Pharmacodynamic evaluation of β-blockade associated with atenolol in healthy dogs

Mari Waterman

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Master of Science

In

Biomedical and Veterinary Sciences

Jonathan A. Abbott, Chair

Andrea C. Eriksson

Jeffrey R. Wilcke

July 30, 2018

Blacksburg, VA

Keywords: atenolol, β-blockade, isoproterenol, dog, pharmacodynamic

Pharmacodynamic evaluation of β-blockade associated with atenolol in healthy dogs

Mari Waterman

ABSTRACT

Objective: Dosing intervals of 12 and 24 hours for atenolol have been recommended, but an evidentiary basis is lacking. To test the hypothesis that repeated, once-daily oral administration of atenolol attenuates the heart rate response to isoproterenol for 24 hours, we performed a double-blind, randomized, placebo-controlled cross-over experiment.

Animals: Twenty healthy dogs

Procedures: Dogs were randomly assigned to receive either placebo (P) and then atenolol (A), [1 mg/kg PO q24h] or vice versa. Treatment periods were 5-7 days; time between periods was 7 days. Heart rates (bpm) at rest (HR_r) and during constant rate [0.2 μg/kg/min] infusion of isoproterenol (HR_i) were electrocardiographically obtained 0, 0.25, 3, 6, 12, 18, and 24 hours after final administration of drug or placebo. A mixed model ANOVA was used to evaluate the effects of treatment (Tr), time after drug or placebo administration (t), interaction of treatment and time (Tr*t) as well as period and sequence on HR_r and HR_i.

Results: Sequence or period effects were not detected. There was a significant effect of Tr ($p < 0.0001$) and Tr*t ($p < 0.0001$) on HR_i. Atenolol significantly attenuated HR_i for 24 hours but did so maximally at 3 hours (least squares means ± SE, A: 146±5 bpm, P: 208±5 bpm); the effect at 24 hours was small (A: 193±5, P: 206±5). Atenolol had a small but significant effect ($p < 0.0001$) on HR_r.

Conclusions and Clinical Relevance: The results of this study support a dosing interval that is less than 24 hours.

Pharmacodynamic evaluation of β-blockade associated with atenolol in healthy dogs

Mari Waterman

GENERAL AUDIENCE ABSTRACT

This thesis was designed to test the effects of the drug atenolol on heart rate in dogs. Atenolol is used to reduce the heart rate of dogs with cardiovascular disease. The study used 20 dogs that were given oral capsules in both a placebo (no drug) and atenolol phase of the experiment. The study was designed to control for other causes of slower heart rate and make sure that the investigator did not know which treatment was given to a dog. Placebo dogs had a high heart rate response to the drug isoproterenol whereas atenolol treated dogs had a statistically significant lower heart rate response compared to placebo over a 24 hours period of time. The difference between treatments was small after 24 hours and further work is needed to determine the best time interval between doses of medication.

DEDICATION

I dedicate this thesis to Dr. Jonathan Abbott. Without his support and expertise, this would never have been completed. He is the World's Best Thesis Advisor and Residency Director.

ACKNOWLEDGEMENTS

I would like to thank my graduate committee, the VMCVM, the graduate school, fellow residents, research support, and the research beagles. The grant provided by the Virginia Veterinary Medical Association Veterinary Memorial Fund made this research financially possible. Especially important to this process were the significant contributions of Maureen Perry, FSVHP, DICVP for drug preparation and Dr. Stephen Werre for statistical consultation. Last but not least, I pledge my endless gratitude to my husband and family for supporting me through this unnecessarily long process.

ATTRIBUTION

The responsibility for the research is entirely mine, but I was aided in the fine-tuning of manuscript preparation for a scientific journal, the study design, and interpretation of statistics by my committee similar to any veterinary resident.

Table of contents

List of figures & tables

Figures

Tables

I. Introduction

Atenolol is a hydrophilic, β1-specific, adrenergic antagonist.[1] It has a variety of common uses in veterinary cardiology, and is prescribed in cases of tachyarrhythmia, hypertrophic cardiomyopathy, outflow tract stenosis (aortic and pulmonic stenosis), systemic hypertension, systolic dysfunction, and systolic anterior motion of the mitral valve.[2] In humans, uses for atenolol include management of heart failure, hypertension, migraine, angina, cardiomyopathy, and tachyarrhythmia.[3] As with other β-antagonists, serum concentrations do not predict the magnitude of clinical effects.[4] There are also inter-species and inter-individual differences that complicate the determination of optimal dose and dose interval. In veterinary medicine, atenolol pharmacodynamics have not been systematically evaluated.

II. Literature Review

A. β-adrenergic receptor antagonist agents - general

Binding of norepinephrine or epinephrine to β-receptors initiates a cascade of intracellular events.[5] There are 3 types of β-receptors: β_1-receptors which are found in highest concentrations in heart muscle, β_2-receptors which are found predominantly in bronchial and vascular smooth muscle, and β_3-receptors which are expressed to the greatest extent in adipose tissue.[6] The heart has all 3 types of β-receptors, the relative proportions of which can change in association with disease, however β_1-receptors are most important for cardiac function.[6] The effects of β-receptor stimulation are mediated through the G-protein coupled adenylyl cyclase system.[5] The end result of β-adrenergic activation of β_1-receptors is cyclic adenosine monophosphate (cyclic AMP or cAMP) production.[5] Cyclic AMP is an intracellular messenger that acts via protein kinases to increase inotropy, lusitropy, dromotropy, and chronotropy through effects on L-type calcium channels and regulatory proteins.[7] Positive inotropy, or increased force of myocardial contraction, is due to increased intracellular calcium entry through greater numbers of "open" calcium channels and increased interactions between actin and myosin heads.[7] Increased lusitropy, or more rapid myocardial relaxation, is mediated by increased reuptake of calcium into the sarcoplasmic reticulum at the end of systole via activation of sarcoplasmic recticulum Ca2+ ATPase (SERCA2a) pumps.[7] Both actions contribute to increased rate of conduction or positive dromotropy. β_1-adrenergic receptor stimulation also increases glucose utilization and decreases fatty acid metabolism.[7] Finally, through complex mechanisms, stimulation of β_1-receptors associated with the sinoatrial node leads to a more rapid heart rate, or increased chronotropy.[7,8] Stimulation of other β-receptor subtypes results in less distinct cardiac effects. Relative to β_1-receptors, β_2-receptors more weakly stimulate cAMP production and have inhibitory effects on

the adenylyl cyclase system, as well as anti-apoptotic effects.[5] β_3-receptors are involved in gastrointestinal motility, lipolysis, and have a weak, negative inotropic effect on myocardium.[5,6] In dogs and humans, the distribution of β-receptors and adenylyl cyclase is similar between the right and left ventricles.[9] However, there is a greater right ventricular than left ventricular cAMP response to β-receptor stimulation due to decreased degradation of phosphodiesterases.[9] This results in increased pressure generated and increased duration of response after stimulation.[9]

β-adrenergic receptor antagonist agents or "β-blockers" are commonly used cardiac drugs. They can be cardio-specific (e.g. selective for β_1-receptor only), nonspecific (activating all β-receptor subtypes), or vasodilatory (nonspecific β-blockade with additional α-adrenergic blockade, nitric oxide stimulation, or intrinsic sympathomimetic activity resulting in peripheral vasodilation).[7] β_1-blockers are competitive antagonists; they cause a dose-dependent effect on β_1-receptors but will bind to β_2-receptors at higher concentrations.[7] Cardiospecific β_1-blockade attenuates β-stimulation resulting in negative chronotropic, negative dromotropic, negative inotropic, antiarrhythmic, and anti-ischemic effects.[7] In some forms of cardiac disease, these effects can be beneficial because they reduce myocardial oxygen demand, increase diastolic filling time, and improve myocardial perfusion.[7]

B. β-blocker Side effects

1. Adverse Effects

Adverse effects of β-blockers result from both direct and indirect adrenergic effects and occur in about 15% of cases.[10] Cardiovascular responses may include shock, bradycardia, angina, heart failure (due to reduced myocardial function or reduced cardiac output), sinoatrial (SA) or

atrioventricular (AV) conduction block, peripheral vasoconstriction, syncope, and hypotension.[10] Other reported negative effects include gastrointestinal signs, fatigue, hypoglycemia, CNS disturbance, bronchospasm, and allergic/hypersensitivity reaction.[7,8,10] Hypoglycemia can occur as a result of blockade of β_2- and α-receptors that control glycogen release in the muscle and liver respectively or can occur as β-blockade affects compensatory mechanisms that respond to hypoglycemia.[10] Metabolic effects include diarrhea, an increased risk of diabetes, and weight gain from inappropriate lipolysis.[7] In humans, β-blockade has been reported to cause dreams, hallucinations, insomnia, fatigue, and depression, but not sedation.[10] Hematologic conditions, oculomucocutaneous syndrome, sclerosing peritonitis, and antinuclear antibodies have also been reported in people.[10] In experimental, high-dose settings using rodent models, some β-blockers – alprenolol, practolol, timolol, tolamolol, pamatolol – have been associated with carcinogenesis, but they are no longer on the market for oral use.[10] Many β-blockers have interactions with other drugs.[7] Dose adjustments need to be performed when used with other drugs and when there is hepatic or renal dysfunction.[1,7]

Some of these adverse effects can be minimized by choice of β-blocker.[7] Adverse effects that specifically result from β_1-blockade include worsening heart failure due to reduced cardiac output and unwanted metabolic effects.[7,8] Excessive β_1-blockade also can cause fatigue, bradycardia, AV block, syncope, and hypotension.[1,7] Fatigue may be caused by effects on metabolism, by reduction in cardiac output, or possibly due to central nervous system (CNS) effects for β_1-blockers that cross the blood-brain barrier.[7] β_1-selective antagonists are not completely selective and can block β_2-mediated bronchodilation and skeletal muscle vasodilation at high doses.[10]

2. Withdrawal

β-blocker withdrawal is associated with clinical effects. This is because β-receptors are upregulated during treatment such that after removing β-blockade, there are more β-receptors and potentially an exaggerated response to endogenous catecholamines.[10] Patients withdrawn from atenolol have increased sensitivity to isoproterenol.[11-13] One study evaluated the response to β-blocker withdrawal in healthy people and found inter-individual variation; some people demonstrated no change in heart rate and others demonstrated changes in resting or exercise-induced heart rate. [10,11] Other effects of β-blocker withdrawal include increased platelet aggregation, elevated thyroid levels, and increased circulating catecholamines.[10] There is conflicting information regarding the risk of myocardial infarction after β-blocker withdrawal in humans.[14] In order to minimize withdrawal symptoms, slow tapering of β-blocker doses is recommended when it is necessary to discontinue drug administration.

C. Atenolol

1. General features

Atenolol is a hydrophilic, β_1-specific, adrenergic antagonist with a molecular weight of 266 Daltons.[1,4] It is not lipid-soluble and in humans is approximately 10% protein-bound.[1,7,15] Atenolol has no sympathomimetic or membrane-stabilizing activity.[1,16] It has minimal first-pass effects in the liver and elimination is primarily renal in dogs.[15] The drug is completely excreted by 3 days post-administration.[15] Atenolol has no active metabolites, but has two enantiomers.[15,17,18] As with all β-blockers, blood concentrations correlate weakly with relevant clinical effects.[19] Atenolol is relatively free from proarrhythmic effects.[20]

2. Atenolol Use in Medicine

Human uses for atenolol include management of systemic hypertension, migraine, angina, cardiomyopathy, heart failure and arrhythmias.[21] Compared to other β-blockers, atenolol has fallen out of favor in human medicine. It has similar activity compared to metoprolol, but metoprolol extended release formulations reduce heart rate and blood pressure with less subjective fatigue and more consistent plasma concentrations.[22] Nadolol is also favored over atenolol because it demonstrates superior "isoprenaline- induced chronotropic response attenuation" relative to atenolol.[23] Carvedilol is also commonly prescribed because of its non-specific β-blockade effects, overall tolerability in humans, and documented beneficial effects in the management of heart failure.[24] In veterinary cardiology, atenolol has a variety of common uses including management of hypertrophic cardiomyopathy, subaortic and pulmonary stenosis, systemic hypertension, hyperthyroidism, tachyarrhythmia, and heart failure.

a.Hypertrophic cardiomyopathy (HCM)

Hypertrophic cardiomyopathy (HCM) is a myocardial disease characterized by hypertrophy of a non-dilated ventricle that occurs in the absence of a hemodynamic or metabolic cause.[25] It is a common disorder in cats but generally believed to be rare in canine patients. HCM is said to be "favorably modified" by atenolol.[26] Theoretically, atenolol might have favorable effects on left ventricular diastolic dysfunction, left atrial dysfunction, left ventricular hypertrophy, myocardial fibrosis, myocardial ischemia, tachycardia, ventricular arrhythmias, thrombosis risk, neurohormonal activation, and dynamic left ventricular outflow (LVOT) obstruction.[15,26-28] However, recently published data fail to demonstrate clinically relevant benefits in feline HCM. In one study of cats with HCM, atenolol was associated with a decrease in left atrial size but another study found an increase in left atrial size.[26,29] Other studies have found that atenolol significantly decreases murmur intensity, heart rate, rate-pressure product, and severity of

dynamic left ventricular outflow tract obstruction in cats.[4,28] It has been suggested that tachycardia predisposes patients with HCM to sudden and life threatening increases in left atrial pressure and decompensation.[30] Based on retrospective analyses, heart rates greater than 200 beats per minute (bpm) negatively influence survival in HCM.[25,31] Atenolol decreases heart rate and may also suppress ventricular arrhythmias.[29] As a result, atenolol should reduce myocardial oxygen demand and reduce ischemic risk.[27,28] However, atenolol has not been shown to reduce concentrations of cardiac biomarkers such as Troponin I or proBNP which are elevated in diseased populations.[26,27] This may be because it decreases left ventricular end diastolic volume and systolic function (negative inotropy).[28] There are no human studies that prove atenolol reduces left ventricular hypertrophy, attenuates ischemia, or improves prognosis.[27] One non-blinded study found no significant mortality difference in cats treated with atenolol compared to those untreated.[26] Adverse effects of atenolol in cats with HCM can include reduced left atrial mechanical function, increased left atrial size, and decreased flow velocity in the left auricle predisposing patients to thrombus formation.[26] In humans, β-blockers are a mainstay of therapy and symptom relief, but may not improve mortality rates.[26,32,33] In dogs, HCM is believe to be rare, and there have been no studies that have evaluated effects of atenolol.

