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The Effects of OPC-18790 on Vascular Capacitance and Conductance in Experimental Heart Failure

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Effects of OPC-18790 on Vascular Capacitance and Conductance in Experimental Heart Failure" submitted by Lisa Marie Semeniuk in partial fulfilment of the requirements for the degree of Master of Science.

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CONDENSED ABSTRACT

The effects of OPC-18790 (OPC) on vascular capacitance and conductance in heart failure were assessed. Because OPC administration reduces left ventricular end-diastolic pressure, we hypothesized that it is also a venodilator. Because of the known arterial effects and the hypothesized venous effects, we compared changes in systemic vascular conductance to changes in venous capacitance. Seven treatment and five control dogs were studied at baseline, after induction of heart failure (microsphere embolization) and then after boluses of OPC. Venous capacitance was measured using a blood-pool scintigraphic method. At the lower doses, capacitance increased more than conductance; these effects were more balanced at the higher doses. Compared to previous studies in the same model, OPC increased capacitance to the same degree as nitroglycerin, increased conductance similarly to hydralazine, and both effects were greater than those obtained with enalaprilat. OPC may prove to be a clinically important alternative to other vasodilators.

Dedication

In loving memory of my father, Dr. Konstantin H. Semeniuk, who passed away suddenly July 18, 1996.

I am writing this upon completion of my thesis with a great feeling of emptiness. Amongst the many articles and books that I accumulated over the last three years are my fathers Medical Physiology, Goodman's and Gilman's, Monitoring in Anaesthesia, and Respiratory Care books to name a few. These were my fathers old text books that he studied from in medicine which he gave to me with pride over the last few years. When I asked him a question he would bring out one of these books, make me read the relevant chapter, discuss it with me and then give me the book to keep. Every day when we were in Mexico last Christmas he would hand me a "Canadian Journal of Anaesthesia" or "New England Journal of Medicine" flagged with a current article that would help strengthen my knowledge. These are only a few of the examples of the things we shared together directly related to the completion of my MSc. The impact he had on my life and the lives of his family, friends, patients and colleagues extends far beyond any words that I can write at this time. It is with great honour that I dedicate this thesis in memory of my father, my "pal", Dr. Konstantin H. Semeniuk.

Acknowledgements

I would like to thank my supervisor, John Tyberg for his guidance throughout my project. In the three years that I have been associated with his lab he has successfully converted me from being a Guytonian and introduced me to new knowledge that truly is forefront in its field. His guidance extends beyond scientific endeavors - although he has not fully cured me of saying "alls thats left," I certainly think twice about saying it now.

I would like to thank my committee members Dr. Belenkie, Dr. MacCannell, and Dr. Wilkens for their suggestions regarding my thesis. I would also like to thank Dr. Belenkie and Dr. MacCannell for their guidance, support and encouragement.

I would like to acknowledge the statistical advice of Dr. Rollin Brant of the Centre for the Advancement of Health.

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Also, it was a pleasure working with Gregg, Huibert, Ellen, and most recently with Takster. The lab will never be the same without the symphonic

echos of chewy. I would also like to thank my late-night study partner and jogging partner Jen machine. I still think that my pheromone hypothesis is the basis of our ability to focus and be so productive when we study together.

I would like to give special thanks to my best friend John Woo. All the thanks you deserve for everything you've done over the past couple of years can be summarized in the phrase we often say to each other in french, "cherie, je t'aime."

Finally, I would like to thank my mother for her love, care, support and advice. She encouraged me to finish my thesis despite the recent loss to our family.

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Chapter 1: Introduction

Heart failure (HF) is a highly prevalent condition associated with a very poor prognosis. Considerable advances in the diagnosis and treatment of HF have been made in the last couple of decades. One of these advancements has been the recognition that to pharmacologically maximize therapeutic effectiveness in the treatment of HF, it is important to optimize the balance between venous and arteriolar effects. Although many agents are classified as vasodilators, the data supporting use of this term are limited to the systemic vascular effects (ie. a decreased systemic vascular resistance). The potential effect of that compound on venous capacitance is often ignored.

In the study which forms the basis of this thesis, the vascular capacitance and conductance effects of OPC-18790, a phosphodiesterase-III inhibitor, have been studied and compared to the effects of equihypotensive doses of nitroglycerin, hydralazine and enalaprilat (results of an earlier study in our lab) using the same model.

Before the actual experiment is presented, the history and importance of the functional coupling between the heart and blood vessels will be reviewed in chapter 2. In particular, the views held by Guyton, Levy and Tyberg will be discussed. This chapter gives the rationale for studying the vascular effects of OPC-18790.

Our experiment involved the use of anaesthetic agents which may

potentially effect the analysis of our results. Chapter 3 reviews the anaesthetics used - sodium thiopental, nitrous oxide and isoflurane. Particular attention was given to the cardiovascular effects of these anaesthetics.

Chapter 4 involves a review of the mechanism of action of phosphodiesterase inhibition. Many of the hemodynamic effects of OPC-18790 obtained in this experiment can be explained by these actions.

Chapter 5, describes the actual experiment "the effects of OPC-18790 on vascular capacitance and conductance in experimental heart failure," including the methods, results, and discussion.

The final chapter, chapter 6, gives a brief overall conclusion to the thesis.

Chapter 2: Functional Coupling of the Heart and Blood Vessels

"...I frequently and seriously bethought me,
and long revolved in my mind, what might be the
quantity of blood which was transmitted, in how
short a time its passage might be effected, and
the like; and not finding it possible that this
could be supplied by the juices of the ingested
aliment without the veins on the one hand becoming
drained, and the arteries on the other getting
ruptured through the excessive charge of blood,
unless the blood should somehow find its way from
the arteries into the veins, and so return to the
right side of the heart; I began to think whether
there might not be A MOTION, AS IT WERE, IN A CIRCLE."

William Harvey (1578-1657)[1]

2.1) Capacitance and Conductance Defined

Capacitance is a general term relating the total volume of the vasculature to the transmural pressure over the physiologic range.[2] Over this operating range where the pressure-volume relationship is considered linear, capacitance is described by both the stressed volume and unstressed volume. The stressed volume is the volume of blood that must be removed from the vasculature to decrease the transmural pressure to zero. It is a virtual volume, calculated by multiplying the compliance (the slope of the pressure-volume curve [$\Delta V/\Delta P$][3]) by the distending pressure. The unstressed volume is the volume present at a transmural pressure of zero. It is a virtual volume obtained by extrapolating the

pressure-volume relationship to zero transmural pressure (the volume-axis intercept of the pressure volume curve [3]). Although the capacitance vessels include all distensible vessels (including the arteries) as well as the spleen and hepatic sinusoids (which have blood-storage function), approximately 70% of the total blood volume is contained in the veins and venules.[2] Thus, changes in capacitance generally reflect changes in venous volume.

Conductance is defined as the inverse of the systemic vascular resistance (Conductance = cardiac output/[mean aortic pressure - mean right atrial pressure]). Traditionally, vascular tone has been expressed as vascular resistance and percentage change in resistance.[4,5] Lautt[4] argues that conductance is the appropriate index if the changes in vascular tone result primarily in changes in blood flow rather than pressure. Under conditions of a constant flow, where altered vascular tone primarily affects pressure, resistance is the appropriate index of vascular tone. However, under most conditions in vivo, local vascular responses result primarily in changes in flow rather than pressure, thus conductance is the best index of vascular tone. We also chose to use the term conductance in our study in order to parallel arterial dilation with venous dilation (vascular smooth muscle relaxation). By doing so, arterial dilation is represented by an increase in conductance and venous dilation by an increase in capacitance. Analogous reasoning holds for constriction.

This study involves the effects of OPC-18790 on vascular capacitance and conductance. In order to understand the impact of these effects on cardiovascular homeostasis, it is important to relate changes in venous capacitance and systemic conductance to cardiac filling and performance.

2.2) Guyton's Explanation

Historically, a graphical procedure for exploring the interplay between the peripheral circulation and the heart in determining cardiac output was suggested by Guyton. The graph is made up of a cardiac output curve (fig 2.1) in which cardiac output is related to right atrial pressure, and a venous return curve that relates venous return to right atrial pressure.

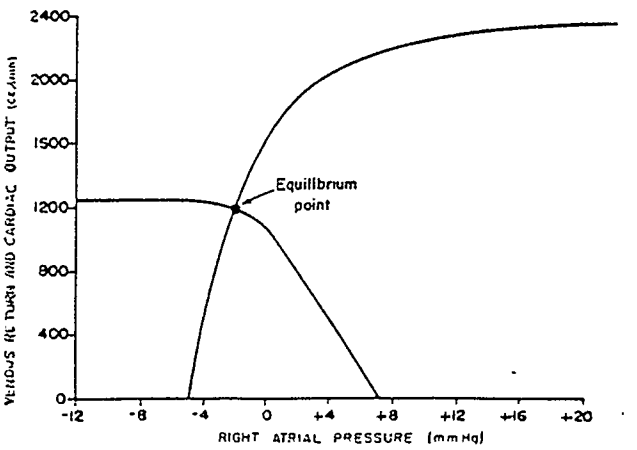

Fig 2.1: Cardiac Output and Venous Return Curves (Reproduced from Hamilton, 1963).[6]

The cardiac output curve is based on the Frank-Starling law which states that the energy of contraction is proportional to the initial length of the cardiac muscle fiber (Ventricular stroke volume vs. end-diastolic volume).[7] The venous return curve is based on the understanding that the return of blood to the heart is determined by the gradient between the peripheral veins (mean systemic filling pressure) and the right atrium. Guyton and his colleagues considered that right atrial pressure was a determinant of venous return. In their right-heart bypass experiments (fig 2.2),[8] blood is taken from the right atrium and returned to the pulmonary artery.

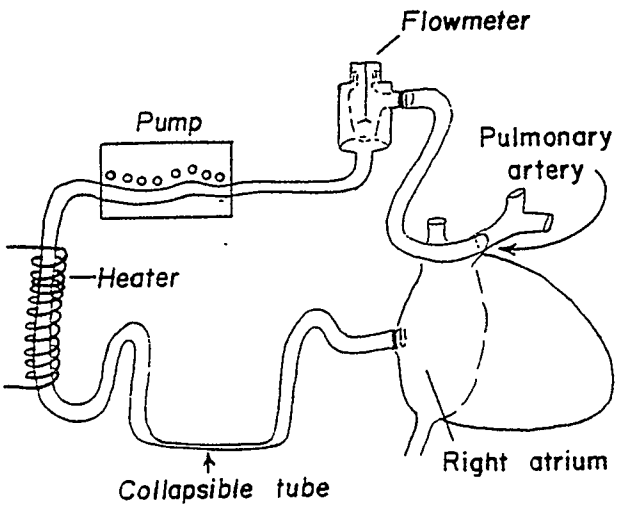

Fig 2.2: Experimental System for Controlling the Right Atrial Pressure (Reproduced from Guyton, 1973).[8]

The collapsible tube segment of the system permits control of the right atrial pressure. The pump was adjusted to keep the pressure on the left-hand side of the tube at -10 to -20 mm Hg and the pressure at the right side was

always equal to 0 mm Hg. Right atrial pressure was altered by raising or lowering the collapsible tube (creating a hydrostatic pressure difference between the zero pressure in the tube and the pressure in the right atrium).

