Histamine H₃ activation depresses cardiac function in experimental sepsis

By

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A Thesis
Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the requirements for the Degree of

Master of Science

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Histamine H₃ activation depresses cardiac function in experimental sepsis

BY

Xing Li

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

Master of Science

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ABSTRACT

In the heart, histamine H₃ receptors may function as inhibitory heteroreceptors that downregulate norepinephine exocytosis in pathological conditions associated with enhanced sympathetic neural activity. In sepsis, there is intense adrenergic stimulation as cardiovascular collapse develops over the course of the illness. Intense adrenergic stimulation may lead to histamine release and activation of cardiac H₃ receptors. Inhibition of cardiac adrenergic responses could lead to a relative decrease in cardiac output contributing to cardiovascular collapse in a gram-negative sepsis. In the present study, it was hypothesized that H₃ receptor blockade might improve cardiovascular function in sepsis. In a canine model of E. coli sepsis, it was observed that after 4 hrs of bacteremia, the H₃ receptor blocker (thioperamide maleate or clobenproprit) caused an increase in cardiac output (3.6 to 5.3 L/min; p<0.05) and blood pressure (mean 76 to 96 mmHg, p<0.05) compared with pretreatment values. In the cardiac mechanics study, H₃ receptor blockade improved left ventricular contractility in sepsis. Plasma histamine concentrations increased in the H₃ blocker/sepsis group as compared with a nonsepsis time control group. In a ventricular trabecular preparation, H₃ receptor blockade caused an increase in the adrenergic response in the presence of septic plasma. The present study shows that activation of H₃ receptors may contribute to cardiovascular collapse in sepsis.
Chapter 1: INTRODUCTION

Despite advances in antimicrobial therapy and medical support, septic shock is still a major cause of morbidity and mortality among hospitalized patients. The pathogenesis of gram-negative sepsis is complex and not well understood. Organisms may invade the bloodstream directly, or may proliferate locally in tissues and release substances, such as endotoxins, into the bloodstream. These endotoxins are high-molecular-weight complexes of lipopolysaccharides (LPS) that are major components of the outer membranes of the cell wall of gram-negative bacteria. Once in the circulation, they may bind to plasma components of the blood such as high-density lipoproteins or LPS binding proteins. The complex then interacts with CD14, a high-affinity receptor on monocytes and macrophages, resulting in the release of a large number of mediators. These include chemokines, prostaglandins, platelet-activating factor (PAF), tumor necrosis factor α (TNF-α), interleukin IL-1, IL-6, IL-8, endothelin and nitric oxide (NO). These mediators have major physiologic effects on all major organ systems, particularly on the heart and the vasculature, cause the tissue injury, lead to secondary release of other mediators, such as catecholamines and histamine.

In animal models, it has been shown that administration of endotoxin produces cardiovascular collapse and that a microvascular inflammatory response is also present (Gilbert, R.P., 1960). Activation of the endothelium by endotoxin stimulates these cells to change from their normal anticoagulant state to a procoagulant state, which increases adhesiveness for leukocytes and platelets. Tissue damage results from reduced perfusion of capillaries, leading to local tissues ischemia. Robert et al. noted that endotoxemia causes a transient hyperdynamic cardiovascular state, that is followed by depressed cardiac function,
lowered peripheral resistance, and hypotension in both patients and experimental animals (Robert et al., 1996).

The mechanisms contributing to the cardiac depression observed in septic shock are unclear. These mechanisms could be related to abnormalities in the function of the sarcoplasmic reticulum (SR), sarcolemma, contractile proteins, energy utilization and alterations in the sympathetic nervous system. It was initially suggested that the myocardial dysfunction of sepsis is a result of inadequate coronary perfusion. However, W. Karzai et al. reported that coronary sinus blood flow was normal or increased in all septic patients, and that cellular oxygen utilization was unaltered (Karzai, 1996). In contrast, other investigators have indicated that myocardial depression might result from the direct or indirect effects of circulating myocardial depressant substances (Lefer, 1970, Carli, 1981). This idea has recently received support by Parrillo et al. who shown that when serum obtained from septic patients was incubated with myocardial cells from newborn rats, the extent and velocity of myocyte shortening was reduced (Parrillo et al., 1985). In our laboratory, Gomez et al.(1990) showed that removal of factors from the circulation of intact animals by continuous arteriovenous hemofiltration was associated with recovery of left ventricular (LV) contractility in a canine model of E coli sepsis. In another study, it was shown that this myocardial depressant substance is a protein, which has a molecular weight of about 20,000 Daltons and an isoelectric point that is between pH of 4.5 to 6 (Jha et al., 1993).

Another factor, which could contribute to cardiac depression in sepsis, may be related to inhibition of the cardiac adrenergic system. Some investigators have reported that
Histamine H₃ receptors are located in the cardiovascular system. Histamine H₃ receptors function as presynaptic receptors that modulate and inhibit norepinephrine release from adrenergic nerve endings (Endou et al., 1994). Imamura et al. (1994) reported that, in the heart, H₃ receptors caused a decrease in norepinephrine release in pathophysiological conditions associated with enhanced adrenergic activity, such as acute myocardial ischemia.

In animal models, sympathetic nerve stimulation has been shown to elicit a frequency-dependent release of cardiac histamine (Gross et al., 1984). However, when adrenergic activity is in the normal range, histamine release appears to be too low to inhibit norepinephrine exocytosis (Endou et al., 1993; Gross et al., 1984), and cardiac H₃ receptors are quiescent. Under these conditions, histamine H₃ receptor blocker thioperamide maleate (TM) fails to increase cardiac norepinephrine release, while the H₃ receptor agonist (R)-α-methylhistamine (RAMH) causes an inhibition of the adrenergic inotropic response under normal adrenergic activity.

However, under pathological conditions, such as myocardial ischemia, Imamura et al. showed that there is a 3.5-fold increase in cardiac histamine release as compared with preischemic conditions, sufficient to fully activate H₃ receptors (Imamura et al., 1994). Under these conditions, RAMH did not modify norepinephrine exocytosis, whereas H₃ receptor blockade TM doubled norepinephrine release. TM also increased the adrenergic chronotropic response in this ischemic model.
Whether enhancement of adrenergic stimulation in sepsis might activate H₃ receptors, and whether H₃ receptor blockade might improve cardiovascular function in sepsis has never been examined. The present study was therefore designed to test whether H₃ receptors are activated in sepsis, and to delineate the relevance of histamine H₃ receptor to the cardiovascular collapse in a canine model of E. coli sepsis.
Chapter 2: LITERATURE REVIEW OF MYOCARDIAL

CONTRACTION

Myocardium can adapt to changing hemodynamic conditions in terms of four distinct physiological mechanisms: preload, afterload, contractility (the inotropic state) and distensibility (the lusitropic state). Cardiac performance is further regulated by many other factors, including neural control, drugs, hormones and metabolic products. However, the mechanisms by which bacterial toxins and host mediators may interact to produce the cardiovascular dysfunction of sepsis are unknown. An understanding of the mechanism of the regulation of cardiac muscle contraction and relaxation is important to further interpret any possible causes of cardiovascular dysfunction in sepsis.

2.1 Structure of myocardial cell

Cardiac muscle cells are made up of sarcomeres that contain thick filaments composed of myosin molecules and thin filaments composed of actin molecules. As in skeletal muscle, shortening of the cardiac sarcomere is caused by the sliding filament mechanism. Actin filaments slide along adjacent myosin filaments by the crossbridge cycle, and bringing the Z lines closer together. Maximal developed force is obtained at resting sarcomere lengths of 2.0 to 2.4 μm. At this point, the number of crossbridge attachments is maximal.
Unlike skeletal muscle, cardiac muscle appears to be a syncytium with branching interconnecting fibers so that a wave of depolarization followed by contraction of the atria or ventricles (all-or-none response) can occur when a superthreshold stimulus is applied. Another striking difference between cardiac and skeletal muscle cells is in the amount of mitochondria. Cardiac muscle is rich in mitochondria, which allows cardiac muscle to synthesize adenosine triphosphate (ATP) for oxidative phosphorylation required to sustain repetitive contraction for a lifetime. In addition, cardiac muscle is also endowed with a rich capillary supply, approximately one capillary per fiber. Such short diffusion distances allow oxygen, carbon dioxide, substrates, and waste material to move easily and rapidly between myocardial cell and capillary.

In the myocardium, the sarcolemma invaginates into the fiber at the Z bands; these constitute the transverse-tubular or T-tubular system. During the passage of the action potential, the T system allows action potential to descend down the T system near the Z bands into triadic junctions, in which a single T system tubule is in extremely close to two terminal cisternae of the sarcoplasmic reticulum (SR), which contain the calcium-release channels of the SR, also called the ryanodine receptor (Fleischer, 1989). Meanwhile, a network of sarcoplasmic reticulum consisting of small-diameter sarcotubules surrounds the myofibrils. Both of them play an important role in myocardial contraction and relaxation.
2.2 Resting membrane and action potentials

As with all other cells in the body, the concentration of potassium ions inside a cardiac muscle cell exceeds the concentration outside the cell; the reverse concentration gradient exists for Na\(^+\) ions and for unbound Ca\(^{2+}\) ions. The resting cell membrane is relatively permeable to K\(^+\), but much less to Na\(^+\) and Ca\(^2\), \(g_k\) is about 10.5 times \(g_{Na}\). Two factors contribute to the resting membrane potential in cardiac muscle cells. One is the consequence of the distribution of ions across the membrane, which is mainly determined by the electrochemical gradient of potassium ions. The other factor is the electrogenic Na\(^+\)-K\(^+\) pump, which extrudes three Na\(^+\) ions out in exchange of two K\(^+\) ions into the cell.

