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The Intrinsic Cardiac Nervous System In Ischemic Heart Disease

By

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Submitted in partial fulfillment of the requirements for the degree of
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at

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Abstract

The intrinsic cardiac nervous system (ICNS) serves an important role in beat-to-beat regulation of cardiac function. It is unknown if the ICNS undergoes "remodelling" when exposed to ischemic injury or therapeutic modalities utilized to treat myocardial ischemia. Chemical stimuli (adenosine and adenosine antagonists) were utilized to study the role intrinsic cardiac neuron P_1 purinergic receptors play in myocardial ischemia and reperfusion. Further investigations examined the influence of spinal cord stimulation (SCS) and transmyocardial laser revascularization (TMLR) on cardiac neuronal control. Lastly, an examination of the human ICNS in patients with ischemic heart disease was performed.

Locally administered adenosine reduced neuronal responses during reperfusion following transient myocardial ischemia in the porcine model. However, adenosine did not affect the ICNS during the ischemic phase. This short-lived effect was mediated via adenosine A_1 receptors. SCS suppressed canine ICNS activity. However, this effect occurred during both ischemic and the reperfusion phase. Additionally, SCS remodelled the ICNS for prolonged periods after its termination. Chronic TMLR (4 weeks) elicited a long-term remodelling of the canine ICNS, resulting in blunting of neuronal responses to potent chemical stimuli. This was associated with hemodynamic instability; ventricular fibrillation occurred in 1 animal. Human intrinsic cardiac neurons generated spontaneous activity and responded to cardiac sensory inputs. Exogenously administered therapeutic agents modified their behaviour in the perioperative period, implying that human intrinsic cardiac neurons could possibly be manipulated to therapeutic advantage. Collectively, these data provided insight into neurocardiologic mechanisms underlying cardiac dysfunction in disease states and the potential for developing novel therapies used in the treatment of ischemic heart disease.

List of Abbreviations

ACE-I	Angiotensin converting enzyme inhibitor medication
AII	Angiotensin II
Ao	Aorta
AMP	Adenosine mono-phosphate
ADP	Adenosine di-phosphate
AP	Aortic pressure
ATP	Adenosine tri-phosphate
AV	Atrioventricular node
ß-blockers	Beta receptor blocking medication
°C	Degrees Celcius
CA	Coronary artery
CABG	Coronary artery bypass graft
cAMP	Cyclic adenosine mono-phosphate
cc	Cubic centimetre(s)
CCS	Canadian Cardiovascular Society
cm	Centimetre(s)
CCA	Circumflex coronary artery
CCB	Calcium channel blocker medication
CPB	Cardiopulmonary bypass
DIV	Dorsal interventricular
Dorsal CA	Dorsal coronary artery
Dorsal A	Dorsal atrial
EAA	Excitatory amino acid(s)
ECG	Electrocardiogram
EF	Left ventricular ejection fraction
$FiCO_2$	Fraction inspired carbon dioxide
FiO_2	Fraction inspired oxygen
G_i	G-protein - inibitory

G_s	G protein - stimulatory
GABA	γ-amino butyric acid
GP	Ganglionated plexus(es)
ICNS	Intrinsic cardiac nervous system
ICU	Intensive care unit
imp	Impulses per minutes
IHD	Ischemic heart disease
i.v.	Intravenous
IVC	Inferior vena cava
IVC – X	Inferior vena cava occlusion
IQR	Interquartile range
hr	Hour(s)
HR	Heart rate
HTN	Hypertension
IAS	Inter-atrial septum
K_{ATP}	ATP-sensitive potassium channels
kg	Kilogram(s)
LA app	Left atrial appendage
LAD	Left anterior descending coronary artery
LMA	Left marginal artery
LV	Left ventricle
LVIMP	Left ventricular intramyocardial pressure
LVIMP (Control)	Leftventricular intramyocardial pressure in control region
LVIMP (Laser)	Left ventricular intramyocardial pressure in lasered region
LVP	Left ventricular pressure
LSG	Left stellate ganglion
LSVC	Left superior vena cava
LSVC-LA	Left superior vena cava – left atrial
mg	milligram(s)
mm	millimetre(s)
mM	millimolar

