Quotes I heard while writing this thesis:

“Daddy, now that you’re done your homework, can we play the Wii?”

– Maxwell Miguel Lih Yeong Gragasin, Age 5

“Go outside, ride bike, ride bike!”

– Aiden Theo Lih Wen Gragasin, Age 2

University of Alberta

ANESTHESIA AND THE AGING VASCULATURE: EFFECTS OF PROPOFOL ON HEMODYNAMICS AND VASCULAR FUNCTION

by

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DEDICATION

I dedicate this work to my nuclear family: to my loving wife Dr. Michelle Chong-Gragasin, to my son number one Maxwell Gragasin, and to my son number two Aiden Gragasin.

ABSTRACT

An increasing life expectancy in Canada has resulted in increasing proportions of elderly patients requiring anesthetic care for surgical procedures. Aging is associated with cardiovascular changes resulting in increased vasoconstriction and hypertension, and medications such as angiotensin converting enzyme (ACE) inhibitors are commonly prescribed for this reason. Propofol, a commonly used general anesthetic agent, is known to cause hypotension that can be exaggerated in the elderly population. Propofol's influence on vascular reactivity has not been investigated in the aging vasculature. Moreover, patients using ACE inhibitors in the perioperative period may exhibit refractory hypotension when given a general anesthetic. Whether propofol differentially alters vascular function in arteries exposed to chronic ACE inhibition is also not known. Therefore, this thesis focused on 1) determining if there is a difference in vasodilating ability between the young and aging vasculature when exposed to propofol, and 2) determining if chronic ACE inhibition differentially alters vasodilation in the presence of propofol in the aging vasculature. Additionally, the utility of Intralipid as a potential treatment for propofol-induced hypotension was investigated in this thesis.

Previous publications suggest that multiple mediators are involved in propofol-induced vascular relaxation. Here, the focus was on nitric oxide (NO) given the age-dependent decrease in NO bioavailability and based on studies

documenting the contribution of NO following propofol administration in young mesenteric arteries, the primary vascular bed studied here. The experiments presented in this thesis demonstrate enhanced bioavailability of NO in the aging vasculature in the presence of propofol. Although NO is important, the aging vasculature chronically treated with ACE inhibitors possesses an enhanced vasodilation in the presence of propofol that results from a non-NO source. Finally, Intralipid is able to reverse propofol-induced vasodilation and hypotension in aging animals, demonstrating its potential to be utilized as a hemodynamic agent following propofol use.

In conclusion, the results presented in this thesis are an important contribution to understanding the effects of propofol, a widely used general anesthetic agent, on the aging vasculature. This is of particular importance given the increasing proportion of the aging population presenting for surgery and requiring the care of the Anesthesiologist.

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACE2	Angiotensin converting enzyme-2
Ang II	Angiotensin II
Ang(1-7)	Angiotensin (1-7)
AT_1	Angiotensin Type 1 Receptor
ANOVA	Analysis of Variance
AUC	Area Under the Curve
BK_{Ca}	Large-conductance calcium-activated potassium channel
Ca^{++}	Calcium ion
Cap	Captopril
cGMP	Cyclic guanosine monophosphate
CNS	Central nervous system
Con	Control
Crem	Cremophor EL®
Cu	Copper
DMSO	Dimethyl sulfoxide
EC	Endothelial cell
EC_{50}	Effective concentration to achieve 50% maximal response
EDH	Endothelial-derived hyperpolarization
EDHF	Endothelial-derived hyperpolarizing factor
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FA	Fatty acid
HUVEC	Human umbilical vein endothelial cell
Inh	Inhibitor
iNOS	Inducible nitric oxide synthase
Intra	Intralipid

Iso	Isoflurane
K^+	Potassium ion
KCl	Potassium chloride
K_{ATP}	ATP-sensitive potassium channel
K_{Ca}	Calcium-activated potassium channel
K_{ir}	Inward rectifier potassium channel
LDL	Low-density lipoprotein
L+M	L-nitro arginine methyl ester + meclofenamate
L-NAME	L-nitro arginine methyl ester
MAP	Mean Arterial Pressure
Meclo	Meclofenamate
Mn	Manganese
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
$O_2{\cdot}^-$	Superoxide anion
$ONOO^-$	Peroxynitrite
PE	Phenylephrine
PG	Prostaglandin
PGI_2	Prostacyclin
PGHS	Prostaglandin H synthase
Prop	Propofol
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
Sal	Saline
sGC	Soluble guanylyl cyclase
SIN-1	3-morpholinosydnonimine
SNS	Sympathetic nervous system
SOD	Superoxide dismutase

TIVA	Total intravenous anesthetic
TXA_2	Thromboxane
VSMC	Vascular Smooth Muscle Cell
Zn	Zinc

CHAPTER 1:

BACKGROUND TO THE DISSERTATION

Portions of this chapter have been published:

Gragasin FS, *Bourque SL, Davidge ST. Vascular aging and hemodynamic stability in the intraoperative period. Front Physiol. 2012;3:74*

Contribution: Gragasin FS and Bourque SL contributed equally to preparation of the manuscript.

1.1 INTRODUCTION

The percentage of the population over the age of 65 is still steadily increasing and is expected to continue in the foreseeable future as life expectancy continues to increase (Figure 1-1),[1] with people over the age of 80 now among the fastest growing subset of the population.[2] This increase in life expectancy is due in part to advancements in medical care and technology. As a result, there is a greater proportion of the aging population seen perioperatively under the care of the Anesthesiologist.

Currently, cardiovascular diseases are the most common cause of death among elderly patients in the Western world, accounting for more than 40% of all mortalities among people aged 65-74, and 60% of people 85 years and older. [3] Moreover, almost 70% of the population over the age of 70 has some degree of hypertension, which reflects the fact that blood pressure increases with age.[4-5] Despite the increased risk of cardiovascular disease associated with aging, evidence indicates that health among the elderly population is improving, as illustrated by falling rates of ischemic heart disease, heart failure, and cerebrovascular disease among people over the age of 80.[6-7] This improvement is undoubtedly due to increased awareness and better management of chronic conditions from health care professionals and patients alike. The inevitable consequence of increasing life expectancy is that the

aging population constitutes a greater proportion of patients undergoing medical treatments and presenting for surgery.

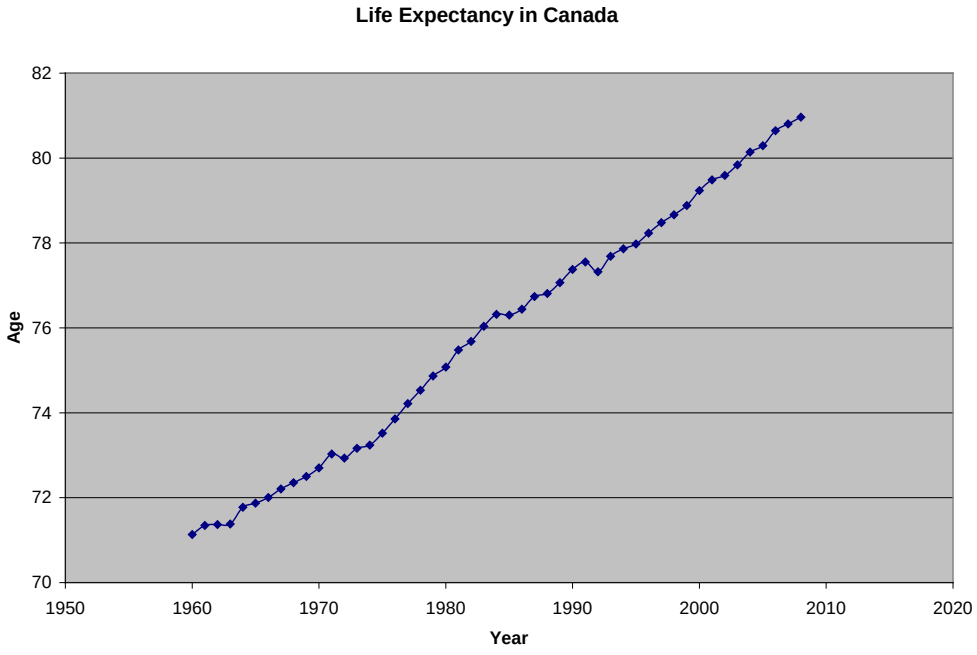

Figure 1-1. Life Expectancy in Canada.

Life expectancy has been steadily increasing over the last 50 years, and this is projected to continue increasing in the future. As a result of this increased life expectancy, increasing proportions of patients in the elderly population are presenting for medical and surgical procedures in the health care system. Therefore, an understanding of the physiologic changes associated with aging are important for individuals involved in the care of these patients.
Figure adapted from information obtained online from data.worldbank.org (2011).

Since the elderly are associated with a higher incidence of disease and concurrent use of medications, perioperative management of these patients is challenging. For example, diabetes and hypertension are highly prevalent in

this population and are known to impact various aspects of circulatory function. Medications such as beta blockers and angiotensin converting enzyme (ACE) inhibitors are commonly used in this age group, and the resultant drug interactions may also compromise hemodynamic stability and lead to untoward events. Even in the absence of these complicating factors, elderly patients are intrinsically more susceptible to the circulatory effects of anesthetic agents and other cardiovascular stressors due to the progressive structural and functional changes in the circulation that invariably occur with aging. Indeed, in cases of trauma, the elderly are less likely to die as a direct result of their primary injury but as a result of underlying co-morbidities, general functional decline, and postoperative complications.[8]

Due to increasing life expectancies in an ever-growing elderly population, studies pertaining to the circulatory changes in these patients are becoming increasingly timely and relevant. The main research focus of this thesis is directed at elucidating the effects of propofol, a very commonly used anesthetic agent, on the aging cardiovascular system. Propofol is an attractive general anesthetic agent because of its desirable neurological recovery profile. Propofol offers a relatively quick induction of anesthesia, a relatively short duration of action, and an advantageous emergence from anesthesia once it is no longer administered to the patient. It is especially useful for patients requiring day surgical procedures where the patients are expected to be discharged home following the conclusion of their procedure. Given its

desirable anesthetic profile, propofol is also used in patients requiring sedation for those in critical care settings, such as the intensive care unit. However, it is well-accepted that the use of propofol in anesthetic care causes hypotension, and this hypotension can be exacerbated in the aging patient population. What is not known in this situation is whether there are effects of propofol on vascular function in aging, when physiological changes in the cardiovascular system occur, compared to the younger cohort. Therefore, I studied the effects of propofol on the aging vascular system at the functional level *ex vivo* as well as in intact physiological systems *in vivo*. To provide a background for this dissertation, I will first discuss the age-associated changes as they relate to the cardiovascular system, highlighting what is currently known about structural and functional changes with the aging vasculature and how this translates to alterations in hemodynamic parameters in the elderly compared to their younger counterparts. I will then discuss the issues regarding anesthesia in the aging population, with a particular focus on hemodynamic stability in the perioperative period. Next, I will review the literature surrounding propofol and its many postulated effects on the cardiovascular system. Of note, propofol is known to have effects on endothelium-dependent vascular function; this background will lead to my initial hypothesis that propofol increases endothelium-dependent vasodilation in aging rats compared to younger rats. I will also discuss the potential interactions between propofol and Intralipid in the cardiovascular system. Intralipid, besides being used for

total parenteral nutrition, is a solubilizing agent for propofol in its clinical formulation, but curiously it may potentially be used to reverse the hemodynamic effects of propofol. This leads to another one of my hypotheses that Intralipid can restore hemodynamic stability after propofol is administered in the aging population. Altogether, I hypothesize that *propofol increases vasodilation in aging* which can be partly explained by changes in the vasculature that is associated with the aging process, and the hemodynamic effects elicited by propofol can be corrected by Intralipid, a novel use for this medication that is used for total parenteral nutrition.

1.2 AGING AND THE CARDIOVASCULAR SYSTEM

Aging is associated with a multitude of physiologic changes including alterations in the mechanics of the cardiovascular system such as arterial remodelling as well as impairment in endothelial function and an increase in oxidative stress.[9] In this section, I will discuss the implications of aging to the structural and functional components of the cardiovascular system, with particular attention to the endothelium and its vasoactive mediators as they change with age.

1.2.1 Structural Changes with Aging

Throughout the entire vascular tree, there are three layers which constitute the blood vessel wall: the intima (endothelial cells [EC]), the media (vascular smooth muscle cells [VSMC] and fibroelastic connective tissue), and the adventitia (fibroblasts and fibroelastic connective tissue). Large elastic arteries, such as the aorta, are conduit arteries and serve to buffer blood flow and the pressure generated by the heart. As a result, these blood vessels have a large amount of elastic fibres in the media and adventitia to generate a recoil force and assist forward flow of blood to end organs, such as the brain and the kidneys, and also particularly to the heart via the coronary arteries during diastole. Collagen also exists in the vascular wall to add extra strength at high pressures.[10-11] As arteries become smaller, they become progressively more muscular relative to the size of the lumen, and although they retain some elasticity, they are not as compliant as their larger counterparts. These smaller muscular arteries are involved in the regulation of blood pressure, since they must regulate the pressure going through the capillary beds into which they feed. That is, too high a pressure may cause capillary damage and excessive leak into the interstitial space. When these arteries constrict, they normalize pressure and flow through the capillary bed but increase systemic vascular resistance, and hence blood pressure, at the same time.

Changes with aging that are intrinsic to the vasculature differ between conduit arteries, such as the aorta, and peripheral arteries, which dictate

systemic vascular resistance. Conduit arteries appear to be more susceptible to age-induced structural changes, which include intimal thickening; VSMC proliferation and migration into the intima; increased collagen content, deposition and covalent cross-linking; breakdown of elastin; and fibrosis.[12-16] The result is that these arteries no longer dampen the forces generated by the cardiovascular system. Specifically, this reduced elasticity and increased arterial stiffness increases pulse pressure and arterial pulse wave velocity.[17-21] The net results of these changes are (1) increased pressures required to maintain organ blood flow during diastole, and (2) increased work on the heart in the wake of refractory waves occurring during late-systole due to increased pulse wave velocities.[22] In other words, the decreased compliance of the vascular system that occurs with aging results in an increase in reflected waves immediately post-cardiac contraction. This results in an increase in cardiac work nearing end-systole since the heart must now pump against forces returning to the heart (i.e. reflected waves return to the heart sooner in aging compared to young individuals). Young compliant conduit vessels store this potential energy to promote forward flow at the end of systole into diastole. Along those lines, in aging there is a lower perfusion pressure exerted on end organs, such as through the coronary arteries of the heart, since forward flow is not augmented as efficiently as it is in the young during diastole. Therefore, there is an additive effect to cardiac compromise that occurs with aging (i.e. increased cardiac work, decreased coronary perfusion). In addition,

hypertension which accompanies aging is a risk factor for developing atherosclerosis, a condition which compromises luminal diameter of blood vessels, further degrading the flow and blood supply to the end organs. Indeed, atherosclerosis is more apparent in the aging patients compared to young.[23] In severe cases associated with disease states, more severe structural changes may manifest, such as vascular calcification, which accurately predicts future adverse cardiovascular events.[24]

Besides the structural changes that occur primarily in the media and adventitia (i.e. alterations of collagen and elastin content), other aspects of vascular wall remodelling also occurs with the aging process. ECs undergo changes in shape and size that are characteristic of a senescent phenotype.[25-26] Appropriate EC function is important because they play a major role in proper vascular function (see section 1.2.2). In addition, endothelial progenitor cells (EPCs), which are important for vasculogenesis and vascular repair and regeneration, are reduced in number and are functionally impaired during the aging process.[27-28] This decrease in EPC function and accelerated senescence may be due in part to the upregulation of the angiotensin system that accompanies aging (see section 1.2.2).[28] In confirmation of this, it has been shown that cultured human mononuclear cells isolated from peripheral blood, when stimulated with angiotensin II (Ang II), resulted in a decrease in EPC numbers compared to no Ang II exposure.[29] This suggests that the angiotensin system, when upregulated with aging, contributes to the deterioration of the

reparative capacity of the vasculature. While the ability of the ECs to regenerate is compromised, the proliferative ability of VSMCs is increased with aging.[30-32] VSMCs in aging arteries have a greater invasive profile compared to younger arteries,[33] and this again may be dependent on the angiotensin system, given that Ang II exposure to cultured VSMC isolated from young rats increases invasive profile to resemble that from VSMC isolated from aged rats; moreover there is an increase in Ang II protein abundance in the arterial wall as these rats age as determined with immunofluorescence.[34-35] However, these migrated smooth muscle cells become senescent[36-37] but increase their secretory function, producing chemokines to promote inflammation.[38-40] In particular, atherosclerosis is considered to be an inflammatory process of the vascular wall (see section 1.2.2) and occurs in aging individuals which results in narrowing of the lumen of arteries that can lead to myocardial infarction or stroke. Of note, VSMCs can migrate to the intima, thereby changing the structural composition of the vascular wall, in the atherosclerotic process.[30,41] These VSMCs change their morphology, and consequently their function, when they migrate to the intima during this process. For instance, migrated VSMC into a thickening intima is associated with an increase in protease activity as determined with *in situ* zymography in aged rats, and Ang II infusion in young rats leads to intima thickening and increased protease activity similar to that seen in aged rats.[42-43] However, to counter these deleterious changes, outward remodelling can occur

which compensates for progressive growth of antherosclerotic plaques to postpone the development of flow-limiting stenoses.[44-46]

Remodelling of the vasculature involves all major cell types in the vessel wall: ECs, VSMCs, and fibroblasts.[47] Of note, oxidative stress is also associated with remodelling in the vasculature.[48] Oxidative stress, which is increased in the aging process,[49] increases deposition of matrix proteins whilst promoting degradation of the basement membrane and elastin through matrix metalloproteinases.[50] Therefore, there is a decreased compliance of the vasculature not only because of the changes in the ratios of collagen and elastin that occur with the aging process but also because of additional structural changes that accompany the remodelling process, leading to an alteration in the dimensions of the blood vessel. Ultimately, this all leads to the aforementioned cardiovascular physiologic changes with aging (widened pulse pressure, increased cardiac end-systolic work because of earlier reflection of waves back from the vasculature and cardiac hypertophy because of decreased vascular compliance).

In smaller arteries, eutrophic vascular remodelling occurs with resistance arteries in the presence of increasing blood pressure with aging; these small resistance arteries mediate autoregulation of blood flow to stabilize capillary pressure.[51] Eutrophic remodelling essentially causes an increase in wall thickness while the lumen diameter is reduced; however, the cross-sectional area of the blood vessel remains unchanged.[51] The hypertrophied

vascular wall offsets the increase in wall stress in the blood vessel, which is generated by the increased blood pressure. This type of remodelling would also require the increased growth of the media layer, populated by VSMCs. Although these VSMCs grow in size and potentially in number in the aging process, their functions are also transformed (from a purely contractile function to a secretory one, as mentioned above).

1.2.2 Functional Changes with Aging

Changes in local and humoral factors, neural signalling, and metabolic function constitute extrinsic mechanisms that affect the vasculature with aging. A noteworthy example is that renin-angiotensin system (RAS) activity increases with age.[52-53] This appears to be an important mechanism by which oxidative stress and particularly superoxide production (via NAD(P)H oxidase) leads to reduced nitric oxide (NO) bioavailability.[42,54-56] In myocardial tissue, it was found that there is an increase in NAD(P)H oxidase activity and expression that accompanies aging which leads to an increase in oxidative stress.[56] In addition, we have previously shown that in cultured EC, Ang II increases NAD(P)H oxidase expression and oxidative stress, which scavenges NO and increases nitrotyrosine formation, a marker of oxidative stress, as determined with immunofluorescence.[54] This increase in oxidative stress is important because it plays a role in precipitating the structural, as described above, and functional changes in the vasculature.[57] Notably, all

aspects of the angiotensin system (Ang II, ACE, angiotensin-type 1 [AT-1] receptor expression in the vascular wall) are increased with age.[33,35,58] Therefore, one can appreciate a role for RAS in the interplay between oxidative stress and aging given this information. Aging is also associated with increased sympathetic nervous system (SNS) activation, leading to an increase in cardiac inotropy and chronotropy. Importantly, there is a close interplay between the SNS and RAS, since both systems influence the activities of one another.[59-62] Due to the importance of the SNS in the regulation of acute hemodynamics, its relevance in the perioperative management of blood pressure is particularly important, as discussed in section 1.3.

In addition to the structural integrity of the vascular system being dependent on structural proteins in the media and adventitia, the tone of the vasculature is maintained by the vasodilating and vasoconstricting activity of smooth muscle. Vascular smooth muscle receives many inputs involved in the control of tone, including neural, adventitial, and endothelial input.[63-67] The studies presented in this thesis focus primarily on the endothelium and its role in the control of vascular tone.

The endothelium plays many roles in the vasculature. These include a barrier role, separating the intravascular space from the perivascular space;[68] its function in hemostasis;[69] its involvement in a variety of inflammatory responses;[70] the role of regulating substrate delivery to tissue, as is the case

with capillary beds;[71] and the role of producing a variety of vasoconstrictors and vasodilators. The relative increase or decrease in production or activity of these mediators dictates the control of vascular tone (Figure 1-2). For instance, an increase in vasoconstrictors or a decrease in vasodilators produced by the endothelium will both ultimately lead to vascular constriction. Endothelial-derived vasoconstrictors include endothelin-1 (ET-1), Ang II (via ACE that is expressed on ECs), and vasoconstricting prostaglandins (such as thromboxane A_2 [TXA_2]). To balance these vasoconstrictors, there are three primary vasodilators produced by the endothelium: nitric oxide (NO), vasodilating prostaglandins (such as prostacyclin [PGI_2]), and endothelium-derived hyperpolarizing factor (EDHF) or simply endothelial-derived hyperpolarization (EDH). Therefore, an impaired endothelium, as can be seen with aging, results in a decreased production/activity of these vasodilators,[9] as will be discussed.

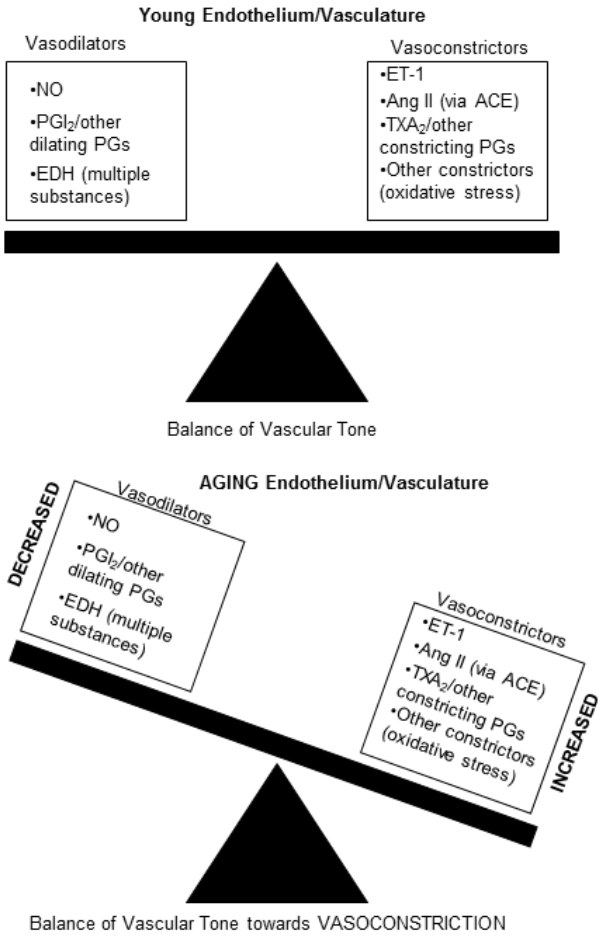

Figure 1-2. Control of vascular tone by the endothelium.

Endothelial production of nitric oxide (NO), dilating prostaglandins (PG) such as prostacyclin (PGI_2), and endothelium-derived hyperpolarization (EDH) cause vasodilation. This is balanced by vasoconstriction originating from the endothelium, which includes production of endothelin (ET-1) and consticting PGs such as thromboxane (TXA_2). Aging results in an imbalance of vascular tone by increasing oxidative stress and thus reducing the contributions of endothelial-derived vasodilators (NO, PGI_2) whilst enhancing the production of vasoconstrictors such as constricting PGs and endothelin-1 (ET-1).

Whereas the peripheral arterial system appears to be largely spared from age-related structural changes in comparison to large conduit arteries,

both small arteries as well as large conduit arteries undergo well-defined functional changes that result in altered secretory profiles and increased responsiveness to endothelial vasoconstrictors.[72-73] As vascular stiffness increases with aging, the arterial dilating capacity concurrently decreases. This was demonstrated in both men and women, where flow-mediated dilation (i.e. endothelial-dependent vasodilation) decreased as age increased, as assessed with high-resolution ultrasonography of the brachial artery.[74]

Also, with aging comes an increase in oxidative stress.[49] As alluded to above regarding RAS and oxidative stress, a defining feature of the aging vasculature is reduced NO bioavailability, which results in part from scavenging by reactive oxygen species such as superoxide anion.[56,75-77] Essentially, superoxide can combine with and inactivate NO to form the more stable product peroxynitrite (Figure 1-3).[78] Besides decreasing the availability of NO, superoxide anion and the subsequent formation of peroxynitrite may also alter the balance of tone through other means. For instance, our laboratory group has previously shown that the addition of 3-morpholinosydnonimine (SIN-1), a peroxynitrite donor, to ECs in culture reduces protein expression of prostacylin synthase, which can result in a decreased production of this vasodilating prostaglandin.[79]

There are multiple sources of reactive oxygen species, most notably through prostaglandin H synthase (PGHS) and NAD(P)H oxidase enzymes which are major sources with the aging process (Figure 1-3).[80-83] In fact,

NAD(P)H oxidase is thought to be the major source of reactive oxygen species in the endothelium.[84] In addition, a study by Ungvari et al. (2008) revealed that mitochondrial sources of reactive oxygen species are also increased in aging as evidenced by confocal microscopy showing a decrease in mitochondrial biogenesis in aged arteries; moreover, they mimicked an aged phenotype of cultured EC by utilizing small-interfering RNA sequences to cause a partial downregulation of cytochrome *c* oxidase subunit IV, a mitochondrial enzyme, and found that the formation of reactive oxygen species are increased in this model.[85] The authors concluded that impaired mitochondrial biogenesis and downregulation of key mitochondrial enzymes contributes to oxidative stress in aging. In addition to these sources of reactive oxygen species, interestingly NO synthase (NOS) enzymes can contribute to the formation of superoxide anion. There are two constitutive forms of NOS, namely endothelial NOS (eNOS) as well as neuronal NOS (nNOS), in addition to an inducible NOS isoenzyme (iNOS).[86] Both eNOS and nNOS play a role in vascular function, since our laboratory has recently shown that nNOS increases *in situ* superoxide anion production in aging as evidenced by increased dihydroethidium fluorescence in thoracic aortas, suggesting an uncoupling of the NO synthase enzyme to produce superoxide anion rather than NO.[87] Furthermore, besides the age-associated increase in production of superoxide anion, there are decreases in endogenous antioxidant enzyme activities (Cu/Zn superoxide dismutase (SOD), Mn SOD, and extracellular

matrix SOD) with aging, which consequently increases superoxide anion in the aging vascular system.[88-89] In addition to the aforementioned scavenging of NO by oxygen free radicals, there is also a reduction in NOS cofactors and substrates (i.e. tetrahydrobiopterin, L-arginine) which contributes to the loss of NO in the aging process and subsequently leads to NOS uncoupling.[75,90-97] Not surprisingly, the loss of vasodilatory NO bioavailability is accompanied by increased activity of vasoconstrictors, including ET-1[98-100] and prostaglandins,[101] since NO is known to counteract the activity of these vasoconstrictors.[102] Therefore, in the aging vasculature, there is an imbalance of vascular tone in part due to endothelial dysfunction, tipping the balance of tone towards vasoconstriction.

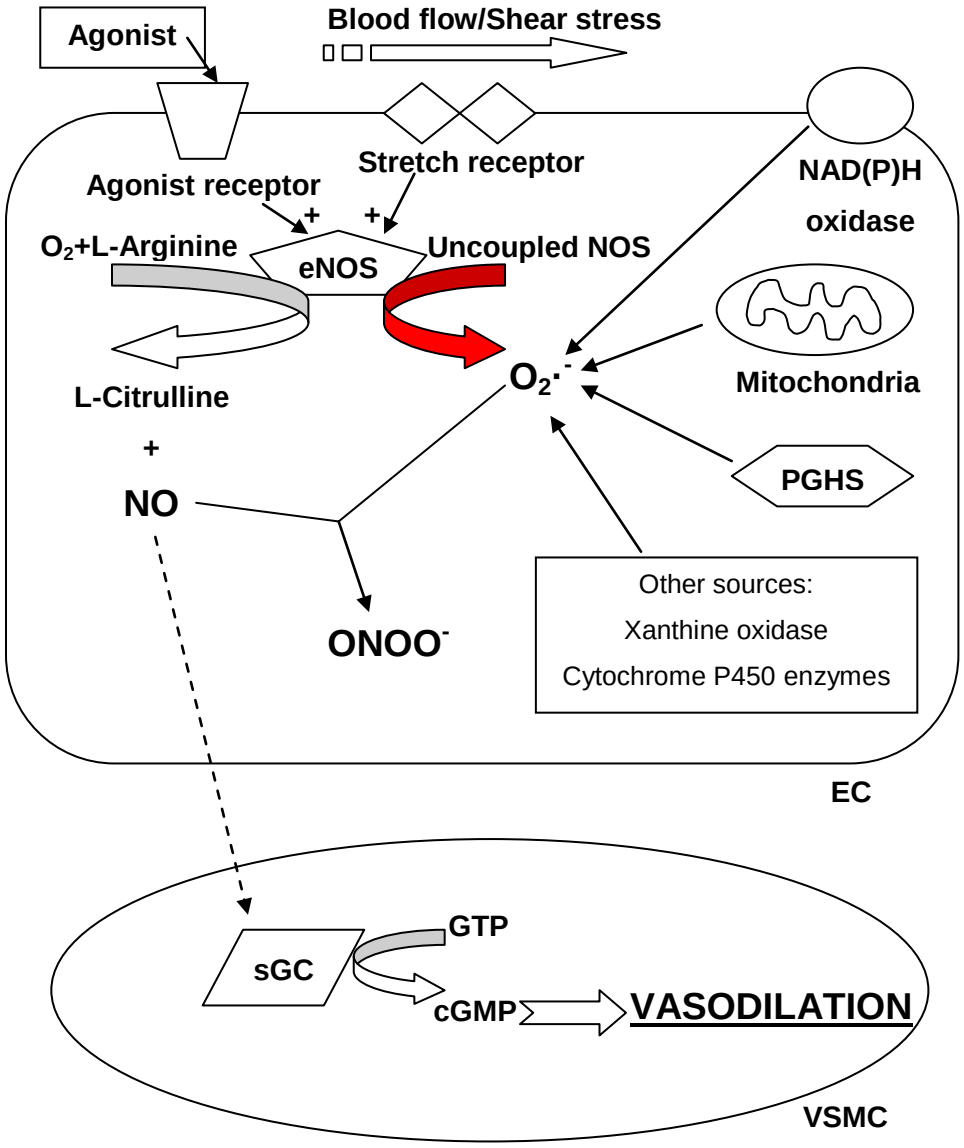

Figure 1-3. NO production and sources of oxidative stress.