Guyton also views preload, afterload, cardiac contractility, and heart rate as the determinants of cardiac output. Factors such as the central venous pressure and aortic pressure which are determinants of preload and afterload, respectively, are thus also considered determinants of cardiac output (blood flow). This widely held view that changes in pressure cause changes in flow has formed the basis of teaching physiology for decades and remains fundamental in many textbooks.

2.3) Levy's Explanation

In 1979, analysis of an experiment conducted by Levy[9] gave an alternative interpretation of the views proposed by Guyton. Levy used a similar experimental system as was used by Guyton (described above, fig 2.2). However, by eliminating the collapsible tubing, Levy made the rate of the blood returning into the pulmonary artery from the right atrium (manipulated by a roller pump) the factor that was altered experimentally as opposed to manipulating the right atrial pressures. The results of this experiment (fig 2.31 and 2.32) showed that changes in total blood flow (Q) evoked concordant

changes in aortic pressure (P_a) and inverse changes in central venous pressure (P_v).

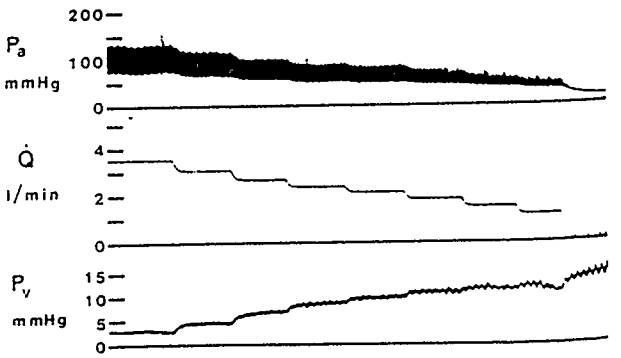

Fig 2.31

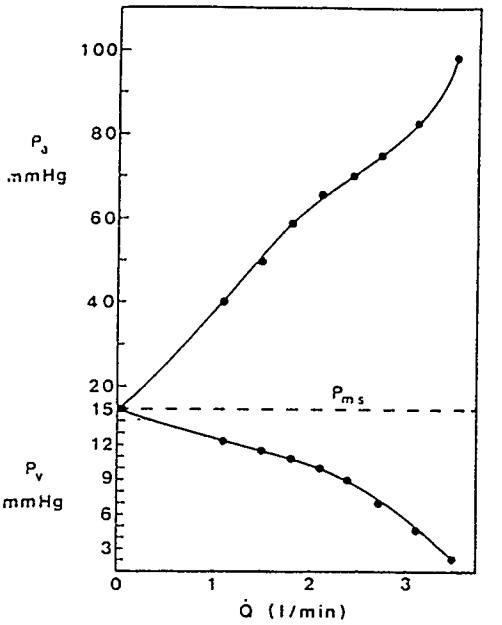

Fig 2.32

The Changes in Arterial (Pa) and Central Venous (Pv) Pressures Produced by Changes in Systemic Blood Flow (Q) (Reproduced from Levy, 1979).[9]

Furthermore, Levy argued that under steady state conditions the cardiac output and venous return are equal. Levy states that "to explain the steady state change in cardiac output on the basis of a change in venous return is a patent example of circular reasoning; it is tantamount to explaining a change in flow on the basis of a change in flow."[9]. Also, the designation of the function curves delineated by Guyton (fig 2.1) is ambiguous because any factor that effects cardiac output has an equal effect on venous return. Thus, the axes must be reversed for one of the curves in order to include the two curves on the same set of axes. As noted by Tyberg[10], the lower portion of fig 2.32

is equivalent to Guyton's venous return curve with the axis reversed. Venous pressure increased as cardiac output decreased due to accumulation of blood in the venous reservoir.

2.4) Tyberg's Explanation

In support of the conclusion that venous pressure falls because cardiac output increases (in accordance with Levy's views), Tyberg[10] has extended the analysis of the vascular system to include pressure-volume relationships. The following arterial and venous pressure-volume diagram (fig 2.4) emphasizes the functional coupling between the heart and the vascular system.

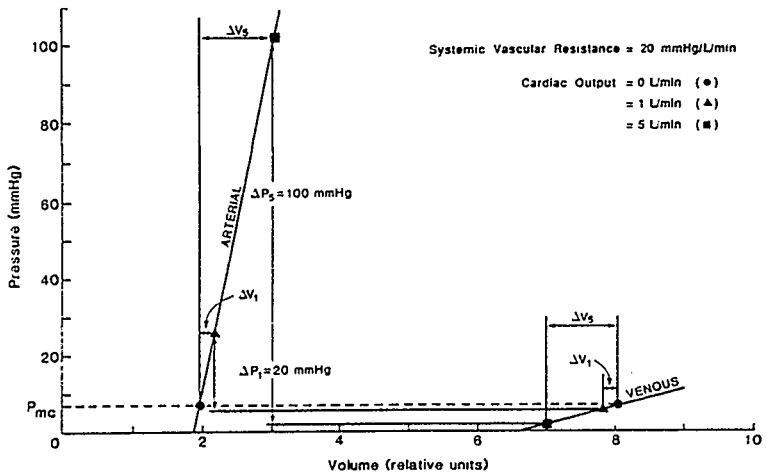

Fig 2.4: Arterial and Venous Pressure-Volume Relationships (Reproduced from Tyberg, 1996).[11]

The slopes of the arterial and venous-pressure volume relationships differ by a factor of 19 (consistent with Levy's venous-to-arterial ratio of

compliances). The intercepts of the two lines are plotted so that approximately 70% of the blood is contained in the veins and 30% in the arterial system at a cardiac output of 5 L/min. Mean circulatory pressure (P_{mc}) is equal to 7 mm Hg when cardiac output is equal to zero. These values are all typical of the human circulation. If the heart pumps a cardiac output of 1 L/min, a volume of blood (ΔV_1) would be removed from the venous circulation and added to the arterial circulation. Because of the venous-to-arterial compliance ratio of 19, this results in a lowering of the venous pressure by 1 mm Hg (to 6 mm Hg) and an increase in the arterial pressure by 19 mm Hg (to 26 mm Hg). Flow back to the venous reservoir is passive and directly proportional to the arteriovenous pressure difference and inversely proportional to the systemic vascular resistance (equal to 20 mm Hg). Thus, because the arterial-venous pressure difference would now be equal to 20 mm Hg (26 mm Hg - 6 mm Hg), it would be sufficient to drive a flow of 1 L/min back through the systemic vascular resistance so that a new equilibrium would be achieved. The same analysis applies when the cardiac output is increased to 5 L/min.

Tyberg[11] has also shown the effects of decreasing venous capacitance (the venous unstressed volume in particular) which results in changes to the system which are analogous to changing the P_{mc} by adding blood volume (fig 2.5).

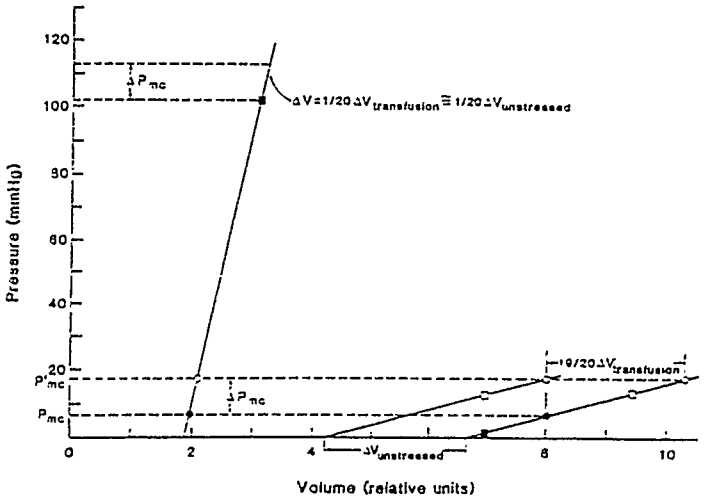

Fig 2.5: Modified Arterial and Venous Pressure-Volume Relationships (Reproduced from Tyberg, 1996).[11]

A leftward parallel shift without a change in compliance (slope changes were not statistically significant in our experiment) in the venous pressure-volume relation, as would occur with venoconstriction, raises the venous pressure without changing the volume. At a cardiac output of 5 L/min, the P_{mc} may be raised from 7 mm Hg to 17 mm Hg, venous pressure would be increased from 2 mm Hg to 12 (17 mm Hg [P_{mc}] - 5 mm Hg [from cardiac output of 5 L/min]), and arterial pressure would be raised from 102 mm Hg to 112 mm Hg (again assuming a venous-to-arterial ratio of compliance of 19). This elevation in P_{mc} allows cardiac output to increase more without producing subatmospheric venous pressures (ie. during exercise). These changes may however be detrimental to a failing heart - the elevated P_{mc} may exacerbate the associated pulmonary congestion. Thus, using this model, one could easily extrapolate the beneficial effects of two traditional therapeutic agents used in the treatment of HF. The administration of diuretics would alleviate the volume

overloaded condition and decrease P_{mc}; and administration of vasodilators (such as OPC-18790) would cause a rightward shift in the venous pressure-volume relation and also effectively decrease the P_{mc}. Furthermore, if the conductance is increased (systemic vascular resistance is decreased) the arteriovenous pressure gradient would decrease,[9] arterial pressure would be decreased and the venous pressure increased (assuming no change in cardiac output or P_{mc}). This effect in itself would tend to increase LV end-diastolic pressure and therefore not be a beneficial effect in the treatment of heart failure. However, in the actual circulation which is dynamic, an increase in venous pressure may also tend to augment the cardiac output (Frank-Starling mechanism). Thus, a decreased SVR would be beneficial. This effect can also be illustrated by using a conceptual model developed by Tyberg[10] in which he has illustrated the coupling between the venous capacitance bed and the heart (fig 2.6).

