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ABSTRACT

Catecholamines and Basal Metabolism in the Myocardium

Sonya A. Baik, M.Sc., 1998
Graduate Department of Physiology
University of Toronto

Blood-based cardioplegia used to arrest the heart for cardiac surgery has elevated levels of endogenous catecholamines. However, myocardial exposure to catecholamines in cardioplegia may increase basal metabolism, or oxygen consumption ($\text{MVO}_2$), in the arrested myocardium and contribute to poor post-ischemic functional recovery. Isolated rabbit hearts ($n=5$) were perfusion-arrested at $37^\circ\text{C}$ with 20 mM K+ Krebs-Henseleit buffer and exposed to 25 nM isoproterenol (Iso), which resulted in a significant increase in basal $\text{MVO}_2$ (0.054±0.006 to 0.088±0.009 mL O$_2$/min/g DW; $p=0.0009$). Hearts exposed to 25 nM Iso in cardioplegia followed by 60 min of normothermic ($37^\circ\text{C}$) ischemia and then reperfusion, demonstrated a significant increase in diastolic pressure compared to control (-21.8±3.0 mmHg vs. -12.5±2.0 mmHg; $p=0.02$). Hypothermia ($20^\circ\text{C}$) during ischemia prevented the deleterious effects of Iso. Furthermore, exposure of the beating myocardium to catecholamines prior to arrest was shown to be deleterious to post-ischemic functional recovery of systolic, diastolic, and developed pressures, which was again prevented by hypothermia during ischemia ($p<0.01$). End-reperfusion myocardial ADP, ATP, and total adenine nucleotide concentrations were significantly better preserved in the hypothermia group.
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A big thanks to all the members of the laboratories of my program committee. From Dr. Rebeyka's lab, the support of Andrea Konig, Jill Waddell, and especially Dr. Yoshi Saiki, was tremendous. I greatly appreciated all the time and advice provided by the members of Dr. Wittnich's laboratory (Karim Bandeli, Mike Belanger, Cathy Boscarino, Shona Torrance, and Jack Wallen) as well as by members of Dr. Backx's laboratory.

Finally, thanks to Claire Coulber, Joan Jowlabar, and Susy Taylor at the Division of Cardiovascular Research at the Hospital for Sick Children for their constant support and help.
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<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>AP</td>
<td>action potential</td>
</tr>
<tr>
<td>atm</td>
<td>atmosphere</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<td>BDM</td>
<td>2,3-butanedione 2-monoxime</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
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<tr>
<td>Ca²⁺</td>
<td>calcium ion</td>
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<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
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<td>Cl⁻</td>
<td>chloride ion</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
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<tr>
<td>CO</td>
<td>cardiac output</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>Cp-Iso</td>
<td>treatment with 25 nM Iso in cardioplegia</td>
</tr>
<tr>
<td>CRC</td>
<td>Ca²⁺ release channel</td>
</tr>
<tr>
<td>d</td>
<td>day</td>
</tr>
<tr>
<td>DevP</td>
<td>developed pressure</td>
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<tr>
<td>DP</td>
<td>diastolic pressure</td>
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<tr>
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<td>E_cl</td>
<td>equilibrium potential of Cl⁻</td>
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<td>equilibrium potential of K⁺</td>
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<td>membrane potential</td>
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<td>Esm</td>
<td>esmolol</td>
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<td>HPLC</td>
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<tr>
<td>I_Kr</td>
<td>rapidly activated component of delayed rectifier K⁺ current</td>
</tr>
<tr>
<td>I_Ks</td>
<td>slowly activated component of delayed rectifier K⁺ current</td>
</tr>
<tr>
<td>I_k1</td>
<td>inward rectifier K⁺ current</td>
</tr>
<tr>
<td>I_to1</td>
<td>Ca²⁺-dependent transient outward K⁺ current</td>
</tr>
<tr>
<td>I_to2</td>
<td>voltage-dependent transient outward K⁺ current</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
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<tr>
<td>i.m.</td>
<td>intramuscular</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>Iso</td>
<td>isoproterenol</td>
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<tr>
<td>K</td>
<td>20 mM K+KHB solution (cardioplegia)</td>
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<tr>
<td>K+</td>
<td>potassium ion</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>KHB</td>
<td>Krebs-Henseleit buffer</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
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<td>min</td>
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</tr>
<tr>
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<td>millilitre</td>
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<tr>
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</tr>
<tr>
<td>mV</td>
<td>millivolt</td>
</tr>
<tr>
<td>MVO2</td>
<td>myocardial oxygen consumption</td>
</tr>
<tr>
<td>Na+</td>
<td>sodium ion</td>
</tr>
<tr>
<td>NAD+</td>
<td>nicotinamide dinucleotide (oxidized)</td>
</tr>
<tr>
<td>NADH</td>
<td>nicotinamide dinucleotide (reduced)</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
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<tr>
<td>nM</td>
<td>nanomole/L</td>
</tr>
<tr>
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<td>oxygen</td>
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<tr>
<td>PFK</td>
<td>phosphofructokinase</td>
</tr>
<tr>
<td>pg</td>
<td>picogram</td>
</tr>
<tr>
<td>P_i</td>
<td>inorganic phosphate</td>
</tr>
<tr>
<td>PK</td>
<td>protein kinase</td>
</tr>
<tr>
<td>Prz</td>
<td>prazosin</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SP</td>
<td>systolic pressure</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
</tr>
<tr>
<td>TAN</td>
<td>total adenine nucleotides</td>
</tr>
<tr>
<td>Tn-C</td>
<td>Troponin C</td>
</tr>
<tr>
<td>Tn-I</td>
<td>Troponin I</td>
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<td>U</td>
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<td>Symbol</td>
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<td>--------</td>
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</tr>
<tr>
<td>μL</td>
<td>microlitre</td>
</tr>
<tr>
<td>μm</td>
<td>micrometre</td>
</tr>
<tr>
<td>μM</td>
<td>micromole/L</td>
</tr>
<tr>
<td>WW</td>
<td>wet weight</td>
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1 INTRODUCTION

Overview of Cardiac Muscle Physiology

Cardiac muscle is comprised of thick filaments, made of myosin, and thin filaments, made of actin [Darnell et al., 1990; Katz, 1992]. Myosin molecules comprising the thick filaments have a long, rod-like tail on one end and two globular heads at the other end. There are two flexible joints, called hinges, along the junction of the head and tail regions. Within the myosin filament, the myosin molecules are arranged laterally and antiparallel to each other, with the globular heads projecting out from the thick filament at regular intervals. The actin-stimulated ATP-ase activity is contained in the myosin heads which project from the thick filaments to form cross-bridges with adjacent actin thin filaments [Darnell et al., 1990; Katz, 1992].

The actin thin filament is composed of a double helix of F-actin which is polymerized globular actin (G-actin) [Darnell et al., 1990; Katz, 1992]. Associated with the actin are four proteins which mediate the regulatory role of Ca^{2+} in contraction. Filamentous tropomyosin molecules lie in one groove of the actin double helix and have specific binding sites on the actin filament. Three troponin proteins called troponin T (Tn-T), troponin I (Tn-I), and troponin C (Tn-C), are bound to specific sites on each tropomyosin molecule. In the absence of Ca^{2+}, troponin and tropomyosin inhibit myosin ATPase activity by inhibiting the interaction of the myosin heads with actin. Tn-I and tropomyosin, together, cause a conformational change in the actin, such that weak binding with myosin heads occurs, thereby preventing the myosin ATPase activity. When Tn-C binds Ca^{2+}, the myosin heads bind to the exposed actin, thus activating myosin ATPase [Darnell et al., 1990; Katz, 1992].

Each cardiac muscle fibre is surrounded by mitochondria which are used for generation of ATP [Darnell et al., 1990; Katz, 1992]. The myosin-catalysis of ATP to ADP and P, acts as the fuel for muscle contraction. The first step in the cyclic process of
muscle contraction can be considered to be the time when ATP binding to myosin weakens binding of myosin heads to actin, thereby relaxing the muscle fibres [Darnell et al., 1990]. The hydrolysis of myosin-bound ATP occurs with little change in free energy as the ADP and P, hydrolysis products remain bound to myosin, producing an "energized" state. The release of these bound products from myosin are strongly exergonic steps, however, and the free energy released is used to power the pivoting movement of the myosin head. The myosin pivots via its hinges to be perpendicular to the actin filament, and then binds to the adjacent actin if the internal Ca2+ concentration is high enough and, concomitantly, P, is released. Once attached to actin, and with the release of ADP, the myosin head pivots again on its hinge, thereby moving the actin filament relative to the fixed myosin and resulting in contraction. The product of this contraction step is called the "rigor complex" because the actin-myosin linkage is inflexible and the two filaments cannot move past each other. Subsequent binding of ATP to the myosin head releases the myosin head from the actin, relaxing the muscle and re-commencing the cycle of contraction. Thus, as long as the intracellular Ca2+ concentration is sufficiently high and ATP is present, the myosin-action cross-bridges will cycle continuously and the muscle will contract [Darnell et al., 1990; Katz, 1992].

Cardiac Action Potential

The high intracellular Ca2+ concentration necessary for contraction is achieved through the cardiac action potential (AP) [Darnell et al., 1990; Katz, 1992; Weiss, 1997]. Briefly, using the Purkinje fibre cell as an example, Phase 0 is the AP upstroke, which is carried predominantly by the Na+ current via voltage-dependent Na+ channels. The inward Na+ current must be large enough to depolarize the adjacent cells to the threshold of their Na+ channels (-60 mV), ensuring rapid propagation of the cardiac impulse [Katz, 1992]. Rapid inactivation ensues to minimize unnecessary Na+ influx because the removal of Na+ depends primarily on the energy-dependent Na+/K+-ATPase pump. Furthermore, when
depolarization reaches the Ca^{2+} current threshold (-35 mV), the voltage-dependent L-type Ca^{2+} channels open to generate a second depolarizing current [Katz, 1992]. After the AP reaches its peak amplitude in Phase 0 (+30 mV), activation of the Ca^{2+}-dependent (I_{m1}) and voltage-dependent (I_{m2}) components of the transient outward K+ current occur. This is Phase 1, or early rapid repolarization [Katz, 1992; Weiss, 1997].

Slow repolarization, however, follows in Phase 2 because the Ca^{2+} current activates and inactivates more slowly than the Na+ current, and thus maintains the membrane in a depolarized state [Weiss, 1997]. This effect creates the plateau in the profile of the cardiac action potential. Early in the plateau phase, the slow inward Ca^{2+} current is high but, toward the end of the plateau, as the Ca^{2+} channels inactivate, the delayed rectifier currents open and are largely responsible for repolarization. The AP plateau of Phase 2 is therefore determined by inactivation of the L-type Ca^{2+} current and activation of delayed rectifier K+ currents, I_{kr} and I_{ks}. I_{kr} activates rapidly and also inactivates, whereas I_{ks} activates slowly with depolarization and does not inactivate during maintained depolarization. The delayed rectifier currents determine the duration of Phase 2 and contribute to Phase 3 [Weiss, 1997].

Towards the end of the plateau phase, the rate of repolarization accelerates, primarily due to a rapid increase in I_{kr} and I_{k1} [Weiss, 1997]. As the membrane potential further repolarizes during Phase 3, or late rapid repolarization, outward current through I_{kr} and I_{k1} increases under positive feedback. I_{kr} controls the initial onset of the rapid depolarization phase and I_{k1} controls the latter portion. I_{k1} is the background strong inward rectifier K+ current which stabilizes the resting membrane potential near E_K due to increased membrane conductance when E_m is near E_K. I_{k1} is inactivated during depolarization and prevents hyperpolarization by currents associated with the Na+/K+-ATPase pump [Weiss, 1997].

Following rapid repolarization to the maximum diastolic potential (-90 mV in Purkinje cells), two time-dependent currents change to contribute to the I_{k1}-maintained

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Phase 4, or diastolic depolarization [Katz, 1992; Weiss, 1997]. An inward Na+/Ca\(^{2+}\) exchange current progressively decays as the intracellular Ca\(^{2+}\) concentration declines, promoting hyperpolarization. In opposition to this is the gradual decay of the delayed rectifier K\(^+\) current, which promotes depolarization [Weiss, 1997].

**Mechanism of Excitation-Contraction Coupling**

The slow inward Ca\(^{2+}\) current of Phase 2 is not, in itself, sufficient to generate contraction throughout the heart. Rather, the Ca\(^{2+}\) influx through the plasma membrane induces an even greater release of Ca\(^{2+}\) stored intracellularly in the sarcoplasmic reticulum (SR), which serves as a reservoir of Ca\(^{2+}\) ions sequestered from the cell cytosol and myofibrils [Darnell et al., 1990]. Structural evidence suggests a direct interaction between the Ca\(^{2+}\)-release channels (CRCs) of the SR and the L-type Ca\(^{2+}\) channels located in invaginations, called T-tubules, of the plasma membrane [Franzini-Armstrong, 1980]. The physical interaction between these two types of Ca\(^{2+}\) channels is believed to account for the mechanism of excitation-contraction coupling [Rios and Brum, 1987]. Depolarization of the plasma membrane induces a conformational change in the voltage-dependent L-type Ca\(^{2+}\) channels which is transmitted to the CRCs, thereby triggering "Ca\(^{2+}\)-induced Ca\(^{2+}\) release" from the SR [Rios and Brum, 1987; Darnell et al., 1990].

**Catecholamines and Beta-Adrenergic Receptor Stimulation**

Catecholamines, or adrenergic agonists, play an integral role in the regulation of myocardial contractility and metabolism. They elicit their effects through alpha-receptors, which mediate a vasoconstrictive response, and through beta-receptors, which mediate a vasodilatory and cardiomyocyte stimulatory response. Although the coronary vascular bed is predominantly characterized by alpha-receptors, the myocardium is predominantly a beta-adrenergic receptor tissue [Katz, 1992; Lindemann and Watanabe, 1995]. In fact, in most mammalian species such as humans [Brodde et al., 1986], dogs [Manalan et al., 1981].
cats [Kaumann and Lemoine, 1985], and rabbits [Tenner et al., 1989]. cardiac beta-adrenergic receptors are of the beta₁-receptor subtype [Bilezikian, 1987; Hieble and Ruffolo, 1991]. The subcellular events following beta-adrenergic receptor stimulation are mediated by cyclic AMP (cAMP) which initiates a cAMP-dependent protein kinase (PK) signalling cascade [Robishaw and Foster, 1989]. A number of sarcolemmal ion channels appear to be regulated by the phosphorylation activity of cAMP-dependent PK. These include the K⁺ (slowly activating component of delayed rectifier) [Walsh and Kass, 1988], Na⁺ [Schubert et al., 1990; Matsuda et al., 1992], Cl⁻ [Harvey et al., 1990] ion channels, and most notably, the Ca²⁺ ion channel [Trautwein and Hescheler, 1990]. Phosphorylation of the sarcolemmal Ca²⁺ channel increases the number of open channels at a given moment, thereby increasing the rate of net Ca²⁺ influx and inward currents [Akera, 1990]. Direct coupling between beta-adrenergic receptor-associated guanine nucleotide stimulatory protein and Na⁺ [Schubert et al., 1989], K⁺ [Yatani et al., 1987], and Ca²⁺ [Yatani et al., 1987b, but refuted by Hartzell et al., 1991] channels have also been reported.

Cyclic AMP-dependent PK also phosphorylates the SR membrane protein phospholamban which upregulates Ca²⁺ sequestration by the SR Ca²⁺-ATPase pumps [Kirchberger and Tada, 1976; Liudemann et al., 1983]. By significantly increasing Ca²⁺ uptake, catecholamines can increase the Ca²⁺ content of the SR, which can lead to greater Ca²⁺ release from the SR and increased activation of the contractile proteins [Hess et al., 1968; Shinebourne et al., 1969; Shinebourne and White, 1970].

Another target for cAMP-dependent PK-mediated phosphorylation is the contractile regulatory protein troponin I which appears to decrease the Ca²⁺ sensitivity of the actomyosin ATPase activity due to an increased rate of Ca²⁺ dissociation from the Ca²⁺-specific binding site on troponin C [Robertson et al., 1982]. As a result of this decreased Ca²⁺ sensitivity as well as the increased Ca²⁺ uptake by the SR, the contractile proteins gain an increased ability to relax [Shinebourne and White, 1970]. Moreover, the decreased Ca²⁺ sensitivity suggests that more of this ion must be released to achieve a given increase in

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force, thereby demanding more Ca\textsuperscript{2+} to be cycled with each beat [Chiu et al., 1989]. Consequently, with the increased intracellular Ca\textsuperscript{2+} levels and the increased rate of Ca\textsuperscript{2+} cycling, catecholamines appear to be able to influence the myofilament cross-bridge cycle by increasing the number of cross-bridges activated per unit time [Hasenfuss et al., 1994], and by decreasing the average force-time integral of the individual cross-bridge cycle due to a decrease in the attachment time and an increase in cycling rate [Hoh et al., 1988; Hasenfuss et al., 1994]. Therefore, the increased Ca\textsuperscript{2+} cycling, the stimulation of contractile protein interaction, and the enhanced relaxant effects mediated by beta-adrenergic stimulation can account for the positive inotropic and chronotropic effects of catecholamines.

**Excessive Catecholamine Stimulation and Myocardial Ischemia**

Normally, catecholamine stimulation is important for the upregulation of myocardial function under conditions of increased stress, such as exercise or trauma, to meet the increased circulatory demands. However, in some situations, the very intensity of the catecholamine response may unwittingly produce a harmful result. In fact, excessive release or administration of catecholamines has been associated with cardiotoxicity [Rona et al., 1959; Rona et al., 1975; Mosinger et al., 1977; Mosinger et al., 1978; Yeager and Iams, 1981; Steen et al., 1982; Muntz et al., 1984; Noronha et al., 1984; Noronha et al., 1985]. Caspi et al. [1993] demonstrated that excessive epinephrine administration to piglets yielded ruptured sarcolemma, mitochondrial swelling, and intramitochondrial dense granules, confirming earlier reports that the characteristic lesion of catecholamine injury was an exaggerated contraction and contraction band formation with mitochondrial granular densities [Bloom and Cancilla, 1969; Csapo et al., 1972]. Furthermore, Rona et al. [1959] discovered that the synthetic catecholamine isoproterenol can produce "infarct-like" myocardial necrosis in experimental animals, where the higher the dose, the more severe the injury [Chappel et al., 1959].
It has been suggested from some studies that the cellular damage resulting from excess catecholamine stimulation is due to myocardial Ca\(^{2+}\) overload secondary to increased sarcolemmal permeability [Rona et al., 1975], whilst others have demonstrated that myocardial Ca\(^{2+}\) overload is crucial in the pathogenesis of catecholamine-induced myocardial necrosis [Bloom and Davis, 1972; Fleckenstein, 1973]. Fleckenstein [1973] documented that isoproterenol administration is followed by increased transport of Ca\(^{2+}\) into the cardiomyocyte. Upon further investigation, Fleckenstein [1983] concluded that the increased myocardial Ca\(^{2+}\) content causes myofilament overstimulation, resulting in increased force and oxygen demand, and deleterious breakdown of high energy phosphate fractions. The exhaustion of high energy phosphates was proposed to determine the catecholamine-induced myocardial necrosis that is not due to ischemia [Fleckenstein, 1983]. In addition, Ca\(^{2+}\)-pump mechanisms at the sarcolemmal and sarcoplasmic reticular membranes are altered in hearts treated with high doses of catecholamines [Dhalla et al., 1983; Makino et al., 1985; Panagia et al., 1985], suggesting a possible contribution to the elevation of the intracellular Ca\(^{2+}\) concentration through membrane leakage.