Primary methods of NO production are through shear stress as well as through agonist stimulation of the appropriate receptor in the EC wall, both of which activate endothelial NOS (eNOS). NO activates soluble guanylyl cyclase (sGC) in the VSMC, ultimately producing cyclic guanosine monophosphate (cGMP) and causing vasodilation. Superoxide ($O2^{\cdot -}$) is produced by a variety of sources including NAD(P)H oxidase, PGHS, mitochondria, and uncoupled NOS; superoxide can then combine with NO to form peroxynitrite ($ONOO^-$). In the aging vasculature, this scavenging of NO further impairs vasodilation, since there is now decreased NO availability to the VSMC.

As NO is also known to have anti-inflammatory and anti-thrombotic actions, the aging vasculature is also associated with increased vascular inflammation and a pro-thrombotic phenotype, as is the case with atherosclerosis,[103] and this exacerbates underlying vascular conditions. Increased oxidative stress also leads to further inflammation, as well as a host of unwanted effects. A noteworthy example is the oxidation of low-density lipoproteins (LDL): reactive oxygen species can transform LDL to oxidized-LDL which can be taken up by macrophages in the vascular wall, changing these macrophages to foam cells which marks the progression of atherosclerosis.[103] Indeed, microscopic examination of advanced atherosclerotic blood vessels reveals the presence of transformed macrophages, ECs, and smooth muscle cells to foam cells.[104] Moreover, because NO is decreased with aging, atherosclerosis will progress because NO also decreases smooth muscle cell proliferation and migration.[103] Therefore, increased oxidative stress accompanying the aging process leads to further perturbations in a cardiovascular system that can already be compromised just by structural changes alone.

1.3 AGING AND ANESTHESIA

Broadly, one of the greatest challenges in perioperative care for elderly patients is the maintenance of stable hemodynamic parameters. In particular,

one of the consistencies in patients receiving general anesthesia for surgical care is the propensity for anesthetic-induced hypotension, which may result in end-organ hypoperfusion. Indeed, given the structural and functional changes discussed in the previous section, maintenance of hemodynamic stability is important, particularly in the aging population. Elderly patients are more prone to developing hypotension in general,[105-108] but this may be exacerbated in surgery due to the fact that many anesthetic agents are, by nature, blood pressure-reducing. These patients may be prescribed multiple medications for the treatment of multiple co-morbidities (e.g. hypertension, ischemic heart disease, peripheral vascular disease, diabetes), therefore drug-drug interactions and exaggerated hemodynamic responses can be a significant concern. Hypoperfusion may be particularly harmful in elderly patients because of a decline in organ function[109] and being accustomed to perfusion at higher pressures. Thus, in a patient with ischemic heart disease and long-standing hypertension, a decrease in blood pressure intraoperatively towards the "low-normal range" of healthy adults may actually be detrimental. Increased vigilance is therefore needed from the Anesthesiologist and other members of the health care team to minimize perioperative complications associated with hypotension.

The Framingham Heart study suggests a steady increase in systolic blood pressure with increasing age, and this increase in blood pressure resulting in hypertension may be due to an increase in vascular stiffness as

well as an increase in systemic vascular resistance.[19,110] Indeed, hypertension has been estimated to occur in up to 70% of the elderly population.[5] Cardiac hypertrophy can develop as a result of this increased afterload. This hypertrophy would offset the increase in wall tension, as dictated by the Law of Laplace, but this would still lead to an increase in myocardial oxygen demand given the increase in muscle mass. In the face of a constant oxygen supply, this can then lead to myocardial ischemia.[111-112] Therefore, further sustained acute reductions in blood pressure (i.e. further decrease in oxygen supply), as can potentially occur during the perioperative period, may substantially increase the likelihood of an untoward cardiovascular outcome.

Given the prevalence of hypertension in the elderly population, it is perhaps counterintuitive that aging patients tend to be more susceptible to hypotension intraoperatively.[113] The hemodynamic instability stems from multiple factors acting in concert. First, the circulation in elderly patients is highly dependent on preload, such that alterations in vascular tone can have a large influence on cardiac output and end organ perfusion.[109] Second, since upregulation of vascular constrictor mechanisms appears prevalent, the sudden removal of these tonically active constrictor mechanisms by anesthetic agents may result in profound hypotension. Third, evidence indicates that "physiologic reserve", broadly referring to the redundancy in physiological systems and therefore the capacity to buffer disturbances in hemodynamics, declines with age.[114] Acute hemodynamic fluctuations are buffered by two

principal mechanisms: 1) baroreceptor reflex signaling, which modulates autonomic nervous system activity, and 2) vascular responsiveness to shear stress, which is coupled to NO production.[115] Both baroreceptor capacity to buffer acute blood pressure changes and shear stress-induced production of NO are markedly attenuated in elderly patients.[116] In other words, despite the age-associated increase in pulse wave velocity and consequent increase in shear stress forces in the vasculature, the lack of NO bioavailability that occurs with aging causes a decreased modulation of these forces in the elderly. The consequence is a more unstable blood pressure, resulting in more exaggerated changes in the wake of agents that influence peripheral resistance and cardiac output.

Another factor that contributes to the hemodynamic instability in the elderly is the down-regulation of vasodilatory mechanisms (e.g. NO, prostaglandins) and concomitant upregulation of vascular constrictor mechanisms (ET-1, SNS stimulation, RAS activity). The sudden removal of these vasoconstrictor signaling mechanisms by anesthetic agents (see below) may contribute to profound and prolonged hypotension. For example, as introduced in section 1.2.2, it is well established that SNS activity is altered with aging. In the face of decreased functional sympathetic neurons as well as decreased receptor responsiveness, the SNS attempts to compensate by increasing plasma norepinephrine concentrations in the elderly.[117-119] Iatrogenic removal of this sympathetic “overstimulation” may result in an

exaggerated reduction in vascular tone, resulting in a loss of blood pressure. In essence, there may be a dramatic decrease in this aspect of "physiologic reserve" whereby removal of this overstimulation results in an exaggerated loss of vascular tone, hence exaggerated hypotension. This effect, coupled with a reduced baroreceptor response, could lead to clinically significant hypotension resulting in unfavourable cardiovascular outcomes.[120]

Clinically, maintenance of hemodynamic parameters and hence organ blood flow is important for ensuring optimal organ function, not only in the heart but also in other major organ systems as well. An example of this is the kidney: renal function declines with age due to a loss of renal parenchyma as well as a progressive decline in renal blood flow and a decreased responsiveness to vasodilating stimuli.[121-122] Therefore, the kidneys of an aging patient may be vulnerable to injury as a result of hypotension. Factors known to predispose renal dysfunction in the surgical patient include preoperative blood pressure elevation (i.e. hypertension) and perioperative blood pressure changes below the preoperative blood pressure level (i.e. hypotension),[123] which are factors particularly relevant in elderly patients. This indicates the importance of maintaining hemodynamic parameters in elderly patients to ensure appropriate perioperative renal function. Another example is in regards to the brain; one of the complications that can occur in the perioperative period, especially in the aging patient population, is post-operative cognitive dysfunction. Anesthetic agents have been implicated in

persistent memory impairment in aging animal models[124-127] which may be linked to direct neurotoxic effects of these agents.[128-130] However, there is suggestion that impaired vascular function may also play a role[131] since cerebral hypoperfusion is associated with cognitive dysfunction.[132] Thus, spinal anesthesia, which is believed to have little or no toxic effects in the brain, is known to cause hypotension by sympathetic blockade and can result in cerebral hypoperfusion, as indicated by decreased cerebral artery velocity, which was shown to be exaggerated in elderly patients.[133] Therefore, hypotension, irrespective of anesthetic technique, may precipitate post-operative cognitive deficiencies.

Anesthetics can influence hemodynamic stability directly by altering cardiac function, vascular reactivity, or by affecting cardiovascular reflexes.[134-139] Because cardiac and vascular function are in many ways compromised with aging, the circulatory effects of anesthetics are exacerbated in the elderly.[140] It is also noteworthy that due to changes in pharmacokinetics and pharmacodynamics, elderly patients are more sensitive to the effects of anesthetic agents administered, which may include the cardiovascular side effects of these drugs in addition to the CNS depressant effects.[141-143] Although there are a multitude of medications that Anesthesiologists utilize for patient care (for example, local anesthetics, analgesic agents, neuromuscular blockers), general anesthetic agents can be considered the foundation by which the specialty originated and subsequently progressed.

Therefore, specialists in the field of Anesthesiology must have an intricate knowledge of the mechanisms of action of these general anesthetic agents, including their effects on the cardiovascular system. Two primary classes of general anesthetics exist: inhalational anesthetics and intravenous anesthetics, which are discussed below.

1.3.1 Inhalational Anesthetic Agents and the Aging Vascular System

While many of the vascular effects of anesthetic agents have been well characterized in young patients and animals, relatively few studies have been done in elderly patients or in animal models of aging. Thus, it is pertinent to conduct research investigating general anesthetics in models of aging. Inhalational anesthetic agents such as sevoflurane, isoflurane, and desflurane are widely acknowledged to cause greater hypotension in elderly patients than in young patients, albeit the scientific studies supporting this observation are generally lacking.[144] The principal cause of hypotension appears to implicate a loss vascular tone and reduction in total peripheral resistance,[145-149] although the specific mechanisms are not clear. There is evidence that inhalational anesthetic agents decrease SNS activity, resulting in reduced vascular tone and diminished baroreceptor responsiveness in the wake of blood pressure fluctuations.[150-151] This is important because sudden decreases in SNS activity may cause excessive and prolonged vasodilation in the elderly, as described

above. Yu *et al.* have also shown that inhalational anesthetics may decrease vascular tone through inhibition of the RAS,[152] which is known to be upregulated in aging.[153-154] Given that Ang II is a strong vasoconstrictor and may lead to increased vascular tone, it is tempting to speculate that the sudden withdrawal of this system may produce a profound hypotension, analogous to the situation involving the SNS. However, evidence that directly implicates the RAS in this exaggerated hypotensive effect is presently lacking.

Inhalational anesthetics have also been shown to have direct vasodilatory effects on the vasculature, which may be implicated in the exaggerated effects in the elderly. In isolated vessels of several species, inhalational anesthetics mitigate the contractile responses to potassium chloride or norepinephrine; this effect was observed whether the endothelium was intact or not,[155] suggesting a direct effect on vascular smooth muscle. Indeed, studies have shown that many such anesthetic agents influence Ca^{++} mobilization and sensitization[155-156] as well as K^+ channel function[157] in VSMCs, with corresponding changes in vessel contractility. Given the importance of Ca^{++} channels and K^+ channels in vascular tone, direct actions on these targets could potentially influence vascular responses to anesthetics in aging patients, although little information is available on the age-related changes with ion channels in the vasculature. There is evidence for diminished expression of large-conductance calcium-activated potassium channels (BK_{Ca})

in aging in both rodents and humans,[158-159] although their contribution to the vascular effects of anesthetic agents in aging is not currently known.

Inhalational anesthetics also have vasodilatory-promoting effects on endothelial function. In an *ex vivo* study of the rat aorta, isoflurane has been shown to decrease ET-1-dependent vasoconstriction,[160] which may have a profound effect in the elderly population given the increased ET-1 contribution to vascular tone with age.[98,161] Inhalational anaesthetics could also cause excessive vasodilation in the elderly via an antioxidant effect. While these agents do not appear to have intrinsic antioxidant effects *per se*, certain agents such as isoflurane and sevoflurane have been shown to increase endogenous antioxidant mechanisms,[162-163] which may have a role in decreasing vascular tone. Indeed, as alluded to, a progressive increase in oxidative stress is a well-defined etiological mechanism of vascular dysfunction and increased vasoconstriction in the aging process.[3,164]

Interestingly, in contrast to those aforementioned studies, inhalational anesthetics have also been shown to increase vascular responsiveness to norepinephrine and potassium chloride in young rat mesenteric arteries, an effect that is entirely dependent on an intact endothelium.[165-168] Similarly, several groups have shown that these agents also inhibit endothelial-dependent vasodilation.[169-173] The mechanisms underlying this latter observation are not clear, although neither NO, EDHF, and PGHS pathways, nor ET-1 or Ang II appear to be implicated; Ca^{++} and K^{+} channels are obvious potential targets.

Taken together, these studies demonstrate that anesthetic effects on the endothelium, are in part, vasoconstrictor in nature. Although puzzling, this apparent disparity may be reconciled by the fact that the net effect of an agent is the sum of all simultaneous effects on the vessel.[169] Therefore, while the endothelial effects of inhalational anesthetics appears to be vasoconstrictor in nature, the net effect, taking into account the effects of inhaled anesthetics on smooth muscle function, SNS, RAS, and other local and humoral factors, is vasodilatory in the young population. Since elderly patients are known to have altered endothelial secretory profiles, it is tempting to speculate that the hypotensive effects of inhalational anesthetics stem, at least in part, from mitigated endothelial vasoconstrictor effects producing an overall enhanced vasodilation.

1.3.2 Intravenous Anesthetic Agents and the Aging Vascular System

As with inhalational anesthetics, information pertaining to intravenous anesthetic effects on vascular physiology in aging is scarce. One intravenous agent which has been investigated in regards to cardiovascular aging is etomidate. In vessels from young rats, etomidate causes direct vasodilation in isolated vessels,[174] involving increased vasodilator prostaglandins[175] and decreased ET-1 production.[176] However, *in vivo*, administration of etomidate has little effect on blood pressure in young patients; in fact, its hemodynamic

stability during induction of general anesthesia make it a preferred agent in many clinical scenarios.[177-178] The hemodynamic stability of etomidate appears to stem from a lack of inhibition of SNS function.[179-180] Thus, despite causing direct vasodilation, hemodynamic perturbations are transient and easily corrected by baroreceptor stimulation of heart rate and contractility. However, when administered to elderly patients, etomidate causes a 20-30% decrease in blood pressure.[181] This hypotensive effect is not well understood, although recent studies have provided insights into its mechanism. The reason may be due in part to the interaction between etomidate and adrenoceptor signalling. Etomidate enhances norepinephrine-induced constriction in mesenteric resistance arteries in young rats, resulting in increased vascular tone; this effect appears to be lost in aged rats.[182] The authors of this study suggested that the loss of blood pressure regulation with etomidate in aged patients is not due to direct actions on the vasculature but due to loss of norepinephrine signalling. Interestingly, Ebert et al. demonstrated that etomidate does not reduce overall release of catecholamines from sympathetic nerve terminals,[179] suggesting that the majority of the blood pressure-lowering effects in the elderly may stem from altering the adrenoceptor responsiveness to catecholamines. Thus, it may be that in aging patients, reduced signalling combined with the progressive decrease in adrenoreceptor responsiveness associated with aging[118-119] results in a more profound vasodilatory and hence hypotensive effect.

Etomidate is also known to cause adrenocortical suppression,[183-184] resulting in diminished cortisol release which can be problematic in certain patients (e.g. septic patients). Cortisol increases blood pressure principally by increasing sensitivity of the vasculature to catecholamines. Thus, etomidate-induced reduction in cortisol production may also play a role in causing hypotension in the perioperative period, again making elderly patients more susceptible to this effect due to the progressive loss of cathecholamine responsiveness with aging. This is supported by a recent case report of an elderly patient given a single dose of etomidate causing refractory hypotension that was only reversible with cortisol administration.[185] It is noteworthy that this mechanism is likely in addition to that described above, since the studies by Shirozu et al. were done in isolated vessels.[182]

Although etomidate can be safely used as an intravenous anesthetic agent, it is currently only available through the Special Access Programme through Health Canada, making its availability somewhat limited for health care providers. Propofol, on the other hand, is a commonly used intravenous anesthetic agent and is noted to be the most widely used agent for anesthetic induction.[186] It should be emphasized that the literature regarding propofol's effects on vascular function in aging models is limited. Therefore, this thesis focuses on the study of propofol as it relates to the vascular system, particularly with aging. The next section focuses on propofol and its interaction with the cardiovascular system.

1.4 PROPOFOL AND ITS INTERACTION WITH THE CARDIOVASCULAR SYSTEM

A previous study suggested that both increasing age of the patient as well as the use of propofol are two predictors of developing clinically significant hypotension in patients receiving general anesthesia.[113] Because propofol use is a predictor of intraoperative hypotension, our laboratory has been involved with studying vascular function in aging as it relates to this very commonly used anesthetic agent, which has not been investigated in the past. Clinically, propofol is thought to cause generalized vascular relaxation. Before addressing the cardiovascular effects of propofol, in this next section I will briefly discuss the history and current formulation of propofol in clinical use.

1.4.1 The History and Composition of Propofol in Clinical Use

From a chemistry standpoint, propofol has a very simple chemical structure. Its anesthetic classification is an alkyl phenol: specifically, it is a phenol with two isopropyl groups substituted at the 2- and 6-positions of the phenol ring (Figure 1-4). The anesthetic properties of this simple molecule were first reported in 1973 in England.[187-188] Given its hydrophobic nature, one of the difficulties in transitioning this drug into clinical trials and ultimately clinical practice was how to solubilize this agent to be administered for human use. Indeed, Cremophor EL®, a modified castor oil, was the first

medium used to solubilize propofol for clinical trials;[189] however, this formulation was withdrawn from development given the high incidence of anaphylaxis reported during these trials.[190] Nonetheless, propofol was a new and promising anesthetic induction agent, which had a similar if not better recovery profile with other anesthetic agents already in use.[191-192]

The optimal formulation of propofol would have to be in a vehicle that poses few side effects but also allows propofol to exert its anesthetic action with very little derangement. That is, the anesthetic properties of propofol should not be hindered by the medium in which it is solubilized. Propofol in a lipid-based emulsion was the next logical step following trials with Cremophor EL. Indeed, clinical trials proceeded in Europe and the United States in the early 1980's.[188] In the lipid-based emulsion, the anesthetic properties of propofol were similar to the properties exhibited when solubilized in Cremophor EL, but anaphylactic reactions like those which occurred with the Cremophor EL formulation were not reported.[193] Because of this success, propofol in lipid emulsion was subsequently launched for clinical use in the United States in 1989.[188]

Although the propofol formulation in lipid emulsion has been a great success, side effects still exist which include pain on injection,[194] risk of peripheral vein thrombophlebitis from the lipid component,[195] and allergic reactions to those who have egg yolk or soybean allergy, which are the main constituents of the lipid emulsion.[196] Efforts are still continuing to find the

"ideal" vehicle to solubilize propofol, including the microemulsion solution Aquafol (which abandons the lipid solvent).[197] However, given that the lipid emulsion formulation is the one that is currently used in the clinical setting, particular focus is given to this agent in my studies. Indeed, all of the experiments detailed in this thesis utilize the current clinical formulation of propofol; that is, propofol that is solubilized in the lipid emulsion Intralipid.

By itself, Intralipid is a medication that is used solely for total parenteral nutrition. The clinical formulation of propofol (Diprivan®) is a 1% propofol solution that is solubilized with 10% Intralipid. A 10% Intralipid solution is composed of 10% soybean oil, 1.2% egg phosphatide, 2.25% glycerol, 0.55% disodium edatate, and water with sodium hydroxide to adjust pH 6.5-8.5.[198] Soybean oil holds the majority of propofol, and the egg phosphatide acts as an emulsifier to allow the soybean oil-propofol droplets to stabilize in aqueous solution (Figure 1-4).[199] Propofol is manufactured by being solubilized in soybean oil first prior to being emulsified with egg phosphatide; this is done in this fashion because it would be very difficult for propofol to cross the charged emulsifier and enter the hydrophobic soybean oil core (written personal communication, Max T. Baker, Ph.D., Associate Professor, Department of Anesthesia, The University of Iowa College of Medicine, Iowa City, Iowa, USA, 16 June 2008). However, once propofol is administered in the blood stream, it is able to diffuse across the emulsified droplet to enlist its actions at the various effect sites, not only in the brain and

the central nervous system but also including components important in the cardiovascular system.

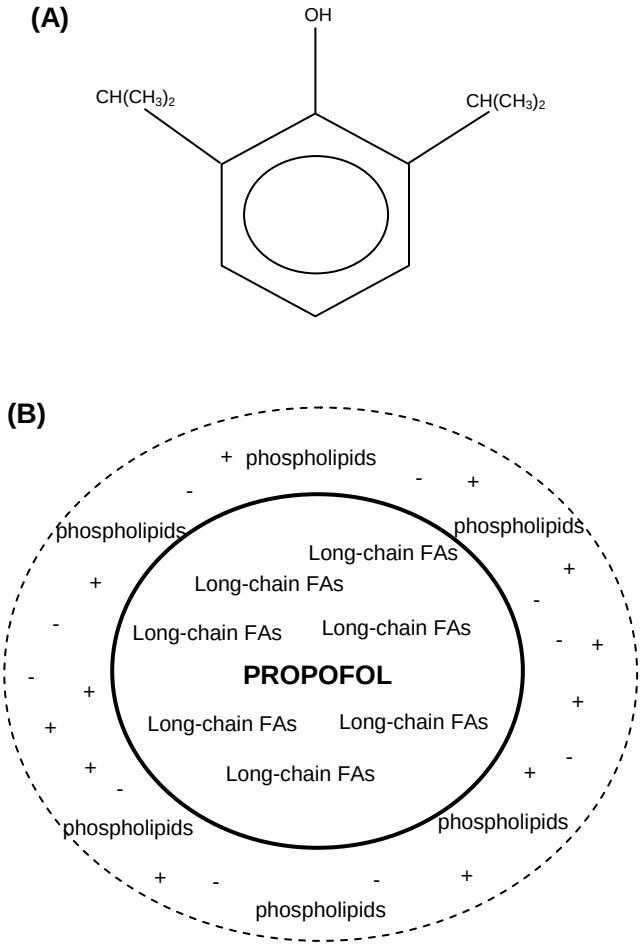

Figure 1-4. Structure of propofol and its mixture in lipid emulsion.

(A) Propofol has a very simple chemical structure with two isopropyl groups on a phenol ring. **(B)** Depiction of a propofol micelle. In lipid emulsion, propofol is solubilized within hydrophobic long-chain fatty acids (FAs). Polar phospholipids act as emulsifiers to create micelles that contain long chain FAs and propofol in the core; the hydrophobic portion of the phospholipids allow for the interaction with the hydrophobic core of the micelle. Propofol cannot easily leave the hydrophobic core and enter the charged region of the micelle (barrier depicted by solid black line) unless the structural integrity of the micelle is compromised as occurs during intravenous injection to induce general anesthesia.

1.4.2 Propofol and the Cardiac System

Induction of anesthesia with propofol often results in hypotension which is particularly apparent in the aging population. Indeed, this hypotension results in decreased organ perfusion and tissue oxygenation and may be detrimental to an elderly patient, given that age is a significant non-modifiable risk factor for ischemic heart disease and peripheral vascular disease. There are many postulated mechanisms by which propofol causes hypotension, each of which may not be exclusive of one another. One of the basic principles of cardiovascular physiology is that blood pressure is dependent on cardiac output and systemic vascular resistance. Determinants of cardiac output are stroke volume and heart rate, and stroke volume can be further broken-down to being dependent on preload, afterload, and inotropy or contractility.

A decrease in myocardial contractility will undoubtedly decrease generated systolic pressures and hence contribute to hypotension. One of the mechanisms through which propofol is suggested to cause hypotension is by myocardial depression resulting in a decrease in contractility. Early reports have suggested that propofol causes a dose-dependent decrease in myocardial contractility. A study by Coetzee et al. (1989) found that increasing plasma levels of propofol correlated with a decrease in myocardial contractility (and a resultant decrease in stroke volume) in a pig model.[200] In humans, a study utilizing transesophageal echocardiography found that an induction dose of

propofol given as a bolus injection results in a decrease in stroke volume and cardiac output, and contractility (as deduced by a decrease in the slope of the end-systolic pressure volume relationship).[201] The mechanisms may involve a decrease in Ca^{++} availability, possibly by inhibiting Ca^{++} channels in cardiomyocytes. In an experimental preparation of ferret ventricular myocardium depleted of Ca^{++} derived from the sarcoplasmic reticulum, it was found that propofol inhibited contractility that was dependent on extracellular sources Ca^{++}.[202] An obvious target is the L-type calcium channel. Indeed, it has been shown that propofol decreases myocardial contractility as well as L-type Ca^{++} channel currents as detected by patch clamp technique in rat cardiomyocytes.[203] An additional mechanism of decreasing myocardial contractility is by decreased Ca^{++} uptake by the sarcoplasmic reticulum as detected by fluorometry in sarcoplasmic reticulum vesicles;[204] however, the authors of this study note that the net effect of propofol on contractility is clinically insignificant because of a simultaneous increase in the sensitivity of the myofilaments to Ca^{++} thereby preserving inotropy. Indeed, other studies have also refuted these early findings regarding propofol inhibition of myocardial contractility, suggesting that propofol in fact minimally affects contractility.[205-207] This has been confirmed in different animal models, including rat,[208] canine,[209] and rabbit models.[210] In fact, another study suggested that propofol increases contractility during sympathetic stimulation, again through an increase in myofilament Ca^{++} sensitivity: this was shown in

isolated ventricular myocytes from adult rat hearts, where the addition of propofol increases myocyte shortening in the presence of phenylephrine and is dependent on protein kinase C activation.[211] Consequently, it has been suggested that the hypotension associated with propofol administration is more likely due to either a central (i.e. SNS) effect or a direct vascular effect.[212]

1.4.3 Propofol and the Sympathetic Nervous System

Another mechanism that has been suggested as a cause of hypotension following administration of propofol is sympathetic blockade.[180,213-214] As mentioned in the previous section, there is enhanced sympathetic activation (albeit accompanied by decreased receptor responsiveness) with aging. By utilizing microneurography of the peroneal nerve in humans, it has been shown that propofol, at typical doses used for induction of general anesthesia, decreases muscle sympathetic nerve activity that is accompanied by a decrease in vascular resistance.[179,215] However, the association of decreased sympathetic activity with decreased vascular resistance does not exclude direct vasodilating effects of propofol, which was not tested directly with these studies. In experiments investigating the effects of escalating doses of propofol injection on renal sympathetic activity in rabbits, it was found that lower doses of propofol minimally affected heart rate but reduced mean arterial pressure that was accompanied with a depression in renal sympathetic

nerve activity.[216] This suggests that vascular sympathetic fibres are likely blocked by propofol more so than cardiac sympathetic fibres (i.e. those involved in chronotropy and inotropy), which is part of the reason why at lower doses propofol causes hypotension with a minimal change in heart rate. At the central nervous system level, propofol can cause inhibition of sympathetic responses: in a study using rat paraventricular neurons, it was found that propofol enhances GABA receptor-mediated currents (i.e. causing neuronal hyperpolarization) and inhibits Ca^{++} entry in these neurons, which has direct implications in causing sympathetic depression.[217]

Regardless of mechanism, the ultimate result of inhibition of sympathetic activity therefore translates to a decrease in vascular tone (i.e. vasodilation) and a resultant decrease in systemic vascular resistance. The key accompaniment to this decrease in systemic vascular resistance is the hypotension which can occur. Although propofol can alter the sympathetic system which is important in modulating systemic vascular resistance, direct effects of propofol on the vasculature (i.e. propofol influencing vascular tone by altering endothelial function and causing vascular smooth muscle relaxation) cannot be overlooked.

1.4.4 Propofol and the Vascular System

A previous study investigated the effects of different plasma concentrations of propofol in post-coronary artery bypass graft surgical

patients, and the cardiac and vascular effects were studied using echocardiography and invasively-derived aortic pressure.[218] The authors found that myocardial contractility did not change as propofol concentrations increased; however, propofol reduced both preload and afterload in these patients. This strongly suggested that propofol lacked direct cardiac depressant effects, and since vascular actions of propofol could be demonstrated, this supports the concept that propofol is a vasodilator. The precise mechanism of action of propofol on the vasculature may involve direct modulation of vascular tone in an endothelial-dependent manner (Table 1-1). In particular, propofol has been reported to cause relaxation dependent on the endothelium by relying on the production of vasodilating prostaglandins. In isolated aortas from spontaneously hypertensive rats, Boillot et al. (1999) demonstrated that propofol attenuation of norepinephrine contraction was suppressed by indomethacin, an inhibitor of PGHS.[219] This suggested that propofol likely produces a vasodilating prostaglandin that mitigates the constriction induced by norepinephrine. These findings were corroborated in the isolated rat renal artery, since propofol-induced vasodilation following pre-constriction with either KCl, norpepinephrine, or the TXA_2 mimetic U46619, was inhibited by indomethacin.[220] In rat coronary arteries, Park et al. (1995) found that propofol caused vasodilation following U46619 preconstriction, which was inhibited by either indomethacin or by NG-nitro-L-Arginine, an inhibitor of NOS.[221] This suggests that propofol, in addition to producing vasodilating prostaglandins,

also causes NO-dependent vasodilation. Therefore, propofol may have more than one mechanism of causing vasodilation which may be dependent on the vascular bed studied. This also suggests that there may be multiple mediators that can contribute to redundancy of propofol-induced vasodilation specifically in rat coronary arteries. Propofol-induced vasodilation that is dependent on NO has also been confirmed in isolated rat extra-pulmonary arteries, since propofol's vasodilating ability was diminished by the presence of NOS inhibition.[222] Independent from the endothelium, propofol can cause vasodilation as has been demonstrated in isolated human radial arteries denuded of endothelium.[223] Propofol may cause this vascular relaxation by altering ion channel function (see below) or by increasing production of cyclic guanosine monophosphate (cGMP) independent of NO, as has been demonstrated in rat mesenteric arteries and in bovine VSMC in culture.[224] Increased production of NO increases cGMP production, so the fact cGMP may increase in the absence of NO suggests that propofol's vasodilating capabilities are complex in nature. Nonetheless, the role of NO (and cGMP) in propofol-induced vasodilation has not yet been established in the aging vasculature.

