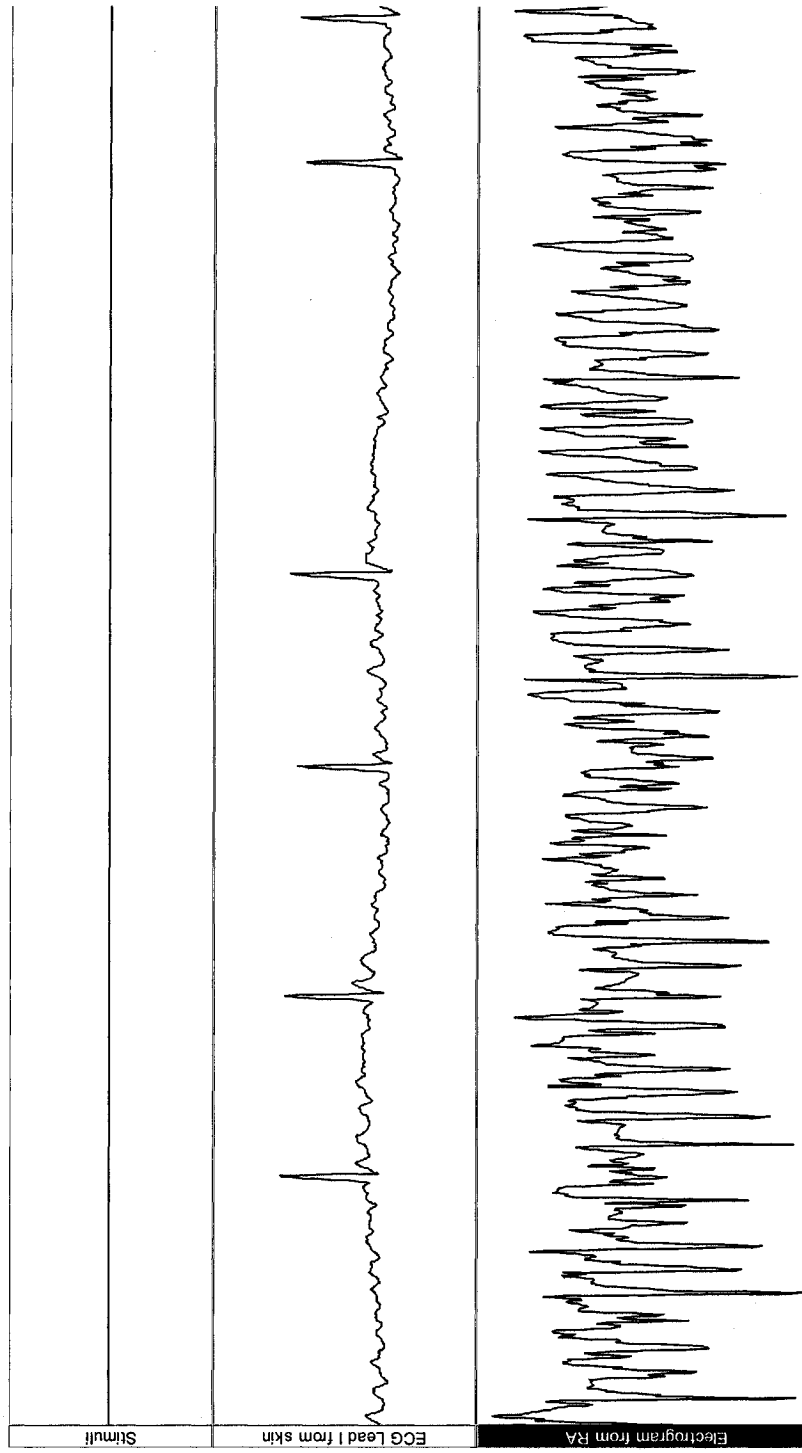


Table 2
In-Vivo Electrophysiology Study

	Young Wistar (n = 7)	Young SHR (n = 6)	Middle- aged Wistar (n = 9)	Middle- aged SHR (n = 9)	Old Wistar (n = 3)	Old SHR (n = 3)	SHHF (n = 3)
ERP₁₀₀	26 ± 1.9	43 ± 3.4*	31 ± 1.3†	34 ± 2.6	30 ± 2.3	28 ± 1.2	37 ± 6.0
ERP₁₂₀	27 ± 2.2	45 ± 3.5*	32 ± 1.6	35 ± 2.5†	30 ± 2.9	29 ± 2	36 ± 6.1
ERP₁₅₀	27 ± 2.4	45 ± 3.4*	31 ± 1.4	36 ± 2.5	29 ± 2.6	31 ± 2.3	34 ± 7.6
AF cycle length (ms)	32 ± 2.7	30 ± 2.9	28 ± 1.8	31 ± 1.7	26 ± 2.7	30 ± 0.5	27 ± 3.9