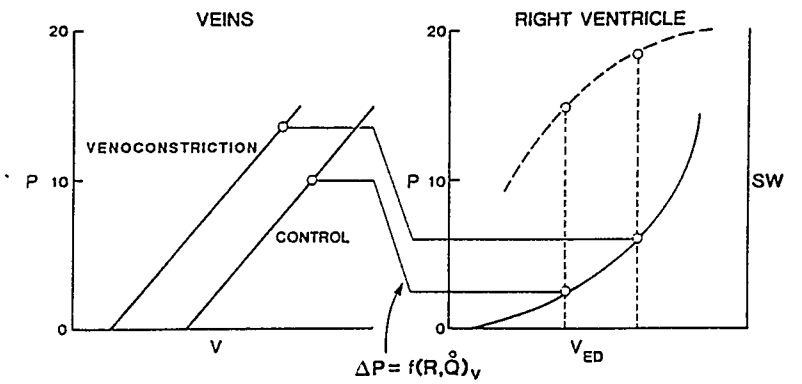

Fig 2.6: Coupling of the Venous Capacitance bed and the Right Ventricle (Reproduced from Tyberg, 1996).[11]

In the control condition, the end-diastolic pressure determines right ventricular end-diastolic volume which defines the level of cardiac output and ventricular stroke work according to the Frank-Starling mechanism (assumptions: the right and left ventricles are coupled and contractility remains constant). With a parallel left-ward shift in the venous pressure-volume relationship, the P_{mc} rises and the pressures rise equally everywhere in the circulation, including the right ventricle. The resultant increase in end-diastolic volume increases the stroke work.

2.5) Conclusion

The importance of studying the effects of venodilation as well as the effects of arteriodilation in the modulation of cardiac function is becoming increasingly apparent. The accomplishments of Guyton laid the groundwork for the role of the vascular system in the control of cardiac output. Levy's work gave an alternative interpretation of the cause and effect relationship of cardiac output and venous pressure. Taking into consideration the results obtained by Levy, Tyberg has gone one step further by creating conceptual models which unify the coupling of the vasculature and heart.

Chapter 3: Anaesthesia

3.1) Introduction

The state of general anaesthesia is a drug-induced absence of perception of all sensations.[12] Although anaesthesia allows us to conduct the necessary surgical procedures, it also alters organ function and therefore may profoundly effect the results of scientific experiments. It is important to have a general understanding of the effects of the anaesthetics used in order to predict how the experimental results may be influenced. Furthermore, because organ alterations are not uniform among all anaesthetics it is important to choose anaesthetics that have minimal influence on the system being studied. The following section will detail the mechanisms of action and cardiovascular effects of the anaesthetics used, and when applicable will give the rationale for the use of a particular anaesthetic. The specific anaesthetics used in this experiment were sodium thiopental, nitrous oxide, and isoflurane.

3.2) Sodium thiopental

Sodium thiopental was introduced as an anaesthetic by Lundy in 1935.[13] It is a barbiturate given as a single intravenous dose that results in unconsciousness in 10 to 20 seconds. The depth of anaesthesia increases for up to 40 seconds and then decreases progressively thereafter. Consciousness returns in 20 to 30 minutes.[12] The drug is initially distributed to the highly

perfused, relatively low volume tissue areas such as the brain and is then quickly redistributed (distribution period plasma half-life = 3 minutes) to other less well perfused lean tissues (muscle). After an initial drop in plasma concentration, further redistribution results in a slower reduction in the plasma concentration which prolongs the duration of action ($t_{1/2\beta}$ = 11.6 ± 6.0 hours). Since thiopental is metabolized slowly in the liver, irreversible removal contributes minimally in the termination of the anaesthetic effects. The effects of small doses of thiopental are terminated by the redistribution to the lean body tissues.[13]

Thiopental likely exerts its anaesthetic effects by activation of the γ-aminobutyric acid (GABA) receptor.[13] Barbiturates bind to their receptors and decrease the rate of dissociation of GABA (the principal inhibitory neurotransmitter in the central nervous system [CNS]) from its receptor. This increases the duration of GABA-activated chloride (Cl_-) ion channel openings, increases Cl^- conductance, and the subsequent hyperpolarization results in inhibition of the post-synaptic neuron. Barbiturates can also directly activate Cl^- channels in the absence of GABA.

Although thiopental is a good anaesthetic agent for rapid induction, it is a poor analgesic agent[12] and inappropriate for maintenance during surgical procedures. For such maintenance, the more potent inhalation anaesthetics

such as isoflurane combined with nitrous oxide are used post-induction.

3.3) Inhalational anaesthetics

A standard comparison for potency of the inhalation anaesthetics is the MAC. "MAC -the minimum alveolar concentration (at 1 atmosphere) of an agent that produces immobility in 50 percent of those subjects exposed to a noxious stimulus."[13] When measured after a short equilibration period, this concentration directly represents the partial pressure of the anaesthetic in the CNS and is independent of the uptake and distribution of the agent to other tissues. Because the concentration of gas is defined as a percentage of one atmosphere, it is independent of barometric pressure and elevation.

It is thought that all inhaled anaesthetics have a common mode of action on a specific molecular hydrophobic region in the CNS.[13] Anaesthetic agents may expand the volume of the hydrophobic region beyond a critical amount and produce anaesthesia by obstructing ion channels or by altering the electrical properties of neurons. The suggested sites of anaesthetic action in the CNS include the brain stem reticular formation (plays a role in altering the state of alertness and in regulating motor activity), cerebral cortex, olfactory cortex, and hippocampus. The anaesthetic action usually depresses excitability of the brain neurons. Excitatory postsynaptic potentials in the ventral root are also depressed.

3.31) Nitrous oxide (N_2O)

Nitrous oxide was discovered in 1772 by Priestley; Davy observed that it had narcotic properties in 1799; and it was used clinically in man in 1844 by Wells.[14] N_2O is probably not metabolized by human tissue but can be reduced in vitro to molecular nitrogen (N_2) by rat and human intestinal bacteria.[13] N_2O is primarily eliminated in the expired gas, and a small amount diffuses out through the skin.[12] A normal adult breathing 70% N2O will achieve 90% equilibration in about 15 minutes. The MAC value is about 105%.[12] N_2O has minimal adverse effects on the physiological functions of the CNS, skeletal muscle, liver, kidney, and gastrointestinal systems.[12] Furthermore, nitrous oxide has minimal effects on cardiovascular dynamics in dogs but can depress contractility in humans.[13] Although N_2O is a powerful analgesic agent, its weak anaesthetic effects prevent it from being used as the sole anaesthetic agent during prolonged surgical procedures. Adequate anaesthesia can be obtained with high concentrations of N_2O, however, the risk of hypoxic organ damage is too great.[12]

The obvious question arises as to why use N_2O at all when the more potent halogenated inhalation anaesthetics are used. In the presence of nitrous oxide in oxygen, the concentration of the potent inhalational agents such as isoflurane can be reduced.[12] Smaller doses result in less cardiovascular and circulatory depression. The use of N_2O not only increases the rapidity of uptake

of the potent inhalational agent (the second-gas effect), but it also increases the alveolar concentration of oxygen (concentration effect) and thus minimizes hypoxia.[12]

3.32) Isoflurane

Isoflurane was discovered in 1965 by Terrell, the same year that Krantz and Rudd observed that it had narcotic properties. It was first used clinically in man in 1971 by Dobkin.[14] Although isoflurane produces fluoride and trifluoracetic acid as metabolic end products, only 0.2% of the isoflurane is metabolized. The small quantities of metabolites generated by isoflurane are insufficient to cause cell damage (it is thought that the toxic effects of anaesthetics are due to their metabolites), thus isoflurane is less toxic than halothane or methoxyflurane which are metabolized to a greater extent (35% and 52%, respectively).[12,14] The MAC value of isoflurane is 1.15% in man and 1.28% in dog.[14]

Isoflurane effects many physiological functions including the CNS (increases cerebral blood flow, reduces cerebral metabolism and intracranial pressure), musculoskeletal system (enhances the neuromuscular blocking effects of muscle relaxants and increases muscle blood flow), kidney (reduces renal blood flow, glomerular filtration rate, and urinary flow), and liver and

gastrointestinal tract (blood flow decreases as the systemic arterial pressure decreases).[12]

The effects of isoflurane on the cardiovascular system, of particular importance in this study, are varied and interfere with the responsiveness at nearly all sites of the system. Isoflurane decreases mean arterial pressure, profoundly diminishes systemic arterial resistance, but has little effect on cardiac output. Isoflurane directly depresses myocardial contraction by decreasing free calcium (Ca^{++}) available and altering the sensitivity of regulatory (troponin and tropomyosin) and contractile (actin and myosin cross-bridge interaction) proteins to available Ca^{++}. There is less free Ca^{++} available due to a decrease in slow calcium inward current through calcium channels in the sarcolemma and a decrease accumulation in sarcoplasmic reticulum (SR) Ca^{++} (greater Ca^{++} release from the SR [increased permeability] and/or decreased SR ATPase activity during loading). The net result is a dose-dependent decrease in the peak developed force and maximum rate of rise of the developed force.[13] Isoflurane decreases phenylephrine-induced contraction in isolated rat aortic strips and may cause endothelial-dependent release of endothelium-derived relaxation factor or facilitate its action on vascular smooth muscle.[12] The decrease in arterial pressure produced by isoflurane results almost entirely from a decrease in systemic vascular resistance. In contrast, the hypotension produced by halothane and enflurane results in part or whole as

a consequence of a decrease in cardiac output. Cardiac output is not affected by isoflurane; it is thought that isoflurane may stimulate the sympathetic nervous system (beta stimulation) and oppose any direct depressant action.[15] A second explanation for the maintenance of cardiac output is that isoflurane may more readily depress the brain and therefore spare the heart.[15] However, this explanation predicted from lipid solubilities is relative to halothane or enflurane and dismisses the observed effect of myocardial depression. Also in favor of the former explanation of beta-adrenoceptor stimulation is the increased heart rate obtained with isoflurane. Isoflurane depresses both parasympathetic and sympathetic activity, however, the parasypathetic depression is greater thus leaving the sympathetic activity less restrained.[15] The possibility that the heart rate increase is a baroreceptor-mediated response may also exist but remains controversial. In some studies, the baroreflex arc was blocked by isoflurane.[15]

Although much of the literature describing isoflurane as a vasodilator focuses on the arterial system, the few studies done on the venous system show that isoflurane causes venodilation as well. McCallum et al.[16] showed that isoflurane directly increased the diameter of mesenteric venous capacitance vessels and attenuated reflex responses of mesenteric veins to activation of the carotid sinus and aortic baroreceptors in intact rabbits. They concluded that isoflurane may influence mesenteric vascular capacitance

directly and through inhibition of the active sympathetic control of the veins, rather than through a passive reduction of arterial inflow into the mesenteric circulation, or through a passive redistribution of blood volume between the vascular beds. Although they made a distinction between active and passive changes, they did not directly measure the influence of active venoconstriction on both unstressed and stressed volume. However, others have shown that the slope of the venous pressure-volume relationship remains fairly constant while unstressed volume changes in response to sympathetic activity.[17]

3.4) Conclusion

The cardiovascular effects of anaesthetics are well known and could potentially influence experimental results. Having a general understanding of how the anaesthetics work not only allows us to use them in a manner that will hopefully limit their adverse effects (combining N_2O and isoflurane), but also allows us to better predict what the influence on the results may be.

