THE HEMODYNAMIC EFFECTS OF AMINOPHYLLINE, ADENOSINE, LOSARTAN AND NITRIC OXIDE ADMINISTERED SHORTLY AFTER RIGHT HEART INFARCT IN A PORCINE MODEL

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2001

Oulu, Finland

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Abstract

Right heart failure may be caused by several etiologic factors such as pulmonary embolism, post coronary bypass, chronic obstructive pulmonary disease (COPD) and right heart infarction. Traditionally, treatment has consisted of fluid loading (volume expansion) and the use of inotropic agents. In the present series of studies, an experimental model of acute right heart failure was developed using right heart infarct. Treatment with drugs chosen to specifically improve right heart performance was then evaluated. The drugs used in this series were aminophylline, adenosine, nitric oxide (NO) and losartan.

Aminophylline transiently improved cardiac index and pulmonary vascular resistance, but simultaneously caused an increase in heart rate and a decrease in stroke volume. Although it may reduce right heart afterload, aminophylline did not improve overall cardiac function in this experimental model of right heart infarction.

Adenosine affected an increase in cardiac index during the adenosine infusion and in stroke index, while pulmonary vascular resistance and mean pulmonary pressure were decreased. There was a marked decrease in systemic vascular resistance as a result of the drug. Heart rate remained unchanged by the infusion. Discontinuation of the drug resulted in a rapid reversal of the hemodynamic changes. The continuous infusion of adenosine therefore appears to cause an effective arterial vasodilation, with a consequent unloading of right heart afterload.

NO treatment significantly reduced right heart afterload. A significant deterioration was observed in cardiac output as well as in left and right ventricle stroke work indices. The use of NO in this model of right heart infarct affected a decrease in both right heart afterload and left heart preload, with an overall deterioration in global hemodynamics.

Losartan was shown to decrease central venous pressure and wedge pressure, while cardiac output, left ventricle stroke work and stroke volume all showed improvement. Compared to the control animals, pulmonary vascular resistance, systemic vascular resistance and systemic pressures were unaffected by the drug, as was heart rate. An inhibition of angiotensin II action may therefore be of benefit in the treatment of right heart failure symptoms during the first hours after right heart infarct.

Keywords: adenosine, losartan, right heart failure, cardiovascular pharmacology
This book is dedicated to my father and mother.
Acknowledgments

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Kemi, May, 2001

Mike Spalding
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Adenosine receptor</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ACEI</td>
<td>Angiotensin converting enzyme inhibitor</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ARDS</td>
<td>Adult respiratory distress syndrome</td>
</tr>
<tr>
<td>ARF</td>
<td>Acute respiratory failure</td>
</tr>
<tr>
<td>AT</td>
<td>Angiotensin receptor</td>
</tr>
<tr>
<td>B</td>
<td>Bradykinin receptor</td>
</tr>
<tr>
<td>CI</td>
<td>Cardiac output index</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CVP</td>
<td>Central venous pressure</td>
</tr>
<tr>
<td>EDVI</td>
<td>End diastolic volume index</td>
</tr>
<tr>
<td>ESVI</td>
<td>End systolic volume index</td>
</tr>
<tr>
<td>ET</td>
<td>Endothelin</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>LVSWI</td>
<td>Left ventricle stroke work index</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MPAP</td>
<td>Mean pulmonary artery pressure</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary capillary wedge pressure</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PVRI</td>
<td>Pulmonary vascular resistance index</td>
</tr>
<tr>
<td>REF</td>
<td>Right ventricle ejection fraction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>RV</td>
<td>Right ventricle</td>
</tr>
<tr>
<td>RVSWI</td>
<td>Right ventricle stroke work index</td>
</tr>
<tr>
<td>SI</td>
<td>Stroke index</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke volume</td>
</tr>
<tr>
<td>SVRI</td>
<td>Systemic vascular resistance index</td>
</tr>
</tbody>
</table>
List of original publications

This thesis was written based on the following original articles. These are referred to in the text by their corresponding Roman numerals.


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1 Introduction

Right heart failure is a clinically important pathological process resulting from one or more of various cardiovascular and/or pulmonary diseases. Failure will present with gradually decreasing cardiac performance accompanied with increases in pulmonary vascular pressure and its concurrent development of pulmonary dysfunction.

Right heart failure may have various etiologies, the more common being failure due to chronically increased pulmonary pressures. These in turn may be pulmonary venous hypertension-caused due to cardiovascular disease (e.g. rheumatic mitral valve stenosis (Setaro et al. 1992)) or pulmonary artery hypertension caused due to pulmonary diseases (e.g. chronic obstructive pulmonary disease (Matthay et al. 1982, Matthay & Mahler 1986, Matthay 1987)). Common to these mechanisms for right heart failure, however, is that they are all chronic processes which will only result in changes in the right heart over an extended period of time. The most common cause of right heart failure is likely left side failure (Thompson & White 1936), which in turn will most probably be due to myocardial infarct.

Right heart infarct, on the other hand, is a process which leads to both an acute decrease in right heart function, as well as chronic compensatory anatomical remodelling. Myocardial infarct of the right ventricle will be at its purest a blockage of branches of the right coronary artery, which will cause little or no direct left ventricle involvement. Occlusion of branches of the anterior interventricular branch of the left coronary will also cause infarction of septal areas of the right ventricle, but this will cause more left ventricle involvement as well.

Another clinically relevant cause of acute right heart failure is pulmonary embolism, which will present with increases in pulmonary vascular resistance and right heart dilation and subsequent impairment of left ventricle function (Lualdi & Goldhaber 1995). In addition, acute right heart failure may be observed after open-heart surgery.

Typical findings in right heart failure are dilation of the right ventricle with a subsequent shift of the intraventricular septum to the left. The pericardial space is reduced and symptoms of tamponade may be evident (Squara et al. 1993). Pulmonary vascular resistance will increase as will mean pulmonary arterial pressure without a concomitant
rise in wedge pressure. Finally, right ventricle stroke volume and cardiac output will be decreased. Treatment of these symptoms of right heart failure is a clinically challenging proposition due to the anatomical and functional differences between this form of heart failure and the more common form of left heart failure. The more salient points of difference between these two distinct processes are the differences in vascular resistance between the pulmonary and systemic circulations and the differences in the muscle mass of the right and left ventricles.

The administration of volume expansion has been prominent in the treatment of right heart failure (Squara et al. 1993), based on its effect on global heart function. Stroke volume - and consequently cardiac output - is increased, as is the mean arterial pressure. According to the Frank-Starling mechanism, optimal ventricular performance can only be assured in the presence of a sufficient preload. This will then stretch myocardial myocytes to their optimal preload pressure, allowing them to contract with maximum force. This results in an improvement in both the systemic and pulmonary circulation. Overloading the right ventricle, however, will cause an increase in ventricular dilation and an attenuation of flow forward through the pulmonary circulation, further impairing filling of the left ventricle and decreasing cardiac output (Romand et al. 1995). Ideally, treatment of right heart failure should be focused on specifically improving right heart function, as well as on treating the cause of the failure if possible. In addition to preload optimization, treatment of right heart failure consists of the use of inotropic agents such as noradrenaline and dobutamine (Squara et al. 1993).

Doyle and associates (1995) summarized the properties desirable in an inotropic drug. According to their report, the drug should increase cardiac output through an increase in stroke volume without increasing heart rate. It should be lusitropic and be vasodilative. It should lower both left and right ventricle afterload through the vasodilation of systemic and pulmonary vascular resistance. Although this may be desirable up to a point, it should be kept in mind that lowering systemic vascular resistance will lead to decreases in systemic pressures and hence reduced tissue perfusion, possibly causing problems in ischemia-prone organs, such as the kidneys and the gastrointestinal tract. Another related problem is that systemic vasodilation will reduce right heart preload somewhat with a subsequent attenuation of cardiac output. Therefore, systemic vasodilation should be avoided in the treatment of heart failure, while pulmonary vasodilation is desirable. To this end, infusable drugs with a very short half-life might be more preferable due to their higher selectivity for the pulmonary circulation. Of the drugs currently available for clinical usage in the treatment of right heart failure, no single medication fulfills all of the above criteria.

The present study was undertaken to elucidate the effects of various treatment regimes on right heart contractility and afterload, as well as on global hemodynamics in general. An experimental model of right heart failure involving right heart infarct in young pigs was developed. Utilizing this model, the effects of intravenous aminophylline (I), adenosine (II), losartan (III), and of inhaled nitric oxide (IV) in fluid-optimized, experimental cases of right-heart failure were examined.
2 Review of the literature

2.1 The anatomy and physiology of the right heart

Although traditionally viewed as being a passive conduit for blood passage between the systemic and pulmonary circulation (Starr et al. 1943), the right ventricle has more recently been shown to play an active role in systemic hemodynamics (Cohn et al. 1974). Many studies since then have shown a decisive role of the right ventricle in overall hemodynamics, which has been especially apparent in cases of right heart failure (e.g. Fisk & Guilbeau 1987, Wingate et al. 1991, Goldstein et al. 1983, Sibbald & Driedger 1983).

Table 1. Morphological considerations of the human right heart (Ganong 1993, Frick et al. 1994).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Right ventricle</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricle volume</td>
<td>Approximately 130 ml at rest</td>
<td>70-90 ml (at rest)</td>
</tr>
<tr>
<td>Stroke volume</td>
<td>15-30 mmHg systole</td>
<td>90-140 mmHg systole</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>Approximately 65% at rest</td>
<td>Approximately 75-85% at rest</td>
</tr>
<tr>
<td>Ventricle pressure</td>
<td>0-8 mmHg diastole</td>
<td>4-12 mmHg diastole</td>
</tr>
</tbody>
</table>

2.1.1 Morphology

Anatomically, the right ventricle can be divided into an inflow area (including the tricuspid valve), a central chamber and an outflow area (including the pulmonary valve) (Farb et al. 1992). This tends to present as more of a sac-shaped formation than as a symmetrical chamber (see Figure 1).
2.1.2 Vascularisation

The myocardium of the right heart receives its blood flow from the right main coronary, the circumflex and the anterior descending (LAD) coronary arteries. The latter two mainly feed the posterior wall (circumflex) and the interventricular septum (circumflex and LAD). The right main coronary is responsible for blood flow to the free wall of the right ventricle (Farb et al. 1992). The right coronary also gives off a small branch which supplies blood flow to the sinoatrial node (see Figure 2).
2.1.3 Autonomic nervous system and the heart

Activation of the sympathetic part of the autonomic nervous system has both positive inotropic and chronotropic effects (Yousef et al. 2000). After infarct, sympathetic activity will lead to ventricular hypertrophy and remodelling of the ventricle itself (Sugden 1999) through activation of the renin-angiotensin system.

2.1.4 Physiology in the healthy state

The right ventricle (RV) is coupled in series with the left ventricle (LV) and ejects its stroke volume (SV) into the pulmonary circulation (Lee 1992). Under steady-state conditions the SV of both ventricles is the same, but the RV ejects its volume into a low-resistance system with a large degree of compliance (Pinsky 1993). Due to its thin freewall, the RV is weak compared to the LV, but is well adapted to wide variations in preload (i.e. compliance is good). Due to its weaker nature and crescent shape, however, the RV cannot adapt to acute increases in pulmonary pressure (i.e. increased afterload), which is thus one
of the major causes of right heart failure (Lee 1992). Early researchers examining the function of the right ventricle hypothesized that it plays only a minimal role in the circulation and that even its total dysfunction will be well tolerated (Starr et al. 1943, Bakos 1950). Not until later were the problems of right heart infarction (Cohn et al. 1974) and right heart failure first begun to be recognized (Doyle et al. 1995).

2.1.4.1 Determinants of right heart function

The functionality of the right heart is governed by various factors, the most important of which are preload, afterload, muscle compliance and muscle contractility. These in turn are affected by previous illness, such as myocardial infarction, pulmonary hypertension or pulmonary disease (e.g. COPD), oxygen availability and myocardial thickness.

Preload is defined as the force stretching the myocardium in a resting state to its precontractile length. This is an experimentally derived figure and can best be demonstrated using a model involving an isolated papillary muscle stretched to its precontractile length by a weight attached to a lever. The weight necessary to stretch the muscle to this length is therefore representative of preload. After this, the lever is prevented of stretching the muscle any further by placing a stop above it, and additional weight is added. When stimulated, the muscle can now contract only when it generates sufficient force to overcome both the preload weight and the additional weight, which is representative of afterload (Freeman et al. 1994). As muscle length can not effectively be measured in vivo, an estimation must be made based on other measurements (Civetta 1999). A good estimate of these models can be obtained by translating them to the force stretching (filling) the myocardium during diastole (preload) and the force against which the myocardium must contract during systole (afterload). In a clinical situation, right ventricle preload is determined by venous return and the distensibility of the RVs thin myocardial walls. Right ventricle contractility depends on the degree to which the RV walls are stretched during the diastole (preload). This in turn defines the ejection fraction and the final right ventricle stroke volume (RVSV), which are important measures of RV efficiency.

2.1.4.2 The Frank-Starling mechanism

The Frank Starling mechanism states that the energy of contraction is proportional to the initial length of the cardiac muscle fiber (Patterson et al. 1914). In practice, this translates into a curvilinear function (see figure below) as cardiac output will at first respond quickly to increases in preload, but as the myocardium is stretched further and further, the response will level off.
Fig. 3. The Frank-Starling relationship

In the normal state, the RV compensates for changes in preload through compliance, which is the stretching of the muscle wall without loss of muscle contractility. A permanent increase in afterload, however, can only be compensated for through an increase in contractility achieved through hypertrophy of the muscle, which will eventually lead to a decrease in compliance. Hypertrophy will also cause an imbalance in the muscle between oxygen delivery and consumption, causing a decrease in contractility. Both of these factors contribute to RV failure.