HCM is associated with systolic anterior motion of the mitral valve (SAM); when SAM is associated with HCM, the condition is known as hypertrophic obstructive cardiomyopathy. SAM results from papillary muscle and left ventricular hypertrophy resulting in abnormal mitral valve movement into the left ventricular outflow tract and dynamic velocity elevation.[2] The overall clinical significance of LVOT obstruction in HCM is unknown– in humans, it has been associated with clinical signs and a negative prognosis, but in cats, it is associated with both positive and neutral outcome.[25,26,30] Atenolol may limit or abolish SAM because it reduces

systolic function and heart rate.[28,29,32,34] Humans with SAM treated with β-blockers respond with favorable clinical improvement in 33-66% of cases.[35] Symptoms from LVOT obstruction can be relieved by atenolol in people despite unchanged function capacity.[36,37,38,39] In an uncontrolled and non-blinded study in dogs with SAM, treatment with atenolol (1 mg/kg orally q24h) was associated with resolution of LVOT obstruction and reduction in ventricular concentric hypertrophy.[35] However, there have been case reports with spontaneous resolution of SAM in dogs when it is caused by underlying right heart changes, hypovolemia, prodromal hypertrophic cardiomyopathy or subaortic stenosis.[35,40-42] There are no double-blind, placebo controlled studies in dogs to ascertain the effects of β-blockade treatment in cases of SAM.

b. Subaortic Stenosis (SAS)

Atenolol is used in the treatment of canine subaortic stenosis (SAS), the rationale is that the negative chronotropic and inotropic effects reduce myocardial oxygen demand and increase time for ventricular filling and coronary perfusion.[43,44] Other reasons that atenolol might be beneficial in SAS include reducing ventricular wall stress and ventricular arrhythmias.[45] One study found that dogs with severe SAS treated with atenolol dosed between 0.46 to 1.5 mg/kg PO q12h had a mortality rate (median survival 56 months) that was similar to that associated with balloon dilation of the left ventricular outflow tract (median survival 55 months).[44] Another study showed no difference between treatment with atenolol dosed between 0.3-1.2 mg/kg PO q12h and no treatment for severe SAS.[43] Overall, there is no evidence to support the use of atenolol in the treatment of SAS, and any benefit is hypothetical.

c. Pulmonary Stenosis (PS)

Atenolol and other β-blockers are also used in the management of moderate and severe canine pulmonary stenosis (PS) although there are no published studies that have evaluated its efficacy.[46,47,48] Because pulmonary stenosis is a mechanical problem, balloon valvuloplasty is the treatment of choice for severe disease.[49,50] After balloon dilation, theoretical benefits of β-blockade in PS include reducing arrhythmias, reducing dynamic right ventricular outflow tract gradient (infundibular gradient), and reducing syncope.[48]

d. Mitral Regurgitation (MR)

In veterinary medicine, atenolol is not consistently prescribed for patients with mitral regurgitation (MR).[51] Other β-blockers such as carvedilol have been used more recently.[52,53] However, there is evidence atenolol might be helpful. In one experimental model of mitral regurgitation with systolic dysfunction in dogs, improvement of contractile function over a 3-5 month period of time was associated with administration of atenolol.[54] This was achieved using atenolol doses of 50 mg/dog/day and heart rate attenuation was confirmed when isoproterenol at 4 ug/kg/min failed to cause an increase in heart rate beyond 5 bpm.[54] Both isolated cardiomyocytes and intact heart function were assessed and showed improvement; measures of improvement that returned to baseline included slope of end ejection stress-volume relationship, end systolic stiffness constant, and stress-volume.[54] Other positive changes in β-blocked dogs included increased myofibrillar density, increased left ventricular mass, reduced heart rate, and reduced pulmonary capillary wedge pressure.[54] Another study demonstrated improved left ventricular myocyte function despite extracellular matrix dysfunction.[55] These reverse remodeling effects imply a reduced burden on the heart. Mechanistic possibilities to explain the reverse remodeling with atenolol include improved viability and protein synthesis in cardiocytes, enhanced responsiveness of depressed β-receptor adenylyl cyclase, reduced toxic effects of

circulating catecholamines, and enhancement of cardiac performance with lower heart rate.[54,55] β_1-blockers may also be helpful in MR because they do not increase total peripheral resistance (which can worsen the severity of regurgitation).[54] Treatment cannot restore the heart to an undamaged state and there are residual changes similar to untreated dogs such as elevated levels of norepinephrine, increased cardiocyte length, and abnormal capillary density.[54] More work is needed to determine the utility of atenolol in diseases affecting the mitral valve.

e.Systemic Hypertension

Atenolol is used in the management of systemic hypertension in people.[56] In dogs, it is recommended as a third-line intervention after ACE-inhibitors and amlodipine or as determined by disease process.[57] Systemic hypertension is associated with excessive glucocorticoids (hyperadrenocorticism), increased mineralocorticoids or sodium chloride (hyperaldosteronism), obesity, diabetes mellitus, kidney disease, hyper- or hypo-thyroidism, excessive erythropoietin, NSAIDs, phenylpropanolamine, pheochromocytoma or idiopathic hypertension.[57] β-blockade is effective in hypertension because they antagonize sympathetic response.[58] General mechanisms of β-blocker anti-hypertensive effects are decreased cardiac output and decreased renin from the juxtaglomerular apparatus (due to blocking the adrenergic-mediated release).[59,60] Plasma concentrations of atenolol are not a reliable predictor of hypotensive effect and chronic use can lead to tissue accumulation in dogs.[60,61] Stress-induced hypertension in rats was reduced by atenolol, partly from inhibition of stress-activated central β-adrenoreceptor transmission directly in brain and also by increased density of cerebral β-adrenoceptors.[62] There may be central nervous system effects even for drugs such as atenolol that are not lipid soluble.[61] Atenolol also reduces blood pressure in rats with primary hypertension by reducing cardiac output, though this may not be helpful as a long term mechanism.[62] Other β-blockers act by a different mechanism,

reducing total peripheral vascular resistance more than atenolol.[29] The use of atenolol compared to other β-blockers is associated with a mildly increased risk of stroke in elderly patients, but not younger patients.[67] However, compared to placebo it reduces stroke risk but not overall cardiovascular risk.[67,68] This increased risk may be related to atenolol's inability to address arterial stiffness and pulse wave dyssynchrony, a common cause of systemic hypertension in elderly patients.[67] In humans, atenolol is most useful for mild to moderate systemic hypertension, but not generally as first-line therapy.[63-66] There have been no systematic evaluations of atenolol in dogs with systemic hypertension.

f. Hyperthyroidism

Hyperthyroid patients might benefit from the heart rate reduction and anti-hypertensive effects of atenolol. A study in hyperthyroid cats found that those treated with atenolol (1-2 mg/kg PO q12 hours) alone had significantly reduced heart rate, but blood pressure (BP) reduction did not meet target value (systolic BP < 160 mmHg to reduce risk of end organ damage) in all cats.[59] There was a positive response in a subset of cats in which BP decreased to less than 160 mmHg, but those cats had lower systemic pressures at baseline.[59] Thus, atenolol is not a reliable single agent therapy for hyperthyroid cats. In hyperthyroid rats, atenolol attenuates increases in heart rate, rectal temperature, and oxygen consumption, but does not alter cardiac hypertrophy, arterial hypertension, or increased β-receptor density caused by increased thyroid activity.[69] Similarly, in human hyperthyroid patients, atenolol attenuates heart rate, but not all hyperthyroid effects on cardiac performance.[70] Thyroid hormone effects persist in the absence of β-receptors as demonstrated in hyperthyroid mice bred without β-receptors.[58] Atenolol therefore has a role in treating hyperthyroid-induced tachycardia and tachyarrhythmias, but as complementary rather than single therapy.

g. Arrhythmias

Arrhythmias are triggered by four primary mechanisms that can be influenced by β-blocker therapy: early afterdepolarizations, delayed afterdepolarization, enhanced automaticity, and re-entry.[2,5] Early afterdepolarizations are caused by an increase in plateau calcium current due to β-stimulation in phase 2.[20] Delayed afterdepolarizations are primarily caused by β-stimulation of inward calcium currents resulting in elevated intracellular calcium(especially in diseased myocardium).[20,71] High intracellular calcium stimulates sodium-calcium exchangers for a net inward positive ion flow during phase 4.[71] If the inward current is strong enough, a depolarization can be triggered. β-blockers decrease the risk of depolarization because they reduce sodium-calcium exchanger activity and upregulate SERCA2a pump removal of calcium, thus preventing depolarization due to ion flux across the plasma membrane.[20] Enhanced automaticity due to sympathetic stimulation also contributes to arrhythmias. β-blockers reduce automaticity by interfering with I_f and I_{Ca} ion channels.[20] In the context of myocardial disease, re-entry occurs when β-adrenergic stimulation causes differential areas of conduction able to sustain a re-entry loop.[71] Arrhythmias due to re-entry can be reduced by β-blockers because β-blockers alter action potential duration and refractory period to reduce "heterogeneity" of depolarization in diseased tissue.[20] All of these actions occur in both myocytes and nodal cells.[71]

In veterinary medicine, atenolol is used for chronic oral therapy in the treatment of ectopic atrial tachycardia, junctional tachycardia, AV nodal re-entry tachycardia, atrial flutter and fibrillation, as well as ventricular arrhythmias/tachycardia.[72-74] β-blockers are commonly used for supraventricular tachyarrhythmias because they slow atrioventricular nodal conduction to reduce ventricular rate response.[2,75] β-blockers prolong the effective refractory period and functional refractory period indirectly through catecholamine inhibition.[76] Cats are commonly treated with

atenolol as first choice therapy for any tachyarrhythmia.[77] In humans, β-blockers provide rate control in atrial fibrillation that is similar to rhythm control in terms of clinically relevant endpoints.[78,79,80] They prevent atrial fibrillation post-operatively and control ventricular response to atrial fibrillation especially during exercise.[20] An experimental study in dogs showed that carvedilol suppressed the induction of atrial fibrillation, but there is no similar study for atenolol.[81] Atenolol has been shown to protect against catecholamine-induced tachycardia and delay depolarization in re-entry pathways to prevent ventricular arrhythmias.[76] Atenolol in experimental beagles decreases the risk of Torsades de Pointe.[82] Unfortunately, atenolol alone does not significantly improve and may increase ventricular arrhythmias in Boxer dogs with arrhythmogenic right ventricular cardiomyopathy (ARVC), however in combination with mexiletine, it does improve control.[83] The action may be related to reducing sympathetically triggered ventricular arrhythmias.[83] German shepherd dogs with inherited ventricular arrhythmias have evidence of longer action potential duration mediated by β_1-receptors predisposing them to calcium overload and after depolarizations.[84] In theory, β-blockers should reduce this arrhythmias. In humans, catecholaminergic polymorphic ventricular tachycardia is also treated with β-blockers but incomplete control necessitates an implantable defibrillator. [85,86] Other arrhythmias that may respond to β-blockers include ventricular fibrillation, hypokalemia-induced arrhythmias, and digitalis toxicity.[76,87]

h. Heart Failure

Heart failure is a clinical syndrome initiated by a decline in cardiac performance.[88] A simplistic model of heart failure starts with cardiac disease that causes a low output state. This results in activation of the sympathetic nervous system and the release of agents such as renin, angiotensin II, and norepinephrine.[2] These neuroendocrine mediators in turn increase blood volume by

retaining sodium and water in the kidneys, increasing heart rate and cardiac output through direct adrenergic receptor stimulation, and increasing peripheral vasoconstriction to maintain blood pressure to important organs.[88] Initially, compensatory mechanisms are beneficial, but in the long run, they contribute to increased morbidity and mortality independent of the hemodynamic status of the patient.[88,89] Chronic sympathetic stimulation results in elevated heart rate, increased afterload, arrhythmias, cardiac hypertrophy, and fibrosis.[90] Ultimately, heart failure can be likened to a vicious cycle of ineffectual myocyte hypertrophy and remodeling, death, fibrosis, arrhythmias, and progressive worsening of myocardial function.[88] β-blockers contribute to cardiovascular stabilization by preventing decline at both a cellular and a hemodynamic level.