Propofol can also modulate vasoconstrictor responses, as summarized in Table 1-2. For instance, constriction with ET-1, which is increased in aging, is dampened by propofol as demonstrated in isolated canine coronary arteries.[225] Additionally, propofol has also been shown to decrease ET-1

production in cultured rat aortic smooth muscle cells as well as in cultured human umbilical vein ECs (HUVECs).[176,226] Therefore, propofol's inhibition of ET-1 release and a decrease in vascular smooth muscle ET-1 signalling would lead to vasodilation. In another study using cultured rat aortic smooth muscle cells, ET-1 stimulation elevated intracellular Ca^{++} which was inhibited by propofol and may be dependent on the L-type Ca^{++} channel.[227] These findings regarding ET-1 were confirmed in an aortic smooth muscle cell line.[228] Propofol's inhibition of increased intracellular Ca^{++} was also suggested to occur in intact isolated human omental arteries and veins and may involve the release of Ca^{++} from intracellular stores rather than from the extracellular space.[229] Therefore, there may be different mechanisms through which propofol decreases intracellular Ca^{++}; however, the end result would be the same, that being inhibition of constriction. In other experiments, again using human omental arteries and veins, propofol-induced vasodilation was inhibited in the presence of tetraethylammonium, an inhibitor of K_{Ca} channels, as well as the presence of KCl to inhibit hyperpolarization, which suggests the role of hyperpolarization in the relaxation response.[230-231] Indeed, cellular hyperpolarization will ultimately result in a decrease in intracellular Ca^{++} concentration. It should be emphasized that a number of vasodilators, including NO, will cause hyperpolarization.

Another notable vasoconstrictor, again associated with the aging process, is Ang II. There are multiple reports demonstrating that propofol

inhibits the Ang II vasoconstrictor response by altering Ang II intracellular signalling, ultimately resulting in a decrease in Ca^{++} mobilization and sensitivity in vascular smooth muscle.[232-234] Indeed, using pharmacogenomic experimental approaches in Brown Norway and Dahl Salt-Sensitive rats, it has been suggested that RAS is very important in the development of hypotension caused by propofol since it was found that the propofol-induced cardiovascular sensitivity and the *in situ* hyperpolarization response was due to differences in the renin gene and AT_1 receptor function.[235] Moreover, propofol inhibits Ang II signalling as it relates to development of cellular hypertrophy in cultured rat cardiomyocytes and apoptosis in cultured human coronary artery ECs, with a pivotal role being the inhibition of oxidative stress in both of these studies.[236-237] Again, this has direct implications in the aging vasculature, which is a setting of increased oxidative stress. A unique property of propofol is that it acts as an antioxidant given its structural similarity to vitamin E[238] and may therefore decrease oxidative stress (Table 1-3). Following the introduction of SIN-1, a peroxynitrite donor, to cultured HUVECs, propofol has been shown to decrease the amount of oxidative stress as measured by tyrosine nitrosylation.[239] Systemically, this was demonstrated as well. In experiments using propofol-anesthetized piglets subjected to aortic bypass and suprarenal aortic clamping, it was found that there was a decreased production of oxygen free radicals and reduced lipid peroxidation when compared to the use of sevoflurane for anesthesia.[240] In an experimental model

of cardiac arrest and cardiopulmonary resuscitation in piglets, it was found that propofol administration resulted in a reduction of oxidative stress, as determined by F2-isoprostane biomarkers, when compared to the Intralipid control.[241] Therefore, antioxidant effects of propofol can be seen both *in vitro* and *in vivo*. Notably, propofol has been shown to decrease superoxide formation in the rat aorta leading to an increase in vascular relaxation, and this is accompanied by a decrease in NAD(P)H oxidase expression.[242] Since NAD(P)H oxidase is a major source for superoxide in the aging vasculature, propofol's effects on vascular tone may be largely related to its ability to mitigate oxidative stress and ultimately increasing NO bioavailability in the vasculature. To support this, a study using computer-assisted videomicroscopy monitoring cerebral parenchymal arterioles in rat brain slices has shown that propofol causes vasodilation and reduces glucose-dependent superoxide formation; this response was abolished in the presence of NOS inhibition, and the NAD(P)H oxidase inhibitor apocynin similarly inhibited the response.[243] Therefore, it can be inferred that propofol increases NO bioavailability (by increasing NO production and reducing scavenging of NO by decreasing the production of superoxide by NAD(P)H oxidase) and hence NO-dependent vasodilation. Whether an antioxidant effect of propofol can be appreciated in the aging vasculature, a state of inherently increased oxidative stress, has also not yet been established.

Regarding NO synthesis in the vasculature, propofol is able to modulate its production (besides altering mechanisms that would result in a reduction in scavenging of NO). In ECs, propofol increases the production of NO. In co-cultures of porcine aortic endothelial and smooth muscle cells, it was found that propofol increases cGMP production, which was inhibited in the presence of NOS inhibition as well as hemoglobin (which binds to NO).[244] In addition, propofol added to cultured HUVECs reduces apoptosis induced by tumor necrosis factor-alpha that is associated with an increase in stable NO end-products found in the culture medium.[245] The mechanism of increased NO production may involve the phosphorylation of eNOS which results in activation of the enzyme to produce increased amounts of NO.[246] In addition, in hydrogen peroxide-stimulated HUVECs in culture, it was found that propofol increases eNOS expression as determined with Western blot.[247] An increased expression of NOS can also play a role in increasing NO production. Furthermore, propofol can increase NO production by restoring tetrahydrobiopterin levels in ECs.[248] As mentioned above, tetrahydrobiopterin levels are decreased in aging,[97] so a restoration of this eNOS cofactor can lead to enhanced vasodilation in the aging vasculature.

A recent study using an experimental model of hypertension in rats demonstrated that propofol inhibits L-type Ca^{++} channels in mesenteric arteris, which the authors suggest can be increased in sensitivity and expression, and this may be one of the reasons for hypotension during propofol

administration.[249] Indeed, as touched on above, a decrease in intracellular Ca^{++} in VSMCs would lead to a reduced contractile response in blood vessels. Other ion channels may be involved (Table 1-4), since it has been suggested that propofol's vasodilating response is dependent on opening of ATP-dependent K^+ channels (K_{ATP}).[242,250] However, opening of K_{Ca} channels, and specifically BK_{Ca} channels, have also been implicated in propofol's vasodilating response,[251-252] which may involve increased Ca^{++} sensitivity of the channel.[253] Regardless of mechanism, opening of K^+ channels would lead to hyperpolarization, reducing Ca^{++} entry into the cell, and causing relaxation of vascular smooth muscle, hence vasodilation. One study utilizing an *in situ* model of rat mesenteric arteries has demonstrated that propofol induces hyperpolarization of vascular smooth muscle, associated with a decrease in blood pressure, which occurs through K_{Ca} and K_{ATP} channels which is a result of enhanced activity of endothelial-derived NO.[254] Indeed, it is known that NO causes hyperpolarization and relaxation by activating K_{Ca}[255-257] and K_{ATP} channels.[258-260] Therefore, it is quite reasonable to presume that the propofol effect in the hyperpolarization-vasodilation-hypotension axis in these small arteries is due to NO. However, whether there is greater vascular relaxation in resistance arteries from aging rats and the mechanisms involved are not known.

Although all of these studies suggest potential multifactorial mechanisms to propofol's vasodilating ability, it must be underscored that

there have been no studies which investigated propofol's actions in the aging vasculature. This information would be important, particularly given the issues surrounding perioperative hemodynamic control in this patient population. This was the impetus for pursuing the studies outlined in this thesis.

Table 1-1. Summary of propofol's effects regarding vasodilation (note: no studies done specifically in an aging model)

Study	Species	Vascular Bed/Tissue	Experimental Technique	Propofol's postulated effects
Boillot[219]	Rat	Aorta	Myography	↑PG
Liu[220]	Rat	Renal artery	Myography	↑PG
Park[221]	Rat	Coronary artery	Myography	↑PG ↑NO
Tanaka[222]	Rat	Extrapulmonary artery	Myography	↑NO
Liu[224]	Rat Bovine	Mesenteric artery	Myography (Rat) VSMC culture (Bovine)	↑cGMP independent of NO
Nakahata[243]	Rat	Cerebral artery	Myography	↑NO ↓ROS
Petros[244]	Pig	Aorta	EC and VSMC culture	↑NO
Luo[261]	Human	Umbilical vein	HUVEC culture	↑NO
Wang[246]	Human	Umbilical vein	HUVEC culture	↑NO
Zhu[248]	Human	Umbilical vein	HUVEC culture	↑NO
Wang[247]	Human	Umbilical vein	HUVEC culture	↑NOS

Legend:
VSMC = vascular smooth muscle cell
HUVEC = human umbilical vein endothelial cell
EC = endothelial cell
PG = prostaglandins
NO = nitric oxide
NOS = NO synthase
ROS = reactive oxygen species
cGMP = cyclic guanosine monophosphate

Table 1-2. Summary of propofol's effects on vasoconstrictors pertinent to aging
(note: no studies done specifically in an aging model)

Study	Species	Vascular Bed/Tissue	Experimental Technique	Propofol's postulated effects
Hayashi[176]	Rat	Aorta	VSMC culture	↓ET-1 production
Cheng[226]	Human	Umbilical vein	HUVEC culture	↓ET-1 production
Samain[232]	Rat	Aorta	VSMC culture	↓Ang II Ca^{++} entry
Samain[233]	Rat	Aorta	Myography	↓Ang II constriction
Zou[236]	Rat	Cardiac	Cardiomyocyte culture	↓Ang II ROS
Chen[237]	Human	Coronary artery	EC culture	↓Ang II ROS

Legend:
VSMC = vascular smooth muscle cell
HUVEC = human umbilical vein endothelial cell
EC = endothelial cell
ET-1 = endothelin-1
Ang II = angiotensin II
ROS = reactive oxygen species

Table 1-3. Summary of propofol's effects regarding oxidative stress (note: no studies done specifically in an aging model)

Study	Species	Vascular Bed/Tissue	Experimental Technique	Propofol's postulated effects
Mathy-Hartert[239]	Human	Umbilical vein	HUVEC culture	↓ROS
Rodriguez-Lopez[240]	Pig	Kidney	*in vivo* propofol	↓ROS
Basu[241]	Pig	Plasma	*in vivo* propofol	↓ROS
Haba[242]	Rat	Aorta	Myography Tissue culture	↓ROS $\uparrow K_{ATP}$

Legend:
HUVEC = human umbilical vein endothelial cell
ROS = reactive oxygen species
$\uparrow K_{ATP}$ = ATP-sensitive K^+ channel activation

Table 1-4. Summary of propofol's effects regarding ion channels (note: no studies done specifically in an aging model)

Study	Species	Vascular Bed/Tissue	Experimental Technique	Propofol's postulated effects
Xuan[227]	Rat	Aorta	VSMC culture	↓L-type Ca^{++}
Lawton[249]	Rat	Mesenteric	Myography	↓L-type Ca^{++}
Wallerstedt[230]	Human	Omental artery and vein	Myography	↑K^+ channel (unspecified)
Bodelsson[231]	Human	Omental artery and vein	Myography	↑K^+ channel (unspecified)
Lam[250]	Rat	Aorta	Myography *in vivo* hemodynamics	↑K_{ATP}
Klockgether-Radke[251]	Pig	Coronary artery	Myography	↑K_{Ca}
Stadnicka[252]	Rat	Mesenteric artery	*in situ* hyperpolarization VSMC patch clamp	↑BK_{Ca}
Liu[253]	Mouse	Cerebral artery	VSMC patch clamp	↑BK_{Ca}
Nagakawa[254]	Rat	Mesenteric artery	*in situ* hyperpolarization *in vivo* hemodynamics	↑NO ↑K_{Ca} ↑K_{ATP}

Legend:
VSMC = vascular smooth muscle cell
↓L-type Ca^{++} = decreased L-type calcium channel function
↑K_{ATP} = ATP-sensitive K^+ channel activation
↑K_{Ca} = Ca^{++}-activated K^+ channel activation
↑BK_{Ca} = large conductance Ca^{++}-activated K^+ channel activation
NO = nitric oxide

1.5 SUMMARY AND HYPOTHESIS

There is a multitude of physiologic changes that occur in the cardiovascular system during the aging process. Anesthetic agents, and in particular propofol, can alter the hemodynamic profile of aging individuals during the perioperative period. Our studies were aimed at identifying mechanisms for enhanced propofol-induced vasodilation in resistance arteries from an aging animal model. The importance of NO in propofol's vasodilating abilities was demonstrated in mesenteric arteries of young rats,[254] so it is possible that an enhanced bioavailability of NO in aging mesenteric arteries would contribute to propofol's hypotensive responses in aging. Therefore, we hypothesize that NO plays a pivotal role in propofol-induced vasodilation in resistance arteries in aging, and this response is exaggerated in aging when compared to young. Given that the angiotensin system is an important pathway that is increased during the aging process, and because patients are commonly on medications that inhibit the angiotensin system, we hypothesize that propofol causes vasodilation in an aging experimental model of angiotensin inhibition which again is dependent on NO. Finally, we hypothesize that Intralipid, a medication that is used for total parenteral nutrition but has shown promise to reverse cardiovascular toxicity of lipophilic drugs, can be used to counteract the hypotensive actions of propofol in our aging animal model.

The specific hypotheses for the studies described in this thesis are as follows:

CHAPTER 2: Propofol increases vascular relaxation in arteries from aging compared to young rats.

Aim 1: To determine whether propofol-induced vasodilation is enhanced in resistance arteries isolated from aging rats compared to young rats and whether the endothelium is important in this response

Aim 2: To determine whether the modulation of endothelium-dependent vasodilation by propofol is enhanced in arteries from aging rats compared to young

Aim 3: To determine the importance of NO in the vascular relaxation response to propofol in aging rats

CHAPTER 3: Propofol increases vascular relaxation in aging rats chronically treated with the angiotensin-converting enzyme inhibitor captopril

Aim 1: To examine whether propofol-induced vasodilation is enhanced in resistance arteries isolated from aging rats chronically treated with an ACE inhibitor compared to untreated aging rats

Aim 2: To examine whether the modulation of endothelium-dependent vasodilation by propofol is enhanced in arteries from ACE inhibitor-treated aging rats compared to untreated aging rats

Aim 3: To determine the importance of NO in the vascular relaxation response to propofol in a state of chronic ACE inhibition

CHAPTER 4: Intralipid reverses propofol-induced vasodilation and hypotension in aging rats

Aim 1: To examine whether propofol-induced vasodilation *ex vivo* is reversed with the administration of substances known to solubilize propofol

Aim 2: To determine whether Intralipid reversal of propofol-induced vasodilation is greater in resistance arteries isolated from aging rats compared to young rats

Aim 3: To assess whether propofol-induced hypotension *in vivo* is reversed with the administration of Intralipid in aging and young rats

Aim 4: To assess whether Intralipid can reverse hypotension caused by other agents besides propofol in aging rats

Aim 5: To determine if Intralipid can be used to reverse propofol-induced hypotension in aging rats chronically treated with an ACE inhibitor compared to untreated aging rats

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CHAPTER 2:

PROPOFOL INCREASES VASCULAR RELAXATION IN ARTERIES FROM AGING COMPARED TO YOUNG RATS

A version of this chapter has been published:

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F. Gragasin contributed to study design, data collection, data analyses, and writing all drafts of the published manuscript. S. Davidge contributed to study design, data analyses, and editing all drafts of the published manuscript.

2.1 INTRODUCTION

Life expectancy has been increasing steadily over the years, and as a result, there is a greater proportion of the aging population seen perioperatively under the care of the Anesthesiologist. Propofol is very widely used not only by Anesthesiologists but also by Emergency Physicians, Intensivists, and other acute care physicians. In the aging patient population, the use of propofol is a strong predictor of developing intraoperative hypotension.[1]

The precise mechanism of action of propofol on the vasculature is controversial and may involve direct modulation of vascular tone in an endothelial-dependent and/or independent manner as discussed in Chapter 1. This may be dependent on the vascular bed studied. However, in the mesenteric arterial bed, it was found that propofol causes vascular relaxation *ex vivo*.[2] In addition, propofol causes mesenteric arterial hyperpolarization *in situ* with a concomitant decrease in blood pressure that may be dependent on nitric oxide (NO).[3] Mesenteric arteries contribute significantly to the alteration of systemic vascular resistance and hence are a major determinant of blood pressure.[4] Therefore, it is important to study this vascular bed when investigating the effects of anesthetic agents on the alteration of blood pressure, which is why we focus on this vascular bed in our studies.

Up until this published manuscript in 2009, there have been no studies specifically addressing the differences in the modulation of vascular tone by propofol between young and aging arteries. The investigation outlined in this chapter evaluates the effects of propofol alone and in combination with a known agonist of endothelial-dependent vasodilation in resistance mesenteric arteries of the rat. A unique property of propofol is that it acts as an antioxidant given its structural similarity to vitamin E,[5-6] so it may decrease the levels of free radicals in the aging vasculature, since aging is associated with increased oxidative stress. We hypothesized that propofol enhances NO-mediated vasodilation by activating NO synthase (NOS) and increasing bioavailability of NO by acting as a free radical scavenger in the aging vasculature leading to an enhanced vasodilation as compared to the young vasculature.

2.2 MATERIALS AND METHODS

This study was approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee and was in accordance with the Canadian Council on Animal Care and National Institutes of Health guidelines.

2.2.1 Animals

Female Sprague-Dawley rats aged 3 to 4 months (Young) or 13 to 15 months (Aging) were obtained from Charles River Breeding Laboratories (Quebec, Canada). Rats were housed in the animal facilities at the University of Alberta until experimentation.

2.2.2 Tissue Isolation

The rats were anesthetized by inhalation of isoflurane (dosed to effect by inhalation) and the mesentery was rapidly excised and placed in iced HEPES-buffered physiological saline solution (sodium chloride 142 mmol/L, potassium chloride 4.7 mmol/L, magnesium sulphate 1.17 mmol/L, calcium chloride 4.7 mmol/L, potassium phosphate 1.18 mmol/L, HEPES 10 mmol/L, and glucose 5.5 mmol/L, pH 7.4, 4°C). The rats were then euthanized by exsanguination via the puncturing of the inferior vena cava. Mesenteric arteries were carefully dissected using a binocular microscope, and arteries with internal diameters ranging 100-200 μm were mounted in an isometric myograph system. Specifically, mesenteric artery segments were divided into sub-segments. Each sub-segment was randomized and subjected to receive either no inhibitor, L-nitro arginine methyl ester (L-NAME; a non-selective inhibitor of NOS isoforms), meclofenamate (meclo; a non-selective inhibitor of PGHS isoforms), or L-NAME+meclo, in separate wire myography baths;

see section 2.2.3 below. Each sub-segment was also subjected to the combination of propofol pre-treatment and acetylcholine (ACh) relaxation, as well as direct vascular relaxation to propofol itself. Four separate baths at 37°C were used to study arterial segments simultaneously.

2.2.3 Protocols for Vascular Reactivity

To determine if propofol has an effect on vasoconstriction, mesenteric arteries from young rats were constricted with cumulative doses of the α-adrenoreceptor agonist phenylephrine (PE; 0.1-100 μmol/L) in the absence and presence of propofol. This constriction was compared to that elicited by high-dose KCl (128 mM) in the absence of propofol. Based on our pilot data, a phenylephrine dose of 10 μmol/L gives us an EC_{90} for constriction in this vascular bed. Therefore, we utilized this dose of phenylephrine for all experiments in this study. By utilizing the value of % relaxation, we attempt to normalize the amount of absolute relaxation between the potential differing pre-constrictions amongst different arteries. Endothelial denudation was achieved by passing a human hair through the arterial lumen[7] and confirmed by lack of acetylcholine (ACh)-induced vasodilation. To determine the extent of endothelium-dependent vasodilation, we compared the reactivity following PE constriction of endothelial-intact and denuded arteries from young rats under three conditions: 1) propofol alone (1-100 μmol/L), 2) lipid emulsion

(Intralipid) vehicle control, and 3) ACh (0.1-10 μmol/L) following 10 minute pre-treatment with and without propofol (1-100 μmol/L). The propofol preparation we used in this study is that which is available clinically (i.e. Diprivan®; AstraZeneca Canada): 10 mg/mL propofol solubilized in a 10% Intralipid solution, which consists of 10% soybean oil, 1.2% egg phosphatide, 2.25% glycerol, 0.55% disodium edatate, and water with sodium hydroxide to adjust pH 6.5-8.5. The doses of propofol used in this study are based on the doses used by others.[2,7-8]

Vascular reactivity was then compared between endothelial-intact arteries from young and aging rats. Relaxation to propofol and ACh followed the same protocol as mentioned above in the presence and absence of L-NAME (100 μmol/L) or meclo (10 μmol/L). The combination of L-NAME and meclo, which would yield residual relaxation attributed to EDHF, was also used. The inhibitors were allowed to incubate in the baths for 15 minutes. Four separate tissue baths were used for the duration of the experiment and allowed for consistent use of endothelial inhibitors for the same sub-segments of arteries. In other words, bath 1 served as control (no propofol, followed by propofol 1 μmol/L, followed by propofol 10 μmol/L, followed by propofol 100 μmol/L pre-treatment), bath 2 had L-NAME added (no propofol, followed by propofol 1 μmol/L, followed by propofol 10 μmol/L, followed by propofol 100 μmol/L pre-treatment), bath 3 had meclo added (no propofol, followed by propofol 1 μmol/L, followed by propofol 10 μmol/L, followed by propofol

100 μmol/L pre-treatment), and bath 4 had L-NAME+meclo added (no propofol, followed by propofol 1 μmol/L, followed by propofol 10 μmol/L, followed by propofol 100 μmol/L pre-treatment).

To investigate the role of free radical scavenging on ACh relaxation in arteries from aging rats, superoxide dismutase (SOD; 50 units/mL) and catalase (500 units/mL) were added to the baths. SOD and catalase in the absence of inhibitors were incubated for 15 minutes prior to stimulation with PE and ACh. In a final series of experiments, arteries were pre-incubated with L-NAME and meclo as above, and relaxation to low-dose KCl (7.2-19.7 mmol/L total in the bath) following pre-treatment with propofol was assessed following PE constriction.

2.2.4 Drugs

PE, ACh, L-NAME, meclo, SOD, and catalase were purchased from Sigma-Aldrich (Oakville, ON, Canada); commercially available propofol in its lipid formulation (Diprivan®) was purchased through our hospital formulary (University of Alberta, Edmonton, Canada); Intralipid was purchased from Baxter Canada (Mississauga, ON, Canada). All drugs were dissolved in purified water unless otherwise stated.

2.2.5 Statistical Analyses

Graphpad Prism 4 software (GraphPad Software, Inc., San Diego, CA, USA) on a Windows XP platform was used for all statistical analyses. Values are expressed as mean±SEM. Since the concentration-response curves for propofol and ACh did not resemble sigmoidal-shaped curves, the data was summarized as area under the curve (AUC) for analyses. AUC was obtained by plotting the individual experimental traces into a relaxation curve, and the summation of the relaxations for each dose was calculated. Intergroup differences were assessed by 1-way analysis of variance (ANOVA) with Tukey post-hoc analysis for multiple comparisons, or Student's t-test, as appropriate. A value of $P<0.05$ was considered statistically significant.

2.3 RESULTS

2.3.1 Effects of propofol alone in endothelial-intact mesenteric arteries from young and aging rats

To determine the extent of endothelial-dependent propofol-induced vasodilation, mesenteric arteries from young rats were denuded of endothelium and the relaxation to propofol was compared to that in arteries

with an intact endothelium. Propofol alone elicited relaxation that is significantly greater in the presence of endothelium than without (83.0±7.2% versus 58.3±6.8% maximal relaxation). Maximal relaxation refers to that relaxation elicited by the highest respective propofol dose used. Additionally, maximal propofol-induced relaxation in these groups was greater than that due to their respective Intralipid volume controls (24.2±2.2% in intact versus 16.1±2.4% in denuded groups) (Figure 2-1A). As can be evidenced in Figure 2-1A, it appears that the majority of endothelial-dependent relaxation occurs at the lower doses (1-10 μmol/L). This was ascertained by the similar change in magnitude of relaxation from 10-100 μmol/L propofol (~52% and 57% in endothelial-denuded and intact vessels, respectively). Therefore, propofol doses of 1-10 μmol/L are shown for the remainder of our study. Additionally, pre-incubation of the arteries with 1-10 μmol/L of propofol did not affect PE-induced constriction, but propofol 100 μmol/L inhibited maximal PE-induced constriction (Figure 2-1B) which confounds the interpretation of relaxation caused by propofol. However, this effect was not due to cellular toxicity, since constriction to PE is restored following washout of propofol 100 μmol/L.

When comparing arteries from aging to young rats, propofol alone in the aging group significantly elicited greater relaxation than in the young group (Figure 2-2A). The maximal relaxation achieved by propofol in arteries from the aging group was 33.4±2.5% whereas in the young it was 26.5±2.4% ($P<0.05$). L-NAME was used to inhibit NO production. In the young group,

the relaxation to propofol was significantly inhibited by L-NAME (Figure 2-2B). The relaxation to propofol was also significantly inhibited by L-NAME in the aging group (Figure 2-2C). However, the inhibition of relaxation in arteries from the aging was greater than in the young (~14% and 24% inhibition in aging and ~2% and 14% inhibition in young at propofol 1 and 10 μmol/L, respectively). These findings suggest a contribution of NO synthesis to propofol-induced relaxation in arteries from aging rats more so than in the young. There was no significant difference when comparing the control group to the meclo-treated group in the arteries from aging or young rats.

2.3.2 Effects of propofol on ACh-induced relaxation in arteries isolated from young and aging rats

There was significantly greater relaxation in arteries from young rats compared to aging rats in response to ACh (95.1±1.3% versus 57.9±5.1% maximal relaxation, respectively; Figure 2-3A). In the young group, there was no significant difference to relaxation caused by ACh in arteries pre-treated with propofol (Figure 2-3B). In contrast, there was a significant increase in relaxation caused by ACh in the arteries from the aging group pre-treated with propofol (80.4±5.0% and 84.6±5.9% maximal relaxation for 1 and 10 μmol/L propofol, respectively; Figure 2-3C). To determine if antioxidant properties of propofol play a role in altered vascular relaxation in aging, we added SOD and

catalase which are known antioxidants. SOD and catalase significantly increased relaxation to ACh in arteries from the aging group (71.7±8.0% maximal relaxation; Figure 2-3D). This relaxation was similar in magnitude to that elicited by ACh in the presence of propofol. This demonstrates a potential antioxidant component to the enhancement of ACh-induced relaxation by propofol.

When elucidating the contribution of endothelial-derived mediators to ACh relaxation in arteries from aging rats, the addition of L-NAME or L-NAME+meclo did not significantly alter relaxation. However, pre-treatment with meclo alone significantly increased relaxation. This demonstrates the inhibition of PGHS-dependent vasoconstriction seen in aging, which our laboratory has previously shown.[9] Propofol pre-treatment resulted in a dose-dependent decrease in relaxation to ACh in the presence of L-NAME and L-NAME+meclo but not in arteries treated with meclo alone (Figure 2-4). Relaxation to ACh in the presence of propofol exceeded that in control arteries and approached that in the meclo-alone group. This suggests that a mechanism exists by which propofol enhances relaxation in arteries from the aging group in spite of the loss of EDHF-dependent relaxation. Since L-NAME alone inhibits ACh-induced relaxation in the presence of propofol, it is likely that propofol is not inhibiting PGHS-dependent vasoconstriction but is in fact enhancing NO-mediated vasodilation.

2.3.3 Effects of propofol on EDHF-mediated relaxation

By inhibiting NOS with L-NAME and PGHS with meclo, the remaining relaxation is attributed to EDHF. EDHF constitutes the majority of ACh-induced relaxation, since there was no significant difference in the relaxation in control versus L-NAME+meclo treated arteries in both the young and aging groups (Figure 2-5A-B). However, propofol dose-dependently inhibited the relaxation produced by ACh in the presence of L-NAME+meclo in both the young and aging groups (Figure 2-5C-D). There is not one universal factor involved in EDHF-mediated relaxation, and its identity is thought to be species and vascular-bed specific. However, K^+ has been postulated to be the EDHF in this type of artery in the same species and strain as our experiments.[10] Propofol did not inhibit the relaxation to low dose K^+ (KCl 7.2-19.7 mmol/L total in the bath; Figure 2-6). Therefore, this suggests it is the release of K^+ from the endothelium following ACh stimulation, and not the action of K^+ on smooth muscle, which is inhibited by propofol.