2.2 Neurohumoral factors governing the cardiovascular system

2.2.1 Angiotensin II

The best-known tissues affected by angiotensin II and the physiologic actions of the hormone in those tissues are listed in Table 2.
Table 2. Actions of Angiotensin II (adapted from Goodfriend et al 1996)

<table>
<thead>
<tr>
<th>Tissue affected</th>
<th>Action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery</td>
<td>Contraction, growth</td>
<td>(Griffin et al. 1991)</td>
</tr>
<tr>
<td>Adrenal zona glomerulosa</td>
<td>Aldosterone secretion</td>
<td>(Laragh et al. 1960)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Inhibits release of renin</td>
<td>(Menard et al. 1991)</td>
</tr>
<tr>
<td></td>
<td>Increases reabsorption of Na+</td>
<td>(Mitchell et al. 1992)</td>
</tr>
<tr>
<td></td>
<td>Stimulates vasoconstriction</td>
<td>(Mitchell et al. 1992, Hall &amp;</td>
</tr>
<tr>
<td></td>
<td>Release of prostaglandin</td>
<td>Granger 1983)</td>
</tr>
<tr>
<td></td>
<td>Affect on embryogenesis</td>
<td>(McGiff et al. 1970)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Aguilera et al. 1994)</td>
</tr>
<tr>
<td>Brain</td>
<td>Stimulates thirst</td>
<td>(Saavedra 1992)</td>
</tr>
<tr>
<td>Sympathetic nervous system</td>
<td>Sympathetic outflow</td>
<td>(Saavedra 1992)</td>
</tr>
<tr>
<td>Heart</td>
<td>Improves contractility</td>
<td>(Sadoshima &amp; Izumo 1993,</td>
</tr>
<tr>
<td></td>
<td>Ventricular hypertrophy</td>
<td>Moravec et al. 1990, Kent et al.</td>
</tr>
<tr>
<td></td>
<td>Release of vasopressin</td>
<td>Foucart et al. 1991)</td>
</tr>
</tbody>
</table>

Most of these actions support or increase arterial blood pressure and maintain glomerular filtration. Actions of angiotensin II which might lower blood pressure if they occurred in isolation, such as the stimulation of prostaglandin production, sustain the perfusion of selected vascular beds despite the powerful vasoconstrictor action of angiotensin II.

Vasoconstriction and the release of aldosterone in response to angiotensin II occur in seconds or minutes, in keeping with their roles supporting the circulation after hemorrhage, dehydration, or postural change. Other actions, such as vascular growth, ventricular hypertrophy, and the pressor effect of very low doses of angiotensin II, take days or weeks (Griffin et al. 1991, Dzau et al. 1991, Gorbea-Oppliger et al. 1994). The slower responses are likely to be more important in the pathogenesis of chronic hypertensive cardiovascular diseases.

### 2.2.1.1 Angiotensin Receptors

The receptor subtypes AT₁ and AT₂ are polypeptides containing approximately 360 amino acids (Sasaki et al. 1991, Murphy et al. 1991, Mukoyama et al. 1993). Despite their similar affinities for angiotensin II, AT₁ and AT₂ receptors are functionally distinct, with a genetic sequence homology of only 30 percent (Szpirer et al. 1993, Koike et al. 1994).
Angiotensin receptors of the AT₁ subtype bind angiotensin II in ways characteristic of other hormone receptors on the cell surface: their structural specificity is high; they have limited binding capacity; they bind angiotensin II with an affinity similar to its circulating concentration, about $10^{-9}$ M; they convert the interaction with angiotensin II into cellular responses; and they are regulated by their rates of biosynthesis and recycling (Goodfriend et al. 1971, Lin & Goodfriend 1970, Carini et al. 1991).

Specific, high-affinity binding of angiotensin II is determined by amino acids located on or near the extracellular surface of the membrane-bound receptor, as well as by sequences in the transmembrane domains (Hjorth et al. 1994). These regions are probably adjacent to one another in the native receptor in which two disulfide bonds hold its helical domains together.

### 2.2.1.2 Neurohormonal effects of angiotensin II

The AT₁ subtype is the target of the new receptor antagonists (Goodfriend et al. 1996) such as losartan. The binding of angiotensin II causes vascular changes, renal changes, hormonal changes, as well as modulating activity in both the central and peripheral nervous system (see Table 2).

Angiotensin II directly stimulates AT₁ and AT₂ receptors causing vasoconstriction, sodium and water retention, cellular remodelling, (AT₁ receptors) cell differentiation and tissue repair (AT₂ receptors). In an elegant study differentiating the action of AT₁ and AT₂ receptors, Smits and colleagues blocked AT₁ receptors with losartan and then stimulated AT₂ receptors with an AT₂-specific agonist DPMA. They found that stimulation of the AT₂ receptors caused dose-related vasodilation in anesthetized rats (Smits et al. 1993). The stimulation of AT₂ receptors also exerts an antiproliferative effect (AT₂ receptors), which may be of consequence in angiogenesis (Willenheimer et al. 1999). In addition, angiotensin II potentiates the actions of other neurohormonal systems, contributing to the remodelling process. It facilitates the activation of the sympathetic adrenergic system, thereby enhancing the release of vasopresin/anti-diuretic hormone, aldosterone and endothelin (Jilma et al. 1997). In addition, it triggers the formation of free radicals, which subsequently accelerate the degradation of nitric oxide (Kumar & Das 1993). Concomitantly, it increases the breakdown of bradykinin, which further reduces levels of nitric oxide (Cody 1997).
2.2.2 Nitric oxide

2.2.2.1 Nitric oxide synthases

Nitric oxide is produced by the oxidation of L-arginine by a family of isoenzymes (nitric oxide synthases NOS) that includes two constitutive isoforms, viz., endothelial NOS (eNOS) and neuronal (or brain) NOS (nNOS), as well as an inducible isoform (iNOS) (Bolli et al. 1998). eNOS produces nitric oxide via a complex reaction which is stimulated by calcium and requires other co-factors. Nitric oxide synthase catalyzes the oxidation of the terminal guanidino nitrogen of L-arginine (Steudel et al. 1999). The reaction consumes oxygen and nicotinamide adenine dinucelotide hydrogen phosphate (NADPH) and produces NO. NOS will not produce detectable levels of nitric oxide unless superoxide dismutase is present. During conditions of L-arginine depletion, NOS generates O₂ (Steudel et al. 1999). The characteristics of the different synthases are depicted in Table 3.

Table 3. Adapted from Steudel et al. (1999). NOS = nitric oxide synthase

<table>
<thead>
<tr>
<th>NOS</th>
<th>Tissues</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal NOS</td>
<td>CNS, peripheral nerve system</td>
<td>Brain development, memory, behavior, pain perception, vasodilation of large cerebral vessels. Regulates smooth muscle relaxation in gastrointestinal, urogenital and respiratory tracts. Modulates skeletal muscle function.</td>
</tr>
<tr>
<td>Inducible NOS</td>
<td>Inflammatory cells, local cells during infection</td>
<td>Promotes wound closing, neovascularization, may cause systemic arterial dilation, may cause deterioration of inflammation.</td>
</tr>
<tr>
<td>Endothelial NOS</td>
<td>Vascular endothelial cells, fetal lung cells</td>
<td>Modulates systemic and pulmonary vascular tone, roles in lung development and disease. Role in the development of post-ischemic conditioning.</td>
</tr>
</tbody>
</table>
2.2.2.2 Metabolism of nitric oxide

Once formed, nitric oxide has a short half-life \textit{in vivo} of a few seconds and is inactivated by its target molecules. The main metabolic pathways are the binding of nitric oxide to O$_2$ and to hemoglobin. In a study examining the uptake of inhaled nitric oxide (Young \textit{et al.} 1996) it was shown that 90\% is absorbed during inhalation. Almost 70\% of this appears within 48 hours in the urine as NO$_3^-$, The remainder is excreted as NO$_2^-$ in the oral cavity, some of which is further converted to nitrogen gas in the stomach and some to ammonia in the intestine (Steudel \textit{et al.} 1999).

2.2.2.3 Protection against ischemic injury

A well-documented effect of nitric oxide is its role in preconditioning against mild ischemia (Bolli \textit{et al.} 1998). This takes place in two phases, generally known as "early" and "late" preconditioning. In their extensive studies on the subject, Bolli and coworkers (1998) have elucidated the method through which this protective mechanism is triggered. In early preconditioning, a brief ischemic stress causes a burst of nitric oxide production via eNOS. This leads to a cascade chain of events involving tyrosine kinases - and probably other kinases - with the ultimate formation of iNOS and an increased generation of nitric oxide (late preconditioning) during subsequent ischemic challenge. According to this model, nitric oxide plays two completely different roles in preconditioning: during the early stage it initiates the development of the cardioprotective mechanism, whereas during the late stage it protects against myocardial stunning.

2.2.2.4 Direct myocardial effects of nitric oxide

Although nitric oxide will effect hemodynamics through vasodilation, it has also been speculated to have a direct effect on myocyte contractility (Michel & Smith 1993, Brady 1993, Brady \textit{et al.} 1993). Later studies have, however, speculated that the observed effect results from the activation of other mechanisms, and is not related to iNOS (Ungureanu-Longrois \textit{et al.} 1995). Some studies have been reported on the myocardial effects of inhaled nitric oxide (see section 2.4.4.1.).

2.2.3 Adenosine

Adenosine is an endogenous nucleoside occurring in all cells of the body, whose exact chemical formula is 6-amino-9-ß-D-ribofuranosyl-9-H-purine. Endogenous adenosine appears to be produced both intra- and extracellularly through the catabolism of adenine
nucleotides by 5'-nucleotidases (ATP -> ADP -> AMP -> adenosine) (Bukoski et al. 1986, Shryock & Belardinelli 1997). In blood, the half-life of endogenous adenosine is very short, ranging from 1 to a few seconds (Moser et al. 1989). Interestingly, during degradation, endogenous adenosine will more likely be “recycled” back to AMP (through phosphorylation) than deaminated to inosine (Sparks & Bardenheuer 1986). Endogenous adenosine acts as a “retaliatory metabolite” i.e. its activation and effect on adenosine receptors causes an inhibition of the target cell (Shryock & Belardinelli 1997). As an example of this action, adenosine will reduce the pacemaking rate of the sinoatrial cells in the heart, thereby slowing heart rate and reducing cardiac work. As adenosine is produced by its own target cell, adenosine formation therefore both signals an imbalance between tissue oxygen supply and demand and initiates responses to correct the problem (e.g. vasodilation, slowing of heart rate, etc.)(Mullane & Bullough 1995).

Adenosine receptors are present in most cells throughout the body and are divided into four sub-groups: A₁, A₂A, A₂B and A₃ (Ralevic & Burnstock 1998). A₁ receptors are mainly found in the brain and spinal cord, in the testis and in other adipose tissues. They are also found to a lesser extent in many other tissues, including kidney, heart and spleen. Stimulation of A₁ receptors will inhibit adenylyl cyclase, thereby attenuating the action of catecholamines. A₂A receptors are mainly found in the brain, while A₂B receptors appear to be located in the colon, esophagus and gastric antrum. Pharmacological studies indicate that A₂B receptors are likely present in the coronary arteries, but they have not yet been located. The action of A₂B receptors appears to stimulate adenylyl cyclase. In humans, A₃ receptors have been identified in the lung and liver. Preliminary reports on this receptor suggest that A₃ receptors inhibit adenylyl cyclase activity (Strickler et al. 1996).

In their recent review of the clinical implications of adenosine and specific receptor agonists/antagonists, Auchampach and Bolli concluded that the therapeutic exploitation of our current knowledge of adenosine and its receptors will require a more specific tissue targeting of specific adenosine receptor agonists or antagonists. This way, for example, the positive inotropic effects of A₂A receptor antagonists will not be offset by a concomitant blocking of A₂A receptor vasodilative activity in the coronaries (Auchampach & Bolli 1999).

2.2.4 Other neurohumoral factors

2.2.4.1 Endothelins

Endothelins (ET) are peptides produced in many cells and tissues. The vascular ET system is represented mainly by ET-1 produced in endothelial cells. The mature peptide ET-1 acts in a paracrine manner on smooth muscle cell ET(A) and ET(B) receptors to induce contraction and growth, and in an autocrine or paracrine manner on endothelial cells to induce the production of nitric oxide and prostacyclin (Schiffrin & Touyz 1998). ET has also been shown to stimulate the release of atrial natriuretic peptides both in vitro and in
vivo (Stasch et al. 1989). ET receptors may be downregulated by ET, especially under conditions in which large amounts of ET are being produced in the vasculature. This has been demonstrated in some models of experimental hypertension and in some forms of human hypertension (Schiffrin & Touyz 1998). Some of the effects of angiotensin II, particularly growth of the smooth muscle media of blood vessels, have been shown under some conditions to be mediated by ET-1 via ET(A) receptors. In their experimental study in pigs with hypoxic pulmonary hypertension, Liska and coworkers (1995) found that at low doses (10-25 ng/kg/min) endothelin-infusion caused a dose-dependent decrease in pulmonary vascular resistance. At a higher dose (100 ng/kg/min) both systemic and pulmonary vascular resistance was increased, while cardiac output was reduced.

2.2.4.2 Eicosanoids

Eicosanoids are the group of compounds including prostaglandins, thromboxanes and prostacyclin. Prostaglandins (PG) are cyclic compounds, of which PGH is the precursor for PGE and PGF (Moncada et al. 1985). PGA-C is derived from PGE. In addition, thromboxane A (TXA) and prostacyclin (PGI2) are also formed from PGH. PGG is a precursor of PGH.