Yet, catecholamine-induced cell damage is not limited to the non-ischemic model. Catecholamines appear to exacerbate the damaging effects of ischemia by accelerating injury in ischemic or hypoxic tissue [Maroko et al., 1971; Maroko et al., 1973; Vatner et al., 1973; Karlsberg et al., 1979; Muntz, et al., 1984; Yoshida and Iimura, 1989]. In isolated rat hearts, Waldenstrom et al. [1978] found that norepinephrine facilitated the spread of ischemic necrosis, while others have found that after 20-40 min of ischemia, the ischemia itself induces norepinephrine release from myocardial nerve terminals [Schomig et al., 1984]. This release can reach the micromolar range, which is 100-1000 times the normal plasma concentration and enough to promote tissue injury [Muntz et al., 1984; Schomig et al., 1984]. Thus, in a vicious cycle, the damaging effect of catecholamines on ischemic myocardium can worsen with further catecholamine stimulation.
Catecholamine Damage and Ischemic Damage

The ultimate damage effected by excessive catecholamine stimulation mimics the damage mediated by ischemia. Thus, an understanding of the ischemic process may shed some understanding of the catecholamine-induced myocardial lesion. The two processes, however, are not mutually exclusive since prolonged ischemia is characterized by an increase in endogenous catecholamines [Muntz et al., 1984; Schomig et al., 1984]. In ischemia, lack or cessation of coronary blood flow and the consequent lack of oxygen that the myocardium experiences induce a cascade of events affecting the energetic, metabolic, and morphologic aspects of the myocardium. Immediately following the onset of global ischemia, the heart's contractile activity is greatly reduced [Jennings et al., 1969]. Lack of coronary artery perfusion followed by metabolic dysfunction contribute to such functional deterioration [Hearse, 1979; Katz, 1992]. The available oxygen that is dissolved in the cytoplasm of the myocytes is expended within the first few seconds after ischemic onset, upon which anaerobic conditions develop within the cell [Katz, 1992].

Anaerobic glycolysis replaces aerobic glycolysis as the main source of energy for the globally ischemic heart [Katz, 1992]. Both humoral and biochemical mechanisms are involved in the transient acceleration of anaerobic ATP production. Glycolysis is accelerated as an increased level of ADP allosterically stimulates the rate-limiting phosphofructokinase (PFK) reaction [Darnell et al., 1990]. Eventually, however, glycolysis is inhibited as the accumulation of reduced NADH and the lack of oxidized NAD+ inhibit PFK and the glyceraldehyde-3-phosphate reduction steps of glycolysis. As lack of oxygen prevents pyruvate entry into the Kreb's cycle, the pyruvate build-up leads to an accumulation of lactate which gradually contributes to acidosis and inhibition of several glycolytic enzymes, including PFK [Darnell et al., 1990]. Thus, anaerobic glycolysis is only a temporary energy source. Furthermore, this anaerobic metabolic pathway has a very low energy yield of only two moles, versus 36 for the aerobic pathway, of ATP per glucose [Darnell et al., 1990]. Consequently, an energy deficit is generated as the ischemic

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stress continues and as the heart is unable to meet energy demands satisfactorily by a means other than oxidative phosphorylation.

Under ischemia, the demand for energy exceeds supply and ATP is broken down eventually to adenosine which can freely diffuse out of the cell unlike its phosphorylated moieties [Wiedmeier et al., 1972; Snow et al., 1973]. Such a loss may contribute to a delay in the repletion of high energy phosphates which eventually may affect functional recovery. In fact, the decline in ATP levels greatly affects the contractility of the myocardium [Kubler and Spieckermann, 1971; Kubler and Katz, 1977; Hearse, 1979], suggesting a critical role in contractile dysfunction. In the initial stages of ischemia, the activation of ATP-dependent sarcolemmal and intracellular Ca\(^{2+}\)-channels that normally accelerate Ca\(^{2+}\) entry into the cell becomes attenuated, thereby contributing to the negative inotropic effect of ischemia [Katz, 1992]. Conversely, the ATP-dependent sarcolemmal and SR Ca\(^{2+}\)-pumps which are responsible for the efficient efflux of Ca\(^{2+}\) from the cytosol are inhibited, resulting in impaired relaxation. The importance of ATP to cell viability is compounded by the fact that ATP confers allosteric effects on the ion pumps, channels, and actin-myosin interactions which regulate contraction and relaxation [Katz, 1992]. Thus, attenuation of the modulatory role of ATP in Ca\(^{2+}\) movement could even reduce contractility and relaxation without having ATP levels necessarily undergoing severe depletion [Kubler and Katz, 1977].

The balance among the Ca\(^{2+}\) fluxes that relax the heart and those that initiate systole is precarious and favours contraction [Katz, 1992] since activation is less energy-requiring than relaxation. Thus, as relaxation becomes compromised, contracture sets in. Actin-myosin rigor complexes form, which cannot disassemble without ATP, as discussed above. Furthermore, after prolonged ischemia, sarcolemmal damage [Yano et al., 1987] can further disrupt the intracellular environment [Jennings et al., 1969; Korb and Totovic, 1969; Krug, 1970; Kloner et al., 1974]. Excessive Ca\(^{2+}\) entry occurs, activating the contractile proteins even more. Thus, with the progression of the ischemic period, many of

*Introduction*
the affected myocytes become irreversibly injured and will die even if the ischemia is eliminated.

*Mitigation of the Effects of Catecholamine Cardiotoxicity and Ischemic Damage*

The deleterious effects of catecholamines via beta-adrenergic activation are consistent with the observed block of these changes by beta-adrenergic antagonists. Beta-antagonists like propranolol prevent the cAMP accumulation and the structural injury induced by epinephrine in the rat heart. Lubbe *et al.* [1978] used the beta-adrenergic antagonist atenolol to inhibit the increase in cAMP and ventricular fibrillation caused by high doses of epinephrine. Kako [1966] reported that propranolol improved ATP levels in hearts that were stimulated with excessive isoproterenol. Such data suggest that the deleterious effects of excess catecholamine stimulation is mediated by beta-adrenergic receptor activation with subsequent depletion of energy.

Beta-adrenoceptor antagonists have also been used to attenuate the deleterious effects of adrenergic drive often associated with acute myocardial ischemia and infarction and other cardiovascular disease states [Frishman and Silverman, 1979; McDevitt, 1979]. Experimental and clinical studies confirm that beta-adrenergic antagonists have beneficial effects during myocardial ischemia. Beta-antagonists have been shown to limit the size of experimentally-produced infarcts [Libby *et al.*, 1973; Group, 1984; Roberts *et al.*, 1984]. while direct infusion of propranolol into an ischemic area of myocardium preserved high-energy phosphate levels [Goodlett *et al.*, 1980]. Propranolol has also been shown to reduce the ultrastructural changes that occur during the first few hours of coronary occlusion such as mitochondrial swelling and microvascular injury [Kloner *et al.*, 1977]. Thus, beta-antagonists are cardioprotective as a result of an energy-sparing effect upon inhibition of the catecholamine response, whereupon tissue integrity is preserved.
Excessive Catecholamine Stimulation of the Arrested Myocardium

The relevance of, and interest in, excessive catecholamine stimulation can be associated with the clinical situation where patients with congestive heart failure are characterized by elevated levels of circulating catecholamines [Ross et al., 1987] and where diseased hearts of patients undergoing surgery will be subject to excessive catecholamine stimulation induced by the surgery itself [Reves et al., 1982]. In addition, exogenous catecholamines are often administered peri-operatively to support compromised myocardial function, which may contribute to poor post-operative recovery.

Experiments reflecting the clinical scenario have also demonstrated poor post-ischemic myocardial functional recovery. Takla et al. [1989] subjected hearts to either dopamine or dobutamine stimulation prior to K+-arrest and 25 min normothermic global ischemia. Recovery of cardiac output ranged from 47% to 66% in the inotropically stimulated hearts, compared to the control recovery of 80%. Furthermore, Komai et al. [1991] demonstrated that pre-ischemic administration of isoproterenol to working rat hearts severely depressed functional recovery that was attributed to a possible catecholamine-potentiated ischemic-reperfusion injury due to aggravated Ca²⁺ overload. As discussed above, catecholamines may directly induce Ca²⁺ overload which can initiate high energy phosphate breakdown, the "marker" of non-ischemic myocardial necrosis of excessive catecholamines [Fleckenstein, 1973; Fleckenstein, 1983].

In some cases, global myocardial ischemia for surgery is induced with a blood-based cardioplegia of which the blood component is extracted from the patient upon institution of the cardiopulmonary bypass circuit. Yet, at this time of surgery, Reves et al. [1982] have demonstrated that plasma catecholamines rise markedly. Basal plasma epinephrine levels in normal, resting man have been measured to range from 0.05 nM-0.14 nM [Kopin, 1986]. If an average value of 0.1 nM is used, then comparison with measurements of plasma epinephrine levels upon the institution of bypass show an increase ranging from 0.7 nM [Reves et al., 1982] to 3.11 nM [Hine et al., 1976], indicating
approximately a seven- to 30-fold rise. In fact, Rebeyka (unpublished data) found that plasma epinephrine levels had elevated to 2.8 nM from basal levels of less than 0.8 nM in blood destined for blood-based cardioplegia. This raises the question of whether the elevated levels of catecholamines presented to the heart upon cardioplegic infusion could affect the basal state, or basal metabolism, of the arrested heart and whether that effect may be deleterious to post-ischemic functional recovery.

**Catecholamines and Myocardial Oxygen Consumption**

The characteristic catecholamine-induced upregulation of Ca$^{2+}$ cycling and contractile protein activity affect the contractile state [Sonnenblick et al., 1965; Graham et al., 1967], tension development [Hasenfuss et al., 1989], heart rate. and activation [Suga et al., 1983; Nozawa et al., 1988] of the heart, all of which are energy-requiring processes. Since the heart is primarily an aerobic organ that can only afford a small oxygen debt [Harden et al., 1979] and which obtains 90% of its ATP energy from mitochondrial oxidative phosphorylation [Crompton, 1990], the rate of myocardial oxygen consumption ($MVO_{2}$) is quite an accurate measurement of the heart's total metabolism. Therefore, the increase in $MVO_{2}$ accompanying catecholamine stimulation can be considered a reflection of the effect catecholamines have on myocardial metabolism [Eckstein et al., 1950; Fisher and Williamson, 1961; Klocke et al., 1965; Sonnenblick et al., 1965; Gibbs et al., 1967; Coleman et al., 1971; Suga et al., 1983]. If total energy expenditure of the heart is divided into basal and beating components, then an increase in energy demand in either component is reflected by an increase in $MVO_{2}$, which, in turn, has basal and beating components.

**Basal Myocardial Oxygen Consumption**

Basal myocardial oxygen consumption is considered to be the energy required for the regenerative processes necessary to maintain structural and functional muscle integrity and for the processes necessary to maintain ionic and electrical homeostasis [Gibbs, 1978:
Suga, 1990]. Thus, by definition, basal metabolism studies require rendering the heart inactive in order to eliminate the active component of MVO2 and isolate the basal activity of the myocardium. This is done by either withholding electrical stimulation of muscle preparations or cardioplegically arresting whole hearts.

**Cardioplegic Arrest Using Hyperkalemia**

The negative transmembrane potential of the resting cardiac cell represents the activity gradient and membrane permeabilities of the various intracellular and extracellular ions in the non-excited state. The resting membrane potential value is attributable primarily to the K⁺ ion gradient and is closely related to the electrochemical gradient for K⁺ across the plasma membrane, because the resting plasma membrane is most permeable to K⁺ relative to the other ions [Kako, 1966; Weiss, 1997]. Thus, any variation in the extracellular K⁺ concentration directly influences the resting potential and the formulation of the cardioplegic solution exploits this fact. The hyperkalemic property of the cardioplegic solution decreases the K⁺ gradient across the plasma membrane, thereby decreasing the resting membrane potential by depolarizing the cell to a new, more positive potential value. Because depolarization not only opens (activates) Na⁺ channels, but also, eventually, closes (inactivates) them in a voltage-dependent manner, the partial depolarization induced by hyperkalemia ultimately inactivates the Na⁺ channels [Katz, 1992]. In the process of becoming inactivated, the channels that can be activated can only generate a slowly rising AP because there is less of a potential difference and less channels available to contribute to the upstroke. With the more slowly rising action potential, more Na⁺ channels have time to inactivate. This contributes further to reducing the rate and extent of the depolarizing Na⁺ current, until finally, all channels are inactivated.

Repolarization of the myocyte to its original resting membrane potential allows for the voltage-dependent Na⁺ channels to recover from inactivation and thereby, re-open, or re-activate [Katz, 1992]. However, with cardioplegia, all the Na⁺ channels become and
remain inactivated as the new, increased extracellular K\textsuperscript{+} concentration reduces the resting membrane potential and deters any outward K\textsuperscript{+} current needed to repolarize the cell. Thus, the myocyte cannot be stimulated to contract and remains in diastolic arrest.

The diastolic arrest induced by the hyperkalemic cardioplegic solution is rapid and by being so, preserves high energy phosphate reserves important for post-ischemic recovery [Hearse, 1980; Hearse et al., 1974]. Potassium-induced membrane-depolarization, however, is not a perfect solution for myocardial protection from ischemia. Similar to its effect on the Na\textsuperscript{+} channels, the partial depolarization of the membrane by hyperkalemia produces calcium influx through the voltage-dependent Ca\textsuperscript{2+} channels, thereby requiring energy-dependent removal of Ca\textsuperscript{2+}. Specifically, the Ca\textsuperscript{2+} or slow-inward, current is activated at more depolarized membrane potentials than the Na\textsuperscript{+} current (-35 mV vs. -60 mV) and, it activates and inactivates much more slowly than the Na\textsuperscript{+} current [Katz, 1992]. Although the normal inward Ca\textsuperscript{2+} current that accompanies normal APs is inhibited, the hyperkalemic-induced depolarization predisposes the myocardium to accumulation of intracellular Na\textsuperscript{+} and Ca\textsuperscript{2+} via activation of voltage-dependent "window currents" (currents defined by the voltage range over which the steady-state activation and inactivation curves overlap) [Weiss, 1997; Lopez et al., 1996]. Thus, with some imperfection, hyperkalemic cardioplegia electrically and mechanically arrests the heart in diastole, maintaining the heart at a level of basal metabolism.

**Measurement of Basal Myocardial Oxygen Consumption**

Basal MVO\textsubscript{2} values tend to vary depending of the technique and animal species used to measure its value. In general, however, basal MVO\textsubscript{2} has been calculated to range between 0.01-0.03 mL/O\textsubscript{2}/g wet weight, which represents as little as 10% of the estimated 0.08-0.1 mL/O\textsubscript{2}/g value for the MVO\textsubscript{2} of the beating, working heart [Gibbs, 1995]. Table I is a summary of a few basal MVO\textsubscript{2} values obtained from the literature. As shown, basal MVO\textsubscript{2} values can range (in considering only the wet weight values) from as low as 0.0049
mL O₂/min/g in the rabbit [Gibbs and Kotsanas, 1986] to as high as 0.03 mL O₂/min/g in the rat [Burkhoff et al., 1990]. Some working and beating heart MVO₂ values are also given with their corresponding basal MVO₂ values to provide a relative comparison.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Contractile State of Heart</th>
<th>MVO₂</th>
<th>Reference</th>
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<tr>
<td>Dog</td>
<td>Working</td>
<td>0.103</td>
<td>[McKeever et al., 1958]</td>
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</table>

Table I. Beating and arrested state myocardial oxygen consumption (MVO₂) values from the literature. The mean values for the working, empty-beating, and arrested states of the heart vary among species and to a lesser degree, within species. Values are expressed as mL O₂/min/g (wet weight). DW indicates a dry weight value.
Not only does basal MVO₂ vary from species to species [Loiselle and Gibbs. 1979], but it can also vary with the arrest conditions [Penpargkul and Scheuer. 1969; Sternbergh et al., 1989; Burkhoff et al., 1990], the status of the heart prior to arrest [Lochner et al., 1968], and with the metabolic substrate used in the cardioplegic perfusate [Chapman and Gibbs, 1974; Burns and Reddy, 1978; Gibbs and Kotsanas, 1986; Loiselle. 1987]. Hearts subjected to depolarized arrest using hyperkalemia have demonstrated a higher basal MVO₂ than hearts subjected to hyperpolarized arrest using the fast sodium channel blocker, tetrodotoxin [Sternbergh et al., 1989]. The lower basal MVO₂ of the latter type of arrest was attributed to a lower ionic flux during arrest. Hyperkalemic arrest MVO₂ has also been compared to acalcemic arrest MVO₂ but considerable discrepancy exists as some have found depolarized arrest to be lower [Penpargkul and Scheuer. 1969; Gibbs and Kotsanas, 1986], some have found acalcemic arrest to be lower [Burkhoff et al., 1990], while others have found no difference [Kohn and Szymanski. 1963]. In addition, Lochner et al. [1968] found that basal MVO₂ of hearts arrested with hyperkalemia depended on the MVO₂ of the beating state prior to arrest. Hearts that had a higher beating MVO₂ induced by a higher perfusion pressure demonstrated a higher basal MVO₂ upon arrest, even though the perfusion pressure had been equalized among all groups for the arrest period [Lochner et al., 1968].

Studies have also revealed that metabolic substrates affect basal MVO₂. Pyruvate, lactate, and acetate have all been shown to increase basal metabolism [Chapman and Gibbs. 1974; Gibbs and Kotsanas, 1986]. Amino acid mixtures administered to isolated cardiac myocytes or whole hearts have demonstrated increases in basal MVO₂ [Burns and Reddy. 1978; Loiselle, 1987]. These studies, however, could not conclude whether the increase in MVO₂ evolved from increased protein synthesis or increased oxidative metabolism via the tricarboxylic acid cycle. However, Kira et al. [1984] showed that protein synthesis could occur during cardiac arrest without affecting basal MVO₂ and likewise, others have
obtained data suggesting the negligible contribution of protein synthesis to basal MVO$_2$ [Loiselle, 1985; Schreiber et al., 1986].

The unique nature of the cardiac muscle, in contrast to its skeletal counterpart, is evident in its basal activity state. Basal MVO$_2$ has been found to be approximately five times greater in cardiac (papillary) than in skeletal (soleus) muscle of the rat [Gibbs, 1978; Loiselle, 1987; Suga, 1990]. Furthermore, the basal metabolism of the striated muscle is only about 2-3% of its active metabolism whereas basal metabolism of the cardiac muscle is about 25-30% of its active metabolism (see Table I) [Loiselle, 1987].

Various investigators have examined the nature of the unusually high basal MVO$_2$ and have tried to estimate what cellular functions of the myocyte would require substantial amounts of energy despite the resting state of the heart. One function that was believed to be a large component of basal metabolism was the membrane-bound ion pumping mechanism which would have to work against the passive leakage of ions (primarily Na$^+$, K$^+$, and Ca$^{2+}$) across the membrane during myocardial arrest. Yet, Gibbs and Chapman [1979] could only account for about 10% of basal MVO$_2$ based on data of Na$^+$ flux. In contrast, Gibbs [1983] suggested a value of as much as 25% which was in accord with estimations made by Ponce-Hornos [1990].

Ca$^{2+}$-ATPase pumps have also been examined for their role in establishing the basal MVO$_2$ value. Based on biochemical data of the sarolemmal Ca$^{2+}$-pump, Ponce-Hornos [1990] estimated that this pump comprises less than 1% of the basal metabolism of rabbit and dog ventricles, and less than 0.1% of that of rat ventricles. In contrast, the SR Ca$^{2+}$-ATPase pump energy consumption is estimated to be more substantial, at about 28% of basal MVO$_2$ [Ponce-Hornos, 1990]. This correlates with the fact that the total membrane area of the SR is much greater than that of the sarcolemma and, consequently, the SR Ca$^{2+}$-ATPase pumps are much greater in number and importance with respect to intracellular Ca$^{2+}$ removal [Katz, 1992].
Clearly, basal metabolism of the myocardium and the processes which may account for its activity have yet to be fully understood. One aspect of basal metabolism that has not been fully elucidated is the effect of catecholamines on oxidative phosphorylation in the arrested myocardium, and therefore on basal MVO$_2$. The fact that basal MVO$_2$ is not fixed and can be influenced by certain factors, as mentioned above, suggests a possibility for an effect by catecholamines.