2.4 DISCUSSION

The goal of this study was to determine if there was a difference between aging and young arteries in the vasodilation caused by propofol either alone or by modulating the activity of ACh. The primary finding in this study

is that propofol enhances vasodilation in arteries from the aging animals compared to the young. This may be one of the mechanisms underlying the hypotension seen when inducing anethesia with propofol in the aging patient population. It has been suggested that the dose used to induce general anesthesia in patients aged > 60 years should be reduced,[11] particularly since patients in this age group have been shown to have decreased clearance of the drug.[12] In addition to this possible mechanism contributing to hypotension with propofol, our study has now shown an increased responsiveness of arteries from aging animals to the vasodilating effects of propofol. The present study gives us insight as to why enhanced vasodilation occurs in aging arteries at the vascular level. Propofol is capable of partly inducing endothelial-independent vasodilation as observed with the highest dose used in our study. However, the focus of this study was to look at the endothelial-dependent effects of propofol, so we chose to analyze the lower doses of propofol used in this investigation. The relaxation achieved by propofol alone was greater in the aging rats compared to the young. More importantly, propofol enhances endothelial-dependent vasodilation by increasing the NO-mediated component whilst inhibiting the component mediated by EDHF. This is prominently observed in the aging vasculature where pre-treatment of propofol partially restores the relaxation response to ACh to that seen in arteries from young animals. Figures 2-7 and 2-8 summarize the postulated changes in the aging vasculature with the addition of propofol.

Although propofol alone causes relaxation in these mesenteric arteries, it is interesting that we also see that the Intralipid vehicle induces a slight relaxation which is greater in an intact artery than one denuded of endothelium. This suggests that either denudation removes any endothelial-derived vasodilators which normally antagonize the constriction caused by PE, or, alternatively, Intralipid causes endothelium-dependent vasodilation. Indeed, it has been suggested that Intralipid itself interacts with the NO pathway.[16] However, the fact that there was a significant difference between propofol- and Intralipid-induced vasodilation in our study suggests that the response is primarily due to propofol. An interesting finding in our study is that L-NAME inhibition of direct propofol-induced dilation in aging arteries is greater when propofol increases from 1 to 10 μmol/L (Figure 2-2C) whereas the potentiation of ACh-induced dilation is no greater when pre-treated with 1 or 10 μmol/L propofol (Figure 2-3C). One explanation can be that ACh substantially increases the NO production when compared to propofol alone. This may be to the point where the difference in ACh-induced production of NO in the presence of 1 versus 10 μmol/L propofol is negligible in the overall scheme. Another explanation is that this may demonstrate an "immediate" and "delayed" effect with the addition of propofol, respectively (i.e. "immediate" release of NO with direct propofol stimulation versus "delayed" enhanced bioavailability of NO upon stimulation with ACh i.e. indicated by a potential antioxidant effect).

Although propofol causes endothelial-dependent relaxation, our study also demonstrates that propofol dose-dependently decreases the EDHF-mediated contribution to vasodilation in systemic arteries, a finding not previously reported to our knowledge. There are many postulated EDHFs, and some candidates include epoxyeicosatrienoic acids,[24] H_2O_2,[25-26] and the K^+ ion.[10] It must be emphasized though that the identity of EDHF is species- and vascular bed-specific, and there is not one universal factor. However, in the same strain of rat used in our study, Edwards et al. demonstrated that K^+ serves as EDHF in the mesenteric artery by activating inward-rectifier potassium channels (K_{ir}) and Na^+-K^+ ATPase.[10] Here, propofol fails to significantly inhibit the relaxation to low-dose KCl. Thus, propofol does not inhibit the hyperpolarization-relaxation response to EDHF but may actually inhibit its release from the endothelium. Nagakawa et al. also used mesenteric arteries from the same strain of rat and demonstrated that propofol-induced hyperpolarization occurs through calcium-activated K^+ channels (K_{Ca}) and ATP-sensitive K^+ channels (K_{ATP}) which can be abolished by inhibiting NO synthesis.[3] These findings suggest that hyperpolarization occurs through NO and not directly by propofol per se. Indeed, it is known that NO causes hyperpolarization and relaxation by activating K_{Ca} [27-28] and K_{ATP} channels [29-30]. EDHF-mediated relaxation in our study contributes to the majority of vasodilation to ACh in arteries from both aging and young rats. Thus, the fact that EDHF-mediated relaxation is inhibited by propofol but relaxation to ACh

is still relatively maintained in the young and even enhanced in the aging suggests this may be due to propofol increasing NO synthesis and/or bioavailability. A consideration, however, is that vascular smooth muscle sensitivity may change with age i.e. relaxation in response to NO may be different in the aging and young vasculature. Although we did not directly determine this in the present study, we have previously shown that relaxation to sodium nitroprusside, a NO donor, does not differ between mesenteric arteries from young and aging rats.[31]

Given its structural similarity to vitamin E,[6] the mechanism by which propofol exerts its effects of increased NO may in part be through an antioxidant mechanism. Aging arteries pre-treated with propofol yield similar results to those pre-treated with SOD and catalase. This is in agreement with previous findings where propofol has been shown to restore endothelial function in septic rats, likely through an antioxidant mechanism.[32] Propofol has also been shown to stimulate release of NO from cultured aortic endothelial cells[33] again suggesting that propofol also directly activates NOS. If it were solely an antioxidant mechanism, this would not explain the inhibition of propofol on EDHF-dependent relaxation but enhanced overall relaxation in arteries from aging animals. Indeed, H_2O_2, a product of SOD, has been proposed to be EDHF in some vascular beds as mentioned above. Hence, the presence of SOD, if anything, would enhance and not inhibit hyperpolarization-relaxation, contrary to what may be happening with

propofol and K^+ as a hyperpolarization component. It is therefore likely an overall enhanced bioavailability of NO (possibly through a combination of increased production of NO and the antioxidant effects of propofol) in the aging vasculature which explains the ACh-induced vasodilating effects of propofol. Moreover, we see that pre-treatment of L-NAME decreases relaxation elicited by propofol alone in the aging vasculature further demonstrating the link with NO production and stimulation with propofol. It is also interesting to speculate that an increase in NOS expression in the aging vasculature may be one of the reasons for increased relaxation in the presence of propofol. That is, propofol, when added acutely, may increase the availability of NO in a state where potential production of NO is already increased. Indeed, Briones et al. utilized Sprague-Dawley rats and found eNOS expression to be increased in mesenteric arteries in aging compared to young rats.[34]

In conclusion, propofol inhibits ACh-induced EDHF-dependent relaxation in small mesenteric arteries, a novel finding not yet described in the systemic circulation. This phenomenon however has been suggested to occur and contribute to propofol-induced constriction in pulmonary arteries.[21] The difference in our study is the finding that relaxation to ACh in the presence of propofol persists despite the majority of relaxation in these arteries is attributed to EDHF. This is due to an increase in NO-mediated vasodilation, a possibly normal physiologic phenomenon regarding this anesthetic agent.

Additionally, propofol enhances vasodilation in small mesenteric arteries from aging rats greater than in the young that is due in part to an increase in NO bioavailability. These findings contribute to gaining further insight as to the mechanism of action of propofol at the vascular level and help explain, at least in part, why there is a higher incidence of hypotension in the aging population with the use of propofol in their perioperative anesthetic care.

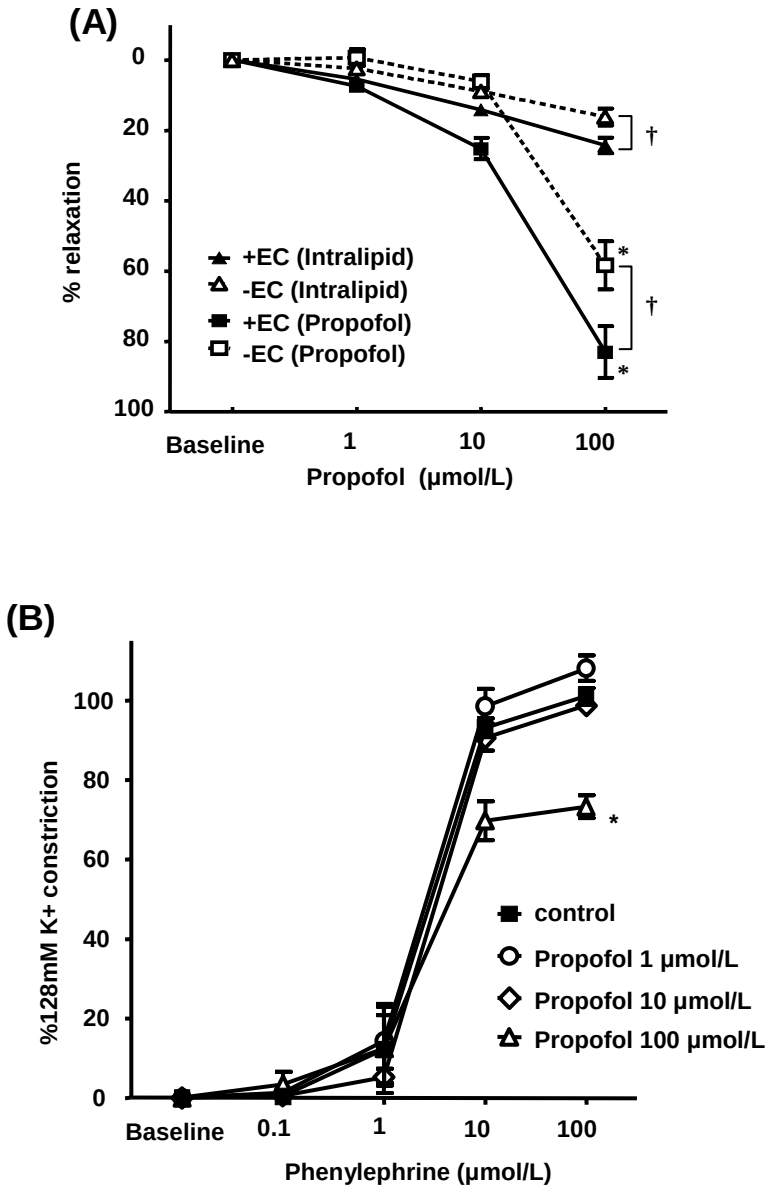

Figure 2-1. Propofol-induced relaxation and its effects on PE constriction in arteries from young rats.

(**A**) Mean data showing propofol-induced dose-dependent relaxation in comparison to Intralipid control in endothelial-intact (+EC) and denuded (-EC) arteries. (**B**) Mean data showing PE constriction in the presence of 0-100 μmol/L propofol. Maximal PE constriction is significantly reduced in the presence of 100 μmol/L propofol. N=5 per group; †$P<0.05$ vs. respective denuded, *$P<0.05$ vs. respective control.

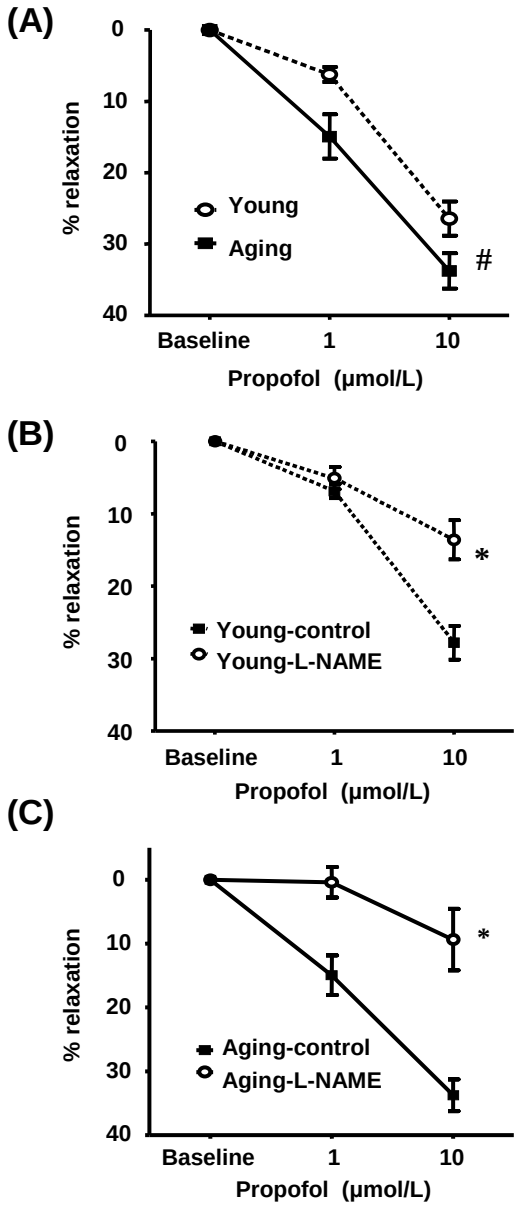

Figure 2-2. Comparison of propofol-induced relaxation in arteries from young and aging rats.

(**A**) Mean data demonstrating the dose-dependent relaxation to propofol in arteries from young compared to aging rats. Mean data demonstrating the dose-dependent relaxation to propofol in the presence and absence of L-NAME in (**B**) young and (**C**) aging arteries. N=6-7 per group; #P<0.05 aging vs. young; *P<0.05 vs. control.

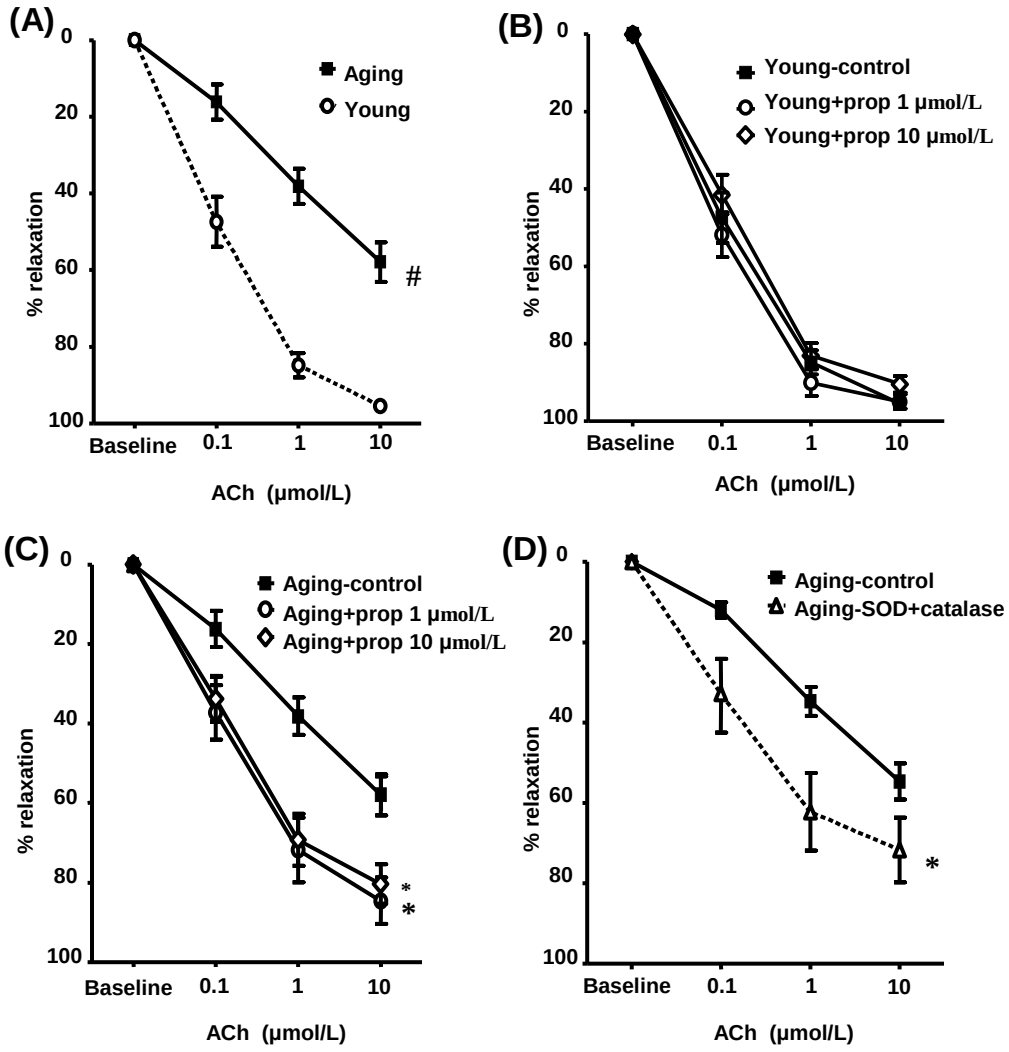

Figure 2-3. Comparison of ACh-induced relaxation in young and aging arteries in the presence or absence of propofol.

(**A**) Mean data showing the dose-dependent relaxation to ACh in arteries from young compared to aging rats. Mean data showing the dose-dependent relaxation to ACh in (**B**) young and (**C**) aging arteries in the absence and presence of increasing doses of propofol. (**D**) Mean data showing ACh-induced relaxation in the presence of SOD and catalase in aging arteries. N=7-8 per group; #p<0.05 aging vs. young; *P<0.05 vs. control.

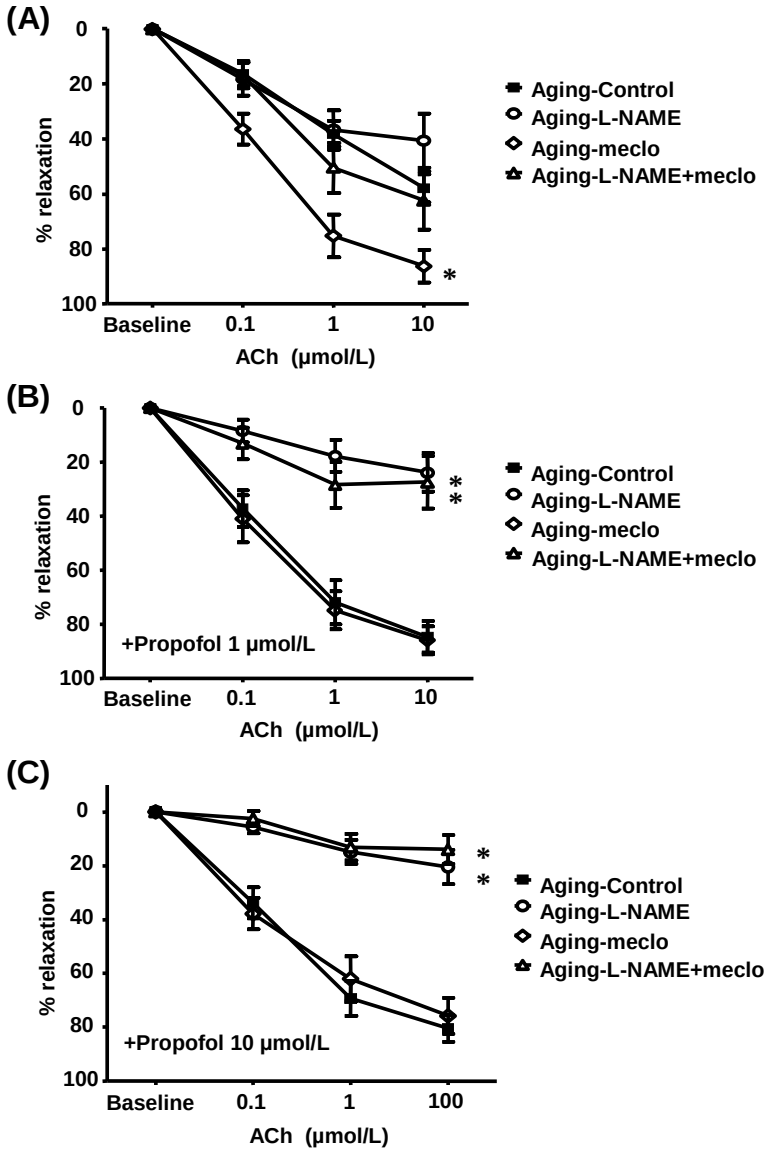

Figure 2-4. Comparison of relaxation to ACh in the presence of increasing doses of propofol in arteries from aging rats.

(**A**) Mean data showing relaxation to ACh in aging arteries in the presence of L-NAME (NOS inhibitor), meclo (PGHS inhibitor), and L-NAME+meclo (relaxation that is attributed to EDHF). Mean data demonstrating relaxation to ACh with (**B**) propofol 1 μmol/L and (**C**) propofol 10 μmol/L in aging arteries in the presence of L-NAME, meclo, and L-NAME+meclo. N=8 per group; *P<0.05 vs. control.

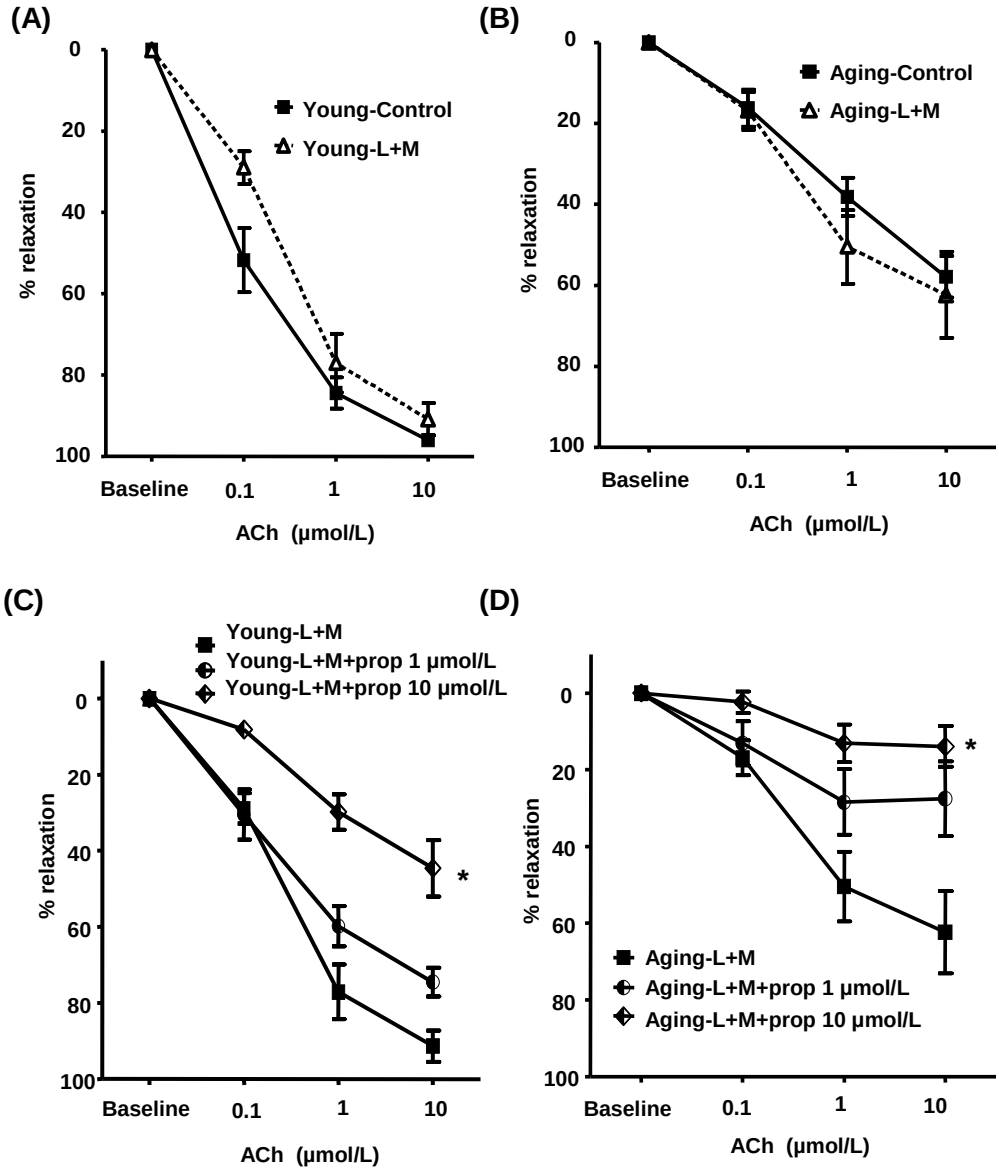

Figure 2-5. Comparison of EDHF-mediated relaxation in the presence of propofol in both young and aging arteries.

Mean data comparing the contributions of EDHF-dependent relaxation to the total relaxation elicited in (**A**) young and (**B**) aging control arteries. Mean data demonstrating the effects of increasing doses of propofol to ACh-induced EDHF-dependent relaxation in (**C**) young and (**D**) aging arteries. L=L-NAME, M=meclo. N=6-8 per group; *P<0.05 vs. control.

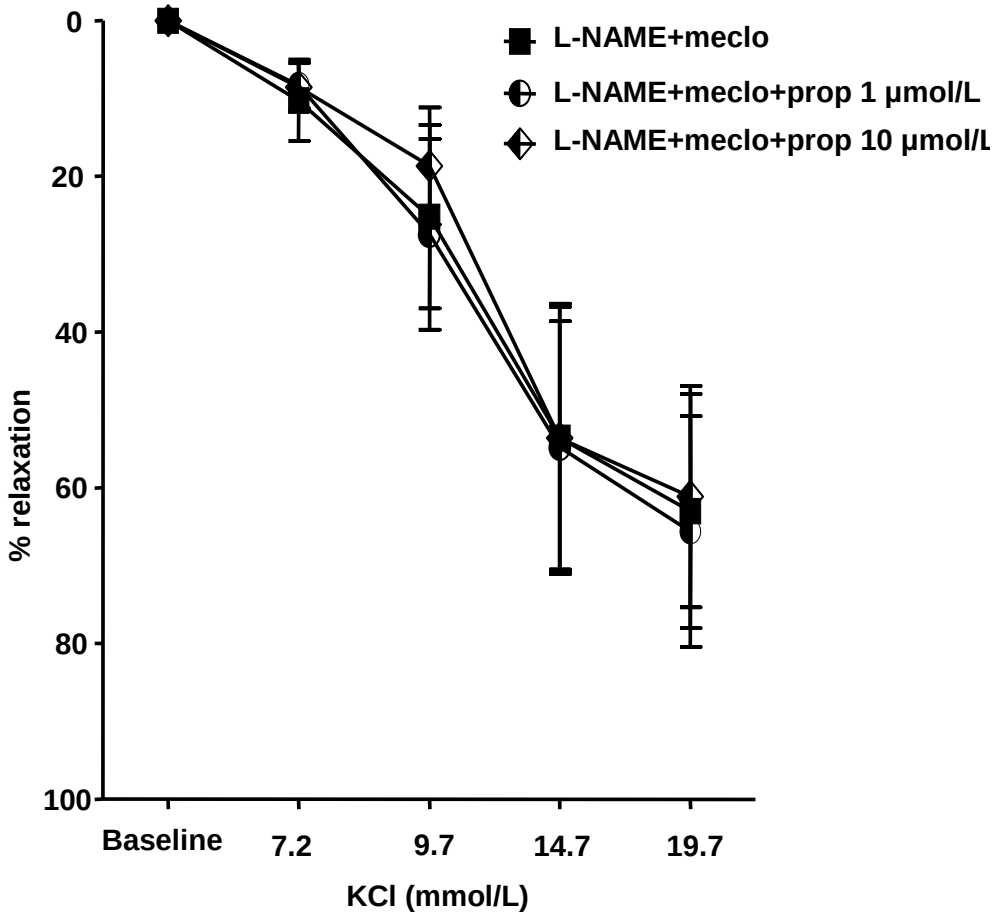

Figure 2-6. Relaxation to low-dose KCl in the presence of propofol.

Mean data demonstrating the effects of increasing doses of propofol to the relaxation of mesenteric arteries elicited by exogenous low-dose KCl. N=5.

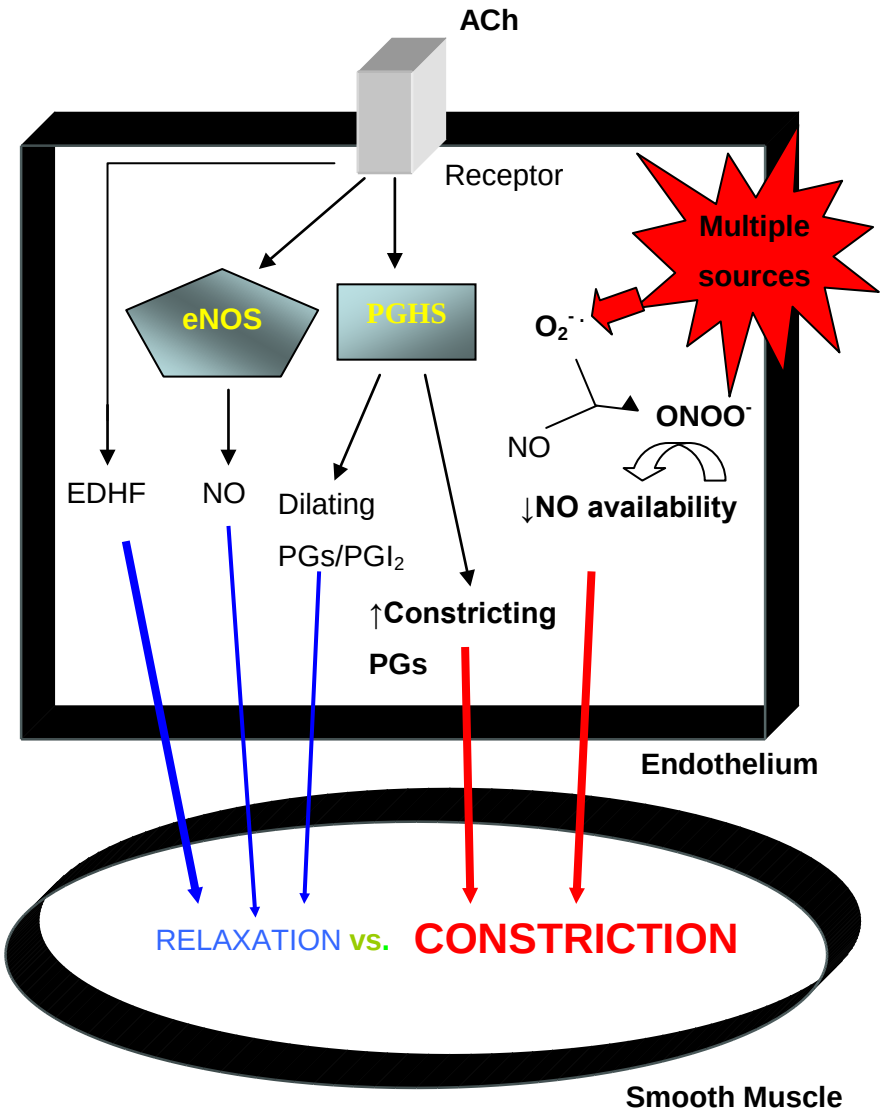

Figure 2-7. Schematic representation of endothelium-dependent dilation in aging.