Chapter 4: Phosphodiesterase Inhibition

4.1) Introduction

The pharmacological regulation of the degradation of cyclic AMP (cAMP) has only began to emerge in the last few decades. cAMP was discovered as a second messenger in the 1960's. Soon after, the multiple molecular forms of phosphodiesterase ([PDE] the enzyme which catalyses the hydrolysis of cAMP to 5'-AMP) were discovered.[18] Since then, many phosphodiesterase inhibitors have been characterized. Only recently has their value as important pharmacological agents in the treatment of congestive heart failure been recognized. The mechanism of action of PDE inhibitors becomes apparent when reviewing their role in the regulation of cAMP.

4.2) cAMP Regulation

The formation of cAMP from ATP is catalyzed by the adenylate cyclase enzyme (fig 4.1). The second messenger cAMP then activates cAMP-dependent protein kinase (PKA) which results in the phosphorylation of intracellular proteins on serine/threonine residues. As a result of cAMP production, specific responses are elicited (discussed below). PDE catalyses the degradation of cAMP into AMP. If PDE is inhibited (ie. by OPC-18790), less cAMP is degraded and there is a resultant increase in the cAMP-dependent responses. A similar pathway is involved in the regulation of cGMP.

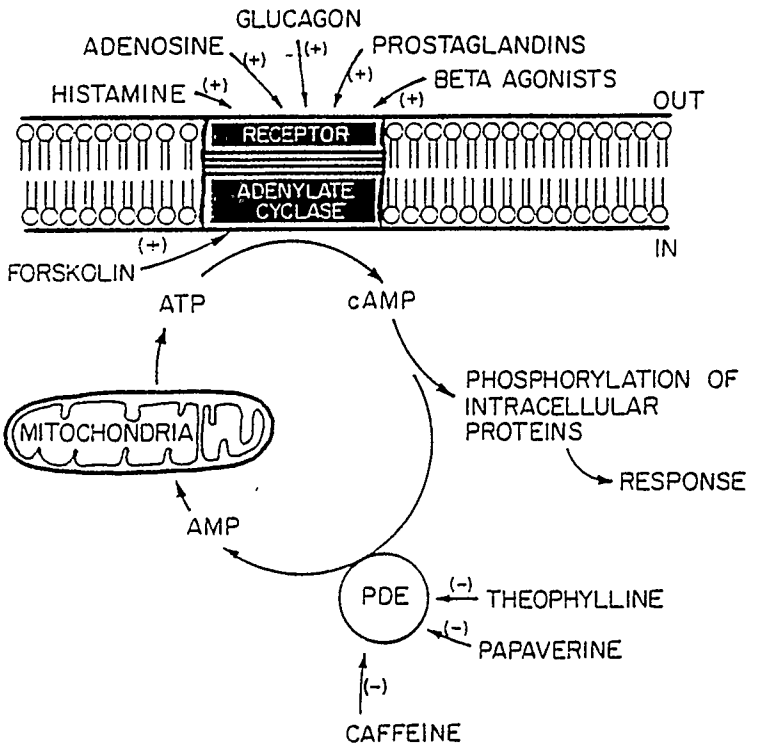

Fig 4.1: Classical model for the involvement of phosphodiesterase in the regulation of intracellular cyclic AMP (Reproduced from Weishaars, 1987).[18]

Although the above pathway depicts a single PDE enzyme regulating cAMP, there are actually many forms of this enzyme. There are three major categories of phosphodiesterase isoenzymes that primarily vary in substrate specificity and kinetic characteristics.

4.3) Phosphodiesterase Isoenzymes

4.31) PDE I

The PDE I isoenzyme has similar high affinities for hydrolysis of both

cAMP and cyclic GMP (cGMP) ($K_m = 1.0$ and 0.8 μM respectively).[19] The K_m values are those characterized for guinea-pig hearts. Because this isoenzyme is Ca^{++}/calmodulin stimulated, it is often referred to as the calmodulin-stimulated phosphodiesterase (CaM-PDE). Although the PDE I isoenzyme can be further divided into 3 subclasses, the characterization of these subclasses is fairly new and rather incomplete. It is not yet known whether these subclasses represent functionally distinct enzymes or subunits of a single enzyme.[18]

4.32) PDE II

The PDE II isoenzyme has similar low affinities for cAMP and cGMP ($K_m = 13.3$ and 26.4 μM respectively).[19] Because this isoenzyme is stimulated by low concentrations of cGMP, many investigators refer to it as the cyclic GMP-stimulated phosphodiesterase (cGS-PDE).[18]

4.33) PDE III

The PDE III isoenzyme has a high affinity for and is very selective for cAMP ($K_m = 0.4$ μM).[19] Because it is inhibited by low concentrations of cGMP, it is often referred to as the cyclic GMP-inhibited phosphodiesterase (cGI-PDE). Subclasses of the PDE III isoenzyme were identified in 1984; one subclass is potently inhibited by cGMP, the other is insensitive to cGMP inhibition.[18]

Other investigators have recently established another class (PDE IV) in addition to the above isoenzymes.[20] They call this class cAMP-PDE of which there is an α and β form. Both forms are selective for cAMP ($K_{m\alpha}=1.8\ \mu M$, $K_{m\beta}=1.3\ \mu M$), and are fairly insensitive to cGMP inhibition. A fifth class has also been reported (PDE V) which is cGMP-specific.[21] In addition, several more subclasses of each isoenzyme have been identified which are differentially expressed and regulated in different cell types.[21]

4.4) Selectivity of Phosphodiesterase Inhibitors

Although nonspecific PDE inhibition with isobutylmethylxanthine (IBMX), for example, can increase the levels of cGMP as well as cAMP, OPC-18790 was found to be selective for PDE III (cGI-PDE, table 1), and thus selective for cAMP hydrolysis.

Table 4.0. Effects of OPC-18790, amrinone, milrinone and 3-isobutyl-1-methyl-xanthine (IBMX) on peak III and peak I phosphodiesterase activities in the canine cardiac muscles (Hosokawa T. et al., 1992)[22]

Compound	Peak III-PDE IC_{50} values (μM)	Peak I-PDE IC_{50} values (μM)
OPC-18790	4.1	100
Amrinone	7.4	100
Milrinone	0.08	100
IBMX	2.8	3.3

The IC_{50} value is defined as the concentration of drug required for 50% inhibition of the enzymatic activity. The fact that different PDE inhibitors are isoenzyme selective is important to mention due to the fact that they will have different cardiotonic effects as a result. For example, it is known that potent inotropic effects are associated with inhibition of the PDE III isoenzyme. However, it is still not known what the cardiotonic effects of inhibiting PDE I,II,IV, and V are. Zaprinast, a specific PDE V inhibitor, has only weak positive inotropic effects and no chronotropic effects. Rolipram, a PDE IV inhibitor, has been found to have no cardiotonic effects in guinea-pig hearts.[19]

4.5) Effects of cAMP

The major effects of an increased level of cAMP include positive inotropic, lusitropic, chronotropic, dromotropic, and arrhythmogenic effects and vasodilation.

4.51) Positive inotropic effect

cAMP activates PKA which phosphorylates the L-type sarcolemmal calcium channels in the heart. There is a resultant increase in the probability that more of these channels open during each action potential and are available for calcium entry. Therefore, calcium influx and calcium-induced Ca^{2+} release from the sarcoplasmic reticulum (SR) produce an increase in force of

contraction. Calcium binds to troponin (TN) C, inducing a conformational change in tropomyosin (bound to the TN complex by TN-T) which then allows cross-bridge formation between actin and myosin with a resultant increase in developed force.[23,24] Phospholamban is a regulatory protein in the cardiac SR that inhibits Ca^{2+}-stimulated ATPase activity. Phosphorylation of phospholamban by PKA removes this constraint on Ca^{2+}-ATPase; as a consequence, Ca^{2+} uptake into the SR is enhanced (increased rate of relaxation) but more Ca^{2+} can be released from the SR in subsequent beats.

4.52) Positive lusitropic effect

The cAMP-stimulated phosphorylation of phospholamban and subsequent rapid reuptake of calcium from the cytoplasm into the SR results in an enhanced rate of myocardial relaxation. An increased cAMP also promotes phosphorylation of TN-I (the inhibitory subunit). Although the exact physiologic role of troponin I phosphorylation is still unknown, it may act to decrease the affinity of TN-C to calcium. This would further shorten the duration of the relaxation process.[23,24]

4.53) Chronotropic, dromotropic, and arrhythmogenic effects

Depolarization of the sinoatrial node pacemaker cells and conduction through atrioventricular nodal cells depends on calcium channel activity. PKA-

induced phosphorylation of Ca^{2+} channels increases the slow inward calcium current ($I_{Ca\text{-}L}$) which increases the slope of phase 4 depolarization. As a result, heart rate and atrioventricular conduction is increased.

The arrhythmogenic effects of cAMP are a result of cAMP increasing cardiac automaticity and triggered activity.[24] Automaticity, the property of spontaneous impulse formation, results from gradual diastolic depolarization during phase 4 of the action potential (calcium inward current). Thus, increased calcium current as a result of elevated cAMP would also effect automaticity. Triggered activity describes impulse formation that is dependent on afterdepolarizations (oscillations in membrane potential). Delayed afterdepolarizations are spontaneous depolarizations occurring after full repolarization and result primarily from intracellular calcium overload. The intracellular calcium overload causes a cyclic, repetitive release of calcium from the sarcoplasmic reticulum. The calcium released then causes inward movement of sodium (Na^+/Ca^{++} exchange mechanism) and inward current through a calcium-activated cation channel. Accordingly, there is an associated fluctuation in the membrane potential. When the afterdepolarizations are large enough to reach threshold, the initiated action potential is considered triggered.[25,26] The cAMP-induced calcium overload will only occur with high levels of cAMP and are unlikely to result from therapeutic doses of PDE inhibition.

4.54) Vasodilation

cAMP activates PKA in vascular smooth muscle which stimulates a sarcolemmal calcium pump. cAMP may also stimulate the sarcolemmal Na^+/K^+-ATPase with subsequent hyperpolarization and removal of intracellular sodium. Sodium is then driven back into the cell (electrochemical gradient) and a Na^+/Ca^{++} antiport pump couples the efflux of calcium to the influx of sodium. In addition, high intracellular levels of cAMP can activate the cGMP-activated protein kinase (PKG). Activation of PKG will produce vasorelaxation by decreased Ca^{2+} influx through voltage-gated (L-type) Ca^{2+} channels as a result of hyperpolarization produced by K^+ channel activation. PKG activation also increases Ca^{2+} sequestration in the SR because of phospholamban phosphorylation.[27] These effects will result in decreased intracellular calcium levels and mediate vascular smooth muscle relaxation.[24] Thus, in contrast to myocardial cells where cAMP promotes an increase in $[Ca^{2+}]_i$, cAMP produces a decrease in $[Ca^{2+}]_i$ in vascular smooth muscle.