**Catecholamines and Basal Myocardial Oxygen Consumption**

The increase in mechanical work of the myocardium in response to beta-adrenergic stimulation is an energy-requiring process and thus, there is an accompanying metabolic demand for an increased supply of energy. Cyclic AMP-dependent PK appears to also regulate an increase in glycogen breakdown with a concomitant decrease in glycogen synthesis [Namm, 1971]. However, Mayer et al. [1963] and Williamson [1964] have shown that catecholamine-induced augmentation of cardiac contractile force can be dissociated from their effect on the phosphorylase enzyme. Alternatively, the increase in Ca$^{2+}$ influx induced by beta-adrenergic stimulation may upregulate oxidative metabolism [Denton and McCormack, 1980a; Denton and McCormack, 1980b; Denton et al., 1980; Denton et al., 1988], thereby providing a mechanism of coordinating increased mechanical performance with energy metabolism. Specifically, Ca$^{2+}$ appears to be able to stimulate three intramitochondrial enzymes: pyruvate dehydrogenase [Denton et al., 1980; Rutter et al., 1989], oxoglutarate dehydrogenase [McCormack and Denton, 1979; Denton et al., 1980], and NAD-linked isocitrate dehydrogenase [Rutter and Denton, 1988], all of which can also be stimulated by high levels of ADP or NADH. In this scenario, Ca$^{2+}$ can act as a communication link between the extramitochondrial increase in mechanical activity and the intramitochondrial energy "factory" of the myocardium. Thus, as catecholamines may increase Ca$^{2+}$ mobilization in the arrested myocardium, this increased level of Ca$^{2+}$ could upregulate mitochondrial metabolic activity.
Numerous studies have been conducted to determine whether catecholamines can directly affect basal MVO\textsubscript{2} by administering catecholamines to the arrested heart. In various studies using papillary muscles [Lee and Yu, 1964; Chandler \textit{et al.}, 1968; Coleman \textit{et al.}, 1971], catecholamines did not affect basal MVO\textsubscript{2}. In cross-circulated, K\textsuperscript+-arrested dog hearts infused with dobutamine [Nozawa \textit{et al.}, 1988] or epinephrine [Suga \textit{et al.}, 1983], basal MVO\textsubscript{2} did not change. In contrast are reports of catecholamine effects on basal MVO\textsubscript{2}. McKeever \textit{et al.} [1958] found that in canine hearts, infusion of norepinephrine raised basal MVO\textsubscript{2} of vagally arrested hearts from 3.3 mL O\textsubscript{2}/min/100 g to 6.4 mL O\textsubscript{2}/min/100 g. Dramatic augmentation of the resting metabolic rate by epinephrine or norepinephrine was also observed in cat papillary muscle [Whalen, 1957] and K\textsuperscript+-arrested canine [Berne, 1958] and rat [Challoner and Steinberg, 1965; Hauge and Oye, 1966b; Hauge and Oye, 1966a] hearts.

Klocke \textit{et al.} [1965] also demonstrated a catecholamine effect on basal MVO\textsubscript{2} in isolated canine hearts. This group compared the effect catecholamine stimulation had on the beating state of the heart with the effect catecholamine stimulation had on the arrested, basal state of the same heart. With the highest dose of catecholamine administered (10 \(\mu\)g bolus injection), the increase in basal MVO\textsubscript{2} was 9\% and 15\% in hearts treated with epinephrine and norepinephrine, respectively, and as much as 32\% in hearts treated with isoproterenol. However, for any given dose of epinephrine, norepinephrine, or isoproterenol, the percent increase in basal MVO\textsubscript{2} induced by catecholamine stimulation was always greater in the beating than non-beating states. Klocke \textit{et al.} [1965] concluded that, in comparison to their effect in the beating heart, catecholamines appeared to have a minimal effect on basal MVO\textsubscript{2}.

Due to the disparity of the available data, evidence for direct effects of catecholamines on oxidative phosphorylation is inconclusive and it is still not clear whether the increase in MVO\textsubscript{2} seen with increased chronotropy and inotropy under catecholamine stimulation is directly proportional and tightly linked, or whether there is a disproportionate

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increase in MVO₂, signifying either uncoupling of or a direct effect on oxidative metabolism. Chandler et al. [1968] addressed this issue by correlating chemical energy use (CP and ATP) with mechanical function. They found that norepinephrine-treated papillary muscles used 115% of the chemical energy of control muscles in only 50% as many contractions while performing only 87% as much work. They concluded that an "oxygen-wasting" effect of norepinephrine resulted from a disproportionately increased use of energy associated with an increased contractile state. However, earlier, Century [1954] had found that epinephrine did not alter the rate of ATPase activity in rat heart homogenates or slices, nor was there any increase in MVO₂. Century [1954] concluded that the epinephrine effect on increased MVO₂ was indirect and resulted from its effect on muscle work rather than a direct effect on oxygen metabolism or ATP breakdown.

As with Chandler et al. [1968], though, Eckstein [1950] observed that oxygen consumption of the beating heart increased disproportionately to an increase in cardiac output (CO). A sizable increase in MVO₂ occurred despite reduction of CO in the presence of sympathetic nerve stimulation, thereby suggesting a catecholamine effect on non-mechanical-related MVO₂. Weisfeldt and Gilmore [1964] also documented a dissociation of the inotropic and oxygen consumption effects of norepinephrine, where low doses of norepinephrine produced changes in contractility with minimal change in MVO₂, whereas with higher doses, contractility did not continue to increase despite a markedly augmented MVO₂. These authors [Weisfeldt and Gilmore, 1964] suggested that the dissociation may have occurred due to the use of a dose which exceeded that required for near maximal inotropic effect, whereupon a direct effect of catecholamines on oxidative metabolism could be distinguished.

*Possible Role for Ca²⁺ in Mediating Catecholamine Effect on Basal MVO₂*

A possible mechanism through which catecholamine action in the arrested myocardium may mediate increased basal MVO₂ may be a type of "diastolic" Ca²⁺ overload.
through beta-adrenergic receptor stimulation [Lappé and Lakatta, 1980; Lakatta and Lappé, 1981]. That is, since beta-adrenoceptor stimulation is known to upregulate the L-type Ca\(^{2+}\) channel current [Trautwein and Hescheler, 1990], it may increase Ca\(^{2+}\) window currents during hyperkalemic arrest. The increased level of cytosolic Ca\(^{2+}\) would not be as high as that seen under contractile states, nor would it induce contraction, but it would be enough to stimulate extra energy expenditure to maintain Ca\(^{2+}\) homeostasis. In fact, Hanley and Loiselle [1992] have demonstrated that basal MVO\(_2\) is sensitive to the intracellular Ca\(^{2+}\) concentration despite the absence of mechanical function. In the presence of ouabain, the Na\(^+\)K\(^+\)-ATPase inhibitor, the basal MVO\(_2\) of K\(^+-\)arrested, guinea pig hearts was augmented from \(7\pm1\) μmol O\(_2\)/min/g DW to \(24\pm3\) μmol O\(_2\)/min/g DW, which was the same value of oxygen consumption when the same hearts were in the empty-beating state. Hanley and Loiselle [1992] proposed that Na\(^+\)/K\(^+\)-ATPase inhibition induced Na\(^+\) accumulation, thereby promoting Na\(^+\)-dependent Ca\(^{2+}\) influx which could be stimulating metabolism at a subcellular level. This was tested by perfusing hearts with Ca\(^{2+}\) -free solution which could reverse the the Na/Ca\(^{2+}\) exchanger to perform Ca\(^{2+}\) efflux. As predicted, basal MVO\(_2\) remained low when the K\(^+-\)arrested, ouabain-treated hearts were perfused with Ca\(^{2+}\) -free solution, indicating that the basal rate of energy expenditure is sensitive to intracellular Ca\(^{2+}\) concentration. Under such conditions of high intracellular Ca\(^{2+}\), ATP energy perhaps may even be wasted if the increased level of Ca\(^{2+}\) induces a low level of actomyosin ATPase activity, but not enough to be manifested as a contraction [Solaro et al., 1974]. Thus, if excessive catecholamine stimulation can exhaust energy stores of the arrested myocardium and the energy level of the myocardium at the end of an ischemic period is a determinant of myocardial functional recovery [Wollenberger and Krause, 1968; Hearse et al., 1974], then post-ischemic myocardial functional recovery would be compromised.
A Possible Link Between the Effect of Catecholamines on Basal Metabolism and Myocardial Functional Recovery Following Peri-Ischemic Excessive Catecholamine Stimulation: Formulation of Hypotheses

In summary, conclusions about any catecholamine effect on basal $MVO_2$ are difficult to establish based on the available conflicting reports. Some groups have demonstrated that catecholamines increase basal $MVO_2$ [Hauge and Oye, 1966a; Hauge and Oye, 1966b; Challoner and Steinberg, 1965; Whalen, 1957; McKeever et al., 1958; Berne, 1958] while others have found no effect of catecholamines on basal $MVO_2$ [Lee and Yu, 1964; Chandler et al., 1968; Coleman et al., 1971; Nozawa et al., 1988; Suga et al., 1983]. Furthermore, evidence of damaging effects of catecholamines in ischemic tissues have mostly used models of regional ischemia and/or low-flow ischemia [Maroko et al., 1971; Neely et al., 1973; Karlsberg et al., 1979; Muntz et al., 1984; Yoshida and Iimura, 1989] where the heart continued to beat. Catecholamine activity under beating heart conditions is better understood than under conditions of myocardial arrest. Even the negative effects of pre-arrest catecholamine stimulation on post-ischemic function, demonstrated by Komai et al. [1991] and Takla et al. [1989], were done by exposing the beating heart to catecholamines prior to arrest.

In the case of blood-based cardioplegia, however, the arrested myocardium is exposed to excessive catecholamines, as discussed above (see pp.11-12). Therefore, one purpose of this study was to determine the effect of catecholamines in the arrested myocardium. It was hypothesized that basal $MVO_2$ would increase upon exposure to excessive catecholamine stimulation. The mechanism of this effect could be explained by beta-adrenergic receptor stimulation and consequent increase in $Ca^{2+}$ mobilization via window currents. Increased diastolic intracellular $Ca^{2+}$ concentration may affect myocardial ion channel pumps, mitochondrial metabolism, and contractile protein interaction, despite the arrested state of the heart. If the catecholamine effect on basal $MVO_2$ is mediated by beta-adrenergic receptors and/or $Ca^{2+}$, then interference with either should be able to inhibit
the effect. Specifically, the beta-adrenergic antagonist, esmolol, and the Ca\textsuperscript{2+}-modulator, BDM, were used as the drug interventions to inhibit the catecholamine effect on basal MVO\textsubscript{2} of the arrested myocardium.

i) Inhibition of Catecholamine Effect on Basal MVO\textsubscript{2}

**Beta-Antagonism of Catecholamine Effect on Basal MVO\textsubscript{2}**

As discussed above, beta-adrenergic antagonists have been shown to mitigate the effects of catecholamine stimulation by preventing cAMP accumulation [Lubbe et al., 1978], reducing structural inhibition [Kloner et al., 1977], preserving ATP levels [Kako, 1966], and limiting ischemic areas [Group, 1984; Libby et al., 1973; Roberts et al., 1984]. If excessive catecholamine stimulation can affect basal MVO\textsubscript{2} of the arrested myocardium by increasing Ca\textsuperscript{2+} mobilization and increasing ATP breakdown, then beta-antagonism should be able to reverse the effect. The beta-adrenoceptor antagonist chosen for this study was esmolol because of its short-acting properties which could be easily controlled within the time limits of the experiment and which, from a clinical standpoint, would not have long-lasting cardiodepressive effects. In the clinical situation, however, patients due for cardiac surgery and its accompanying period of cardiac ischemic arrest, already have increased levels of circulating catecholamines, as much as 20 times basal serum levels (based on calculation from data of [Kopin, 1986] and [Hine et al., 1976]). Thus, to simulate the clinical situation in these experiments, esmolol was added after the arrested myocardium was exposed to catecholamines to determine whether the catecholamine effect on basal MVO\textsubscript{2} could be reversed.

**Esmolol, A Short-Acting Beta-Adrenoceptor Antagonist**

Despite evidence supporting the beneficial use of beta-antagonists, the relatively long duration of action of most available beta-antagonists, with elimination half-lives ranging from 2 to 6 hours, limits their use [Ritschel, 1980]. The cardiodepressive effects of the long-acting beta-adrenergic antagonists may linger and clinically, patients with acute
myocardial infarction or those undergoing cardiac surgery are at risk of adverse effects such as cardiac failure, bradycardia, hypotension, or AV block.

In 1982, Zaroslnski et al. [1982] introduced the concept of ultrashort-acting beta-adrenergic antagonists, envisioning a compound that is extensively and rapidly metabolized to inactive products in a similar fashion to catecholamines or nitroglycerin. Such a compound could therefore be administered by constant i.v. infusion to provide for controlled levels of beta-antagonism that could be titrated and quickly altered if necessary. An ultrashort-acting beta-antagonist was expected to be much safer to use in critical care situations. If undesirable hemodynamic effects or cardiac failure resulted from beta-antagonism with an ultrashort-acting beta-antagonist, rapid recovery of function could be achieved within minutes by reducing or eliminating its administration.

Consequently, Zaroslnski et al. [1982] developed the ultrashort acting beta-adrenergic antagonist drug esmolol, originally identified as ASL-8052. The ultrashort beta-antagonistic nature of esmolol is a consequence of the rapid and extensive metabolism of esmolol in whole blood. In both dog and human blood, hydrolysis of esmolol's methyl ester to yield methanol and the primary acid metabolite, ASL-8123, occurs in the cytosol of the red blood cell as well as in the liver [Sum et al., 1982]. Clinical studies have shown that the half life of elimination of esmolol in humans is about 9 min and the duration of action of esmolol is very brief, with no trace of beta-antagonism activity 30 min after cessation of infusion of even very high doses of esmolol (400 μg/kg/min) [Sum et al., 1983].

Various experiments have been conducted to further characterize the nature of esmolol. Studies were done to compare the action of esmolol with that of propranolol, a long-acting beta-antagonist, on anesthetized dogs given a submaximal bolus dose of isoproterenol [Gorczynski et al., 1983]. Steady-state levels of beta-antagonism were achieved within 10 to 20 minutes of each beta-antagonist infusion. Esmolol caused a dose-dependent decrease in tachycardia responses. Blockade with propranolol, however.
progressively increased throughout most of the infusion period. Twenty minutes after termination of esmolol infusion, no significant beta-antagonism was detectable regardless of dose, whereas only minimal recovery from beta-antagonism was observed after propranolol infusion was ceased.

The effect of esmolol on experimental myocardial infarct size has also been investigated. In two separate models of coronary occlusion, esmolol reduced myocardial infarct size and prevented early functional deterioration after coronary artery reperfusion. Zaroslinski et al. [1982] showed that esmolol reduced myocardial infarct size when coronary occlusion was maintained for one hour followed by one day of reperfusion. In an extension of this study, Lange et al. [1983] treated dogs with a continuous infusion of esmolol begun 15 min after coronary occlusion, which lasted for three hours and was followed by three hours of reperfusion. In control animals, 73±6% of the ischemic area at risk became necrotic, while in treated animals only 48±7% became necrotic (p<0.025). The untreated animals also showed a decrease in cardiac function in the early phases of reperfusion, unlike the esmolol-treated group which showed no change in function. Therefore, esmolol presents itself as a possible candidate that can potentially reverse the increase in basal MVO₂ induced by excessive catecholamine stimulation of the arrested heart.

*Ca²⁺-Modulation of Catecholamine Effect on Basal MVO₂*

To further investigate the nature of the catecholamine effect on basal MVO₂ of the arrested myocardium, another substance, BDM was chosen for its effects on myofibril interaction with Ca²⁺. If catecholamines can mobilize Ca²⁺ in the arrested myocardium and thereby increase basal MVO₂, then any substance that can negatively modulate the effects of Ca²⁺ may consequently reduce basal MVO₂. To parallel the protocol with esmolol, the administration of BDM occurred after the arrested myocardium was exposed to catecholamines, to determine whether BDM could reverse the catecholamine effect.
Effects of BDM on Cardiac Muscle

BDM, or 2,3-butanedione-2-monoxime, is a rapidly-acting, reversible, contractile inhibitor which has been shown to decrease contractility in both cardiac [Li et al., 1985; Gwathmey et al., 1991; Marijic et al., 1991; Watkins et al., 1992; Boban et al., 1993; O'Brien et al., 1993; Backx et al., 1994] and skeletal [Li et al., 1985; Higuchi and Takemori, 1989; Higuchi et al., 1989] muscles. Similar to the quick elimination of esmolol following cessation of infusion, the effects of BDM are rapidly reversed on washout [Sada et al., 1985]. The precise mechanism of action of BDM remains to be elucidated but evidence strongly suggests a predominant effect on the contractile proteins. In vitro experiments on rabbit skeletal muscle [Higuchi and Takemori, 1989; Higuchi et al., 1989] indicated that BDM acts directly on the myosin molecule, suppressing ATPase activity and energetically stabilizing the unattached state of the myosin molecule. Consequently, the number of force-generating myosin molecules is reduced as the rate of myosin binding to actin is reduced. In support of this theory, studies in rat cardiac muscle have shown that maximal force, twitch force, the rise of force development, and twitch duration were all diminished in the presence of BDM in a dose-dependent manner [Backx et al., 1994].

BDM also appears to decrease myofilament sensitivity to Ca\textsuperscript{2+} [Gwathmey et al., 1991; Backx et al., 1994]. Not only does this contribute to a decrease in maximal force but also, more Ca\textsuperscript{2+} must bind to the troponin-tropomyosin complex for cross-bridge attachment to occur in the presence of BDM compared to control. However, troponin and tropomyosin studies have indicated that BDM does not directly affect these regulatory proteins, further suggesting BDM activity at the level of the contractile proteins [Higuchi and Takemori, 1989; Higuchi et al., 1989].

Other experiments show that BDM may also be implicated in the inhibition of Ca\textsuperscript{2+} influx via the slow inward channel [Bergey, 1978; Wiggins et al., 1978; Wiggins et al., 1980; Coulombe et al., 1990; Gwathmey et al., 1991]. The decrease in transsarcolemmal

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Ca\textsuperscript{2+} flux would weaken the stimulation for Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release and lessen SR Ca\textsuperscript{2+} loading, ultimately reducing the amount of Ca\textsuperscript{2+} available for force generation.

A consequence of such Ca\textsuperscript{2+}-modulatory activity suggests that BDM may attenuate the effects of Ca\textsuperscript{2+} overload characteristic of excessive catecholamine stimulation [Fleckenstein, 1973; Fleckenstein, 1983]. Moreover, BDM has been shown to significantly reduce the Ca\textsuperscript{2+} gained during reperfusion after 30 min of ischemia in isolated perfused rat hearts [Elz and Nayler, 1988]. Addition of 30 mM BDM to the reperfusate during Ca\textsuperscript{2+} reperfusion afforded significant protection against Ca\textsuperscript{2+} paradox-induced Ca\textsuperscript{2+} gain in isolated rat hearts [Daly et al., 1987]. Studies have even shown the preservation of ATP after reperfusion with BDM [Elz and Nayler, 1988; Nayler et al., 1988] and in hearts stored with 30 mM BDM cardioplegic solution [Stringham et al., 1993]. Studies on myocardial stunning of canine hearts [O'Brien et al., 1993] and global ischemia of isolated rabbit hearts [Stringham et al., 1992; Stringham et al., 1993] have shown that BDM greatly improved post-ischemic recovery compared with control hearts. In reflection of the aforementioned discussion on BDM, O'Brien et al. [1993] have suggested that the mechanisms of the beneficial action of BDM on the post-ischemic reperfused myocardium could involve preservation of myocardial ATP levels and a decrease in Ca\textsuperscript{2+} overload through effects on the sarcoplasmic reticulum or contractile proteins.

The energy-sparing effects resulting from the Ca\textsuperscript{2+} modulation by BDM suggest a potential for myocardial protection from peri-ischemic excessive catecholamine stimulation. If catecholamines can unnecessarily increase basal MVO\textsubscript{2} by increasing Ca\textsuperscript{2+} mobilization, then, in the arrested heart, BDM may preserve basal MVO\textsubscript{2} through inhibition of the Ca\textsuperscript{2+} overload effect.

ii) Catecholamine Effect on Basal MVO\textsubscript{2} Under Ischemic Conditions

The effect of excessive catecholamine stimulation on the arrested myocardium has clinical relevance to blood-based cardioplegia. However, in the clinical setting.
cardioplegia is associated with an ischemic period. In consideration of this, it was proposed that the excessive catecholamine-induced increase in basal MVO₂ may contribute to poor post-ischemic functional recovery. The excessive catecholamines may be increasing Ca²⁺ mobilization and, as discussed above, this may affect myocardial ion channel pumps, mitochondrial metabolism, and contractile protein interaction. All of these activities can affect ATP levels in the myocardium, which will influence post-ischemic recovery [Wollenberger and Krause, 1968; Hearse et al., 1974]. In further consideration of the clinical situation, hypothermia is often used during cardioplegic arrest and cardiopulmonary bypass to improve myocardial protection and this effect may also protect the heart from any catecholamine effect.