mmHg	millimetres of mercury
µg	microgram(s)
µM	micromolar
mv	millivolt(s)
min	minute(s)
msec	millisecond(s)
n.s.	non-significant
Nico	Nicotine
NIH	National Institute of Health
occl.	Occlusion(s)
p	p-value
P_1	Purinergic subtype 1 receptor(s)
PA	Pulmonary artery
PAP	Pulmonary artery pressure
PTCA	Percutaneous transluminal coronary angioplasty
RA	Right atrium
RAGP	Right atrial ganglionated plexus
RCA	Right main coronary artery
RMA	Right marginal coronary artery
RSG	Right stellate ganglion
RV	Right ventricle
RVC-RA	Right vena cava – right atrial
RVIMP	Right ventricular intramyocardial pressure
RVIMP (Control)	Right ventricular intramyocardial pressure in control region
RVIMP (Laser)	Right ventricular intramyocardial pressure in lasered region
RSVC	Right superior vena cava
SA	Sinoatrial node
SCS	Spinal cord stimulation
sec	Second(s)
SEM	Standard error of the mean
SIF	Small intensely fluorescent (cells)

TMLR	Transmyocardial laser revascularization
VCM	Ventral cranial medial
VIV	Ventral interventricular ventricular coronary artery
VRA	Ventral right atrial
X-clamp	Cross-clamp

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Chapter 1 Introduction

1.1 Historical Perspective and General Introduction

The traditional functional model of autonomic neuronal control of the heart is a dichotomous one, consisting of a "brake" and an "accelerator". This classical model put forth by Langley in 1921[1] and further characterized anatomically by others[2,3] consists of two major efferent divisions: the sympathetic and parasympathetic nervous systems. These systems act in a reciprocal fashion to regulate the heart rate and myocardial contractility[4,5]. While both system are tonically active, when one efferent limb is activated the other becomes largely suppressed[5,6]. This neuronal action is analogous to antagonistic muscle pairs, such as the biceps brachii and triceps muscles of the arm. Both muscles have a basal level of tone, however, when flexion of the forearm is required, the triceps relaxes to allow this appropriate movement response of the biceps brachii.

Sympathetic efferent nerve cells located principally in the rostral ventrolateral medulla oblongata (under the influence of the nucleus of the tractus solitarius and intervening interneurons), project their axonal outputs to the interomediolateral nucleus of lamina VII in the spinal cord[5,7]. In this traditional neuronal framework, the sympathetic efferent nervous system, generally acts as the "accelerator" of heart function. These preganglionic neurons, in turn, send projections to paravertebral ganglia in the thoracic cavity (middle cervical, stellate and T2-5 ganglia) to synapse with postganglionic somata, which in turn, innervate the heart principally via the sinoatrial (SA), the atrioventricular (AV) node and the cardiomyocyte directly. The cardiac parasympathetic efferent

nervous system, acting as the "brake" of cardiac function, also originates in the medulla oblongata. Its preganglionic neurons (also influenced by projections from the tractus solitarius and intervening interneurons) are located in the dorsal motor nucleus and (principally) the nucleus ambiguus[5,7]. These preganglionic projections then synapse with parasympathetic postganglionic somata near or on the heart. Several other regions of the brain (e.g., cerebral cortex, basal ganglia, hypothalamus), in addition to these sympathetic and parasympathetic nuclei located in the brainstem, can also exert influence on autonomic neuronal output[5].

In keeping with this model, the heart itself has been thought to contain only two types of neurons: i) parasympathetic efferent postganglionic neurons and ii) small intensely fluorescent cells (SIF cells)[8]. While the function of intrinsic cardiac SIF cells remains enigmatic, it has been believed the parasympathetic efferent postganglionic neurons represent simple relay stations transferring information from medullary centres to cardiac end-effectors[5,9]. This elegant, yet, simple model therefore relegates afferent cardiovascular sensory processing and subsequent efferent neuronal influence of cardiac functioning to reside solely within the central nervous system. Current evidence however, now indicates this model is an oversimplification of the actual neural control of the heart.