Eicosanoids participate in the regulation of blood pressure as vascular tone is subject to the continuous relaxing influence from endogenous vasodilating prostaglandins (PG’s A, B, E and F) or related substances such as bradykinin (Moncada et al. 1985). Prostacyclin (PGI2) decreases blood pressure with a concomitant increase in cardiac output and a reduction of systemic vascular resistance related to the peripheral vasodilation. Splanchnic, pulmonary and coronary vasodilation are also observed with increased blood flows in the mesenteric, renal and coronary beds. These changes in regional blood flows have been linked to the inhibition by PGI2 of the vasoconstrictor response to sympathetic stimulation and pressor hormones (noradrenaline, angiotensin II). TXA exerts a strong vasoconstrictive effect (Moncada et al. 1985). It appears that prostaglandins stabilize hemodynamics through an increase in systemic vascular resistance (Vanlersbergh et al. 1992).

Eicosanoids potentiate the cardiovascular effects of bradykinin and are in fact generated by bradykinin through the formation of arachidonic acid. In vascular tissue, most of the available arachidonic acid is converted into vasodilator prostaglandins, i.e., prostacyclin (PGI2) and prostaglandin E2 (PGE2). These prostaglandins are involved in vasodilator actions of the kinins. The biological significance of kinin-related prostaglandin formation becomes apparent after inhibition of kinin breakdown by ACE inhibitors. These compounds prevent the generation of vasoconstrictor angiotensin II and stimulate endothelial eicosanoid formation via local kinin accumulation. There is evidence suggesting that kinin-induced prostaglandin generation contributes to the anti-ischemic, inotropic, and blood pressure-lowering effects of the compounds (Schror 1992).

PG’s and TXA also participate in platelet release and aggregation in injury (Moncada et al. 1985). Interestingly, PGE and PGD are inhibitors of platelet aggregation, while TXA2 is a potent inducer of aggregation. PGI2 is also a potent inhibitor of platelet activity.
2.2.4.3 Kallikrein-kinin system

Kinins are peptides synthesized *de novo* at sites of tissue damage and function as inflammatory mediators, participating in the acute inflammatory response and aiding in tissue repair (Hall 1997). Bradykinin is the most common of the kinins and interact with bradykinin receptors on the cell surface. Regoli and colleagues (1980) first described the two subtypes of bradykinin receptors, B₁ and B₂, but it wasn’t until the 1990’s that specific bradykinin antagonists have been developed (Hall 1992). The kinins mediate a great variety of responses, including: contraction or relaxation of smooth muscle in the gastrointestinal, the respiratory and the urogenital tracts, effects on blood pressure and circulation (vasodilation) and a putative response in the central nervous system (hyperlgesia). The most extensively researched area to date is bradykinin’s role in the inflammatory response, where they act on the microvasculature to promote vasodilation and the extravassation of plasma into the damaged tissue (Hall 1992). This response appears to be a B₂-mediated event. B₁ receptors also contribute to the inflammatory response, producing cytokinins. The response is unique in that both bradykinin and the B₁ receptors involved in the event are synthesized *de novo* at the damage site (Marceau *et al.* 1997).
Table 4. Neurohumoral factors in the cardiovascular system and their more important properties.

<table>
<thead>
<tr>
<th>Factor/properties</th>
<th>Effect on myocardium</th>
<th>Effect on vasculature</th>
<th>Effect on peripheral nerves</th>
<th>Effect on CNS</th>
<th>$T_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>Attenuates pacing</td>
<td>Mediates neurotransmitter release</td>
<td>Mediates neurotransmitter release</td>
<td>1–8 sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Attenuates catecholamines</td>
<td>Vasodilation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>Potentates contractility</td>
<td>Vasoconstriction / growth</td>
<td>Augments nerve transmission</td>
<td>Stimulates thirst</td>
<td></td>
</tr>
<tr>
<td>Bradykinin</td>
<td>Potentates beta-adrenergic activity</td>
<td>vasodilation</td>
<td>Promotes algesia / hyperalgesia</td>
<td>Increases blood pressure</td>
<td></td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>None</td>
<td>vasodilation</td>
<td>None</td>
<td>Memory, pain perception</td>
<td>-10 sec</td>
</tr>
<tr>
<td>Endothelin</td>
<td>No direct action</td>
<td>vasoconstriction</td>
<td>None</td>
<td>None</td>
<td>1-2 min</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>Potentates contractility</td>
<td>vasodilation</td>
<td>Pain mediation?</td>
<td>Stimulant / depressant</td>
<td>5-30 sec</td>
</tr>
</tbody>
</table>

2.3 Right heart failure: its etiology and pathophysiology

Right heart failure is a comparatively common process which may be caused by several etiologic factors (Matthay et al. 1982, Matthay 1987, Clauss & Ray 1968, Herity et al. 1994) (see Table 5). Typically, pulmonary vascular resistance will increase as will mean pulmonary arterial pressure. Increasing pulmonary circulation resistance leads to over-distension of the right ventricle, which is clinically apparent as a reduction in right ventricle stroke volume as well as in cardiac output (Squara et al. 1993).

The anatomical disposition and geometry of the right ventricle allow it to adapt very well to wide variations in preload, but poorly to increases in afterload. In the presence of increased afterload, RV stroke volume decreases linearly with increasing resistance and the ventricle eventually dilates (Lee 1992). This dilation is then responsible for further RV failure, due to decreased right coronary artery flow (from systolo-diastolic to diastolic
only) at a time when myocardial oxygen consumption is increased. Furthermore, RV
dilation shifts the interventricular septum to the left, decreasing left ventricular preload and
compliance and, hence, the cardiac output. An often-lethal vicious circle is induced. The
main therapeutic goals aimed at breaking this circle are restoration of adequate oxygen
delivery to the myocardium and diminution of RV afterload (Romand et al. 1995).

Table 5. Etiologic factors contributing to both acute and chronic right heart failure.

<table>
<thead>
<tr>
<th>Cause of RHF</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic damage</td>
<td>Infarct</td>
</tr>
<tr>
<td>Primary pulmonary hypertension</td>
<td>Unknown</td>
</tr>
<tr>
<td>Secondary pulmonary hypertension</td>
<td>Left heart failure, valve disorder</td>
</tr>
<tr>
<td>Pulmonary artery stenosis</td>
<td>Congenital</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>Thrombosis/Fat-embolism</td>
</tr>
<tr>
<td>Chronic pulmonary disease</td>
<td>Vasoconstriction through hypoxia</td>
</tr>
</tbody>
</table>

2.3.1 Treatment modalities for acutely reduced right heart function

Essential cornerstones in treating acute RV failure are the optimisation of preload and
afterload, and the use of inotropic agents to increase myocardial contractility.

2.3.1.1 Optimize preload

By optimizing the degree to which the right ventricle will stretch during diastole, the
RVSV can be increased. This is accomplished through the use of fluid infusion to increase
venous return. In this, as in all instances of fluid infusion, careful monitoring is necessary
to avoid a concomitant increase in afterload, impeding flow into the pulmonary circulation.
Fig. 4. The effects of myocardial compliance and contractility on the Frank-Starling mechanism. Figure reproduced from Internal Medicine, 4th edition, 1994, W.B. Saunders with permission

Over-stretching of the ventricle can also occur, at which point contractility will decrease (Freeman et al. 1994) as can be seen from loading curves depicting the Frank-Starling principle (see figures above). Another limiting factor in the use of fluid loading is the pericardium surrounding the heart. As the pericardium is a relatively stiff and non-elastic structure, it will form a barrier to expansion of the right ventricle. This in turn will cause an increase in diastolic transmural pressure on the left ventricle, thereby reducing its compliance (Doyle et al. 1995).

2.3.1.2 Reduce right heart afterload

Right heart afterload, i.e. the pressure gradient present in the pulmonary circulation is the result of several different factors, namely the ability of the right heart to pump blood forward, the ability of the left heart to move that blood on from the pulmonary circulation into the main circulation, the degree of volume loading present in the total circulation at any given time, as well as localised impediments in the pulmonary circulation (e.g. pulmonary embolism, COPD, primary pulmonary hypertension) (Romand et al. 1995). The main aim of reducing right heart afterload is to reduce pulmonary vascular resistance. This can be accomplished, for example, by improving LV-function (contractility) through the use of vasoactive medication (e.g. digitalis or an adrenergic drug such as dobutamine). This has the benefit of increasing performance on both sides of the heart so that not only does LV-function improve, but RV-function does, as well. Another important method is the use of vasodilating drugs such as those counteracting α- and/or β-adrenergic actions, angiotensin converting enzyme inhibitors (ACEI) or nitroglycerin-based drugs (see Figure
There are also some drugs which have both an adrenergic and a peripheral vasodilating effect (e.g. milrinone, dobutamine) (McCall 1994).

![Fig. 5. The effect of vasoactive drug treatment on output/pressure curves in heart failure. Unloading of the heart and inotropic intervention improve cardiac performance (displace the curve upwards). The area to the extreme right of the figure indicates the left ventricular end diastolic pressure area at which symptoms of pulmonary congestion appear. The bottom area (cardiac index less than 2.5 l/min/m$^2$) indicates low cardiac output. Figure reproduced from Internal Medicine, 4th edition, 1994, W.B. Saunders with permission]

2.3.2 Fluid loading

Treatment of right heart failure has traditionally consisted of fluid loading (volume expansion) and the use of inotropic agents such as dobutamine (Squara et al. 1993). As explained above, optimal ventricular performance can only be assured in the presence of sufficient preload. This will then stretch myocardial myocytes to their optimal preload pressure, allowing them to contract at maximum force. One limiting factor in the use of preload optimization when treating heart failure is the resultant distension of the ventricle, especially of the right ventricle. As the Frank-Starling principle shows, over-extension of the myocytes will eventually result in an attenuation of myocardial contractile strength (see Figure 6).
Fig. 6. The effect of fluid loading and lusitropy on stroke volume. $E_a =$ effective arterial elastance, $Ees =$ left ventricular end-systolic elastance, $EDV =$ end systolic volume, $ESP =$ end systolic pressure, $EDV =$ end diastolic volume. A. Increasing preload improves stroke volume ($EDV - ESV$). B. Increasing afterload decreases stroke volume by increasing $ESV$ more than $EDV$. C. Increasing contractility improves stroke volume, arterial elastance is decreased. D. Increasing ventricular wall stiffness results in a lower $EDV$ and stroke volume. E. An increase in lusitropy improves stroke volume by increasing $EDV$. (Adapted from Pinsky & Dhainaut 1993)
Perhaps the most detrimental sequela of fluid loading is the passage of intravascular fluid into the extravascular space and the ultimate reduction in perfusion of both peripheral and central (i.e. pulmonary) tissues which results. Due to these limiting factors, fluid loading must be used with caution in the treatment of heart failure, and care must be taken to avoid over-distension of the right ventricle and the exaggerated extravasation of the fluid.

2.4 Review of the drugs under study

2.4.1 Aminophylline

Theophylline is a methylxanthine which acts as a non-specific phosphodiesterase inhibitor, thereby increasing tissue levels of cyclic-AMP. Chemically, theophylline is related to the other methylated xanthines, caffeine and theobromine. Xanthine is a dioxypurine which is structurally related to uric acid (Hardman et al. 1995). Theophylline itself is poorly soluble, but solubility is much enhanced by the formation of complexes, the clinically most important of these being the complex of theophylline and ethylenediamine, which forms aminophylline. Given intravenously, aminophylline converts to theophylline at about a 70 to 85 percent rate of efficiency, with a half-life thereafter from 3.5 to 9 hours (Kaplan 1988).

In addition to its phosphodiesterase inhibiting effect, theophylline has also been shown to exert other effects in vivo, the most important of these being its antagonist action on adenosine receptors (Hendeles et al. 1985) and has a catecholaminic effect, acting as a dopamine-agonist (Hillis & Been 1982). In addition, an increased binding of cAMP to cAMP-binding protein has also been reported (Miech et al. 1979) and an action as a prostaglandin antagonist (Hendeles et al. 1985). The end-point of the action as a phosphodiesterase inhibitor, however, is an increase in intracellular calcium levels, which in turn contributes to positive inotropy (Colucci et al. 1986). The proportional magnitude of the mechanisms of action attributed to theophylline has not been ascertained, however, and there is some disagreement as to whether the effects observed are the result of a mainly phosphodiesterase inhibition or of a direct catecholaminic effect (Hillis & Been 1982, Matthay et al. 1978, Falicov et al. 1973). There is also some controversy as to the degree theophylline improves hemodynamics through an improvement in myocardial contractility as compared to its effect on afterload via broncho- and vasodilatation in the pulmonary circulation (Matthay 1987).

The pharmacologic effects of intravenous aminophylline include bronchodilation, vasodilatation, positive chronotropy, positive inotropy as well as an increase in renal activity. In addition, respiratory muscle strength has been reported to improve with intravenous aminophylline (Siafakas et al. 1993), and diaphragmatic muscle fatigue to be reduced (Aubier et al. 1981, Bukowskyj et al. 1984). An improvement in mucociliary clearance has also been reported (Guyton 1991).
2.4.1.1 Cardiovascular effects of aminophylline

The earliest reports of the cardiovascular effects of aminophylline were from studies conducted during the first decades of the 1900's (e.g. Thompson & White 1936, Marvin 1926, Starr et al. 1937, Howarth et al. 1947, Fowell et al. 1949, Werkö & Lagerlöf 1950, Borst et al. 1957). The findings in these early reports showed a slight chronotropic effect, a short-lived drop in venous pressures and (in post-Second World War studies) increases in cardiac output. Parker and his associates conducted experiments on the cardiovascular effects of aminophylline in various pathological states in the 1960's (Parker et al. 1967, Parker et al. 1966, Parker et al. 1966). Their findings showed an increase in cardiac output along with a decrease in the filling pressures of both right and left ventricles. Interestingly, even though they used a rather large dose of aminophylline (1 gram infused intravenously over 30 minutes), there was no change in heart rate in their patients with heart failure (Parker et al. 1966), while a substantial increase was observed in those with chronic obstructive pulmonary disease (Parker et al. 1967). Murphy and associates performed a study (1968) based on observations concerning the inotropic effect of aminophylline in various forms of heart disease. They showed that aminophylline effected no improvement in cardiac output in twenty-one patients with valvular heart disease (both mitral and aortal), even though there was an improvement in inotropy (dP/dt). They also observed significant reductions in pulmonary vascular resistance, which they concluded was the most favorable hemodynamic effect of the drug in this subset of patients who generally suffer from concurrent pulmonary edema.