**Protective Effect of Hypothermia**

Hypothermia is used in conjunction with cardioplegic arrest to provide additional myocardial protection during ischemia. The additional protective effect of using lower temperatures during ischemic arrest has been well documented. Numerous studies show how hypothermic arrest slows down ATP depletion during ischemia and improves functional recovery during reperfusion [Hearse et al., 1974; Tyers et al., 1977; Jones et al., 1982]. The protective effect of hypothermia is primarily attributed to the temperature dependency of metabolic rate, leading to energy conservation [Bigelow et al., 1954; Blair, 1965]. Arrested hearts at 20°C have been demonstrated to have MVO₂ values that are about 50% of those at 37°C [Blair, 1965]. Hypothermia slows down the ATP-requiring enzymatic processes and reduces transmembrane Ca²⁺ fluxes, including those at the mitochondrial level [Ferrari et al., 1990]. By reducing tissue and mitochondrial Ca²⁺ accumulation, hypothermia can consequently reduce the amount of energy-dependent processes necessary to maintain cell homeostasis and preserve the ATP-producing function of mitochondria. Hypothermia contributes to the goal of optimal myocardial protection which aims to induce immediate electromechanical arrest and minimize metabolic needs during ischemia.
The cardioprotective benefits of hypothermia during ischemia may not only afford protection of the myocardium against catecholamine stimulation during arrest, but may also be protective of hearts challenged with catecholamines prior to arrest. It has already been demonstrated that hearts exposed to catecholamines in the beating state prior to arrest have compromised post-ischemic functional recovery [Takla et al., 1989; Komai et al., 1991], but the effect of lowering the ischemic temperature was not examined. Improved recovery may be reflective of improved preservation of high energy stores by hypothermia during ischemia.

Thus, it was hypothesized that hypothermia could also attenuate the deleterious effects on post-ischemic myocardial functional recovery of hearts stimulated with catecholamines in the beating state prior to arrest.
The Hypotheses:

In summary, the hypotheses were as follows:

1. Excessive catecholamine stimulation of the arrested myocardium will increase basal MVO₂.

2. The catecholamine-induced increase in basal MVO₂ of the arrested myocardium can be reversed by either a beta-adrenoceptor antagonist or by a Ca²⁺-modulator.

3. The catecholamine-induced increase in basal MVO₂ of the arrested myocardium will be deleterious to post-ischemic functional recovery and can be attenuated by hypothermia during ischemia.

4. Poor post-ischemic functional recovery following catecholamine stimulation of basal MVO₂ is associated with decreased levels of myocardial adenine nucleotides.

5. The detrimental effect of pre-ischemic catecholamine stimulation of the beating heart on post-ischemic functional recovery will be attenuated by hypothermia during ischemia. Hypothermia will contribute to improved preservation of myocardial adenine nucleotide concentrations.

Overview of Experiments

As a preliminary study, and to serve as the basis of the ischemia studies, experiments were done to determine basal metabolism of the heart under varying arrest conditions. This part of the study was designated under the title, "Basal Metabolism Under Varying Arrest Conditions." Myocardial oxygen consumption was used as the parameter to define the energy requirements of the heart and to observe how that energy requirement would be affected by various arrest conditions, such as in the presence of isoproterenol, esmolol, and/or BDM. Arterial and venous oxygen contents and coronary flow, all of which are necessary to calculate MVO₂, had to be obtained from the inflow and
the outflow of the heart and thus, the MVO₂ studies required constant perfusion of the myocardium even under arrest conditions.

Upon determining whether catecholamines could affect basal MVO₂, in contrast to their hemodynamic/mechanical effect on total MVO₂, further studies were done in ischemic conditions to simulate the clinical situation. Thus, normothermic and hypothermic ischemia conditions were used. This part of the study was designated under the title. "Peri-Ischemic Catecholamine Stimulation and Myocardial Functional Recovery." In the first part of the ischemia studies, the purpose was to determine whether the effect of catecholamines on the metabolic rate of the arrested, ischemic myocardium could be deleterious to post-ischemic myocardial functional recovery. This section was entitled, "Post-Ischemic Functional Recovery Following Catecholamine Stimulation of Arrested Myocardium."

The second part of the ischemia studies was based on previous findings described in the literature, where hearts stimulated with catecholamines in the beating state prior to arrest have decreased post-ischemic function [Takla et al., 1989; Komai et al., 1991]. To determine whether hypothermia could improve recovery, two additional groups of hearts were studied. This part of the study was entitled, "Post-Ischemic Functional Recovery Following Pre-Ischemic Catecholamine Stimulation of Beating Myocardium."

All experiments were conducted using isolated, Langendorff-perfused rabbit hearts. Modified, crystalloid Krebs-Henseleit buffer (KHB) was used as the coronary perfusate. In the MVO₂ studies, hearts were subjected to perfusion arrest with 20 mM K⁺ KHB ("K"). Isoproterenol was used as the catecholamine, while esmolol and BDM were used as the beta-blocker and contractile inhibitor, respectively. Either isoproterenol, esmolol, or BDM, or a combination of the inotropic agonist with an antagonist was added to the arrest perfusate during certain portions of the entire arrest period. Myocardial oxygen consumption was evaluated under these varying conditions to determine any change in basal metabolic rate of the arrested heart.
The first part of the ischemia studies was divided into two temperature groups: 37°C normothermia for 60 min or 20°C hypothermia for 120 min. Two groups of hearts were tested at each ischemic temperature. The control group of hearts (Control) was not given any catecholamine stimulation. Group Cp-Iso was challenged with catecholamines within the cardioplegic solution. All hearts underwent the same basic protocol of equilibration, cardioplegic arrest, ischemia, and reperfusion, upon which recoveries of systolic and diastolic function, and developed pressure were evaluated.

In the second part of the ischemia studies, one group of hearts subjected to normothermic ischemia conditions (Group Pre-Iso/37) was compared to another which was subjected to hypothermic ischemia conditions (Group Pre-Iso/20). These hearts followed the same basic protocol as described above, except that both groups were challenged with catecholamines in the beating state just prior to arrest.

Myocardial adenine nucleotide concentrations were also measured from biopsies taken at the end of each ischemia experiment. Functional recovery could then be correlated to the biochemical energy status of the myocardium and therefore, the effects of excessive catecholamine stimulation of ischemic myocardium could possibly be correlated to a decreased level of high energy phosphates.
2 MATERIALS AND METHODS

2.1 Preparation of Perfusates

Modified Krebs-Henseleit control buffer (KHB) [Rebeyka et al., 1990] was prepared with the following composition: 118.4 mM NaCl, 25.0 mM NaHCO₃, 11.1 mM D-(-)-glucose, 4.7 mM KCl, 1.27 mM CaCl₂·H₂O, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄ (anhydrous), 2 U/L insulin (bovine-porcine, Novopharm, ), 0.06 mM EDTA, then filtered with a 5 µm filter. The perfusate was filtered once again (5 µm filter) in-line of the Langendorff perfusion system. Any modifications made to the control buffer were made after the first filtration.

Modifications made to the KHB perfusate are designated by the following abbreviations. Concentrations used were either as specified below or within the Experimental Protocol sections if various concentrations were used.

a) K: addition of 15.3 mM K⁺ to bring total concentration of K⁺ to 20 mM

b) Iso: addition of 25 nM isoproterenol (isoproterenol hydrochloride injection, 0.2 mg/mL; Sabex, Inc., Canada). In the beating heart, 30 min of perfusion with KHB and this dose of Iso was found to increase heart rate (by visual inspection) as well as increase beating heart MVO₂ by over 50% (Figure B.1, Appendix B. p.93), without having a toxic effect as MVO₂ returned to its pre-stimulated values upon reperfusion with just KHB.

Initially, epinephrine (Epi) was used as the candidate catecholamine but pilot experiments testing various doses of Epi on beating heart MVO₂ did not yield expected results of increased MVO₂ [Challoner and Steinberg, 1965; Vasu et al., 1978]. Starting with a dose of 2 nM Epi to mimic the clinical situation (Rebeyka. unpublished data, see p. 12), doses of 25 nM and 50 nM Epi were also tested separately in beating hearts (Appendix A, Figures A.2, A.3, and A.4, respectively. pp. 91-92). None of the three doses demonstrated the expected increase in beating
heart MVO₂. The possible reasons for the lack of an effect are discussed in the Discussion section. In this study, however, the focus was on the beta effect of catecholamines and whether that effect can affect basal MVO₂. Iso clearly demonstrated a beta-effect with increased MVO₂ (Figure B.1, Appendix B. p.93), and thus, was used instead.

c) Esm: addition of esmolol hydrochloride (Brevibloc, Zeneca Pharma, Canada). Esmolol was chosen as the candidate beta-adrenoceptor antagonist for this study due to its cardioselectivity and short-acting properties. Initially, 2 mg/L was chosen, based on doses administered to patients [Sum et al., 1983]. This dose had no effect in the catecholamine-stimulated, arrested heart (Figure 3.2, p.50). As it was uncertain whether the lack of an effect was truly due to an inability to reverse the catecholamine-effect or because this dose is ineffective in the model, 2 mg/L Esm as well as 10 mg/L and 100 mg/L were tested in pilot studies in the beating, non-working heart, to study activity (Appendix C, pp.94-95). The dose of 10 mg/L appeared to decrease MVO₂ without any lingering effects on washout. The highest dosage of 100 mg/L demonstrated a marked decrease in MVO₂ that remained upon washout. Thus, to ensure an effect without cardiotoxicity, an intermediate dose of 25 mg/L Esm (which is about 10⁻⁴ M) was used in the MVO₂ study. These studies on Esm are elaborated upon in the Discussion.

d) BDM: addition of 30 mM 2,3-butanedione-2-monoxime (BDM) (Sigma Chemical Company, Canada). This dose has been previously demonstrated to be effective in affording myocardial protection without cardiotoxicity [Daly et al., 1987; Stringham et al., 1992; Hebisch et al., 1993; Stringham et al., 1993]

e) Epi: addition of epinephrine (adrenaline chloride injection U.S.P. 1:1000, Parke-Davis, Canada). Epinephrine was initially used as the catecholamine challenge but then, was replaced by isoproterenol (see (b), above). The reasons for this switch and the preliminary results using Epi are given in the Discussion and Appendix A.

Materials and Methods
f) Prz: addition of 1 μM prazosin hydrochloride (Sigma Chemical Company, Canada).

This dose has been previously demonstrated to be effective in potentiating the beta-receptor-mediated inotropic response by inhibiting alpha-adrenergic stimulation [Youngson and Talesnik, 1985].

2.2 Langendorff Perfusion System

The isolated rabbit heart in Langendorff mode [Langendorff, 1895; Doring and Dehnert, 1988] was used for all experiments. A schematic diagram of this perfusion system is given in Figure 2.1. Tygon tubing from the main (primary) reservoir ran through a peristaltic pump (Duney Incorporated Piper Pump, Canada), through a small, in-line filter, up to a secondary, air-tight reservoir which was set at 75 cm H₂O to provide a perfusion pressure of 55 mmHg. Tubing leading out of the secondary reservoir then ran down to a heating coil. Tubing between the heating coil and the aortic cannula was accommodated with a T-joint, with a stopcock, to allow for "arterial" MVO₂ measurements or cardioplegia infusion. The perfusion pressure was kept constant by continuous replenishment of the secondary reservoir from the primary reservoir. The perfusate was not recirculated and was oxygenated with 95% O₂:5% CO₂ using a bubble oxygenator placed in the primary reservoir.

The glassware (Radnoti Glassware, Canada) was water-jacketed and connected to a water bath with a circulating pump (Haake 001-3954, Berlin). The system was primed prior to every experiment to ensure absence of air bubbles. After allowing the water bath to warm up, the temperature of the perfusate exiting the aortic cannula was tested to ensure a constant temperature of 37.5°C. A secondary water bath with circulating pump was kept at 20°C and connected to a separate heart chamber for use in the ischemia study.

Materials and Methods
Figure 2.1. Langendorff perfusion system. The Langendorff, isolated rabbit heart system was used to conduct experiments in both the basal metabolism and ischemia studies. This diagram briefly outlines the main features of the system used. See Materials and Methods section for details. A: Perfusate reservoir. B: Roller pump. C: In-line filter. D: Secondary air-tight reservoir set at 75 cm H$_2$O. E: Heating coil. F: Side-arm for obtaining "arterial" samples or for infusing cardioplegic solution. G: Isolated, rabbit heart. H: Heart chamber. I: Coronary effluent. J: Myocardial temperature probe monitor with attached probe.

Materials and Methods
2.3 Basal Metabolism Under Varying Arrest Conditions

2.3.1 Heart Preparation

All animals received humane care in accordance with guidelines of the Canadian Council on Animal Care. In each experiment, New Zealand white rabbits weighing 600-1200 g (30 d-45 d old) were pre-anesthetized with 50 mg/kg ketamine (i.m.), heparinized (300 U.S.P. units/kg, i.v.), and then anesthetized with sodium pentobarbital (40 mg/kg, i.v.). Bilateral sternotomy was performed and the inferior and superior vena cavae were ligated near their insertions into the right atrium. Hearts were excised and immediately cannulated via the aorta to be perfused in Langendorff mode. Perfusion occurred within 10-15 seconds of excision. The pulmonary artery was cannulated for coronary effluent collection and this cannula, fitted with a stopcock on the end for sample extraction, was propped up for support in order to minimize disturbance of the heart. A small temperature probe inserted into the myocardium was used to ensure that the water-jacketed apparatus and perfusate maintained a constant myocardial temperature of 37.5°C. The hearts were left to beat spontaneously in all experiments and were randomly assigned to the experiments.

2.3.2 Experimental Protocol

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<th>Figure</th>
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Table II. Experimental protocol for basal MVO₂ studies. Schematic outline of chronology of perfusion intervals for each experiment. "Perf. "=Perfusion. Time denotes length of perfusion interval. Experiment name describes arrest conditions and results can be found in corresponding Figure.
All MVO₂ experiments followed the same general protocol pattern, as described in Table II. After isolation and attachment to the Langendorff system, the hearts were stabilized for 30 min with KHB perfusion before being perfused for 15 min with the high potassium arrest solution, "K" (i.e. KHB + 20 mM K⁺). This "control arrest" period was then followed by a 15 min interval of perfusion arrest with an added drug. Afterwards, the hearts were re-introduced to 15 min perfusion with the original arrest perfusate, K, followed by perfusion with the drug added to K again. Thus, the protocol followed an "A B C B C A" pattern of perfusion, where A, B, and C would each represent a different perfusate/perfusion interval, and A would always be KHB. When more than one drug (e.g. Iso and Esm) was added, the first drug was added to the arrest perfusate immediately after the 30 min equilibration period, while the second was added in the next perfusion interval. Less than two minutes before the end of each perfusion interval, arterial and venous samples were taken and coronary flow for one minute was recorded before switching to the next perfusion interval.

2.3.3 Measurement of Oxygen Consumption

Myocardial oxygen consumption (MVO₂) was calculated as follows:

\[
MVO₂ (\text{mL O}_2/\text{min/g}) = \frac{\text{arterial} - \text{venous} \text{ O}_2 \text{ tension (mmHg)}}{760 \text{ mmHg/atm}} \times \text{solubility of O}_2 \times \text{coronary flow (mL/min)} \times \text{weight of heart (g)}
\]

where the solubility of oxygen in water at 37.5°C is: \(0.02370 \text{ mL O}_2/\text{mL solution/atm}\) [Daggett et al., 1987]

"Arterial" samples were drawn, by a syringe, from a short tube connected to the T-joint leading to the aortic cannula. "Venous" samples were drawn, by a syringe, from a stopcock in-line with the cannulated pulmonary artery. Both arterial and venous samples were collected under air-tight conditions, capped, and placed on ice. Oxygen tension measurements were then immediately obtained on a Radiometer Copenhagen ABL 330
blood-gas machine. Coronary effluent was collected over a one minute interval to obtain measurement of the rate of coronary flow. Samples for MVO₂ calculation were taken at the end of every perfusion interval. At the end of the experiments, hearts were blotted, then weighed to obtain wet weight (WW) values and then freeze-dried overnight to obtain dry weight (DW) values.

2.3.4 Dosage Studies

The protocol used to determine the effect of a certain dosage of drug on the beating, non-working heart followed similar protocol to that used for the basal MVO₂ study. After a 30 min stabilization period with KHB perfusion (and MVO₂ measurement), hearts were exposed to the drug (Epi, Iso, or Esm) for 30 minutes. MVO₂ data was collected at the 15 min and the 30 min time points. Hearts were then reperfused with KHB for a final 30 min. at the end of which MVO₂ was measured. As above, coronary flow and heart weight were also recorded as required.

2.4 Peri-Ischemic Catecholamine Stimulation and Myocardial Functional Recovery

2.4.1 Heart Preparation

In each experiment, New Zealand white rabbits weighing 600-1200 g (30 d-45 d old) were pre-anesthetized with 50 mg/kg ketamine (i.m.), heparinized (300 U.S.P. units/kg, i.v.), and then anesthetized with sodium pentobarbital (40 mg/kg, i.v.). Bilateral sternotomy was performed and the inferior and superior vena cavae were ligated near their insertions into the right atrium. Hearts were excised and cannulated via the aorta onto the Langendorff perfusion system, as described above. A small incision was made in the pulmonary artery to relieve pressure in the right ventricle and to allow for collection of coronary flow.

Myocardial function was evaluated by using a balloon in the left ventricle. To prevent air from entering the left ventricle during balloon insertion, the heart was immersed
in a warm water bath before a small incision was made in the left atrium. As air is compressible, any air pockets between the ventricle and the balloon, or within the balloon, would give an inaccurate measure of pressure. A latex intraventricular balloon, to record left ventricular pressure, was attached to the end of polyethylene tubing and was filled with water to eliminate any air. After minimizing its volume, the intraventricular balloon was inserted through the left atrium into the left ventricle and secured with a suture around the remaining left atrial tissue. The balloon and tubing were connected to a pressure transducer which was linked to a Harvard Apparatus Universal Oscillograph for the recording of pressure. Balloon volume was set during the stabilization period to provide a pre-ischemic baseline end-diastolic pressure of 10 mmHg.

A small temperature probe inserted into the myocardium was used to ensure that the water-jacketed apparatus maintained a constant myocardial temperature of 37.5°C. The hearts were left to beat spontaneously in all experiments and were randomly assigned to the groups.

2.4.2 Experimental Protocol

2.4.2a Post-Ischemic Functional Recovery Following Catecholamine Stimulation of Arrested Myocardium

A schematic of the chronology of the experimental protocol is given in Figure 2.2. Briefly, hearts in the control group, Control (Figure 2.2a), had an initial equilibration period of 30 min with KHB, followed by infusion with 20 mM K+ KHB cardioplegic solution (K) to initiate ischemic arrest after perfusion through the aortic cannula was clamped off. All hearts were given 20 mL of K to induce arrest. This volume was found to be adequate for the experimental model. The cardioplegia was infused by a syringe pump (Syringe Infusion Pump 22, Model 221/W, Harvard Apparatus Canada) at a rate of 6 mL/min through the side-arm of the T-joint just above the aortic cannula. The temperature of the cardioplegia was 37°C for hearts to be subjected to normothermic ischemia and 20°C for hearts to be subjected to hypothermic ischemia. That is, each of the two groups were
a. CONTROL (Control Group)

- 30 min Stabilization
- Ischemia (60 min at 37°C or 120 min at 20°C)
- 30 min Reperfusion

Cardioplegia

b. CARDIOPLEGIC CATECHOLAMINE CHALLENGE (Group Cp-Iso)

- 30 min Stabilization
- Ischemia (60 min at 37°C or 120 min at 20°C)
- 30 min Reperfusion

Cardioplegia with 25 nM Iso

Figure 2.2. Post-ischemic functional recovery following catecholamine stimulation of arrested myocardium. Control Group: After a 30 min stabilization period, hearts were subjected to cardioplegic arrest and normothermic or hypothermic ischemia. Hearts were then reperfused for 30 min. Change in systolic, diastolic, and developed pressures were measured pre- and post-ischemically (black arrows) to evaluate functional recovery. Group Cp-Iso hearts differed from Control only in that they were exposed to 25 nM Iso within the cardioplegia (shaded box). Tissue biopsies were taken at the end of reperfusion (*) to measure myocardial adenine nucleotide concentrations.
a. Group Pre-Iso/37

15 min Stabilization  15 min Iso  Normothermic Ischemia (60 min at 37°C)  30 min Reperfusion
Cardioplegia

b. Group Pre-Iso/20

15 min Stabilization  15 min Iso  Hypothermic Ischemia (120 min at 20°C)  30 min Reperfusion
Cardioplegia

Figure 2.3. Ischemia temperature effect on post-ischemic functional recovery following pre-ischemic catecholamine stimulation of beating myocardium. After 15 min of stabilization, beating hearts were stimulated with 25 nM Iso for 15 min just prior to arrest with cardioplegia and ischemia. Group Pre-Iso/37 hearts were subjected to normothermic ischemia at 37°C for 60 min. Group Pre-Iso/20 hearts were subjected to hypothermic ischemia at 20°C for 120 min. Hearts were then reperfused for 30 min. Systolic, diastolic, and developed pressures were measured pre- and post-ischemically (black arrows) to evaluate functional recovery. Tissue biopsies were taken at the end of reperfusion (*) to measure myocardial adenine nucleotide concentrations.
studied at two different temperatures of ischemia. In the normothermic ischemia study, hearts were subjected to cardioplegic arrest and global ischemia at 37°C for 60 min. In the hypothermic ischemia study, hearts were subjected to cardioplegic arrest and global ischemia at 20°C for 120 min. After ischemia, hearts were reperfused for 30 min.