The concept of antagonism between the two efferent limbs of the cardiac nervous system has been revised since it has been demonstrated that both efferent limbs can be either enhanced or suppressed in parallel[10,11]. Over the last several years, details of the complexity of cardiovascular reflex arcs involved in the beat-to-beat coordination of regional cardiac function have begun to

emerge. Evidence indicates that in addition to the central nervous system, further processing and integration of cardiovascular sensory information also occurs within intrathoracic ganglia[12,13] including a component directly on the heart[14-17]. The integration of cardiac sympathetic[18] and parasympathetic[19] efferent preganglionic neuronal inputs to the intrinsic cardiac nervous system involves complex neuronal processing at the level of the heart[12]. Efferent postganglionic neurons in each part of the intrinsic cardiac nervous system exert control over functionally discrete regions throughout the heart[20]. This independent neuronal system of the heart, the intrinsic cardiac nervous system, contains afferent neurons[15,21,22], sympathetic efferent postganglionic neurons[22-27] and parasympathetic efferent postganglionic neurons[28-30]. In addition, populations of local circuit neurons, analogous to those found in the spinal cord, function to interconnect neurons within and between the separate aggregates of ganglia that form the various intrinsic cardiac ganglionated plexuses[23,25-27,31].

Local circuit neurons form an essential element for the overall coordination of activity within the intrinsic cardiac nervous system[12,16], including the generation of basal activity in the absence of higher centre inputs[12,15,31,32]. These local circuit neurons permit interactions to occur not only among adjacent intrinsic cardiac neurons, but also among distant clusters of intrinsic cardiac neurons and with intrathoracic extracardiac neurons[12,15].

This intricate neuronal system with afferent, efferent and local circuit neuronal components allows for more complex two-way communication between neurons in the heart and brain than previously believed in the Langley paradigm.

It is felt that this elaborate neuronal arrangement acts as a "short-feedback loop" to permit finer, "real-time" coordination of regional cardiac function[12,33,34]. The intrinsic cardiac nervous system has the potential to act as a "low pass" filter to mitigate potential imbalances in neural control of regional cardiac function arising from extracardiac neuronal inputs to the heart or as the result of pathophysiological events within the heart such as myocardial ischemia.

In addition to the importance of the intrinsic cardiac nervous system in the hierarchy of the neuronal system that coordinates regional cardiac function, evidence has demonstrated that the heart retains a functional nervous system following surgical interruption of its extrinsic neuronal inputs[15] or following cardiac transplantation[16]. Given the fact the heart possesses its own nervous system capable of independent function during each cardiac cycle, it is an appropriate question to ask whether the intrinsic cardiac nervous system is altered or "remodelled" when exposed to ischemic injury or to therapeutic modalities utilized to treat myocardial ischemia.

1.2 Myocardial Ischemia and the Intrinsic Cardiac Nervous System

Ganglionated plexuses within the intrinsic cardiac nervous system of large mammalian hearts have an identifiable arterial blood supply[35]. At least two arteries supply blood to each major intrinsic cardiac ganglionated plexus, forming an interconnection *rete* of vessels surrounding ganglia therein[35-37]. Presumably, this anatomical arrangement minimizes the effects of reducing blood flow in one of the arteries perfusing a ganglionated plexus. Despite this anatomical

arrangement, transient occlusion of a principal coronary artery supplying blood to a single intrinsic cardiac ganglionated plexuses can directly modify the activity generated by many of its neurons[35,37].

Various populations of cardiac chemosensory afferent neurons situated in intrathoracic ganglia[38,39], on the heart[35], in dorsal root ganglia[40] or in nodose ganglia[41,42] transduce local myocardial events. Although the mechanisms underlying cardiovascular reflexes initiated during myocardial ischemia are not fully understood, it is apparent that when the blood flow in a coronary artery perfusing an intrinsic cardiac ganglionated plexus becomes compromised the function of its neurons are affected[35]. It has been postulated that metabolites that accumulate locally when the regional coronary arterial blood supply of intrinsic cardiac neurons is compromised influence the somata and dendrites of such neurons in a direct manner[35,37].

In addition to direct neuronal ischemia, activity generated by intrinsic cardiac neurons can also be modified by focal ventricular ischemic events that occur distally to the more cranially located atrial and ventricular neurons (i.e., myocardial ischemia that does not directly involve the blood supply of investigated neurons somata[35]. Focal ventricular ischemia appears to influence the chemical milieu of sensory axonal and neurite functioning in the affected myocardium. This altered neuronal activity during ischemia likely reflects the function of intracardiac feedback loops that are evoked subsequent to the activation of sensory neurites within the myocardial ischemic zone[35]. It has also been demonstrated that during ventricular ischemia, locally liberated adenosine,

ATP, and other chemicals can affect the sensory neurites associated with afferent neuronal somata in nodose, dorsal root or intrathoracic ganglia[8,41,43,44]. Upon subsequent myocardial reperfusion, the various metabolites that accumulate can further influence intrinsic cardiac neurons and their sensory neurites and some cases, in excess of those neuronal effects during myocardial ischemia[35,45].