AF duration (S)	7.0 ± 7.4	6.1 ± 3.2	14.7 ± 6.7	15.5 ± 6.1	195 ± 167.8	21.3 ± 3.6	29.2 ± 4.9
Sustained AF (%)	29	17	33	44	100	100	100

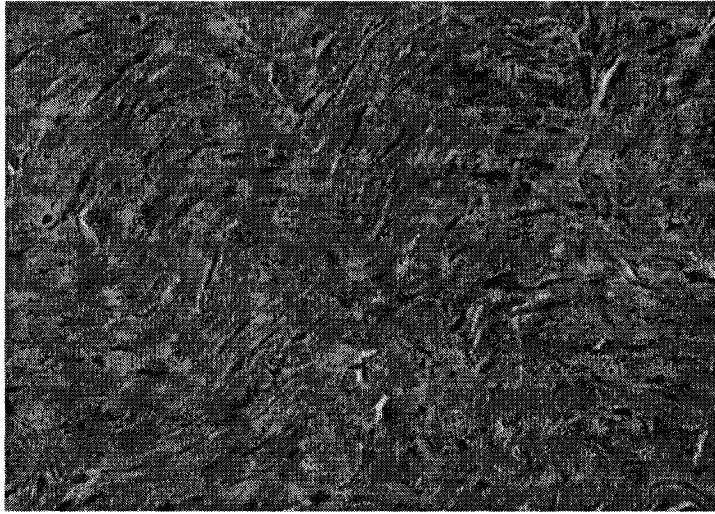
Values are given as mean ± SEM.

* : P < 0.01 (condition effect, Wistar vs. SHR); † : P < 0.05 (age effect)

ERP: effective refractory period measured at three different cycle length of 100, 120, and 150 ms; AF cycle length: mean cycle length of 10 atrial signals during atrial fibrillation; AF duration: Duration of atrial fibrillation; Sustained AF: Animals with episodes of sustained AF.

Figure 3:

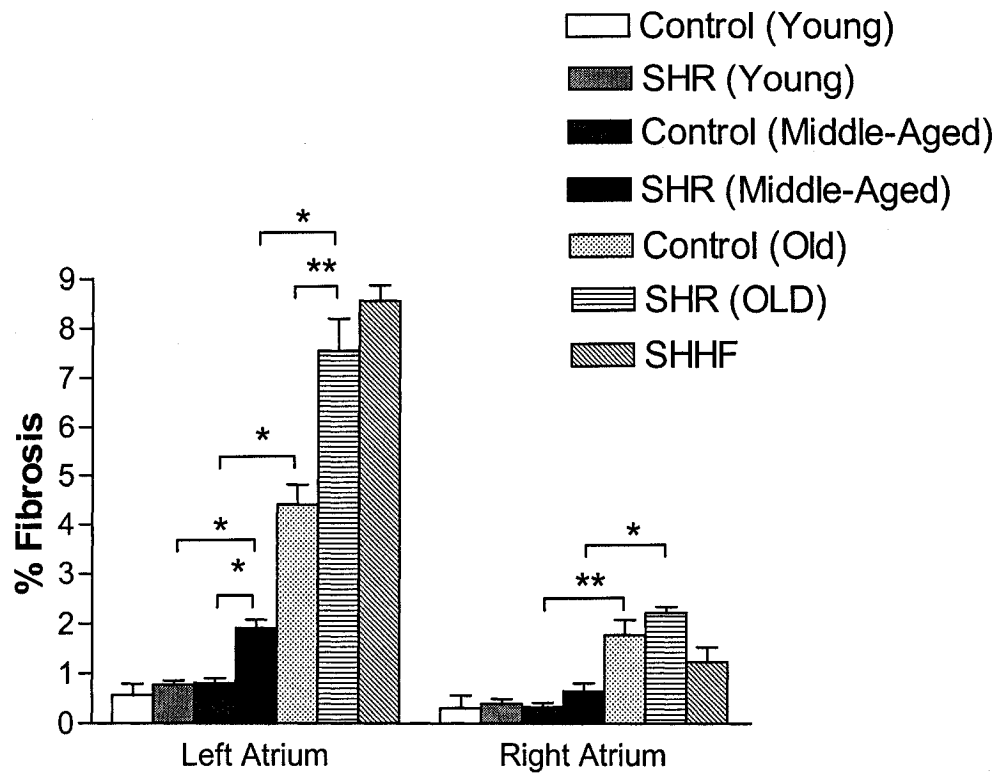
A.



B.



Figure 4:



*: $P < 0.01$, **: $P < 0.05$

Figure Legends:

Figure 1. (A) Systolic blood pressure before and after constriction of the thoracic aorta measured in three rats with atropine injection. Change in blood pressure is comparable to the difference in blood pressure between normal Wistar rats and SHRs. **(B)** ERP measured before, 10, 30, and 60 minutes after constriction of aorta in three rats with atropine injection at basic cycle length of 120 msec. BP: blood pressure; ERP: effective refractory period; AB: aortic banding.

Figure 2. This figure shows a representative example of an episode of atrial fibrillation after burst pacing. The top trace shows the atrial stimulation signal. The middle trace is the Lead I ECG from skin where the irregularity of the ventricular rhythm with no clear P waves could be seen, and the bottom trace is the electrogram from the right atrium recorded during an episode of atrial fibrillation with a mean cycle length of 29ms.

Figure 3. Representative atrial tissue sections with no interstitial fibrosis in a young rat **(A)** and with increased interstitial fibrosis in an old rat **(B)**. The sections are magnified at $\times 400$ and are stained with trichrome Masson. The atrial myocytes and the fibrotic tissue are stained red and green respectively.

Figure 4. Mean $\pm$ SEM of % interstitial fibrosis in the atria of different groups. SHR: Spontaneously hypertensive rat; SHHF: Spontaneously hypertensive heart failure.

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4. Discussion

4.1. General Findings of this Study

In the present study, we have found no difference in AF sustenance between the normal and hypertensive rats at different age groups. At young age, AF was induced in only 17% of hypertensive rats in the absence of LVH, LAE, and myocardial damage (accumulation interstitial fibrosis) compared to 29% of normal rats. Moreover, at young age, the result of the electrophysiology study shows that ERP was higher in the SHR rats compared to control rats. This difference in ERP disappears between the two groups at older ages. Higher ERP at young age in the SHR rats compared with their controls is not considered a “pro-arrhythmic” phenomenon. Therefore, hypertension by itself in the absence of structural damage to the myocardium did not provide a substrate for maintenance of AF.

At middle-age, LVH was present in the hypertensive rats compared to their age-matched controls. At this age, no significant LAE was observed except the enlargement that occurred in the left atrium of both SHR rats and their controls as a result of aging. The left atrial diameter was smaller in the SHR rats compared to the controls up to this age. An important change that was observed at this age was the increase in the amount of interstitial fibrosis in the SHR rats compared to young hypertensive rats, while such an increase was absent in the control rats. Sustained AF was induced in 44% of SHR rats compared to 33% of controls. Therefore, a mere hypertrophy of the left ventricle and a small change in the amount of interstitial fibrosis was not sufficed to cause a statistical difference between the AF sustainability of the two groups.