Stimulation produces a propagated action potential that is responsible for initiating contraction. A typical action potential of a cardiac muscle cell includes: (1) rapid upstroke, which is due to a rapid increase in Na\(^+\) conductance; (2) immediately after the upstroke there is a brief period of partial repolarization, which is due to closure of Na\(^+\) channels and Cl\(^-\) influx; (3) followed by a plateau, which is due to a slower but prolonged opening of voltage-gated Ca\(^{2+}\) channels; (4) the potential becomes progressively more negative until the resting state of polarization is again attained, which is due to closure of the Ca\(^{2+}\) channels and K\(^+\) efflux through two kinds of K\(^+\) channels. Changes in the external K\(^+\) concentration mainly affect the resting membrane potential of cardiac muscle; changes in the external Na\(^+\) concentration affect the magnitude of the action potential; and changes in Ca\(^{2+}\) concentration result in change of contractile force.
2.3 Excitation-contraction coupling in cardiac muscle

In contrast to skeletal muscle, cardiac muscle has an absolute requirement for extracellular calcium in order to contract. The calcium ion (Ca$^{2+}$) plays a central role in excitation-contraction coupling (Langer, 1984; McDonald, 1984; Ebashi, 1991). When depolarization permits trigger Ca$^{2+}$ to enter the myoplasma through voltage-gated L-type calcium channels located in the sarcolemma, this trigger Ca$^{2+}$ opens the ryanodine receptor to release a much larger quantity of activator Ca$^{2+}$ from sarcoplasmic reticulum. This process is called calcium-induced calcium release. The released Ca$^{2+}$ binds with troponin C, which is one of the regulatory proteins (tropomyosin-troponin) in the thin filament that normally inhibits actin from interacting with myosin in the thick filament. When Ca$^{2+}$ is bound to troponin C, this inhibition is removed and sites of actin can now interact with myosin, permitting the sarcomere to contract. There are three proteins in the troponin complex: troponin C, which binds Ca$^{2+}$; troponin I, which interacts with tropomyosin so that actin is unable to associate with myosin; and troponin T, which attaches these units to tropomyosin.

Intracellular Ca$^{2+}$ homeostasis is maintained by a few other mechanisms, including a sodium-calcium exchange mechanism, an energy-dependent Ca$^{2+}$ pump in the sarcolemma that extrudes Ca$^{2+}$ into the extracellular space, and an energy-dependent Ca$^{2+}$ pump in the sarcoplasmic reticulum that extrudes Ca$^{2+}$ into SR.

Relaxation occurs when the rise in cytosolic Ca$^{2+}$ increases the uptake of Ca$^{2+}$ into the SR, after which Ca$^{2+}$ dissociates from troponin C (Callewaert, 1992; Carafoil, 1985; 1988;
1991; Lepeuch, 1989; Schatzmann, 1989; Missiaen, 1991). Ca\textsuperscript{2+} is taken up by the calcium pump, located mainly on the SR, and diffuses back to the terminal cisternae within the SR. A smaller amount of Ca\textsuperscript{2+} is pumped back into the interstitial space by a plasma membrane calcium pump (PMCA) (Caroni, 1981; Siri, 1991). Although some of the Ca\textsuperscript{2+} stored in the SR is in a free ionized form, most appears to be associated with Ca\textsuperscript{2+} - binding protein within this intracellular membrane system. Most important of the Ca\textsuperscript{2+} - binding protein is calsequestrin, a 45-KDa protein that traps calcium within this membrane system. Other Ca\textsuperscript{2+} - binding proteins found in smaller amounts include the 44-KDa protein calreticulin and a 170-KDa histidine-rich calcium-binding protein (Lytton, 1991). Another protein, phospholamban (PLB), increases the Ca\textsuperscript{2+} uptake by sensitizing the SR ATPase to Ca\textsuperscript{2+}. Recently a study has shown that both basal and stimulated myocardial functions in the intact animal are highly dependent on the relative level of PLB gene expression (Lorenz, 1997).

There are other factors besides Ca\textsuperscript{2+} that modulate the cardiac contractile state. Changes in the responsiveness of the myofilaments to Ca\textsuperscript{2+} can play an important regulatory role, which include alteration in the affinity of troponin-C for Ca\textsuperscript{2+}, phosphorylation of the myosin light chains, phosphorylation of protein kinase C, or changes in the lattice structure of the myoflaments.
2.4 The Frank-Starling principle

Frank et al. published in 1895 the fundamental concepts of what would be called the Frank-Starling law of the heart, that an increase in ventricular performance is induced by increased filling. Sarnoff and Berglund investigated various modifications of the Frank-Starling principle in the 1960s and suggested that ventricular function can be plotted as the work-preload relationship, which was the first representation of the Frank-Starling law of the heart. They described a family of Starling curves instead of a single one to account for changes in contractility (Sarnoff, 1962). Recently Glower et al. revisited this relationship (Glower, 1985). In their approach, vena caval occlusion was used for varying preload, which was defined by diastolic fiber length. Steady-state infusion of phenylephrine in the presence of autonomic blockade was considered be the standard of reference for varying afterload in the intact circulation. They concluded that the slope of the relationship between stroke work and end-diastolic dimensions, which they termed the preload recruitable stroke work (PRSW) in the normal conscious dog was highly linear and indeed preload independent. It is also seemed insensitive to physiologic changes in afterload. This means that myocardial contractile performance could be described reliably by the slope \((M_w)\) of PRSW.

2.5 Protein kinase C

Protein kinases regulate numerous biological processes; among these is the regulation of cardiac contraction. The effects of protein kinases on cardiac contraction involve
regulation at two levels. First, they affect handling of Ca\textsuperscript{2+} by the sarcolemma and by the sarcoplasmic reticulum, and also the handling of other ions (Na\textsuperscript{+}, Cl\textsuperscript{−}) is affected. Secondly, there is modulation at the level of the myofibril and the phosphorylation of the regulated MLC has already been mentioned (Sugden, 1995).

Numerous protein kinases have been so far identified and roughly classified into two groups, namely serine/threonine and tyrosine kinases on the basis of the target amino acid. Protein kinase C is a serine/threonine kinase originally identified in brain and their functional significance in the heart has been recently reviewed (Pucéat, 1994). It is a family of at least eight isoenzymes, some of which are calcium dependent (α, βI, βII, and γ) and some calcium independent (δ, ε, ζ, and η). PKC may phosphorylate troponinI and troponin C protein, the resulting decreases the maximal activity of the Ca\textsuperscript{2+}-activated actomyosin ATPase. The latter could lead to negative inotropism (Venema, 1993). Activation of PKC may also influence the activity of a variety of other ion channels with inotropic consequences. These include L-type Ca\textsuperscript{2+}, Na\textsuperscript{+}, Cl\textsuperscript{−} channels, but effects are complex. Phosphorylation through PKC is potentially positively or negatively inotropic depending on the model chosen.

Young et al. (1998) found that cytosolic PKC activity was activated in rat heart during the early hyperdynamic phase of sepsis. Because PKC mediated phosphorylation plays an important role in regulating myocardial contractility, they concluded that activation in cytosolic PKC might contribute to the development of a hypercardiodynamic state during the early phase of sepsis. Takeishi et al. (1993) used a PKCbeta2 overexpressing mouse, an animal model of heart failure, and found that \textit{in vivo} PKCbeta2-mediated phosphorylation of
troponin I may decrease myofilament $Ca^{2+}$ responsiveness, and thus causes cardiomyocyte dysfunction.

### 2.6 Regulation of cardiac norepinephrine release

The sympathetic nervous system is an important component of myocardial contraction and may be altered in pathological conditions. In septic shock, there is an increase in sympathetic stimulation. Evidence of sympathetic stimulation during developing septic shock is based in part on depletion of catecholamines in heart, spleen, and adrenal medulla (Pardini et al., 1979; 1982). Pardini et al. (1983) also reported that norepinephrine turnover was increased in the heart during endotoxicosis.

Sympathetic nervous activity enhances myocardial contractility. When a sympathetic nerve is stimulated, an action potential causes an influx of $Ca^{2+}$ into the sympathetic nerve terminal, with subsequent fusion of the vesicle with the plasma membrane and then norepinephrine is released by exocytosis. Neurally released norepinephrine interacts with $\beta$-adrenergic receptors on the cardiac cell membranes. The two $\beta$-adrenergic receptors are coupled to a stimulatory G-protein ($G_s$), which activate adenylyl cyclase (AC), leading to an increase in cyclic adenosine monophosphate (cAMP). Consequently protein kinases are activated and promote phosphorylation. Phosphorylation of specific sarcolemmal proteins augments the opening of calcium channels in the myocardial cell membranes. Thus $Ca^{++}$ influx increases during each action potential plateau, and more $Ca^{++}$ is released from the
sarcoplasmic reticulum in response to each cardiac excitation. Thereby, the contractile strength of the heart is increased.

There are two different mechanisms by which norepinephrine is released from sympathetic neurons in human heart (Kurz et al., 1995; Imamura et al., 1996). Kurz et al. reported that under normal conditions norepinephrine release, evoked by electrical stimulation, is strongly dependent on calcium, and the N-type calcium channel is the predominant pathway for calcium entry causing exocytotic norepinephrine release from sympathetic neurons. In contrast, ischemia-induced norepinephrine release in human cardiac tissue is characterized by a calcium-independent carrier-mediated release mechanism.

Norepinephrine release is in part regulated by autoreceptors. Norepinephrine in the synaptic cleft can activate presynaptic $\alpha_2$ receptors, which will inhibit further exocytotic release of norepinephrine. In addition to autoreceptors, there are heteroreceptors, which also can regulate the release of norepinephrine. In the heart, rich concentrations of histamine are present in sympathetic nerves (Von Euler, 1966; Ryan and Brody, 1970, 1972). When sympathetic nerves to guinea pig hearts are stimulated, in addition to norepinephrine, the concentration of endogenous histamine increases 3-fold compared with prestimulation (Gross, 1984). Histamine has been shown to decrease the quantity of [$^3$H]norepinephrine released by field stimulation from guinea pig atria and canine arteries and veins (Rand et al., 1982; Powell, 1979) which negatively modulates adrenergic responses in cardio-vascular tissues (Arrang et al. 1983). The proposed mechanism is that prejunctional $H_3$ receptors function as inhibitory heteroreceptors that downregulate norepinephrine exocytosis (Imamura et al., 1994).
Parrat (1973) reported that the inotropic and chronotropic response to an infusion of norepinephrine in cats was markedly reduced 2-3 hours after endotoxin administration. Increased sympathetic nerve activity, decreased response of the heart to adrenergic stimulation, and eventual decreased ventricular function with circulatory collapse suggest that the basic phenomenon of adrenergic desensitization could be occurring in the heart during the development of septic shock. Jones et al. (1990) reviewed the evidence for alterations in myocardial β-adrenergic receptor function during developing septic shock. They suggested that desensitization of adrenergic agonists occur in a sequential order beginning with uncoupling of the receptor from adenylate cyclase. This may involve phosphorylation of the receptor but without changing the number of surface receptors. As sepsis progress, the processing of internalized receptors decreases the surface receptor population.