The imbalance of tone leading to vasoconstriction partly due to endothelial dysfunction is seen with aging. In particular, despite an increase in NOS expression, there is a decrease in NO bioavailability secondary to increased production of free radicals (superoxide [$O_2^{-\cdot}$], which is formed by multiple sources, combines with NO to form peroxynitrite [$ONOO^-$]) and an increase in formation of constricting prostaglandins in the aging vasculature. PG=prostaglandin

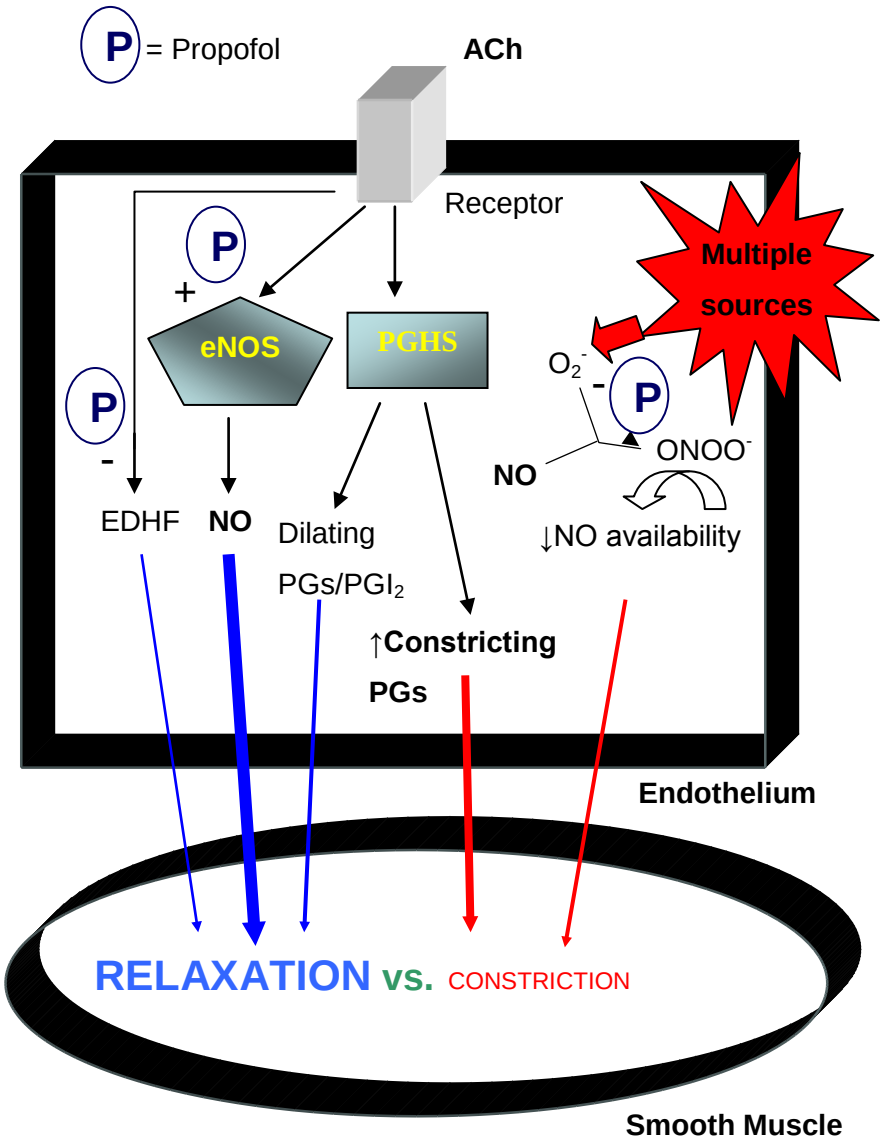

Figure 2-8. Schematic representation of endothelium-dependent dilation in aging and the changes seen with the addition of propofol.

Although propofol inhibits EDHF-dependent relaxation, it shifts the balance of tone to vasodilation in aging arteries by increasing nitric oxide (NO) availability. PG=prostaglandin, PGHS=PG H synthase, eNOS=endothelial NO synthase, P=propofol.

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CHAPTER 3:

PROPOFOL INCREASES VASCULAR RELAXATION IN AGING RATS CHRONICALLY TREATED WITH THE ANGIOTENSIN CONVERTING ENZYME CAPTOPRIL

A version of this chapter has been published:

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F. Gragasin contributed to study design, data collection, data analyses, and writing all drafts of the published manuscript. S. Bourque and S. Davidge contributed to study design, data analyses, and editing all drafts of the published manuscript.

3.1 INTRODUCTION

As alluded to in the previous chapters, propofol is widely used for patient care in the perioperative setting. Perioperative hypotension is associated with unfavourable cardiovascular outcomes.[1] Again, increasing age of the patient as well as the use of propofol in anesthetic care are predictors of intraoperative hypotension in patients receiving general anesthesia for their surgical care.[2] As discussed in Chapter 2, we demonstrated that, in systemic resistance arteries from the aging rat, vasodilation is increased in the presence of propofol. This was due in part to an increase in NO-mediated vasodilation, a possibly normal physiologic phenomenon regarding this anesthetic agent. These findings gave further insight regarding the mechanism of action of propofol at the vascular level and helped explain, at least in part, the hypotension that can be seen in the aging population with the use of propofol in their perioperative care.

On the other end of the spectrum, blood pressure increases as we age, and it has been estimated that hypertension occurs in up to 70% of the elderly population.[3] Angiotensin II antagonists such as angiotensin converting enzyme (ACE) inhibitors are commonly prescribed medications for treating hypertension in the elderly and for patients with impaired left ventricular function. It has been documented that patients on these medications may

exhibit greater hypotension under general anesthesia.[4-7] However, the reasons for this have not been fully investigated, particularly in the aging population. Ang II antagonism has been shown to improve vascular function in pathophysiological states by potentially increasing NO bioavailability and reversing oxidative stress.[8-10] Therefore, given our functional findings in the aging vasculature, there is the likelihood of interactions between the two pathways initiated by angiotensin II and propofol. Indeed, it is suggested that the concomitant use of nitroglycerin to alter intraoperative hemodynamics with the perioperative use of ACE inhibitors can cause severe hypotension in patients under general anesthesia.[11] Additionally, it has been reported that NOS uncoupling occurs with aging such that the enzyme produces superoxide rather than NO, and this uncoupling can be reversed with blocking the Ang II pathway with an ACE inhibitor to restore NO availability.[12-13] Thus, we hypothesized that, in aging rats chronically treated with the ACE inhibitor captopril, the administration of propofol enhances NO-mediated vasodilation.

3.2 MATERIALS AND METHODS

This study was approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee and was in accordance with the

Canadian Council on Animal Care and National Institutes of Health guidelines.

3.2.1 Animals

Female Sprague-Dawley rats aged 3 months were obtained from Charles River Breeding Laboratories (Quebec, Canada). Rats were housed and aged in the animal facilities at the University of Alberta until experimentation.

3.2.2 Treatment Groups

Rats were aged to 12 to 13 months and were treated with the ACE inhibitor captopril (2 g/L) dissolved in drinking water, which was available ad libitum, for a duration of 7 to 8 weeks. Control animals received no treatment in their drinking water. The final age of the animals at the time of experimentation was 14 to 15 months. The concentration and duration of captopril treatment were chosen based on previous studies that showed a lowering of plasma Ang II levels with this dosage.[14-16]

3.2.3 Blood Pressure Measurement and Tissue Isolation

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (70 mg/kg). Once surgical anesthesia was achieved, the left

carotid artery of each rat was exposed and invasively cannulated with an ultra-miniature Mikro-Tip® pressure transducer catheter (Millar Instruments, Houston, TX, USA). The systolic and diastolic blood pressures were recorded continuously for 10 minutes. The peak blood pressures recorded during these time periods were considered as baseline blood pressure. The mesentery was then rapidly excised and placed in iced HEPES-buffered physiological saline solution, as previously described in section 2.2.2. The rats were then euthanized by exsanguination via the puncturing of the inferior vena cava. Mesenteric arteries (100-200 μm internal diameters) were dissected and mounted in an isometric myograph system, as described in section 2.2.2. Separate tissue baths at 37°C were used to study arterial segments simultaneously. For this study, we have assessed 7 control and 7 captopril-treated rats. After each rat was euthanized, the mesenteric artery segments were divided into sub-segments, and randomized to receive inhibitors of endothelial-dependent vasodilation as described in section 2.2.2. Each sub-segment was subjected to the combination of propofol pre-treatment and methacholine (MCh) relaxation, as well as direct vascular relaxation to propofol itself.

3.2.4 Protocols for Vascular Reactivity

Cumulative doses of the α-adrenoreceptor agonist phenylephrine (PE; 0.1-100 μmol/L) were administered at the start of each experiment to determine the EC_{80} dose to be used for the remainder of each protocol. By utilizing the value of % relaxation, we attempted to normalize the amount of absolute relaxation between the potential differing pre-constrictions amongst different arteries. Vascular reactivity was compared between arteries from rats treated with and without captopril. Intact endothelium was confirmed with a relaxation response to MCh, the stable analog of acetylcholine. We assessed the reactivity following PE constriction under two conditions: 1) propofol alone (0.1-100 μmol/L), and 2) MCh (0.01-3 μmol/L) following 10 minute pre-treatment with and without propofol (1 and 10 μmol/L). Vascular tone measurements were taken at the end of each time point. The propofol preparation we used in this study is that which is available clinically. Since we have shown that there is a significantly greater vasodilation with propofol compared to its vehicle Intralipid in Chapter 2, we did not add an Intralipid arm in this study. In order to determine the contributions of endothelial-dependent pathways of relaxation to propofol and MCh, L-NAME (100 μmol/L), an inhibitor of NOS, or meclo (10 μmol/L), an inhibitor of prostaglandin H synthase (PGHS), was added. The combination of L-NAME and meclo, which would result in residual relaxation attributed to endothelium-derived hyperpolarizing factor (EDHF), was also used. The

inhibitors were allowed to incubate in the baths for 15 minutes. Four separate tissue baths were used for the duration of the experiment and allowed for consistent use of endothelial inhibitors for the same sub-segments of arteries. In other words, bath 1 served as control (no propofol, followed by propofol 1 μmol/L, followed by propofol 10 μmol/L pre-treatment), bath 2 had L-NAME added (no propofol, followed by propofol 1 μmol/L, followed by propofol 10 μmol/L pre-treatment), bath 3 had meclo added (no propofol, followed by propofol 1 μmol/L, followed by propofol 10 μmol/L pre-treatment), and bath 4 had L-NAME+meclo added (no propofol, followed by propofol 1 μmol/L, followed by propofol 10 μmol/L pre-treatment).

3.2.5 Drugs

All drugs were purchased from the same sources mentioned in section 2.2.4. Captopril was purchased from Sigma-Aldrich (Oakville, ON, Canada).

3.2.6 Statistical Analyses

Graphpad Prism 4 software (GraphPad Software Inc., San Diego, CA, USA) on a Windows 7 platform was used for all statistical analyses. Values are expressed as mean±SEM. The logarithm of the drug concentration eliciting 50% of the maximum relaxation response (EC_{50}) was calculated using nonlinear regression analysis by fitting the concentration-response for

methacholine to a sigmoidal-shaped curve. For data that did not resemble sigmoidal-shaped curves, area under the curve (AUC) was used to summarize the data, as described in section 2.2.5. Intergroup differences were assessed by Student's t-test, or 1-way ANOVA with Tukey post-hoc analysis or 2-way ANOVA with Bonferroni post-hoc analysis, as appropriate, for multiple comparisons. A value of $P<0.05$ was considered statistically significant.

3.3 RESULTS

3.3.1 Blood pressure is decreased in rats chronically treated with captopril

Both the systolic and mean arterial pressures were significantly lower in rats treated with captopril compared to control (95.7±8.3 mmHg versus 120.3±6.4 mmHg, $P<0.05$; and 77.3±8.0 mmHg versus 98.6±5.5 mmHg, $P<0.05$, respectively, Figure 3-1). There was a trend for lower diastolic blood pressure in the captopril-treated rats compared to control, but it did not achieve statistical significance (68.1±8.0 mmHg versus 87.9±5.2 mmHg, $P=0.06$). Despite this lower blood pressure, it should be noted that the rats did not have changes in behaviour while housed in our animal facility which would have suggested the presence of symptomatic hypotension.

3.3.2 Effects of propofol alone in mesenteric arteries from rats treated with and without captopril

Results of the arterial relaxation experiments are shown in Figure 3-2. Cumulative doses of propofol resulted in greater arterial relaxation in rats chronically treated with captopril compared with control animals. The AUC revealed the captopril dose-arterial relaxation response to propofol was greater than the control response. Maximal arterial relaxation was significantly greater in rats treated with captopril than without (97.8±0.7% versus 63.7±11.3%, $P<0.05$). Maximal relaxation refers to that elicited by the highest respective propofol dose used (100 µmol/L) i.e. the percent reversal of PE-induced constriction back towards baseline tension prior to constriction. When assessing endothelial-derived mediators involved in this response, statistical significance was achieved with the addition of L-NAME and L-NAME+meclo, but not meclo alone, in regards to decreased relaxation in arteries from captopril-treated but not from control rats (Figure 3-2C and 3-2D).

3.3.3 Effects of propofol on MCh-induced relaxation in arteries from rats treated with and without captopril

In the absence of propofol, there was no difference in relaxation in response to MCh in rats treated with captopril versus control rats (Figure 3-

3A). Upon the addition of 1 or 10 µmol/L propofol to the tissue bath, sensitivity to MCh was significantly increased in captopril-treated rats greater than in control (Figure 3-3B and 3-3C). The contribution of endothelial-derived mediators to MCh relaxation in arteries from aging rats treated with or without captopril were investigated by assessing relaxation in control and captopril-treated animals in the presence of L-NAME, meclo, and the combination of L-NAME+meclo as shown in Figure 3-4. The presence of L-NAME decreased relaxation in arteries from both control and captopril-treated rats when compared to meclo. However, L-NAME decreased relaxation to MCh only in the control group compared to no inhibitor (Figure 3-4A). With propofol pre-treatment, we hypothesized that the contribution of NO to vascular relaxation is increased in both control and in captopril-treated rats. In the presence of propofol, the contribution of NO to vascular relaxation was increased in both control and in captopril-treated rats. Propofol pretreatment at a dose of 1 µmol/L resulted in a decrease in relaxation to MCh in the presence of L-NAME in control and captopril-treated rats when compared to no inhibitor or meclo. L-NAME+meclo inhibition of MCh relaxation reached statistical significance in only the control group when compared to meclo (Figure 3-5). Propofol pretreatment at a dose of 10 µmol/L also resulted in a decrease in relaxation to MCh in the presence of L-NAME in control and captopril-treated rats when compared to no inhibitor or meclo (Figure 3-6). In the presence of L-NAME+meclo, there was decreased relaxation to MCh in

the control group only when compared to meclo whereas it was decreased in the captopril-treated group when compared to no inhibitor or meclo (Figure 3-6). Arteries from both control and captopril-treated rats were not affected in the presence of meclo when compared to no inhibitor in the presence or absence of propofol.

The differences in contribution of endothelial-derived mediators in the captopril-treated animals and controls were evaluated by observing the EC_{50} shift in the presence of L-NAME, meclo, and L-NAME+meclo as shown in Figure 3-7. We found that in the presence of meclo, there was no shift in EC_{50} when comparing captopril treatment to control or in the presence or absence of propofol. In the presence of L-NAME, the EC_{50} shift was different in control rats compared to captopril-treated rats. Additionally, the presence of propofol achieved a statistically significant alteration of the EC_{50} shift of L-NAME. A similar response is seen in the presence of L-NAME+meclo in these arteries.

3.4 DISCUSSION

The goal of this study was to determine if there was an increase in vascular relaxation in the presence of propofol in aging rats chronically treated with ACE inhibitors. The primary finding in this study is that, based on the concentration response curves, propofol enhances vasodilation in arteries from

the aging animals chronically treated with captopril compared to those not treated. This enhanced relaxation to propofol can be seen both by its direct stimulation and by its modulation of endothelium-dependent relaxation to MCh. It appears that NO is the primary endothelial-derived mediator involved in the enhanced relaxation to propofol in the captopril-treated group, since L-NAME significantly inhibits direct propofol-induced relaxation. When assessing the modulation of endothelial-dependent vasodilation (i.e. dilation to MCh), and in light of propofol's ability to modulate endothelial dependent vasodilation, it is intriguing to see that there is no difference between control and captopril-treated groups in relaxation to MCh in the absence of propofol. This finding is supported by others in the literature.[17] When assessing the endothelial-dependent vasodilators involved in this response, propofol increases NO modulation in both groups as evidenced by the EC_{50} shift to L-NAME. However, the enhanced relaxation to MCh in the presence of propofol in the captopril-treated animals is not attributed to NO since propofol similarly increased the proportion of the EC_{50} shift to L-NAME in both groups. Therefore, a non-NO-dependent vasodilation accounts for this differential increase in MCh-induced relaxation in the presence of propofol in the captopril-treated group.

It has been shown that ACE inhibition ameliorates endothelium-dependent impairment of relaxation in hypertension,[18] and this effect may be a result of improving EDHF-dependent relaxation which declines with

increasing age.[19] Indeed, our data suggests that, in the absence of propofol, control rats possess more NO-dependent vasodilatory capacity than in captopril-treated rats, but given that there is similar overall relaxation, this is likely due to enhanced EDHF in captopril-treated rats to compensate for this. Moreover, the fact that there is little effect of L-NAME+meclo on relaxation in the absence of propofol suggests that the majority of baseline relaxation is attributed to EDHF in arteries from captopril-treated rats.

Previous clinical studies regarding anesthesia and ACE inhibition include a meta-analysis which suggests that patients receiving immediate preoperative ACE inhibition are more likely to develop hypotension requiring intervention intraoperatively.[20] Additionally, perioperative angiotensin antagonism has been associated with increased 30-day mortality in patients undergoing abdominal aortic aneurysm repair.[21] The authors of this latter study speculate that perioperative hypotension may be a possible contributing factor, although their data set did not allow for this analysis. In regards to propofol specifically, a small study has suggested that hypotension with the use of propofol is worse in patients treated with the ACE inhibitor enalapril.[22] It has also been suggested that increasing induction doses of propofol in patients on chronic ACE inhibitors leads to an increased number of hypotensive episodes requiring intervention.[23] However, another recent paper suggested that there is not a significant decrease in blood pressure with the use of propofol in patients on ACE inhibitors undergoing minor surgery; part of

the reason for this could be the use of a slow induction rather than a bolus induction.[24] It has been suggested that patients on chronic ACE inhibitor therapy receiving ACE inhibitors less than 10 hours prior to surgery was a risk factor for developing hypotension intraoperatively.[5] It would be interesting to see if there is a change at the vascular level that may account for this finding. Therefore, a potential future direction of study would be to see if removing the availability of captopril the day prior to experimentation in these rats would reverse the enhanced vasodilation we see in the presence of propofol.

Terlipressin, a vasopressin analog, may be used to restore intraoperative systemic vascular resistance in patients chronically treated with angiotensin system antagonists.[25] Indeed, a recent clinical trial suggests that the use of low-dose vasopressin infusion restores vascular tone in patients chronically treated with ACE inhibitors undergoing cardiac surgery.[26] It was speculated that this study would propose that other medications, specifically those interacting with the NO pathway such as methylene blue, may potentially be utilized in patients chronically treated with ACE inhibitors during the intraoperative period when circumstances dictate conventional treatment to restore blood pressure to be insufficient. An additional speculation we had was that this study would suggest that NO donors, such as nitroglycerin or nitroprusside, should be avoided in patients chronically treated with ACE inhibitors during the intraoperative period (as has been anecdotally suggested[11]). Our data does suggest that the direct vasodilation induced by

propofol may be affected by medications interacting with the NO pathway in patients on chronic ACE inhibitors. However, when looking at the modulation of endothelial-dependent relaxation by propofol, these speculations cannot be substantiated with the results of this study. Although direct propofol relaxation is increased because of NO, the difference in modulation of MCh-induced relaxation between the control and captopril-treated groups is not due to NO. Our data showed increasing magnitude of inhibition by L-NAME in both groups with increasing pre-treatment doses of propofol, suggesting increased NO; however, the proportions of increasing L-NAME EC_{50} shifts are similar between the two groups. It is known that ACE inhibitors are kininase inhibitors,[27] so potentiation of endothelial-derived vasodilation via NO and non-NO (i.e. EDHF) pathways is a strong possibility in contributing to acute vascular responses to propofol *in vivo*. Our data points to EDHF being upregulated with captopril treatment and thus may be the dilator involved. However, the major issue is that there is no universal mediator involved in EDHF-dependent vasodilation, and its identity is considered to be species- and vascular bed-specific. Therefore, EDHF in the human vasculature involved in the regulation of systemic vascular resistance would need to be identified in order to relate these findings. ACE inhibition may increase relaxation by increasing H_2O_2 in the wake of increased eNOS expression[28] or by increasing epoxyeicosatrienoic acids,[29] two substances which have been identified as mediators involved in EDHF-dependent relaxation. A conjecture that can be

made based on this information and relating our findings in Chapter 2 regarding K^+ and propofol is that ACE inhibition may increase another non-NO and non-PG vasodilator besides K^+, which is not readily altered by propofol. Nonetheless, further investigation is required to determine the reasons for propofol's differential response between control and captopril treatment.

In summary, chronic ACE inhibition in aging rats results in an alteration in vascular reactivity in resistance mesenteric arteries that makes them more sensitive to propofol's vasodilating effects, both directly and through modulation of endothelial-dependent vasodilation. Surprisingly, NO plays more of a role in endothelial-dependent vasodilation in rats not on chronic ACE inhibition, and the increased relaxation in the presence of propofol observed in the captopril-treated animals may be due to an upregulation of an EDHF-like response. This may be one of the mechanisms underlying hypotension that can be seen in patients chronically treated with ACE inhibitors. It would be interesting to see if this effect can be seen with other general anesthetic agents, including inhalational anesthetics. Indeed, inhalational anesthetics are commonly used for maintenance of anesthesia, and they can cause vasodilation ultimately by decreasing intracellular calcium and by inhibiting protein kinase C which can decrease the activity of the angiotensin type 1 (AT_1) receptor and therefore decrease vascular tone.[30-31] Nevertheless, although further investigation is required to further elucidate the

reasons for this differential response between control and captopril-treated rats, these findings may help explain in part the increased incidence of anesthesia-induced hypotension in this subset of patients.

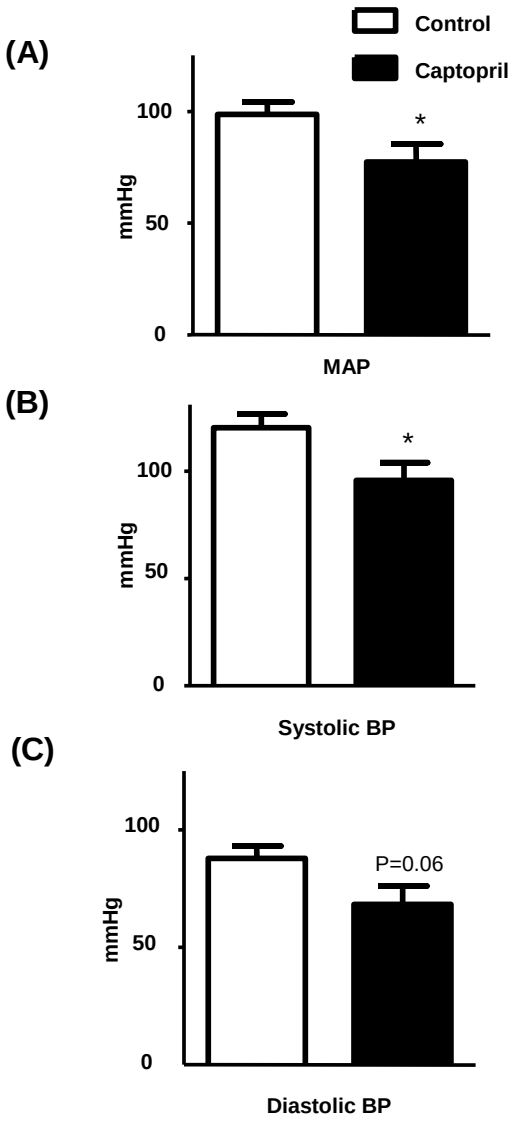

Figure 3-1. Blood pressures in control and captopril-treated rats.

(**A**) Systolic and (**B**) mean arterial blood pressures, as measured by invasive carotid cannulation, are significantly lower in captopril-treated rats compared to control. (**C**) Diastolic blood pressure, although not reaching statistical significance, was lower in the captopril-treated rats compared to control. N=7 per group; *P<0.05 vs. control

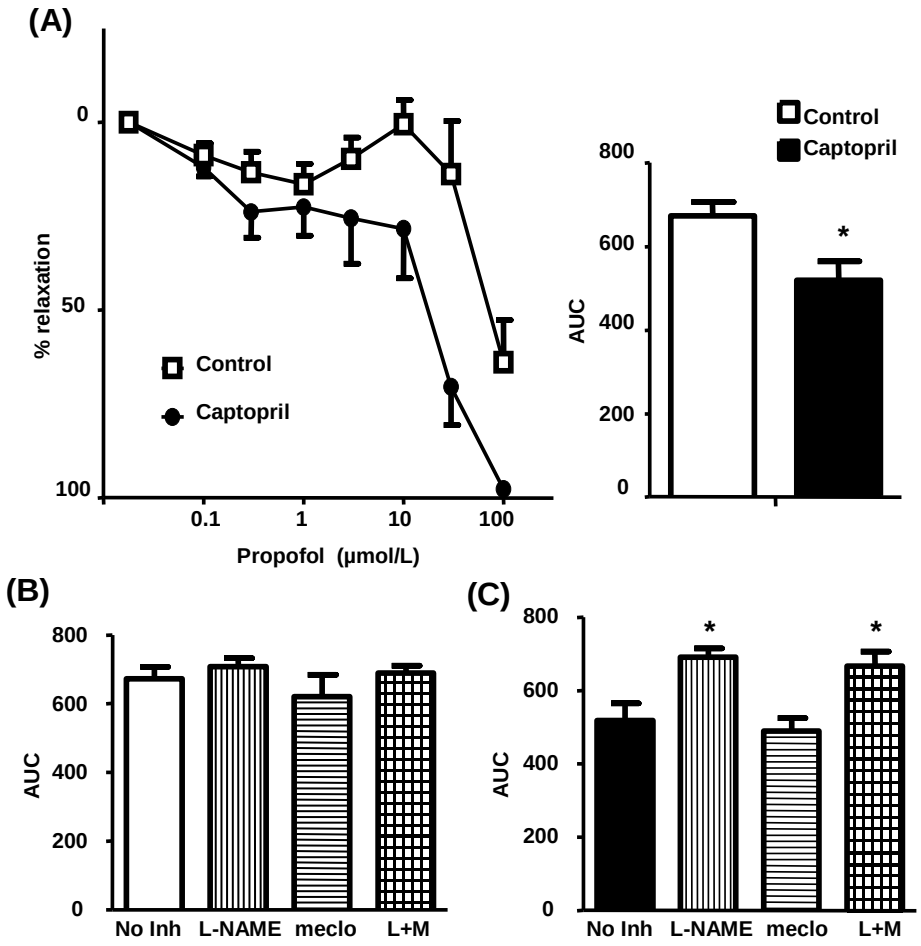

Figure 3-2. Comparison of propofol-induced relaxation in arteries from control and captopril-treated rats.

(**A**) Concentration-response data and cumulative data represented as area under the curve (AUC) were different when comparing relaxation in captopril-treated rats versus control. (**B**) Direct propofol relaxation does not show statistical significance when looking at the alteration by L-NAME, meclo, or L-NAME+meclo in control rats. (**C**) There is a statistically significant reduction in direct propofol relaxation by L-NAME and L-NAME+meclo in captopril-treated rats. N=7 per group. † $P<0.05$ vs. control; *$P<0.05$ for L-NAME vs. No inhibitor or L-NAME vs. meclo; #$P<0.05$ for L-NAME+meclo vs. No inhibitor or L-NAME+meclo vs. meclo.

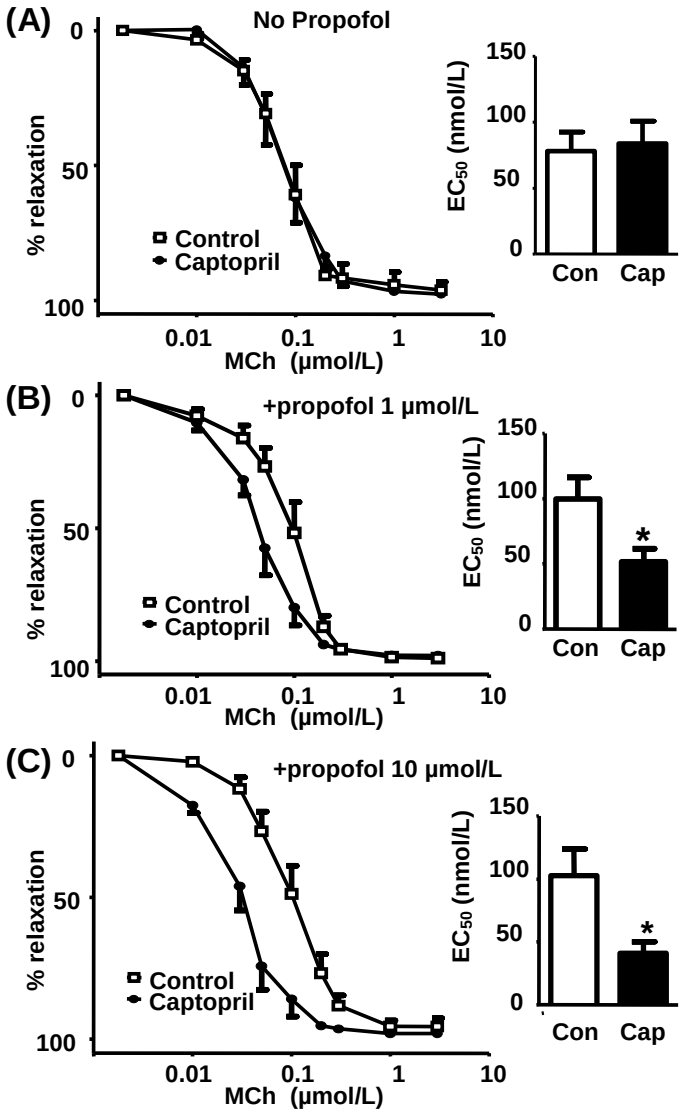

Figure 3-3. Comparison of MCh-induced relaxation with no propofol or the presence of increasing doses of propofol in arteries from control and captopril-treated rats.