Sustained AF was observed in all the rats at old age regardless of their blood pressure. At old age, significant LVH was present in the hypertensive rats compared to controls. While there was no statistical difference between the left atrial sizes of the two groups of rats, interstitial fibrosis was significantly increased in both groups compared to their younger counterparts. However, such an increase was greater in the SHR. The fact that sustained AF was induced in all the normotensive and hypertensive animals could lead to the conclusion that myocardial damage in the form of accumulation of interstitial fibrosis is the determining factor in providing a substrate for maintenance of AF in this model. On the other hand statistical difference was not obtained between the percentages of rats with sustained AF at old age compared to that in younger ages. However, it should be noted that only 3 old animals were used per group due to the unavailability of the strains of rats used in these experiments in ages above three months old. In fact both aging and increased amount of interstitial fibrosis have been related to AF in the literature before.

4.1.1. Relationship between Atrial Fibrillation and Aging

Aging has been proposed to be one of the most important predictors of AF in several studies^{13;98}. It has been associated with atrial dilation¹⁰² and increased nonuniform atrial interstitial fibrosis in a rat model of aging¹²⁰. Moreover, in a study performed on dogs aging is also linked with a change in distribution of gap junctions in a way that they are polarized to the cell termini¹²¹. In terms of electrophysiological changes associated with aging that would promote AF, various data exist in the literature.

With regards to the effects of aging on atrial conduction velocity, prior animal studies of aging-related changes in electrophysiology have demonstrated development of conduction slowing, frequently associated with the presence of interstitial fibrosis. In a study of the cellular electrophysiology of old canine atria, Anyukhovsky et al.¹²² showed a reduction in the conduction velocity with an increased window of conduction slowing in response to premature stimuli but not during normal beats. Similarly, Hayashi et al.¹²⁰ observed that interatrial conduction time was prolonged in aged rat hearts. Moreover, in a recent clinical study, Kistler et al.¹²³ have provided evidence of generalized conduction slowing in the aged human atria.

Several prior studies have evaluated the specific effects of increased age on atrial refractoriness. There have been conflicting results with this regard, some studies showing an increase^{124;125}, while others have found no prolongation in atrial effective refractory period with aging¹²⁶. Evidence for lack of change in action potential duration and therefore the effective refractory period is also present in animal studies performed on rats^{127;128}. However, Kistler and his colleagues have indicated an increase in atrial ERP with aging in human in a recent study¹²³.

4.1.2. Fibrosis and AF

Atrial fibrosis is a common pathological finding in the atria of patients with AF and is increased with age in humans. Theoretical models have implicated atrial interstitial fibrosis as a substrate for AF^{133;134}. Furthermore, atrial fibrosis as a substrate for AF sustenance has been observed in animal models of aging¹²⁰, mitral regurgitation¹³⁵, and CHF¹³⁰ as mentioned before.

Increased interstitial fibrosis makes the atria a structurally heterogeneous substrate. Increased heterogeneous interstitial fibrosis and the resulting non-uniform atrial cellular uncoupling provide a suitable substrate for activation wavefront breakups leading to increased incidence of pacing-induced AF. Heterogeneity is a major mechanism of wavebreak that converts single re-entrant wavefront to fibrillation (multiple wavefronts)¹³⁶. Atrial fibrosis and its concomitant decrease in side-to-side electrical coupling in the myocytes, can cause a shift from uniform anisotropy to non-uniform anisotropy in atrial conduction¹³⁴. In non-uniformly anisotropic tissue, slow and heterogeneous conduction may occur during transverse propagation in the absence of variations in intrinsic membrane properties, making it possible for re-entry to occur in relatively small circuits leading to multiple wavelets and the associated AF in the atrial tissue.