These ischemia-induced changes in the intrinsic cardiac nervous system can affect afferent feedback to the intrathoracic nervous system[8,46]. When such neuronal activity becomes excessive, ventricular arrhythmias may ensue[47].

1.2.1 Generation of cardiac arrhythmias

It has been previously demonstrated that activity generated by cardiac mechanosensory neurites becomes altered in the presence of atrial or ventricular arrhythmia/fibrillation[39,48,49]. It has also been proposed that injury to intrinsic cardiac neurons may be involved in the genesis of cardiac electrical instability following cardiac surgery[50].

In some instances, the activity generated by cardiac afferent neurons becomes enhanced in the ischemic state just before the onset of ventricular fibrillation. This suggests that some cardiac afferent neurons are sensitive to chemicals liberated during myocardial ischemia before gross alterations in cardiac function become apparent. The increased cardiac afferent neuronal activity that subsequently inputs into various components of the cardiac neuronal hierarchy can result in excessive activation of cardiac adrenergic efferent

neurons projecting to or located within the intrinsic cardiac nervous system. This excessive adrenergic activity subsequently contribute to the formation of ventricular tachycardia or even ventricular fibrillation secondary to enhanced release of catecholamines from regional sympathetic efferent nerve terminals[45,47,51]. Thus, cardiovascular reflexes associated with myocardial ischemia involve a number of neuronal factors and the generation of cardiac dysrhythmias in ventricular ischemia is also due in part to alterations in feedback regulation within this neuronal system.

1.3 The Responses of the Intrinsic Cardiac Nervous System During Reperfusion Following Regional Myocardial Ischemia

Early re-establishment of oxygenated blood flow following coronary occlusion is essential to halt the progression from reversible to irreversible ischemic damage in cardiac tissue. This re-oxygenation or reperfusion however, is not without hazard. Mounting evidence suggests that "reperfusion-induced injury" may lead to lethal cellular damage.

The hypothesis that reperfusion might further exacerbate the injury sustained during a period of ischemia was initially proposed in the 1930s by Tennant and Wiggers[52] who observed that myocardial reperfusion induced ventricular fibrillation in the canine heart. In the 1960s, Jennings[53] and co-workers were able to show the re-establishment of blood flow after a transient occlusion of a coronary artery in the dog resulted in leakage of myocyte enzymes, ultrastructural injury and myocardial contracture. Hearse et al[54] later

put forth the concept of the "oxygen paradox", in which it was observed that the damage that occurred during the early minutes of reperfusion appeared to be far greater than would be expected than if the ischemic episode had been maintained.

Reperfusion injury results in four major categories of consequences: 1) reperfusion induced arrhythmias, 2) myocardial stunning, 3) lethal reperfusion injury and 4) accelerated necrosis[55]. Each one of these events has been the source of intense study. For the purposes of this section of the dissertation, I will focus on the intrinsic cardiac neuronal system and arrhythmogenic sequalae of the reperfusion response.

1.3.1 Reperfusion induced arrhythmias

A potentially lethal consequence of reperfusion injury is the induction dysrhythmias that range from ventricular premature beats to ventricular fibrillation[52,55-58]. It has been shown in several species, including humans, that the generated arrhythmias occur within seconds of the onset of "re-oxygenation" of ischemic myocardial tissue[59-61]. Clinically, reperfusion arrhythmias have been observed in patients undergoing cardiac surgery, percutaneous transluminal angioplasty and stenting, after thrombolytic therapy and have been suggested as a cause of sudden cardiac death following the spontaneous relief of coronary spasm[55,62-66]. Many possible mechanisms have been identified for these reperfusion responses[55,61]: stimulation of adrenergic receptors, increase in cyclic

AMP, disturbances of lipid metabolism, disturbances of ionic homeostasis and the release of various chemical metabolites.

As stated above, ischemia and locally administered chemical excitation of specific populations of intrinsic cardiac neurons can induce ventricular arrhythmias[47] or even ventricular fibrillation[51]. It is less clear what effect myocardial reperfusion following transient coronary occlusion has on the intrinsic cardiac nervous system and more importantly if resultant activity can be modified. Adenosine, liberated by the ischemic myocardium, influences populations of intrinsic cardiac neurons[47,67] and may play a modulatory role in these circumstances.