4.6) Conclusion

In conclusion, OPC-18790 is a potent, selective PDE III inhibitor which may achieve its noted effects primarily by increasing cAMP levels.

Chapter 5: Vascular Effects of OPC-18790 on Vascular Capacitance and Conductance

5.1) Abstract

Background: OPC-18790 (OPC), a phosphodiesterase-III inhibitor similar to vesnarinone, enhances cardiac contractility and is an arterial dilator. However, its effects on the venous system have not yet been clarified. Because OPC administration reduces left ventricular (LV) end-diastolic pressure, we hypothesized that it is also a venodilator. Because of the known arterial effects and the hypothesized venous effects, we compared changes in systemic vascular conductance (the inverse of resistance) to changes in venous capacitance.

Methods and Results: In 12 (7 treatment, 5 control) anaesthetized, splenectomized dogs, pressures were measured in the right atrium, aorta, portal vein, and LV. A cuff constrictor was placed around the portal vein. Cardiac output was measured by thermodilution and splanchnic venous capacitance was measured by a blood-pool scintigraphic method. Data were collected at baseline, after induction of heart failure (microsphere embolization into the left coronary artery), and then after OPC boluses of 0.1, 0.2, 0.4, and 0.8 mg/kg. Heart failure was associated with decreased capacitance and conductance (to $90 \pm 3\%$ and $64 \pm 6\%$ of baseline values, respectively, $p < 0.05$). After administration of the lower doses of OPC, capacitance increased more than conductance; however, the effects were more balanced at the higher doses. Compared to equihypotensive doses of nitroglycerin, hydralazine and enalaprilat

(results of an earlier study) in the same model, OPC increased capacitance to the same degree as nitroglycerin, increased conductance similarly to hydralazine, and both effects were greater than those obtained with enalaprilat.

Conclusions: OPC is a potent balanced venous and arterial dilator in experimental acute heart failure. These prominent effects suggest that it may prove to be a clinically important alternative to other vasodilators.

5.2) Introduction

OPC-18790 [(±)-6-[3-(3,4-dimethoxy)benzylamino-2-hydroxy]-propyl-2(1H)-quinolinone] (Otsuka Pharmaceuticals Co. Ltd., Tokushima, Japan) (OPC), a phosphodiesterase III inhibitor,[22] has been shown to increase contractility and cardiac output, with only slight changes in heart rate and mean blood pressure in both experimental[22] and clinical[28-31] studies. Although OPC is known to be a vasodilator,[28-30] previous studies have focused on arterial dilation with little being known about its venous effects. Our current understanding of its effects on veins is based on indirect hemodynamic measurements. LV end-diastolic volume decreased substantially when a low dose (5 mg/kg/min) of OPC was given to patients with dilated cardiomyopathy; a higher dose (10 mg/kg/min) reduced systemic vascular resistance and mean and systolic arterial pressures.[28] To our knowledge, no direct assessment of the effects of OPC on venous capacitance has been reported. Since OPC decreases LV end-diastolic

pressure in heart failure, we hypothesized that it may have important venodilator properties.

We used a previously described[32] experimental model to study the effects of OPC on splanchnic venous capacitance and systemic vascular conductance (the inverse of systemic vascular resistance) in acute heart failure. Splanchnic venous capacitance was measured using a blood-pool scintigraphic technique.[33] These results were compared to those in our previous report of the vascular effects of other vasodilators in the same model.[34] Our data indicate that OPC has substantial venodilator effects which may have important clinical implications.

5.3) Methods

5.31) Animal Preparation

Adult mongrel dogs (7 experimental, 5 control, weighing 13-21 kg) were initially anaesthetized with sodium thiopental (25 mg/kg IV) (Abbott Laboratories, Montreal, PQ) and intubated. Anaesthesia was maintained by ventilation with a mixture of oxygen and nitrous oxide (30:70) and isoflurane (Anaquest, Mississauga, ON), using a constant-volume respirator (model 607, Harvard Apparatus, Millis, MA). Blood gases and body temperature were maintained at physiological levels throughout the experiment. To ensure

adequate hydration, a 15 ml/kg infusion of 3.3% dextrose in 0.3% NaCl was given 2 hours before the experimental protocol was begun. A splenectomy was performed through a midline abdominal incision to minimize changes in haematocrit. A pneumatic cuff was placed around the portal vein. To measure portal venous pressure, a fluid-filled catheter (exterior diameter 1.5 mm, interior diameter 1.00 mm, Dural Plastics Engineering, Dural, NSW) was introduced into an arcade branch of the portal vein and positioned so that the tip lay just upstream from the pneumatic cuff. To correct for radioactivity from the ventral abdominal wall, a 4x4-cm sheet of radiographic apron material was fixed to the ventral surface of the liver (Vetbond, 3M Animal Care Products, St. Paul, MN) just under the ventral abdominal wall.[33] The abdominal wall was reapproximated using towel clips. Fluid-filled catheters (7F, Abbott, North Chicago, IL) were inserted into the proximal aorta through the right femoral artery and right atrium through the jugular vein to measure aortic and right atrial pressures, respectively. LV pressure was measured with an 8F micromanometer-tipped catheter (model SPC-485A, Millar Instruments, Houston, TX) introduced through the left carotid artery. Cardiac output was measured by thermodilution using a triple-lumen balloon thermister catheter (7F, Abbott, North Chicago, IL) placed in the pulmonary artery via the right internal jugular vein. A catheter for removing reference blood samples for radioactivity analysis was inserted into the right femoral vein. The fluid-filled catheters were connected to pressure transducers (model P23Ib, Stratham-Gould, Oxnard, CA).

The electrocardiogram and pressures were recorded using a multichannel recorder (model VR-16, Electronics for Medicine-Honeywell, White Plains, NY). Hemodynamic data were acquired and analyzed using a custom-designed program (CVSOFT®, Odessa Computer Systems Ltd, Calgary, AB).

OPC was dissolved using sonication in a 1.5×10^{-2} M solution of d,l-lactate (Sigma, St. Louis, MO) in distilled water to yield a final concentration of 10^{-2} M.

5.32) Induction of Heart Failure

Heart failure was induced by repeated microsphere (Dupont, Wilmington, DE, 50 μm diameter, 4 mg/ml) embolization into the left coronary artery through a 5F left Judkins coronary artery catheter as previously described[32] until LV end-diastolic pressure was at least 20 mm Hg or cardiac output was decreased by at least 50% of the baseline value. Induction of failure took 110 ± 24 (SD) minutes.

5.33) Measurement of Splanchnic Blood Volume (SBV)

In-vivo red blood cell labelling was accomplished by injecting 7 mg of stannous pyrophosphate intravenously followed by 20 mCi of ^{99m}Tc pertechnate.[35] This was performed at least 30 minutes before data collection was begun to minimize the amount of unbound radionuclide. Scintigrams were

recorded from 5 cm above the abdomen for 45 seconds with a gamma camera (model DYNA-MO 4, Picker, Northford, CT) interfaced to a nuclear medicine computer system (model DPS-3300, ADAC Laboratories, San Jose, CA). The number of counts per pixel were obtained from manually-defined mesenteric regions of interest (excluding the liver, kidneys, bladder, and large blood vessels). The regions of interest were computer-duplicated and redefined only if the camera or the dog was moved. We made corrections for physical decay (^{99m}Tc half-life, 6.02 hours), "biological decay" (using the count rates of 100 μL reference blood samples taken every 3-5 minutes throughout the experiment) and the contribution of the ventral abdominal wall.[33]

Abdominal scintigrams were obtained at three different portal pressures including baseline (7 ± 2 mm Hg), 13 ± 2 mm Hg, and 18 ± 2 mm Hg, the latter two by inflating the cuff around the portal vein to raise the pressure to the desired level.

5.34) Experimental Protocol

In the OPC treatment group, hemodynamic (heart rate, cardiac output, and aortic, right atrial, LV, and portal venous pressures) and radionuclide data were obtained in duplicate at baseline, after the induction of heart failure, and 5, 10, 15, 20, and 30 minutes after each IV bolus (administered over 1 minute) of OPC (0.1, 0.2, 0.4, 0.8 mg/kg). The same protocol was followed in the control dogs except that only the OPC vehicle (d,l-lactate) was administered,

in the same volume as that which had OPC dissolved in it. Of the data collected following each placebo dose, only the 15- and 30-minute values were analyzed and compared to heart failure values and only the 30-minute values are shown in the table.

5.35) Analysis of Data

Systemic Conductance. Systemic conductance was calculated as the inverse of the systemic vascular resistance (ie. conductance = cardiac output/[mean aortic pressure - mean right atrial pressure]).

Vascular Capacitance. Portal pressure-splanchnic blood volume (PP-SBV) relations, linear fits of the SBV data points measured at the 3 portal pressures, were defined for each set of data (duplicate measurements taken at baseline and after induction of heart failure, and single measurements after administration of each dose of OPC).

Using interpolation, vascular capacitance was defined as the SBV at PP = 7.5 mm Hg. One hundred percent capacitance was defined as the mean of the 2 baseline SBVs and subsequent values were expressed as percentages of that value. Rightward or leftward shifts of the PP-SBV relations respectively reflect increased or decreased capacitance compared to the baseline value. Thus, we measured relative rather than absolute changes in capacitance. The reported values are the maximum changes in capacitance, 5 to 30 minutes after

the administration of the drug.

Comparison with other Vasodilators. OPC doses were compared with equihypotensive doses of nitroglycerin (30-50 μg/kg/min; MW = 227.1 g/mol), hydralazine (1 mg/kg; MW = 196.6 g/mol) and enalaprilat (0.15 mg/kg; MW = 348.4 g/mol) (results of a previous study).

5.36) Statistical Analysis

Mean baseline values were compared to the values obtained after induction of heart failure using the Student's paired t test. OPC values were compared to the control values using repeated-measures, two-way ANOVA and the Student-Neuman-Keuls test. Because of a slight difference in baseline values of cardiac output between the treatment and control groups, analysis was performed using percentage changes from the means of the two baseline values. Absolute values were used for all other data except for venous capacitance which is always relative to baseline. To assess hemodynamic stability after induction of heart failure in the control group, OPC-vehicle data were compared to their heart failure values using repeated-measures, one-way ANOVA and the Student-Neuman-Keuls test. Equality of slope coefficients of the PP-SBV relations were compared using a partial F test for a multiple regression model. Data are expressed as mean ± SEM with statistical significance accepted at the 95% confidence level ($p < 0.05$).