Rall and West (1963) demonstrated that theophylline potentiates contractility in isolated cardiac preparations, and these findings have later been clinically supported (Falicov et al. 1973, Rutherford et al. 1981). Several studies in the 1970's and 1980's in humans indicated that either aminophylline used intravenously or theophylline orally will improve hemodynamics in right heart failure (Matthay & Mahler 1986, Falicov et al. 1973, Conradson 1984). Other studies showed an improvement in both global myocardial performance (Falicov et al. 1973, Rutherford et al. 1981) and right heart performance with the use of intravenous aminophylline (Matthay et al. 1978, Bisgard & Will 1977).

Aminophylline and its effects on cardiovascular performance has still been the subject of research in the 1980's and 1990's. Many of the more recent studies have concentrated mainly on the consequential effects of per oral methylxanthines on hemodynamics (Matthay & Mahler 1986, Matthay 1987, Dini et al. 1993, Matthay 1985, Ueno et al. 1990), showing improvement in pump function and reductions in systemic and pulmonary vascular resistances.

Elliot and his coworkers (1985) examined the effect of intravenous aminophylline on cardiac performance during tread-mill exercise in healthy human subjects, finding no significant improvement after drug administration.

Conradson (1986) showed that theophylline only produced minor improvements in healthy male human subjects, in his study on theophylline and enprofylline. He concluded that with plasma concentrations of 3.8 to 12.6 mg/l of theophylline there was some degree of vasodilation, but only weak inotropic response.

Gomez and associates (1988) examined the effect of blood oxygen saturation on the hemodynamic response of aminophylline in dogs. They showed that aminophylline exerted
a positive inotropic effect under normoxic conditions as well as increasing stroke volume, but had little effect on left ventricle performance during hypoxia. Hakim and coworkers (1988) continued to examine the effects of aminophylline on hemodynamics during hypoxia in their study using pigs and dogs. They found that therapeutic doses of aminophylline (1-10 mg/kg) administered intravenously during hypoxia attenuated pulmonary vascular resistance without effecting systemic vascular resistance. They concluded that xanthines can only be effective as vasodilators when there is already some vasoconstriction.

Schreiber and associates (1992) examined the response of preconstricted pulmonary vascular smooth muscle to aminophylline in newborn lambs. They could show no relaxation by the drug, however, and concluded that the proposed use as a vasodilator in the treatment of persistent pulmonary hypertension in newborns could not be recommended. In another study (Mols et al. 1993) showed a dose-dependency for inotropic and vasodilator effects of aminophylline in COPD patients.

Various studies on the effect of aminophylline on coronary blood flow have been reported (Roig et al. 1996, Crea et al. 1994, Granato et al. 1990), with somewhat conflicting results. Granato and companions (1990) found that aminophylline had no significant effect on hemodynamics or coronary blood flow in an open-chest study using dogs. Crea and associates (1994) performed a similar study on dogs examining the effects of aminophylline on myocardial blood flow. They concluded that aminophylline may improve myocardial ischemia through 1) an unloading of the left ventricle and 2) constriction of subepicardial vessels with a consequent redistribution of flow to the subendocardium. They did not observe any improvement in total coronary flow. More recently, Roig and coworkers (1996) ascertained an improvement in coronary flow by aminophylline in patients with coronary occlusion. Exercise-induced ischemia was delayed by the drug through an improvement in the collateral circulation. Despite the ambiguous results reported above, aminophylline is regularly used to dilate coronary arteries during medical imaging (Nahser et al. 1996, Johnston et al. 1995, Kahn et al. 1990, Miller et al. 1989, Mohiuddin et al. 1992).

In a study in open-chest dogs Minamino and coworkers (1995) were recently able to ascertain that aminophylline had a biphasic action in myocardial ischemia. They found that the drug (0.8 mg/kg infused over 10 minutes intravenously) improved mild ischemia but worsened severe ischemia. They attributed this finding to the fact that aminophylline acts through both an increase in plasma catecholamine concentrations and a blockade of adenosine receptors. They conclude that the administration of aminophylline may result in a worsening of myocardial ischemia through inhibition of adenosine receptors because adenosine mediates cardioprotection in the ischemic and reperfused myocardium.

Aminophylline has been used to decrease pulmonary arterial pressure and increase cardiac performance in chronic pulmonary hypertension (Matthay 1985, Maskin et al. 1984, Panuccio et al. 1984). Several studies have shown it to be an effective reducer of pulmonary pressures (Matthay et al. 1978, Parker et al. 1966, Parker et al. 1984, Panuccio et al. 1984), while some more recent studies have put this into question (Mols et al. 1993). Although other, more specific phosphodiesterase inhibitors are available (i.e. amrinone, milrinone, enoximone), aminophylline is still in use in some centers as a pulmonary...
vasodilator. Due to its positive inotropic and vasodilative effects, this type of drug would appear to be a natural choice for the treatment of right heart failure.

2.4.2 Adenosine

2.4.2.1 Cardiovascular effects of adenosine

Adenosine has long been recognized as a clinically effective anti-arrhythmic agent (Belhassen & Pelleg 1984, Parker & McCollam 1990, Faulds et al. 1991, Rankin et al. 1992) and has been used to produce controlled hypotension (Sollevi et al. 1984). Wainwright and Parratt (1993) reported on the effects of the administration of a selective A<sub>1</sub> adenosine agonist (R-PIA) during left main coronary occlusion in an open-chest pig model. They reported a high incidence of ventricular fibrillation (70%) in animals not receiving the drug, the incidence being 20% to which it was given. They concluded that the A<sub>1</sub> adenosine agonist markedly reduced the incidence and severity of ischemic arrhythmias. They also concluded that the A<sub>1</sub> adenosine agonist was a much more powerful antiarrhythmic agent than a similar A<sub>2</sub>-specific adenosine agonist reported in an earlier study (Wainwright & Parratt 1991).

Wang and coworkers (1994) examined the effects of adenosine on guinea pig atrial myocytes, showing that the mechanism involved in the negative inotropic effect of the agent is a potassium current on the cell membrane activated by adenosine type A<sub>1</sub> receptor stimulation which hyperpolarizes the cell membrane and shortens the action potential in supraventricular tissues. This leads to negative dromotropy, negative chronotropy and, ultimately, negative inotropy. No similar effect on ion currents has been demonstrated in ventricular tissue, but attenuation of the effects of catecholamines on ion currents have been reported (Belardinelli et al. 1995). Stimulation of A<sub>2</sub> receptors causes vasodilation in the coronaries, increasing coronary flow, appearing to act on both the endothelium and vascular smooth muscle (Shryock & Belardinelli 1997).

Several researchers have reported on the cardioprotective potential of adenosine over the past decade (Lasley & Mentzer 1995, Ely & Berne 1992, Belardinelli et al. 1989). Current evidence suggests that stimulation of A<sub>1</sub> and A<sub>2</sub> receptors with exogenously administered adenosine may enhance myocardial tolerance to ischemia and attenuate damage to the myocardium during reperfusion of the stunned heart. The exact mechanism involved is the subject of some discussion, but the activation of both A<sub>1</sub> and A<sub>2</sub> receptors appears to be inherent in the phenomenon. Lasley and Mentzer (1993) reported on the use of exogenous and endogenous adenosine in isolated rat hearts during low-flow ischemia. Their findings indicated that the mechanism through which adenosine enhanced myocardial tolerance to ischemia may be in part due to a modulation of glucose metabolism. In a later study of theirs (Lasley & Mentzer 1995) using a different model, they noted several important points on using adenosine in the stunned heart. First, the degree of ischemia involved is of great importance when considering the mechanism by
which the adenosine will act. As an example, in their earlier study the model of ischemia involved low-flow through the myocardial tissue, but not total ischemia, and a modulation of glucose metabolism was found to be important. Second, the timing for administering the adenosine is of critical importance, and must be begun before the ischemic event to achieve maximal effect. They concluded that adenosine acts in the myocardium through A1 receptors, but also appears to modulate phosphorylation potential. The former takes place during ischemia, while the latter occurs during reperfusion. It has been theorized (Shryock & Belardinelli 1997) that the stimulation of A1 receptors causes an attenuation of pacing, a slowing of cellular metabolism, an inhibition of catecholamine effects and of the cellular release of noradrenaline, while A2 receptor stimulation causes inhibition of platelet aggregation, the formation of protective superoxide anions and the activation of neutrophils. De Jong and associates applied this to use in cardioplegia (de Jong et al. 1990) and found that low-dose adenosine as an adjunct to potassium cardioplegia resulted in a shortened arrest time in their model while improving post-ischemic recovery.

Adenosine is a vasodilator and will cause dilation of the coronary vessels as well. It has therefore been used in coronary imaging (Marwick 1997, Ogilby 1997) to identify vessels which are occluded as opposed to those with low flow due to constriction. One of the pitfalls associated with this method, however, is the coronary steal sometimes associated with a vasodilatation extending to the systemic circulation. In addition, ischemia may also occur during adenosine infusion due to a decrease in distal coronary perfusion, causing collapse of the vessel at the site of the stenosis (Ogilby et al. 1993). Anderson and coworkers (1997) therefore recommended that the adenosine infusion be titrated to suit the individual patient in their examination of hemodynamic response to adenosine infusion before and after coronary bypass surgery.

Iskandrian and associates (1997) reported on the use of adenosine as an aid in coronary imaging. They administered a 140 µg/kg/min infusion to patients unable to participate in exercise stress tests due to difficult symptoms of angina pectoris. They concluded that the infusion was well tolerated, although mild side-effects such as flushing, shortness of breath, dyspnea and chest pains were quite common (70-80%). Dysrhythmias such as first or second degree AV block were well tolerated and transitory. The report concluded that this method of myocardial perfusion imaging is a viable alternative to exercise stress-tests.

2.4.2.2 Pulmonary effects of adenosine

Jolin and associates (Jolin et al. 1992) examined the effects of a continuous infusion of adenosine on pulmonary hemodynamics and fluid filtration in isolated rat lungs with either no damage or an ARDS-simulating fat emulsion-induced injury. They found that the drug effected a decrease in both the intact and the injured lung in pulmonary vascular resistance, as well as modulating the fluid filtration rate in the lung tissue. Öwall and companions (1991) demonstrated a similar effect on pulmonary vascular resistance in anesthetized pigs with hypoxia-induced pulmonary hypertension, as well as an increase in cardiac output. The prostaglandin prostacyclin was also used in that experiment and was shown to be a more effective vasodilator than adenosine, while adenosine increased cardiac output more.
Konduri and coworkers (1992) also demonstrated that adenosine infusion caused an effective decrease in pulmonary hypertension in hypoxic newborn lambs. Schrader and coworkers (1992) demonstrated a significant reduction in pulmonary vascular resistance (37%) in patients with pulmonary hypertension of varied etiology. They also showed that adenosine increased cardiac output by as much as 57%. Compared to the calcium channel blocking agent nifedipine, adenosine proved to be the more effective pulmonary vasodilator. In a later study (Inbar et al. 1993), the same authors examined the combination of calcium channel blockers (nifedipine or diltiazem) and an adenosine infusion (mean 180±63 µg/kg/min). They concluded that adenosine could further decrease pulmonary vascular resistance in patients already receiving an optimum dose of a calcium channel blocker. Of five patients not responding to the calcium channel blocking agent with a decrease in pulmonary vascular resistance, three did respond to the adenosine infusion.

Adenosine and prostacyclin were compared with respect to reductions in pulmonary vascular resistance and cardiac output in a study by Nootens and coworkers (1995) involving patients with primary pulmonary hypertension. They showed that the two agents produced similar reductions in pulmonary vascular resistance and increases in cardiac output, with no untoward effects on systemic or pulmonary pressures. They concluded that – due to its short half-life – adenosine is not suitable as a therapeutic agent in these patients, but may be useful as a test for patient suitability for prostacyclin treatment in this disorder. They further theorized that prostacyclin as a second vasodilating agent may be useful in cases where calcium channel blocking alone does not produce sufficient reductions in pulmonary hypertension, referring to Inbar and associates’ earlier publication (Inbar et al. 1993).

Another effect of adenosine in the pulmonary circulation is to protect the pulmonary tissue from injury such as that induced by ARDS (Jolin et al. 1992, Jolin et al. 1994, Adkins et al. 1993). Adkins and coworkers (1993) demonstrated that pretreatment with an infusion of adenosine or of an A2 agonist completely prevented the hemodynamic changes (increase in pulmonary vascular resistance and in capillary permeability) seen in lung injury associated with phorbol myristate acetate (PMA) challenge. Jolin and associates (1994) showed that adenosine infusion also helped to preserve the morphological ultrastructure of lung tissue after fat emulsion injury.