2.7 Ion channels and receptors

2.7.1 Calcium channels in cardiovascular tissues

Calcium ions play an important role in the regulation of contraction of cardiac muscle. Calcium channels are mainly pathways for calcium entry from extracellular into intracellular space. The patch clamp technique has enabled the characterization and identification of new families of Ca^{2+} channels. Overall, two categories of Ca^{2+} channels in cardiac tissues can be distinguished on the basis of their activation threshold. The first category is low-voltage
activated, which includes T-type (transient) channels. They have similar permeability for Ba\(^{2+}\) and Ca\(^{2+}\) ions and are inactivated very rapidly. The second category is high-voltage-activated, called L-type Ca\(^{2+}\) channels, which have larger permeability for Ba\(^{2+}\) than Ca\(^{2+}\). This channel inactivates very slowly, and is inhibited by different classes of Ca\(^{2+}\) antagonists (Nargrot, 1997).

L-type (Slow) and T-type (fast) Ca\(^{2+}\) channels are common and located in most excitable cells, where they are often co-expressed. The T-type likely plays a role in pacemaking (Vassort G, 1994). The L-type current is directly responsible for excitation-contraction coupling of cardiac cells. The activity of L-type Ca\(^{2+}\) channels is strongly dependent on the frequency of stimulation, and on cardiac chronotropism. The acceleration of stimulation frequency induces enhancement of the amplitude and a slowing of the inactivation time course of the L-type current, and thereby increases the Ca\(^{2+}\) influx (Tiaho et al., 1994, Piot et al., 1996), resulting in a positive inotropic effect.

The N-type Ca\(^{2+}\) channel is exclusively found in the nervous system and is specifically blocked by \(\omega\)-conotoxin GVIA (McCleskey, 1994). It has been shown that its role is related to liberation of neurotransmitters (Turner et al., 1992; Takahashi et al., 1993; Uchitel et al., 1992).

Many neurotransmitters or hormones are able to modulate the opening of cardiac Ca\(^{2+}\) channels, such as histamine and \(\beta\)-adrenergic agonists. They interact with \(\beta\)-adrenergic receptors located in the cardiac cell membranes, stimulate the membrane bound enzyme
adenylyl cyclase via a heterotrimer G protein, and raise the intracellular concentration of cyclic adenosine monophosphate (cAMP). This change increases the activation of protein kinases A, which increases activation of the L-type Ca$^{2+}$ channels in the cell membrane via the phosphorylation of either the Ca$^{2+}$ channel protein itself or another closely associated protein (Hartzell, 1988) and augments the influx of Ca$^{2+}$ into cells.

2.7. 2 Receptor signaling in the heart

In myocardium, signals that control the rate and force of contraction are initiated at the cell surface by the interaction of hormones, neurotransmitters and para- or autocrine factors with their specific receptors. Many investigators have focused their research on modification of G protein-coupled receptor-initiated transmembrane signaling because it is related to pathophysiologic conditions of heart disease that contribute to human morbidity and mortality.

G protein-coupled receptors functionally interact with a wide variety of G proteins. Three types of G protein have been found in cardiovascular tissue (Robishaw, 1989). Gs protein mediates stimulation of adenylate cyclase and production of second messenger cAMP via both β1- and β2-adrenergic receptors. Gs also mediates β-adrenergic modulation of voltage-dependent ion channels (Brown, 1990). Gi protein mediates inhibition of β-adrenergic-stimulated adenylate cyclase activity by muscarinic cholinergic, α1-adrenergic, and adenosine A1-receptor agonists (Bohm et al., 1986; Neumann et al., 1989). Gi also
directly couples muscarinic receptors to activate atrial and pacemaker cell K\(^+\) channels. G\(_o\) is also present in cardiovascular tissue, but its role is unclear.

G protein is a heterotrimer consisting of \(\alpha\)-, \(\beta\)-, and \(\gamma\)-subunits. The \(\alpha\)-subunit is unique for each G protein. G proteins undergo a regulatory cycle consisting of two states. The inactive G protein exists as the \(\alpha\beta\gamma\) heterotrimer, with GDP in its nucleotide-binding site. The interaction of the heterotrimeric G protein with a ligand-bearing receptor causes a conformational change in the \(\alpha\) subunit, which has a high affinity for GTP and a low affinity for the \(\beta\gamma\) pair. Therefore the activated \(\alpha\) subunit exchanges GTP for GDP, dissociates from \(\beta\gamma\), and then interacts with the effector. Termination of the cycle occurs with the hydrolysis of GTP to GDP and reassociation of \(G\alpha\)-GDP with \(G\beta\gamma\) to form the inactive complex (Birnbaumer, 1987; Moss and Vaughan, 1988; Weiss, 1988).

Receptor regulation is now known to occur at multiple levels, i.e., transcriptional, translational, and posttranslational (Ramkumar, 1988; Parsons, 1987; Longabaugh, 1989;). One of the physiologically and therapeutically important modes of regulation is the phenomenon of agonist-induced desensitization. When a receptor is stimulated by an agonist, the effect is usually of limited duration. Most G protein-coupled receptors have a number of serine and threonine residues in the cytoplasmic loops and carboxyl terminus of the receptor. These residues can be phosphorylated by several kinases, and these phosphorylation results in diminished interaction between receptor and G protein, known as receptor desensitization. Also, after prolonged agonist activation, the number of receptors in the plasma membrane
could be regulated by a process of receptor internalization (receptor down-regulation (Barnes, 1995; Stiles, 1990; Sibley, 1987).

Adrenergic receptors (ARs) belong to the large family of G protein-coupled receptors that form the interface between the sympathetic nervous system and the cardiovascular system. Using transgenic and gene-targeted “knockout” mouse models, Rockman et al. (1997) has shown that ARs are importantly involved in regulation of myocardial contractility; ARs are themselves regulated by a set of specific kinases, termed the G protein-coupled receptor kinases (GRKs) that phosphorylate activated receptors. GRKs initiate a two-step process known as homologous or agonist-specific desensitization by phosphorylating only agonist-occupied receptors. Once phosphorylated, ARs bind to inhibitory proteins, which inhibit further activation of G proteins (Lefkowitz, 1993; Lohse, 1996).

The sympathetic system may be altered in sepsis. The exact cellular mechanisms of this depressed cardiac performance in sepsis may lie within the β-adrenergic receptor system, and may be due to a decrease in the number of receptors, a decrease in the affinity of receptors for agonists, an uncoupling of the the β-adrenergic receptor from adenylate cyclase, or a dysfunction in the regulatory protein unit or adenylate cyclase system. Henry demonstrated that β-adrenergic receptor stimulation of cyclic adenosine monophosphate levels is lower in septic shock patients, which is associated with myocardial hyporesponsiveness, suggesting that β-adrenergic receptor dysfunction may contribute to the reduced myocardial performance (Silverman, 1993). However, there are other kinds of receptors, which are
related to myocardial dysfunction in sepsis, for example, the histamine receptors (see chapter 3).

2.8 Field stimulation

Field stimulation is a method to increase sympathetic activity that will be used in the present study. Brady et al. first (1960) found that they could greatly increase the sympathomimetic effects of field stimulation by applying trains of intense stimuli to heart muscle during the refractory period of its regular beats, evidently stimulating the nerves repeatedly without reexciting the muscle. Later, West and his coworkers have shown that high-intensity pulses of brief duration will excite the autonomic nerve without stimulating the muscle within which they lie (West, 1961). Blink confirmed this finding in later study: field pulses too brief to excite the myocardium were shown to produce pronounced sympathomimetic and parasympathomimetic effects, demonstrating that it is possible to release a large amount autonomic neurotransmitters by the application of pulses without disturbing the rate or rhythm of contraction (Blink, 1966). A single field pulse can influence the strength of contraction of heart muscle by at least three different mechanisms, (1) by a parasympathomimetic effect; (2) by a sympathomimetic effect; (3) by synchronizing the contractions of the various parts of the preparation.

In addition, Blink et al. found that only negligible amounts of transmitters are released by stimuli used to drive preparations of isolated heart muscle if those stimuli are of barely threshold intensity. This finding is consistent with two other observations: (1) that
spontaneously beating preparations in which it was found that stimuli strong enough to excite the muscle had no effect on the strength of contraction if they were timed to coincide with the naturally occurring beats; (2) that the concentration of atropine or propranolol sufficient to prevent or greatly diminish the parasympathomimetic or sympathominetic effects of field stimulation does not affect the strength of contraction of preparation driven with threshold stimuli (Blinks, 1966).

Mammalian ventricular myocardium is commonly thought to have little or no parasympathetic nerve supply (Blinks and Koch-Weser, 1963). However, Whalen (1958) and Vincenzi (1963) have found a slight parasympathomimetic effect to field stimulation in the cat papillary muscle. Nevertheless, the parasympathomimetic effect of field stimulation is much less pronounced in the cat papillary than in atrial tissue. It is possible that this difference is due to sensitivity of muscle to the muscarinic effect of acetylcholine in different animal models
Chapter 3: LITERATURE REVIEW OF HISTAMINE RECEPTOR IN THE HEART

3.1 Distribution of histamine

Histamine (HA) is called β-aminoethylimidazole, which molecule is comprised of an imidazole ring and an amino group connected by two methylene groups. Almost all mammalian tissues contain histamine in amounts ranging from less than 1 μg/g to more than 100 μg/g. In the CNS, this amine is synthesized in neurons located in the tuberomammillary nucleus of the posterior hypothalamus. These neurons project diffusely to most cerebral areas and have been implicated in various functions of the brain of mammalian species, including sleep/wakefulness, hormonal secretion, and cardiovascular control (Schwartz et al., 1991). In peripheral neural tissues, histamine is stored in mast cells, basophils, enterochromaffin cells and probably in specific neurons. Histamine has also long been known to be present in the mammalian heart and sympathetic nerves. The human heart contains as much as 3 μg/g histamine. There is a good correlation between the concentrations of histamine and norepinephrine in the human and guinea pig heart (Levi, 1991).
3.2 Biological effects of histamine on the cardiovascular system

When Dale and Laidlaw (1910,1911) first investigated the function of histamine, they discovered that it stimulated the smooth muscle and had an intense vasodepressor action. Histamine was released during immediate hypersensitivity reactions and caused cellular injury. Histamine has direct effects on the vasculature. Histamine produces the dilation of the finer blood vessels, decreases the total peripheral vascular resistance and lowers the systemic blood pressure. Histamine also increases capillary permeability.