5.4) Results

Of 8 dogs originally in the OPC treatment group, data from one were excluded because they differed from the means by greater than 4 SDs. Of the 7 remaining animals, 2 received only 3 of the 4 doses of OPC (0.1, 0.2, 0.4 mg/kg). Of the 5 control dogs, one was given a lidocaine bolus (1 mg/kg, iv) during heart failure induction to suppress an idioventricular rhythm. The hemodynamic measurements were not different between this and the other 4 control dogs.

TABLE 5. Hemodynamic Data

		baseline	HF	0.1 mg/kg	0.2 mg/kg	0.4 mg/kg	0.8 mg/kg
n		7	7	7	7	7	5
AoP(mm Hg)	OPC	106±5	82±5*	92±4	84±4	77±3	63±6†
	C	112±2	106±5	104±7	98±8	97±1	94±1
HR (beats/min)	OPC	131±3	154±6*	156±8	152±7	154±12	134±9
	C	133±4	148±6*	151±6	151±8	153±1	153±1
RAP (mm Hg)	OPC	2.5±0.4	3.8±0.4*	2.9±0.5	2.8±0.4	2.5±0.5	2.2±0.7†
	C	2.2±0.5	3.7±0.7*	4.2±0.6‡	4.6±0.5‡	5.0±0.6‡	5.2±0.7‡
LVEDP (mm Hg)	OPC	8.7±1.0	22.2±1.1	22.7±0.8	20.0±1.5	17.9±1.9	18.4±2.6
	C	10.9±1.4	24.9±1.1	27.6±1.0	27.7±1.3	28.1±1.4	27.6±1.9
CO (L/min)	OPC	4.1±0.2	2.0±0.2*	2.6±0.2	2.8±0.2	3.2±0.3	3.2±0.1†
	C	5.6±0.6	3.0±0.2*	3.1±0.3	3.0±0.3	3.0±0.4	2.9±0.3
COND (%)	OPC	100	64±6*	78±7	92±10	111±12	139±13†
	C	100	65±5*	63±3	65±5	66±5	69±6
CAP (%)	OPC	100	90±3*	110±6†	116±5†	127±9†	119±9†
	C	100	85±2*	83±3	80±1	90±3	85±1

HF, heart failure; AoP, mean aortic pressure; HR, heart rate; RAP, mean right atrial pressure; LVEDP, LV end-diastolic pressure; CO, cardiac output; COND, conductance; CAP, capacitance; OPC, treatment group; C, control group. Mean±SEM; *$p<.05$ (baseline vs HF values); †$p<.05$ (OPC vs OPC-vehicle values); ‡$p<.05$ (HF vs OPC-vehicle values).

5.41) Hemodynamic Changes due to Heart Failure

As seen in Table 5.0, heart failure was associated with significant increases in heart rate, right atrial and LV end-diastolic pressures, and significant decreases in cardiac output, conductance and capacitance in both the control and treatment groups. The only difference between the two groups was that the decrease in aortic pressure from 112 ± 2 to 106 ± 5 mm Hg was not statistically significant in the control group ($p = 0.42$), and that the decrease from 106 ± 5 to 82 ± 5 mm Hg in the treatment group was significant ($p = 0.03$).

5.42) Hemodynamic Effects of OPC

As seen in Table 5.0, OPC increased cardiac output from 2.0 ± 0.2 to 3.2 ± 0.1 L/min, decreased mean aortic pressure from 82 ± 5 to 63 ± 6 mm Hg, right atrial pressure from 3.8 ± 0.4 to 2.2 ± 0.7 mm Hg, and LV end-diastolic pressure from 22.2 ± 1.1 to 17.9 ± 1.9 mm Hg (all $p < 0.05$, two-way ANOVA). The decrease in heart rate was not statistically significant. In the control group, the only further significant hemodynamic changes after the induction of heart failure were right atrial pressure and LV end-diastolic pressure, which increased from 3.7 ± 0.7 to 5.2 ± 0.7 mm Hg and from 24.9 ± 1.1 to 28.1 ± 1.4 mm Hg, respectively ($p < 0.05$, one-way ANOVA).

5.43) Vascular Capacitance

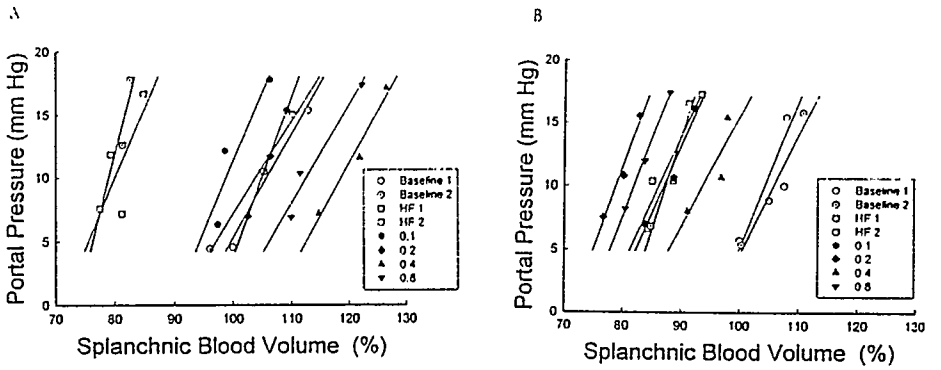

Fig 5.1: Panel A illustrates plots of portal pressure vs splanchnic blood volume relations from a representative experiment showing the effects of heart failure and subsequent administration of OPC. Heart failure (□) was associated with a significant shift of the relations to the left compared to the baseline (○). Subsequent administration of OPC (filled symbols) produced a significant rightward shift from heart failure toward and beyond the baseline position. 0.1, 0.2, 0.4, 0.8 indicate OPC doses (mg/kg).

Panel B shows similar plots from of a representative control experiment showing the effects of heart failure and subsequent administration of the OPC vehicle on the portal pressure-splanchnic blood volume relations. Heart failure was associated with a significant shift of the relations to the left compared to the baseline. Subsequent administration of the OPC vehicle did not produce any further significant shifts. 0.1, 0.2, 0.4, 0.8 indicate equivalent OPC vehicle doses.
HF, heart failure.

As illustrated in Fig 5.1A, heart failure was associated with a parallel leftward shift in the PP-SBV relation (venoconstriction). Subsequent administration of OPC was associated with parallel rightward shifts in the relation (venodilation) beyond baseline values. In the control group (fig 5.1B), there was a similar leftward shift of the curves after the induction of heart failure, but the OPC vehicle had no significant effects on these relations. There were also no significant changes in the slopes in both the OPC treatment

($p = 0.34$) and control ($p = 0.99$) groups. These data are summarized in Fig 5.2.

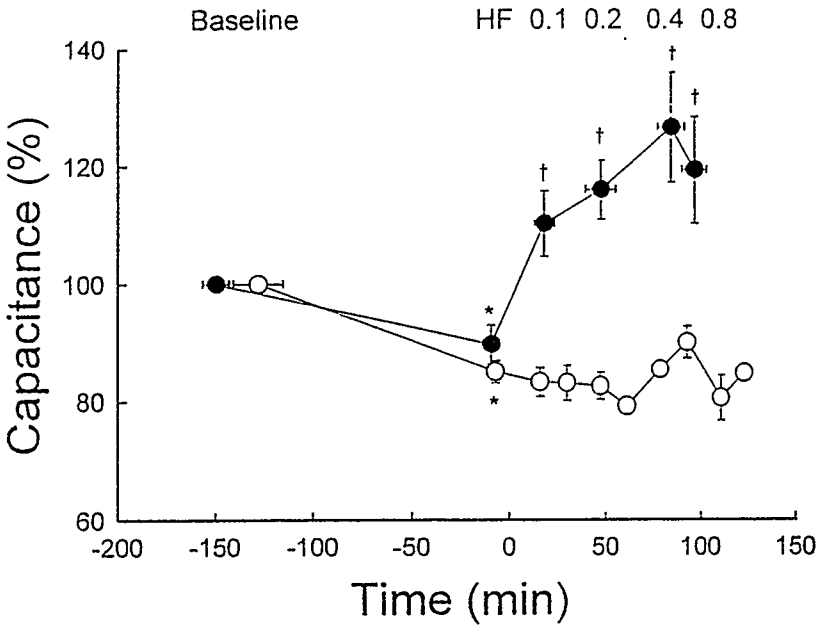

Fig 5.2: Plots of venous capacitance at baseline, after induction of heart failure and after administration of OPC doses (•) or OPC vehicle (○). Heart failure was associated with a significant decrease in capacitance in both the OPC and control experiments. Administration of OPC increased capacitance compared to that after induction of heart failure; in the control experiments, capacitance did not change following development of heart failure after administration of the OPC vehicle. The time axis is defined with respect to the time of administration of the first dose of OPC.
HF, heart failure. *$p < 0.05$ vs baseline; †$p < 0.05$ vs control.

After induction of heart failure, there was a significant decrease in capacitance to $90 \pm 3\%$ compared to baseline ($p = 0.02$). Subsequent OPC administration caused significant increases in capacitance to $110 \pm 6\%$, $116 \pm 5\%$, $127 \pm 9\%$, and $119 \pm 9\%$ for the 0.1, 0.2, 0.4, and 0.8 mg/kg doses, respectively. In the control animals, there were similar changes in capacitance after the induction of heart failure ($85 \pm 2\%$ versus baseline, $p = 0.002$), but in contrast to the treatment group, there were no further significant changes for the duration of the experiment.

5.44) Systemic Conductance

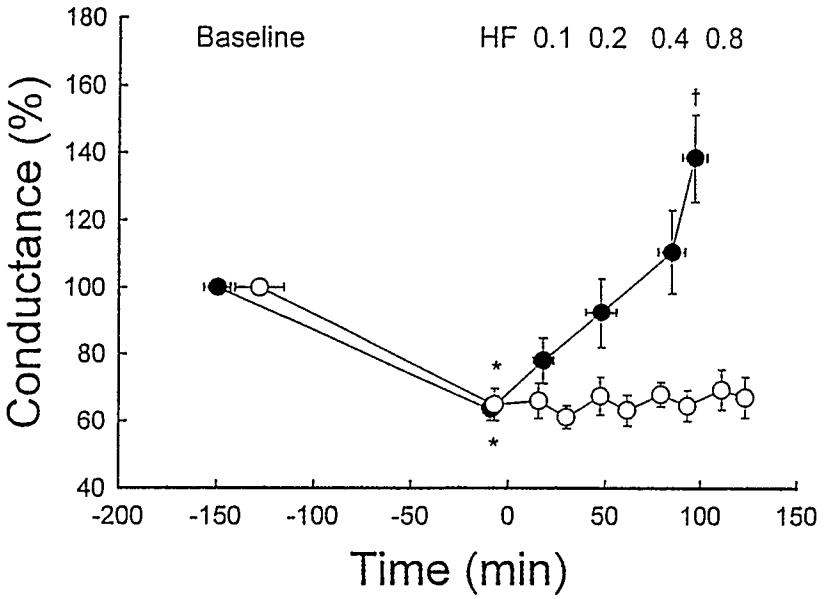

Fig 5.3: Plots of conductance at baseline, after induction of heart failure and after administration of OPC doses (•) or OPC vehicle (○). Heart failure was associated with a significant decrease in conductance in both the OPC and control experiments. Administration of OPC increased conductance compared to that after induction of heart failure; in the control experiments, conductance did not change following development of heart failure after administration of the OPC vehicle. The time axis is defined with respect to the time of administration of the first dose of OPC.
HF, heart failure. *$p<0.05$ vs baseline; †$p<0.05$ vs control.