(**A**) Concentration-response curves showing captopril treatment fails to alter relaxation to MCh. The mean EC_{50} for MCh relaxation between control and captopril-treated rats were not different. In the presence of (**B**) 1 µmol/L and (**C**) 10 µmol/L of propofol, the concentration-response curves show enhanced relaxation to MCh in the captopril-treated group in relation to control, with lower mean EC_{50} values for MCh in these groups. N=7 per group; *P<0.05 vs. control for 1 µmol/L and 10 µmol/L.

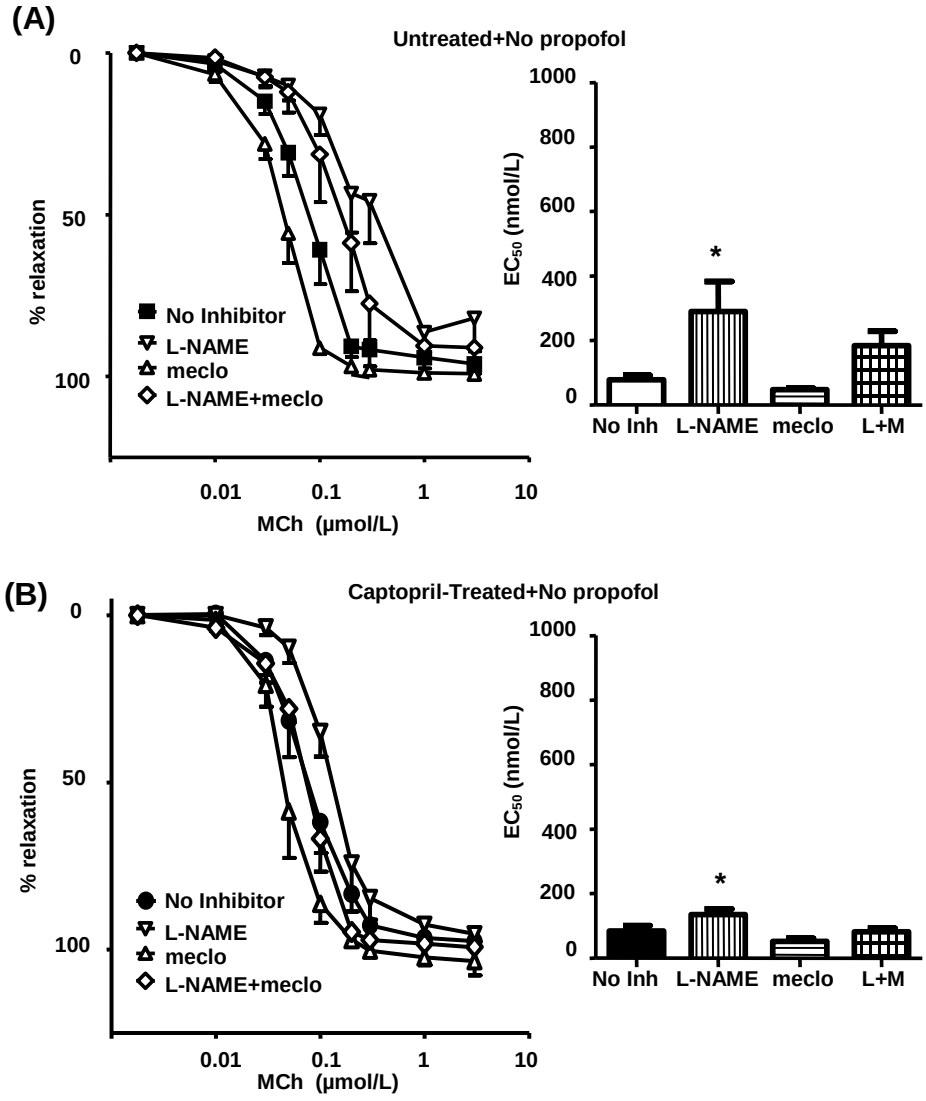

Figure 3-4. Comparison of relaxation to MCh in the presence of inhibitors of endothelial-dependent vasodilation in arteries from control and captopril-treated rats.

Concentration response curves and corresponding mean EC_{50} values in arteries from (**A**) control and (**B**) captopril-treated rats in the presence of L-NAME, meclo, and L-NAME+meclo. L-NAME decreased relaxation to MCh when compared to No inhibitor or meclo in the control group, whereas L-NAME decreased relaxation to MCh when compared to meclo in the captopril group. N=7 per group; *P<0.05 L-NAME vs. No inhibitor (control); L-NAME vs. meclo (control); and L-NAME vs. meclo (captopril).

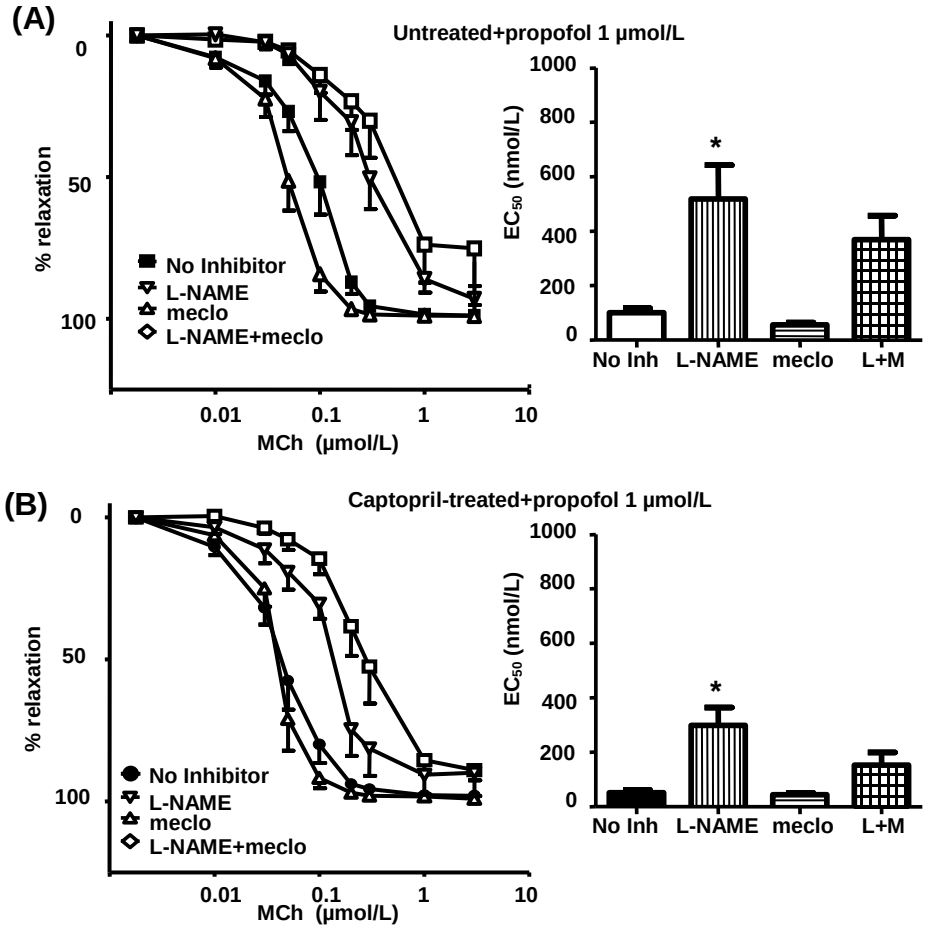

Figure 3-5. Comparison of relaxation to MCh following pre-treatment with 1 µmol/L propofol in the presence of inhibitors of endothelial-dependent vasodilation in arteries from control and captopril-treated rats.

Concentration response curves and corresponding mean EC_{50} values following pre-treatment with 1 µmol/L propofol in arteries from (**A**) control and (**B**) captopril-treated rats in the presence of L-NAME, meclo, and L-NAME+meclo. In the control group, L-NAME decreased relaxation to MCh when compared to no inhibitor or to meclo whereas L-NAME+meclo significantly decreased relaxation to MCh only when compared to meclo. In the captopril group, only L-NAME significantly decreased relaxation to MCh when compared to no inhibitor or to meclo. N=7 per group. *P<0.05 L-NAME vs. No inhibitor or L-NAME vs. meclo (control); and L-NAME vs. No inhibitor or L-NAME vs. meclo (captopril). #P<0.05 L-NAME+meclo vs. meclo (control).

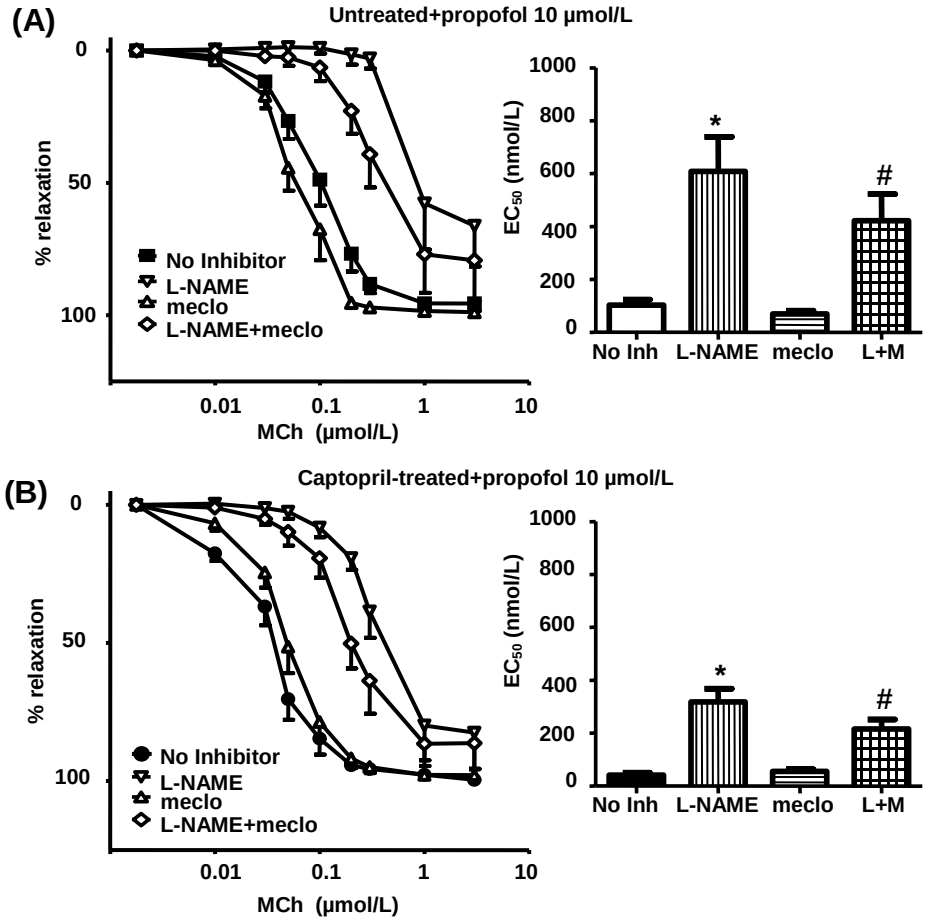

Figure 3-6. Comparison of relaxation to MCh following pre-treatment with 10 µmol/L propofol in the presence of inhibitors of endothelial-dependent vasodilation in arteries from control and captopril-treated rats.

Concentration response curves and corresponding mean EC_{50} values following pre-treatment with 10 µmol/L propofol in arteries from (**A**) control and (**B**) captopril-treated rats in the presence of L-NAME, meclo, and L-NAME+meclo. In the control group, L-NAME decreased relaxation to MCh when compared to no inhibitor or meclo, and L-NAME+meclo decreased relaxation to MCh when compared to meclo. In the captopril group, both L-NAME and L-NAME+meclo decreased relaxation to MCh when compared to no inhibitor or to meclo. N=7 per group. *P<0.05 L-NAME vs. No inhibitor or L-NAME vs. meclo (control); and L-NAME vs. No inhibitor or L-NAME vs. meclo (captopril). #P<0.05 L-NAME+meclo vs. meclo (control); and L-NAME+meclo vs. No inhibitor or L-NAME+meclo vs. meclo (captopril).

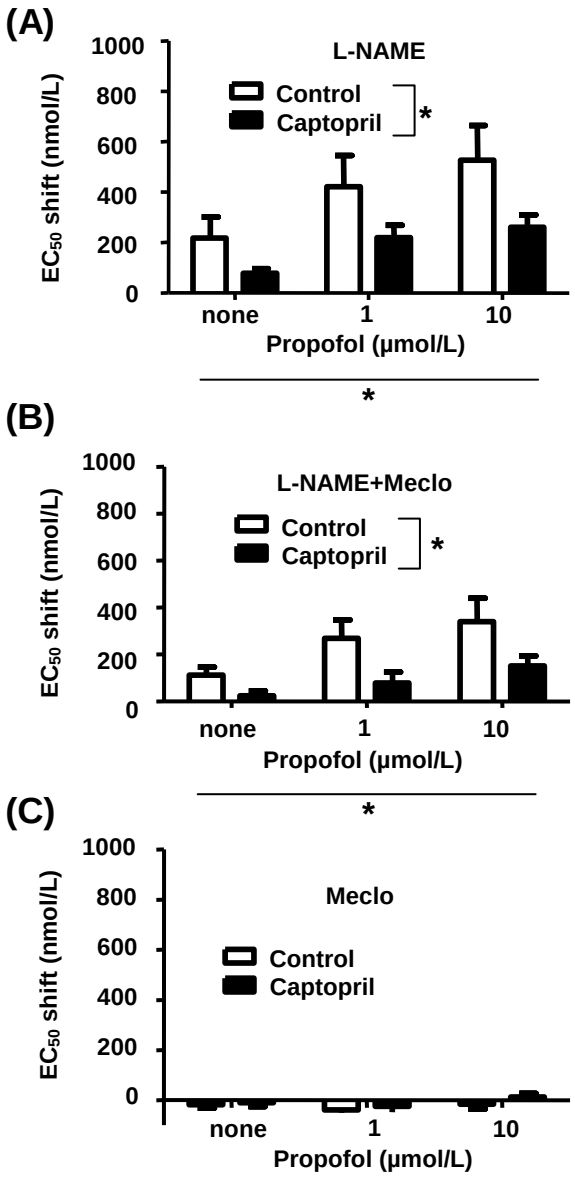

Figure 3-7. Comparisons of EC_{50} shift between arteries from control and captopril-treated rats in the presence of increasing doses of propofol pre-treatment and in the presence of inhibitors of endothelial-dependent vasodilation.

In the presence of (**A**) L-NAME and (**B**) L-NAME+meclo, both increasing doses of propofol pre-treatment and chronic captopril treatment altered the EC_{50} shift. (**C**) The presence of meclo did not affect the EC_{50} shift. N=7 per group; *P<0.05 for both captopril treatment and propofol pre-treatment.

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CHAPTER 4:

INTRALIPID REVERSES PROPOFOL-INDUCED VASODILATION AND HYPOTENSION IN AGING RATS

A portion of this chapter has been published:

***Gragasin FS**, Davidge ST, and Tsui BC. The potential use of intralipid to minimize propofol's cardiovascular effects. Can J Anaesth. 2009 Feb;56(2):170-1.*

A version of this chapter has been submitted for publication:

***Gragasin FS**, Bourque SL, Mansour Y, Tsui BC, and Davidge ST. Intralipid reverses propofol-induced vasodilation and hypotension in aging rats. Anesthesiology (submitted 2013).*

F. Gragasin contributed to study design, data collection, data analyses, and writing all drafts of the manuscripts. Y. Mansour was a summer student supervised by F. Gragasin and contributed to data collection and data analyses. B. Tsui contributed to data analyses and editing drafts of the manuscripts. S. Bourque and S. Davidge contributed to study design, data analyses, and editing all drafts of the published (SD) and submitted (SB, SD) manuscripts.

4.1 INTRODUCTION

The aging population presenting for anesthetic care poses inherent challenges due to the higher incidence of comorbidities and the greater likelihood of being treated with medications, such as ACE inhibitors for blood pressure control (as discussed in Chapter 3), compared to younger patients. Perioperative hypotension is associated with increased morbidity and mortality in surgical patients[1] and is particularly problematic in the elderly due to various physiological changes that occur with aging. Moreover, the majority of general anesthetics, and in particular propofol, induces hypotension, and this effect is exacerbated in aging.

Intralipid is a lipid emulsion formulation that is used for total parenteral nutrition but is also used as a vehicle to solubilize propofol. Recently, Intralipid has shown promise as a resuscitation adjunct in a variety of cardiovascular toxidromes, most notably with cardiotoxicity induced by lipophilic ***local*** anesthestics such as bupivacaine.[2-4] However, whether Intralipid may serve as a therapeutic intervention for lipophilic ***general*** anesthetics is not known. Based on preliminary work, we were able to demonstrate a proof-in-principle that Intralipid is able to reverse the direct vasodilation caused by propofol in isolated mesenteric arteries in rats (Figure 4-1). In this chapter, we sought to determine whether Intralipid could reverse the hypotension elicited by propofol administration in aging rats. Furthermore,

the doses of Intralipid employed in our preliminary work were high (2-4%), and therefore we sought to determine whether lower doses could effectively reverse the vasodilatory effects of propofol. In addition to the *ex vivo* experiments, we sought to determine whether Intralipid could reverse the hypotension elicited by propofol administration in young and aging rats *in vivo*. Therefore, we also hypothesized that Intralipid will fully reverse hypotension caused by propofol in aging rats, which will be greater than the reversal seen in young rats. Moreover, we investigated whether Intralipid can reverse hypotension caused by other medications, namely hydralazine, a common anti-hypertensive used in the perioperative period, as well as the inhalational anesthetic isoflurane. Given the relative lipophilic nature of isoflurane (oil:gas partition coefficient 98) compared to water-soluble hydralazine, we hypothesized that Intralipid will reverse hypotension caused by isoflurane, but not hydralazine, to a similar extent as reversal of propofol-induced hypotension. Finally, as discussed in Chapter 3, perioperative use of antihypertensive medications such as ACE inhibitors can lead to refractory hypotension in patients under general anesthesia, and we demonstrated that propofol enhances vascular relaxation in arteries from rats chronically treated with the ACE inhibitor captopril. Our aim for the ACE inhibitor arm of this study was to determine if Intralipid may potentially be utilized as a hemodynamic agent to reverse the refractory hypotension during general anesthesia that can occur with perioperative angiotensin antagonism.

Therefore, we also hypothesized that Intralipid would cause a complete reversal of propofol-induced hypotension in captopril-treated rats.

4.2 MATERIALS AND METHODS

This study was approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee and was in accordance with the Canadian Council on Animal Care and National Institutes of Health guidelines.

4.2.1 Animals

Male and female Sprague-Dawley rats aged 3 months were obtained from Charles River Breeding Laboratories (Quebec, Canada). Rats were housed and aged in the animal facilities at the University of Alberta until experimentation. Rats aged 3 months (young) and 14-16 months (aging) were used for experiments. Food and water were available *ad libitum* while rats were housed in the animal care facility. Wire myograph experiments were first conducted in isolated resistance mesenteric arteries to establish the *ex vivo* responses of Intralipid to reverse propofol-induced vasodilation and to determine if the response was greater in arteries from aging compared to young rats. Based on the outcomes from the *ex vivo* arms of this study, we

then proceeded to establish whether this translated to *in vivo* hemodynamic interactions between Intralipid and propofol (i.e. reversal of hypotension) in both young and aging rats.

4.2.2 Captopril Treatment

A subset of aging male rats were treated with the ACE inhibitor captopril dissolved in drinking water (2 g/L), which was made available *ad libitum* for 7 to 8 weeks, as described in Chapter 2. The final age of these animals at the time of experimentation was 15 to 16 months.

4.2.3 Tissue Isolation and Protocols for Vascular Reactivity

The first aim of the study was to determine if Intralipid (0.002-0.2%) would be effective to fully reverse direct propofol-induced vasodilation. Rats were anesthetized by isoflurane (dosed to effect by inhalation), and the mesentery was rapidly excised and placed in ice-cold HEPES-buffered physiological saline solution as described in section 2.2.2. The rats were then euthanized by exsanguination, and mesenteric arteries (with internal diameters 100-200 μm) were carefully dissected and mounted in an isometric myograph system as described in section 2.2.2. Preconstriction was achieved with phenylephrine using the calculated EC_{80} dose (see section 3.2.4). Following preconstriction, vasodilation was induced with either propofol (100 μmol/L) or

methacholine (MCh, 3 μmol/L); these doses were based on our work in Chapter 3. Doses (0.002-0.2%) of either Intralipid, dimethyl sulfoxide (DMSO), or Cremophor EL were then added to the baths in a cumulative manner. DMSO and Cremophor EL were chosen as comparators to Intralipid because both substances are known to solubilize propofol.[5-6] The propofol preparation used in the present study is that which is used clinically available (see section 2.2.3).

4.2.4 Hemodynamic Measurement and Protocols for Intravenous Drug Delivery

Rats were anesthetized by isoflurane (3-5% in 100% oxygen) and maintained on a warming pad. Once surgical anesthesia was achieved, a laparotomy was performed and the abdominal aorta was exposed with the aid of a binocular microscope. The aorta was invasively cannulated with a 22 gauge winged catheter (BD, Mississauga, Ontario, Canada) and connected to a pressure transducer. The systolic, diastolic, and mean arterial (MAP) blood pressures were recorded continuously for the duration of the experiment. The left femoral vein was exposed and cannulated with a 24 gauge winged catheter (BD, Mississauga, Ontario, Canada) for intravenous drug delivery. A tracheostomy was also performed, and each rat was connected to a Harvard Model 683 Small Animal Ventilator (Harvard Apparatus Canada, St. Laurent,

Quebec, Canada) and mechanically ventilated with a tidal volume of 2.5 mL/kg and a respiratory rate of 80. Inspired isoflurane was set at 1.5% and administered in 100% oxygen once intubation was completed. Following arterial cannulation, blood pressure was allowed to equilibrate for at least 25 minutes prior to the injection of intravenous drugs.

We sought to determine if Intralipid or its volume control normal saline can reverse hypotension elicited by propofol. Hypotension was defined as a decrease in mean arterial pressure >20% from baseline. Propofol was injected at a dose of 10 mg/kg (equivalent to 1 mL/kg), a dose used in this animal model in the literature.[7-8] This dose was also chosen because it resulted in a sustained decrease in blood pressure in our pilot experiments. Following a one-minute equilibration period post-propofol injection, an equivalent volume of Intralipid was administered four times sequentially every minute. In other words, the chosen Intralipid dose was based on the propofol dose administered for each animal and given in a 1:1 ratio, and this ultimately resulted in 1 mL/kg of Intralipid given four times successively every minute during the protocol (i.e. final administered volume of 4 mL/kg). In other rats, normal saline was administered in the same fashion (1 mL/kg given four times successively every minute post-propofol injection, equating to a final volume of 4 mL/kg).

We also sought to determine if Intralipid has a non-selective effect of restoring blood pressure following other agents that cause hypotension.

Therefore, the ability of Intralipid to reverse hypotension caused by other agents—namely hydralazine and isoflurane—was also tested in young and aging male and female rats. Hydralazine was administered at a dose of 0.6 mg/kg (based on pilot data), and inspired isoflurane was administered at 5% during mechanical ventilation. As in the propofol protocol, Intralipid was given at a dose of 1 mL/kg four times successively every minute after hypotension was induced by hydralazine or isoflurane. In some experiments, isoflurane was administered following propofol injection (and correction of hypotension) or *de novo*; hydralazine was always administered at the conclusion of the experiment (i.e. following propofol injection, following high-dose isoflurane inhalation, or both).

Finally, to determine if Intralipid had an intrinsic effect on blood pressure, 1 mL/kg was injected four times successively every minute in the absence of any agent. To determine if Intralipid can prevent hypotension induced by 10 mg/kg propofol, a 2:1 volume ratio of Intralipid to propofol was administered. This equated to 2 mL/kg of intralipid given 30 seconds prior to propofol injection.

4.2.5 Drugs

All drugs were purchased from the same sources mentioned in section 3.2.5. Cremophor EL, DMSO, and hydralazine were purchased from Sigma-Aldrich (Oakville, ON, Canada).

4.2.6 Statistical Analyses

Graphpad Prism 4 software (GraphPad Software Inc., San Diego, CA, USA) on a Windows 7 platform was used for all statistical analyses. Values are expressed as mean±SEM. Dose-response data for reversal of propofol-induced vasodilation was summarized as area under the curve (AUC). For *ex vivo* experiments, AUC was obtained as described in section 2.2.5. For *in vivo* experiments, AUC was obtained by plotting the individual MAP values into a curve representing percent-reversal of blood pressure decrease with each dose of Intralipid, and the summation of these values was calculated. Data were analyzed using a Student's t-test or 1-way analysis of variance (ANOVA) with Tukey post-hoc analysis for multiple group comparisons, as appropriate. In all analyses, a value of $P<0.05$ was considered statistically significant.

4.3 RESULTS

4.3.1 Propofol-induced vasodilation is reversed with Intralipid in aging rats

Propofol-induced vasodilation was completely reversed by Intralipid as well as by Cremophor EL ($P<0.05$ each versus DMSO; Figure 4-2A); Intralipid-mediated reversal of vascular tone was greater than reversal by Cremophor EL ($P<0.05$). To determine whether this effect was due to reversal of propofol-induced vasodilation per se or an unrelated effect on the vasculature, we performed several experiments. First, we assessed constrictor capacity of Intralipid (Figure 4-2B). Intralipid caused no direct vasoconstriction, nor did it potentiate the vasoconstriction induced by PE (Figure 4-2B). Next, we assessed whether Intralipid could reverse endothelium-dependent vasodilation by MCh (Figure 4-2C). Intralipid had no effect on MCh-induced vasodilation, whereas both Cremophor EL and DMSO effectively reversed MCh-induced vasodilation.

4.3.2 Comparison of Intralipid reversal of propofol-induced vasodilation in aging versus young

We next assessed whether propofol differentially affected propofol-induced vasodilation in aging and young rats (Figure 4-3). Time control

groups were also included, and there was no statistically significant effect of time in regards to spontaneous resolution of propofol-induced vasodilation (data not shown). Intralipid had a greater effect in reversing vasodilation to propofol in arteries from aging versus young rats. Maximum reversal by Intralipid (0.2% dose) was 53.0±23.7% (young) versus 111.6±4.8% (aging).

4.3.3 Intralipid reversal of hypotension caused by propofol, isoflurane, and hydralazine

We next sought to extend our findings *in vivo* by assessing hemodynamic responses to intravenous propofol followed by Intralipid. Intralipid reversed propofol-induced hypotension in all groups, albeit this effect was greatest in aging males (Figure 4-4). There was no statistically significant difference between aging females, young males, and young females. Since there was no statistical significance achieved in these latter three groups, the remaining analyses were conducted comparing aging males and females. In aging males and females, saline control did not reverse the hypotension caused by propofol (Figure 4-5). Heart rate was increased to a greater extent in aging males when compared to females in response to Intralipid following propofol administration: heart rate (as a percentage of

baseline heart rate) following the final dose of Intralipid was 110.4±3.3% in aging males versus 99.3±2.8% in aging females.

Reversal of propofol-induced hypotension by Intralipid was also compared to the reversal of hypotension caused by isoflurane and hydralazine. Whereas Intralipid completely reversed propofol-induced hypotension, Intralipid had modest effects on isoflurane-induced hypotension, and no effect in hydralazine-induced hypotension in aging males (Figure 4-6). Intralipid also increased heart rate in propofol-treated rats (110.4±3.3%) compared to isoflurane (92.8±2.9%; $P<0.05$), but not in hydralazine-treated rats (105.5±4.3%; $P=0.48$). Similar effects were seen in aging females: there was an overall difference in the ability of Intralipid to reverse hypotension induced by the various agents, with Intralipid showing the greatest reversal of propofol compared to isoflurane and hydralazine ($P<0.05$). In aging females, the heart rate increase did not achieve statistical significance when comparing Intralipid administration following propofol-, isoflurane-, and hydralazine-induced hypotension. Percent of baseline heart rate values were as follows: 99.3±2.8% (propofol), 91.6±4.2% (isoflurane), and 101.2±7.2% (hydralazine).

Finally, in the absence of propofol, Intralipid alone had little effect on MAP (+5.1±6.8 mmHg), compared to a robust effect (+77.8±3.3 mmHg) when given in the wake of propofol administration (Figure 4-8A). To determine if Intralipid could be used prophylactically, a 2:1 volume ratio was administered as a bolus dose 30 seconds prior to the administration of

propofol. Pre-treatment with Intralipid in aging males prevented the hypotension at the 5 minute mark following propofol injection, corresponding to a maintenance of 114.2±9.9% of baseline blood pressure, compared to 18.9±8.2% of baseline pressure when no Intralipid was given (Figure 4-8B).