Adenosine has lately been the cause of considerable interest due to its vasodilative and cardiac protective properties. Studies have been performed in recent years on its use in such diseases as pulmonary hypertension (Fullerton et al. 1996, Haywood et al. 1992, Morgan et al. 1991, Reeves et al. 1991) and reperfusion injury (Mullane & Bullough 1995, Ely & Berne 1992, Randhawa et al. 1993, Engler 1994, Granger 1997, Lee & Lineaweaver 1996) and have shown that it causes a clear reduction in pulmonary vascular resistance. Its effect on right heart and global hemodynamics, however, is still the subject of some controversy (Haywood et al. 1992). As an effective vasodilating drug with a very short half-life, adenosine is drug of a good deal of theoretical interest with respects the treatment of right heart failure. It can be centrally infused and its infusion rate titrated to render its effects specific to the pulmonary circulation. Its negative chronotropic and dromotropic effects may also be of benefit.
2.4.3 Losartan

Losartan (DuP 753, 2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2’-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole) is a specific angiotensin II type 1 receptor (AT, receptor) inhibitor. It acts as an angiotensin II inhibitor, mainly on AT, receptors. Losartan is primarily metabolized through oxidation and glucuronidation (Lankford et al. 1997), with a major oxidative product being the carboxylic acid metabolite EXP3174, which has been shown to be approximately 33-41 times as potent an AT, receptor inhibitor than is the parent molecule, losartan. Lankford (1997) found the expression of this metabolite to be species-dependent, with peak EXP3174 plasma concentrations in humans and rats being twice that of losartan, while those in pig and dog are almost non-existent. The half-life of losartan also shows inter-species variability, being about 2 hours in humans, with EXP3174’s half life being 6.3 hours (Lo et al. 1995), while losartan’s T1/2 was only 40 minutes in pigs, with a mere 2% of that being oxidized to EXP3174 (Lankford et al. 1997).

2.4.3.1 Cardiovascular effects of losartan

Hypertension. Losartan was primarily developed for use in the treatment and control of systemic arterial hypertension, and at present this is its only approved indication. The inhibition of AT, receptor expression causes vasodilation and stimulates renal excretion of sodium and water. It also improves renal blood flow (Symons & Stebbins 1996).

Myocardial infarct. The effect of angiotensin II inhibition after myocardial infarct is the subject of some debate. Hartman and coworkers reported (1993) that direct angiotensin II stimulation or inhibition had no effect on the degree of infarct necrosis in an experimental, open-chest rabbit model. They also reported that ACE inhibition using ramiprilat had a protective effect. Capasso and associates (1994) performed a similar study comparing captopril and losartan in rats with a large myocardial infarct of the left ventricle. They found that both drugs improved cardiac performance, but through different mechanisms. Whereas captopril reduced systemic pressures, thereby unloading the heart on both sides, losartan was shown to improve myocardial contractility, with a consequential decrease in ventricular workload. They also determined that losartan prevented ventricular remodelling after infarct. They concluded that losartan may improve long-term survival after myocardial infarct. Another study on the subject (Milavetz et al. 1996), found no difference in the survival of infarcted rats receiving either captopril or losartan. No differences were found in heart weight, left ventricular pressures, dP/dt, cardiac index or left ventricular volume or dimensions. Sladek and associates (1996) performed an experimental study on myocardial infarct of the left ventricle in rats, in which they were able to ascertain that losartan improved cardiac performance, reduced right ventricle loading and improved angiogenesis in the infarction area (38% improvement compared to controls).
**Heart failure.** Fitzpatrick and coworkers (1992) induced heart failure in sheep with 7 days of rapid ventricular pacing, after which intravenous losartan was given followed by captopril on separate days. Their findings indicated that losartan was similar to captopril in its efficacy in the treatment of heart failure with both drugs reducing systemic pressures, while glomerular filtration and renal perfusion pressures were maintained. Dickstein and coworkers (1994) examined the effects of per oral losartan in sixty-six patients with heart failure (NYHA II-IV, ejection fraction <40%), observing favorable vasodilatory effects as well as dose-related increases in plasma renin activity, angiotensin II levels and moderate reductions in serum aldosterone and plasma noradrenaline levels. Crozier and others (1995) reported a large, multicenter study on the use of per oral losartan in symptomatic heart failure. Patients received 25 or 50 mg losartan. In addition to the neurohumoral effects observed by Dickstein et al, these researchers were able to report beneficial changes in systemic vascular resistance and blood pressure, as well as improvements in pulmonary wedge pressure, cardiac index and heart rate after 12 weeks of drug administration. They found the drug to be well tolerated, with the beneficial effects not diminishing over time.

**Coronary circulation.** Richard and coworkers reported (1993) on the effects of Exp3174 on the coronary circulation in dogs. They measured regional myocardial blood flow using a radioactive imaging technique, and found no improvement in coronary circulation after administration of the metabolite. In a later study, Nunez and associates (1997) were able to demonstrate an improvement in coronary hemodynamics either alone or in combination with enalapril in spontaneously hypertensive rats. This was associated with a reduction in cardiac mass and a reduction in systemic blood pressure. In that study, it was noted that enalapril alone had no effect on coronary blood flow. Similarly, Kaneko and coworkers (1996) showed that losartan decreased left ventricular mass and improved coronary blood flow in a study on exercise in spontaneously hypertensive rats. In a study of exercise-induced angiotensin II release (1996), Symons and associates showed that losartan effectively increased blood flow and/or decreased vascular resistance during exercise in the myocardium, as well as in the vasculature for the stomach, small intestine, and colon. It also improved renal blood flow in their study using mini-swine running on treadmills. Recently, Zhu and associates (1999) performed an interesting study to evaluate the protection from reperfusion injury offered by losartan. They showed that losartan significantly improved coronary flow and left ventricular developed pressure in isolated rat hearts after 15 minutes of ischemia and 30 minutes of post-ischemic reperfusion. Interestingly, they also found that this effect appeared to be bradykinin-dependent.

**Pulmonary hypertension.** Angiotensin II can contribute to the development and exacerbation of pulmonary hypertension through several paths. Left ventricle ischemia will result in elevated levels of angiotensin II, which cause vasoconstriction and ventricular remodelling, leading to left heart failure. This impedes flow through the pulmonary system, resulting in pulmonary hypertension and – ultimately – right heart hypertrophy and insufficiency.

Morrell and associates (1995) examined the role of angiotensin II in the development of hypoxia-induced pulmonary hypertension in rats. They found that the changes in these animals after 14 days in a hypobaric hypoxic chamber (increased pulmonary artery
pressure, right ventricular hypertrophy, medial thickening and muscularization of the small pulmonary arteries) could be attenuated using losartan or captopril. Furthermore, a bradykinin B2 receptor inhibitor failed to reverse the protective effects of the ACE inhibitor, indicating that the protective effects were mediated by reductions in angiotensin II levels, rather than increases in levels of bradykinin. They also ascertained that AT2 receptor expression did not contribute to the development of pulmonary hypertension.

Kiely and coworkers (1997) performed a clinical study in patients with hypoxemic cor pulmonale and found that a 50 mg dose of per oral losartan significantly reduced MPAP and TPR (total pulmonary resistance). MAP and SVR were also reduced, while cardiac output was increased.

2.4.3.2 Losartan and right heart failure

To an increasing degree, angiotensin converting enzyme inhibitors (ACEI) have been one of the mainstays in the treatment of heart failure after myocardial infarct (Capasso et al. 1994, Lindpaintner & Ganten 1991, Pfeffer 1991). Angiotensin II levels rise after myocardial infarct (Lindpaintner & Ganten 1991, Baker et al. 1990, Hirsch et al. 1991, Olivetti et al. 1990), causing a subsequent increase in systemic pressure and pulse (Yang et al. 1997). The beneficial effects of ACEI consist of both their immediate improvement of hemodynamics by decreasing the effects of angiotensin II and the long-term attenuation of angiotensin II-induced cardiac remodelling (Smits et al. 1992). In addition, however, ACEI’s also cause an inhibition of nitric oxide, bradykinin and prostaglandins (Willenheimer et al. 1999), which effect may be detrimental in heart failure. Angiotensin II type 1 receptor (AT1 receptor) antagonists have therefore been used for a more selective blockade of the renin-angiotensin system by blocking the activated renin-angiotensin system selectively and effectively at the receptor level (Milavetz et al. 1996, Pitt et al. 1997).

Earlier studies have demonstrated the beneficial effects of losartan in heart failure in both acute (Fitzpatrick et al. 1992, Smits et al. 1992) experimental models and clinical trials (Crozier et al. 1995, Dickstein et al. 1995) involving patients with chronic heart failure. In an experimental model involving acute left-heart infarct in rats, Capasso et al. (1994) studied losartan’s effects on cardiac pump performance, finding an improvement in myocardial contractility as well as in afterload, with a resultant improvement in cardiac output. Pit and coworkers (1997) found that the long-term use of losartan was associated with a lower mortality than that for captopril in elderly patients with heart failure.

Therefore, with its vasodilative effects and attenuation of ventricular remodelling, as well as its non-inhibition of the actions of prostaglandins and bradykinin, losartan would appear to be a potentially effective drug for the treatment of post-infarct right heart failure.
2.4.4 Nitric oxide

2.4.4.1 Cardiovascular effects of inhaled nitric oxide

Nitric oxide has effect on both early and late preconditioning of the myocardium (Bolli et al. 1998) (see section 2.2.2.3.). With reference to right heart function, the administration of inhaled nitric oxide in RV failure has been shown to dramatically reduce pulmonary pressure, thereby improving right heart performance (Hillman et al. 1997). It has also been shown that due to its short half-life, while decreasing pulmonary vascular resistance, inhaled nitric oxide has little or no effect on systemic arterial pressure. This results in an improvement in right ventricle performance without compromising systemic pressure (Ziegler et al. 1998). Nitric oxide has been shown to effectively reduce pulmonary vascular resistance (for example Snow et al. 1994, Chen et al. 1997, Rich et al. 1994, Semigran et al. 1994). Adrie and coworkers (1996) showed that inhaled nitric oxide improves coronary blood flow. Several studies have concentrated on the treatment of pulmonary vascular hypertension such as persistent pulmonary hypertension in the newborn (PPHN) (Kinsella et al. 1992, Roberts et al. 1992, Roberts et al. 1997, Kinsella et al. 1997). Although these studies have demonstrated clinical improvement in the patients, as well as a reduced need for extracorporeal membrane oxygenation (ECMO), overall survival was not improved.

Ishibashi and associates (1998) examined the roles of potassium channels, adenosine receptors and nitric oxide in coronary vasodilation. They found that vasodilation by nitric oxide was reduced to approximately one quarter of its normal exercise-induced level in the presence of K+-(ATP) channel and adenosine receptor blockade. Their conclusion was that K+-(ATP) channels are critical for maintaining coronary vasodilation at rest and during exercise but that when K+-(ATP) channels are blocked, both adenosine and nitric oxide act to increase coronary blood flow during exercise.

Several studies have been published in recent years concerning the effects of exogenous nitric oxide on the myocardium. Prendergast and coauthors (1998) reported that nitric oxide enhanced the inotropic response of isolated preparations of the guinea-pig heart to dobutamine. Somewhat in contrast to these findings, however, there is a growing body of evidence that nitric oxide exerts a depressive effect on the myocardium (Baigorri et al. 1999, Goldstein et al. 1997). Some studies have associated the use of inhaled nitric oxide with increases in pulmonary capillary wedge pressure which the authors have attributed to an attenuation in left ventricular function (Bocchi et al. 1994, Kelm et al. 1997), while others feel that this change is merely the result of changes in pulmonary vasodilation and not of changes in myocardial contractility (Loh et al. 1999).

2.4.4.2 Pulmonary effects of inhaled nitric oxide

The clinical use of inhaled nitric oxide has concentrated mainly on its use in the treatment of pulmonary disorders such as adult respiratory distress syndrome (ARDS) (Benzing et al.
Matthay and coworkers (1998) reported in their editorial on the subject that the consensus of trials concerning the use of nitric oxide in ARDS was that the treatment was of little or no benefit. They concluded that inhaled nitric oxide may still be of value as a rescue therapy for some patients with refractory hypoxemia. Nitric oxide has also been clinically tested in other conditions such as chronic pulmonary arterial hypertension (Sitbon et al. 1995), COPD (Högman et al. 1993), lung transplantation (Date et al. 1996) and heart surgery (Curran et al. 1995).

More recent reports on the clinical use of nitric oxide have been less optimistic than those published in the early 1990’s. Manktelow and coworkers (1997) reported that ARDS patients were less likely to respond to nitric oxide-treatment (60-70% being hyporesponsive). They attributed this phenomenon to the increased levels of endogenous nitric oxide and of systemic and infused catecholamines present in these subjects due to septic vasodilation.

In their exhaustive review of the use of inhaled nitric oxide, Steudel and coworkers concluded that the clinical usefulness of inhaled nitric oxide remains unclear. In their paper they established that the use of inhaled nitric oxide in the treatment of ARDS has not improved mortality rates, but whether this is due to the complexity and broad spectrum of the disease, inappropriate dosing, inappropriate study design, inefficacy of nitric oxide or even to the counterbalancing toxic effects of nitric oxide is unknown (Steudel et al. 1999).

At present, nitric oxide therapy has its main clinical use in the treatment of neonatal respiratory distress syndrome (Kinsella et al. 1997).

### 2.4.4.3 Systemic effects of inhaled nitric oxide

Högman and coworkers demonstrated that inhaled nitric oxide increases bleeding time by inhibiting platelet function (Högman et al. 1994) in their study in a rabbit model of inhaled nitric oxide. This same effect was also demonstrated by Adrie and associates (1996) in a model using open-chest dogs. In that same study, the authors also reported that nitric oxide improves healing of vascular injury.