The effect of excessive catecholamine stimulation during arrest was determined by subjecting some hearts to catecholamine stimulation within the cardioplegia (Group Cp-Iso), where 25 nM Iso was added to the cardioplegic solution (Figure 2.2b). Equilibration, ischemia and reperfusion were identical to the control (Control).

2.4.2b Post-Ischemic Functional Recovery Following Pre-Ischemic Catecholamine Stimulation of Beating Myocardium

A schematic of the chronology of the experimental protocol is given in Figure 2.3. The basic structure of these experiments was identical to the arrest study (Section 2.4.2a), except that 25 nM Iso was administered to beating hearts in the 15 min prior to arrest (i.e. last half of stabilization period). Groups were then divided according to the temperature of the ischemic period. Hearts were subjected to either normothermic ischemia at 37°C for 60 min (Group Pre-Iso/37, Figure 2.3a) or hypothermic ischemia at 20°C for 120 min (Group Pre-Iso/20, Figure 2.3b). After ischemia, hearts were reperfused for 30 min. The effect of ischemia temperature on excessive catecholamine stimulation of the beating heart prior to arrest was determined in Group Pre-Iso/37 and Group Pre-Iso/20.

2.4.3 Evaluation of Myocardial Function

Pre-ischemic (baseline) values of systolic pressure (SP) and diastolic pressure (DP) were recorded at the end of the stabilization period. Post-ischemic values of these same parameters of cardiac function were recorded at the end of the reperfusion period. Developed pressure (DevP) was calculated as the difference between SP and DP. Post-ischemic functional recovery was calculated as the absolute change (Δ) in systolic pressure, diastolic pressure, and developed pressure from baseline to post-ischemic values.

Materials and Methods
2.4.4  **HPLC Analysis For Post-Ischemic Myocardial Adenine Nucleotide Concentrations**

The HPLC procedure used is similar to that described by Weisel *et al.* [1989]. Briefly, at the end of each experiment, a biopsy from the left ventricular free wall was immediately taken and immersed in liquid nitrogen, to be freeze-dried overnight at -50°C. The freeze-dried muscle was stored in a -70°C freezer for subsequent HPLC analysis of adenine nucleotides and their degradation products. The myocardial tissue was cleaned of connective tissue. The muscle was homogenized for 10 minutes on ice with 20 μL 0.5 M perchloric acid/mg tissue. An internal standard, 2'-O-methyladenosine, was added at a known concentration to each sample. After a 10 min centrifugation at 2500 rpm, the supernatant was neutralized with 2 M potassium hydroxide to pH 7.6, then reacidified with 0.1 M perchloric acid to pH 6.8. The sample was centrifuged again for 10 min at 2500 rpm, after which the supernatant was frozen in liquid nitrogen to be freeze-dried overnight. The freeze-dried samples were stored at -70°C until all heart biopsies were ready to be analyzed on the HPLC machine.

A modification of the step-gradient technique described by Hull-Ryde *et al.* [1986] was used to measure the levels of adenine nucleotides and their degradation products by high performance liquid chromatography [Weisel *et al.*, 1989]. Samples were re-suspended in 100 mM ammonium phosphate buffer (pH 5.7) just prior to analysis. Sample injection was done by an auto-injector (Model 700 Satellite WIS, Waters Associates, Mississauga, Canada). A reciprocating pump (Models 501 and 510, Waters Associates) performed step-gradient solvent delivery. The chromatographic column, a Radial-Pak Resolve C18 Column (Waters Associates) with a 5 μM particle size, was operated in a 175 bar-radial compression module (Model RCM 100, Waters Associates). Using a programmable multiwavelength detector (Model 490, Waters Associates), the system measured uric acid, adenosine triphosphate (ATP), inosine monophosphate (IMP), adenosine diphosphate (ADP), hypoxanthine (HXN), xanthine (XN), adenosine
monophosphate (AMP), and inosine at a peak absorbance of 254 nm. Creatinine phosphate was measured at a peak absorbance of 229 nm. The total adenine nucleotide (TAN) concentration was calculated as the sum of the concentrations for AMP, ADP, and ATP. The results are expressed as µmol/g DW myocardial muscle. A sample trace from the HPLC analysis of myocardial tissue is shown in Figure 2.4.

2.5 Statistical Analysis

2.5.1 Basal Metabolism Under Varying Arrest Conditions

The perfusion arrest experiments addressed changes in MVO₂ of the arrested state of the heart. After the control arrest period, a drug was added to the arrested heart to determine its effect on basal MVO₂. These two intervals were then repeated in sequence. Thus, for each study, two-tailed, paired t-tests were used to test for any significant change in basal MVO₂ of the arrested heart from the control arrest period to the following "drug-added" period. A two-tailed, paired t-test was also used to test for any significant difference between the two control arrest MVO₂ values. A significance level of p<0.05 was used. The equilibrium and reperfusion periods where the heart was in a beating state were considered as control periods only, to ensure that the heart was functional.

Experiments testing the effect of a certain drug on the beating heart addressed changes in MVO₂ of the beating heart. After recording MVO₂ of the control beating period, the drug was administered and MVO₂ was recorded every 15 min for 30 min before returning to the control perfusate. Thus, ANOVA for repeated measures, followed by post-hoc Bonferroni t-tests to isolate significant differences between any two groups, was used to test for any significant changes among the initial control, beating period MVO₂ and the two MVO₂ values of the "drug-added" period. A significance level of p<0.05 was used. Also, a two-tailed, paired t-test was used to test for any significant

Materials and Methods
Figure 2.4. HPLC sample trace of end-reperfusion myocardial tissue. Biopsies taken at the end of reperfusion following ischemia were analyzed by high performance liquid chromatography (HPLC) to calculate tissue concentrations of AMP, ADP, and ATP. Retention times in the HPLC column are indicated along the x-axis and emission activity at 254 nm is indicated along the y-axis. The ratio of the area under the peak for each adenine nucleotide to the area under the peak of the internal standard (last tall peak) was used to calculate the concentration of the nucleotide. This sample taken from a heart in Group Cp-Iso.
difference between the initial and final control periods, using a significance level of p<0.05.

Statistical analyses were performed on StatView 4.51 (Abacus Concepts, 1995) and Instat 1.12 (GraphPad Software, 1992).

2.5.2 Peri-Ischemic Catecholamine Stimulation and Myocardial Functional Recovery

Baseline values and post-ischemic functional recovery values were compared by two-tailed, unpaired t-tests to test for any significant differences (significance level of p<0.05) between Groups Control and Cp-Iso, for each temperature study (i.e. normothermia and hypothermia). Parameters compared to evaluate post-ischemic change in function between the two groups were: change in systolic pressure, change in diastolic pressure, change in developed pressure. The concentration of each adenine nucleotide was also compared between each group (Control vs. Cp-Iso) for each separate temperature study (normothermic ischemia and hypothermic ischemia).

The same method of statistical analysis was used to compare functional and adenine nucleotide differences between Groups Pre-Iso/37 and Pre-Iso/20.

In an additional analysis of data, the ischemia temperature effect on post-ischemic change in diastolic pressure was compared in the Control and Cp-Iso groups (e.g. change in DP for normothermia Control vs. change in DP for hypothermia Control). Two-tailed, unpaired t-tests were used, with a significance of p<0.05.

Statistical analyses were performed on StatView 4.51 (Abacus Concepts, 1995) and Instat 1.12 (GraphPad Software, 1992).

Materials and Methods
3 RESULTS

3.1 Basal Metabolism Under Varying Arrest Conditions

All values are given as mean±SEM and statistical significance, as described in Materials and Methods, is indicated as a significant difference with p<0.05 using two-tailed, paired t-tests.

3.1.1 Perfusion Arrest With Catecholamine Stimulation

The effect of catecholamines on basal metabolism of the myocardium was evaluated by exposing perfusion-arrested hearts to isoproterenol (Figure 3.1). Hearts (n=5) exposed to 25 nM Iso in the arrested state (i.e. 25 nM Iso added to K) demonstrated a significant (p=0.0009) increase in basal MVO\textsubscript{2} from perfusion arrest without Iso (Figure 3.1). Specifically, the change was from 0.054±0.006 mL O\textsubscript{2}/min/g DW to 0.088±0.009 mL O\textsubscript{2}/min/g DW. Subsequent repetition of these two intervals (second Intervals B-C, Figure 3.1) appeared to demonstrate a similar increase (0.034±0.004 to 0.063±0.008 mL O\textsubscript{2}/min/g DW) but the change was not statistically significant (p=0.07). However, the MVO\textsubscript{2} of the second control arrest period (second Interval B, Figure 3.1) was statistically different from the MVO\textsubscript{2} of the initial control arrest period (first Interval B, Figure 3.1) (p=0.02). Recovery of the beating state is shown in the shaded area of the graph (Figure 3.1), where pre-arrest MVO\textsubscript{2} was 0.342±0.048 mL O\textsubscript{2}/min/g DW and post-arrest MVO\textsubscript{2} was 0.350±0.052 mL O\textsubscript{2}/min/g DW.

3.1.2 Perfusion Arrest with Catecholamine Stimulation and Beta-Adrenoceptor Antagonist

Since an increase in basal metabolism was observed during perfusion arrest with isoproterenol, the ability of the beta-adrenoceptor antagonist, esmolol, to reverse the effect was questioned. Initially, however, experiments were done to evaluate the effect of esmolol alone in the perfusion-arrested heart (Figure 3.2). The MVO\textsubscript{2} of hearts (n=13)
arrested with K (0.062±0.004 mL O₂/min/g DW) did not significantly change with the addition of 2 mg/L esmolol to the arrest perfusate (0.052±0.005 mL O₂/min/g DW; p=0.08). Repetition of these two perfusion intervals yielded similar results (0.052±0.005

![Graph](image-url)

Figure 3.1. Effect of 25 nM isoproterenol on basal MVO₂. Basal MVO₂ significantly increased upon the addition of 25 nM isoproterenol to the arrest perfusate. The shaded area shows the recovery of the beating state MVO₂. Values are mean±SEM (n=5). Perfusion intervals: A, KHB; B, K; C, K + 25 nM Iso. *p=0.0009 vs. prior control arrest interval (B); †p=0.02 vs. initial Interval B; two-tailed, paired t-test.

to 0.051±0.004 mL O₂/min/g DW; second Intervals B-C, Figure 3.2; p=0.8). The shaded area of the graph (Figure 3.2) shows recovery of the beating heart MVO₂ from a pre-arrest value of 0.246±0.033 mL O₂/min/g DW to a post-arrest value of 0.218±0.029 mL O₂/min/g DW.
Because the lack of any effect of 2 mg/L esmolol may have been due to an ineffective dosage, the effects of 2, 10, and 100 mg/L esmolol in the beating heart were determined in pilot studies (Appendix C, pp.94-95). Whereas 10 mg/L esmolol tended to decrease MVO$_2$ of the beating heart (Figure C.2. Appendix C, p.94), 100 mg/L esmolol markedly reduced contractile function (visual inspection) and MVO$_2$, both of which tended to remain depressed after discontinuation of esmolol perfusion (Figure C.3. Appendix C, p.95). Thus, an intermediate dose of 25 mg/L esmolol was chosen for use in subsequent experiments.

![Figure 3.2. Effect of 2 mg/L esmolol on basal MVO$_2$. Basal MVO$_2$ did not significantly change upon the addition of 2 mg/L esmolol to the arrest perfusate. The shaded area shows the recovery of the beating state MVO$_2$. Values are mean±SEM (n=13). Perfusion intervals: A, KHB; B, K; C, K + 2 mg/L Esm.](image-url)
Experiments were then done with a dose of 25 mg/L Esm to determine whether esmolol could reverse, that is, eliminate, the effect Iso had on basal MVO₂ (Figure 3.3). After equilibration with KHB for 30 minutes, hearts (n=11) were arrested with K + Iso.

Figure 3.3. Effect of esmolol on basal MVO₂ in the presence of isoproterenol. Basal MVO₂ stimulated with 25 nM isoproterenol did not significantly change upon the addition of 25 mg/L esmolol to the arrest perfusate. The shaded area shows the recovery of the beating state MVO₂. Values are mean±SEM (n=11). Perfusion intervals: A, KHB; B, K + 25 nM Iso; C, K + 25 nM Iso + 25 mg/L Esm.

After 15 minutes of arrest in the presence of Iso, mean MVO₂ of the hearts was 0.089±0.010 mL O₂/min/g DW. MVO₂ did not significantly change after 25 mg/L Esm was added to the perfusate of K + Iso (0.085±0.014 mL O₂/min/g DW; p=0.8). Similarly, return to the original arrest perfusate of K + Iso to repeat the arrest conditions without and
then with 25 mg/L Esm demonstrated no significant change (0.071±0.004 to 0.063±0.009 mL O₂/min/g DW; p=0.3). Recovery of MVO₂ of the beating state is shown in the shaded area of the graph (Figure 3.3) where pre-arrest MVO₂ was 0.421±0.014 mL O₂/min/g DW and post-arrest MVO₂ was 0.387±0.027 mL O₂/min/g DW.

3.1.3  **Perfusion Arrest With Catecholamine Stimulation and Ca²⁺-**

**Modulator**

Studies were also done using BDM to determine whether this negative inotrope could reverse the change in MVO₂ induced by isoproterenol in the arrested myocardium. As with the esmolol studies, experiments were initially done to determine the effect of BDM alone in the arrested heart (Figure 3.4). The MVO₂ of hearts (n=6) arrested with K did not significantly change with the addition of 30 mM BDM to the hyperkalemic perfusate (0.076±0.020 to 0.045±0.006 mL O₂/min/g DW; p=0.1). Likewise, a subsequent repetition of these two intervals of arrest (without and then with BDM) demonstrated no change in basal MVO₂ (0.060±0.010 to 0.057±0.013 mL O₂/min/g DW; p=0.9). The shaded area of Figure 3.4 shows the recovery of the beating heart MVO₂ (pre-arrest MVO₂: 0.378±0.040 mL O₂/min/g DW; post-arrest MVO₂: 0.361±0.044 mL O₂/min/g DW).
Figure 3.4. Effect of 30 mM BDM on basal MVO₂. Basal MVO₂ did not significantly change upon the addition of 30 mM BDM to the arrest perfusate. The shaded area shows the recovery of the beating state MVO₂. Values are mean±SEM (n=6).

Perfusion intervals: A. KHB; B. K; C. K + 30 mM BDM.

Following the protocol outlined for K + 25 nM Iso + 30 mM BDM in Table II, the effect of BDM during arrest in the presence of catecholamines was determined (Figure 3.5). After equilibration with KHB for 30 min, hearts (n=9) were arrested with K+Iso. 15 min after which MVO₂ was 0.079±0.018 mL O₂/min/g DW. No significant change in MVO₂ occurred with the addition of 30 mM BDM to the perfusate (0.058±0.008 mL O₂/min/g DW) (Figure 3.5), despite an apparent pattern of decrease in MVO₂ (p=0.2). Subsequent repetition of these two intervals of arrest (without and then with BDM, both in the presence of Iso) demonstrated no change in basal MVO₂ (0.080±0.014 to 0.050±0.012...
mL O₂/min/g DW) (p=0.2). Recovery of MVO₂ of the beating state is shown in the shaded region of the graph (Figure 3.5: pre-arrest MVO₂: 0.379±0.022 mL O₂/min/g DW; post-arrest MVO₂: 0.401±0.024 mL O₂/min/g DW).

![Graph showing recovery of MVO₂](image)

Figure 3.5. Effect of BDM on basal MVO₂ in the presence of isoproterenol. Basal MVO₂ stimulated with 25 nM isoproterenol did not significantly change upon the addition of 30 mM BDM to the arrest perfusate. The shaded area shows the recovery of the beating state MVO₂. Values are mean±SEM (n=9). Perfusion intervals: A. KHB; B. K + 25 nM Iso; C. K + 25 nM Iso + 30 mM BDM.

3.1.4 Summary of Results for Basal Metabolism Study

In this model, catecholamine stimulation significantly increased basal MVO₂. However, the addition of either esmolol or BDM did not significantly reverse this catecholamine effect on basal MVO₂.
3.2 Peri-Ischemic Catecholamine Stimulation and Myocardial Functional Recovery

3.2.1 Post-Ischemic Functional Recovery Following Catecholamine Stimulation of Arrested Myocardium

As described in Materials and Methods, hearts were randomly assigned to one of two groups: 1) Control Group: control hearts with no excessive catecholamine stimulation during cardioplegic arrest and global ischemia; 2) Group Cp-Iso: hearts given cardioplegia with 25 nM Iso (i.e. 20 mM K+ KHB + 25 nM Iso). The two groups in each of the normothermic and hypothermic ischemia studies did not differ in their baseline values for systolic pressure, diastolic pressure, and developed pressure (Table III). All values are given as mean±SEM and statistical significance, as described in Materials and Methods, is indicated as a significant difference with p<0.05 using a two-tailed, unpaired t-test.

<table>
<thead>
<tr>
<th>Ischemia</th>
<th>Group</th>
<th>SP</th>
<th>DP</th>
<th>DevP</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>Control (11)</td>
<td>116.2±3.0</td>
<td>9.9±0.3</td>
<td>106.3±2.8</td>
</tr>
<tr>
<td></td>
<td>Cp-Iso (12)</td>
<td>117.1±3.2</td>
<td>9.6±0.2</td>
<td>107.5±3.1</td>
</tr>
<tr>
<td>20°C</td>
<td>Control (8)</td>
<td>118.4±2.4</td>
<td>9.0±0.5</td>
<td>109.4±2.4</td>
</tr>
<tr>
<td></td>
<td>Cp-Iso (10)</td>
<td>114.0±4.0</td>
<td>9.1±0.5</td>
<td>104.9±4.0</td>
</tr>
</tbody>
</table>

Table III: Pre-ischemic baseline values. SP: Systolic Pressure; DP: Diastolic Pressure; DevP: Developed Pressure. All values are mean±SEM, mmHg; numbers in brackets indicate sample sizes.

3.2.1a Normothermic Ischemia Study

Groups in the normothermia study had sample sizes of: Control=11; Cp-Iso=12. Normothermic ischemia was designated as an ischemic period of 60 minutes at 37°C. Details of the experimental protocol are in Materials and Methods, and are depicted in Figure 2.2 (p.41).

i) Change in Systolic Pressure After Normothermic Ischemia

Recovery of systolic function was evaluated as the absolute change (Δ) in systolic pressure of each group, from baseline values just before arrest to systolic pressure values after 30 min reperfusion following 60 min normothermic ischemia (Figure 3.6). Hearts which had 25 nM Iso added to the cardioplegia (Group Cp-Iso) had a -21.6±2.5 mmHg
change in systolic pressure after 60 minutes of normothermic ischemia. This was not significantly different (p=0.6) from the Control Group which was not subjected to excessive catecholamine stimulation by Iso (-19.7±2.7 mmHg).