In the heart, the effects of HA mainly include positive chronotropic, inotropic and negative dromotropic effects, coronary vasoconstriction and vasodilation, as well as multiform arrhythmias (Endou, 1995). H₂-mediated adenylate cyclase activation in sino-atrial node cells causes a positive chronotropic effect; in atrial and ventricular myocytes, it elicits a positive inotropic effect. In contrast, H₁-receptor activation may elicit negative inotropic and chronotropic effects (Göthert et al, 1995). The effects of H₃ receptor will be discussed in below.

3.3 Histamine receptors and location in the cardiovascular system

Histamine acts through at least three distinct classes of receptors for histamine, designated H₁ (Ash and Schild, 1966), H₂ (Black et al., 1972), and H₃ (Arrang et al., 1983). 2-Methylhistamine elicits responses mediated by H₁-receptors and 4(5)-methylhistamine has a preferential effect on H₂ receptors, whereas (R)α–methylhistamine is the agonist at H₃-
receptor sites. H₁-receptors and H₂-receptors are located on different cell types in the vascular bed. Vascular H₁-receptors are distributed in the smooth muscle cell and in the intima (Göthert, 1995) while H₂-receptors are located on the smooth muscle cell. In the heart, the predominant histamine receptor is the H₂-receptor. H₃-receptors were first found on presynaptic nerve endings of histaminergic neurones in the brain where they function as autoreceptors to regulate the synthesis and release of histamine (Arrang et al., 1983). More recently, they have been found to be located on noradrenergic nerve endings that function as heterogeneous receptors to mediate noradrenaline release in the heart and vessels (Arrang et al., 1987).

3.4 Histamine H₃-receptors

3.4.1 Localization of H₃-receptors

The H₃-receptor was first described as a presynaptic autoreceptor present on histaminergic nerve ending in the CNS of rat brain, which exerts feedback regulation of histamine synthesis and release (Arrang et al., 1983). The receptor density is low (30±3 fmol/mg protein) and much lower than for H₁ and H₂ (Arrang et al., 1987). Later, H₃-receptor was found in human brain (Arrang et al., 1988). The results of autoradiographic and membrane binding studies have shown that the H₃-receptor is heterogenous. The H₃-receptors are not only restricted to histaminergic neurones, but also exist on serotoninergic and noradrenergic nerve terminals (Schlicker et al., 1989) in the rat brain. Moreover, H₃-receptor was found in the periphery. They have a physiological role in controlling the
peripheral circulation and gastrointestinal movement. In the guinea pig lung, H3-receptor is scattered in the parenchyma and, in a denser manner, around the airway, where the density is approximately 5 fmol/mg protein (Arrang et al., 1987). It strongly inhibits noradrenergic, non-cholinergic bronchoconstriction by reducing neurotransmitter release (Schwartz et al., 1990).

Ishikawa and Sperelakis first demonstrated that H3 receptors were present on the vascular postganglionic sympathetic neurones in 1987. They found that H3 receptor agonist depressed perivascular sympathetic neurotransmission, inhibited the amplitude of the electrically elicited junction potentials and caused vasodilation in the guinea pig mesenteric artery. "In vitro" studies have confirmed the presence of prejunctional H3 receptor on the noradrenergic nerve terminals in the guinea pig (Luo et al., 1991, 1993; Ea Kim et al., 1988; Hey et al., 1992; Mcleod et al., 1993).

Subsequently, H3 receptors were discovered on the sympathetic nerve terminals in the human saphenous vein, where H3 receptor agonists inhibited NA release (Molderings et al., 1992). Histamine also inhibited NA release in response to electrical stimulation in the human saphenous vein. The effect of histamine was mimicked by (R)-α-methyhistamine, but H1 and H2 receptor agonists did not influence NA release. The effect of histamine was prevented by the H3 receptor antagonist thioperamide, but not affected by H1 and H2 receptor antagonists (Schlicker et al., 1994). They concluded that H3 receptors controlled NA release in the human saphenous vein. Activation of H3 receptors did not affect the basal diastolic blood pressure while (R)-α-methyhistamine inhibited the rise in diastolic blood pressure.
induced by electrical stimulation. However, (R)-α-methyhistamine did not affect the rise in diastolic blood pressure induced by exogenous NA. In conclusion, H₃ receptor is a presynaptic receptor that inhibits the NA release in response to electrical stimulation on the postganglionic sympathetic neurones.

Endou et al. (1994) further investigated whether H₃ receptor inhibited NA release in guinea pig atria. They concluded that presynaptic H₃ receptors modulated NA release from the sympathetic nerve terminals of the guinea pig heart. They further concluded that the H₃ receptor is coupled to a pertussis toxin sensitive G₅/G₆ protein and that receptor activation may induce a decrease in Ca²⁺ current. After this, Imamura (1995) first demonstrated that H₃ receptors are present in sympathetic nerve endings in the human heart, where they modulate the adrenergic responses by inhibiting NA release.

Cardiac H₃ receptors appear to be quiescent in normal physiologic conditions, yet available for activation. During stresses such as myocardial ischemia, H₃ receptors become activated by an endogenous ligand to inhibit NA release. Since cardiac H₃ receptors induce attenuation of sympathetic neurotransmission, H₃ receptor activation may play an important role in causing cardiovascular collapse in sepsis.

3.4.2 Selective ligands for the histamine H₃ receptors

In most H₃ receptor systems, histamine is a highly active agonist. For inhibition of the stimulated [³H] histamine release from rat cortex preparations, a pD₂ value of 7.4 has been
reported (Arrang et al., 1983; Van der werf et al., 1987). Mono- or dimethylation of the terminal amino function results in compounds that are more active and H3 selective with regard to H1 and H2 receptors, than histamine (Arrang et al., 1983; Van Der Werf et al., 1987). Methylation of the α-carbon atom of the ethylamine side chain drastically increases the potency at the H3 receptor (Arrang et al., 1985b; 1987). This increased activity completely resides in the R-isomer; the corresponding S-isomer appears to be approximately 100-fold less potent than the histamine. Since α-methylation of histamine leads to highly reduced activity at both the H1 and H2 receptor (Leurs et al., 1991), (R)-α-methylhistamine is a highly selective agonist at the H3 receptor. The pD2 value of this agonist is 8.4 (Arrang et al., 1987). Tritated forms of Nα-methyhistamine and (R)-α-methylhistamine are currently available as radiolabelled agonists for the H3 receptor (Leurs et al., 1995). More interesting is the finding that the amine function of histamine can be replaced by an isothiourea group. The resulting compound, termed imetit, appears to be a full and highly potent H3 receptor agonist (Garbarg et al., 1992; Howson et al., 1992; Van Der Goot et al., 1992). The amine function can also be incorporated in ring structures to produce compounds such as immepip. This compound is effective in vitro and in vivo (Vollinga et al., 1994) with a pD2 value of 8.0. Since for the H1 and H2 receptor only weak activities were observed, immepip is an important new selective H3 receptor agonist, and will be a valuable tool for structure future quantitative -activity relation and molecular modeling studies.

A potent and highly selective H3 receptor antagonist, thioperamide, was found in 1987. This compound is active in various in vitro H3 receptor assays and shows H3 receptor antagonistic activity in vivo because it penetrates the CNS (Arrang et al., 1987). Later, in
Leurs' laboratory, based on the H₃ receptor agonist imetit, the highly potent antagonist clobenpropit and iodophenpropit were developed (Van Der Goot et al., 1992; Leurs and Timmerman, 1992). The resulting compound, clobenpropit, displaced [³H] N¹-methylhistamine binding to rat cortex/hippocamapal membranes (pKᵢ=9.77±0.03) and antagonised the inhibitory responses to (R)-α-methylhistamine against electrical field stimulation in the isolated longitudinal smooth muscle preparation of guinea-pig ileum (pKᵢ=9.95±0.07). Clobenpropit is 10 to 50-fold more potent in H₃ receptor antagonistic activity than is thiopermide, and will become a value tool in vitro studies (Kathmann, 1993, Julie et al., 1993). Recently, from this compound, an iodinated analogue of clobenpropit has been prepared. It also appears to be a potent H₃ receptor antagonist, and can be used as a radioligand for H₃ receptor (Menge et al., 1992).

3.4.3 Molecular properties of the histamine H₃ receptors

Both the histamine H₁ and H₂ receptors belong to the large family of G-protein-coupled receptors. The deduced amino acid sequence of H₁ receptor revealed a 491 amino acid protein of 56 KD molecular weight. The human H₁ receptor gene resides on chromosome 3 (Yamashita 1991, Leconiat 1994), while the H₂ receptor is a 356 amino acid protein (Gantz et al., 1991), gene for which resides on chromosome 5 (Traiffort et al., 1995). Due to the low abundance of histamine H₃ receptors in various tissues, a highly sensitive radioligand has only recently been developed, and the molecular properties of the H₃ receptors are therefore less well described. From 1992, Zweig et al. began to use [³H] histamine as radioligand in the membranes of bovine whole brain. They reported that [³H] histamine labels a binding site
with a $K_d$ value of 4.6±1.3nM and a $B_{max}$ of 78±20 fmol/mg protein (Zweig et al., 1992). The
$[^3H] \text{histamine}$ binding to bovine membranes could be inhibited by guanine nucleotides,
indicating that the $H_3$ receptor is coupled to a G-protein. They also found that guanine
nucleotide sensitivity was retained after solubilization, indicating a relatively tight coupling
of the $H_3$ receptor with a G-protein.