### 2.4.4.4 Inhaled nitric oxide in the treatment of right heart failure

Inhaled nitric oxide may cause an unloading of the right heart, which should be of benefit in cases of right heart failure. Its effects on right heart failure due to right ventricle infarction have not previously been documented.
3 Purpose of the present study

The present study was undertaken to examine the hemodynamic effects of various drugs after experimental right heart infarct. An open-chest porcine model was developed for the experiment due to the similarities between the cardiovascular system of man and pigs and the repeatability of this model. More specifically, the aims of the present study were as follows:

1. To examine the effects of intravenous aminophylline on hemodynamics when administered shortly after right heart infarct.
2. To examine the hemodynamic effects of a continuous intravenous infusion of adenosine at four different infusion rates in a similar model.
3. To examine the effect of a bolus dose of the angiotensin II inhibitor losartan on hemodynamics in this model, and
4. To examine the hemodynamic effects of progressive concentrations of inhaled nitric oxide in this experimental model of right heart infarct.
4 Materials and Methods

4.1 Experimental animals

The animals used in the studies involved were young, healthy mongrel pigs of either sex and of uniform size and age (27-31 kg, approximately 4 months of age). All animals received humane care in compliance with the “Guide for the care and use of laboratory animals” (NIH Publication no. 85-23, 1985). The local committee governing ethical considerations in experimental animal studies approved the protocol involved in the four separate parts of the study. All in all, 70 animals were anesthetized for the study, of which ten were included in the aminophylline group, ten in the adenosine group, four in the losartan group, ten in the nitric oxide group and seven in the control group. In addition, 5 animals were used in two separate pilot studies for the losartan protocol. The remaining 28 animals were lost during the experiment due to (in order of frequency): drug incompatibility, early death (i.e. infarct too large), uncontrollable hemorrhage during cannulation, previous illness (e.g. pericarditis, pneumonia), anesthesia-related malignant hyperthermia, and other reasons (e.g. insufficient infarct).

All animals were treated similarly prior to the experiment. They were chosen from the farm based on their size and apparent health and thereafter transferred to the Experimental Animal Center of the University of Oulu’s Medical Faculty, where they were acclimatized for approximately one week before the experiment. The animals were kept in separate pens with unlimited access to water and were fed twice daily. On the morning of the experiment the animals were sedated with intramuscular midazolam (1 mg/kg), ketamine (10 mg/kg) and atropine 0.5 mg in their pens and then transferred to the operating theater. An intravenous cannula was introduced into a peripheral vein, usually in the left ear. Thiopental (100-150 mg) was administered intravenously and muscle relaxation induced with 8 mg pancuronium, after which the animals were intubated. Mechanical ventilation with the Siemens 730 ventilator (Siemens-Elema AB, Sweden) using 30% oxygen and 70% nitrous oxide was begun and maintained with normoventilation for the duration of the experiment. Supplemental anesthesia was given in the form of inhaled enflurane at 1-2%, intermittent bolus doses of pancuronium and a continuous intravenous infusion of
thiopental (5 mg/kg/h). An exception to these last two points was the nitric oxide group of animals who were ventilated using the Dräger Evita-4™ respirator device (Drägerwerk AG, Lübeck, Germany) with 30% oxygen in an oxygen/air mixture and maintained with normoventilation for the duration of the experiment. Anesthesia was maintained in this group using a total intravenous anesthesia (TIVA) technique via a continuous infusion of a sodium thiopental-fentanyl-pancuronium mixture.

4.2 Monitoring protocol

Monitoring was carried out similarly in all studies using the Datex AS/3 anesthesia monitor (Datex Inc., Espoo, Finland). Subsequent to the induction of anesthesia and intubation, the femoral vein and artery were exposed and catheters introduced through them for the monitoring of systemic pressures and drawing blood samples. A thermodilution ejection fraction catheter (93A-43H-7.5 Baxter Health Care Co. Ltd., CA, USA) was then placed via the right internal jugular vein for the measurement of heart function. Hemodynamic measurements were performed at this point and thereafter at regular intervals. Values were obtained and recorded for heart rate (HR), mean systemic arterial pressure (MAP), central venous pressure (CVP), mean pulmonary arterial pressure (MPAP), pulmonary capillary wedge pressure (PCWP) and cardiac index (CI). Right ventricle end-systolic volume index (ESVI), right ventricle end-diastolic volume index (EDVI), left ventricle stroke volume index (SVI), pulmonary vascular resistance index (PVRI), systemic vascular resistance index (SVRI) and right (RVSWI) and left (LVSWI) ventricle stroke work indices were then calculated from the above (see Appendix I). An arterial blood-gas analysis was also performed for the first time at this point and a total of eight times during the protocol to ensure normoventilation.

4.3 Study protocol

4.3.1 General

Pre-sternotomy-level hemodynamic measurements were obtained after catheterization and before any further intervention. Animals with a pulmonary capillary wedge pressure outside the range of 8-10 mmHg received fluid loading at this point (100-200 ml hydroxyethyl starch solution and/or Ringer’s solution 500 ml as needed), after which these first measurements were performed again. A sternotomy was performed and hemodynamic measurements taken, the results of which served as a baseline for later measurements. Branches of the right coronary artery on the free wall of the right ventricle were then ligated. Ligation was judged to be successful if a distinct blanching of the myocardial wall could be observed.
New baseline measurements were performed 60 minutes later after allowing the circulation to accommodate to the new hemodynamic state. A 250 ml infusion of a hydroxyethyl starch solution (Plasmafusin®, Pharmacia AB, Sweden; average molecular weight ~120,000 daltons) was then given over 15 minutes, with hemodynamic measurements one half hour after the infusion.

A final bolus dose of 200 ml hydroxyethyl starch solution was given to the animals in every group (including controls), followed by hemodynamic measurements, after which the animal was exsanguinated. The heart was removed and the free wall of the right ventricle was dissected and examined macroscopically to determine the extent of the infarct. All animals included in the study were determined to have a transmural infarct of the free wall of the right ventricle. No infarct extended to the septum.

### 4.3.2 Controls

Animals in the control group (N=7) were treated similarly to those in the study groups with the exception that they received no drug during the post-loading period. All animals received a continuous infusion of a 0.9% saline solution throughout the follow-up to maintain the circulating volume.

### 4.3.3 Aminophylline

In contrast to the other groups, post-infarct loading was performed in this group of experimental animals (N=10) in five aliquots of a hydroxyethyl starch solution (Plasmafusin®, Pharmacia AB, Sweden, average molecular weight ~120,000 daltons), 50 ml each (i.e. 250 ml) at ten-minute intervals. Hemodynamic measurements were performed after each injection. A final measurement of hemodynamics and blood gases was performed 30 minutes after the fluid loading.

Aminophylline was subsequently administered intravenously at 30 minute intervals in graduating doses of 50, 100, and 200 mg and its effects on hemodynamics observed with measurements every 15 minutes.

### 4.3.4 Adenosine

In study II (N=10), an adenosine infusion (Adenocor®, adenosine 3 mg/ml, Sanofi-Winthrop AB, Helsinki, Finland) was begun as a continuous infusion at 25 µg/kg/min via the atrial port of the pulmonary catheter subsequent to loading. The infusion rate was subsequently raised at one half hour intervals to 50, 75 and 100 µg/kg/min. Hemodynamic measurements were performed during this time at 15 minute intervals. After adenosine had
been infused at 100 µg/kg/min for 30 minutes, the infusion was discontinued and hemodynamics followed for another half hour with measurements at 15 minute intervals. One animal was excluded from the final analysis due to a high HR (> 180/minute), as this made calculations for REF impossible. A further three animals were excluded due to insufficient (less than 25%) changes in REF, CI and PVRI subsequent to right heart infarct, in spite of significant macroscopic changes being observed in the free wall of the right ventricle. This left six animals in the study group.

4.3.5 Losartan

In this group (N=5), a bolus dose of losartan (30mg/kg) was administered intravenously. This dose had been calculated using the literature available (Lankford et al. 1997) and information obtained in two pilot studies performed by our group previously (animals received a 50 mg and a 30 mg/kg dose in the two studies). This dose was shown to effectively block all angiotensin receptor activity for at least 45 minutes. After administration of the drug, hemodynamic measurements were performed at 15-minute intervals for one hour.

4.3.6 Nitric oxide

In the study animals (N=10) of study IV, nitric oxide gas was introduced into the inhaled gases at progressive concentrations (5, 10, 15 and 20 ppm) for 30-minute periods at each concentration. Hemodynamic measurements were performed during this time at 15-minute intervals. After nitric oxide had been inhaled at 20 ppm for 30 minutes, the insufflation was discontinued and hemodynamics followed for another half hour with measurements at 15-minute intervals.

4.4 Statistical Analysis

It was intended that an analysis of variance (ANOVA) for repeated measurements would be performed on data from each group in order to minimize type II error. Due to the small groups, however, this was not possible in the losartan part of the study (III) (four animals in the drug group), and the Mann-Whitney U-test for non-parametric data was performed in that group. Data from all other groups (I, II, IV) was examined using ANOVA. Bonferroni's t-procedure (II) and Scheffé's multiple comparison (IV) were used for a post hoc examination of the ANOVA results. All statistical analyses were performed using the StatView for Macintosh™ software package (SAS Institute Inc, North Carolina, U.S.A., version 5.0). A p-value less than 0.05 was considered to denote statistical significance.
Differences between post-infarct and post-fluid loading hemodynamic values were examined using the two-tailed t-test. A p-value less than 0.05 was considered statistically significant.
5 Results

5.1 Aminophylline

Aminophylline had no significant effect on any hemodynamic parameters at the 50 mg dose (I), although slight and transitory changes were seen in CI (p = 0.1097) and HR (p = 0.1382) (see Figure 7). The 100 mg dose effected a positive chronotropic effect (p = 0.0020), as well as causing a decrease in left heart afterload. This was reflected in decreases in MAP (p = 0.0041) and SVRI (p = 0.0012) (see Figures 7 and 9). Again, changes in CI (p = 0.1409) and PVRI (p = 0.2156) tended towards a clear, but transitory improvement, which was not statistically significant and had reverted within 30 minutes of the dose.

At 200 mg, the aforementioned changes were amplified, but the shift in CI (p = 0.0112) and PVRI (p = 0.0460) were still short-lived. There was a significant positive chronotropic effect (p < 0.0001), which remained even after the changes in CI and PVRI had reverted. A transient, non-significant decrease was also observed in SVRI (p = 0.0653), but no change in MAP.

The increase in HR was reflected in a gradual decrease in SI (see Figure 9), which although not significant between adjacent measurements (i.e. after any single dose), had decreased significantly between administration of the first and last dose (p < 0.0001).

5.2 Adenosine

Both right ventricle preload (CVP) and left ventricle preload (PCWP) remained unchanged during the adenosine infusion (II). The following changes could be observed in the parameters describing right ventricle afterload (MPAP and PVRI). MPAP was reduced by the adenosine infusion, although this difference was not significant (p=0.3). PVRI was also reduced, which change was statistically significant (p<0.01)(see Figures 7 and 8).
Similarly, the changes in left ventricle afterload were as follows. MAP was reduced by the adenosine infusion, which was especially significant (p<0.01) at the 75 and 100 µg/kg/min infusion rates. ANOVA showed a significant decrease (p<0.01) in SVRI during the infusion (see Figure 10).

There was a general improvement in pump performance during the adenosine infusion. CI remained elevated after the fluid loading, while values in the control group dropped back down to the baseline, indicating an inotropic effect of the infusion (see Figure 7). This between-group difference was not, however, statistically significant. RVSWI remained unchanged by the infusion, while a decrease was observed in LVSWI at the higher infusion rates (Scheffe p<0.01, see Figure 9). An improvement was observed in SI (p<0.01). All changes observed during the drug infusion reverted after cessation of the drug (p<0.01).

No change was observed at any infusion rate in HR, while it increased (p<0.01) after cessation of the drug (See Figure 7).

5.3 Losartan

Right ventricle preload (CVP) exhibited a significant decrease (p<0.05) after the administration of losartan (III) as did left ventricle preload (PCWP) (p<0.05), which latter change had reverted, however, by the 45 minute mark. No change was affected in HR by the drug in comparison to the control animals (see Figure 7).

Concurrently, no statistically significant changes were observed in the parameters describing right ventricle afterload (MPAP and PVRI) (see Figures 7 and 9). Similarly, the left ventricle afterload parameters (MAP and SVRI) were not significantly affected by the bolus dose of losartan (see Figures 7 and 9).

A significant enhancement in cardiac performance took place after the administration of losartan. CI, LVSWI and SI were all increased, remaining elevated for the first 30 minutes of the follow-up period (see Figures 6 and 8).

5.4 Nitric oxide

Insufflation of nitric oxide decreased right ventricle (RV) preload (IV), which was seen as a progressive drop in CVP (p<0.05 at 15 ppm), and in EDVI (p<0.01 at 15 ppm). A slight and progressive decrease in left ventricle (LV) preload (PCWP) (p<0.05 at 15 ppm) was observed during the nitric oxide treatment.

RV afterload was monitored through MPAP, PVRI and ESVI. A significant decrease (p<0.01 at all levels of insufflation) was observed in MPAP during nitric oxide treatment (see Figure 8). PVRI, on the other hand did not exhibit any significant changes during the nitric oxide-insufflation (see Figure 10). This is likely due to the progressive deterioration (p<0.05 at all levels) in cardiac index (PVRI = [MPAP-PCWP]x80/CI). ESVI remained stable throughout the drug treatment.
The parameters characterizing LV afterload (SVRI, MAP) were in partial discord, as MAP tended to decrease, especially at the higher levels (p<0.01 at 15 ppm), while SVRI increased steadily (p<0.01 at 15 ppm) (see Figures 7 and 9).