In general, atrial fibrosis in itself can be sufficient to form a substrate for AF as confirmed by a recent animal model¹³⁷. This indicates that atrial fibrosis would be a significant predictor for AF vulnerability in patients with pronounced atrial interstitial fibrosis, even in the absence of other pro-arrhythmic factors and it might play a pivotal role in association of hypertension and increased risk of AF.

4.1.3. Heart Failure, Hypertension and AF in this Model

In a separate study that was performed on SHHF rats, AF was induced in all the animals in the presence of heart failure at old age while severe LV hypertrophy, LA enlargement, and LA interstitial fibrosis was noted in this group. Interestingly, in a study by Kannel et al.⁹⁸ hypertension unaccompanied by cardiac enlargement on x-ray films, electrocardiographic evidence of LVH, or cardiac failure was only weakly related to the

occurrence of atrial fibrillation, suggesting that myocardial damage was a prerequisite as observed in our study. Congestive heart failure by itself is one of the main risk factors for AF in the literature.

4.1.4. Relationship between Atrial Fibrillation and CHF

Congestive heart failure is a major public health problem in the western world and is a common cause of atrial fibrillation¹²⁹. Mechanisms underlying AF associated with CHF are still incompletely understood. However, structural remodeling such as atrial enlargement and increased interstitial fibrosis seems to play a major role¹³⁰. Also, the possible atrial electrophysiological characteristics that predispose to AF in animals and patients with CHF have been determined.

Animal studies of atrial electrical remodeling in CHF have demonstrated conflicting results. While in the study by Li et al.¹³⁰, no apparent change in atrial ERP is observed in the presence of CHF in dogs, the work of Cha et al.¹³¹ shows that CHF significantly increases the atrial ERP. However, in both studies discrete regions of slow conduction are associated with the development of interstitial fibrosis. In addition, in the research performed by Sanders et al.¹³², atrial remodeling due to CHF is characterized by abnormalities of conduction, and increased refractoriness.

4.1.5. Electrical Remodeling and AF in this Model

In the electrophysiology studies that we performed in these experiments, ERP does not seem to have a role in arrhythmogenic remodeling. It is conceivable that hypertension, aging, and heart failure cause changes in cellular electrophysiology of atrial myocytes as noted before. We do not have enough evidence to be able to exclude

the presence of alterations in cellular electrophysiology, which could contribute to AF vulnerability. However, the lack of pro-arrhythmic alteration in ERP should be noted. Moreover, an acute increase in the afterload and the immediate effect of elevated LV pressure on atrial pressure and stretch did not lead to pro-arrhythmic changes in the ERP and development of AF.

4.2. Conclusion

Hypertension by itself in young animals without the presence of LVH and atrial damage does not predispose the atria to AF in this model. This is supported by the clinical studies in which increased risk of AF in hypertensive patients is associated with LVH and age. Despite the importance of LVH as an underlying risk factor for AF in hypertensive patients, in our study on hypertensive rats, LVH alone did not cause an increase in AF sustainability. This could be indicative of the fact that structural damage is necessary in the atria to provide a suitable substrate for AF maintenance in hypertensive patients.

In this rat model of hypertension, structural remodeling especially in the form of increased amount of interstitial fibrosis seems to be the major contributing factor to the AF sustainability. Moreover, it can be concluded that hypertension could accelerate the accumulation of fibrosis which occurs during the process of aging as atrial interstitial fibrosis occurred much earlier in the SHRs compared to their controls. Although the role of LA enlargement is not clear in the Wistar rats and SHRs up to the age studied, its enlargement by approximately 40% in the SHHFs at old age compared to old controls could contribute to the fact that all the animals from this group developed sustained AF.

In general, given the well-recognized relationship between hypertension and AF, it is also possible that the rat model is not an optimal one for man. The reasons for this are unclear, but may have to do with general differences in cardiac electrophysiology, with processes that simply require a longer period of time than available in the rat life span, or with differences in heart size between species.