Figure 3.6. Post-normothermic ischemia change (Δ) in systolic pressure. Hearts exposed to 25 nM Iso (Group Cp-Iso, n=12) prior to arrest and global ischemia at 37°C for 60 min demonstrated no difference in recovery of systolic function compared to the Control Group (n=11). Values are mean±SEM.

ii) Change in Diastolic Pressure After Normothermic Ischemia

Recovery of diastolic function was evaluated as the absolute change (Δ) in diastolic pressure of each group, from baseline values just before arrest, to diastolic pressure values after 30 min reperfusion following 60 min normothermic ischemia (Figure 3.7). Group Cp-Iso demonstrated decreased diastolic function reflected by a significant increase in diastolic pressure (p=0.02) from its baseline value compared to the mean increase of 12.5±2.0 mmHg in the Control Group. Specifically, Group Cp-Iso experienced an increase of 21.8±3.0 mmHg as diastolic pressure of hearts in this group changed from a mean baseline value of 9.6±0.2 mmHg prior to normothermic ischemia to 31.3±3.0 mmHg afterwards (Figure 3.7).
iii) Change in Developed Pressure After Normothermic Ischemia

Recovery of developed pressure was evaluated as the absolute change (Δ) in developed pressure of each group, from baseline values just before arrest, to developed pressure values after 30 min reperfusion following 60 min normothermic ischemia (Figure 3.8). The Control Group experienced a -32.3±3.8 mmHg change in developed pressure and Group Cp-Iso did not significantly differ (p=0.1) from the Control Group, with a recovery of -43.3±5.2 mmHg.

Figure 3.8. Post-normothermic ischemia change (Δ) in developed pressure. Administration of 25 nM Iso within the cardioplegia (Group Cp-Iso, n=12) did not have any significantly detrimental effect on recovery of developed pressure, compared to Control Group (n=11). Global ischemia was at 37°C for 60 min. Values are mean±SEM.
iv) **End-Reperfusion Myocardial Adenine Nucleotide Concentrations After Normothermic Ischemia**

Biopsies of the myocardium were taken after the 30 min reperfusion period following normothermic ischemia to evaluate adenine nucleotide concentrations (Figure 3.9). Concentrations of AMP, ADP, and ATP were measured, and total myocardial adenine nucleotides (TAN) were calculated. Comparisons were made for each adenine nucleotide between the two groups. Control Group (n=4) concentrations for AMP, ADP, ATP, and TAN were 0.29±0.10, 3.25±0.55, 11.18±0.78, and 14.73±1.22 μmol/g DW, respectively. The corresponding values for Group Cp-Iso (n=6) were not significantly different (0.26±0.08, p=0.8; 2.40±0.34, p=0.2; 8.68±1.51, p=0.2; and 11.34±1.82, p=0.2; μmol/g DW, respectively).

![Bar chart](image)

**Figure 3.9.** Post-normothermic ischemia myocardial adenine nucleotide concentrations. The presence of 25 nM Iso in the cardioplegia (Group Cp-Iso, n=6) did not affect end-reperfusion myocardial adenine nucleotide levels. Global normothermic ischemia was at 37°C for 60 min. n=4 for Control Group. AMP: adenosine monophosphate; ADP: adenosine diphosphate; ATP: adenosine triphosphate; TAN: total adenine nucleotides. Values are mean±SEM.
3.2.1b Hypothermic Ischemia Study

Groups in the hypothermia study had sample sizes of: Control=8; Cp-Iso=10. Hypothermic ischemia was designated as an ischemic period at 20°C for 120 minutes. Details of the experimental protocols are in Materials and Methods and shown in Figure 2.2 (p.41).

i) Change in Systolic Pressure After Hypothermic Ischemia

Recovery of systolic function was evaluated as the absolute change (Δ) in systolic pressure of each group, from baseline values just before arrest, to systolic pressure values after 30 min reperfusion following 120 min hypothermic ischemia (Figure 3.10). The Control Group experienced a decrease of 12.8±2.5 mmHg from its original systolic pressure. Group Cp-Iso had similar results with a change of -15.0±3.3 mmHg (Figure 3.10). Thus, there was no difference (p=0.6) in recovery of systolic function between the two groups following hypothermic ischemia.

![Figure 3.10. Post-hypothermic ischemia change (Δ) in systolic pressure. Hearts stimulated with Iso within the cardioplegia (Group Cp-Iso, n=10) regained systolic function, after 120 min of 20°C ischemia, comparable to the control hearts. Control Group (n=8). Values are mean±SEM.](image)

ii) Change in Diastolic Pressure After Hypothermic Ischemia

Recovery of diastolic function was evaluated as the absolute change (Δ) in diastolic pressure of each group, from baseline values just before arrest, to diastolic pressure values
after 30 min reperfusion following 120 min hypothermic ischemia (Figure 3.11). In the Control Group, diastolic function fully recovered, reflected by a mean average change of -0.6±1.5 mmHg. Similarly, Group Cp-Iso had a mean change of 0.1±1.3 mmHg in diastolic pressure (p=0.7).

![Graph showing diastolic pressure change](image)

**Figure 3.11. Post-hypothermic ischemia change (Δ) in diastolic pressure.** Both Control (n=8) and Group Cp-Iso (n=10) hearts maintained their original diastolic tone following 120 min ischemia at 20°C. Values are mean±SEM.

iii) **Change in Developed Pressure After Hypothermic Ischemia**

Recovery of developed pressure was evaluated as the absolute change (Δ) in baseline developed pressure of each group, from baseline values just before arrest, to developed pressure values after 60 min reperfusion following 120 min hypothermic ischemia (Figure 3.12). Group Cp-Iso did not significantly differ from the Control Group (p=0.6). Group Cp-Iso experienced a decrease of 13.9±3.2 mmHg compared to the 12.1±2.3 mmHg decrease in Control Group hearts.

iv) **End-Reperfusion Myocardial Adenine Nucleotide Concentrations After Hypothermic Ischemia**

AMP, ADP, ATP, and total adenine nucleotide (TAN) concentrations in the myocardium after 30 min reperfusion following 120 min hypothermic ischemia of Group Cp-Iso were not significantly different from their corresponding values in the control group (p=0.4, 0.5, 0.7, and 0.6, respectively; Figure 3.13). The values for the Control Group

*Results*
Figure 3.12. Post-hypothermic ischemia change (Δ) in developed pressure. Administration of 25 nM Iso within the cardioplegia (Group Cp-Iso, n=10) had no effect on recovery of developed pressure compared to the Control Group (n=8), after 120 min hypothermic ischemia at 20°C. Values are mean±SEM.

Figure 3.13. Post-hypothermic ischemia myocardial adenine nucleotide concentrations. The presence of 25 nM Iso in the cardioplegia did not affect end-reperfusion myocardial adenine nucleotide levels. Global normothermic ischemia was at 20°C for 120 min. n=8 for Control Group. AMP: adenosine monophosphate; ADP: adenosine diphosphate; ATP: adenosine triphosphate; TAN: total adenine nucleotides. Values are mean±SEM.
(n=8) were 0.37±0.12, 3.56±0.47, 15.64±2.25, and 19.57±2.56 μM/g DW, respectively. Those for Group Cp-Iso (n=10) were 0.54±0.15, 3.91±0.28, 16.44±1.07, and 20.88±1.17 μM/g DW, respectively.

3.2.2 Post-Ischemic Functional Recovery Following Pre-Ischemic Catecholamine Stimulation of Beating Myocardium

As described in Materials and Methods, hearts were randomly assigned to one of two groups: 1) Group Pre-Iso/37 (n=12): hearts stimulated with 25 nM Iso in the beating state prior to arrest and 37°C ischemia for 60 min; 2) Group Pre-Iso/20 (n=10): hearts stimulated with 25 nM Iso in the beating state prior to arrest and 20°C ischemia for 120 min. These two groups did not differ in their baseline values for systolic pressure, diastolic pressure, and developed pressure (Table IV). All values are given as mean±SEM and statistical significance, as described in Materials and Methods, is indicated as a significant difference with p<0.05 using a two-tailed, unpaired t-test.

<table>
<thead>
<tr>
<th>Group</th>
<th>SP</th>
<th>DP</th>
<th>DevP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Iso/37 (12)</td>
<td>113.6±3.1</td>
<td>10.0±0.4</td>
<td>103.6±2.8</td>
</tr>
<tr>
<td>Pre-Iso/20 (10)</td>
<td>120.4±3.0</td>
<td>9.6±0.4</td>
<td>110.8±3.2</td>
</tr>
</tbody>
</table>

Table IV: Pre-ischemic baseline values. SP: Systolic Pressure; DP: Diastolic Pressure; DevP: Developed Pressure. All values are mean±SEM, mmHg; numbers in brackets indicate sample sizes.

3.2.2a Ischemia Temperature Effect on Change in Systolic Pressure

Recovery of systolic function was evaluated as the absolute change (Δ) in systolic pressure of each group, from baseline values just before arrest to systolic pressure values after 30 min reperfusion following ischemia (Figure 3.14). Hypothermia during ischemia significantly protected systolic function of the Group Pre-Iso/20 hearts (p=0.003). These hearts experienced only a 15.4±3.2 mmHg decrease in systolic pressure, compared to a decrease of 31.6±3.5 mmHg experienced by the Group Pre-Iso/37 hearts.
Figure 3.14. Effect of ischemia temperature on change (Δ) in systolic pressure. Beating hearts stimulated with 25 nM Iso prior to arrest and hypothermic ischemia (20°C, 120 min: Group Pre-Iso/20, n=10) had significantly improved systolic function compared to hearts subjected to normothermic ischemia (37°C, 60 min; Group Pre-Iso/37, n=12). Values are mean±SEM. *p=0.003 vs. Group Pre-Iso/37; two-tailed, unpaired t-test.

3.2.2b Ischemia Temperature Effect on Change in Diastolic Pressure

Recovery of diastolic function was evaluated as the absolute change (Δ) in diastolic pressure of each group, from baseline values just before arrest to diastolic pressure values after 30 min reperfusion following ischemia (Figure 3.15). Hypothermia during ischemia significantly protected diastolic function of the Group Pre-Iso/20 hearts (p<0.0001). These hearts experienced only a 8.8±1.6 mmHg increase in diastolic pressure, compared to

Figure 3.15. Effect of ischemia temperature on change (Δ) in diastolic pressure. Beating hearts stimulated with 25 nM Iso prior to arrest and hypothermic ischemia (20°C, 120 min; Group Pre-Iso/20, n=10) had significantly improved diastolic relaxation compared to hearts subjected to normothermic ischemia (37°C, 60 min). Values are mean±SEM. *p<0.0001 vs. Group Pre-Iso/37; two-tailed, unpaired t-test.

Results
an increase of 21.0±1.5 mmHg experienced by the Group Pre-Iso/37 hearts.

3.2.2c Ischemia Temperature Effect on Change in Developed Pressure

Recovery of developed pressure was evaluated as the absolute change (Δ) in developed pressure of each group, from baseline values just before arrest to developed pressure values after 30 min reperfusion following ischemia (Figure 3.16). Hypothermia during ischemia significantly attenuated the decrease in developed pressure that occurred under normothermic conditions (p<0.0001). Developed pressure of Group Pre-Iso/37 hearts decreased by 52.6±4.0 mmHg whereas in Group Pre-Iso/20 hearts, the decrease was by 24.2±3.4 mmHg.

![Figure 3.16. Effect of ischemia temperature on change (Δ) in developed pressure. Beating hearts stimulated with 25 nM Iso prior to arrest and hypothermic ischemia (20°C, 120 min; Group Pre-Iso/20, n=10) had significantly improved pressure development, compared to hearts subjected to normothermic ischemia (37°C, 60 min). Values are mean±SEM. *p<0.0001 vs. Group Pre-Iso/37; two-tailed, unpaired t-test.](image)

3.2.2d Ischemia Temperature Effect on End-Reperfusion Myocardial Adenine Nucleotide Concentrations

End-reperfusion ADP (p=0.0002), ATP (p<0.0001), and total adenine nucleotide (TAN) (p<0.0001) concentrations in the myocardium were significantly preserved in hearts subjected to hypothermic ischemia following catecholamine stimulation in the beating state (Group Pre-Iso/20, n=8), compared to hearts under normothermic conditions (Group Pre-Iso/37, n=11) (Figure 3.17). AMP, ADP, ATP, and TAN values for Group Pre-Iso/20

Results
were 0.31±0.06, 3.66±0.42, 15.62±1.49, and 19.58±1.93 μmol/g DW, respectively. Those for Group Pre-Iso/37 were 0.21±0.06, 1.89±0.12, 5.24±0.68, and 7.35±0.76 μmol/g DW, respectively.

Figure 3.17. Ischemia temperature effect on end-reperfusion myocardial adenine nucleotide concentrations. Hearts treated with 25 nM Iso in the beating state prior to 37°C arrest for 60 min (Group Pre-Iso/37, n=11) were characterized by significantly lower ADP, ATP, and TAN tissue concentrations than hearts under hypothermic ischemia conditions (20°C, 120 min; Group Pre-Iso/20, n=8). Values are mean±SEM. *p≤0.002 vs. Group Pre-Iso/37; two-tailed, unpaired t-test. AMP: adenosine monophosphate; ADP: adenosine diphosphate; ATP: adenosine triphosphate; TAN: total adenine nucleotides.

3.2.3 Ischemia Temperature Effect on Change in Diastolic Pressure in Control and Cp-Iso Groups

Post-normothermic ischemia diastolic function in hearts exposed to catecholamines during arrest (Group Cp-Iso) was the only significantly compromised parameter in the first part of the ischemia study (i.e. Control vs. Cp-Iso experiments, Section 3.2.1). To further evaluate the protective effect of hypothermia, the change in diastolic pressure following normothermic ischemia was compared to that following hypothermic ischemia for both the Control and Cp-Iso groups (Figure 3.18). Statistical analysis showed that hypothermia...
during ischemia significantly attenuated the deleterious effects of normothermic conditions during ischemia in both untreated (p=0.0001) and catecholamine-exposed hearts (p<0.0001).

**Figure 3.18.** Effect of ischemia temperature on post-ischemic change (Δ) in diastolic pressure following catecholamine stimulation during arrest. Diastolic function of hearts subjected to 20°C hypothermic ischemia demonstrated improved recovery over hearts subjected to 37°C normothermic ischemia. Normothermia n-values: Control=11, Group Cp-Iso=12; hypothermia n-values: Control=8, Group Cp-Iso=10. Values are mean±SEM. *p<0.0001 vs. corresponding group in normothermia; two-tailed, unpaired t-test.

### 3.2.4 Summary of Ischemia Study Results

In this model, catecholamine stimulation during arrest significantly compromised post-normothermic ischemia diastolic functional recovery. The diastolic functional compromise was dramatically attenuated by lowering ischemic temperature and was not linked to decreased levels of myocardial adenine nucleotides. In contrast, preserved levels of myocardial adenine nucleotides accompanied the protective effect of hypothermic ischemia in hearts stimulated with catecholamines in the beating state prior to arrest.
4 DISCUSSION

Excessive catecholamine stimulation has been documented to be deleterious to the non-ischemic [Rona et al., 1959; Rona et al., 1975; Mosinger et al., 1977; Steen et al., 1982; Caspi et al., 1993] and ischemic myocardium [Maroko et al., 1971; Maroko et al., 1973; Karlsberg et al., 1979; Muntz et al., 1984; Yoshida and Iimura, 1989]. Based on such evidence, the effect of an elevated level of catecholamines, such as that seen in blood-based cardioplegia, on the globally ischemic myocardium, was questioned. Thus, it was hypothesized that catecholamines can affect basal metabolism of the heart, and that this effect could be linked to a compromised post-ischemic functional recovery.

The Langendorff system was used because of its widely accepted use in whole heart studies. The modifiability, simplicity, and stability of this preparation allow for the studying of a large number of hearts quite efficiently. The system allows for ease of drug administration and removal and evaluation of heart function without the potential complications arising from the body system in an in vivo preparation.

Myocardial oxygen consumption (MVO₂) is largely represented by the energy required for the contraction process of the beating state of the heart. However, the energy required to maintain the integrity of the heart even in the arrested state is an important determinant, although to a lesser degree, of MVO₂. Basal energy requirements include energy required for maintaining protein synthesis and compartmentalized differences in ion concentrations against membrane leaks. Optimal cardioplegic arrest should maximize myocardial protection from ischemia-induced injuries and minimize metabolic and mechanical energy requirements while the myocardium is in its basal state. However, if an elevated level of catecholamines is present within the cardioplegia, such as with blood-based cardioplegias, the protective nature of the cardioplegia may be compromised.

Studies in arrest metabolism have reported basal metabolism to range from about 12% in rats [Sternbergh et al., 1989] to 46% in dogs [Gibbs et al., 1980] of the empty-
beating heart metabolism (see Table 1, p.15). Arrest MVO₂ values in this study ranged from 15.8% (Figure 3.1, p.49) to 27.9% (Figure B.1, p.93). Comparably, a study by Gibbs and Kotsanas [1986] on rabbit hearts demonstrated a 77% drop in the beating, non-working MVO₂ upon arrest with 30 mM K⁺ solution. Considering the variations in experimental conditions, the values presented in this study do roughly correspond with MVO₂ values found by others and it appears that in this model, basal MVO₂ constitutes about one-sixth to over one-quarter of empty-beating MVO₂. However, one thing to consider in the variability of the MVO₂ values is the fact that it is calculated as a unit per dry weight. Dry weight of the hearts were, on average, 11% of wet weights (Appendix D, pp.96-97). This indicates a 89% water content which may or may not have greatly influenced the MVO₂ results. If the majority of edema, due to the low oncotic property of crystalloid perfusate, occurred in the beginning of the experiment, then the MVO₂ value would be equally "erroneous" throughout all subsequent intervals. However, if the edema occurred sometime throughout the perfusion intervals, then MVO₂ results could vary largely from one perfusion interval to another.

4.1 Basal Metabolism Under Varying Arrest Conditions

4.1.1 Reasons for not Using Epinephrine as the Catecholamine

Although the protocol describes using isoproterenol as the catecholamine challenge, initially, epinephrine had been chosen as the candidate catecholamine. Results using epinephrine are given in Appendix A (pp.90-92). Pilot studies (unpublished data. I.M. Rebeyka) had shown that plasma epinephrine levels increased from less than 0.8 nM to more than 2.8 nM after institution of the cardiopulmonary bypass apparatus. Thus, to simulate the elevated catecholamine levels of the clinical situation, a dose of 2 nM Epi was initially used. However, 2 nM Epi in the presence of potassium arrest (Figure A.1. Appendix A, p.90) did not significantly change MVO₂. Although the focus of the study was to determine the effect of the presence of catecholamines during arrest, the effect of 2
nM Epi on the beating heart was questioned. That is, the absence of any apparent effect of 2 nM Epi may have been due to a masking effect of the potassium arrest, or due to the lack of any effect at all. To address this issue, further experiments were done to confirm that the dose of 2 nM Epi was experimentally measurable in this model of the beating, non-working rabbit heart. The results are given in Figure A.2, Appendix A (p.91). No significant change in MVO₂ was observed in the beating, non-working heart upon exposure to 2 nM Epi.

Considering the nature of epinephrine and its known positively inotropic and chronotropic effects [Kaufman et al., 1951; Lee and Yu, 1964; Suga et al., 1983; Endoh and Blinks, 1988], an increase in MVO₂ was expected in the beating heart model. The lack of any effect of administering 2 nM Epi may be that the model is not sensitive enough to detect the increase in MVO₂ caused by this particular dose of Epi, or that the human sympathetic response is different from the rabbit's [Downing and Chen, 1985] and so, the increased dose of 2 nM Epi found in humans may not have been transferable to the rabbit model. Furthermore, alpha₁-receptor stimulation by epinephrine has been shown to have a positive inotropic effect in the rabbit myocardium [Endoh et al., 1991]. Generally, catecholamine cardiotoxicity has been considered to be through the beta-adrenergic receptor pathway, as increased inotropy, chronotropy and energy demand exceed supply, thereby creating "ischemic-like" injury. However, a study by Downing and Chen [1985] investigating the effect of catecholamine cardiotoxicity in the rabbit myocardium revealed that activation of the alpha-adrenergic pathway is the dominant mechanism of injury in the rabbit myocardium. Yet, in the rabbit, the maximal effects of beta-stimulation were demonstrated to be greater than the maximal effects of alpha-stimulation [Endoh and Blinks, 1988]. The importance of the alpha₁-response to positive inotropy in the rabbit suggests that the alpha-mediated catecholamine effects seen in this experimental model may not be as great as they could be.

Discussion
Alpha₁-receptor activation has been shown to increase intracellular Ca²⁺ through cAMP-independent pathways, and thus, can result in inotropic stimulation of the myocardium [Bruckner et al., 1984; Scholz et al., 1988; Endoh et al., 1991]. If so, the combined alpha and beta effects of epinephrine would be expected to increase MVO₂. The effects of 25 nM Epi and 50 nM Epi (Figures A.3 and A.4, Appendix A, pp.91-92) were also tested in the empty-beating heart, and there was a tendency for MVO₂ to increase with these higher doses but these changes were not statistically significant.