β-blockers at the cellular level reduce cardiovascular dysfunction and can reverse the cardiac remodeling that is associated with heart failure. Excessive β-stimulation in heart failure causes reversible myocardial changes in gene expression and detrimental neurohormone production. β-blockers reverse changes in the type of myosin heavy chain (α-MHC upregulation blocked), undo cues for programmed cell death, increase SERCA2a, reduce matrix metalloproteinase, and alter natriuretic peptide production.[91] α-myosin heavy chains are detrimental because they use more ATP to perform the same work when the heart is already struggling to meet energy demands.[5] β-blockade upregulates the gene bcl2 which protects against apoptosis and prevents activation of pro-apoptotic caspases.[92,91] β-blocker therapy in experimental canine heart failure induced by coronary microembolization reduces cardiomyocyte apoptosis.[92] Reduced β-stimulation by norepinephrine and reduced transmyocardial norepinephrine directly decrease myocyte apoptosis.[88,92,93,94] Apoptosis may also be decreased because of the favorable hemodynamics and decreased myocardial oxygen demand associated with β-blockade.[92] Heart failure is associated with increased intracellular calcium levels which can be toxic to myocytes.[54]

β-blockade decreases calcium leakage from cardiac ryanodine receptors in the sarcoplasmic reticulum.[95] β-blockade restores activities of SERCA2a, sodium-calcium exchanger, and calreticulin to those seen in normal animals.[96] These changes remove calcium from the intracellular space after contraction by sequestration into the sarcoplasmic reticulum or pumping it out of the cell.[5] β-blockade also increases phosphorylated phospholamban, reverses hyperphosphorylation of ryanodine receptors (RyR2), normalizes elF2α-P (marker of endoplasmic reticulum stress), and reduces DNA damage.[96] All of these cellular changes improve calcium handling in the failing myocardium and contribute to reverse left ventricular remodeling.[95] β-blockade with metoprolol downregulates mRNA for matrix metalloproteinases (MMPs) and natriuretic peptides A and B.[91] MMPs are important because changes in concentrations signal dysregulation of the extracellular matrix and worsening systolic myocardial function.[97,98] Both A-type and B-type natriuretic peptides are upregulated in heart failure and improve with β-receptor attenuation of the hemodynamic abnormalities.[5]

The primary neuroendocrine actor in heart failure is the renin-angiotensin-aldosterone system (RAAS). β-blockade directly decreases renin release, an effect mediated by antagonism of juxtaglomerular β-receptors.[99] Blunting of RAAS activation might also occur indirectly through improved hemodynamic status. In one study, β-blockade resulted in the following histologically documented changes: decreased interstitial fibrosis (54%), decreased replacement fibrosis (46%), increased capillary density, and reduced cardiomyocyte hypertrophy (20%).[94] These changes contribute to increased myocardial oxygen delivery.[94] However, the degree of improvement may vary between β-blockers. Both atenolol and metoprolol prevent accumulation of fibrosis and myocyte hypertrophy and increase capillary density compared to control dogs.[100] However, metoprolol has a greater "extent of normalization" compared to atenolol with larger effects on

SERCA2a and BNP gene expression.[100] The reason for these differential treatment effects may be explained by the nonlipophilicity of atenolol or other pharmacodynamics factors.

β-blockers also alter pathways of adrenergic stimulation over time. In heart failure, high catecholamine concentrations are associated with downregulation of β_1-receptors and upregulation of β_2- and α_1-receptors. β-adrenergic receptor kinase (βARK) is integral to these processes and prevents β-receptor activation of the G protein-coupled pathway in a process called desensitization and uncoupling of the receptor-G-protein complex.[89,101] This process is reversible, but prolonged β_1-receptor stimulation results in internalization and digestion of the receptors by proteolytic enzymes.[5] Norepinephrine is more active at β_1-receptors which may be why they are preferentially down-regulated.[101] β_2-receptors become relatively more prevalent because their numbers remain constant as β_1-receptor numbers decrease.[95] The shift in receptor subtype contributes to depressed systolic function and detrimental norepinephrine release.[89] In dilated cardiomyopathy (DCM), there is an increase in the inhibitory G-protein 1α causing reduced sensitivity to adrenergic stimulation and increased norepinephrine release contributing to further systolic dysfunction.[102] Excessive norepinephrine causes cell death.[93] β-blockers reverse these changes; this results in improved β-agonist response and restoration of appropriate β-receptor density.[54,95] Recently, it was shown that β_3-receptors are also upregulated in heart failure and may contribute to progressive left ventricular systolic and diastolic dysfunction through inhibitory G-proteins (G_i).[103,104] β-blockers also bind to β_3-receptors reducing the negative effects.[103] β_1-selective agents are better tolerated in heart failure than nonspecific β-blockers because β_2-receptors are unblocked and can support myocardial function; there is also less reflex vasoconstriction (peripheral β_2-receptors mediate vasodilation).[89] Overall, β-blockers have positive long term effects on mortality, morbidity, and symptoms in human heart failure.[89]

At the hemodynamic level, β-blockers can influence heart rate and arrhythmias in patients with heart failure. In experimentally induced canine mitral regurgitation, improvement in left ventricular function is not observed if bradycardia from β-blockade is prevented by an electronic pacemaker.[105] In a separate experiment, if heart rate reduction was achieved through the sinus node agent cilobradine, some improvements similar to β-blockers in heart failure patients were seen.[106] Thus, many of the benefits of β-blockade are related to their heart rate lowering effects. Reduced heart rate alone may improve function by reducing energy utilization, reducing cell injury due to calcium influx, or restoring the ventricle to a more effective position on its force-frequency curve.[95] Interestingly, in two experimental canine studies, there was no significant decrease in heart rate on atenolol compared to controls, although many measurements were obtained under anesthesia.[94,100] However, some patients with heart failure cannot tolerate β-blockers because of the negative chronotropic effect.[2] Unlike other negative chronotropic agents, treatment with β-blockers reduces heart rate, but also improves all time domain heart rate variability indices suggesting restored responsiveness to sympathetic and parasympathetic influences more similar to normal individuals that those with heart failure.[107] Decreased heart rate variability is an independent predictor of sudden death related to arrhythmias.[107] β-blockers also reduce arrhythmias in humans with heart failure and there is a mortality benefit of β-blockade mediated by fewer episodes of ventricular tachycardia.[107] β-blockade reduces ventricular arrhythmias, the incidence of ventricular fibrillation, and overall heart rate which have a mortality benefit for coronary artery disease, ischemic disease and heart failure in people.[20] This benefit is significant – for instance, in the MERIT–HF study, β-blockade reduced mortality by 38% .[108] Other studies have shown a mortality risk reduction of 32-50% in

humans.[109] Data related to chronotropic effects of atenolol in spontaneous canine heart failure are lacking.

In humans, β-blockers represent the standard of care for heart failure because they improve cardiovascular hemodynamics. β-blockers improve cardiac output, heart size, systolic function, and overall patient mortality. Bisoprolol, carvedilol, and metoprolol reduce all-cause mortality in patients with heart failure by 33-35 %, sudden death by 31- 40%, and hospitalizations for heart failure by > 30 % compared to standard therapy without β-blockade.[109,110] Though atenolol is less studied in human heart failure patients, in one study when added to baseline enalapril therapy, atenolol improves mortality, worsening heart failure, and hospitalization rates.[111] With all β-blockers, there are long-term improvements in systolic function, regression in myocardial mass, and normalization of ventricular shape (reverse remodeling) despite short term negative ionotropic effects.[89] This may be related to improved sensitivity of β-receptors, G protein effects, improved heart rate control, decreased myocardial oxygen demand, reduced renin levels, or protection from cardiotoxic effects of norepinephrine.[112] Although there is a theoretical concern of reduced cardiac output due to β-blockade because of negative inotropic effects and vasodilatory action, β-blocker therapy discontinuation is not recommended in the setting of acute decompensated heart failure in human studies.[113] Brief discontinuation does not improve patient outcome, interferes with the ability to reintroduce β-blockers after hospitalization, and prevents the benefits of long term treatment.[113] β-blockers do not work in all patients because there are variations in response due to dose, liver or kidney dysfunction, abnormal genetic factors, stage of heart failure, race, and sex.[89,108]

β-blockade is associate with improved hemodynamics in canine experimental models but this has not been demonstrated in spontaneous disease. Left ventricular function, sphericity, and size

predict mortality and morbidity in heart failure due to systolic dysfunction.[94] Over time, β-blockers reduce ventricular sphericity, wall stress, and functional mitral regurgitation in experimental heart failure.[88,72,77] Metoprolol reduces systemic vascular resistance, decreases afterload, and attenuates heart rate increases.[114,115] In dogs with experimentally-induced heart failure, metoprolol 25 mg PO BID improves or stabilizes ejection fraction, cardiac output, and left ventricular dimensions and prevents the progressive decline in functional variables observed in untreated dogs.[114,115] Treatment with metoprolol restores responsiveness to isoproterenol (β-agonist) stimulation.[55,105,106] Improved systolic function may be due to increased responsiveness to β-agonists, improved myocardial oxygen consumption, attenuation of tachycardia or improved wall stress.[114] It may also be related to the cellular effects discussed previously. [89,94] β-blockers may also be beneficial in right heart failure because they reverse β-receptor downregulation and improve systolic performance.[9] However, there are likely to be differences between drugs. For instance, atenolol is not as effective as metoprolol at reversing the effects of heart failure on dogs with experimental coronary embolization. In one comparison, atenolol stabilized the heart failure changes compared to the untreated dogs (there was a reduction in systolic function and increased left ventricular internal chamber dimensions), but metoprolol significantly improved wall stress and myocardial function compared to both atenolol and the control.[100] More study is necessary to determine the differential effects of these β-blockers.

A benefit of β-blockade in naturally occurring canine heart failure has not been established.[112] There are no large prospective or retrospective studies of spontaneous canine heart failure treated with atenolol, but metoprolol, propranolol, and carvedilol are tolerated in naturally occurring diseases including degenerative valve disease and dilated cardiomyopathy.[53,90,116,117] A retrospective analysis found that the overall survival did not differ between these two disease

groups, but regardless of etiology, dogs with heart failure did not live as long (412 vs. 217 median days).[116] Of dogs that received carvedilol, there was a subset with DCM that had, relative to a control group, stable cardiovascular variables but this result was not statistically significant.[117] Multivariable analysis of data retrospectively acquired from dogs with valvular disease that received carvedilol revealed that higher heart rates were associated with a lower survival, but for some of these dogs carvedilol administration was discontinued or dose was decreased when they developed heart failure.[52] Heart rates in dogs decreased after starting β-blockers only in some studies and side effects resulted in discontinuation of the drug in 0-16%.[116,117] No canine study has shown improvements in morbidity and mortality comparable to that demonstrated by studies of humans.[52,53,112,117] This may be because the doses used in dogs with acquired disease have been lower than those used in experimental studies or studies of humans with heart disease. In addition, it has been suggested that dogs with spontaneous disease may have irreversible myocardial remodeling by the time they are diagnosed.[116,117] Further work needs to be done to determine the place for β-blockers in canine heart failure treatment.

D. Pharmacokinetics

1. General

Therapeutic drug action is determined by both pharmacokinetic and pharmacodynamic relationships. Pharmacokinetic factors are those that determine drug disposition including absorption, distribution, metabolism, and excretion.[8,17] At steady state, drug concentration at an active site and in circulation should be in equilibrium.[16] In general, pharmacokinetic factors that determine plasma concentrations of antiarrhythmic drugs include protein binding, metabolite

activity, absorption, elimination, myocardial uptake, and bioavailability.[16,19] Plasma concentration reflects both unbound drug that can elicit action (the "active portion") and drug that is bound to protein.[16] The degree of protein binding determines the proportion of drug that is active.[16] Protein binding can vary between patients and even within a given patient based on physiologic changes, concurrent drugs, disease processes, and specific binding characteristics.[16] The activity of metabolites determines whether the drug is active upon absorption and the proportion of drug that is available for utilization.[19] Some metabolites are more potent than the parent compound, while others are antagonistic, and still others have a different volume of distribution or even different excretion or storage properties.[16] Absorption and elimination are some of the most important pharmacokinetic parameters of a drug. For some drugs, cellular uptake reflects the amount of the drug that can reach the target endpoint (e.g. cardiac muscle); it is influenced by binding characteristics, cellular transport mechanisms, plasma vs. myocardial concentration, and patient variability.[16] Finally, the bioavailability determines the amount of drug taken into circulation, the length of time it remains in systemic circulation, and the amount transformed into working components.[16] Oral bioavailability depends on the molecular weight of the drug, lipophilicity, ionic charge, and binding to dietary components.[118] It also is influenced by intestinal absorption/degradation, drug efflux by transporters, and first pass effects from metabolism by the liver and intestines.[118]

Collectively, pharmacokinetics are most commonly expressed in terms of plasma drug concentration. All factors that determine concentration can be expressed by a plasma concentration range or C_{max} – the peak plasma dose concentration determined by the rate of absorption versus the speed of elimination.[118,16,19] However, the most appropriate model accounts for the mathematical distribution of the drug in the body and is called a one-, two-, or

three-compartment model.[8] Drugs that do not conform to these models can be assessed using non-compartmental analyses. Pharmacokinetic modeling is complicated and expensive. There are also many factors that affect pharmacokinetics in clinical patients including concurrent medications, illness, metabolic status, and inter-individual variation.[8,17] There are significant species and breed differences that veterinarians have to account for in practice.[8] Many antiarrhythmic drugs have an "optimal plasma concentration" where the arrhythmia is controlled with minimal adverse side effects.[16] Unfortunately, the pharmacokinetics of β-blockers do not reliably predict antiarrhythmic efficacy because there is no consistent relationships between therapeutic range and relevant clinical effects.[19]

2. Atenolol Pharmacokinetics

The elimination half-life of atenolol varies considerably between species and individuals. One study of oral atenolol administration in cats showed an average of half-life of 3.66 hours (range 2.9 - 4.1 hours)[21] while another found a half-life of 11.4 hours (range 3.6 - 21.9 hours).[4] In dogs, the half-life is 3.2 hours to 7 hours compared to a half-life of 5-9 hours in humans.[1,7,21,119] Plasma levels of atenolol peak after 1 hour when fasted and approximately 90% is bioavailable in cats after oral administration.[21] In dogs, its peak concentration is 1-2 hours after oral dosing; oral and IV formulations achieve similar peak blood concentrations and the linear pharmacokinetics suggest no drug accumulation with good bioavailability (65%).[119] Atenolol is found in CNS despite low lipid solubility.[61] Transdermal preparations have too much variation in absorption in cats for a useful half-life assessment; one study found that only 2 of 7 cats had atenolol concentrations in "therapeutic range" at 2 hours and none had therapeutic levels at 12 hours.[4] In humans, there is no difference in plasma concentration between single and chronic daily dosing of atenolol.[23] However, there may be variation in clinical response over time

because β-blockers may alter receptor sensitivity and density.[54,95] In rats, atenolol causes an increase in the density of β_1 and β_2- receptors on a cellular level, but a decrease in the levels of β_1-receptors mRNA levels.[120] In guinea pigs, atenolol causes no increase in left ventricular β-receptors.[121] In one study of beagles, there is no sex or dose-dependency in dogs.[119] However, another study showed cardioselectivity in male dogs, unlike in humans.[122] There is quite a range in pharmacokinetic parameters across species and responses in dogs cannot be predicted from any available information on other species. For instance, the bioavailability of 43 different drugs between people and dogs was poorly correlated (r=0.51). There may also be differences between dog breeds. Furthermore, different β-blockers have differences in size, metabolism, lipophilicity, volume of distribution, receptor binding, and half-life such that attempting to extrapolate the pharmacokinetic variables of atenolol based on data from other β-blockers is likely to be inaccurate.[10] Although atenolol has not been systematically evaluated in canine clinical patients, β-blocker pharmacokinetics are not a reliable way of determining antiarrhythmic efficacy because there is no reliable correlation between therapeutic range and relevant clinical effects.[19]