4.3. Future Directions

Due to the limited efficacy and the nontrivial adverse effects of the presently available drug therapies for AF, the prevention of arrhythmogenic remodeling is emerging as a potential new treatment strategy. The prominent role of structural versus electrophysiological remodeling as a substrate for AF sustenance in the context of aging and hypertension has been underlined in this work and various other studies. Therefore, drug therapies that start at young age and prevent the structural damage to the heart tissue, which is a process that occurs over time, might be found useful.

Various pathways have been suggested over the years in the literature that are responsible for the myocardial damage due to hypertension and senescence. The RAA system has emerged as a potential candidate mediating the regulation of the cardiac myocyte growth and the accumulation of collagen in cardiac fibroblasts¹³⁸⁻¹⁴¹. Both angiotensin II and aldosterone increase perivascular collagen deposition and interstitial fibrosis *in-vivo*¹⁴². Therefore, modulation of the RAA system by angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists or aldosterone antagonists can alter the progression of myocardial fibrosis and its consequences.

Ample evidence exists in the literature for the cardioprotective effects of the ACE inhibitors and aldosterone blockers against the structural remodeling of the myocardium. Protective effects of long-term enalapril, an ACE inhibitor, treatment against myocardial damage due to aging has been shown in various animal studies^{143;144}. Enalapril prevention of myocardial fibrosis has been related to enzymatic-mitochondrial or cellular cycle modifications¹⁴⁵. Cardiac protective effects of spironalactone, an aldosterone blocker, has been shown in a recent study on old normotensive rats by significant prevention of cardiac fibrosis¹⁴⁶.

Beneficial effects of ACE inhibitors and aldosterone blockers for the heart have also been shown in pathophysiologic conditions such as hypertension. Long-term treatment of SHR with an ACE inhibitor has been shown to prevent the structural remodeling^{147;148}. Inhibition of RAA system provides tissue protection even when the origin of hypertension is not an overstimulation of RAA¹⁴⁹. This suggests that ACE inhibitors can exert direct tissue effects that are not a consequence of their hemodynamic actions. Moreover, treatment of SHR with spironalactone in combination with an ACE inhibitor has been shown to preserve the myocardium against damage¹⁵⁰.

Other substances that seem to be involved in structural remodeling are advanced glycation endproducts (AGEs). These are the late products of the modification of proteins, lipids, and nucleic acids by reducing sugars, and have been shown to accumulate slowly in cardiovascular tissue with normative age and at accelerated rates in patients with hypertension¹⁵¹. It has been suggested that accumulation of AGE can alter the structure and function of the cardiovascular system by crosslinking of collagen^{152;153}, crosslinking of circulating proteins such as lipoproteins¹⁵⁴, and cellular signalling leading to vascular and myocardial stiffening, endothelial dysfunction, and

atherosclerotic plaque formation¹⁵¹. Receptors for AGE have been found on cardiac fibroblasts, macrophages, mesangial cells, smooth muscle cells, as well as endothelial cells¹⁵⁵. Therefore, long term inhibition of AGEs could be regarded as a therapeutic option for preventing cardiac remodeling.

Despite the evidence for the cardioprotective effects of ACE inhibitors, aldosterone blockers, and AGE inhibitors, no work exists in the literature investigating the long term effects of these treatments on the arrhythmogenic remodeling in the atria leading to sustained AF during the process of aging and hypertension. Considering the great potential for preventive methods as the first line of therapy against structural changes that provide a substrate for AF, it will be of great value to investigate the outcome of therapies with these substances starting at young age. Rats could be used in these studies considering the structural damage that occurs in their myocardium as a result of senescence and hypertension.

On the other hand, our inability to find a clear relationship between hypertension and AF in this rat model was disappointing. Further work in other species and other models of hypertension would be interesting in order to define an adequate animal model for the relationship between hypertension and AF in man. This would allow for a study of the relevant basic mechanisms and potentially for the development of improved preventive approaches.

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