In contrast to the possible positive inotropic effects of alpha₁-stimulation, Oleksa et al. [1996] and Chen et al. [1996] have recently found an inhibitory effect of alpha₁-adrenergic stimulation on beta-adrenergic responses. Using norepinephrine, Oleksa et al. [1996] found that the activation of the Cl⁻ current through beta-adrenoceptor stimulation was limited by the intrinsic ability of norepinephrine to also activate alpha₁-adrenergic receptors. (Normally, because E_Cl is about -50 mV, cAMP-dependent activation of the Cl⁻ channel has two roles in beta-stimulation: it enhances excitability by helping to depolarize the cell, and it shortens AP duration to preserve diastole, and coronary blood flow, at faster heart rates [Weiss, 1997]). Similarly, Chen et al. [1996] found that the L-type Ca²⁺ current stimulated by beta-adrenergic agonists was inhibited by alpha₁-adrenergic activation. With the possibility that alpha₁-adrenergic stimulation may be inhibitory to beta-adrenergic responses, and considering how beta-adrenergic stimulation increases MVO₂ in the beating heart, any alpha₁-stimulation may confound the MVO₂ effect of beta-stimulation in the myocardium. In summary, the precise role the alpha-adrenergic response has in the myocardium and how it interacts with the beta-adrenergic response is yet to be elucidated. Thus, whether an alpha₁-adrenergic antagonist could depress the possible inhibitory effect of alpha₁-stimulation on beta-stimulation or block the vasoconstrictive response to alpha₁-stimulation, an increase in MVO₂ would be expected upon isolating the beta-effects of epinephrine.

Discussion
Another possible reason for the lack of any expected effect from Epi may have stemmed from a possible complication of its beta-adrenergic effects by its vasoactive alpha-adrenergic effects [Mohrman and Feigl, 1978; Feigl, 1987]. That is, it may have been difficult to detect the beta-effect of Epi since the parameter measured, \( \text{MVO}_2 \), is directly proportional to coronary flow, which is negatively affected by the vasoconstrictive alpha-action of Epi [Berne, 1958; Imai et al., 1975].

An additional study was therefore conducted with epinephrine to determine whether its alpha-effects may indeed have substantially affected the \( \text{MVO}_2 \) results. In order to determine whether the lack of any change in \( \text{MVO}_2 \) (in the beating hearts experiments) was secondary to \( \alpha_1 \)-stimulation, prazosin was used to block the \( \alpha_1 \)-effects of epinephrine while isolating its beta-effects. Prazosin (Prz) is a competitive \( \alpha_1 \) (post-synaptic)-selective adrenergic antagonist which causes less tachycardia than other equieffective vasodilating drugs [Cambridge et al., 1977]. Thus, the effect of prazosin will focus on its vasorelaxant properties with minimal interference with the other mechanical determinants of \( \text{MVO}_2 \) since the experiments with epinephrine and prazosin were done in the beating heart model. The results of the effect of epinephrine in the presence of prazosin in the beating, non-working heart are shown in Figure A.5 (Appendix A, p.92). After 30 min equilibration with KHB, the \( \text{MVO}_2 \) of hearts did not change with the addition of 25 nM Epi (0.450±0.041 mL O\(_2\)/min/g DW to 0.461±0.026 mL O\(_2\)/min/g DW). Prazosin was then added to the KHB+Epi perfusate and \( \text{MVO}_2 \) of hearts significantly decreased to 0.372±0.030 mL O\(_2\)/min/g DW (p<0.05, Figure A.5, Appendix A, p.92).

Due to the conflicting reports on the action of \( \alpha_1 \)-adrenergic receptors, it is difficult to determine why such an effect was seen using prazosin. If \( \alpha_1 \)-receptors do indeed have a positive inotropic effect, then perhaps prazosin inhibited the positive inotropic action of \( \alpha_1 \)-stimulation complementary to beta-stimulation. However, since epinephrine did not have any augmentative effect at all on \( \text{MVO}_2 \) in this model, the results do not support this possibility. If, in contrast, \( \alpha_1 \)-adrenergic receptor stimulation can
suppress the upregulation of Ca^{2+} and Cl^{-} currents by beta-stimulation. then prazosin should have potentiated the beta-effect of epinephrine. Yet, no effect was seen in these experiments. Statistical analysis of the change in coronary flow (Table V(b), Appendix D. p.97) from the Epi perfusion interval to the Epi+Prz perfusion interval (Intervals B to C. Figure A.5, Appendix A. p.92) indicates a significant increase in coronary flow in the presence of prazosin (p<0.02, two-tailed, paired t-test). With the increased coronary flow, the hearts decreased the amount of O_2 extracted from the perfusate, and this decrease was enough to significantly lower the MVO_2 value of the interval (Interval C). Hence, although the dose of prazosin used was effective in this model, the dose of epinephrine used may still not have been enough to demonstrate marked beta-adrenergic effects on MVO_2.

The most plausible explanation for the lack of an Epi effect is that the dosage of Epi may have been too low. The dosage study up to 50 nM Epi showed no significant effect, indicating the possibility that even higher doses were required for a clear beta-effect to occur. This possibility is supported by studies which have shown that low doses of Epi have a greater alpha-effect with very little beta-effect, while higher doses of Epi demonstrate a stronger beta-effect [Benfey and Varma, 1967; Endoh and Blinks, 1988]. In fact, most studies using Epi have used doses around the micromolar range [Century. 1954: Lee and Yu, 1964; Challoner, 1968; Endoh and Blinks, 1988] compared to the nanomolar value used in this study to simulate the clinical situation (see p.68). If the dose is too low for a beta-effect, then any positive inotropy effected by the alpha-response would be suppressed by prazosin, which may explain the decrease in mean MVO_2 as prazosin was added to the KHB+Epi perfusate (Interval C, Figure A.5, Appendix A. p.92).

The Epi experiments provided interesting insight into the complicated mechanisms of catecholamine stimulation. Until further experiments can be done with proper dosages, no conclusions can be drawn except that upregulation of contractile function may arise from the interaction of both alpha- and beta-stimulatory effects which regulate each other to ultimately regulate myocardial function.
4.1.2 Catecholamine Stimulation of Arrested Myocardium

In consideration of the ambiguous results with epinephrine, the catecholamine of this experimental model was changed to a drug that was simpler in action for a more unifactorial effect. Specifically, isoproterenol was considered because it has only a beta-effect [Erlij and Mendez, 1964; Endoh and Blinks, 1988]. Since the heart is predominantly a beta₁-receptor tissue, in both humans and rabbits [Brodde et al., 1986; Tenner et al., 1989], the focus was on the beta-effect of catecholamines in the myocardium. Isoproterenol could be used to do so and thereby, to eventually determine whether any decreased post-ischemic functional recovery due to peri-ischemic catecholamine stimulation could possibly be attributed to the beta-effect of catecholamines and whether this beta-effect affected basal MVO₂ despite the absence of mechanical activity.

Prior to starting with isoproterenol in the arrested state, its effect in the beating, non-working heart was determined to confirm its activity (Figure B.1, Appendix B, p.93). Unlike the results with epinephrine, 25 nM Iso demonstrated an unequivocal increase in MVO₂ (p=0.007 vs. initial KHB perfusion interval). MVO₂ significantly increased by over 50% of its control period value (0.404±0.067 vs. 0.622±0.049 mL O₂/min/g DW). Furthermore, this dose of Iso did not appear to be cardiotoxic, as pre-Iso MVO₂ values were recovered after 30 min of reperfusion with KHB following a 30 min perfusion interval with Iso (Figure B.1, p.93). Thus, further experiments were continued using isoproterenol.

The marked increase in MVO₂ observed in the isoproterenol study is not surprising as isoproterenol, a synthetic catecholamine, is a known potent beta-receptor agonist that is structurally related to epinephrine [Kaufman et al., 1951]. Isoproterenol increases both the heart rate (chronotropy) [Kaufman et al., 1951; Kassebaum and Van, 1966; Hasenfuss et al., 1989] and the contractility (inotropy) [Kaufman et al., 1951; Hasenfuss et al., 1989; Futaki et al., 1991] of the heart, both of which affect myocardial oxygen consumption of
the beating heart [Sonnenblick et al., 1965; Suga et al., 1983; Hasenfuss et al., 1989; Futaki et al., 1991].

The fact that the myocardial response to Iso was a clear increase in basal MVO₂, compared to the response to Epi, could be attributed to both the dose and mechanism of action. Whereas 25 nM Iso demonstrated a definitive increase in MVO₂ of beating hearts (Figure B.1, Appendix B, p.93), the same dosage for Epi did not (Figure A.3. Appendix A, p.91). This is not surprising as Epi is 10-40 times less potent at beta₁-adrenoceptors and 3-15 times less potent at beta₂-adrenoceptors than Iso [Bilezikian, 1987]. In fact, Klocke et al. [1965] showed that Iso could increase beating heart MVO₂ by 55% whereas the same dose of Epi only increased MVO₂ by 5%. As discussed in detail above, Epi has alpha-effects as well as beta-effects. In fact, Epi has a stronger alpha- than beta-action, except at higher doses [Benfey and Varma, 1967; Endoh and Blinks, 1988] and thus, the alpha-effects may be confounding any beta-effect.

Clearly, beta-adrenoceptor-mediated mechanical stimulation increases the energy expenditure of the heart, reflected in increased myocardial oxygen consumption [Eckstein et al., 1950; Fisher and Williamson, 1961; Klocke et al., 1965; Sonnenblick et al., 1965; Gibbs et al., 1967; Coleman et al., 1971; Suga et al., 1983]. However, whether catecholamines have any direct effect on myocardial metabolism, independent of their cardiodynamic effects, is yet to be fully understood. The present study examining the effect of isoproterenol on MVO₂ of the arrested myocardium addressed this question. By administering catecholamines to a quiescent heart, any effect the beta-adrenergic agonist would have on energy state would be reflected in a change in basal metabolism. As hypothesized, isoproterenol did significantly affect basal MVO₂ (Figure 3.1, p.49), increasing it by over 60% of the control arrest MVO₂ value.

Thus, results in both the beating (Figure B.1, Appendix B, p.93) and arrest experiments (Figure 3.1, p.49) with Iso demonstrated a more than 50% increase in MVO₂ from the control beating and control arrested states, respectively. Klocke et al. [1965].
however, found that a dose of Iso which increased beating heart MVO₂ by 55% (0.30 μg) only increased basal MVO₂ by 5%. An interesting observation is that Klocke et al. [1965] found that their arrested heart MVO₂ averaged 78% of the beating heart MVO₂, which is in sharp contrast to the aforementioned 16-28% values found in the literature and these studies. The unusually high basal MVO₂ could be attributed to the fact that the Klocke group had also stimulated the hearts with Iso in the beating state prior to arrest. As Lochner et al. [1968] had previously shown, basal MVO₂ is influenced by the state of the beating heart prior to arrest and thus, the hearts in Klocke et al.'s [1965] study had a high basal MVO₂. Perhaps by raising basal MVO₂ so much with high pre-arrest activity, any effect of Iso was relatively small, and therefore masked in the basal state.

Hanley and Loiselle [1992] have documented the great influence basal intracellular Ca²⁺ concentration has on basal MVO₂. Thus, a possible explanation for the increase in basal MVO₂ effected by Iso may be through an increase in Ca²⁺ mobilization within the myocyte despite the absence of mechanical activity. This increased Ca²⁺ would not be to the extent seen in conjunction with the contractile state, but enough to affect the basal metabolism of the heart as more energy must be used to maintain intracellular Ca²⁺-homeostasis, especially considering that the SR Ca²⁺-ATPase may contribute 28% to the basal MVO₂ value [Ponce-Hornos, 1990].

The active nature of the resting state of the myocardium with respect to intracellular Ca²⁺ concentration was demonstrated by Lappé and Lakatta [1980], who found that resting force varied linearly with Ca²⁺ concentration in resting rat right ventricular papillary muscle exposed to stepwise increases and decreases in Ca²⁺ concentrations (0.4-4 mM) in the bathing fluid. Further studies by this group supported their initial findings and sought to determine the relationship between diastolic Ca²⁺ flux and subsequent twitch force in isolated rat and cat papillary muscles [Lakatta and Lappé, 1981]. Their results suggested that an increase in the Ca²⁺ concentration during diastole could predict an increased twitch force up to a certain concentration. This effect could possibly be explained by a "priming"
effect of the Ca\(^{2+}\) concentration on the contractile proteins or, the increasing concentrations could be inducing greater release of Ca\(^{2+}\) from the SR upon inward current activation, since the elevated Ca\(^{2+}\) concentration already present in the cytosol would make it that much closer to the threshold of release. Thus, in diastole, or the resting state of the heart, increased Ca\(^{2+}\) activity may be occurring, enough to stimulate increased mitochondrial respiration [Denton and McCormack, 1980a; Denton et al., 1980; Denton and McCormack, 1980b; Denton et al., 1988; McCormack and Denton, 1979; Rutter and Denton, 1988; Rutter et al., 1989] or the SR Ca\(^{2+}\)-ATPase pumps, but not enough to cause a contraction. This is what may have occurred in the Iso-treated arrested myocardium and may explain how Iso increased basal MVO\(_2\) in the arrested heart (Figure 3.1, p.49).

Further support of this possibility comes from Solaro et al. [1974] who conducted experiments in purified canine cardiac myofibrils to determine the amount of calcium required for myofibrillar activation. For the most part, ATPase activity correlated almost exactly with isometric tension activation except for in the lower Ca\(^{2+}\) concentrations of 10\(^{-8}\) to 10\(^{-6}\) M Ca\(^{2+}\), where ATPase activity demonstrated elevated activity over tension development. Solaro et al. [1974] suggested that at these low Ca\(^{2+}\) concentrations, not enough to cause a true contraction, cross-bridge formation might be discontinuous along the length of the filament, where ATP may be getting hydrolyzed but overall, no tension is generated at the fibre ends.

Although the results of the effect of catecholamine stimulation in the arrested myocardium demonstrated an increase in basal MVO\(_2\), one cannot conclude whether the catecholamine effect in the arrested heart in this model was one that resulted from direct stimulation of oxidative metabolism or whether the increase in basal energy expenditure was secondary to an increase in intracellular Ca\(^{2+}\). The augmented MVO\(_2\) may even have been a combination of both.

*Discussion*
4.2 Peri-Ischemic Catecholamine Stimulation and Myocardial Functional Recovery

If, as it was hypothesized, catecholamines can substantially affect basal MVO$_2$, then this effect may be deleterious to the ischemic heart. Thus, catecholamines were administered within the cardioplegia (Group Cp-Iso) to see if the basal effect of catecholamines seen in the perfusion arrest experiments could be representative of the ischemic situation. The control group (Control) had no catecholamine stimulation. Interestingly, hearts that were stimulated with 25 nM Iso in their arrested state (i.e. Group Cp-Iso) and then were subjected to normothermic ischemia had a significantly elevated post-ischemic diastolic tone (Figure 3.7, p.57).

Many studies have shown that diastolic relaxation is incomplete and stiffness is increased under conditions of ischemia [Mathey et al., 1974; McLaurin et al., 1973; Palacios et al., 1978; Serur et al., 1976; Weisfeldt et al., 1974; Diamond and Forrester, 1972]. Relaxation is an energy-dependent process, requiring the activity of the ATP-dependent Ca$^{2+}$ pumps of the SR. In contrast, activation is a passive process where Ca$^{2+}$ can diffuse down its concentration gradient from the extracellular space and the sarcoplasmic reticulum, where the Ca$^{2+}$ concentration is in the millimolar range, into the cytosol where the Ca$^{2+}$ concentration is about 0.2 μM [Ahn et al., 1994; Cobbold and Rink, 1987; Katz, 1992; Lee et al., 1987]. The maximal rate at which Ca$^{2+}$ can be removed from the cytosol to relax the heart is about an order of magnitude lower than the rate at which influx occurs to activate the heart [Katz, 1992]. Thus, an energy-starved heart is easily susceptible to Ca$^{2+}$ overload which would compromise the relaxation and tone of the myocardium during diastole.

Although the relaxation property per se of the heart (i.e. $-dP/dt_{max}$) was not measured, Mathey et al. [1974] demonstrated that an exponential relationship exists between diastolic stiffness and relaxation ability. Stiffness is estimated as dP/dV and since the experimental model used isovolumic conditions, an increase in diastolic pressure would

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indicate increased stiffness. Katz [1992] indicates that stiffness may be considered an index of the extent of Ca\(^{2+}\) removal from troponin. If so, the increased diastolic pressure seen in hearts in Group Cp-Iso of normothermic ischemia and Group Pre-Iso/37 may be indicative of increased levels of cytosolic Ca\(^{2+}\) that may have been induced by the excessive catecholamine stimulation.

In Lakatta and Lappé's [1981] study on diastolic Ca\(^{2+}\) concentration and twitch force, a decline in twitch force was seen with supra-optimal Ca\(^{2+}\) concentrations during the resting state, indicating that if diastolic tone was too elevated with the presence of too much Ca\(^{2+}\), mechanical function would be compromised. The higher levels of diastolic myoplasmic calcium concentration could be a type of "diastolic Ca\(^{2+}\) overload" which would contribute to a decline in mechanical function as diastolic tension would be greater [Lakatta and Lappé, 1981]. If the heart is too stiff, it cannot preload sufficiently and, in relation to the force-length relationship curve of muscle, its ability to generate maximal force is impaired at shorter lengths [Katz, 1992]. This is reflected in decreased systolic pressure development that accompanies increased diastolic stiffness, according to the Frank-Starling relationship [Katz, 1992]. Eventually, if too much Ca\(^{2+}\) enters the cell, such as during the reperfusion process, the cell may undergo complete Ca\(^{2+}\) overload, reminiscent of that resulting from catecholamine-induced myocardial injury. Appropriate intracellular Ca\(^{2+}\) concentration, therefore, is crucial not only for proper relaxation but also for proper contractile function.

It is interesting to note that the changes in SP, DP, and DevP experienced by the Group Pre-Iso/37 hearts (excessive catecholamine stimulation in beating state before arrest) were even greater than those experienced by either the hypothermia Control or Group Cp-Iso hearts. Furthermore, even though the change in diastolic pressure for Group Pre-Iso/20 was significantly attenuated by hypothermia (Figure 3.15, p. 63), this change was in contrast to the lack of any change seen with the Control Group and Group Cp-Iso under hypothermic ischemia conditions (Figure 3.11, p. 60). This suggests that excessive

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catecholamine stimulation exacerbates ischemic damage to a greater degree if the myocardium is exposed to the catecholamines in the beating state just prior to arrest (such as with Groups Pre-Iso/20 and Pre-Iso/37), rather than during arrest. The amount of Ca\(^{2+}\) mobilized with each beat would be greater than the amount mobilized during arrest (eg. with Group Cp-Iso) and thus, the myocardium would be "pre-loaded" with an increased amount of Ca\(^{2+}\) via the cAMP-dependent upregulation of Ca\(^{2+}\) influx and SR Ca\(^{2+}\) uptake. Because the level of basal metabolism is influenced by the metabolic level of the preceding beating state [Lochner et al., 1968], hearts stimulated with Iso in the beating state prior to arrest would be predisposed to a higher basal metabolism during arrest. Thus, despite the fact that coronary blood supply was eliminated during arrest, energy demand would be at an elevated level.

With increased energy demand, ATP breakdown would occur. As ATP gets catabolized to adenosine, then inosine, then hypoxanthine, energy levels would decrease and Ca\(^{2+}\) homeostasis would be more difficult to maintain. The rise in intracellular Ca\(^{2+}\) would activate a Ca\(^{2+}\)-dependent protease in the cytosol, which attacks xanthine dehydrogenase. Xanthine dehydrogenase normally reduces NAD\(^{+}\) to NADH while oxidizing hypoxanthine to xanthine. However, when proteolytically modified, a new enzyme activity emerges, xanthine oxidase, which reduces molecular oxygen to produce the superoxide radical [McCord, 1984]. Thus, re-introduction of oxygen upon reperfusion will trigger the production of superoxide radicals which react with lipid molecules and lead to the disruption of the cellular and intracellular membranes [McCord, 1984]. Consequently, further increase in intracellular Ca\(^{2+}\) concentration would occur through the disrupted membranes, thereby exacerbating the damage.