The hemodynamic parameters characterizing inotropy (CI, SI, LVSWI, RVSWI) displayed a progressive deterioration during the nitric oxide treatment (see Figures 6 and 8). This was statistically significant at all levels of the insufflation (p<0.05).

A slight rise in heart rate was observable during the drug inhalation (p<0.05 at 15 ppm) (see Figure 7).

The final bolus fluid-loading dose effected an improvement in both right heart and global cardiac performance (see Figure 7).
Fig. 7. Percentual changes in cardiac index and rate heart in the drug and control groups during the study period.
Fig. 8. Percentual changes in mean arterial pressure and mean pulmonary artery pressure in the drug and control groups during the study period.
Fig. 9. Percentual changes in stroke volume and left ventricular stroke work indices in the drug and control groups during the study period.
Fig. 10. Percentual changes in pulmonary vascular resistance and systemic vascular resistance indices in the drug and control groups during the study period.
Table 6. The qualitative effects of the drugs under study on different hemodynamic parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aminophylline</th>
<th>Losartan</th>
<th>Adenosine</th>
<th>Nitric oxide</th>
<th>Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>↑ (50 mg)</td>
<td>↑</td>
<td>↑</td>
<td>↓ (10 ppm)</td>
<td>↑</td>
</tr>
<tr>
<td>CVP</td>
<td>↓ (200 mg)</td>
<td>↓</td>
<td>↔</td>
<td>↓ (15 ppm)</td>
<td>↑</td>
</tr>
<tr>
<td>EDVI</td>
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<td>↓ (50 µg/kg/min)</td>
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Minimal drug dosage at which the effect was statistically significant are given in parentheses. See original articles I-IV. ↑ = increase, ↓ = decrease, ↔ = no change.
6 Discussion

6.1 Aminophylline

Several earlier studies in humans have indicated that aminophylline used intravenously or theophylline orally will improve hemodynamics in right heart failure (Matthay & Mahler 1986, Falicov et al. 1973, Rutherford et al. 1981, Conradson 1984). Improvement in both global myocardial performance (Matthay & Mahler 1986, Falicov et al. 1973, Parker et al. 1966, Rutherford et al. 1981, Matthay 1985) and right heart performance have been demonstrated with the use of intravenous aminophylline (Matthay et al. 1978, Bisgard & Will 1977). It is likely that the improvement observed in these earlier studies were to some extent reliant on an improvement in left ventricle function, which will have subsequently helped unload the right ventricle and thereby improved right ventricle performance.

Theophylline has been shown to improve hemodynamics to some extent through an augmentation of myocardial contractility as well as through its effect on afterload via broncho- and vasodilatation (Matthay 1987). The present study was therefore designed to examine the effects of aminophylline on left and right heart separately. The results demonstrated a decrease in overall cardiac performance induced by aminophylline, which was seen as reductions in ventricular stroke work and stroke volume, and an increase in heart rate. These can best be explained as reflecting a reduction in left-side preload through pulmonary vasodilatation.

An important factor confounding an analysis of hemodynamic data in studies on right heart failure is that the failure itself is often secondary to a concomitant respiratory disease (COPD, ARDS, ARF) (Matthay 1985). The drop observed in PVR in these studies may therefore be more a result of vasodilatation secondary to bronchodilation.

The results from some earlier studies on the use of aminophylline have demonstrated either a decrease in PVR (Murphy et al. 1968, Bisgard & Will 1977) or no change (Rall & West 1963, DiBianco et al. 1980, Harasawa & Rodbard 1961) in studies involving pulmonary hypertension and/or chronic right heart failure. Matthay et al (1982) showed that orally ingested theophylline moderately improved both right and left ventricle performance in COPD patients. These changes were similar after long-term treatment
More recent reports from studies performed in lambs (Schreiber et al. 1992, Konduri et al. 1992), however, suggest that aminophylline is a poor pulmonary vasodilating agent and that it inhibits the pulmonary vasodilator effect of endogenous adenosine. The present study employed animals with an otherwise healthy vasculature, in which the wall of the pulmonary arteries contain very little muscle (Ganong 1993).

Some earlier studies have mirrored the findings from the present report that hemodynamic changes only become apparent when using larger doses of aminophylline (Mols et al. 1993). The results demonstrated an only modest decrease in pulmonary vascular resistance and a similar increase in cardiac output at a 200 mg dose. These changes were reflected in the systemic circulation as a decrease in systemic vascular resistance. The effect of the previously healthy pulmonary vasculature on the response to aminophylline can only be speculated on.

An interesting observation made in this study was how short-lived the effect of even a relatively high bolus dose of aminophylline was. All improvements observed in hemodynamic parameters were visible ten minutes after the dose, but – for the most part – had reverted by 30 minutes from the dose. Heart rate remained elevated, however, through to the end of the experiment. DiBianco (1980) made similar observations in his study comparing aminophylline to sodium nitroprusside, showing that hemodynamic changes had reverted within 60 minutes of cessation of its infusion.

The more recent studies of the drug have concentrated on its global hemodynamic effects, not on specific models of right heart failure of infarct, nor has the examination of the results been weighted towards right heart function. Brown and coworkers (1991) examined the relative effects of noradrenaline, theophylline and dibutyryl cAMP on isolated rat atria and ventricle tissue. Their findings indicated that, while each of the substances had a similar chronotropic effect on the tissue, noradrenaline’s inotropic effect was significantly more pronounced than that for theophylline or dibutyryl cAMP.

6.2 Adenosine

Due to adenosine’s extremely short half-life (approximately 10 seconds), the drug must be infused centrally to obtain the desired effect on the pulmonary vasculature. By titrating the infusion rate, its vasodilative effects can be localized in the pulmonary circulation without concurrent systemic changes (Haywood et al. 1992, Morgan et al. 1991, Utterback et al. 1994). In these studies, the decrease in PVRI has been thought to be due to unloading of the right heart, which has consequently lead to an increase in cardiac output. In Utterback’s study (1994), however, the increase in cardiac output only occurred after a corresponding decrease in mean arterial pressure. The maximum infusion rate without decreases in systemic vascular resistance or blood pressure (i.e. a high transpulmonary extraction rate) has been reported in the literature as varying between 30 (Morgan et al. 1991, Utterback et al. 1994) and 100 µg/kg/min (Haywood et al. 1992). In the present study, a significant decrease was observed in SVRI in all animals when the infusion rate was increased to 75
µg/kg/min (see figure in Results). Similar variations were observed in the reduction in MAP, but not in the improvement in introply.

Haywood’s results (1992) showed no change in pulmonary arterial pressure, but a significant increase in wedge pressure and cardiac index, which in turn lead to the observed decrease in PVR. No unloading of the right ventricle was observed in that study, as judged by the figures given for right side preload. Interestingly, no change was observed in SVR in that study, even at an infusion rate of 100 µg/kg/min adenosine. Fullerton, however, demonstrated a distinct decrease in MPAP (right heart afterload) without a concomitant decrease in MAP (systemic afterload) (Fullerton et al. 1996). This was accompanied by a significant increase in cardiac output. In the present study, infusion rates above 50 µg/kg/min caused systemic vasodilation, which were apparent as decreases in MAP, LVSWI and SVRI.

Adenosine has been shown to improve hemodynamics in other ways than through decreasing afterload by vasodilation. According to Berne’s “adenosine hypothesis” (1963), endogenous adenosine regulates coronary vasodilation in reduced myocardial oxygen supply, increasing coronary flow. Although several other mediators of coronary flow have been suggested (e.g. nitric oxide, bradykinin, endothelin), adenosine appears to be an important factor in this respect (Mubagwa et al. 1996). Adenosine also exerts negative chronotropic and dromotropic effects (Pelleg & Belardinelli 1993), which was apparent in this study subsequent to the discontinuation of the infusion when a significant increase in HR was observed.

Adkins and associates (1993) demonstrated in an isolated canine lung model that the PVRI-attenuating effect of adenosine appears to be associated with the A2 receptors, which is the main type of adenosine receptors in the pulmonary circulation (McIntyre et al. 1997). Extrapolating these results to intact models or clinical situations is problematic, however, as the isolated-lung model does not take into account the other hemodynamic effects associated with adenosine. These may include improved pump performance (Öwall et al. 1991, Fullerton et al. 1996, Öwall et al. 1988, Reid et al. 1990), and the systemic vasodilation observed at higher infusion rates, as observed in the present study.

### 6.3 Losartan

Despite the fact that the vasodilative effect of losartan is well documented (Smits et al. 1993, Duan et al. 1995), no changes were observed in our study in systemic or pulmonary pressure or in systemic vascular resistance. Compensation must have taken place to counteract the vasodilation in the form of an increase in cardiac output. In agreement with this, Capasso and associates showed an improvement in inotropy (dP/dt) in animals treated with losartan, but not in those treated with captopril or in the controls in their study on the effects of losartan and captopril after left ventricle infarction in rats (Capasso et al. 1994). In addition, while captopril caused a significant reduction in afterload, this was not observed with losartan, which is also in agreement with our findings. Those authors hypothesized that the improvement in inotropy might have been partially brought about by
a prevention of angiotensin II’s indirect negative inotropic effect on the heart (Capasso et al. 1994), though the decrease in afterload will undoubtedly have also contributed to the improvement in pump performance.

Smits and coworkers (1992) showed no improvement in cardiac output in their experimental study of losartan in rats with myocardial infarct, although myocardial remodelling was somewhat attenuated by the drug. Several other experimental studies have also examined the effect of angiotensin II inhibition in different models of heart failure (Richard et al. 1993, Nunez et al. 1997, Irlbeck et al. 1997, Stebbins & Symons 1995) and found no improvement in myocardial contractility or cardiac output. These latter studies examined non-infarct models, however, in which the circulating levels of angiotensin II can be expected to differ from those in an infarct study model. Other studies have shown varying degrees of improvement in cardiac output during losartan treatment (Fitzpatrick et al. 1992, Dickstein et al. 1994, Crozier et al. 1995). In some of these (Dickstein et al. 1994, Crozier et al. 1995), however, a dose-dependent decrease in left heart afterload was also observed, which was not observed in the present study. Dickstein (1994) concluded that losartan should be of benefit to patients suffering congestive heart failure. The results from the present study are in good agreement with Dickstein’s and Crozier’s studies with respect to the maintenance of heart rate and left ventricle afterload as well as the increase in cardiac output.

An interesting observation in this study was that while the increase in cardiac performance was relatively short-lived (approximately 30 minutes), the decrease in right heart preload (CVP) persisted for the entire 60 minute follow-up. It might be conjectured that the higher levels of losartan circulating immediately after its injection caused a total inhibition of angiotensin II action, thereby improving inotropy and inducing vasodilation. As the level of circulating losartan decreased, it may be that - while there was not sufficient angiotensin II inhibition to maintain the improvement in inotropy - perhaps that required to cause vasodilation is sufficiently less for the effect to still be observable. In a recent study (Lankford et al. 1997) examining the pharmacokinetics of losartan in pigs, it was demonstrated that EXP3174, losartan’s most active metabolite in humans, was formed from less than 2% of the losartan dose administered intravenously. In addition, while in humans EXP3174 has a half-life three times that of losartan (2.1 vs. 6.3 hours), in swine the two have a half-life roughly equal to each other, and are otherwise much shorter than in humans (approximately 40-50 minutes). The consequence of this is that losartan has a relatively short-lived effect in the pig, whereas in humans it is substantially longer.

One of the arguments for the use of a specific AT1-inhibitor is that even when adequate ACE-inhibition is attained, circulating levels of angiotensin II are still found in some patients, which may even attain pre-ACEI levels (Willenheimer et al. 1999). This phenomenon has raised speculation of other enzymes capable of converting angiotensin I to angiotensin II and human heart chymase has been suggested to be potentially important in this respect (Urata et al. 1993). Another reason may be that tissue ACE may not be effectively inhibited (Willenheimer et al. 1999). On the other hand, the use of AT1 receptor blockers may be less efficient than ACE-inhibition due to not inhibiting the breakdown of bradykinin with its inherent benefits to the cardiovascular system (Willenheimer et al. 1999). Some evidence exists, however, which indicates that bradykinin may cause an increased release of noradrenaline from sympathetic nerves in the ischemic myocardium, in
which case increases in bradykinin levels would be deleterious (Seyedi et al. 1997). In contrast to the above studies, Stauss and coworkers (1994) have presented evidence supporting the use of ACE-inhibitors after infarct in their study on rats. They administered an ACE-inhibitor or losartan to rats before and after experimental infarct and found a decrease in infarct size, a prevention of any increase in cardiac weight and a decrease in end-diastolic pressure in ACEI-treated rats compared to the losartan group. A similar study by Hartman and coworkers (1993) came to the same conclusions.

6.4 Nitric oxide

In other studies examine the effects of nitric oxide on pulmonary vascular resistance. Semigran and associates (1994) compared the inhalation of nitric oxide via a positive expiratory end-pressure (PEEP) mask at 20, 40 and 80 ppm to the use of iv nitroprusside in patients with NYHA III-IV (left-)heart failure symptoms awaiting consideration for heart transplant. They showed that nitric oxide significantly reduced PVR at 80 ppm, this change exceeding that obtained using nitroprusside at the maximum tolerated dose. They also observed that the reduction in PVR was specific to the pulmonary circulation (i.e. no concomitant reduction in left heart function), which was not the case for nitroprusside. They concluded that it offers a viable alternative as a specific pulmonary vasodilator. At present, however, significantly smaller doses of nitric oxide are in use in clinical practice, with 20 ppm being generally recognized as the maximum used for patients with pulmonary distress such as ARDS. One of the basic precepts of our protocol was that we wished to only examine the hemodynamic effects of nitric oxide at clinically relevant doses. In their recent study, Matsumoto et al (1997) came to similar conclusions using exercise capacity during nitric oxide inhalation in patients with chronic heart failure. The doses used in that study were 0, 10, 20 and 40 ppm. Our results demonstrated a reduction in mean pulmonary pressure somewhat similar to Semigran’s and Matsumoto’s, but with the important difference that this was accompanied by a concomitant decrease in cardiac index. This change was even more pronounced than that observed in the animals in the control group (see Figure 7). Unfortunately, the findings from these two groups cannot be directly compared due to differences in the anesthesia techniques used for the nitric oxide-treated animals and for all others in this study.