E. Pharmacodynamics

1. General

Pharmacokinetic measures do not quantify the actions of a drug, only its concentration. Pharmacodynamics describe the "biochemical and physiological effects of drugs"; it is a "study of actions of drugs on the body".[17,123] Pharmacodynamic responses have significant variability due to inter-individual factors.[124] The general response of the body to a drug is determined by target receptors, binding affinity, intrinsic responsiveness, transducer pathways, and the

regulatory effects of a drug.[8,17] Drugs can act as agonists or antagonists and their initial binding is determined by receptor location, number, isoform, and selectivity.[17,123] Binding and tissue concentrations may also be affected by stereoisomer composition and affinity.[18] Agonists cause a conformational change in the receptor that can activate a signaling cascade whereas antagonists prevent conformational change or stimulate an inhibitory pathway of the signaling cascade.[123] The effects of drug-receptor binding involve direct cellular changes mediated by effector proteins and cell signaling molecules.[17] Affinity is the "property of attraction between a drug and a receptor" or the rate of attachment/detachment (binding) to the receptor.[123] The activity of a drug may not be determined by binding alone, especially when signaling cascades are responsible for physiologic effects. The intrinsic responsiveness of a drug is defined by its interaction with the target tissue.[16] A drug's pharmacodynamics action is measured by efficacy (a number between 0 to 1 quantifying the max response to that particular ligand) and potency (concentration of drug needed to achieve a given level of response).[123,124] All factors that determine concentration can be expressed by a therapeutic plasma concentration range or a drug concentration-response curve that is determined by trough plasma concentration and measures of toxicity (plasma level-effect relationship).[16,19] Finally, a patient or patient class's sensitivity to a drug is expressed as the slope of the concentration/dose-response curve.[123,124] Unfortunately, in veterinary medicine there are frequently differences in pharmacodynamics that result from both species and sometimes breed.[125] Laboratory studies are performed on dogs whose response to drugs may differ from that of pet dogs because they do not adequately represent the range of breeds available.[125] Receptor polymorphism also leads to variation in β-blocker response. For example, dogs with ADRB1 gene deletions have a reduced response to atenolol.[126]

Pharmacodynamic antagonism occurs when the antagonist blocks the effect of an agonist by using the same pathway.[123] There are three types of antagonism: reversible competitive antagonism, irreversible competitive antagonism, and non-competitive antagonism.[123] Reversible antagonists bind to receptors temporarily and their effect can be overcome if the agonist concentration is sufficiently high, while irreversible antagonists form a permanent bond with receptors.[123] Non-competitive antagonism occurs when the agonist response is blocked not at the receptor but at a subsequent step in a functional pathway and is usually reversible with a sufficiently high concentration of agonist.[123] The clinical response of a drug can be expressed as a graded dose relationship (e.g. increased epinephrine dose leads to increased blood pressure).[8] Drug concentration-effect relationships can also be graphically displayed allowing comparison of potency, efficacy, and individual variation.[8] There are also dose relationships that can be assessed in terms of a "quantal" or all or nothing response. Quantal and graded responses can be expressed as a frequency distribution curve, therapeutic index, or standard safety margin.[8]

β-blockers are reversible, competitive antagonists.[127] The most common methods of evaluating the dose- response relationship for β-blockers include resting heart rate assessment, exercise testing, and assessment of the response to isoproterenol (β-agonist) administration. Alternatives include administration of other sympathomimetic drugs, monitoring of ambulatory blood pressure, continuous ECG, serum concentrations of catecholamines (catecholamine responsiveness index), or direct nerve stimulation.[127]

Resting heart (HR) rate is an easy method of assessment, but because HR is influenced by both sympathetic and parasympathetic input, there is considerable inter-individual variation.[127] It is also a poor monitoring tool because environmental and individual variables confound assessment of treatment response. One study found that stress was associated with resting rate elevation and

the "in hospital ECG heart rate" exceeded the average Holter heart rate by an average of 31 bpm.[128] Another Holter study in normal dogs of different breeds found that heart rates ranged from 47-182 bpm over the course of a day with statistically significant breed differences regarding mean heart rate.[129] Even after appropriate therapy, heart rate varies according to external factors. For instance, dogs with atrial fibrillation and heart disease "well controlled" by administration of digoxin and diltiazem had heart rates from Holter recordings that ranged from 93-158 bpm.[130] In both dogs and people, sinus arrhythmia also contributes to baseline variability in heart rate.[131,132] Therefore, short duration assessments of resting heart rate are a replicable way to evaluate β-blockade and many studies with atenolol (or other β-blockers) show minimal to no significant difference in resting heart rate.[132] However, measuring heart rate variability over time using a continuous ECG monitor may be a more sensitive method of examining the effects of atenolol on resting heart rate and provide a surrogate measure of autonomic influences.[133] This has not been systematically evaluated for atenolol in dogs.

Exercise testing is an ideal way to measure heart rate elevation due to endogenous catecholamines.[23,127] There are different measures of response including percent change vs. absolute change in heart rate or percent inhibition of maximum heart rate.[127] Since it is difficult to distinguish parasympathetic effects on heart rate from β-blocker effects, atropine administration prior to testing is sometimes used to remove parasympathetic influence.[127] In one meta-analysis, survival was correlated with the magnitude of heart rate reduction rather than with β-blocker dose.[134] There is also support for heart rate reduction improving cardiovascular outcome as cited by Tardif, et al.[135] Unfortunately, exercise testing is not practical for veterinary patients because it requires additional training. Isoproterenol is a mixed β-agonist used to increase heart rate that can be used to simulate the effects of exercise in the laboratory setting. It

is non-physiologic and sometimes results do not mirror response to endogenous catecholamines and results of exercise testing.[127] In one canine study, there was a marked discrepancy in magnitude of response to exercise compared to isoproterenol stimulation between different β-blockers.[136] There are also variations in individual response which makes dosing controversial.[127] However, it is a practical and accepted method. Other ways to measure β_1-blockade include glycerine trinitrate, tilt test, and Valsalva maneuver.[127] Glycerine trinitrate and other nitrates cause vasodilation and a fall in blood pressure triggering a tachycardia response from the heart. This technique is unaffected by atropine, but uses other receptors in addition to β-adrenergic/sympathetic stimulation to mediate effects.[127] Tilt tests and Valsalva also rely on blood pressure triggered heart rate elevation, but are impractical for dogs and results can vary based on time of day and patient hydration status.[127] β_2-receptor blockade can be assessed with inhaled isoproterenol and measurements of peak spirometric flow rate.[127,137] However, these latter techniques lack repeatability and are not as well accepted. Cardiac biomarkers may also gauge success of β-blockade in clinical patients, but they are not helpful in the context of healthy experimental dogs.[138]

2. Atenolol Pharmacodynamics

Atenolol is a β-adrenergic receptor antagonist that blocks activation of the G-protein binding signal transduction cascade.[8] Atenolol pharmacodynamics are evaluated through effects on heart rate, electrocardiogram (ECG), or blood pressure.[19] Previous studies of sympathetic stimulation to assess degree of β-blockade primarily have been performed in cats. One such study found that for cats with resting heart rates between 180-240 bpm (average 208 bpm), oral atenolol administration resulted in statistically significant decreases in resting heart rate by 20 bpm at 6 hours and 26 bpm at 12 hours.[21] In that same study, there was no significant differences in heart

response to four different isoproterenol doses (0.25, 0.5, 1 and 2 mg/kg), however the response to isoproterenol did differ with respect to the time after oral administration (decreased response to isoproterenol of 15 bpm at 12 hours vs 18 bpm at 6 hours).[21] These heart rate blunting effects were associated with widely varying atenolol plasma concentrations between 260-897 ng/ml despite consistent heart rates less than or equal to 170 bpm.[4] In healthy people there is no compounding of β-effects or increased risk of β_2-stimulation with repeated administration of atenolol.[23] In a study of laboratory dogs, 6 mg/kg oral or repeated intravenous injections of atenolol did not significantly affect blood pressure or basal heart rate.[139] Atenolol did modify the response to isoproterenol-induced tachycardia, but the dose used was 6 times higher than the published oral dose recommendations for clinical use.[139] There is some correlation between β-blocker plasma dose and effect, however there is increased species and individual variation with regard to given plasma concentrations and the associated quantitative heart rate response.[110,127] No studies have compared breed-associated differences in β-blocker effects. Further studies are needed to elucidate atenolol pharmacodynamics in dogs.

F. Heart rate measurement in dogs

In health, heart rate is determined by the primary pacemaker of the heart, the sinoatrial node. The rate of spontaneous depolarization is determined by sympathetic and parasympathetic inputs as well as medication effects.[72] Heart rate can be determined by auscultation or more typically from ECG. Instantaneous heart rate, determined from the reciprocal of the RR interval, is representative if the cardiac rhythm is regular.[140] The mean heart rate is defined by the number of complexes that occur in an interval, for example 6 seconds, that is a divisor of 60.[140]

G. Isoproterenol

Isoproterenol (Isoprenaline) is a mixed β-agonist (β1 > β2 stimulation) administration of which mimics some effects of exercise including tachycardia, bronchodilation, peripheral vasodilation and increases in inotropic state.[7,137,141,142] It is primarily administered intravenously (constant rate infusion at 0.01 to 0.1 ug/kg/min), but has been used as an inhaled preparation.[137,140] Intravenous doses are primarily excreted unchanged in the urine.[143] Adverse effects include tachycardia, hypotension, and cardiac arrhythmias.[7] Isoproterenol is used clinically for the treatment of β-blocker overdose, severe bradycardia (especially 3rd degree AV block), low heart rate with high peripheral resistance, and other conditions of poor systolic function.[7,140]

Isoproterenol is used experimentally to test β-receptor effects. One study of healthy, mixed breed, anesthetized dogs found that heart rates plateaued 15 minutes after initiation of a constant rate of infusion (CRI) of isoproterenol and it took 10-15 minutes for heart rates to return to baseline after CRI was discontinued.[137] Bolus doses of 0.27 to 0.64 ug/kg increased heart rate from 22-80 bpm above resting heart rate and half- life ranged from 0.5 to 2.5 minutes.[143] Studies of the effects of isoproterenol in humans suggest that isoproterenol activity occurs through direct cardiac β-agonist stimulation as opposed to vagal withdrawal.[131] The dose required to increase heart rate by greater than 40 bpm varies across studies from 0.05 to 1 ug/kg/min.[137] In dogs, infusions of isoproterenol from 0.007 – 0.175 ug/kg/min CRI are associated with heart rates that range from 120-250 bpm.[137] Individual variation exists for human response to isoproterenol, however there is a maximum heart rate increase of greater than 40-42 bpm beyond which there is an increase in arrhythmias.[131] A maximum response is not known for dogs. There is no

bronchodilator effect or heart rate tachyphylaxis seen in healthy dogs, however there may be experimental conditions or patient populations where this is not the case.[137] Isoproterenol's tachycardia response is due to direct stimulation of the sinoatrial node and due to indirect increases in heart rate to compensate for isoproterenol's vasodilatory effects (reflex mediated tachycardia).[136] β-blockers studies may not yield the same results when tested on exercising subjects compared to isoproterenol treated subjects because reflex-mediated tachycardia is not blocked by β-blockers.[136] Isoproterenol treatment with aggressive β-blockade may not have the same positive inotropic effect as exercise because the myocardium needs both increased heart rate and contractive response to generate an increase in systolic performance during sympathetic stimulation.[144] In one study, atenolol 0.3 mg/kg IV inhibited a chronotropic response to 0.3 ug/kg IV isoproterenol when administered to anesthetized healthy dogs (this dose of isoproterenol increased heart rate > 50% of basal rate).[145] Isoproterenol response is often performed as a constant rate infusion to ensure a consistent heart rate response during the ECG recording.[54,146,147]

H. Literature Review Conclusion

Atenolol has been used based on the theory that it should limit the cardiotoxic effects of catecholamines, reduce oxygen demand, improve left ventricular function, and improve quality of life.[91] The efficacy of β-receptor blockade depends on a variety of factors. In people, multiple studies have compared the effects of different β-blockers. Despite a theoretic similarity of action, there is a marked difference in response between β-blocker drugs. Atenolol has no

experimentally confirmed duration of effect in dogs. I propose a study using isoproterenol to assess the effects of atenolol on heart rate in healthy dogs over 24 hours.

III. Primary Research Manuscript

Duration of β-blockade associated with once-daily oral administration of atenolol in healthy dogs

A. Abstract

Objective: Dosing intervals of 12 and 24 hours for atenolol have been recommended, but an evidentiary basis is lacking. To test the hypothesis that repeated, once-daily oral administration of atenolol attenuates the heart rate response to isoproterenol for 24 hours, we performed a double-blind, randomized, placebo-controlled cross-over experiment.

Animals: Twenty healthy dogs

Procedures: Dogs were randomly assigned to receive either placebo (P) and then atenolol (A), [1 mg/kg PO q24h] or vice versa. Treatment periods were 5-7 days; time between periods was 7 days. Heart rates (bpm) at rest (HR_r) and during constant rate [0.2 μg/kg/min] infusion of isoproterenol (HR_i) were electrocardiographically obtained 0, 0.25, 3, 6, 12, 18, and 24 hours after final administration of drug or placebo. A mixed model ANOVA was used to evaluate the effects of treatment (Tr), time after drug or placebo administration (t), interaction of treatment and time (Tr*t) as well as period and sequence on HR_r and HR_i.

Results: Sequence or period effects were not detected. There was a significant effect of Tr ($p < 0.0001$) and Tr*t ($p < 0.0001$) on HR_i. Atenolol significantly attenuated HR_i for 24 hours but did so maximally at 3 hours (least squares means ± SE, A: 146±5 bpm, P: 208±5 bpm); the effect at 24 hours was small (A: 193±5, P: 206±5). Atenolol had a small but significant effect ($p < 0.0001$) on HR_r.