Later, Cherfi et al. (1992) reported that the histamine $H_3$ receptor agonist (R) $\alpha$-
methylhistamine inhibited basal and carbachol-stimulated inositol phosphate formation in the
human gastric tumoral cell line HGT1-clone 6. Using $[^3H] \text{N}^\alpha$-methylhistamine, they
demonstrated high affinity binding sites with a $B_{max}$ of 54±3 fmol/mg of protein and a $K_d$
value of either 0.61±10.04 or 22±0.4nM, in the absence or presence of 50 $\mu$m GTP[$\gamma$]s,
respectively. After gel filtration, Sepharose-famotidine and Sepharose-thioperamide affinity
chromatography, the $[^3H] \text{N}^\alpha$-methylhistamine binding site was 90,225-fold purified. Binding
was competitively displaced by $\text{N}^\alpha$-methylhistamine ($IC_{50}=5.8\pm0.7nM$), R) $\alpha$-
methylhistamine ($IC_{50}=9\pm1nM$), and thioperamide ($IC_{50}=85\pm10nM$), but not by famotidine
($H_2$ antagonist) or by mepyramide ($H_1$ antagonist). The purified sites had a molecular mass of
approximately 70KD molecular weight in SDS-PAGE (Cherifi et al., 1992). They concluded
that $H_3$ receptor protein is negatively coupled to phosphatidylinositol turnover through an
unidentified G protein.
3.4.4 Transmembrane signalling of the histamine H₃ receptors

The signal transduction mechanisms for the three histamine receptors appear to different. H₁-receptors have been shown to be coupled to phospholipase C (PLC). The resulting increase in phosphoinositide (PI) turnover generates more inositol trisphosphate (IP₃) and diacylglycerol (DAG) in the cell membrane. IP₃ causes a rapid release of Ca²⁺ from the endoplasmic reticulum. DAG activates protein kinase C while calcium ion activates Ca²⁺/calmodulin-dependent protein kinases, phospholipase A₂ and nitric oxide synthase in the target cell to generate the characteristic response (Carswell, 1985). For example, in the smooth muscle of large blood vessels, bronchi and intestine, the activation of H₁ receptors and resultant IP₃-mediated release of intracellular Ca²⁺ from the SR causes activation of the Ca²⁺/calmodulin-dependent myosin light chain kinase and nitric oxide synthase. The former increases the cross-bridge cycling and contraction. The latter enhances nitric oxide production that stimulates guanylyl cyclase. The accumulation of cyclic GMP activates a cyclic GMP-dependent protein kinase and decreases intracellular Ca²⁺, resulting in muscle relaxation.

In contrast to the H₁-receptors, H₂-receptors are linked to adenylate cyclase (Hegstrand, 1976) which leads to activate cyclic AMP-dependent protein kinase in the target cell.

The precise mechanism involved in stimulus-response coupling following H₃-receptor activation is not completely clear. The H₃ receptor is thought to belong to the superfamily of G-protein-coupled receptors. From the binding studies, interaction with a G-protein is
suggested (Arrang et al., 1990; West et al., 1990; Cherifi et al., 1992; Cumming et al., 1991; Jansen et al., 1992; Zweig et al., 1992). The possibility of G-protein involvement is strengthened by the attenuated effects on H3 receptor function, which was caused by the G-protein toxins, cholera and PTX (Cherifi et al., 1992; Nozaki and Sperelakis, 1989; Endou, et al., 1994). One possible mechanism is that presynaptic H3-receptors mediate an inhibition of the evoked NA release from the sympathetic nerve terminals of the guinea-pig myocardium. These receptors are probably coupled to a pertussis toxin sensitive G\textsubscript{i}/G\textsubscript{o} protein and may be assumed to induce a decrease in Ca\textsuperscript{++} current (Endou et al.1994).
Chapter 4: MATERIALS AND METHODS

4.1 In-vivo protocols

This study was approved by the Central Animal Care Committee at University of Manitoba. In the first experiment, the effect of H3 blockade on hemodynamics was examined in four study groups (Hemodynamic study). These groups included 1) H3 blockade /sepsis group (n= 8) in which the H3 antagonist (TM, 2mg/kg, n= 6, or clobenpropit 0.6 mg/kg n= 2 mixed in 250 cc, 5% dextrose in water (D5W)) was administered over 30 minute after 4 hours of E. Coli infusion; 2) Sepsis-group (n=8) in which placebo treatment (250 cc, 5% D5W) was administered after 4 hours of E. coli infusion; 3) H3 antagonist group (n=6) in which H3 antagonist (either TM, 2mg/Kg, n=4; or clobenpropit 0.6 mg/Kg, n=2 mixed in 250 ml D5W) was given at the 4 hours period in nonseptic dogs; and 4) a time control group in which placebo (250 ml D5W) was administered at the 4 hours period in nonseptic dogs (n=6). Since the findings obtained with both H3 receptor antagonists (i.e. TM and clobenpropit) were the same, the results were averaged as a single group. Clobenpropit was used in the latter experiments because it has a longer duration of action (Kathman et al., 1993).

In the hemodynamic study, measurements were obtained at presepsis (baseline); after 4 hour of sepsis during which hypotension occurs in this model (Gomez et al., 1990); immediately treatment or placebo was administered and then at 30 minutes, 1 hours and 2 hours posttreatment respectively.
The schema of the experimental design and its time course is given below.

1. Presepsis (baseline)

2. After one-hour stabilization, infection was induced by infusion of live E.Coli.

3. After four hours sepsis, hypotension occurs.


5. One-hour after treatment.


Because hemodynamic differences between the H₃ antagonist/sepsis group and the sepsis group were found, a cardiac mechanics study was subsequently performed in separate dogs in these groups. In the cardiac mechanics study, sonomicrometric techniques were used to determine LV volumes (n=4). The objective of this study was to examine whether LV contractility increased when a histamine H₃ antagonist was administered in sepsis. The sequence of measurements in this study was the same as that used in the hemodynamic study except that measurements were not obtained after the one-half hour
posttreatment period, because the changes due to H₃ receptor antagonist were found to occur early after treatment in the hemodynamic study.

4.2 Preparation and measurements

In all studies, mongrel dogs (20 to 30 Kg) were anesthetized with thiopental sodium (20 mg/Kg intravenously) which was followed by a constant infusion of sufentanil citrate (1 µg/min) and midazolam (5µg/Kg/min). The rates were adjusted to abolish the palpebral reflex. The animals were placed in the supine position. The trachea was intubated with an endotracheal tube, and lungs were mechanically ventilated at a tidal volume of 20 ml/kg (Harvard Apparatus) with a rate of approximately ~10 breaths/min that was changed as necessary to maintain blood pH with in a range of 7.3 to 7.4. Oxygen at 3 to 4 L/min was inspired to maintain arterial oxygen tension (PO₂) above 100 Torr over the duration of the entire experiment. Hemodynamic measurements were obtained at end-expiration with ventilator turned-off for 10 seconds.

In the sepsis groups, infection was induced by intravenous infusion of 10¹⁰ colony forming units of live E.Coli (designation 011.1:B4) (Gomez, 1990). The bacteria were suspended in normal saline solution that was given over 0.5 hour. A constant infusion of approximately 5x10⁹ colony forming units /hour of E.Coli was then maintained for the remainder of the experiment. In the nonsepsis groups, the same amount of normal saline solution was given over this period.
In all groups, a thermodilution Swan-Ganz thermistor tipped catheter was advanced from the jugular vein into the pulmonary artery to measure pulmonary artery pressure (Ppa), mean pulmonary capillary wedge pressure (Pwp), right atrial pressure (Rap), and cardiac output (CO). Cardiac output was measured by the thermodilution technique (Columbus Instruments, Ohio). A polyethylene catheter was placed into the femoral artery and was used to measure mean blood pressure (BP) and to withdraw samples of blood. All catheters were connected to transducers (Cobe laboratories) and were referenced relative to the left atrium. All transducers were connected to a chart recorder (Astra-Med, Inc, West Warwick, RI).

In the cardiac mechanics study, in addition to the above procedures, LV end-diastolic and end-systolic dimensions were determined by sonomicrometry in order to determine whether contractility was improved in sepsis with H3 receptor antagonist (Gomez et al., 1990; 1992). In these experiments, a sternotomy was performed and the chest was widely opened. The airway was placed on 5 cmH2O end-expiratory pressure. The pericardium was opened. Pairs of hemispheric crystal transducers (piezoelectric crystals: Channel Industries, Santa Barbara, CA) were placed subendocardially along the anterior-posterior (AP) and apex-base (AB) dimensions of the LV as previously described by Gomez (Gomez et al., 1990,1994). Dimensional signals were processed by a sonomicrometer (Sonomicrometer 120, Triton Technology, San Diego, CA).

A carotid artery incision was made, and a high fidelity transducer-tipped catheter (Millar Instruments, Houston, TX) was advanced into the LV for measurement of LV end-diastolic pressure (LVEDP) and systolic pressures. LVEDP was defined as the point in the cardiac cycle where dp/dt increased by 150 mmHg/sec with the increase sustained for at least 50
msec (Walsh et al., 1985). LVEDP was related to the simultaneous LV dimension tracing to define end-diastolic dimension (LVEDD). LV end-ejection dimension (LVEED) was defined by the maximum negative LV pressure decline (-dP/dt max) (Walsh et al., 1985).

A Fogarty catheter was inserted into the inferior vena-cava, so that when the balloon was inflated, preload was reduced. SW was calculated at multiple values of LV end-diastolic volume (LVEDV). This enabled us to calculate a preload recruitable stroke-work relationship (PRSWR) in which the slope of this relationship, derived by linear regression analysis, defines the contractile state of the LV (Glower et al., 1985). This index of LV contractility was compared between the different groups (H₃ antagonist/ sepsis group and sepsis group).

4.3 Data Collection and analysis

LV end-diastolic volume and LV end-ejection volume were calculated from the respective crystal dimension (D) as described by Sodums et al from:

\[ \text{Volume} = \frac{\pi}{6} \times D_{AP} \times D_{SL} \times D_{AB} \]

In this equation, the septal-lateral (SL) dimension is equal to the AP (anterior-posterior) dimension (Gomez et al., 1990), AB represents apex-base.

In this analysis, SV was derived from:

\[ (\text{LVEDV-LV end-ejection volume}) \]

and SW was calculated from the equation (Glower et al., 1985)

\[ \text{SW} = (\text{mean LV ejection pressure-LVEDP}) \times \text{SV} \]

Heart rate (HR) was measured from the recorder tracing.
Systemic vascular resistance (SVR) was calculated from the equation:

\[ \text{SVR} = \frac{(B P - \text{RAP})}{C O}. \]

Pulmonary vascular resistance (PVR) was calculated from the equation:

\[ \text{PVR} = \frac{(P A P - \text{PWP})}{C O}. \]

**4.4 Histamine Assays**

In the hemodynamics study, samples of blood were taken from the femoral artery catheter. Histamine immunoanalysis was performed by competition between modified histamine in the sample and the iodinated histamine tracer for the binding to the antibody coated on the tubes (Immunotech International, AMAC Inc, Westbrook, ME, USA)(Morel et al., 1988). In order to increase histamine recognition by antibodies, the sample is acylated by a reagent that allowed simple rapid and reproducible derivatization of histamine. This acylation procedure allows a sensitivity of the assay to 0.2 nM, has minimal cross reactivities with other products, and has within (intra-assay) and between (inter-assay) coefficients of variation of 8.4% and 8.2%, respectively.
5.1 preparation

Mongrel dogs (15-20 Kg) of either sex were anesthetized with pentobarbitone sodium (30 mg/Kg). The heart was rapidly excised and placed in a dissection dish filled with oxygenated Krebs-Henseleit solution. The composition of the solution was (in mM): NaCl 118, KCl 4.7, KH₂PO₄ 1.4, MgSO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25, dextrose 11.