In a report by Bocchi et al (1994), the authors found that nitric oxide caused pulmonary edema in cases of severe heart failure, possibly associated with an increase in pulmonary capillary wedge pressure during nitric oxide-inhalation. They suggest that nitric oxide will not cause any improvement in cardiac index, but may improve pulmonary ventilation and hemodynamics. Other studies have also raised concern as to whether nitric oxide might have a negative inotropic effect (Kelm et al. 1997), but Goldstein and associates (1997) concluded that the drug does not impair ventricular contractility. Their study protocol involved thrombaxane-induced pulmonary hypertension, however, and it might be argued that any conclusions drawn from observations of the combined effects of these two vasoactive neurohumoral drugs will be complicated at best. The results from the present
study tend to support a depressive effect for nitric oxide, especially with regards cardiac index and the pulmonary circulation (MPAP).

6.5 Methodological aspects

In the study under review, the following points concerning methodological questions should be considered.

6.5.1 Animals

The animals chosen for the study were young pigs (3-4 months old), of mixed sex and breed. They were raised on a nearby farm for the purpose of meat production and the only criteria for inclusion in this study was that they be of apparent good health and that they weigh between 25 and 30 kilograms for the sake of conformity. The rationale behind choosing the pig as a study animal is that - due to its many inherent physiological similarities with man - this animal has been used with increasing frequency over the past years in many fields of experimental medicine. The use of this animal therefore eases both the comparison of results with other studies and the extrapolation of the data obtained to clinical practice.

A species-specific drawback associated with the use of the pig was the very short metabolism of losartan compared to that seen in humans. This is partially due to a shorter half-life in pigs of losartan itself and partially due to the non-formation of EXP3174, losartan’s active metabolite in humans (Lankford et al. 1997). The effect of an intravenous bolus dose of losartan shown in our previous pilot study to completely block angiotensin II activity therefore wore off within 30 to 45 minutes. A dose of similar effect in humans (50 mg orally) will be effective at least 8 hours (Lo et al. 1995).

Apart from these drawbacks, the pig proved to be a highly suitable animal for this study. There is a high degree of similarity between the anatomy and physiology of the pig and human cardiovascular systems. In addition, the animal is readily available, relatively inexpensive to procure, and due to its large size can be monitored in a manner similar to that used in the clinical monitoring of human subjects.

6.5.2 Anesthesia

The balanced anesthesia used in the present series of studies was that generally acceptable in clinical practice. The use of intravenous bolus doses of a barbiturate (tiopentothal) will cause some attenuation in cardiac performance (Rosenberg et al. 1995), but this will have corrected itself by the start of the study - the first pre-sternotomy hemodynamic
measurements generally being performed one hour or more after the induction of anesthesia. Care was taking in choosing the pre-induction drugs (ketalamine, midazolam and atropine) that they would cause as little attenuation of hemodynamics as possible. The use of enflurane is also associated with minimal changes in hemodynamics, although other inhalational agents (e.g. sevoflurane) might have had less effect in this respect (Yamada et al. 1994).

A different mode of anesthesia was necessary in the nitric oxide study, as the respiratory device used in that study (the Dräger Evita-4 respirator device [Drägerwerk AG, Lübeck, Germany]) did not allow the use of an inhaled anesthetic. This in turn rendered a comparison between this group and the control group inexact and such a comparison was therefore abandoned in that part of the study.

6.5.3 Choice of right heart infarct model

The purpose of this study was to examine the effects of various drugs on hemodynamics during right heart failure. The choice of right heart infarct was made as this provided an acute model of right heart failure in which the hemodynamic effects of the treatment regimes could be examined in its very early stages. This in turn addresses a distinct clinical subset of patients suffering from acute heart failure after right heart infarct, as opposed to those with valvular or pulmonary disease, in whom the disease and symptoms will have gradually developed over an extended period of time. This distinction is important when considering the clinical implications of the results of this study, as the etiology and morphology of acute and chronic heart failure differ from each other in important ways. Perhaps the most important of these with regards overall hemodynamics are the changes in the right heart and pulmonary blood vessels associated with heart failure. These include a thickening of the right heart myocardium and an enlargement of the right ventricle. The walls of the pulmonary arteries will thicken and become more muscular over time, until they eventually resemble the systemic arteries. This allows for a higher degree of effect by medication on both the right ventricle and the pulmonary vessels, resulting in, for example, a more effective vasodilatation and unloading of the right heart by vasodilators and a greater degree of inotropy by beta-adrenergic agents. While there are some reports supporting the use of nitric oxide in cases without significant pulmonary hypertension (De Backer et al. 1996), other studies suggest that pulmonary circulation will only benefit from nitric oxide if a previous pathology exists (Snow et al. 1994, Rich et al. 1994, Bigatello et al. 1994, Loh et al. 1994, Wilson et al. 1997). Our results show a decrease in mean pulmonary arterial pressure and would thus appear to support this latter thesis.

In this model of acute right heart failure after right heart infarct, the animals included in the final analysis were uniformly healthy prior to the study with normal right ventricles and minimal vascular resistance in the pulmonary system. It must be assumed, that the drugs in this study would likely have had more effect on the right heart and on the pulmonary vasculature if the animals had had a larger muscle mass on which the drug to take effect.
Future studies might therefore be targeted to examine the effects of these treatment regimes in a chronic model of right heart failure.

6.5.4 The open chest model

All animals in this study underwent sternotomy and pericardiotomy prior to induction of right heart infarct. The chest and pericardium was subsequently left open, although the sternum was covered with saline-solution-soaked cloths to prevent volume loss through evaporation. It is important to recognize, however, that this model therefore avoids the constricting effect normally exerted by the pericardium and the chest wall. In a clinical situation, as right heart failure develops and the right ventricle expands, the pericardium will cause growth restriction with signs of tamponade and a shifting of the septal wall over to the left side, with a consequential deterioration in left ventricle performance (Romand et al. 1995). In this experimental model, any expansion in the right ventricle would be unimpeded and symptoms of tamponade and attenuation in left ventricle performance would not appear. It is unlikely, on the other hand, that any such significant expansion in ventricle size would have appeared in such a short period, but this mechanism should be kept in mind when analyzing this data for any possible clinical extrapolations. The model itself approximates the situation in which right heart failure occurs after open-heart surgery when coming off perfusion.

6.6 An overview of the results obtained in the present study

The main criteria used for the choice of drugs used in the present study were their pharmacological action with respect to vasodilation, inotropy, chronotropy and half-life. The drug should improve right heart function through an increase in inotropy and a decrease in pulmonary vascular resistance, but should not increase heart rate by any significant degree. In addition, the drug should have a relatively short half-life to render it pulmonary-specific, as vasodilation in the systemic circulation will lead to a decrease in right heart preload, with its attendant attenuation of cardiac performance, as well as a decrease in peripheral perfusion. Using these criteria as a measure, the following conclusions can be drawn regarding the effectiveness of the drugs under study.

6.6.1 Inotropy

Of the four drugs examined, only aminophylline has been shown to exert any direct inotropic effect (Hillis & Been 1982, Crea et al. 1994), while adenosine has been shown to have a negative inotropic effect (Wang & Belardinelli 1994, Belardinelli et al. 1989). Of
the other two drugs under study, nitric oxide should have no effect on myocardial contractility, while reports on the inotropic effects of losartan are somewhat conflicting. Inhibition of angiotensin II should reduce myocardial contractility, and some studies have reported results to this effect (Milavetz et al. 1996, Sladek et al. 1996). Capasso, on the other hand (1994) reported that losartan improves myocardial contractility.

Inotropy (dP/dt) was not directly measured in the present study, but ventricular stroke work indices will closely approximate this measurement (Nelson & Rutherford 1993). Only losartan (III) could be shown to significantly and specifically improve ventricle stroke work in this study (see Figure 9), the other drugs having no effect (I, II) or an attenuating effect (IV) on LVSWI.

6.6.2 Vasodilation

All of the drugs examined in the present study have been shown to exert some degree of vasodilation in previous studies (Matthay 1987, Steudel et al. 1999, Dickstein et al. 1994, Mosqueda-Garcia 1992). The desired effect in this examination was a reduction in pulmonary vascular resistance without a significant drop in systemic vascular resistance. Theoretically, therefore, the drug with the shortest half-life should be the most preferable in this respect. Adenosine and nitric oxide best fit this description and the former did in fact effectively attenuate pulmonary vascular resistance. At higher infusion rates, however, adenosine also had a significant effect on systemic vascular resistance. Aminophylline (200 mg dose) also reduced pulmonary vascular resistance in this study, causing a slight drop in systemic vascular resistance at the same dose.

6.6.3 Chronotropy

With the exception of aminophylline, none of the substances used in this study have been shown to exert a direct enhancing effect on heart rate. The results support this, with a significant increase in heart rate only observed in the aminophylline group after the 100 mg bolus dose.

6.6.4 Cardiac output

Concentrating on any single hemodynamic parameter can be a major pitfall in any examination of the effects of a drug on hemodynamics. The end-point of any analysis of a drug should concentrate on the overall well-being of the subject rather than on isolated parameters. To illustrate this, the effect of simple vasodilation can be considered. If system-wide vasodilation occurs, pulmonary vascular resistance will decrease, which
might well be considered a desirable outcome in cases of right heart failure. At the same time, however, the attenuation of systemic vascular resistance will result in a reduction of right heart preload, which – according to the Frank-Starling principle – will result in a consequent reduction in myocardial contractile force. This can easily result in an overall deterioration of hemodynamics. Of the hemodynamic parameters followed in this study, cardiac output is widely regarded as best reflecting overall cardiac performance. Apart from myocardial contractility, preload, afterload and heart rate also greatly effect cardiac output. It is therefore appropriate to include this parameter as an indicator of overall improvement or deterioration in cardiovascular performance in this study. Adenosine infusion improved cardiac index, especially at the higher infusion rates. This despite the drop in systemic vascular resistance observed at these same doses. Aminophylline and losartan also improved cardiac output somewhat (see Figure 7), while cardiac output deteriorated in the nitric oxide-treated animals.

6.7 The ideal drug in the treatment of right heart failure (due to myocardial infarct)

In their exhaustive review of therapeutics in heart failure, Bonarjee and Dickstein (1996) concluded that optimal therapy will consist of a combination of several drugs. This is due to the wide range of effects desirable in a drug used for the treatment of heart failure. Doyle and associates summed up the characteristics desirable in such a drug (1995), including in this list (1) inotropy without chronotropy, (2) vasodilation and (3) lusitropy.

Based on the findings of the present study, the following recommendations can be made.

1. The ideal drug should increase myocardial contractility and reduce afterload without any increase in energy expenditure. In addition, it should improve myocardial oxygenation through an improvement in coronary blood flow. To this end the drug should be both inotropic and (preferably) lusitropic, as well as being a vasodilator.

2. The ideal drug should not decrease preload, nor should it be chronotropic as - while an increase in heart rate will initially result in an increase in cardiac output (stroke volume x heart rate) - contractility and therefore stroke volume will begin to suffer as the pulse increases to 140-150 beats per minute (Freeman et al. 1994).

Of the drugs examined in the present study, none of them meet the above requirements. It might be concluded that adenosine comes close, being a short-lived vasodilator with negative chronotropic and dromotropic properties, and having been demonstrated to improve coronary blood flow. It does not, however, exert any direct inotropic effect.

The angiotensin II inhibitor losartan also provides an interesting alternative in the treatment of right heart failure. This drug is a potent vasodilator and appears to improve inotropy as well. Its effect is not pulmonary-specific, however, and it has a relatively long effective half-life in humans, which complicates its use in a titrated manner on the ICU.
7 Summary and conclusions

In this study of previously healthy young swine with experimental right ventricle infarct, the following conclusions can be drawn:

1. Aminophylline was demonstrated to cause an appreciable transitory decrease in pulmonary vascular resistance, and a similar short-lived increase in cardiac index. Coupled with an increase in heart rate, however, the overall effect was a decrease in left-side preload and subsequently in cardiac performance. It is recommended that aminophylline be used with caution for pulmonary vasodilation in patients suffering right heart failure due to infarct and that left-heart preload be carefully optimized in these subjects.

2. A continuous infusion of adenosine at various rates was shown to effectively decrease both systemic and pulmonary afterload and thereby improve cardiac output after right heart infarct. The effects on pulmonary pressure were observable, but not as marked. Discontinuation of the infusion led to an increase in both systemic and pulmonary resistances, as well as a decrease in cardiac index. The infusion of adenosine therefore appears to cause an unloading of the right heart and an improvement in inotropy. The vasodilative effect extended to the systemic vasculature at higher doses.

3. Losartan induced a significant improvement in cardiac performance with a concurrent reduction in both right and left heart preload. Of equal importance, both systemic pressures and heart rate were maintained. Due to the longer half-life of this drug in humans, it may be an effective regime in the treatment of heart failure after right heart infarct.

4. Nitric oxide appears to effectively unload the right heart, even in subjects with normal pulmonary vasculature. It also effects a decrease in left-side preload, however, as well as a deterioration in cardiac pump performance and systemic pressures. When using nitric oxide in a clinical situation, special attention should be paid to its effects on left heart preload and myocardial performance.
8 References


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