Conclusions and Clinical Relevance: The results of this study support a dosing interval that is less than 24 hours.

B. Introduction

Atenolol is a selective antagonist of β_1-receptors that limits myocardial oxygen consumption and might preserve myocardial function by decreasing heart rate and contractility.[1–3] In humans, atenolol does not have an appreciable effect on resting heart rate at clinically used doses, but it attenuates tachycardia that develops in response to sympathetic stimulation.[1] Cardiac diseases in veterinary patients that are treated with atenolol include aortic stenosis, pulmonic stenosis, tachyarrhythmias, hypertrophic obstructive cardiomyopathy, systemic hypertension, hyperthyroidism, and systolic dysfunction.[4–9]

Canine doses of 0.2-1 mg/kg PO and dosing intervals of both 12 and 24 hours have been recommended.[10] The elimination half-life of atenolol in healthy dogs is 3-7 hours and peak plasma concentration occurs 1-2 hours after oral dosing.[11] Atenolol is excreted unchanged in the urine.[12] However, pharmacokinetic variables do not accurately predict pharmacodynamic effects of β-blockers, and serial evaluation of serum concentration is not a recommended monitoring strategy.[13,14] Optimal dosing intervals are determined by the duration of β-blockade after oral atenolol administration. The magnitude of increase in heart rate induced by isoproterenol administration is a surrogate measure of β-blockade that has been used in investigations of β-adrenergic antagonists.[15–17] Determination of an appropriate dosing interval is important because abrupt withdrawal of β-blockade is associated with adverse effects including the development of tachycardia, and has a negative impact on prognosis in patients with cardiac disease. More specifically, deleterious cardiovascular effects of abrupt β-blocker withdrawal have been

documented in humans with hypertrophic cardiomyopathy and with systemic hypertension.[18–20] The pathogenesis of these effects is believed to involve a hypersensitivity to catecholamines that reflects the upregulation of adrenergic receptors associated with chronic β-blockade.[21] Studies of the use of β-blockers for heart rate and blood pressure control in human patients emphasize the importance of consistent pharmacologic activity rather than a specific dose.[22,23] Although the pharmacokinetics of atenolol are known, pharmacodynamics of β-blockade for dogs receiving oral atenolol at commonly prescribed doses have been incompletely described.

This study's hypothesis is repeated, once-daily oral administration of 1 mg/kg atenolol attenuates the heart rate response to isoproterenol for 24 hours in healthy dogs. The secondary hypothesis was: atenolol administration does not affect resting heart rate.

C. Materials and Methods

Ten male and ten female, purpose-bred, young adult mixed breed dogs weighing between 8.8 and 12.4 kg (average 10.85 kg) were used in a double-blind, randomized, placebo-controlled crossover experiment. All dogs were determined to be healthy based on the results of physical examination and evaluation of a complete blood count, a plasma chemistry panel, and urinalysis. Dogs were randomized to receive either placebo (P) and then 12.5 mg atenolol (A) orally once daily, or vice versa. Atenolol, and the methylcellulose that served as placebo, were administered in identical gelatin capsules with a small volume of commercial dog food in the morning at approximately the same time every day. Heart rate data were acquired after 5-7 days of repeated oral administration of atenolol. The washout period between treatment periods was 7 days. The investigation was approved by the Institutional Animal Care and Use Committee (IACUC) of Virginia-Maryland College of Veterinary Medicine.

All dogs were acclimated to electrocardiographic examination before collection of heart rate data. On the day of data acquisition, a resting ECG was recorded at time 0. Then, a 20G, 1.25" intravenous catheter was placed in a cephalic vein of each dog, and atenolol or placebo was orally administered. For each subsequent recording, 30 seconds of electrocardiographic data were collected after which isoproterenol was intravenously infused at a constant rate of 0.2 μg/kg/min for 5-7 minutes before a 90 second electrocardiographic recording. The assessment of heart rate response to isoproterenol was at 15 minutes, and then at 3, 6, 12, 18, and 24 hours after oral administration of atenolol or placebo.

ECG Analysis

ECGs were recorded and digitally stored for later analysis[a]. Individual recordings were randomly selected from the dataset and the investigator who evaluated the ECG was unaware of dog identity, time of recording, and treatment group assignment. ECG variables consisted of resting heart rate and maximum heart rate after isoproterenol. The software[b] detects successive peaks or nadirs of the ECG signal to determine rate, but each interval was confirmed by visual inspection. Resting heart rate (HR_r) was the average heart rate per minute calculated from a 20-30 second interval. Maximum HR after isoproterenol (HR_i) was the greatest average heart rate from any successive 6 second interval during isoproterenol infusion.

Statistical Analysis

Data were analyzed using a linear mixed model that included the fixed effects of time (t), treatment (Tr), period, and sequence as well as the random effects of dog on the response variable, heart rate (HR_r or HR_i). The interaction between time and treatment were further analyzed to compare treatment groups at each time point and to compare time points within each

treatment group. P-values from two-way comparisons were adjusted for multiple comparisons using Tukey's procedure. Least squares mean (LSM) and standard error (SE) are reported for all data except where noted. A $p \leq 0.05$ was considered significant. Analysis of residuals confirmed that the assumptions of the analyses were met. Analyses of data were performed using commercially available computer software[c].

D. Results

None of the dogs developed clinical signs of illness during the study. Isoproterenol administration was associated with the occurrence of occasional premature ventricular and supraventricular complexes, but sustained pathologic tachyarrhythmia was not recorded. Neither the effects of sequence nor period were significant (Table 1).

response variable	sequence	period	time	treatment	time*period	time*treatment
HR_i	0.734	0.6321	<0.0001	<0.0001	0.2691	<0.0001 ^
HR_r	0.9995	0.3309	<0.0001	<0.0001	0.0672	0.1324

Table 1: A mixed model ANOVA was used to evaluate the effects of fixed explanatory variables on response variables recorded after oral administration of atenolol. HR_r = resting heart rate, HR_i = heart rate during isoproterenol infusion, sequence = sequence of atenolol or placebo administration, period = periods of drug or placebo administration, time = time after oral administration of atenolol, treatment = assignment to atenolol or placebo, time*period = interaction of time and period, time*treatment = interaction of time and treatment assignment

The relationships between time and HR_i and between time and HR_r are graphically demonstrated in Figures 1 and 2.

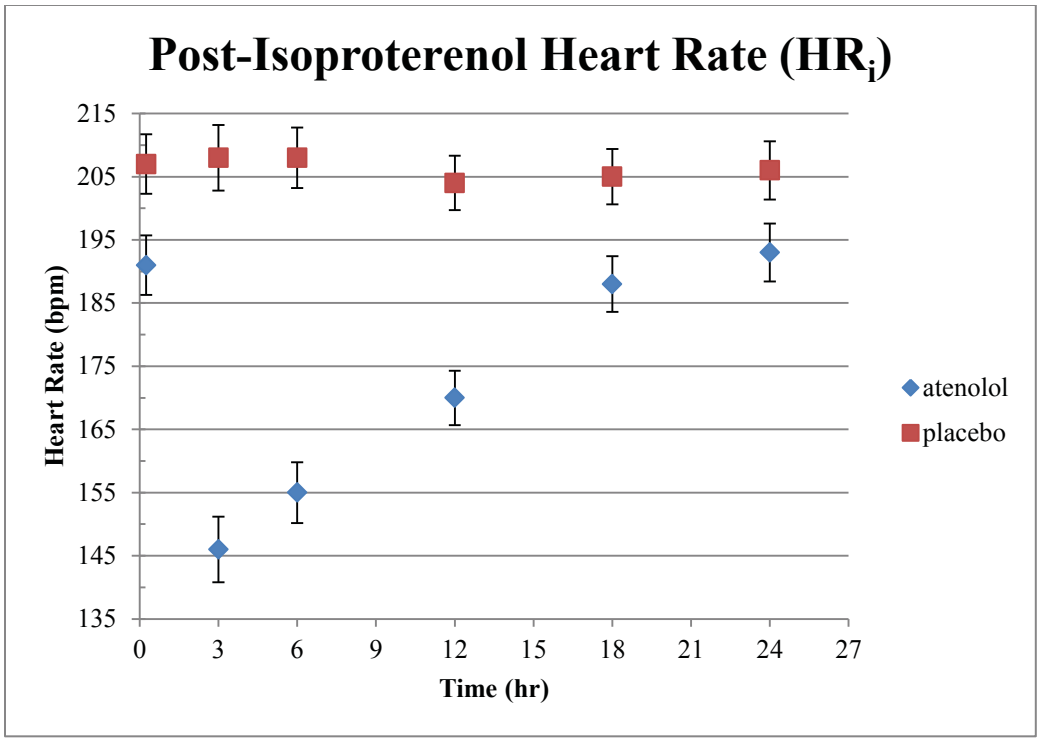

Figure 1 – This figure demonstrates the relationship between heart rate during isoproterenol infusion and time in healthy dogs. Data points are least square means (maximum heart rate) with standard error bars. Atenolol treatment resulted in significant heart rate attenuation compared to placebo at all time points.

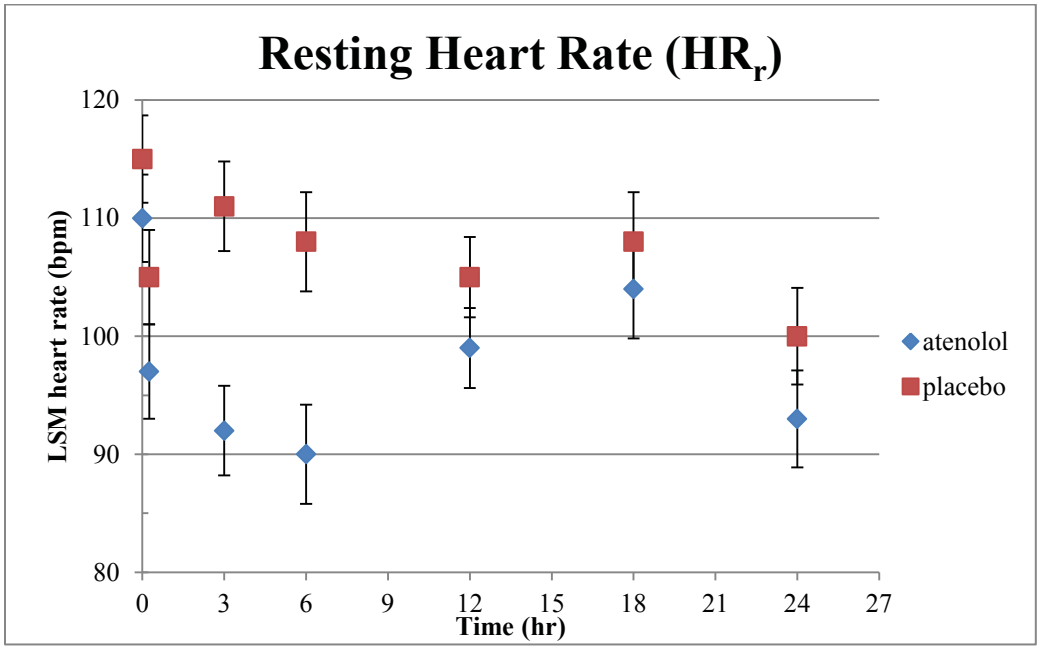

Figure 2 – This figure demonstrates the relationship between resting heart rate and time in healthy dogs. Data points are least square means (resting heart rate) with standard error bars. Atenolol treatment significantly attenuated resting heart rate only at 3 and 6 hours after administration.

There was a significant interactive effect of treatment and time on HR_i (Pr <0.0001). Atenolol significantly attenuated heart rate response to isoproterenol at all time points (0.25, 3, 6, 12, 18, and 24 hours after atenolol administration). The magnitude of heart rate attenuation was greatest at 3 hours. For dogs that received atenolol, HR_i at 3, 6, and 12 hours differed significantly from heart rates at all other times ($p<0.0001$, except for the comparison of HR_i at 3 and 6 hours, for which $P = 0.3395$). Heart rate during isoproterenol challenge did not significantly vary with time for dogs that received placebo (Table 2). There were statistically significant effects of time ($p<0.0001$) and treatment ($p<0.0001$) on HR_r, but treatment and time did not have a significant interactive effect ($p=0.1324$). Atenolol treatment resulted in lower HR_r, but only at hours 3 ($p<0.0001$) and 6 ($p=0.0008$) were statistically significant differences from placebo detected (Table 3).

Time (h)	**atenolol HR_i (bpm)**	**placebo HR_i (bpm)**	**p-value**
0.25	191 ± 4.7	207 ± 4.7	0.0023
3	146 ± 5.2	208 ± 5.2	<0.0001
6	155 ± 4.8	208 ± 4.8	<0.0001
12	170 ± 4.3	204 ± 4.3	<0.0001
18	188 ±4.4	205 ± 4.4	0.0002
24	193 ± 4.6	206 ± 4.6	0.0061

Table 2: Heart rate during isoproterenol (HR_i) infusion expressed as least square means ± SE. Time = time from last oral administration of atenolol or placebo. HR_i = heart rate during isoproterenol infusion, p-values relate to the difference between HR_i for atenolol and placebo at each time point from a mixed model ANOVA.

time	atenolol HR_r (bpm)	placebo HR_r (bpm)	p-value
0	110 ± 3.7	115 ± 3.7	0.17
0.25	97 ± 4.0	105 ± 4.0	0.1
3	92 ± 3.8	111 ± 3.8	<0.0001*
6	90 ± 4.2	108 ± 4.2	0.0008*
12	99 ± 3.4	105 ± 3.4	0.0757
18	104 ± 4.2	108 ± 4.2	0.3469
24	93 ± 4.1	100 ± 4.1	0.146

Table 3: Resting heart rate expressed as least square means ± SE. HR_r = resting heart rate, time = time from last oral administration of atenolol or placebo. p-values relate to the difference between HR_r for atenolol and placebo at each time point from a mixed model ANOVA.