Thin right ventricular trabeculae (<1mm diameter) were tied with 6-0 silk thread and dissected, then mounted vertically in a 5-ml double-walled Pyrex chamber filled with Krebs-Henseleit solution. This solution was bubbled with a mixture of 95% O₂ and 5% CO₂ to maintain a pH of 7.4 at a room temperature of 37°C. The upper end of the trabeculae was connected to an isometric force-displacement transducer (model FT03C, Grass Instrument). The lower end of the trabeculae was hung in a glass holder containing a pair of stimulating electrodes. Rest tension of 0.5g was applied, the length kept constant thereafter.
5.2 Experiment protocols

During study, the right ventricular trabeculae were continuously paced at 0.5Hz with a pulse of 2 msec in duration and an intensity of 100% greater than threshold by a computer-driven stimulator. The preparation was allowed to stabilize for at least 1 hour.

Sympathetic stimulation was produced by intense field stimulation. Intense field stimuli were delivered at a frequency of 0.5 Hz by a pair of platinum electrodes and consisted of pulses of 20 msec in duration. The pulse number was increased from 4 to 24. When the maximum inotropic response was obtained, electric field stimulation was discontinued. The adrenergic response % was calculated from maximal tension minus basal tension during stimulation / basal tension x 100.

In preliminary experiments, it was also determined that the increase in the strength of contraction that was produced by field stimulation was due to norepinephrine release (NE). This positive inotropic response was abolished by propranolol (5x10^-6 M) (n=5). In addition, atropine (1 μmol) has no effect on the adrenergic response (n=3) which indicated that muscarinic receptors were not activated during field stimulation in our preparation.

In initial experiments, the H₃ agonist RAMH was added to the muscle bath in concentrations of 0.01, 0.1, and 1 μM, and the percent decrease in adrenergic activity was determined in normal trabeculae (n=5). This was followed by the addition to the bath of the
H₃ receptor blocked clobenpropit in concentrations of 0.01, 0.1, 1uM, and the extent to which adrenergic activity increased was assessed.

In other experiments, the effect of septic plasma on the adrenergic response was determined. A plasma fraction<30,000 Dalton was obtained by pore filtration techniques in which preseptc and then postseptic plasma samples were passed through a 30,000 Dalton filter (Amicon). The reason the <30,000 dalton fraction was chosen relates to previous studies that suggested that this plasma fraction contained a substance which contributes to cardiac depression in sepsis (Gomez et al., 1990; Jha et al., 1993).

The effect of septic plasma fraction (<30,000 Dalton) on the adrenergic response was examined in trabeculae obtained from nonseptic animals (n=4); this effect was compared with the response when nonseptic plasma fraction (<30,000 Dalton) was added to the trabecular bath (n=4). Separate trabeculae were used when pre and postsepsis plasma fractions were compared, but the trabeculae were obtained from the same donor dog. In these experiments, 0.5 ml of nonseptic and septic plasma fractions (30,000 Dalton) were placed into respective organ baths and the effect of clobenpropit (0, .001, 0.01, 0.1 and 1 uM) on modulating the adrenergic response was ascertained.

Finally, the effect of increasing concentrations (10⁻¹¹-10⁻³ M) of histamine on basal isometric tension and sympathetic stimulation was examined to determine whether different concentrations of histamine activated the H₃, H₂, and H₁ receptors, also to further confirm
whether an H3 receptor antagonist could attenuate the specific histamine H3 effect. The effect of histamine at $10^{-11}$-10$^{-3}$ M on basal tension and the adrenergic response were determined.

5.3 Statistical Analysis

The result was expressed as mean±SD. When two comparisons were obtained, paired or unpaired t-tests were used in the appropriate circumstances. When multiple comparisons were obtained, the analysis includes one and two way ANOVA for repeated measures and Student-Newman-Keul’s multiple comparison tests. In the hemodynamic and cardiac mechanics studies, the respective conditions between groups were compared by two-way ANOVA for two repeated measures (factor A, different treatment groups; factor B, different time periods), in which the interaction between the two factors was assessed. In this analysis, significance in the interaction term controls for experiment-wise error and repeated measurements (Snedecor, 1967). If a significant interaction was present, then the treatments behaved differently over time. In that case, a Student-Newman-Keuls multiple-range test was used to determine at which specific time periods a difference among groups was present. A P value of <0.05 was considered to be statistically significant.
Chapter 6: RESULTS

6.1 Results in vivo experiments

6.1.1 Effect of H₃ antagonist on hemodynamic variables

After 4 hours infusion of E. Coli, Mean blood pressure (BP) fell significantly (P<0.05, n=8) in both sepsis groups as compared with baseline measurements. BP remained unchanged over the course of the experiment in the nonseptic groups. Administration of H₃ blocker in the H₃ blockade/sepsis group immediately increased the BP and BP remained higher than values measured in the placebo treated sepsis-group for the remainder of the experiment. In the placebo-treated sepsis group, BP did not increase and continued to fall through the rest of the study. There was no effect of the H₃ blocker on BP in the nonseptic group (Figure 1).

At baseline, mean value of the cardiac output in the four groups was similar. After 4 hours infusion of bacteria, CO decreased significantly compared with nonsepsis groups. H₃ blocker significantly increased the mean CO over the remaining 2-hr interval as compared with values found in the sepsis group (P<0.05, n=8). There were no changes in CO observed with H₃ blocker treatment in the nonsepsis group (Figure 2).

HR was unchanged with H₃ blocker treatment in both sepsis and nonsepsis. Because HR was unchanged with H₃-blocker treatment in sepsis and nonsepsis groups, the changes in SV followed those in CO (Table 1).
The changes in SVR are shown in figure 3. Compared with baseline measurement, SVR in the both sepsis groups decreased significantly at the 4-hour interval. There was no effect of the H₃ blocker on SVR in either the nonsepsis or sepsis groups.

In both sepsis and nonsepsis groups, Pwp showed no significant change (Table 1) after 4 hours. Administration of H₃ blocker immediately increased Pwp in the blocker/sepsis group, but at ½ and 1 hour posttreatment, Pwp was not different in the two sepsis groups. In the nonsepsis group, Pwp remained higher after H₃ blocker treatment than the value found in the time control group for the remainder of the study.

At baseline, histamine concentrations were similar in all four groups. There was a modest increase in plasma histamine concentrations through the rest of experiment in the H₃ blocker/sepsis group compared with other groups. In the time control group, histamine concentrations fell over the course of study (Table 2).

6.1.2 Effect of H₃ antagonist on cardiac contractility

In the cardiac mechanics study, sonomicrometric techniques were used to determine whether the higher CO observed with treatment in the H₃-blocker-sepsis group reflected an increase in LV contractility. PRSWR was used to assess LV systolic performance. In this analysis, LVEDV vs. SW coordinates were examined over a similar range of LVEDV between conditions and linear regression analysis was used to determine whether there was a change in slope between conditions.
In the nontreated dog (Figure 4A), after 4 h of sepsis, there was a shift in the relationship downward and to the right compared with the baseline relationship and no change in the relationship when placebo was administered. In contrast, in a H₃ blocker/sepsis dog (Figure 4B), H₃ receptor blockade caused an improvement in LV contractility compared with the untreated sepsis dog. The mean slopes are shown in Table 3. There were no changes in the intercepts observed in the H₃ blocker/sepsis group (21±6, 15±13, 23±5, and 23±5 ml) or in the sepsis group (21±12, 18±9, 13±7, and 15±5 ml) over the four measurement periods, although there was wide variability in the individual dogs, which accounts for the apparent changes in intercepts in figure 4. Furthermore, in the linear regression analysis, R²>0.92 in all experiments.

6.2 Results of in vitro experiments

6.2.1 Positive inotropic response of canine ventricular trabeculae to cardiac sympathetic nerve stimulation.

Electrical field stimulation caused an increase in isometric contraction in ventricular trabeculae. Representative tracings from one experiment are shown in figure 5A, and the mean increase in adrenergic response was 51±31 % (n=6). The positive inotropic response to the electric field stimulation was abolished by the β-receptor antagonist propranolol (10⁻⁵ M) (Figure 5B). This indicates that positive inotropic response was caused by norepinephine
released from sympathetic nerve ending elicited by field stimulation. The course of the experiment usually lasted 1 to 1.5 hours. In the time control experiments, basal tension slightly decreased over the course of the experiment while the average change in response to field stimulation over the course of study in time-control experiments was less than 2% as compared with the beginning of the experiment.

6.2.2 Effect of α-MeHA on the positive inotropic response of ventricular trabeculae to cardiac sympathetic nerve stimulation.

The administration of α-MeHA reduced the contractile force elicited by field stimulation, which was significant at 1 μm. The concentration-response curve for the α-MeHA-induced inhibition of the positive inotropic response to adrenergic nerve stimulation is shown in figure 6. When the selective H₃ receptor antagonist clobenpropit was added to the bath in the presence of α-MeHA (1 μM), the reduction in adrenergic response was completely reversed by the H₃ antagonist at a dosage of 1 μM.

The selective H₃ receptor antagonist clobenpropit alone has no effect on the adrenergic response in the trabecular preparation. In the experiments (n=5), the mean ±SD adrenergic response was 37±29% in the beginning of experiment, 34±31% after 1 nM, 35±33% after 10 nM, 28.9±26% after 100 nM, and 31±26% after 1 μM.
6.2.3 Effect of H₃-receptor antagonist on adrenergic stimulation in the presence of septic and nonseptic plasma fraction.

There was a reduction in adrenergic response when septic plasma was added to the bath compared with control plasma (n=5) (Figure 7). In the presence of nonseptic plasma fraction (<30,000), H₃-receptor antagonist did not change the adrenergic response compared with the pretreatment value. In contrast, in the presence of the septic plasma fraction (Figure 8, 9), the addition of the H₃-receptor antagonist to the ventricular trabecular preparation caused an increase in the adrenergic response.