As illustrated in Fig 5.3, heart failure was associated with a significant decrease in conductance to 64±6% compared to baseline ($p<0.001$). After administration of OPC, conductance increased to 78±7%, 92±10%, 111±12%, and 139±13% for the 0.1, 0.2, 0.4, and 0.8 mg/kg doses, respectively ($p<0.05$ for the 0.8 mg/kg dose). In the control group, heart failure was associated with a similar decrease in conductance (65±5% versus

baseline, p=0.018), but there were no further significant changes during the remainder of the experiment.

In Fig 5.4, the effects of OPC (closed circles) on capacitance and conductance from the present study are compared to the data obtained in a previous study of the effects of hydralazine (H), enalaprilat (E), and nitroglycerin (N) in the same model.[34]

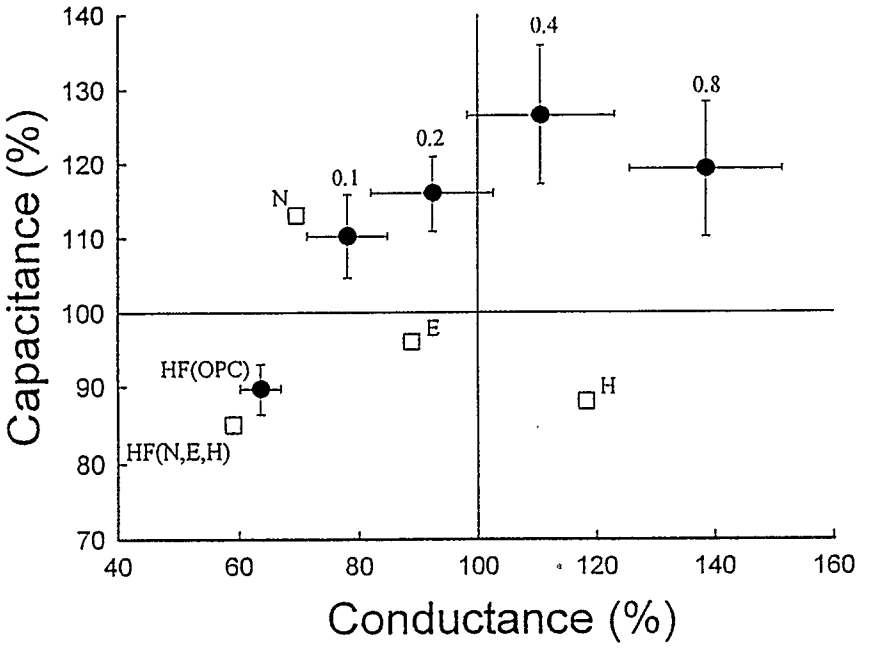

Fig 5.4: Plots of capacitance vs conductance at baseline (100%), after the induction of heart failure (HF) and subsequent administration of OPC (0.1, 0.2, 0.4, 0.8 mg/kg), nitroglycerin (N), hydralazine (H) and enalaprilat (E). Heart failure was associated with significantly decreased capacitance and conductance in all experiments.[8] OPC increased capacitance with little effect on conductance at the lower doses, and had a more balanced dilator effect at the higher doses, increasing both capacitance and conductance. At lower doses, OPC was similar to nitroglycerin and had a greater effect on capacitance than conductance. At higher doses, the balanced effect of OPC was similar to, but greater than, enalaprilat. Also, at higher doses, the effect of OPC on conductance was similar to that of hydralazine.

In all groups of animals, heart failure was associated with a decrease in both capacitance and conductance. Lower doses of OPC (0.1 and 0.2 mg/kg) caused a greater increase in capacitance than conductance. This effect was similar to that obtained with nitroglycerin. At higher doses (0.4 and 0.8 mg/kg), there was a pronounced and more balanced effect which was similar to but greater than that obtained with enalaprilat. Hydralazine increased conductance comparatively to OPC but had no apparent effect on capacitance.

In Fig 5.5, the effects of a single dose of OPC on capacitance and conductance are plotted (n = 1). Heart failure was associated with a decrease in capacitance and conductance. After administration of OPC, capacitance and conductance increased to 106% and 163%, respectively. The effects on capacitance and conductance were diminished but continued for an hour.

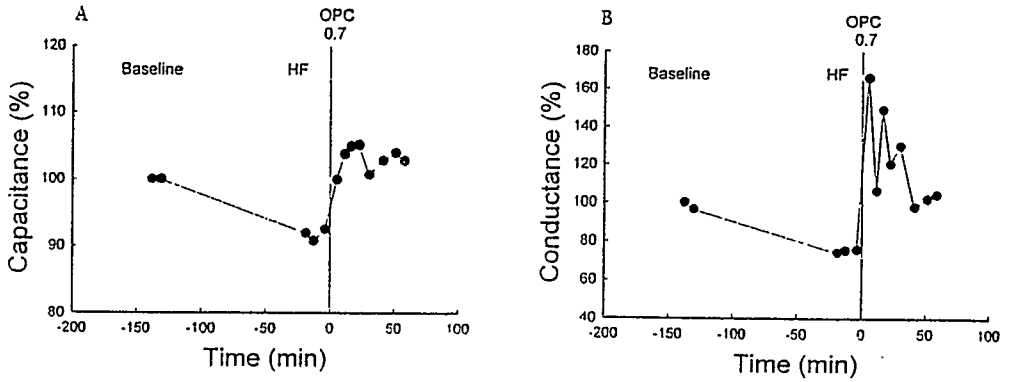

Fig 5.5: Panels A and B illustrate plots of capacitance and conductance, respectively. Data are shown at baseline, after induction of heart failure and after administration of a single dose of OPC (0.7 mg/kg). Heart failure was associated with a decrease in capacitance and conductance. Administration of OPC increased capacitance and conductance compared to that after induction of heart failure and the effects remained present after an hour. The time axis is defined with respect to the time of administration of OPC.
HF, heart failure.

5.5) Discussion

In this study of anaesthetized dogs with acute heart failure caused by coronary embolization, OPC resulted in the expected reduction in right atrial pressure and LV end-diastolic pressure and increased systemic vascular conductance. However, there was also substantial venodilation which appeared to be greater than that observed with equihypotensive doses of nitroglycerin and enalaprilat in a previous study in the same model. Thus, our data clearly demonstrated that the arterial dilator effects of OPC are associated with substantial venodilation. The parallel displacements of the pressure-volume curves (Fig 5.1A) suggest that OPC increased splanchnic venous volume by increasing venous unstressed volume which implies an active reduction in smooth muscle tone.[33,34,36] The increased conductance which resulted from OPC administration clearly reflects reduced arteriolar tone.

The effects of OPC on capacitance and conductance are in keeping with previously reported findings. Thus, both the decrease in LV filling pressure after OPC administration in patients with heart failure[28,30,31,37] and the decrease in right atrial pressure and LV end-diastolic pressure in this study are consistent with venodilation. Increased capacitance and cardiac output tend to decrease LV end-diastolic pressure while decreased systemic vascular resistance tends to increase central venous pressure [ie, raise it toward mean circulatory pressure] and therefore LV end-diastolic pressure.[9] Thus, the venodilation was more than sufficient to negate the tendency of arteriolar dilation to raise filling

pressure. Furthermore, since there was a statistically significant trend for LV end-diastolic pressure to increase in the control animals after the induction of heart failure (implying that the model has some tendency to deteriorate spontaneously), the decrease in LV end-diastolic pressure observed in the treatment group is even more important.

Although OPC dilates both veins and arterioles after the development of heart failure, the relative effects appeared to be dose dependent [see Fig 5.4]. Thus, at lower doses, capacitance increased more than conductance while, at the higher doses, both effects were prominent. These data are consistent with observations in patients with heart failure.[28] Although capacitance was not measured directly, decreases in right atrial pressure, peak pulmonary artery pressure, and LV end-diastolic volume were also more prominent at a lower dose, whereas decreased systemic vascular resistance was most evident with a higher dose. It is possible that the lack of a further increase in capacitance at higher doses may have been due to maximum or near maximum venodilation at the lower dose.[17] Another possible explanation is that the baroreceptor activity was decreased by the fall in aortic blood pressure and caused reflex venoconstriction. However, this mechanism appears unlikely because heart rate did not increase nor did conductance decrease.

Although the precise mechanism of action of OPC has not yet been fully

elucidated, the increase in capacitance and conductance may be partially explained by its phosphodiesterase (PDE) inhibition, it being somewhat selective towards the PDE III isoenzyme (cGI-PDE).[20,22] The accumulation of cyclic AMP which results from PDE III inhibition enhances calcium extrusion across the sarcolemma by two possible mechanisms: i) cyclic AMP-dependant protein kinase present in vascular smooth muscle is known to stimulate the sarcolemmal calcium pump; ii) cyclic AMP stimulation of sarcolemmal Na^+,K^+-ATPase causes hyperpolarization and removal of intracellular sodium. Extracellular sodium then exchanges with intracellular Ca^{2+} (Na^+/Ca^{2+} exchanger) resulting in relaxation of both arterial and venous smooth muscle.[24]

Although PDE III inhibition can explain many of the hemodynamic effects of OPC, the mechanism responsible for the lack of heart rate change is uncertain and discriminates OPC from other PDE III inhibitors and conventional β-agonists.[28] Several investigators have found that OPC prevents an otherwise-expected reflex-mediated increase in heart rate.[28,30,31,38]

5.51) Comparison of OPC with Other Venodilators

We have recently described the different effects of several vasodilators in the same model which provides us with the opportunity to compare the effects of OPC to the previously studied drugs.[34] At equihypotensive doses, the degree of venodilation obtained with OPC is comparable to that caused by

nitroglycerin, the degree of arterial dilation is comparable to that of hydralazine, and the balanced effect is greater than that of enalaprilat. Thus, the substantial venodilating effects of OPC have the potential for being clinically beneficial in treating heart failure, and if vesnarinone has similar effects, may contribute to the demonstrated reduction in mortality by that drug in congestive heart failure.[39] Furthermore, the effects of OPC on increasing capacitance and conductance seem to last at least an hour [see Fig 5.5] which would also be clinically beneficial.