E. Discussion

Relative to placebo, repeated once-daily administration of atenolol significantly attenuates heart rate response to isoproterenol for 24 hours. The magnitude of heart rate attenuation varies over the 24 hours period with peak effect at 3 hours. Although the magnitude of heart rate attenuation that is associated with beneficial effects of β–blockade has not been determined, the effect of atenolol 24 hours after dosing is small. Atenolol also had a small, but statistically significant effect relative to placebo, on resting heart rate obtained 3 and 6 hours after oral administration.

Atenolol is a competitive antagonist of β-adrenergic receptors.[1] The antagonist effect is selective, in that β_1-receptors, which are expressed to the greatest extent in cardiac tissue, are affected to a greater degree than are β_2-receptors. Predictably, pharmacodynamic effects of β-

blockade include bradycardia, slowing of atrioventricular conduction and negative inotropism although of course, these effects are indirect. In fact, β-blockade prevents the positively chronotropic, dromotropic and inotropic effects that would otherwise result from β-adrenergic agonism.[1] Atenolol in the dog has moderate bioavailability and is excreted largely unchanged in the urine.[11,12] The elimination half-life after oral administration is 3.2- 6 hours in the dog and is independent of dose.[11,12] Atenolol is used as an anti-hypertensive agent in humans, although anecdotal evidence suggests that it has limited efficacy in the management of canine hypertension. In dogs, atenolol has been used in the medical management of subvalvular aortic stenosis, pulmonic stenosis and ventricular tachyarrhythmias associated with arrythmogenic right ventricular cardiomyopathy. In most clinical studies, a dosing interval of 12 hours was used but once daily dosing has also been described.[5,6,24] A veterinary formulary provides 12 and 24 hour dosing intervals.[10]

Although resting heart rate was also determined, the effect of atenolol in this study primarily was inferred from the heart rate response to isoproterenol infusion. This study was designed as a placebo-controlled, blinded cross-over experiment. Relative to parallel studies, cross-over designs increase statistical power, but are subject to period and sequence effects. The former relate to within-subject changes that occur over time but independent of an intervention. Sequence effects occur when the order of treatment has an effect on response variables and in pharmacodynamics studies, generally reflect inadequate withdrawal times resulting in carryover of drug effect into the subsequent treatment period. In this study, the two treatment periods were separated by 7 days (28 half-lives). The effect of period and sequence were included in the statistical model and significant effects were not detected.

Heart rate response to isoproterenol is a practical way to quantify β_1–blockade. Alternate methods include administration of other sympathomimetic drugs and exercise testing. Isoproterenol response was evaluated during a constant rate infusion to ensure a consistent heart rate response and the infusion rate was selected based on previously published data. More specifically, it has been determined that infusion of isoproterenol at rates between 0.13 and 0.28 μg/kg/min causes heart rates to exceed 180 bpm in healthy dogs.[16] Other investigators have used considerably higher infusion rates to evaluate β-blockade, but in these studies, isoproterenol was administered only to dogs that had received β-adrenergic antagonists and we were mindful of the possible adverse effects of high doses of sympathomimetic drugs to subjects that had received placebo.[17,25] At all time points, mean heart rate of subjects receiving placebo exceeded 200 bpm during isoproterenol infusion, while mean resting heart rate was close to 110 bpm; these observations provide indirect evidence of substantive β-adrenergic stimulation.

There are few published data that are directly comparable to our own. A canine model of induced mitral valve regurgitation has been used to demonstrate beneficial effects of chronic oral atenolol administration.[25] Although the bodyweights of the dogs were not provided, it is likely that atenolol dose of 50 mg/dog/day was higher than the one we used, because 100% attenuation of heart rate increase in response to a 4 ug/kg/min isoproterenol infusion was observed. Based on the reported left ventricular volumes at baseline, it is likely that the dogs included in this investigation weighed approximately 27 kg and that the daily atenolol dose was therefore close to 2mg/kg orally; we studied a dose of 1 mg/kg.

Relative to placebo, once daily doses of atenolol significantly decreased resting heart rate only at 3 and 6 hours after administration. Relative activities of the sympathetic and parasympathetic nervous systems modulate heart rate and because of this, the effect of β-

blockade on resting heart rate is dependent not only on pharmacodynamic effect, but also on prevailing autonomic tone. Vagal effects predominate in resting dogs; the intrinsic sinus rate – that is, sinus rate during complete autonomic blockade – is higher than resting heart rate, and the magnitude of the heart rate effect of parasympatholysis exceeds that of sympatholysis.[26] It is therefore unsurprising that we observed only a small decrease in heart rate after administration of atenolol. This finding is consistent with other investigations of β-blockade.[15,16] Recently published data provide evidence that heterogeneity of the canine genome may explain variable responses to adrenergic stimulation and therefore, β- blockade.[27] Genetic screening was not performed during our study, but it is possible that this factor contributed to variation in our data.

The results of this study must be considered in the context of its limitations. Our study sample consisted of healthy, mixed-breed dogs; the results may not be applicable to genetically diverse population of older clinical patients that might concurrently receive medications or have co-morbidities that interfere with the action, elimination or absorption of atenolol. Patients with some forms of cardiac disease have adrenergic activation that is unopposed by vagal restraint, while in contrast, parasympathetic activity dominates in healthy dogs.[26,28,29] β-agonism resulting from an infusion of isoproterenol might not replicate the endogenous adrenergic stimulation in clinical cases. Furthermore, while attenuation of the effect of an exogenous agonist is a practical method of quantifying β –blockade, there is no direct correspondence between this attenuation and clinically relevant effects such as a decrease in resting heart rate that might be observed in patients with heart disease. Ambulatory electrocardiographic monitoring might have better reflected average resting heart rate, but it was impractical for our study. We also investigated only effects on heart rate, not other cardiovascular functional variables. However, despite, these limitation, based on what is known of the pharmacokinetics of atenolol, it seems unlikely that the

duration of drug effect would differ markedly in patients with heart disease, except perhaps when there is concurrent renal dysfunction.[10,11,12,14]

In healthy, conscious beagle hounds, orally administered atenolol significantly attenuates heart rate response to β-adrenergic receptor stimulation for 24 hours, but the effect after 12 hours is relatively small. Oral administration of atenolol causes a time-dependent decrease in resting heart rate such heart rates at 3 and 6 hours after administration are significantly different when compared to those after placebo administration. Based on knowledge that clinical endpoints for therapy require more of a reduction in heart rate, our findings suggest that the dose interval of atenolol should be less than 24 hours.

Footnotes:

a. MP150 Analogue to Digital Converter, BioPac Systems Inc., Goleta, CA

b. AcqKnowledge Software, BioPac Systems Inc., Goleta, CA

c. SAS Ver.9.2; SAS Institute Inc. Cary, NC

F. References

1. Westfall TC, Westfall DP. Adrenergic Agonists and Antagonists. In: Brunton L, Lazo JS, Parker KL, eds. Goodman and Gilman's Pharmacologic Basis of Therapuetics. 11th ed. New York: McGraw-Hill; 2006:237–296.

2. Bristow MR. beta-adrenergic receptor blockade in chronic heart failure. Circulation 2000;101:558–569. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10662755.

3. Cleland JG. Beta-blockers for heart failure: why, which, when, and where. Med Clin North Am 2003;87:339–371. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12693729.

4. Eason BD, Fine DM, Leeder D, et al. Influence of Beta blockers on survival in dogs with severe subaortic stenosis. J Vet Intern Med 2014;28:857–862. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24597738.

5. Monnet E, Orton EC, Gaynor JS, et al. Open resection for subvalvular aortic stenosis in dogs. J Am Vet Med Assoc 1996;209:1255–1261. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8837645.

6. Meurs KM, Spier AW, Wright NA, et al. Comparison of the effects of four antiarrhythmic treatments for familial ventricular arrhythmias in Boxers. J Am Vet Med Assoc 2002;221:522–527. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12184702.

7. Schober KE, Zientek J, Li X, et al. Effect of treatment with atenolol on 5-year survival in cats with preclinical (asymptomatic) hypertrophic cardiomyopathy. J Vet Cardiol 2013;15:93–104. Available at: http://www.sciencedirect.com/science/article/pii/S1760273413000532.

8. Brown S, Atkins C, Bagley R, et al. Guidelines for the Identification, Evaluation, and Management of Systemic Hypertension in Dogs and Cats. J Vet Intern Med 2007;21:542–558. Available at: http://dx.doi.org/10.1111/j.1939-1676.2007.tb03005.x.

9. Abbott JA. Beta-blockade in the management of systolic dysfunction. Vet Clin North Am - Small Anim Pract 2004;34.

10. Papich MG. Saunders Handbook of Veterinary Drugs: Small and Large Animal. 3rd ed. St. Louis Missouri: Elsevier Saunders; 2011.

11. McAinsh J, Holmes BF. Pharmacokinetic studies with atenolol in the dog. Biopharm Drug Dispos 1983;4:249–261.

12. Reeves PR, Barnfield DJ, Longshaw S, et al. Disposition and Metabolism of Atenolol in Animals. Xenobiotica 1978;8:305–311. Available at: https://doi.org/10.3109/00498257809060955.

13. Jurgens G, Graudal NA, Kampmann JP. Therapeutic Drug Monitoring of Antiarrhythmic Drugs. Clin Pharmacokinet 2003;42:647–663.

14. Talbert RL. Pharmacokinetics and pharmacodynamics of beta blockers in heart failure. Hear Fail Rev 2004;9:131–137. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15516861.

15. Abbott JA, Broadstone R V, Ward DL, et al. Hemodynamic effects of orally administered carvedilol in healthy conscious dogs. Am J Vet Res 2005;66:637–641. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15900944.

16. Gordon SG, Arsenault WG, Longnecker M, et al. Pharmacodynamics of Carvedilol in Conscious, Healthy Dogs. J Vet Intern Med 2006;20:297–304. Available at: http://dx.doi.org/10.1111/j.1939-1676.2006.tb02860.x.

17. Uechi M, Sasaki T, Ueno K, et al. Cardiovascular and renal effects of carvedilol in dogs with heart failure. J Vet Med Sci 2002;64:469–475. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12130829.

18. Houston MC, Hodge R. Beta-adrenergic blocker withdrawal syndromes in hypertension and other cardiovascular diseases. Am Heart J 1988;116:515–523.

19. Swedberg K, Hjalmarson A, Waagstein F, et al. Adverse effects of beta-blockade withdrawal in patients with congestive cardiomyopathy. Br Hear J 1980;44:134–142. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=6107091.

20. Gilligan DM, Chan WL, Stewart R, et al. Adrenergic hypersensitivity after beta-blocker withdrawal in hypertrophic cardiomyopathy. Am J Cardiol 1991;68:766–772. Available at: http://www.sciencedirect.com/science/article/pii/000291499190651Z.

21. Ross PJ, Lewis MJ, Sheridan DJ, et al. Adrenergic hypersensitivity after beta-blocker withdrawal. Br Heart J 1981;45:637–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6114739%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC482576.

22. Flannery G, Gehrig-Mills R, Billah B, et al. Analysis of Randomized Controlled Trials on the Effect of Magnitude of Heart Rate Reduction on Clinical Outcomes in Patients With Systolic Chronic Heart Failure Receiving Beta-Blockers. Am J Cardiol 2008;101:865–869. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0002914907022527.

23. McAlister FA, Wiebe N, Ezekowitz JA, et al. Meta-analysis: beta-blocker dose, heart rate reduction, and death in patients with heart failure. Ann Intern Med 2009;150:784–794.

24. Meurs KM, Lehmkuhl LB, Bonagura JD. Survival times in dogs with severe subvalvular aortic stenosis treated with balloon valvuloplasty or atenolol. J Am Vet Med Assoc 2005;227:420–424. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16121608.

25. Tsutsui H, Spinale FG, Nagatsu M, et al. Effects of chronic beta-adrenergic blockade on the left ventricular and cardiocyte abnormalities of chronic canine mitral regurgitation. J Clin Invest 1994;93:2639–2648. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7911128.

26. Cavero I, Riggenbach H, Wall M, et al. Analysis of cardiac chronotropic responses to some autonomic blocking agents in conscious trained dogs. Eur J Pharmacol 1976;39:193–202.

27. Meurs KM, Stern JA, Reina-Doreste Y, et al. Impact of the canine double-deletion beta1 adrenoreceptor polymorphisms on protein structure and heart rate response to atenolol, a beta1-selective beta-blocker. Pharmacogenet Genomics 2015;25:427–431. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26065525.

28. Ware WA, Lund DD, Subieta AR, et al. Sympathetic activation in dogs with congestive heart failure caused by chronic mitral valve disease and dilated cardiomyopathy. J Am Vet Med Assoc 1990; 197:1475-1481. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2272879.

29. Zucker IH, Wang W. Modulation of baroreflex and baroreceptor function in experimental heart failure. Basic Res Cardiol 1991; 86 Suppl 3: 133-148. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1781763.

IV. CONCLUSION

Atenolol significantly attenuates heart rate response to β-adrenergic stimulation for 24 hours. The pharmacodynamic response parameter heart rate was assessed using isoproterenol CRI administration because β-adrenergic blockade does not have a marked effect on resting heart over time. This is just one step to aid practical prescribing of atenolol in veterinary medicine. Many future studies need to be performed to determine the dose, administration interval, and whether there is a beneficial effect for atenolol in different disease processes as well as individual breeds of dogs.