4.3.4 Intralipid reversal of propofol-induced hypotension in control and captopril-treated rats

Since we saw a greater reversal of hypotension in aging males compared to females, and because ACE inhibition is associated with enhanced vasodilation in the presence of propofol (as evidenced in Chapter 2), we sought to determine if Intralipid can reverse the propofol-induced hypotension in aging male rats treated with the ACE inhibitor captopril. Captopril treatment resulted in a lower baseline MAP (122.0±4.8 mmHg in control versus 95.7±9.1 mmHg in captopril-treated; Figure 4-9). Propofol caused a greater hypotensive effect in captopril-treated rats (MAP following propofol injection was 44.3±2.3 mmHg in control versus 30.4±2.6 mmHg in captopril-treated rats; Figure 4-9B). Reversal of propofol-induced hypotension with Intralipid was blunted in captopril-treated rats compared to controls (AUC 224.3±37.3 in control versus 84.7±19.3 in captopril-treated rats; Figure 4-9C). The effect of Intralipid on heart rate was similar between control and

captopril-treated rats (percent of baseline heart rate was 100.3±3.4% in control versus 99.2±5.6% in captopril-treated rats, P=0.88).

4.4 DISCUSSION

The primary findings in this study are that 1) Intralipid reverses direct propofol-induced vasodilation *ex vivo*, and 2) Intralipid reverses propofol-induced hypotension *in vivo* in aging rats. This has direct clinical implication because Intralipid is already used in the clinical setting, not only for maintenance of nutrition but also in more dire situations requiring resuscitation following cardiovascular toxicity from a number of drugs, including local anesthetics[2,9-10] as well as a variety of psychiatric medications.[11-14] Propofol is the most widely used anesthetic induction agent worldwide,[15] and with both propofol use and advanced age of the patient being predictors for intraoperative hypotension,[16] these findings suggest that Intralipid may potentially be used to rectify hemodynamic changes that can occur in the perioperative setting for an aging patient population in whom propofol is used for their clinical care.

In the *ex vivo* arm of this study, we were able to show that Intralipid reverses propofol-induced vasodilation in aging rats. Intralipid did not cause direct vasoconstriction by itself, and it did not enhance phenylephrine-induced constriction. We compared the ability of Intralipid to reverse propofol-induced vasodilation to the reversal by Cremophor EL and DMSO, two substances known to solubilize propofol. The fact that both Intralipid and Cremophor EL, but not DMSO, reversed the vasodilation suggests that a lipid-sink

phenomenon may not be the reason for this response. We also did not see substantial reversal of MCh-induced vasodilation by Intralipid in our experiments. Since Cremophor EL was able to reverse vasodilation to both propofol and MCh, it is possible that this may be due to Cremophor EL's own intrinsic ability to cause vasoconstriction.[17]

We compared the *ex vivo* responses between arteries from young and aging rats. Our primary goal with this arm of the *ex vivo* study was solely to determine if aging arteries have a greater response to Intralipid than in young. It is interesting that the response is more robust in arteries originating from aging animals. This suggests that there is an intrinsic change in the vasculature with aging that renders these arteries more sensitive to Intralipid's effects on vasodilation caused by propofol. Indeed, as mentioned in Chapter 1, a number of structural and functional vascular changes occur with aging, including increased vascular tone due to a shift in a variety of endothelial-derived mediators as well as increased oxidative stress. It has been suggested that Intralipid enhances α-receptor response, which can account for its ability to increase blood pressure.[18] However, we were unable to detect this response in the *ex vivo* setting since Intralipid did not increase tone when it was given following phenylephrine pre-constriction (i.e. in the absence of propofol). Interestingly, given the negative AUC value in Figure 4-2B, it appears that Intralipid may cause some vasodilation following phenylephrine constriction. This was also seen when we used Intralipid as a volume control in our

experiments in Chapter 2. Despite there being suggestion that Intralipid, by itself, may actually decrease vascular tone through a NO pathway,[19] it was recently demonstrated that lipid emulsion can inhibit NO release in an isolated vessel preparation.[20] In vascular endothelial cells *in vitro*, free fatty acids can inhibit NO bioavailability.[21] In particular, lipids can inhibit phosphatidylinositide 3-kinase, which in turn would decrease phosphorylation of endothelial NOS, effectively decreasing the production of NO.[22] Indeed, we have demonstrated in Chapters 2 and 3 the importance of the NO pathway in propofol-induced vasodilation, so it is possible that Intralipid is having some effect on the NO pathway to re-establish tone following vasodilation to propofol.

The *in vivo* results unexpectedly revealed a sex difference in aging. We had originally intended to combine the results of the aging males and females for analyses, but the *in vivo* studies revealed that there is a greater reversal of propofol-induced hypotension in males compared to females. These findings also suggest again that a lipid sink phenomenon does not fully explain Intralipid's mechanism of action. When looking solely at propofol, although the percent-change in heart rate caused by Intralipid was greater in males compared to females (+10.4% compared to -0.6%), it is likely that the 10% increase in heart rate does not account for the 1.6-fold difference in reversal of propofol-induced hypotension by Intralipid in males and females. It is possible that the sympathetic system is activated by Intralipid and contributes to this

response by increasing the heart rate; however, this also does not fully explain the results, particularly since post-hoc analyses revealed only a significant difference between propofol and isoflurane in aging males in regards to heart rate, whereas we did not see this effect with either aging females or with hydralazine. Regarding hydralazine, it is possible that it inhibits the action of Intralipid at a downstream point because hydralazine may have more than one cellular target to cause vasodilation and decrease blood pressure (e.g. opening of K^+ channels, decreasing influx of Ca^{++}, decreasing intracellular Ca^{++} release).[23-24]

Previous human studies have suggested that Intralipid increases blood pressure and heart rate when infused over hours.[25] It is questionable whether this response is due to altered baroreflex sensitivity, since there is evidence for[26] and against[27] this. Norpepinephrine levels are not elevated with Intralipid infusion.[28] However, in both young and aging humans, it appears that central sympathetic activation may be involved, at least in part, for the increase in blood pressure following Intralipid infusion, which is coincident with an increase in aldosterone and F_2-isoprostane levels.[29-30] F_2-isoprostanes are biomarkers of oxidative stress and can cause vasoconstriction.[25,31] Indeed, in isolated vascular smooth muscle and endothelial cells, free fatty acids can stimulate production of reactive oxygen species and increase oxidative stress,[32] which would have important implications in the aging population.

One major difference in this current study is that we are seeing hemodynamic effects of Intralipid injection within minutes of administration. In particular, it appears that the increase in blood pressure is selective following propofol administration. The studies discussed above were investigating the effects of Intralipid infusion over a span of hours to days, whereas what we are proposing is an almost instantaneous response that becomes apparent within minutes, at least in our animal model and with the use of propofol. Intralipid may also increase blood pressure by increasing myocardial contractility.[33] Although there is some evidence that propofol may alter myocardial contractility,[34] it has been shown not to decrease, and may in fact increase,[35] contractility in experimental rat heart preparations.[36-38] In addition, it was found that in isolated human myocardium, propofol minimally affects contractility.[39] Therefore, propofol's hypotensive effects, and Intralipid's reversal, likely stem from mechanisms besides solely altering cardiac contractility.

In regards to potential mechanisms, it would appear that an arm of the RAS is involved, at least in part, in the Intralipid reversal of propofol-induced hypotension, since captopril-treated rats did not show the same reversal as in control rats. It would also appear that this differential effect is not due to heart rate, since both control and captopril-treated rats had similar return to baseline heart rates following Intralipid administration. Contrary to what we anticipated, Intralipid may not be efficacious as a vasopressive agent after

propofol administration in a setting of chronic ACE-inhibitor exposure. Interestingly, studies have documented that there is no alteration of renin or Ang II concentrations following lipid infusion.[29-30,40] An additional explanation is that since we and others have suggested that chronic ACE inhibition leads to an upregulation of an EDHF (i.e. non-NO and non-prostaglandin)-like relaxation response in arteries (please refer to Chapter 3), it is conceivable that Intralipid does not affect this relaxation in captopril-treated rats. Once again, it can be speculated that Intralipid affects the NO pathway to restore blood pressure following propofol administration, but because another vasodilating pathway besides NO persists with chronic ACE inhibition (i.e. upregulation of an EDHF-like response), we do not see the same effect of Intralipid in this particular condition as we do with those not exposed to ACE inhibitors.

The doses of lipid emulsion used for our *ex vivo* work (0.002-0.2%) were lower than those which were used, in Sprague-Dawley rats and using similar experimental techniques, in a recently published study (0.2-1.4%) looking at bupivacaine-induced vasodilation.[20] Additionally, the doses used in our *in vivo* work were also lower than those which were used in animal studies investigating reversal of bupivacaine-induced toxicity, with the cumulative dose ranges approximating 10-20 mL/kg.[3,41-43] In the current study, the cumulative Intralipid dose was 4 mL/kg, suggesting that it may have a role at a lower dose compared to when resuscitation is needed following cardiac arrest.

Therefore, we propose that Intralipid, at lower doses than those recommended for resuscitation (as can be found on lipidrescue.org), may potentially have a role in the clinician's daily practice for controlling intraoperative hemodynamics, particularly when propofol is used. Clinical studies will need to be done to address this hypothesis. Moreover, another important question is whether Intralipid alters propofol's anesthetic/awareness effects, which may be contributing to the hemodynamic changes that we see in this study. It should however be noted that only small amounts of Intralipid cross the blood-brain barrier,[44] which suggests that most of the effect that we see is occurring at the vascular level. Nonetheless, although Intralipid does not appear to alter thiopental's anesthetic effects,[45] this may not be the case with propofol. Another future direction of study is to determine what mechanisms are involved at the vascular and cellular level, in particular if the NO pathway is involved with Intralipid's reversal of propofol-induced vasodilation and hypotension as previously mentioned. It has also been suggested that propofol's inhibition of vascular mitochondrial function may contribute to its hypotensive effects.[46] Indeed, an interesting crossover between propofol and bupivacaine is that they both inhibit mitochondrial function, in particular fatty acid transport.[47-48] It is tempting to speculate that Intralipid may also ameliorate impaired fatty acid utilization in mitochondria which contributes to its restorative effects that can be seen following bupivacaine and propofol administration. Of note, propofol infusion syndrome, a rare but life-

threatening condition, is thought to be due to impairment of mitochondrial fatty acid oxidation by propofol, and perhaps Intralipid may have a role in this situation despite the recommendation that administration of lipids should be avoided in these patients.[49]

In summary, Intralipid reverses propofol-induced vasodilation in aging rats more than in young rats. Propofol-induced hypotension, on the other hand, can be reversed by Intralipid in both young and aging rats; however, in aging rats, Intralipid reversal of propofol-induced hypotension is greater in males than in females. The reversal of hypotension by Intralipid appears to be a selective response to propofol, and interestingly the RAS is involved in this response. The doses used in this current study are lower than those used in the studies of resuscitation following bupivacaine-induced cardiovascular collapse. Intralipid may therefore potentially be a future addition to the Anesthesiologists' armamentarium of drugs to maintain hemodynamic stability in the perioperative period, in particular for aging patients given propofol as a component of their anesthetic care.

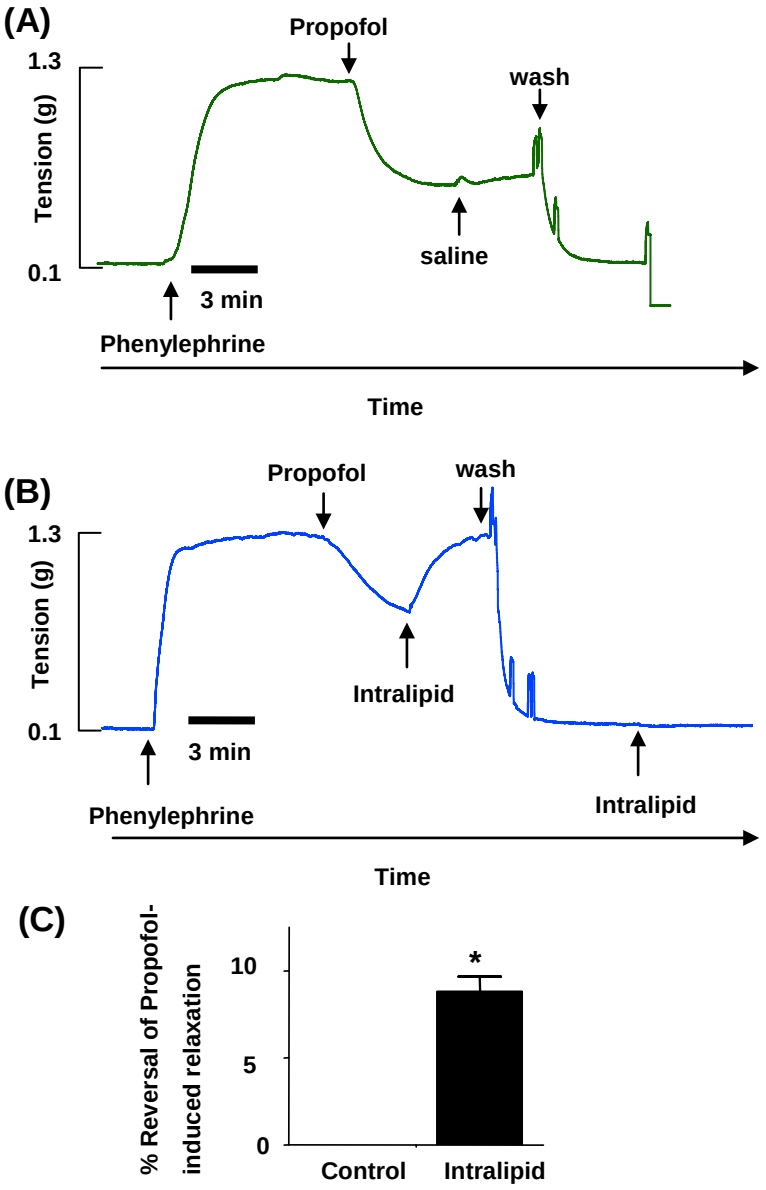

Figure 4-1. Intralipid, but not the equivalent volume of saline, reverses propofol-induced vasodilation.

Representative traces for the effects of (**A**) saline control versus (**B**) Intralipid, and (**C**) mean data demonstrating the significant reversal of propofol-induced relaxation in resistance mesenteric arteries by the addition of Intralipid. N=4 per group, *P<0.05 Intralipid vs. control.

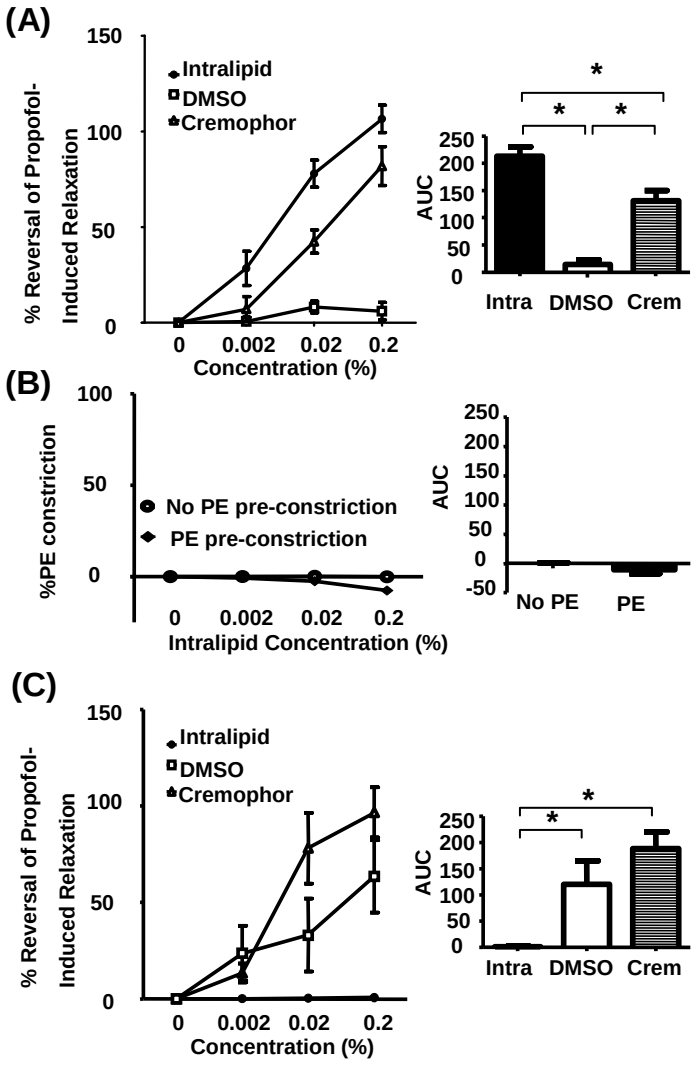

Figure 4-2. Comparisons of Intralipid, Cremophor, and DMSO reversal of propofol-induced vasodilation.

(**A**) Cumulative data and corresponding area under the curve (AUC) values demonstrating that both Intralipid and Cremophor reverse propofol-induced vasodilation greater than DMSO. Moreover, Intralipid reverses propofol-induced vasodilation more than Cremophor. (**B**) The cumulative data and corresponding AUC demonstrating lack of effect of Intralipid on vascular tone in the absence of propofol. (**C**) Cumulative data and corresponding AUC values demonstrating that both Cremophor and DMSO reverse MCh-induced vasodilation greater than Intralipid. No statistical significance was seen between Cremophor and DMSO. N=5-7 per group, *P<0.05

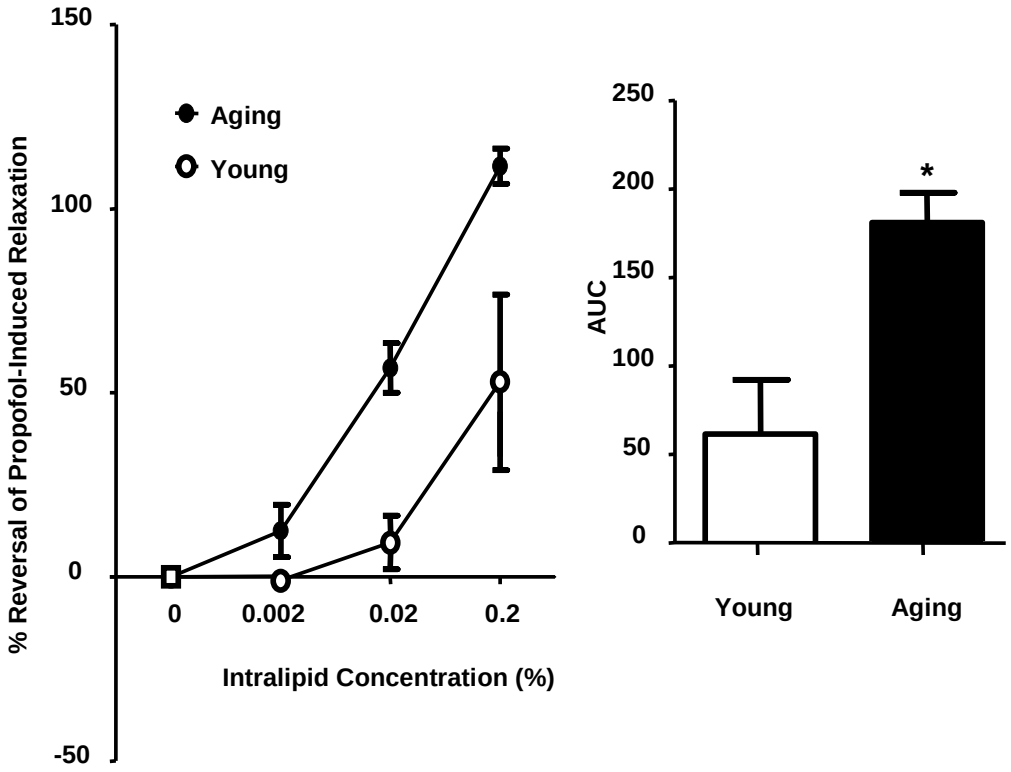

Figure 4-3. Comparison of Intralipid reversal of propofol-induced vasodilation in arteries from aging and young rats.

Cumulative data and corresponding AUC values demonstrating that Intralipid reversal of propofol-induced vasodilation is greater in arteries from aging rats than in arteries from young rats. N=5 per group, *P<0.05

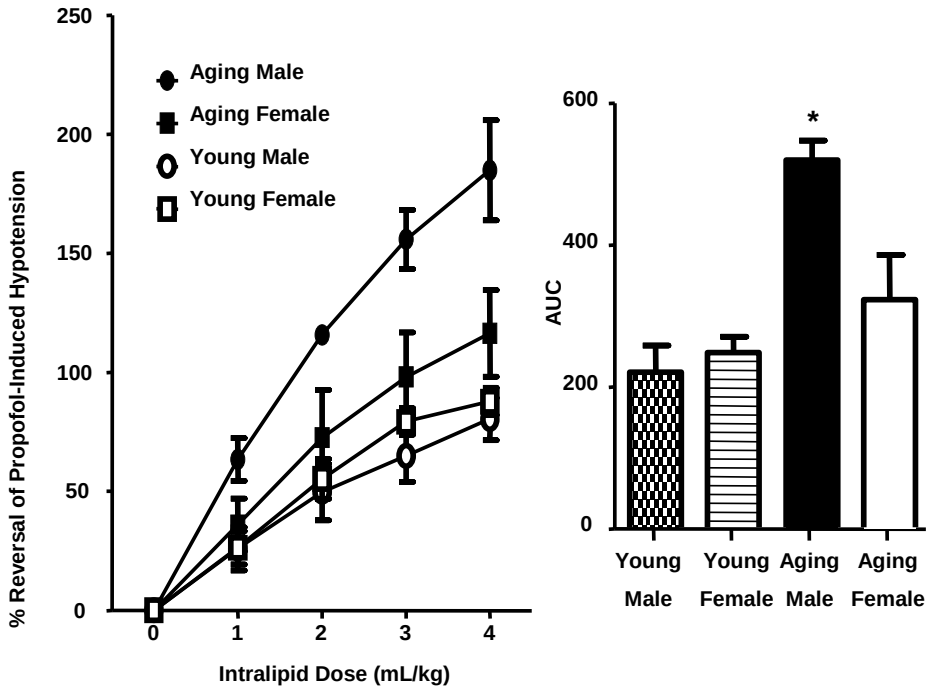

Figure 4-4. Comparisons of Intralipid reversal of propofol-induced hypotension in aging and young rats.

Cumulative data and corresponding AUC values demonstrating that Intralipid reverses propofol-induced hypotension in all groups. However, the effect is greatest in aging males ($P<0.05$ aging male vs. aging female, aging male vs. young female, and aging male vs. young male). Statistical significance was not achieved when comparing aging female, young female, and young male. N=5-6 per group, *$P<0.05$.

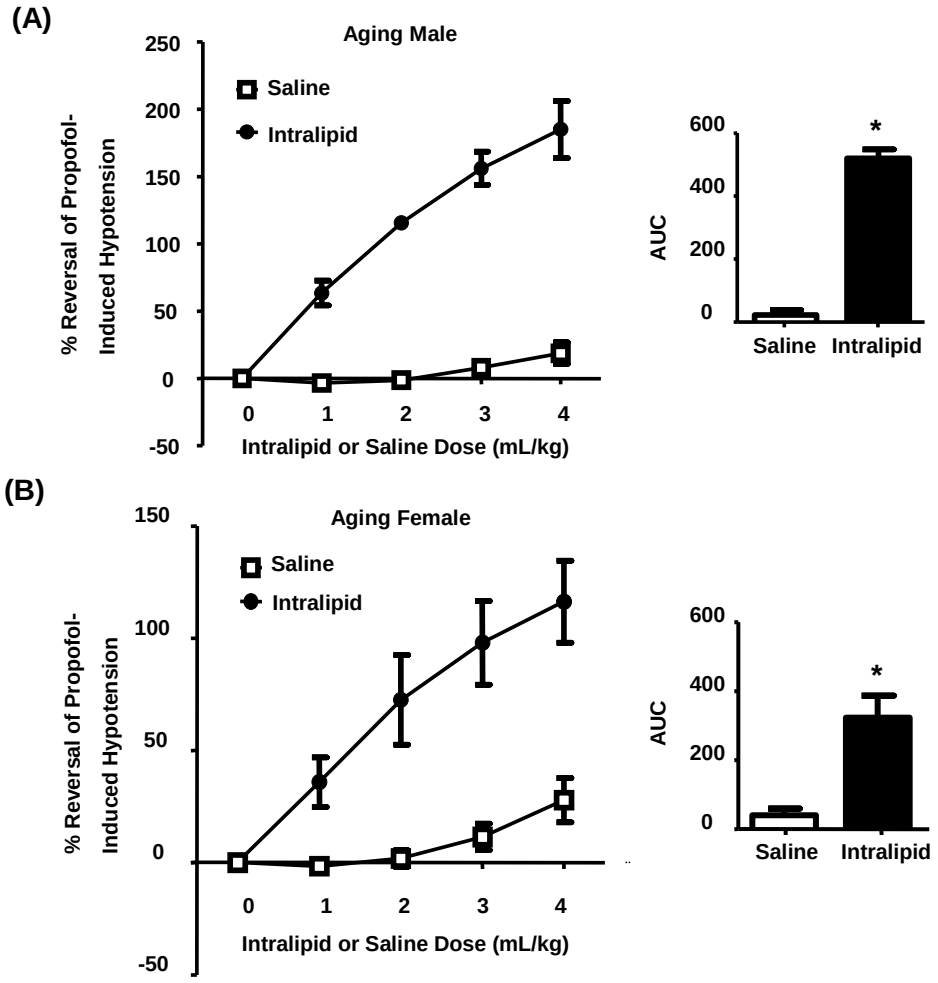

Figure 4-5. Comparisons of Intralipid and saline reversal of propofol-induced hypotension in aging males and females.

In (**A**) aging males and (**B**) aging females, an equivalent volume of normal saline does not restore blood pressure to the same extent as Intralipid following propofol administration. N=5-6 per group, *P<0.05

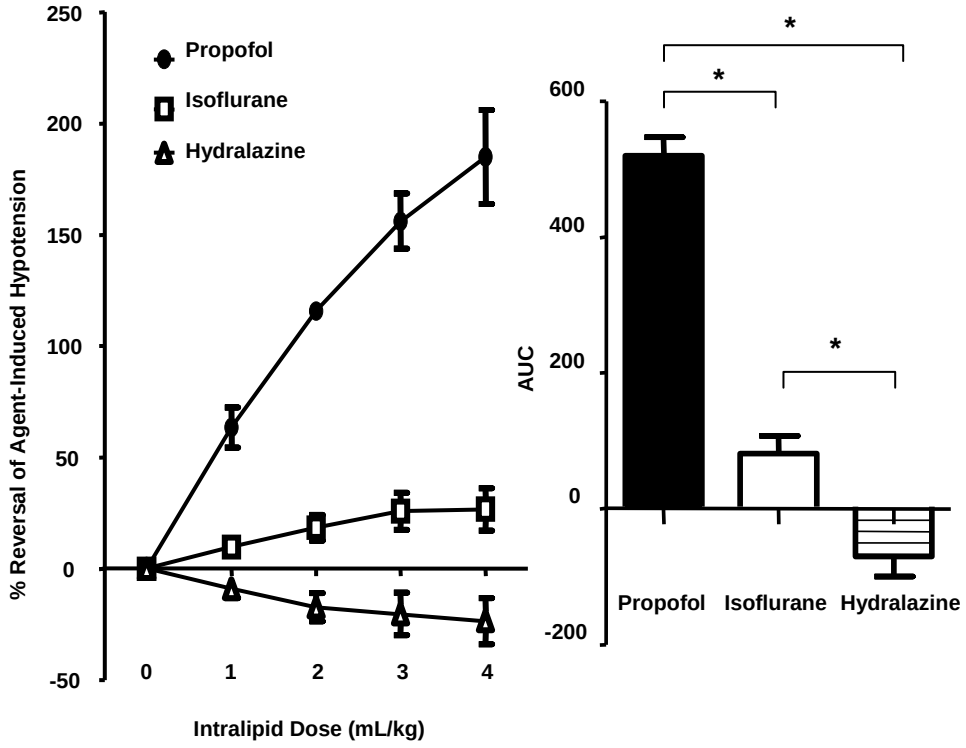

Figure 4-6. Comparisons of Intralipid reversal of hypotension caused by propofol, isoflurane, and hydralazine in aging males.

Cumulative data and corresponding AUC values demonstrating that Intralipid reverses propofol-induced hypotension to a greater extent than hypotension due to isoflurane and hydralazine. $P<0.05$ propofol vs. isoflurane, propofol vs. hydralazine, and isoflurane vs. hydralazine. N=5-6 per group, *$P<0.05$

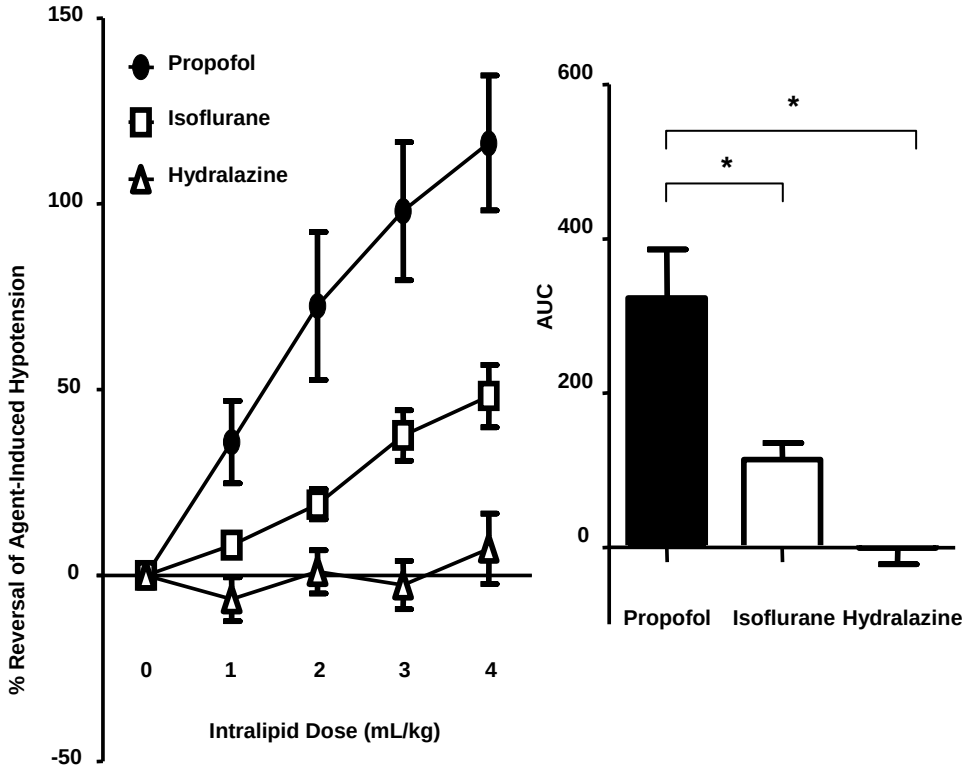

Figure 4-7. Comparisons of Intralipid reversal of hypotension caused by propofol, isoflurane, and hydralazine in aging females.