5.52) Consideration of the Model

The model used in the present study has important limitations which need to be considered. Clearly, the anaesthetic itself has the potential of substantially altering the vasculature. Because of the vasodilator effect of the anaesthetic, the potential vasoactive effects of OPC may be even greater when used in conscious subjects with heart failure, given that vasoconstriction may be prominent in this syndrome. As well, acute heart failure induced by microsphere embolization may be quite different in some respects than clinical acute or chronic congestive failure. Despite these considerations, the vasculature responded to induction of failure in the expected fashion and OPC proved to be a potent arterial and venous dilator. Thus, while our findings cannot be assumed to apply to conscious patients with all forms of heart failure, there are clear and prominent effects in our model and there are clinical

reports which suggest that similar effects will be observed in patients. It therefore appears reasonable to do similar studies of OPC in patients with congestive heart failure.

5.6) Conclusions

The hemodynamic effects of OPC were substantial in our model of acute heart failure and included a large increase in capacitance (in addition to the expected increase in conductance), greater than that previously observed with enalaprilat - another balanced vasodilator. These prominent balanced effects suggest that OPC has the potential to be a clinically important drug in the treatment of heart failure. Its characteristics may contribute to both the improved hemodynamics and survival in patients with heart failure who are administered this class of agents.

Chapter 6: Conclusion

OPC-18790, a selective PDE III inhibitor (chapter 4), had significant dilating effects on both the arterial and venous vasculature. Determination of the increase in splanchnic venous capacitance using pressure-volume relations showed that OPC-18790 altered unstressed volume as opposed to stressed volume. The conceptual models discussed in chapter 1, the arterial and venous pressure-volume relations and the coupling of the venous capacitance bed and the right ventricle allow us to better understand the benefits of an increased conductance and capacitance. To recapitulate, a rightward shift in the venous pressure-volume relation effectively decreases the mean circulatory filling pressure and would alleviate the pulmonary congestion often associated with congestive heart failure. Increasing systemic conductance would decrease the arteriovenous pressure gradient, increase venous pressure and therefore augment the cardiac output via the Frank-Starling mechanism. Although the venodilating effects seem to conflict with the augmentation of cardiac output (pericardial effects ignored), it becomes evident that it is important to have a balanced vasodilator such as OPC-18790.

Knowing that the anaesthetics used effect the responsiveness of the cardiovascular system (chapter 3) at many sites in the system potentially interferes with proper analysis of the experimental results. The obvious solution

to this problem is to extend this study to include congestive heart failure patients and thus eliminate the confounding effects of the use of anaesthetics.

References

1. Willius FA, TE Keys: *Classics of Cardiology I*. New York, Dover Publications, Inc., 1941, pp 47

2. Rothe CF: Reflex controls of veins and vascular capacitance. *Physiol Rev* 1983;63:1281-1341

3. Shoukas AA, Sagawa K: Control of total systemic vascular capacity by the carotid sinus baroreptor reflex. *Circ Res* 1973;33:22-33

4. Lautt WW: Resistance or conductance for expression of arterial vascular tone. *Microvasc Res* 1989;37:230-236

5. d'Almeida MS, Lautt WW: Expression of vascular escape: conductance or resistance? *Am J Physiol Heart Circ Physiol* 1992;262:H1191-H1196

6. Guyton AC: Venous Return, in Hamilton WF, Dow P (eds): *Handbook of Physiology: Circulation II*. Washington, D.C., American Physiological Society, 1963, pp 1099-1133

7. Ganong WF: The Heart as a Pump, in *Review of Medical Physiology*. Norwalk, Appleton and Lange, 1989, pp 475-485

8. Guyton AC, Coleman T: , in *Circulatory Physiology: Cardiac Output and its Regulation 2nd edition*. Philadelphia, Saunders, 1973, pp 189

9. Levy MN: The cardiac and vascular factors that determine systemic blood flow. *Circ Res* 1979;44:739-747

10. Tyberg JV: Venous modulation of ventricular preload. *Am Heart J* 1992;123:1098-1104

11. Tyberg JV, Wang SY, Scott-Douglas NW, Robinson VJB, Chihara E, Semeniuk LM, Isaac DL, Belenkie I, Manyari DE: The veins and ventricular preload, in Maruyama Y, Hori M, Janicki JS (eds): *Cardiac-Vascular Remodeling and Functional Interaction*. Tokyo, Springer-Verlag Tokyo, Inc., 1996

12. *Goodman & Gillman's The Pharmacological Basis of Therapeutics*. New York, McGraw Hill, 1996

13. *Anaesthesia*. New York, Churchill Livingston Inc., 1990

14. *Veterinary Anaesthesia*. Philadelphia, Lea & Febiger, 1984

15. Eger EI: The pharmacology of isoflurane. *Br J Anaesth* 1984;56:71S-99S

16. McCallum JB, Stekiel TA, Bosnjak ZJ, Kampine JP: Does isoflurane alter mesenteric venous capacitance in the intact rabbit. *Anesth Analg* 1993;76:1095-1105

17. Shoukas AA, Bohlen HG: Rat venular pressure-diameter relationships are regulated by sympathetic activity. *Am J Physiol Heart Circ Physiol* 1990;259:H674-H680

18. Weishaar RE: Multiple molecular forms of phosphodiesterase: an overview. *J Cyclic Nucleotide Prot Phosph Res* 1987;11(7):463-472

19. Leyen H, Schmitz W, Scholz H, Scholz J: New positive inotropic agents acting by phosphodiesterase inhibition or alpha1-adrenergic stimulation. *Pharm Res* 1989;21(4):329-337

20. Sugioka M, Ito M, Masuoka H, Ichikawa K, Konishi T, Tanaka T, Nakano T: Identification and characterization of isoenzymes of cyclic nucleotide phosphodiesterase in human kidney and heart, and the effects of new cardiotonic agents on these isoenzymes. *Naunyn-Schiedeberg's Arch Pharmacol* 1994;350:284-293

21. Beavo JA, Reifsnyder DH: Primary sequence of cyclic nucleotide phoshodiesterase isozymes and the design of selective inhibitors. *TiPS* 1990;11:150-155

22. Hosokawa T, Mori T, Fujika H, Kinoshita S, Takemoto K, Imaizumi T, Noda T, Ohura M, Tominaga M, Yabuuchi Y: Cardiovascular actions of OPC-18790: a novel positive inotropic agent with little chronotropic action. *Heart Vessels* 1992;7:66-75

23. Colucci WS, Wright RF, Braunwald E: New positive inotropic agents in the treatment of congestive heart failure Mechanisms of action and recent clinical developments. *N Engl J Med* 1986;314:290-299

24. Honerjager P: Pharmacology of positive inotropic phoshodiesterase III inhibitors. *Europ Heart J* 1989;10(Suppl C):25-31

25. Kleber AG: Mechanisms of ventricular arrythmias: a perspective. *J Cardiovasc Pharm* 1991;17(Suppl 6):S1-S8

26. Janse MJ: The premature beat. *Cardiovasc Res* 1992;26:89-100

27. Schmidt HHHW, Lohmann SM, Walter U: The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. *Biochim Biophys Acta* 1993;1178:153-175

28. Feldman MD, Pak PH, Wu CC, Haber HL, Heesch CM, Bergin JD, Powers ER, Cowart TD, Johnson W, Feldman AM, Kass DA: Acute cardiovascular effects of OPC-18790 in patients with congestive heart failure. Time- and dose-dependent analysis based on pressure-volume relations. *Circulation* 1996;93:474-483

29. Hosono H, Abe A, Tanabe N, Takahasi M, Hanawa H, Kodama M, Izumi T, Shibata A, Tsuda T: Acute hemodynamic effects of a newly developed inotropic agent, OPC-18790. *J Mol Cell Cardiol* 1993;25:S.26(Abstract)

30. Mishima M, Hirayama A, Sakai A, Nishida K, Masue T, Nanto S: Beneficial inotropic effects of OPC 18790, a newly synthesized agen (quinolinone derivative), in patients with severe congestive heart failure. *Proc Fifth World Conf Pharmacol Therap* 1992;174(Abstract)

31. Hoit BD, Burwig S, Eppert D, Bhat G, Walsh RA: Effects of a novel inotropic agent (OPC-18790) on systolic and diastolic function in patients with severe heart failure. *Am Heart J* 1994;128:1156-1163

32. Smiseth OA, Mjos OD: A reproducible and stable model of acute ischaemic left ventricular failure in dogs. *Clin Physiol* 1982;2:225-239

33. Scott-Douglas NW, Manyari DE, Smiseth OA, Robinson VJB, Wang SY, Smith ER, Tyberg JV: Measurement of intestinal vascular capacitance in dogs: an application of blood-pool scintigraphy. *J Appl Physiol* 1995;78:232-238

34. Wang SY, Manyari DE, Scott-Douglas NW, Smiseth OA, Smith ER, Tyberg JV: Splanchnic venous pressure-volume relation during experimental acute ischemic heart failure: differential effects of hydralazine, enalaprilat, and nitroglycerin. *Circulation* 1995;91:1205-1212

35. Pavel DG, Zimmer MA, Patterson VN: In vivo labelling of red blood cells with 99mTc: a new approach to blood pool visualization. *J Nucl Med* 1977;18:305-308

36. Manyari DE, Wang Z, Cohen J, Tyberg JV: Assessment of the human splanchnic venous volume-pressure relation using radionuclide plethysmography: Effect of nitroglycerin. *Circulation* 1993;87:1142-1151

37. Cody RJ, Leier CV, Bristow MR, Fifer MA, Binkley PF, Haas GJ, Starling RC, Abraham WT, Lowes BD, Dec GW, Cowart TD: OPC-18790 produces titrable hemodynamic benefit in hospitalized patients with severe congestive heart failure. *Circulation* 1993;88 (Suppl. I):I-300(Abstract)

38. Itoh S, Mori T, Tominaga M, Ishikawa M, Koga K, Yabuuchi Y: Differential effects of OPC-18790, amrinone and dobutamine on cardiac function and energy metoabolism in the guinea-pig isolated ischaemic heart. *Br J Pharmacol* 1995;114:1090-1096

39. Feldman AM, Bristow MR, Parmley WW, Carson PE, Pepine CJ, Gilbert EM, Strobeck JE, Hendrix GH, Powers ER, Bain RP, White BG: Effects of vesnarinone on morbidity and mortality in patients with heart failure. *N Engl J Med* 1993;329:149-155