V. REFERENCES

1. Plumb DC. Plumb's veterinary drug handbook. Stockholm, Wis.; Ames, Iowa: PharmaVet ; Distributed by Blackwell Pub.; 2008.
2. Fox PR, Sisson D, Moïse NS. Textbook of canine and feline cardiology : principles and clinical practice, 2nd ed. Philadelphia: Saunders; 1999;xiv. 955 p.
3. Tenormin/atenolol (2016). In: PDR Digital Drug Database. Retrieved from http://www.pdr.net/.
4. MacGregor J, et al. Comparison of pharmacodynamic variables following oral versus transdermal administration of atenolol to healthy cats. Am J Vet Res 2008;69:39-44.
5. Katz AM. Physiology of the Heart, 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2011.
6. Najafi A, Sequeira V, Kuster DW, et al. β-adrenergic receptor signalling and its functional consequences in the diseased heart. Eur J Clin Invest 2016;46:362-374.
7. Opie LH, Gersh BJ. Drugs for the heart, 7th ed. Philadelphia: Saunders; 2009;ix, 502 p.
8. Hsu W. Handbook of Veterinary Phamacology. Ames, IA: Wiley-Blackwell; 2008.
9. Molina C, et al. Interventricular differences in beta-adrenergic responses in the canine heart: role of phosphodiesterases. J Am Heart Assoc 2014;3:2-15.
10. Frishman W. Beta-adrenergic receptor blockers: adverse effects and drug interactions. Hypertension 1988;suppl II:II21-II29.
11. Walden RJ. Withdrawal phenomena after atenolol and bopindolol: haemodynamic responses in healthy volunteers. British Journal of Clinical Pharmacology 1990;30:557-565.
12. Walden RJ. Withdrawal of beta-blocking drugs. The American Heart Journal 1982;104:515-520.
13. Chang DHT, Einstein R. Changes in cardiovascular responsiveness to dopexamine and β1- and β2-adrenoceptor function after the chronic treatment of β-adrenoceptor antagonists and agonists in anaesthetized dogs. Journal of Autonomic Pharmacology 1996;16:269-280.
14. Teichert M, Hofman A, Witteman J, et al. Discontinuation of beta-blockers and the risk of myocardial infaction in the elderly. Drug Safety 2007;30:541-549.
15. Reeves PR, Barnfield DJ, Longshaw S, et al. Disposition and Metabolism of Atenolol in Animals. Xenobiotica 1978;8:305-311.
16. Kates R. Plasma level monitoring of antiarrhythmic drugs. Am J Cardiol 1983;52:*C-13C.
17. Goodman LS, Gilman A BL, Lazo JS, et al. Goodman & Gilman's the Pharmacologic Basis of Therapeutics, 11th ed. New York: McGraw-Hill; 2006.
18. Mehvar R, Brocks D. Stereospecific pharmacokinetics and pharmacodynamics of beta-adrenergic blockers in humans. J Pharm Pharmaceut Sci 2001;4:185-200.
19. Jurgens G, NA G, Kampmann J. Therapeutic Drug Monitoring of Antiarrhythmic Drugs. Clin Pharmacokinet 2003;42:647-663.
20. Zicha S, Tsuji Y, Shiroshita-Takeshita A, et al. Beta-Blockers as Antiarrhythmic Agents. Handbook of Experimental Pharmacology 2006;171:235-266.
21. Quinones M, Dyer DC, Ware WA, et al. Pharmacokinetics of atenolol in clinically normal cats. Am J Vet Res 1996;57:1050-1053.
22. Blomqvist I, et al. Pharmacokinetics and pharmacodynamics of controlled-release metoprolol: a comparison with atenolol. Eur J Clin Pharmacol 1988;33:s19-24.
23. Lipworth B, Irvine N, McDevitt D. The effects of time and dose on the relative β1- and β2-adrenoceptor antagonism of betaxolol and atenolol. British Journal of Clinical Pharmacology 1991;31:154-159.
24. Bauman JL, Talbert RL. Pharmacodynamics of beta-blockers in heart failure: lessons from the carvedilol or metoprolol European trial. J Cardiovasc Pharmacol Ther 2004;9:117-128.
25. Rush J, Freeman L, Fenollosa N, et al. Population and survival characteristics of cats with hypertrophic cardiomyopathy: 260 cases (1990-1999). J Am Vet Med Assoc 2002;220:202-207.
26. Schober KE, Zeintek J, Li X, et al. Effect of treatment with atenolol on 5-year survival in cats with preclinical (asymptomatic) hypertrophic cardiomyopathy. Journal of Veterinary Cardiology 2013:93-104.

27. Jung S, Kittleson M. The effect of atenolol on NT-proBNP and troponin in asymptomatic cats with severe left ventricular hypertrophy because of hypertrophic cardiomyopathy: a pilot study. Journal of Veterinary Internal Medicine 2011;25:1044-1049.
28. Riesen S, Schober K, Cervenec R, et al. Comparison of the Effects of Ivabradine and Atenolol on Heart Rate and Echocardiographic Variables of Left Heart Function in Healthy Cats. Journal of Veterinary Internal Medicine 2011;25:469-476.
29. Jackson B, Adin D, Lehmkuhl L. Effect of atenolol on heart rate, arrhythmias, blood pressure, and dynamic left ventricular outflow tract obstruction in cats with subclinical hypertrophic cardiomyopathy. J Vet Cardiol 2015;17:S296-S305.
30. Payne J, Luis-Fuentes V, Boswood A, et al. Population characteristics and survival in 127 referred cats with hypertrophic cardiomyopathy (1997 to 2005). Journal of Small Animal Practice 2010;51:540-547.
31. Atkins C, Gallo A, Kurzman I, et al. Risk factors, clinical signs and survival in cats with a clinical diagnosis of idiopathic hypertrophic cardiomyopathy: 74 cases (1985-1989). J Am Vet Med Assoc 1992:613-618.
32. Sherrid M. Drug therapy for hypertrophic cardiomyopathy: physiology and practice. Current Cardiology Reviews 2016;12:52-65.
33. Ball W, et al. Long-term survival in patients with resting obstructive hypertrophic cardiomyopathy: comparison of conservative versus invasive treatment. J Am Coll Cardiol 2011;58:2313-2321.
34. Blass K, Schober K, Li X, et al. Acute effects of ivabradine on dynamic obstruction of the left ventricular outflow tract in cats with preclinical hypertrophic cardiomyopathy. J Vet Intern Med 2014;28:838-846.
35. Connolly D, Boswood A. Dynamic obstruction of the left ventricular outflow tract in four young dogs. Journal of Small Animal Practice 2003;44:319-325.
36. Cotrim C, Lopes L, Almeida A, et al. Efficacy of beta-blocker therapy in symptomatic athletes with exercise-induced intra-ventricular gradients. Cardiovasc Ultrasound 2010;8:1-5.
37. Al-Nasser F, Duncan A, Sharma R, et al. Beta-blocker therapy for dynamic left-ventricular outflow tract obstruction. Int J Cardiol 2002;86:199-205.
38. Ozaki K, Sakuma I, Mitsuma K, et al. Effect of cibenzoline and atenolol administration on dynamic left ventricular obstruction due to sigmoid-shaped septum. Circulation Journal 2008;72:2087-2091.
39. Nistri S, Olivotto I, Maron M, et al. Beta-blockers for prevention of exercise-induced left ventricular outflow tract obstruction in patients with hypertrophic cardiomyopathy. Am J Cardiol 2012;110:715-719.
40. Aoki T, Sunahara H, Sugimoto K, et al. Dynamic left ventricular outflow tract obstruction secondary to hypovolemia in a German Shepard Dog with splenic hemangiosarcoma. Journal of Veterinary Medical Science 2015.
41. Paige C, Abbott J, Pyle R. Systolic anterior motion of the mitral valve associated with right ventricular systolic hypertension in 9 dogs. J Vet Cardiol 2007;9:9-14. Epub 2007 Apr 2006.
42. Loureiro J, Smith S, Fonfara S, et al. Canine dynamic left ventricular outflow tract obstruction:assessment of myocardial function and clinical outcome. J Small Anim Pract 2008;49:578-586.
43. Eason B, Fine D, Leeder D, et al. Influence of beta blockers on survival in dogs with severe subaortic stenosis. J Vet Intern Med 2014;28:857-862.
44. Meurs K, Lehmkuhl L, Bonagura J. Survival times in dogs with severe subvalvular aortic stenosis treated with balloon valvuloplasty or atenolol. J Am Vet Med Assoc 2005;227:420-424.
45. Johnson A. Aortic stenosis, sudden death, and the left ventricular baroreceptors. Br Heart J 1971;33:1-5.
46. Johnson MS, Martin M. Results of balloon valvuloplasty in 40 dogs with pulmonic stenosis. Journal of Small Animal Practice 2004;45:148-153.
47. Francis A, Johnson M, Culshaw G, et al. Outcome in 55 dogs with pulmonic stenosis that did not undergo balloon valvuloplasty or surgery. J Small Anim Pract 2011;52:282-288..

48. Estrada A. Pulmonic Stenosis. In: Bonagura J, Twedt D, eds. Kirk's Current Veterinary Therapy XIV, XIV ed. Philadelphia: W.B. Saunders; 2009:752-756.
49. Schrope D. Balloon valvuloplasty of valvular pulmonic stenosis in the dog. Clin Tech Small Anim Pract 2005;20:182-195.
50. Johnson M, Martin M, Edwards D, et al. Pulmonic stenosis in dogs: balloon dilation improves clinical outcome. J Vet Intern Med 2004;18:656-662.
51. Atkins C, Bonagura J, Ettinger S, et al. Guidelines for the Diagnosis and Treatment of Canine Chronic Valvular Heart Disease. Journal of Veterinary Internal Medicine 2009;23:1142-1150.
52. Gordon S, Saunders A, Hariu C, et al. Retrospective review of carvedilol administration in 38 dogs with preclinical chronic valvular heart disease. J Vet Cardiol 2012;14:243-252.
53. Marcondes-Santos M, Tarasoutchi F, Mansur A, et al. Effects of Carvedilol treatment in dogs with chronic mitral valvular disease. J Vet Intern Med 2007;21:996-1001.
54. Tsutsui H, Francis GS, Nagatsu M, et al. Effects of Chronic β-Adrenergic Blockade on the Left Ventricular and Cardiocyte Abnormalities of Chronic Canine Mitral Regurgitation. J Clin Invest 1994;93:2639-2648.
55. Pat B, Killingsworth C, Denney T, et al. Dissociation between cardiomyocyte function and remodeling with beta-adrenergic receptor blockade in isolated canine mitral regurgitation. Am J Physiol Heart Circ Physiol 2008;295:H2321-H2327.
56. Berg T, Piercey B, Jensen J. Role of B1-3-adrenoceptors in blood pressure control at rest and during tyramine-induced norepinephrine release in spontaneously hypertensive rats. Hypertension 2010;55:1224-1230.
57. Brown S, Atkins C, Bagley R, et al. Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. J Vet Intern Med 2007;21:542-558.
58. Bachman ES, Hampton TG, Harveen D, et al. The metabolic and cardiovascular effects of hyperthyroidism are largely independent of beta-adrenergic stimulation. Endocrinology 2013;145:2767-2774.
59. Henik R, Stepien R, Wenholz L, et al. Efficacy of atenolol as a single antihypertensive agent in hyperthyroid cats. Journal of feline medicine and surgery 2008;10:577-582.
60. Ishizaki T, Oyama Y, Suganuma T, et al. A dose ranging study of atenolol in hypertension: fall in blood pressure and plasma renin activity, beta-blockade and steady state pharmacokinetics. Br j clin pharmac 1983;16:17-25.
61. Davies MK, McAinsh J. Tissue atenolol levels following chronic β-adrenoceptor blockade using oral atenolol in dogs. Journal of Pharmacy and Pharmacology 1986;38:316-319.
62. Takita M, Oda Y, Kigoshi S, et al. Effects of propranolol and atenolol on immobilization stress-induced hypertension and down-regulation of central beta-adrenoceptors in rats. Pharmacology Biochemistry and Behavior 1995;50:225-232.
63. Ibrahim M, Mossallam R. Clinical evaluation of atenolol in hypertensive patients. Circulation 1981;64:368-374.
64. Hansson L, Aberg H, Karlberg B, et al. Controlled study of atenolol in treatment of hypertension. Br Med J 1975;2:367-370.
65. Wiysonge C, Bradley H, Volmink J, et al. Beta-blockers for hypertension. Cochrane Database Syst Rev 2017;Jan 20:CD002003.
66. Ripley T, Saseen J. Beta-blockers: a review of their pharmacological and physiological diversity in hypertension. Annals of Pharmacotherapy 2014;48:723-733.
67. Kuyper L, Khan N. Atenolol vs. nonatenolol beta-blockers for the treatment of hypertension:a meta-analysis. Can J Cardiology 2014;30:S47-S53.
68. Dahlof B, Devereux R, Kheldsen S, et al. Atenolol as a comparator in outcome trials in hypertension:a correct choid in the past, but not for the future? Blood Pressure 2007;16:6-12.
69. Amos G, Kerr D, Sernia C, et al. Beta-adrenoceptor antagonism and the hyperthyroid rat heart. J Cardiovasc Pharmacol 1994;24:336-343.