6.2.4 Effect of histamine on the basal tension and on the adrenergic response to cardiac sympathetic nerve stimulation.

At low concentrations (10⁻¹¹-10⁻⁷ M), histamine inhibited the adrenergic response and had no effect on basal tension (Figure 10). Before histamine was added to the bath, the increase in adrenergic response was 40±33%, decreasing to 20±10% at 10⁻¹¹ M histamine, further declining to 10±15% with 10⁻⁷ M histamine, and finally decreased to zero with 10⁻⁵ M histamine. This inhibition could be blocked by H₃ blocker (clobenpropit or TM). When the concentrations of histamine increased to 10⁻³ M, histamine H₁ receptors and H₂ receptors
were activated and basal tension was altered (Guo, 1984). Furthermore, $H_1$ and $H_2$ effects could be modulated by $H_1$ receptor antagonist pyrilamine maleate ($10^{-5}$ M) and $H_2$ receptor antagonist imetadine metiamide ($10^{-5}$ M), respectively, but not by $H_3$ blockade.
Table 1. Cardiovascular parameters in the hemodynamic study

(mean± SD)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 hrs</th>
<th>Treatment</th>
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<th>1 hr</th>
<th>2 hr</th>
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<td>SV</td>
<td>47±13.5ᵃ</td>
<td>34±15³</td>
<td>53±15ᵃ,³,e</td>
<td>43±15³</td>
<td>44±19³</td>
<td>35±14ᵃ,³,e</td>
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<td>Pwp</td>
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<td>6.4±2.2³</td>
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<td>Ppa</td>
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<tr>
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<td>31±19ᵇ</td>
<td>29±14ᵇ</td>
<td>26±12ᵇ</td>
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<td>Rap</td>
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<td>131±22</td>
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### H₃ blocker group

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<td>Pwp</td>
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<td>8.9±3.2ᵈᵉ</td>
<td>9.0±3.4ᵇ</td>
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<tr>
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<tr>
<td>HR</td>
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<td>106±19</td>
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### Control Group

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<td>SV</td>
<td>55±20</td>
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<tr>
<td>Pwp</td>
<td>6.7±2.3</td>
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<td>7.6±2.4</td>
<td>6.6±2.5ᵉ</td>
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<tr>
<td>Rap</td>
<td>2.9±1.5</td>
<td>3.3±1</td>
<td>3.6±1.1</td>
<td>3.5±1.5</td>
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<tr>
<td>Ppa</td>
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<td>15.1±3.5</td>
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<td>15.7±2.7</td>
<td>15±1.3</td>
</tr>
<tr>
<td>HR</td>
<td>114±24</td>
<td>116±19</td>
<td>92±12</td>
<td>98±16</td>
<td>103±11</td>
<td>99±10</td>
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</table>
SV is stroke volume (ml), Pwp is mean pulmonary capillary wedge pressure (mmHg), Rap is mean right atrial pressure (mmHg), Ppa is mean pulmonary artery pressure (mmHg), HR is heart rate (beats/min). By ANOVA and tudent-Newman-Keul’s test, $p^a < 0.05$ vs. 4h within a group, $p^b < 0.05$ vs. other groups, $p^c < 0.05$ vs. nonsepsis groups, $p^d < 0.05$ vs. control group, $p^c < 0.05$ vs. sepsis group.
Table 2. Plasma histamine concentrations (nM) in the hemodynamic study (mean±SD)

<table>
<thead>
<tr>
<th>Group</th>
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<th>Treatment ½ hr</th>
<th>1 hr</th>
<th>2 hr</th>
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<tbody>
<tr>
<td>I</td>
<td>0.9±0.3</td>
<td>1.1±0.5</td>
<td>1.6±1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6±1.1</td>
<td>1.7±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>1.0±0.6</td>
<td>1.6±0.9</td>
<td>1.0±0.3</td>
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<td>1.1±0.3</td>
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<tr>
<td>III</td>
<td>1.4±0.9</td>
<td>0.8±0.4</td>
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<td>0.8±0.4</td>
<td>0.8±0.5</td>
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<tr>
<td>IV</td>
<td>0.8±0.3</td>
<td>0.8±0.4</td>
<td>0.6±0.3</td>
<td>0.6±0.3</td>
<td>0.7±0.3</td>
</tr>
</tbody>
</table>

I. H<sub>3</sub> blocker – sepsis group
II. Sepsis group
III. H<sub>3</sub> blocker alone
IV. Time control

By 2-way ANOVA and Student Newman-Keul's test, p<sup>a</sup><0.05 vs. time control group,
p<sup>b</sup><0.05 vs. all other groups.
Table 3. Slope of PRSWR in the cardiac mechanics study

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>4h</th>
<th>Treatment ½ H</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3 blocker – sepsis</td>
<td>90±28a</td>
<td>47±30</td>
<td>97±30a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>107±18ab</td>
</tr>
<tr>
<td>Sepsis</td>
<td>96±46</td>
<td>72±12</td>
<td>61±10</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>63±10</td>
</tr>
</tbody>
</table>

Values are mean±SD in mmHg, n=4, PRSWR is preload recruitable stroke-work relationship. By 1-way repeated –measures ANOVA and Student-Newman-Keul’s test, Pa<0.05 vs. 4 h within a group. By 2-way ANOVA and Student-Newman-Keul’s test, p<0.05 vs. sepsis group.
Figure 1. Mean blood pressure for the four groups in the hemodynamic study. After treatment, there was a significant increase in BP in the H₃ sepsis/blocker group. By one way ANOVA repeated measures and SNK, \( p^* < 0.05 \) vs 4 hours measurement within a group. By two way ANOVA for two repeated measures and SNK, \( p^\# < 0.05 \) H₃ sepsis/blocker group vs sepsis group; \( p^\dagger < 0.05 \) vs nonseptic group.
Mean blood pressure

Figure 1.
Figure 2. Cardiac outputs are shown for the four groups in the hemodynamic study. After treatment, there was a significant increase in cardiac output in the H₃ sepsis/blocker group. By one way ANOVA repeated measures and SNK, p*<0.05 vs 4 hours measurement within a group. By two way ANOVA for two repeated measures and SNK, p#<0.05 H₃ sepsis/blocker group vs sepsis group; p'<0.05 vs nonseptic group, p*<0.05 vs. time-control group.
Figure 2.

Cardiac output
Figure 3. Systemic vascular resistances are shown for the four groups in the hemodynamic study. There was a decrease in SVR after sepsis in both H₃ sepsis/blocker and Sepsis groups and no change with treatment in H₃ sepsis/blocker group. By one way ANOVA repeated measures and SNK, p<0.05 vs 4 hours measurement within a group. By two way ANOVA for two repeated measures and SNK, p'<0.05 vs nonseptic group.
Systemic vascular resistance

Figure 3.
Figure 4. Stroke work plotted against left ventricular end-diastolic volume for different measurement conditions in cardiac mechanics study. A: dog in sepsis group, after 4 h of sepsis, relationship was shifted downward and to right compared with baseline, and there was no response to placebo treatment. —□— Baseline, —△— 4 hr sepsis, —○— Placebo treatment, —★— ½ hour post treatment. B: dog in H₃ sepsis/blocker, treatment was associated with a return in slope to presepsis value. —□— Baseline, —△— 4 hr sepsis, —○— H₃ blocker treatment, —★— ½ hour post treatment.
Figure 4.
Figure 5. A: Isometric tension plotted against time in *in vitro* trabecular preparation. When sympathetic nerve stimulation was applied, there was an increase in isometric tension. Adrenergic response is indicated by interval between arrows. The small decrease in isometric tension at the beginning of stimulation and the slight increase in tension immediately after stimulation are thought to represent the effect of synchronization as described by Blinks (1966). This may be due to abnormal conduction of action potential when sympathetic stimulation is initially applied and then stopped. B: After added propranolol (10⁻⁵ M) to the bath, an increase in adrenergic response was abolished.
Figure 5.
**Figure 6.** In trabecular preparation, H₃ receptor activator (R)-α-methylhistamine decreased adrenergic response relative to pretreatment, whereas in presence of RAMH (1µM), H₃ blocker clobenpropit (1µM) attenuated effect of RAMH, $p^* < 0.05$ vs. other conditions by 1-way ANOVA and Student–Newman-Keuls test.
Effect of α-MeHA induced inhibition of the positive inotropic response

Figure 6.
Figure 7. In trabecular preparation, when nonseptic plasma was added into the bath, there was no change in adrenergic response as compared with before added nonsetic plasma, while there was a significant reduction in adrenergic response when septic plasma was added to the bath as compared with before given septic plasma.
Figure 7.
Figure 8. In presence of nonseptic plasma fraction, there was no effect of H₃ receptor blocker clobenprofat on adrenergic response in trabecular preparation. However, in presence of septic plasma fraction, effect of H₃ receptor blocker was to increase adrenergic response compared with pretreatment. By one way ANOVA repeated measures and SNK, $p^*<0.05$ vs. pretreatment. By two way ANOVA for two repeated measures and SNK, $p^*<0.05$ septic plasma fraction vs nonseptic plasma fraction.
Effect of $H_3$-blockade on adrenergic response in presence of plasma fraction

Figure 8.
Figure 9. In presence of septic plasma, addition of H₃ receptor blocker clobenpropit increased adrenergic response in in vitro preparation. When greater concentration of H₃ receptor blocker was added to preparation, adrenergic response increased (i.e., greater vertical distance from horizontal dashed line).
Figure 9.
Figure 10. Isometric tension (ordinate) plotted against time (see Fig 6. for scales). Interval between arrows indicates application of sympathetic nerve stimulation. In presence of $10^{-9} - 10^{-7}$ M histamine, adrenergic response decreased compared with prehistamine, whereas basal isometric tension was unchanged. On the other hand, higher histamine concentration (●, $10^{-3}$ M) caused a decrease in basal isometric tension, which was followed by an increased in tension, but no adrenergic response was observed.
Figure 10.
Chapter 7: Discussion

The purpose of this study was to assess the relevance of histamine H₃ receptors to cardiovascular function in sepsis. It was found that, in an in vivo model of sepsis, H₃ receptor blocker was associated with an improvement in hemodynamics. In an in vitro ventricular preparation, the results showed that specific H₃ agonist α-MeHA attenuates the inotropic response to transmural stimulation of adrenergic nerve endings. This attenuation was associated with a reduction in norepinephrine release. The antiadrenergic effects of α-MeHA were also prevented by the H₃ receptor antagonist thioperamide. Furthermore, a substance in contained plasma fraction from septic dogs caused an inhibition of the cardiac adrenergic response that was amenable to the H₃ receptor blockade. Thus, the in-vivo and in vitro data suggest that prejunctional H₃ receptors are activated during adrenergic stimulation, and that H₃ receptor blockade may restore cardiac adrenergic response in sepsis.