Degradation of ATP past AMP (i.e. to adenosine and its catabolites) results in the loss of precursors for re-synthesis [Wiedmeier et al., 1972; Snow et al., 1973; Berne and Rubio, 1974]. In rabbits, where the mitochondrial synthesis of adenine nucleotides is primarily due to the salvage pathway which uses such precursors (in contrast to the de

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"novo pathway using ribose-5-phosphate (Rossi, 1975), the loss of preformed precursors could therefore have a profound effect. Not only will loss of precursors limit ATP resynthesis but also, the mitochondria may also be too damaged to synthesize ATP. Thus, the decreased level of ADP in Group Pre-Iso/37, could have limited ATP re-synthesis via the creatine kinase-catalyzed reaction using phosphocreatine and ADP. Also, it may be possible that the Ca\(^{2+}\) overload effect in Group Pre-Iso/37 led to depletion of ATP and production of superoxide radicals upon reperfusion, both of which contributed to disabling mitochondrial energy metabolism by consequently overloading the mitochondria with Ca\(^{2+}\) as well.

Decreased levels of adenine nucleotides, however, cannot account for the compromised diastolic function of Group Cp-Iso following normothermic ischemia. In this case, the protection afforded by cardioplegia, despite the concomitant presence of catecholamines, could have also minimized the extent of ATP depletion, thereby allowing for an easier recovery of ATP stores upon reperfusion. Engelman et al. [1979] studied the time course of myocardial high energy phosphate degradation under conditions of both normothermic and hypothermic (8-15°C) cardioplegic arrest. They found that ATP decay was much more dramatic in the normothermic group, despite hyperkalemic cardioplegia, compared to hypothermic potassium arrest, but both had better ATP preservation than groups without cardioplegia at all. In this study, because the myocardium was sampled for adenine nucleotide concentrations at the end of the 30 min reperfusion period, the hearts in Group Cp-Iso may have been able to restore their ATP levels. It may have been more informative, therefore, to obtain biopsies immediately after the ischemic period, for a better idea of the extent of ATP decay under each condition. Such data would also provide information about the catecholamine effect on ATP during ischemia (eg. Group Cp-Iso data). Due to the small size of the hearts used, though, this could not be feasible.

In considering the ATP data, a few factors should be taken into account, however. ATP and its intermediates have been shown to be compartmentalized within the cell but the

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method used to measure their concentrations only allowed for determination of the average tissue content. The ATP which determines tissue survival may be in relatively small compartments whose contents may be masked by changes (or lack thereof) in other compartments upon homogenization of the myocardial tissue [Miller and Horowitz. 1986]. This theory is supported by reports that ischemic hearts can suddenly lose contractile activity despite maintenance of relatively high ATP levels [Gudbjarnason et al., 1970]. Gudbjarnason et al. [1970] documented that contraction ceased when the ATP concentration had dropped from only 5.7 to 4.5 μmol/g during ischemia. Thus, depletion of ATP stored in a critical subcompartment of the cytosol may have occurred but could not be detected upon analysis of the tissue homogenate of the normothermic ischemia Group Cp-Iso hearts.

Although only Group Pre-Iso/37 demonstrated any significant depletion of adenine nucleotides, one cannot conclude that the control groups had fully preserved adenine nucleotide concentrations since control levels were not recorded prior to the ischemic period. Vanoverschelde et al. [1994], however, found that isolated, Langendorff-perfused rabbits hearts at 37°C had the following concentrations: AMP=1.0±0.3; ADP=7.0±0.7; ATP=25.8±1.7; TAN=33.7±1.9 μmol/g DW, and Hearse et al. [1975] found similar ATP concentrations in the Langendorff-perfused rat heart. Comparison with the normothermic ischemia Control Group values indicates a decrease of about 50% for all myocardial adenine nucleotides.

In consideration of the protective effect of hypothermia and K+ -cardioplegia, it is not surprising that only the hearts which experienced an excessive catecholamine challenge under normothermic conditions in addition to normothermic ischemia (i.e. normothermia Group Cp-Iso and Pre-Iso/37) demonstrated significantly compromised diastolic function. Even Group Pre-Iso/20, which was exposed to Iso under normothermic beating conditions had an elevated DP compared to the hypothermic ischemia Control Group. The magnitude of change in DP in Group Pre-Iso/20, however, was not as great as with Group Cp-Iso/37.

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Perhaps the elevated basal MVO₂ may have been suppressed enough by the hypothermic conditions of ischemia to minimize ischemic energy expenditure and allow for improved post-ischemic reperfusion recovery compared to Group Cp-Iso/37.

The protection provided by hypothermia is also evident upon comparing the diastolic functional recovery of Groups Control and Cp-Iso in normothermia with their corresponding group in hypothermia (Figure 3.18). Hypothermic ischemia was found to very significantly (p≤0.0001) preserve diastolic function for both groups compared to the corresponding groups under normothermic ischemia. Similarly, the reason that no significant increase in diastolic pressure was observed in hearts given Iso within the cardioplegia and then subjected to hypothermic ischemia may be due to the fact that cardiac effects of beta-stimulation are reduced or even abolished in hypothermic conditions [Price et al., 1967; Lauri, 1996]. This may be partly attributed to the decreased fluidity of the membrane proteins and phospholipids at lower temperatures [Darnell et al., 1990], which would affect the mobility of the stimulatory G-protein essential in beta-adrenoceptor signal transmission. Furthermore, the combined protective power of hyperkalemic cardioplegia and hypothermia has been well documented to improve preservation of high energy phosphates [Engelman et al., 1979; Hearse et al., 1980; Ahn et al., 1994], myocardial integrity [Rosenfeldt et al., 1980], and myocardial function [Hearse et al., 1975; Hearse et al., 1980; Rosenfeldt et al., 1980].

4.3 Reversal of Catecholamine Effect on Basal MVO₂

Esmolol was chosen as the candidate beta-adrenergic antagonist for this study due to its cardioselectivity and short-acting properties. Initially, no significant effect of esmolol on basal MVO₂ (without any excess catecholamine influence) was observed (Figure 3.2, p.50). Since the heart was already in its basal state, any further depression of MVO₂ that esmolol could have afforded on the heart may have been too small to measure, or perhaps the dose was not high enough, or was indicative of minimal endogenous catecholamine.

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action. Thus, the effect of this dose (2 mg/L) on the beating, non-working heart was determined (Appendix C, p.94). Because esmolol has been demonstrated to be a cardioselective beta1-antagonist which has the ability to depress both resting heart rate [Gorczynski et al., 1983; Iskandrian et al., 1985] and exercise- [Iskandrian et al., 1985] or catecholamine- [Zarosliński et al., 1982; Gorczynski et al., 1983] induced tachycardia, a decrease in MVO₂ was expected in concert with functional depression. However, a significant increase in beating MVO₂ was observed after 30 min perfusion with KHB + 2 mg/L Esm. Closer inspection of individual hearts revealed that both coronary flow (Table V(b), Appendix D, p.97) and arteriovenous oxygen difference, both of which are directly proportional to MVO₂, tended to increase with Esm administration. Murthy et al. [1983] found that esmolol seemed to have an inexplicable hypotensive effect independent of its beta-blocking activity. Furthermore, esmolol has been found to have some intrinsic sympathomimetic activity at low concentrations of 10⁻⁹ to 10⁻⁵ M [Gorczynski et al., 1983] and the dose of 2 mg/L Esm is less than 10⁻⁵ M, which may account for the increased arteriovenous oxygen difference. Indeed, higher doses of 10 and 100 mg/L Esm seemed to have a more depressant effect on MVO₂ (Figure C.2 and C.3, Appendix C, p.95). With 100 mg/L Esm, MVO₂ decreased dramatically but the sample size was much too small to evaluate statistical significance (Figure C.3).

An intermediate dose of 25 mg/L Esm (which is about 10⁻⁴ M) was then administered to hearts which were stimulated with 25 nM Iso while in the arrested state (Figure 3.3, p.51). No significant change in MVO₂ resulted, indicating that the subsequent addition of a beta₁-antagonist to a catecholamine-exposed arrested heart cannot reverse the catecholamine-induced increase in basal MVO₂. Evidently, the pre-administration of Iso was enough to elicit an effect which a large dose of esmolol in the order of about five magnitudes larger than the dose of Iso could not reverse. Thus, even though the antagonist was able to "out-compete" the agonist for receptor sites, the action of the antagonist came...
too late as the agonist had already stimulated the cell. Furthermore, because a beta₁-antagonist was used, the possibility of a beta₂-mediated effect exists as well.

BDM was another drug used to possibly reverse the deleterious effects of excessive catecholamine stimulation in the arrested myocardium. Yet, whereas Esm was primarily used to directly target the action of Iso at the site of the beta-adrenergic receptor, BDM was used to intervene at the intracellular level. BDM has been shown to inhibit myofibrillar ATPase activity [Higuchi and Takemori, 1989; Higuchi et al., 1989; Ebus and Stienen, 1996], and decrease force production through increased detachment and decreased attachment of actin-myosin cross-bridges [Ebus and Stienen, 1996]. BDM energetically stabilizes the unattached state of the myosin molecule [Higuchi and Takemori, 1989; Higuchi et al., 1989]. In addition, BDM has been implicated in the inhibition of Ca²⁺ influx via the slow inward channel [Bergey, 1978; Wiggins et al., 1978; Wiggins et al., 1980; Coulombe et al., 1990; Gwathmey et al., 1991]. This could counteract the upregulation of Ca²⁺ influx via beta-stimulation. BDM also appears to decrease myofilament sensitivity to Ca²⁺ [Gwathmey et al., 1991; Backx et al., 1994]. Not only does this contribute to a decrease in maximal force but also, more Ca²⁺ must bind to the troponin-tropomyosin complex for cross-bridge attachment to occur in the presence of BDM compared to in its absence. Thus, if intracellular Ca²⁺ increases with Iso stimulation of the arrested myocardium, then BDM could interfere with the deleterious effects of increased diastolic Ca²⁺ concentration.

The MVO₂ results demonstrating the effect of BDM on basal MVO₂ show that BDM had no significant effect on basal MVO₂ (Figure 3.4, p.53), indicating that basal metabolism achieved with hyperkalemic cardioplegia reduced MVO₂ low enough that any effect BDM had was insignificant. Likewise, there was no significant effect of BDM on the catecholamine-stimulated arrested myocardium (Figure 3.5, p.54). Statistically, BDM did not change the elevated basal MVO₂ of the Iso-stimulated arrested heart, although the error accompanying each interval mean value is fairly large and there appears to be a pattern
pattern of decrease in MVO$_2$ induced by the addition of BDM. Further experiments would have to be done to determine whether this effect is indeed a significant one. If, however, BDM did significantly reverse the catecholamine-induced increase in basal MVO$_2$, then this would suggest that increased intracellular Ca$^{2+}$ and ATP breakdown are the mechanisms by which catecholamines can stimulate basal MVO$_2$ of the arrested myocardium.
CONCLUSIONS

Excessive catecholamines increase basal metabolism of the arrested myocardium. This effect cannot be reversed by the subsequent addition of a beta-adrenoceptor antagonist or a Ca²⁺-modulator. Exposure of the arrested myocardium to excessive catecholamines is deleterious to post-normothermic ischemia recovery of diastolic function. But hypothermia during ischemia attenuates this effect and a decreased level of myocardial adenine nucleotides is not a factor contributing to the compromised function. Hypothermia during ischemia also improves post-ischemic function of hearts stimulated with catecholamines in the beating state prior to arrest, and this protective effect is associated with preserved adenine nucleotide concentrations.

By gaining a better understanding of the mechanism of excessive catecholamine stimulation and its relevance to both non-ischemic and ischemic myocardium, measures for myocardial protection during compromised states of disease or surgery can be optimized.
LIMITATIONS

Various aspects of this study limited the full scientific value of the results:

1. **Sample size**: The sample size calculation indicates that a sample of at least 9 hearts per group would allow for detection of an intergroup difference of 10% with 95% confidence and a power of 85%. However, most of the MVO₂ data, and some of the data in the ischemia study, were represented by groups that were less than 9 in number. Although statistical analysis was still performed for these smaller groups, the smaller sample size tended to violate the basic rules of parametric statistical analysis such as the requirement of random sampling from a normally distributed population, and the requirement of having enough data within each group to form a normal distribution.

2. **Drug administration**: A more thorough dose-response study with all drugs used should have been done to minimize uncertainty about the effectivity of a dose.

3. **Heart rate (HR)**: HR is a determinant of MVO₂ and thus, if the hearts were paced, perhaps a more fair comparison could be made among the hearts.

4. **Wet weight vs. dry weight**: As discussed in the Discussion, edema formation may have greatly influenced MVO₂ values if it occurred throughout the entire experiment.

5. **Animal model**: Although the rabbit heart is primarily a beta₁-receptor tissue [Tenner et al., 1989] and the maximal effects of beta-stimulation have been demonstrated to be greater than the maximal effects of alpha-stimulation [Endoh and Blinks, 1988]. Downing and Chen [1985] reported that the alpha-adrenergic pathway is the dominant mechanism of injury in the rabbit myocardium. Thus, the rabbit myocardium may not provide clear, indisputable results with respect to the effect of beta₁-stimulation and its effects during arrest and ischemia (see p.69).
FUTURE STUDIES

Further studies would need to be conducted to investigate the mechanism of the catecholamine effect in arrested myocardium. If, by pre-treating hearts with a beta-antagonist, the hearts remain unaffected upon catecholamine administration, then this would indicate beta-adrenoceptor-mediation of catecholamine-induced increase in basal MVO₂ of arrested myocardium. If Ca²⁺ is (also) a mediator of increased basal MVO₂, then hearts pre-treated with Ca²⁺-antagonists, or modulators, or substances which can chelate intracellular Ca²⁺, should maintain their basal MVO₂ upon exposure to catecholamines.

Increased intracellular Ca²⁺ concentration and decreased ATP levels may be the main factors of decreased post-ischemic functional recovery mediated by excess catecholamine stimulation. Yet, these experiments do not reveal whether, or how much, these two are related. To clarify their roles in compromising myocardial function, the ischemia experiments could be done using Ca²⁺-free solution during reperfusion. If diastolic tone is just as high as with the original perfusate (KHB), then the poor diastolic functional recovery may be attributed to ATP depletion, rather than to an overload of Ca²⁺ in the cytosol. This may explain why Group Cp-Iso had compromised diastolic function after normothermic ischemia, even though its levels of myocardial adenine nucleotides were not different from the Control Group. However, in normocalcemic perfusion with KHB, one cannot distinguish whether the increased diastolic tone was due to an already present Ca²⁺ overload, or one that was due to reperfusion injury.

Another way to distinguish between ischemic injury and reperfusion injury could be to use an adenosine deaminase inhibitor and an adenine nucleoside transport blocker to inhibit adenosine deamination and block nucleoside release, respectively. Use of these two interventions would result in the site-specific entrapment of intramyocardial adenosine and inosine generated during ischemia, thereby preventing degradation to free-radical substrates during reperfusion. Thus, the effect of catecholamine stimulation during arrest, or prior to
arrest, on myocardial functional recovery can be separated from reperfusion injury. Subsequently, the extent of catecholamine-induced injury can be measured by pre-treatment of the hearts with a beta-antagonist, Ca²⁺ antagonist, or Ca²⁺ modulator. Any improvement demonstrated with these interventions would be indicative of the extent of the catecholamine effect. Finally, to differentiate between the damage incurred by ischemia and that incurred by catecholamines, experiments could be repeated using hearts depleted of catecholamine stores. Post-ischemic recovery can be assessed and the catecholamine contribution to damage could be quantified.
8.1 Appendix A: Effect of Epinephrine on MVO₂

Figure A.1. Effect of 2 nM epinephrine on basal MVO₂. Basal MVO₂ did not significantly change (p=0.7 and p=0.6 for each B-C sequence; paired t-test) upon the addition of 2 nM epinephrine to the arrest perfusate. The shaded area shows the recovery of the beating state MVO₂. Values are mean±SEM (n=4). Perfusion intervals: A. KHB; B. K; C. K + 2 nM Epi.
Figure A.2. Effect of 2 nM epinephrine on beating, non-working MVO₂. MVO₂ of the beating, non-working heart did not significantly change upon the addition of 2 nM epinephrine to the coronary perfusate (p=0.6, ANOVA for repeated measures). Values are mean±SEM (n=4). Perfusion intervals: A, KHB; B, KHB + 2 nM Epi.

Figure A.3. Effect of 25 nM epinephrine on beating, non-working MVO₂. MVO₂ of the beating, non-working heart did not significantly change upon the addition of 25 nM epinephrine to the coronary perfusate (p=0.1, ANOVA for repeated measures). Values are mean±SEM (n=6). Perfusion intervals: A, KHB; B, KHB + 25 nM Epi.
Figure A.4. Effect of 50 nM epinephrine on beating, non-working MVO₂. Values are mean±SEM (n=3; n-value too small for statistical analysis). Perfusion intervals: A, KHB; B, KHB + 50 nM Epi.

Figure A.5. Effect of 25 nM epinephrine and 1 μM prazosin on beating, non-working MVO₂. MVO₂ of the beating, non-working heart did not significantly change upon the addition of 25 nM epinephrine to the coronary perfusate. Subsequent addition of 1 μM prazosin (Prz) did significantly decrease MVO₂. Values are mean±SEM (n=5). Perfusion intervals: A, KHB; B, KHB + 25 nM Epi; C, KHB + 25 nM Epi + 1 μM Prz. *p<0.05 vs. Intervals A and B, ANOVA for repeated measures.
8.2 Appendix B: Effect of 25 nM Isoproterenol on Beating Heart MVO₂

**Figure B.1.** Effect of 25 nM isoproterenol on beating, non-working MVO₂. MVO₂ of the beating, non-working heart significantly increased upon the addition of 25 nM isoproterenol to the coronary perfusate. Values are mean±SEM (n=4). Perfusion intervals: A, KHB; B, KHB + 25 nM Iso. *p<0.01 vs. Interval A; ANOVA for repeated measures.
8.3 Appendix C: Effect of Esmolol on Beating Heart MVO$_2$

Figure C.1. Effect of 2 mg/L esmolol on beating, non-working MVO$_2$. MVO$_2$ of the beating, non-working heart significantly increased upon the addition of 2 mg/L esmolol to the coronary perfusate. Values are mean±SEM (n=6). Perfusion intervals: A, KHB; B, KHB + 2 mg/L Esm. *p<0.05 vs. Intervals A and B. ANOVA for repeated measures; †p=0.03 vs. initial KHB interval; two-tailed, paired t-test.
Figure C.2. Effect of 10 mg/L esmolol on beating, non-working MVO$_2$. MVO$_2$ of the beating, non-working heart did not significantly change upon the addition of 10 mg/L esmolol to the coronary perfusate (p=0.5, ANOVA for repeated measures). Values are mean±SEM (n=5). Perfusion intervals: A, KHB; B, KHB + 10 mg/L Esm.

Figure C.3. Effect of 100 mg/L esmolol on beating, non-working MVO$_2$. Values are mean±SEM (n=2; n-value too low for statistical analysis). Perfusion intervals: A, KHB; B, KHB + 100 mg/L Esm.
8.4 Appendix D: Coronary Flow and Heart Weight Data

<table>
<thead>
<tr>
<th>Figure</th>
<th>Expt.</th>
<th>n</th>
<th>Coronary Flow (mL/min)</th>
<th>Heart Weight (g)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
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<tr>
<td>3.1 (49)</td>
<td>K+ 25 nM Iso</td>
<td>5</td>
<td>39.4±5.1</td>
<td>21.9±2.6</td>
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<td>3.2 (50)</td>
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<td>13.6±1.6</td>
<td>7.9±0.8</td>
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<tr>
<td>3.3 (51)</td>
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<td>28.7±5.5</td>
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<td>35.9±3.2</td>
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<td>A.1 (90)</td>
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<td>8.7±2.0</td>
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Table V(a). Coronary flow and heart weight data for MVO₂ study. The average coronary flow of each perfusion interval, indicated by a letter (as marked in the corresponding Figure) is shown. The corresponding Figure is given, with page reference in brackets. Values are mean±SEM. Expt. = experiment. n = sample size. WW = wet weight. DW = dry weight. Table V(b) is on following page.
<table>
<thead>
<tr>
<th>Figure</th>
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<th>Heart Weight (g)</th>
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<td></td>
<td></td>
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<td>B</td>
</tr>
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<td>A.2 (91)</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>B</td>
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<tr>
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</tbody>
</table>

Table V(b). Coronary flow and heart weight data for MVO₂ study (cont'd). See legend for Table V(a).
REFERENCES


Erlij D., Mendez C. Adrenergic actions on heart rate, atrio-ventricular refractory period and intraventricular conduction in dogs. *Archives Internationales de Physiologie et de Biochimie* (1964) 72: 44-65.


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