70. Mintz G, Pizzarello R, Klein I. Enhanced left ventricular diastolic function in hyperthyroidism: noninvasive assessment and response to treatment. J Clin Endocrinol Metab 1991;73:146-150.
71. Marriott H, Conover M. Advanced Concepts in Arrhythmias, Third ed. St. Louis: Mosby, Inc; 1998.
72. Meurs KM, et al. Association of Dilated Cardiomyopathy with the Striatin Mutation Genotype in Boxer Dogs. J Vet Intern Med 2013;28:12163.
73. Ware WA. Cardiovascular disease in small animal medicine. London: Manson/The Veterinary Press; 2007.
74. Wright K. Assessment and Treatment of Supraventricular Tachyarrhythmias. In: Bonagura J, Twedt D, eds. Kirk's Current Veterinary Therapy XIV. Philadelphia: W.B. Saunders; 2009:722-726.
75. Conolly M, Kersting F, Dollery C. The clinical pharmacology of beta-adrenoceptor-blocking drugs. Progress in Cardiovascular Diseases 1976;XIX:203-234.
76. Uprichard A, Harron D. Atenolol, but not mexiletine, protects against stimulus-induced ventricular tachycardia in a chronic canine model. Br J Pharmac 1989;96:220-226.
77. Cote E, MacDonald K, Meurs K, et al. Chapter 29: Which Drug for Which Disease? In: Feline Cardiology. Ames: Wiley-Blackwell; 2011:437.
78. Wyse D, Waldo A, DiMarco J, et al. A comparison of rate control and rhythm control in patients with atrial fibrillation. N Engl J Med 2002;347:1825-1833.
79. Carlsson Jö, Miketic S, Windeler Jü, et al. Randomized trial of rate-control versus rhythm-control in persistent atrial fibrillation: The Strategies of Treatment of Atrial Fibrillation (STAF) study. Journal of the American College of Cardiology 2003;41:1690-1696.
80. Roy D, Talajic M, Nattal S, et al. Rhythm control versus rate control for atrial fibrillation and heart failure. N Engl J Med 2008;358:2667-2677.
81. Kishihara J, Niwano S, Niwano H, et al. Effect of carvedilol on atrial remodeling in canine model of atrial fibrillation. Cardiovasc Diagn Ther 2014;4:28-35.
82. Van der Linde H, Van Deuren B, Somers Y, et al. The Electro-Mechanical window:a risk marker for Torsades de Pointes in a canine model of drug induced arrhythmias. Br J Pharmacol 2010;161:1444-1454.
83. Meurs K, Spier A, Wright N, et al. Comparison of the effects of four antiarrhythmic treatments for familial ventricular arrhythmias in Boxers. J Am Vet Med Assoc 2002;221:522-527.
84. Sosunov EA, Gainullin RZ, Moise NS, et al. β1 and β2-adrenergic receptor subtype effects in German shepherd dogs with inherited lethal ventricular arrhythmias. Cardiovascular Research 2000;48:211-219.
85. Sumitomo N, Harada K, Nagashima M, et al. Catecholaminergic polymorphic ventricular tachycardia: electrocardiographic characteristics and optimal therapeutic strategies to prevent sudden death. Heart 2003;89:66-70.
86. Sy R, Gollob M, Klein G, et al. Arrhythmia characterization and long-term outcomes in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 2011;8:864-871.
87. Menken U, Wiegand V, Bucher P, et al. Prophylaxis of ventricular fibrillation after acute experimental coronary occlusion by chronic beta-adrenoceptor blockade with atenolol. Cardiovasc Res 1979;13:588-594.
88. Mann DL, Bristow MR. Mechanisms and Models in Heart Failure: The Biomechanical Model and Beyond. Circulation 2005;111:2837-2849.
89. Bristow MR. β-Adrenergic Receptor Blockade in Chronic Heart Failure. Circulation 2000;101:558-569.
90. Tidholm A. Survival in dogs with dilated cardiomyopathy and congestive heart failure treated with digoxin, furosemide and propranolol: A retrospective study of 62 dogs. Journal of Veterinary Cardiology 2006;8:41-47.
91. Sabbah H. Biologic rationale for the use of beta-blockers in the treatment of heart failure. Heart Failure Reviews 2004;9:91-97.
92. Sabbah H, Sharov V, Gupta R, et al. Chronic therapy with metoprolol attenuates cardiomyocyte apoptosis in dogs with heart failure. J Am Coll Cardiol 2000;36:1698-1705.

93. Mann DL, Kent RL, Parsons B, et al. Adrenergic effects on the biology of the adult mammalian cardiocyte. Circulation 1992;85:790-804.
94. Morita H, Suzuki G, Mishima T, et al. Effects of long-term monotherapy with metoprolol CR/XL on the progression of left ventricular dysfunction and remodeling in dogs with chronic heart failure. Cardiovasc Drugs Ther 2002;16:443-449.
95. Reiken S, Wehrens X, Vest J, et al. Beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. Circulation 2003;107:2459-2466.
96. George I, Sabbah H, Xu K, et al. B-Adrenergic receptor blockade reduces endoplasmic reticulum stress and normalizes calcium handling in a coronary embolization model of heart failure in canines. Cardiovascular Research 2011;91:447-455.
97. Moesgaard SG, Aupperle H, Rajamäki MM, et al. Matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs) and transforming growth factor-β (TGF-β) in advanced canine myxomatous mitral valve disease. Research in Veterinary Science 2014;97:560-567.
98. Aupperle H, Disatian S. Pathology, protein expression and signaling in myxomatous mitral valve degeneration: Comparison of dogs and humans. Journal of Veterinary Cardiology 2012;14:59-71.
99. Martin M. Treatment of congestive heart failure - a neuroendocrine disorder. J Small Anim Pract 2003;44:154-160.
100. Zaca V, Rostogi S, Mishra S, et al. Atenolol is inferior to metoprolol in improving left ventricular function and preventing ventricular remodeling in dogs with heart failure. Cardiology 2009;112:294-302.
101. Bristow M, Ginsburg R, Umans V, et al. Beta 1- and Beta 2-adrenergic-receptor Subpopulations in Nonfailing and Failing Human Ventricular Myocardium: Coupling of Both Receptor Subtypes to Muscle Contraction and Selective Beta 1-receptor Down-Regulation in Heart Failure. Circulation Research 1986;59:297-309.
102. Bohm M, Gierschik P, Jakobs K, et al. Increase of Gialpha in human hearts with dilated but not ischemic cardiomyopathy. Circ J 1990;82:1249-1265.
103. Morimoto A, Hasegawa H, Cheng H-J, et al. Endogenous β3-adrenoreceptor activation contributes to left ventricular and cardiomyocyte dysfunction in heart failure. American Journal of Physiology - Heart and Circulatory Physiology 2004;286:H2425-H2433.
104. Cheng H, Zhang Z, Onishi K, et al. Upregulation of functional beta3-adrenergic receptor in the failing canine myocardium. Circ Res 2001;89:599-606.
105. Nagatsu M, Spinale FG, Koide M, et al. Bradycardia and the Role of β-Blockade in the Amelioration of Left Ventricular Dysfunction. Circulation 2000;101:653-659.
106. Cheng Y, et al. Bradycardic therapy improves left ventricular function and remodeling in dogs with coronary embolization-induced chronic heart failure. J Pharmacology and Experimental Therapeutics 2007;321:469-476.
107. Aronson D, Burger A. Effect of beta-blockade on heart rate variability in decompensated heart failure. Int J Cardiol 2001;79:31-39.
108. MERIT-HF study group, Berthe C, Boutefeu J, et al. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL randomised intervention trial in congestive heart failure. Lancet 1999;353:2001-2007.
109. Al-Gobari M, Khatib CE, Pillon F, et al. Beta-blockers for the prevention of sudden cardiac death in heart failure patients: a meta-analysis of randomized controlled trials. BMC Cardiovascular Disorders 2013;13:52-52.
110. Talbert RL. Pharmacokinetics and pharmacodynamics of beta blockers in heart failure. Heart Fail Rev 2004;9:131-137.
111. Sturm B, Pacher R, Strametz-Juranek J, et al. Effect of β1 blockade with atenolol on progression of heart failure in patients pretreated with high-dose enalapril. European Journal of Heart Failure 2000;2:407-412.
112. Abbott J. Beta-blockade in the management of systolic dysfunction. Veterinary Clinics of North America: Small Animal Practice 2004;34:1157-1170.

113. Jondeau G, Neuder Y, Eicher J, et al. B-CONVINCED: Beta-blocker CONtinuation vs. INterruption in patients with Congestive heart failure hospitalizED for a decompensation episode. European Heart Journal 2009;30:2186-2192.
114. Sabbah HN, Shimoyama H, Kono T, et al. Effects of long-term monotherapy with enalapril, metoprolol, and digoxin on the progression of left ventricular dysfunction and dilation in dogs with reduced ejection fraction. Circulation 1994;89:2852-2859.
115. Dillon A, Dell'Italia L, Tillson M, et al. Left ventricular remodeling in preclinical experimental mitral regurgitation of dogs. J Vet Cardiol 2012;14:73-92.
116. Rush J, Freeman L, Hiler C, et al. Use of metoprolol in dogs with acquired cardiac disease. Journal of Veterinary Cardiology 2002;4:23-28.
117. Oyama MA, Sisson D, Prosek R, et al. Carvedilol in Dogs with Dilated Cardiomyopathy. J Vet Intern Med 2007;21:1272-1279.
118. Trepanier L. Applying the pharmacokinetics to veterinary clinical practice. Vet Clin North Am Small Anim Pract 2013;43:1013-1026.
119. McAinsh J, Holmes B. Pharmacokinetic studies with atenolol in the dog. Biopharm Drug Dispos 1983;4:249-261.
120. Horinouchi T, Morishima S, Tanaka T, et al. Different changes of plasma membrane β-adrenoceptors in rat heart after chronic administration of propranolol, atenolol and bevantolol. Life Sciences 2007;81:399-404.
121. Elfellah MS, Reid JL. Regulation of Beta1 - and Beta2-adrenoceptors Following Chronic Treatment with Beta-adrenoceptor Antagonists. European Journal of Pharmacology 1989;173:85-92.
122. Harms HH, Spoelstra AJG. Cardiac and bronchial β-adrenoceptor antagonistic potencies of atenolol, metoprolol, acebutolol, practolol, propranolol, and pindolol in the anaesthetized dog. Clinical and Experimental Pharmacology and Physiology 1978;5:53-59.
123. Lees P, Cunningham F, Elliott J. Principles of pharmacodynamics and their applications in veterinary pharmacology. J Vet Pharmacol Therap 2004;27:397-414.
124. Toutain P, Lees P. Integration and modelling of pharmacokinetic and pharmacodynamic data to optimize dosage regimens in veterinary medicine. J Vet Pharmacol Therap 2004;27:467-477.
125. Toutain P, Ferran A, Bousquet-Me'Lou A. Species differences in pharmacokinetics and pharmacodynamics. In: Cunningham F, al e, eds. Comparative and Veterinary Pharmacology, Handbook of Experimental Pharmacology. Berlin: Springer-Verlag; 2010.
126. Meurs K, Stern J, Reina-Doreste Y, et al. Impact of the canine double-deletion B1 adrenoreceptor polymorphisms on protein structure and heart rate response to atenolol, a B1-selective beta-blocker. Pharmacogenetics and genomics 2015;24:427-431.
127. McDevitt D. The assessment of beta-adrenoceptor blocking drugs in man. br j clin pharmac 1977;4:413-425.
128. Miller R, Lehmkuhl L, Bonagura J, et al. Retrospective analysis of the clinical utility of ambulatory electrocardiographic (Holter) recordings in syncopal dogs: 44 cases (1991-1995). journal of Veterinary Internal Medicine 1999;13:111-122.
129. Rasmussen C, Vesterholm S, Ludvigsen T, et al. Holter monitoring in clinically healthy cavalier king charles spaniels, wire-hair dachshunds, and cairn terriers. Journal of Veterinary Internal Medicine 2011;25:460-468.
130. Gelzer A, Kraus M, Rishniw M, et al. Combination therapy with digoxin and diltiazem controls ventricular rate in chronic atrial fibrillation in dogs better than digoxin or diltiazem monotherapy: a randomized crossover study in 18 dogs. Journal of Veterinary Internal Medicine 2009;23:499-508.
131. Cleaveland C, Rangno R, Shand D. A standardized isoproterenol sensitivity test: the effects of sinus arrhythmia, atropine and propranolol. Arch Intern Med 1972;130:47-52.
132. Mignatti A, Sims DB, Colombo PC, et al. Resting Heart Rate Does Not Reflect the Degree of Beta-Blockade in Subjects with Heart Failure on Chronic Beta-Blocker Therapy. Cardiovascular Therapeutics 2009;27:42-48.

133. Panina G, Khot U, Nunziata E, et al. Assessment of autonomic tone over a 24-hour period in patients with congestive heart failure:relation between mean heart rate and measures of heart rate variability. Am Heart J 1995;129:748-753.
134. McAlister F, Wiebe N, Ezekowitz J, et al. Meta-analysis: beta-blocker dose, heart rate reduction, and death in patients with heart failure. Ann Intern Med 2009;150:784-794.
135. Tardif J. Heart rate as a treatable cardiovascular risk factor. British Medical Bulletin 2009;90:71-84.
136. Adam KR, Pullman LG, Scholfield PC. Isoprenaline- and exercise-induced tachycardia in the assessment of β-adrenoceptor blocking drugs; a comparison between tolamolol, practolol and propranolol. British Journal of Pharmacology 1973;49:560-563.
137. Minatoya H, Spilker B. Lack of cardiac or bronchodilator tachyphylaxis to isoprenaline in the dog. Br J Pharmac 1975;53:333-340.
138. Stanek B, Frey B, Hülsmann M, et al. Prognostic evaluation of neurohumoral plasma levels before and during beta-blocker therapy in advanced left ventricular dysfunction. Journal of the American College of Cardiology 2001;38:436-442.
139. Himora N, Honma S, Izumi A, et al. Duration and selectivity in adrenoceptor blocking action of a adrenoceptor blocking drug, D-32 in conscious dogs. Naunyn Schmiedebergs Arch Pharmacol 1981;316:19-23.
140. Kittleson MD, Kienle RD. Small animal cardiovascular medicine. St. Louis, MO: Mosby; 1998.
141. Moss AJ, Allen HD. Moss and Adams' heart disease in infants, children, and adolescents : including the fetus and young adult. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.
142. Krasnow N, Rolett EL, Yurchak PM, et al. Isoproterenol and cardiovascular performance. The American Journal of Medicine 1964;37:514-525.
143. Conolly ME, Davies DS, Dollery CT, et al. Metabolism of isoprenaline in dog and man. British Journal of Pharmacology 1972;46:458-472.
144. De Pauw M, Vilaine JP, Heyndrickx GR. Role of force – frequency relation duringAV–block, sinus node block and betaadrenoceptorblock in conscious animals. Basic Research in Cardiology 2004;99:360-371.
145. Coram W, Olson R, Beil M, et al. Effects of metoprolol, alone and in combination with lidocaine, on ventricular fibrillation threshold: comparison with atenolol, propranolol, and pindolol. J Cardiovasc Pharmacol 1987;9:611-621.
146. Gordon S, Arsenault W, Longnecker M, et al. Pharmacodynamics of carvedilol in conscious, healthy dogs. J Vet Intern Med 2006;20:297-304.
147. Abbott J, Broadstone R, Ward D, et al. Hemodynamic effects of orally administered carvedilol in healthy conscious dogs. Am J Vet Res 2005;66:637-641.