In the H₃ blocker-sepsis group, it was found that H₃ receptor blockade increased CO and BP as compared with a nontreated sepsis group. The higher BP in the H₃ blocker-sepsis group was due to an increase in CO, since SVR did not change with H₃ receptor blockade. There has been some controversy about the role of H₃ receptors on nerve fibers innervating blood vessels. McLeod et al. (1993) found that activation of peripheral H₃ receptors resulted in lower basal SVR in a guinea pig preparation. They suggested that activation of H₃ receptors might cause a decrease in tone in arterial resistance vessels. Ishikawa & Sperelakis (1987) showed that histamine can depress sympathetic neurotransmission in the mesenteric artery by interacting with prejunctional H₃ receptors.
that are located on the perivascular nerve terminals. Although Schneider et al. (1991) found no evidence for H₃ receptor-mediated inhibition of [³H]-noradrenaline release in the rat vena cava in vitro, Molderings et al. (1991) reported that activation of prejunctal H₃ receptors inhibited electrically-induced release of [³H]-noradrenaline from human saphenous vein. However, in the present study, SVR did not increase in sepsis with H₃ receptor blockade. This may indicate that the reduction in peripheral resistance observed in sepsis is not caused by activation of H₃ receptors in a major way. Other mediators released during sepsis, for example, products of the postaglandin pathway (Jacobs, 1982), vascular nitric oxide production (Hollenberg, 1993), or other pathways, may account for the lower SVR found in sepsis.

In the H₃ blocker-sepsis group, although preload (Pwp) was not different from that found in the sepsis group over most of the measurement intervals, Pwp increased transiently after treatment compared with the 4 hours value (Table 1). Histamine H₃ receptors have been identified in splanchnic tissues, such as guinea pig ileum and duodenum (Hew, 1990; Poliz, 1994). H₃ receptor blockade may have reduced vascular compliance in the splanchnic circulation, leading to an increase in venous return and to a higher Pwp after treatment. Thus, to some extent, an increase in preload may have contributed to the higher CO found in the H₃ blocker-sepsis group, but whether contractility also increased was not clear from these data alone.

By most definitions, an index of contractility must assess the capacity of the heart to perform work and must also be independent of preload and afterload. Sarnoff and
Berglund showed that myocardial contractile performance could be described by a plot of work against volume (or fibre length), which would be close to a straight line. Glower et al. (1984) used vena caval occlusion as a method to vary preload. Steady-state infusion of phenylephrine in the presence of autonomic blockade was used to vary afterload. They reported that the relationship between stroke work and end-diastolic segment (PRSWR) was highly linear and that the slope, which is proposed as a potential measure of intrinsic myocardial performance, was independent of preload and afterload. Accordingly, in the present cardiac mechanics study, as determined by PRSWR (Glower, 1985), it was found that after H₃ receptor blockade was administered, the slope of PRSWR was shifted leftward as compared with the 4-hour measurement, which indicated that H₃ receptor blockade improved LV contractility in sepsis.

The presence of modulatory H₃ receptors on adrenergic nerve endings in the heart infers their possible activation by an endogenous ligand, possibly histamine (Endou, 1993; Gross, 1984; Imamura, 1994). Gross et al. (1984) previously showed that, under conditions of sympathetic nerve stimulation, there was a frequency-dependent release of cardiac histamine. Later, Yoshitomi et al. (1989) showed that ischemia promotes the release of cardiac histamine in experimental models., Brackett et al. (1990) also found that plasma histamine concentrations increased from 10 to 30 ng/ml over 4 h of sepsis while there was no change in the control group. Recently, Imamura et al. (1994) showed that sympathetic nerve stimulation caused a 1.5-fold increase in histamine overflow, which was insufficient to activate H₃ receptors. In myocardial ischemia, histamine overflow increased 3.5-fold. (R)α-methylhistamine did not modify norepinephrine
release, whereas thioperamide doubled it. This indicated that those H3-receptors appear to be fully activated by an endogenous ligand in the ischemic myocardium, probably by histamine. In the hemodynamic study, histamine concentrations were measured from blood taken from the femoral artery catheter. In the H3 blocker-sepsis group, concentrations of histamine increased ~2.5 times over the course of the experiment compared with baseline (p<0.11) and were significantly different from the time-control group (Table 2). It appears that enough histamine is released in sepsis to cause H3 receptor activation in this model.

That histamine modulates adrenergic responses in cardiac-vascular tissues was known before histamine H3 receptors were discovered a decade ago by Arrang et al. (1983). Histamine has been shown to decrease the quantity of [3H]norepinephrine released by field stimulation from guinea pig atria and canine arteries and veins (McGrath and Sheperd, 1976; Powell, 1979). Histamine has also been found to reduce the tachycardia caused by sympathetic nerve stimulation in the dog, but not that caused by the administration of norepinephrine. However, the histamine receptor subtype that mediated these responses has remained unclear until recently. H1-antagonists were initially claimed to inhibit the modulatory effects of histamine in the dog heart (Kimura and Satoh, 1983). It was also reported that histamine concentrations below threshold for direct cardiac stimulation elicited a marked postjunctional H2-receptor-mediated reduction in sympathetically evoked increases in heart contractility (Gross et al., 1984). Recently Schlicker et al. reported that neither H1- nor H2- receptor antagonists modify histamine’s inhibition of [3H]norepinephrine release from rat (Schlicker et al., 1989) and mouse brain
cortex (Schlicker et al., 1992) and pig retina (Schlicker et al., 1990). Binding studies have demonstrated that many “selective” histamine receptor antagonists can interact with histamine H3-receptors to modulate adrenergic responses. Accordingly, as part of the in vitro study, the concentration of histamine that was required for histamine H3-receptor activation was determined. The present study shows that H3 receptor activation can be produced by concentrations of histamine as low as 10^{-9} M. This activation can be blocked by TM, but not by H1 and H2 antagonists. During H3 receptor activation, basal isometric tension was unchanged. Furthermore, at high histamine concentrations of 10^{-3} M, histamine caused a decrease in basal isometric tension, which was followed by an increase in tension, while no adrenergic response was observed (Figure 7). Guo (1984) reported that H1 and H2 antagonists could block these changes in basal tension. The present data indicate that the concentrations of histamine that activate H3 receptors is much lower than the concentrations required to activate H1 and H2 receptors.

In sepsis, basal depression in myocardial function has been shown in human subjects and animal models (Gomez et al., 1994) and has been related to the release of cytokines and the product of an inducible nitric oxide synthase (Finkel et al., 199; Schulz et al., 1992). As part of the in vitro study, the <30,000 molecular weight plasma fraction was used to represent septic plasma, because a previous study in our laboratory showed that it contains a factor that causes a depression in basal contraction in sepsis (Jha, 1993). Histamine would also be found in this fraction. The present study shows that the histamine concentration found in the fraction would not be large enough to effect basal contractility in this sepsis, but would be sufficient to cause H3 activation in sepsis.
In terms of the effect of histamine $H_3$ activation on norepinephrine release, Imamura et al. (1994) found that the detectable norepinephrine overflow during sympathetic stimulation was relatively small in an isolated heart preparation. Furthermore overflow increased from an undetectable basal level to a maximum of approximately 40 pmol/g 15/sec during each of three consecutive stimulation periods, then decreased to 30 pmol/g (25%) during $H_3$ activation. In a sepsis model, Brackett et al. also showed that plasma norepinephrine concentrations increased from 250 pg/ml at baseline to 1,500 pg/ml over a 4-h interval, in which this increase would come from all sources. Since the total amount of plasma norepinephrine concentrations in sepsis may be large compared with the amount due to sympathetic neural overflow per se, in the design of the in vivo study, rather than to directly measure norepinephrine release, our approach was to examine the effect of $H_3$ receptor blockade on improving hemodynamics, which was the important end point of study.

In the present study, both TM and clobenpropit were used to cause $H_3$ receptor blockade. Initially TM was used on the basis of the work of Mcleod et al. (1993). Subsequent work showed that clobenpropit may have a longer period of action and higher potency, so it was decided to switch to TM (Kathmann et al., 1993). However, it was not the purpose of this study to obtain a dose-response relationship of the different agents, but only to determine whether histamine $H_3$ activation was present in sepsis. Both agents showed similar effects in our model.
In the present study, a ventricular preparation was used to corroborate the *in vivo* findings. However, these results must be interpreted cautiously. In the *in vitro* preparation, field stimulation was used to produce sympathetic stimulation, but this approach is a very unphysiological way of causing norepinephrine release. A more physiological approach would be to use an innervated preparation in which the sympathetic nerve could be directly stimulated. Furthermore, the results showed that propranolol blocked the increase in isometric tension observed during field stimulation, and the conclusion was that the sympathetic nervous system caused the changes in muscle tension. However, propranolol has numerous effects, including direct membrane stabilization (Hoffman, 1998), and therefore it must be cautiously viewed that sympathetic stimulation accounted for the entire contractile effect during field stimulation.

Schwartz et al. (1990) have reported that the specific binding of [*H]α-MeHA to rat cerebral cortex is selectively inhibited in the presence of guanyl nucleotides. This indicated that, as with other receptors, a G protein couples the H3-receptor to its effector system, a concept supported by recent H3-receptor purification (Zweig et al., 1992). Endou et al. (1994) have found that H3-receptors are probably coupled to a PTX-sensitive G protein and probably effect a reduction in Ca++ current through N-type Ca++-channel, resulting in inhibition of norepinephrine release. Further study is needed to understand intracellular signalling for the H3 receptor activation.
In summary, in an *in vivo* study of sepsis, H₃-receptor blockade was associated with an improvement in hemodynamics and increase in LV contractility. In an *in vitro* ventricular preparation the results showed that a substance in the septic plasma fraction caused an inhibition of the cardiac adrenergic response that was amenable to H₃-receptor blockade. Although the present study favors the idea that histamine H₃-receptor blockade augments norepinephrine release from adrenergic nerve endings, it is also possible that histamine H₃-receptor blockade may improve hemodynamics in sepsis by other as yet undefined mechanisms. Furthermore, it is important to recognize that the findings obtained in this animal model and the in vitro preparation may not reflect those in human disease and that the animals were studied under anesthesia, which may also have affected the results. However, we conclude that activation of H₃-receptors may contribute to cardiovascular collapse in sepsis.
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Characterization of a digitonin-solubilized bovine brain H3 histamine receptor