Cumulative data and corresponding AUC values demonstrating that Intralipid reverses propofol-induced hypotension to a greater extent than hypotension due to isoflurane and hydralazine. $P<0.05$ propofol vs. hydralazine and propofol vs. isoflurane; statistical significance was not achieved when comparing isoflurane and hydralazine. N=5-6 per group, *$P<0.05$

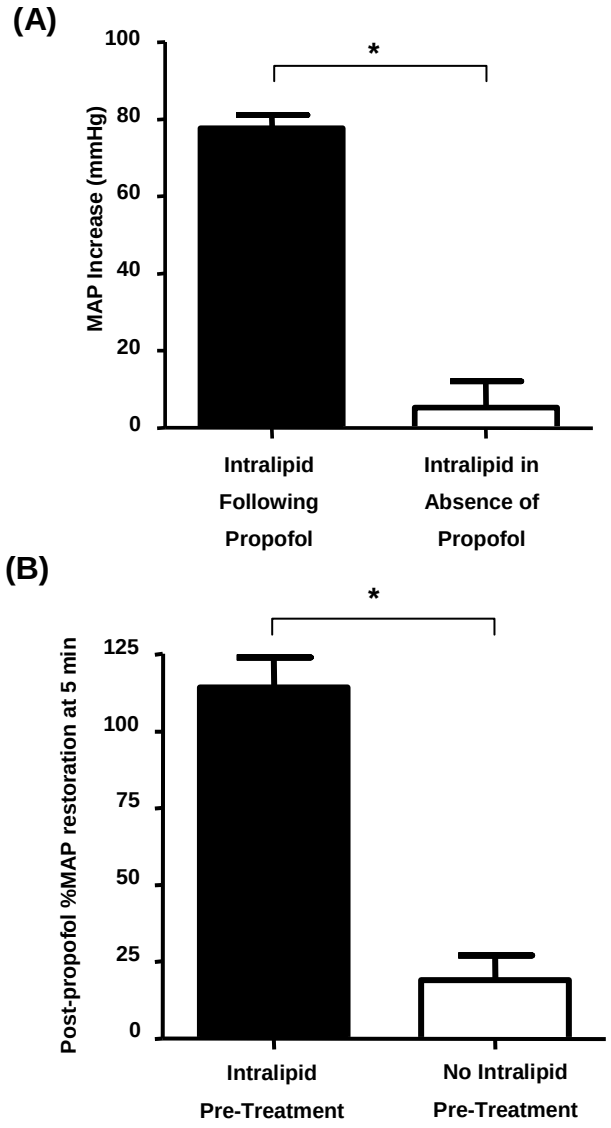

Figure 4-8. Intralipid increases MAP following propofol exposure, and pre-treatment with Intralipid prevents propofol-induced hypotension.

(**A**) Cumulative data demonstrating that mean MAP increase by Intralipid is greater when following propofol exposure than in the absence of propofol. (**B**) Pre-treatment with Intralipid restores MAP to baseline at the 5-minute mark post-propofol injection. Statistical significance was achieved when compared to no Intralipid pre-treatment. N=5-7 per group, *P<0.05

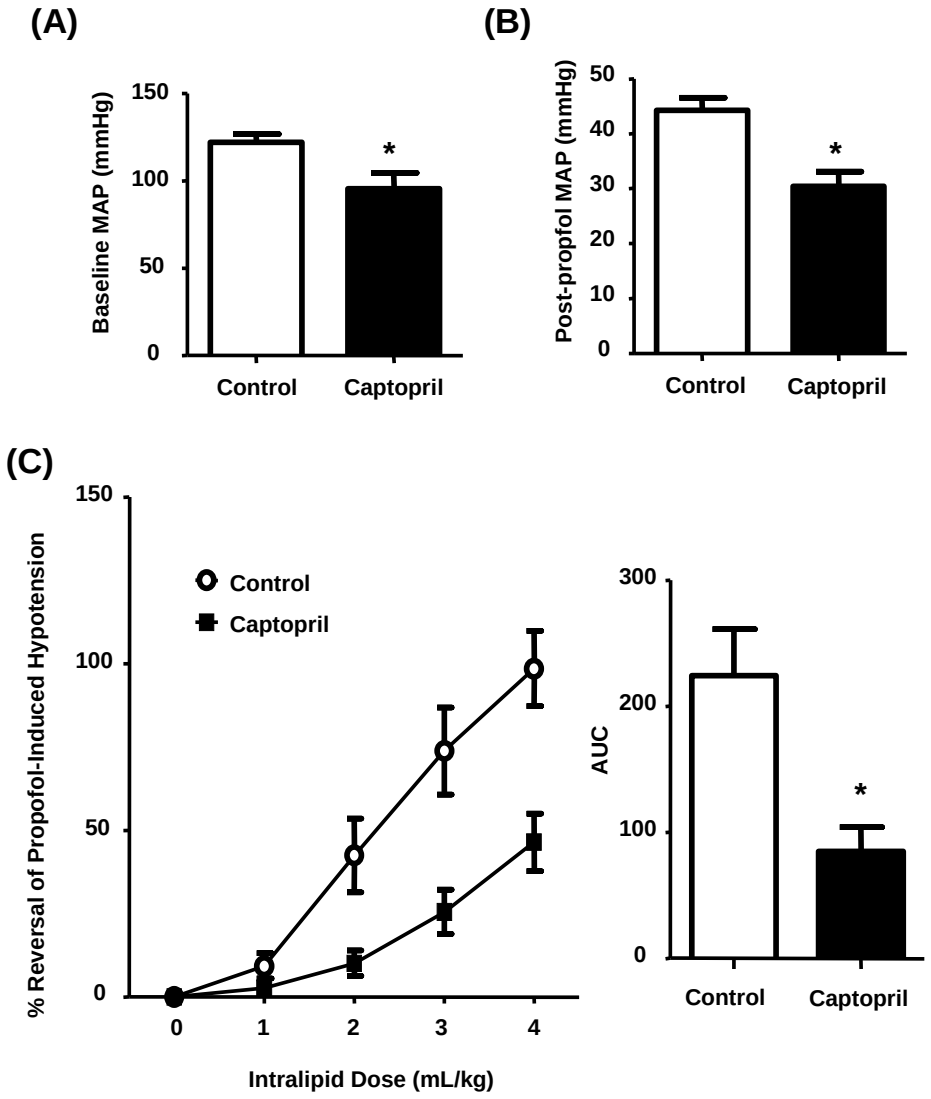

Figure 4-9. Intralipid loses its ability to reverse propofol-induced hypotension in aging male rats treated with captopril.

(**A**) Baseline MAP is lower in captopril-treated rats compared to control. (**B**) Following propofol administration, MAP is lower in captopril-treated rats compared to control. (**C**) Cumulative data and corresponding AUC values demonstrating that the ability of Intralipid to reverse of propofol-induced hypotension is decreased following captopril treatment. N=7 per group, *P<0.05

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CHAPTER 5:

GENERAL DISCUSSION

5.1 SUMMARY

Aging is associated with a multitude of physiologic changes that occur in many different organ systems. Here, the focus was on the cardiovascular system. As stated in Chapter 1, many changes occur in the cardiovascular system with advancing age, which includes structural and functional alterations in the aging vasculature that ultimately leads to hemodynamic changes that are congruent with the aging process. Notably, this includes vascular stiffening, increased oxidative stress, and imbalance of vascular tone towards vasoconstriction, all of which promotes an increase in blood pressure with increasing age. However, as enforced in Chapter 1, contrary to what may be expected in an aging vascular system that seems overtly stiff and vasoconstrictive in nature, a precipitous decline in blood pressure (and potentially end-organ perfusion) can result in aging patients during the perioperative period when exposed to general anesthetics, especially propofol. Given propofol's antioxidant profile and capabilities to increase NO production in the vasculature as reported in the literature, and given the information regarding increased oxidative stress and associated decreases in NO bioavailability in the aging vasculature, I hypothesized that increased NO bioavailability plays a critical role in the vasodilation of aging blood vessels following propofol exposure. In Chapter 2, a role of NO-dependent vasodilation in the aging vasculature was established, since L-NAME was able

to inhibit direct propofol-induced vasodilation and ACh-induced vasodilation in the presence of propofol. This served as a foundation for my following study, which investigated the role of chronic ACE inhibitor treatment *in vivo* on the *ex vivo* vascular response to propofol. In Chapter 3, I found that although the vascular relaxation response to propofol was increased in the aging vasculature exposed to chronic ACE-inhibitor treatment (as evidenced by lower MCh EC_{50} values with increasing doses of propofol pre-treatment), NO-dependent vasodilation was likely not responsible for the observed differential response. Specifically, although L-NAME inhibited MCh relaxation in control more than in captopril-treated rats, the proportional increase in the L-NAME EC_{50} shift was similar between control and captopril-treated rats. Nevertheless, this suggested for the first time that, at the vascular level, inherent differences may exist between arteries exposed to chronic ACE inhibition and those not exposed to ACE inhibitors which may contribute to the refractory hypotensive responses seen clinically during general anesthesia in patients treated with ACE inhibitors. Finally, I sought to determine if Intralipid can reverse the vasodilating and hypotensive responses to propofol administration in the aging cardiovascular system. Based on serendipitous findings demonstrating Intralipid reversal of propofol-induced vasodilation *ex vivo*, in Chapter 4 I established that Intralipid also reverses propofol-induced hypotension in an aging model, showing promise as a potential treatment for propofol-induced hypotension in the aging population. Moreover, given the

information introduced in Chapter 3 regarding refractory hypotension in ACE-inhibitor patients given a general anesthetic in the clinical setting, I sought to determine if Intralipid can be used as a potential treatment to restore blood pressure following propofol-induced hypotension in the presence of chronic ACE inhibition. Contrary to my hypothesis, Intralipid does not appear to be an efficacious treatment for propofol-induced hypotension in the setting of chronic ACE inhibition. Nonetheless, I was able to determine that the ability of Intralipid to reverse hemodynamic perturbations appears selective for hypotension induced by propofol.

The information obtained in the studies presented in this thesis has allowed me to gain a further appreciation regarding propofol's effects on the aging vasculature in normal physiological aging as well as in a setting of chronic ACE inhibition, a common situation seen in the clinical setting. In addition, these studies allow me to embark upon studying the exciting interactions between propofol and its clinical solvent Intralipid.

5.2 PROPOFOL AND AGING

To determine if propofol has an effect on the aging vascular system, we utilized a rat model of aging. In this model, we examined the potential mechanisms whereby propofol influences vascular relaxation. We chose to

investigate the effects specifically on the mesenteric circulation, primarily because this circulation plays a major role in influencing systemic vascular resistance, and therefore it is a major factor determining blood pressure.[1] Indeed, hypertensive crises can result from overt vasoconstriction of mesenteric arteries as can occur with conditions such as autonomic dysreflexia,[2] demonstrating the importance of this vascular bed in determining blood pressure in physiological and pathophysiological states. Since my initial question for this thesis revolved around the fact that both age and propofol use are predictors for developing intraoperative hypotension,[3] and based on my clinical observations regarding hypotension with propofol use in my elderly patient population, the logical step in translating these clinical findings back to bench research was to utilize an animal model of aging to determine if there is a differential effect at the vascular level (i.e. in an isolated resistance artery, devoid of influences from other physiologic systems such as the SNS).

In these isolated resistance arteries, I demonstrated novel evidence that 1) propofol enhances vasodilation in aging arteries when compared to young, and 2) NO plays a major role in this response. In particular, although EDHF-dependent vasodilation is decreased whilst NO-dependent vasodilation is increased by propofol, there is an overall enhanced vasodilation in arteries isolated from aging compared to young rats in the presence of propofol, again reinforcing an enhanced bioavailability of NO in the aging vasculature that contributes to endothelium-dependent vascular relaxation. This vascular

relaxation may be part of the reason why hypotension occurs in the aging patient population when exposed to propofol.

5.2.1 NO and Propofol

As detailed in the background to this dissertation (section 1.4.4), multiple mechanisms may exist by which propofol causes vascular relaxation. The focus of my study was on the NO pathway, primarily because of its alteration in the aging vasculature given its decreased bioavailability secondary, in part, to scavenging in the presence of increased oxidative stress which occurs in the aging cardiovascular system. The fact that propofol possesses antioxidant capabilities[4-5] also suggested that NO may play a substantial role in propofol's vasodilating capabilities due to its potential to decrease oxidative stress that is already inherent in the aging vasculature. In addition, the study that demonstrated the dependence of NO in the hyperpolarizing responses in mesenteric arteries when given propofol (which was accompanied by a decrease in blood pressure),[6] albeit in a young animal model, served as part of the basis for my hypotheses regarding propofol, NO, and the aging vasculature.

Although NO-dependent vasodilation is decreased with aging, propofol enhances this component of relaxation in the aging vasculature, which likely contributes to the hemodynamic effects that we see in the clinical situation

when propofol is administered to elderly patients. My studies demonstrated the role of NO in enhanced vasodilating effects of propofol by the use of L-NAME to inhibit NO synthesis. Although, we saw a clear effect of NO in the vasodilatory response in the presence of propofol, one area of criticism is the fact that we did not measure, in real-time, the actual production of NO in these vascular reactivity studies. Nonetheless, it can be inferred based on our results that NO is important in vasodilation in the presence of propofol.

To demonstrate the importance of oxidative stress that is present in the aging vasculature in mediating the vascular tone, in my experiments in Chapter 2 we administered the combination of antioxidant enzymes SOD and catalase to these isolated resistance arteries and found that there was a similar relaxation response to arteries administered propofol. Although we did not directly detect a reduction in oxidative stress in the presence of propofol in these arteries, these findings nonetheless suggest that the antioxidant effects of propofol may also play a role, at least in part, in enhancing NO-mediated vasodilation.

Given the many sources of oxidative stress associated with aging, namely NAD(P)H oxidase, PGHS, and mitochondria, NOS uncoupling can occur by which there is enhanced production of superoxide anion rather than NO. This has particular relevance in the aging vasculature since the uncoupling of NOS enzymes occurs with aging.[7-8] A single study exists that shows propofol inhibits NOS uncoupling: propofol restored levels of

tetrahydrobiopterin, increased eNOS phosphorylation, and decreased levels of superoxide anion and peroxynitrite in cultured HUVECs stimulated with high glucose.[9] Moreover, inhibition of NOS also decreased superoxide production in this cell culture model,[9] which suggested that NOS uncoupling was the source of increased superoxide formation. The addition of propofol may ultimately lead to increased NO production and bioavailability, possibly by restoring NOS function (i.e. "re-coupling"[10]) in addition to direct antioxidant effects, in the aging vasculature. Further studies in an aging phenotype are needed to test this hypothesis. Nevertheless, the NO pathway plays a critical role in the vasodilating response seen in the presence of propofol during normal physiologic aging.

5.2.2 EDHF and Propofol

Interestingly, propofol inhibited EDHF-dependent relaxation, as shown in Chapter 2. EDHF constituted the majority of baseline endothelium-dependent vasodilation in both young and aging rats. However, the fact that it is inhibited by propofol but there is still an enhanced vasodilation in aging suggests that there is a substantially increased NO response perhaps to compensate for this loss of EDHF-dependent activity in these presence of propofol in these arteries. It is difficult to ascertain whether propofol inhibits EDHF-dependent vasodilation by inhibiting its "release" from the endothelium

versus its "action" on smooth muscle as originally suggested in Chapter 2, since in Chapter 3 we are still seeing an enhanced vasodilation in the presence of chronic ACE inhibition. To elaborate, my findings implied that the reason for this differential response with ACE inhibition in aging was not due to NO given similar proportional changes of the EC_{50} shifts in the presence of NOS inhibition as demonstrated in Chapter 3; this suggested that there is an upregulation of an EDHF-type response with chronic ACE inhibition, a phenomenon that has been reported in the literature.[11] Therefore, a speculation that can be made based on these observations is that chronic ACE inhibition either increases the K^+ component of EDH to overcome propofol's inhibition (as suggested in section 2.2.3) or, perhaps, there is an upregulation of another factor to cause EDH (e.g. epoxyeicosatrienoic acids, hydrogen peroxide, gap junctions) which may not be affected by propofol and then constitutes the majority of the vascular relaxation that is seen with chronic ACE inhibition. Further studies are warranted to investigate this speculation.

5.3 RAS AND PROPOFOL

A fascinating intersection between my studies is the importance of the angiotensin system. As discussed in Chapter 1, the role of RAS is upregulated in aging. What I found in Chapter 3 is that chronic ACE inhibition led to an

enhanced vasodilatory response in the presence of propofol. In Chapter 4, I found that ACE inhibition caused a decreased response of Intralipid to reverse propofol-induced hypotension. These data suggest that intact RAS function is beneficial to maintain vascular tone following propofol treatment and may play a role in Intralipid's ability to reverse propofol-induced hypotension, despite the perceived deleterious nature of increased RAS in aging (and hence the prescription of drugs inhibiting the angiotensin system for this patient population). Indeed, ACE inhibitors and AT_1 receptor blockers are first-line treatment for hypertension in the elderly.[12] Given my *in vivo* findings, it would be interesting to determine if there is an effect, at the vascular level, comparing arteries from aging animals that are treated with ACE inhibitors versus animals that are untreated, to Intralipid reversal of vasodilation.

Chronic ACE inhibition can also lower blood pressure by increasing Angiotensin-(1-7) (Ang(1-7)) production,[13] which may play a role in the *in vivo* response to propofol. Ang(1-7) is primarily produced by angiotensin converting enzyme-2 (ACE2); Ang(1-7) ameliorates endothelial dysfunction and improves endothelium-dependent vasodilation.[14] Interestingly, a recent study showed that propofol increased mRNA levels, protein expression, and activity of ACE2 in cultured human pulmonary artery ECs.[15] This study is currently the only one which has looked at propofol and ACE2 and suggests that there may be an increased production of Ang(1-7) which can cause vasodilation. Whether propofol causes an increase in Ang(1-7)-dependent

vasodilation will need to be established, as this has not yet been investigated. Therefore, an exciting future direction of this research is to determine if ACE2 has a role in vascular reactivity studies/hemodynamics involving propofol.

In regards to the role of NO and potential upregulation of EDHF-like response in the setting of chronic ACE inhibition, an additional explanation regarding an alteration of the NO pathway (and not NO *per se*) can be offered regarding ACE inhibition. Although there is an increased amount of cGMP, the downstream mediator involved in NO-dependent vasodilation, in arteries from rats chronically treated with ACE inhibitors,[16-17] a presumed reason is that there is increased NO bioavailability in the vasculature as a result of ACE inhibition. This may be true, however it is unclear whether cGMP content is augmented in ACE inhibition due to increased NO production/availability directly, increased sCG expression/activity (which would produce cGMP), decreased phosphodiesterase expression/activity (which would degrade cGMP), or a combination of the three possibilities. Another explanation is that increased sGC has been implicated to cause an increase in cGMP despite lower levels of NO;[18] this mechanism may also be involved given what I have found in my Chapter 3 study regarding a possible decreased effect of NO in captopril-treated rats compared to control rats.

5.3.1 Inconsistencies with Aging Rats in ACE Inhibitor Study

In regards to my data investigating direct propofol-induced vasodilation and NO, some discrepancies can be seen between Chapters 2 and 3. To elaborate, L-NAME inhibited direct propofol-induced vasodilation in mesenteric arteries from aging rats (section 2.3.1), and this was not repeated *per se* in my following study in the control aging rats (section 3.3.4). It is difficult to reconcile this disparity, although it is noteworthy that L-NAME was found to attenuate the relaxation at the lowest doses of propofol (i.e. before the onset of the second phase constrictor response after 1 μmol/L; Student's t-test $P<0.05$) in Chapter 3. These findings suggest that NO may modulate propofol-mediated vascular effects depending on the dose. In addition, the MCh EC_{50} values in the presence of propofol were similar in Chapter 3, whereas we were able to detect a difference in ACh AUC values in the presence of propofol in Chapter 2. Although animal weights were similar between the two sets of aging animals (mean weights 433.0 g in Chapter 2 versus 435.7 g in Chapter 3), we must acknowledge that a multitude of factors may also account for these differences that we see between the two studies. These include the dose ranges of drugs used, the number of cumulative doses used, time intervals between doses, and the use of EC_{90} versus EC_{80} of phenylephrine constriction. In addition, a notable difference between the studies presented in Chapters 2 and 3 was that sodium pentobarbital was used for anesthesia (Chapter 3) whereas isoflurane was previously used (Chapter 2).

Sodium pentobarbital has different effects compared to isoflurane, including differential gene expression profiles and alterations in second messenger systems. For instance, in a rodent burn model, it was found that isoflurane differentially alters cytokine production as opposed to sodium pentobarbital.[19] Particularly relevant to my studies, it was found that isoflurane decreased cGMP content, whereas sodium pentobarbital increased cGMP content, in the rat CNS.[20] Therefore, there may be an alteration in vascular second-messenger systems involved in the NO pathway which can result in altered vascular reactivity between two seemingly similar groups of animals. Future studies are therefore needed to assess the role of anesthetic agents on vascular reactivity to fully test this hypothesis. Notwithstanding this disparity, it should be noted that for both studies, there was a clear differential response between aging and young rats as well as control and captopril-treated aging rats in the respective groups of animals.

5.4 INTRALIPID AND PROPOFOL

It is quite interesting that Intralipid was able to reverse propofol-induced vasodilation and hypotension in our animal model. Given that it is a substance that is already used in the clinical setting for total parenteral nutrition, translating its use from “bench-to-bedside” is a logical step

following the studies presented in this thesis as well as in my future studies that I plan to embark upon.

Although I did not directly investigate the mechanisms by which Intralipid is able to reverse propofol-induced vasodilation and hypotension, some possibilities can be entertained based on some information in the literature. I utilized the clinical formulation of propofol (i.e. solubilized in Intralipid) in all of the studies presented in this thesis. Given the possibility that Intralipid causes NO-dependent vasodilation in an experimental model utilizing instrumented dogs,[21] whether the NO response is altered due to propofol alone or possibly in combination with Intralipid was not directly studied in my experiments. However, the fact that Intralipid did not cause substantial vascular relaxation in Chapters 2 and 4 following phenylephrine constriction suggests that Intralipid, on its own (and at the same concentration of the Intralipid in the propofol formulation), likely contributes minimally to the overall relaxation response we see with the propofol solution that I administered in these studies. Indeed, using HUVEC culture, it was refuted that lipid emulsion reduces production of NO since the levels of phosphorylated eNOS (induced by bupivacaine administration) are decreased within minutes following treatment with lipid.[22] Therefore, the interaction we see between Intralipid and propofol-induced hypotension may in fact be a result of interfering with the NO pathway.

Determining the mechanism(s) by which Intralipid reverses vasodilation and hypotension following propofol administration is of great importance. Moreover, determining the components of Intralipid important in this response is also important, since there are a variety of substances (i.e. multiple fatty acids, phospholipids, glycerol) that constitute the Intralipid solution. Therefore, potential future experiments can be tailored to elucidating which component(s) of Intralipid are involved in the vascular and hemodynamic responses seen in Chapter 4 of this thesis, and perhaps this can lead to future studies of that single component of Intralipid in vascular function studies as well as studies investigating Intralipid's hemodynamic effects.

An interesting and serendipitous finding in Chapter 4 is the fact that we were able to demonstrate sex differences in aging regarding the reversal of hypotension with Intralipid. Of note, it is known that the milieu of female sex hormones, namely estrogen, has a cardioprotective role in females[23] and may involve altered structure and function of the vasculature;[24] this suggests that vascular reactivity may differ between young males and young females. However, the cardioprotective effect in females is lost during reproductive senescence that occurs with aging since there are lower levels of circulating estrogen, increasing cardiovascular risk.[25] Although elucidating sex differences in vascular and hemodynamic function was not a study aim of the

thesis (i.e. my primary goal was the investigation of aging and propofol), these issues will nonetheless be incorporated in my future studies.

5.5 GENERAL LIMITATIONS

To address my hypotheses regarding propofol and increased vasodilation in aging and in the setting of chronic ACE inhibition, an *ex vivo* experimental approach (i.e. wire myography) was utilized. Although the *ex vivo* studies in this thesis addressed propofol effects specifically at the vascular level, one should not immediately assume that this *ex vivo* system behaves like an intact system *in vivo*. Indeed, my *in vivo* studies in Chapter 4 take into account the interplay of multiple factors in the control of vascular tone (e.g. SNS). Another benefit of incorporating *in vivo* studies in Chapter 4 is the fact that entire organ systems remain intact. A previous study utilizing rat thoracic aortas with or without perivascular tissue has documented the role of perivascular adipose tissue in propofol's vasodilating ability,[28] so the results seen in my *ex vivo* experiments (where mesenteric perivascular adipose tissue was removed) may only reveal a partial story regarding propofol's vasodilating effects in aging, in the setting of chronic ACE inhibition, and with reversal by Intralipid. Fortunately, perivascular adipose tissue was still retained in the *in vivo* work which addresses this issue.

An additional limitation from the experiments completed in this thesis is that the findings may not be generalized to all vascular beds. That is, even though we were able to find NO-dependent responses to propofol in resistance mesenteric arteries, NO may or may not play role in other vascular beds such as in the coronary, carotid, and femoral arteries in this animal model. Importantly, although we found that Intralipid can reverse propofol-induced vasodilation in mesenteric arteries and hypotension, which may primarily be mediated by a return in vascular tone in the mesenteric arterial bed (as discussed section 5.2), this return of vascular tone in other vascular beds may be harmful. For instance, specifically in coronary arteries, if Intralipid does reverse propofol-induced vasodilation, this may prove to be undesirable in a setting of ischemic heart disease when coronary flow is already compromised. Future studies will need to be completed to determine if this is the case.

The propofol doses primarily used in the *ex vivo* studies are 1-10 μmol/L. It has been estimated that plasma concentrations of propofol in humans ranges from 2-10 μg/mL[23-24] which is similar to that reported in rats (up to 14 μg/mL in whole blood, ~80 μmol/L).[25] It has been conveyed that protein binding of propofol may be in the range of 97-98%[26] thereby bringing the concentration of the free fraction to ~2.5 μmol/L. However, it has recently been suggested that the peak plasma concentration of propofol may approach 35 μg/mL (~200 μmol/L), yielding a free fraction of 6 μmol/L.[27] Another potential point of criticism is regarding the dose of propofol used by bolus

injection for intravenous anesthesia (10 mg/kg) for the *in vivo* studies. Although this dose may be seemingly high by clinical standards, it must be highlighted that this dose was based on our pilot work as well as the dose used by others in the literature utilizing rat models for their studies.[29-30] However, since these studies were focused on the cardiovascular effects of propofol, it would also be important to determine what the anesthetic effects of this dose would be on the brain (i.e. if this dose causes burst suppression or isoelectrical activity on electroencephalogram).

5.6 OTHER FUTURE DIRECTIONS

One key question which needs to be addressed is whether Intralipid alters the anesthetic effects of propofol (in addition to the hemodynamic changes demonstrated in Chapter 4). One key area of ongoing research is about awareness during a general anesthetic ("anesthesia awareness"), which can be devastating to patients undergoing surgery.[31-33] Although much effort is devoted to determining efficacious interventions to prevent anesthesia awareness, the question posed by my studies is whether Intralipid can improve recovery from a general anesthetic (i.e. following anesthetic induction and/or maintenance with propofol) to accelerate emergence/awareness. This is particularly important in difficult airway scenarios where rapid recovery from

a general anesthetic in a "cannot intubate-cannot ventilate" scenario is paramount, as described in the American Society of Anesthesiologists Difficult Airway Algorithm.[34] Another important clinical scenario where this would be beneficial is following neurosurgical procedures, when total intravenous anesthesia (TIVA) is maintained with a continuous propofol infusion, such as following craniotomy surgery or spinal surgery utilizing spinal cord monitoring,[35] and assessment of neurological function as soon as possible following the conclusion of surgery is very necessary. This may also reduce the "guesswork" in reduction of propofol infusion rates towards the end of surgery utilizing TIVA, a time when clinicians try to balance appropriate anesthetic depth during surgery with desirable emergence times at the end of surgery, since there is no gold standard "awareness" monitor for TIVA. On the other hand, if it is found that Intralipid does not affect the anesthetic effects of propofol, this would also be beneficial as it would suggest that Intralipid may be useful for the sole purpose of hemodynamic modulation following propofol use. Regardless of outcome, determining if Intralipid interferes with propofol's anesthetic effects (in addition to cardiovascular effects) is of utmost importance.

5.7 SIGNIFICANCE OF THE THESIS RESULTS

The results of the experiments presented in this thesis help explain, at least in part, why using propofol in the aging population results in significant hypotension. A potential use of this information may lead to novel therapies (e.g. Intralipid) involving propofol or an alteration of anesthetic practice in regards to the aging patient, exemplifying the bench-to-bedside approach in experimental medicine. Much like understanding the effects of general anesthetics at the CNS level with studies devoted to understanding the mechanisms of action of notable general anesthetics (with implications of eliminating the phenomenon of anesthesia awareness), the focus of this thesis was devoted to further understanding the cardiovascular effects of propofol, a widely used general anesthetic, in the aging vasculature. This is very important given the increasing life expectancy in Canada and the ever-increasing elderly population presenting for surgical procedures requiring general anesthesia. This thesis relays further insights to our understanding of the mechanisms of action of anesthetic agents (in this case, propofol at the vascular level), and will hopefully lay the foundation for future studies in vascular research tying together anesthesia and aging.

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