1.4 Role of Adenosine in Ischemia Reperfusion

Adenosine is a ubiquitous cellular purinergic nucleoside involved in cellular metabolism, cardiovascular (coronary dilatation) and in neuronal modulation. It acts as an effector for myocardial cells, sympathetic nerve cells, endothelial cells, leucocytes and platelets[68-70].

1.4.1 Metabolism of adenosine

Details of adenosine metabolism have been described elsewhere (see Pelleg, 1990[71] for review). For the purposes of this dissertation, only adenosine metabolism as it pertains to ischemia and reperfusion will be discussed.

Adenosine is a catabolic by-product of the ischemic cell. The heart produces adenosine when there is a net breakdown of ATP. Removal of the two-

high energy phosphates from ATP produces AMP. The latter is dephosphorylated by 5'-nucleotidase to produce free adenosine that can easily exit the cell. Once in the interstitial space, adenosine binds to the surface receptors on cardiomyocytes[72]. Adenosine likely then binds to one of three specific purinergic receptors on the cell surface that are coupled to the extracellular domain of a complex membrane-spanning protein receptor, termed G-protein. The G-protein once activated causes a number of intracellular events, including the activation of protein kinases.

Adenosine is rapidly detached or taken up by other cells (by both active and passive mechanisms) and enzymatically metabolized intracellularly. The $t_{1/2}$ of adenosine is approximately 0.5-10 seconds[73,74]. The enzymatic action is then either deaminated by adenosine deaminase to the protein inosine or phosphorylated to adenosine monophosphate by adenosine kinase.

1.4.2 Cardiovascular effects of adenosine

Adenosine has potent cardiovascular, neurohumoral and coronary circulation effects. Adenosine physiological effects result from its interaction with extracellular purinergic receptors type 1 (P_1) receptors[75,76]. This class of receptor can be subdivided into at least four sub-types of adenosine receptors: A_1, A_{2a}, A_{2b} and the newly identified A_3 receptor[77]. Adenosine A_1 receptors have been located on neutrophils and on cardiomyocytes[78]. The binding of adenosine to A_1 receptors activates ATP sensitive potassium (K_{ATP}) channels via inhibitory G-protein-mediated (G_i) transduction (reduction of adenyl cyclase activity), which

results in a net potassium outward conductance[78-81]. This induces hyperpolarization of the cell, inhibits calcium conductance and consequently reduces the effect calcium overload. The physiological effects of adenosine A_1 receptor stimulation include negative chronotropy and dromotropy, anti-adrenergic effects, stimulation of glycolysis, and stimulation of neutrophil adherence[78,82]. Adenosine A_{2a} receptors are localized on neutrophils, endothelial cells, vascular smooth muscle, and platelets. Receptor-ligand interaction stimulates adenylate cyclase through a stimulatory G protein (G_s) transduction mechanism, which result in vasodilation, renin release, the inhibition of neutrophil superoxide generation and adherence to endothelium. Adenosine A_{2b} receptors are likely localized on the ventricular myocyte[83,84]. Similar to A_{2a} receptors, A_{2b} receptors are linked to adenylate cyclase and appear to antagonize the anti-adrenergic effects of the A_1 adenosine receptor. The A_{2b} receptor has also been implicated in the inhibition of cardiac fibroblasts and possibly in modulation of cardiomyocyte hypertrophy[85,86]. Adenosine A_3 receptors have been localized in heart tissue as being associated with the endothelium and myocytes, although firm data are not available[77,78]. The adenosine A_3 receptor is similar to the adenosine A_1 receptor in that it inhibits adenylate cyclase and stimulates protein kinase C translocation[78,87,88].

1.4.3 Adenosine and myocardial reperfusion dysrhythmias

The genesis of reperfusion associated ventricular dysrhythmias is not clear. Adenosine production, a by-product of catabolism during ischemia and

hypoxia, increases 50-fold within the ischemic cell[74,89-91]. Adenosine, therefore, has received considerable attention in this regard as it is known to exert modulatory effects on a number of cellular mechanisms associated with this ischemic/reperfusion response. These include interaction with α-adrenergic receptors[92 69] alterations in intracellular cyclic AMP[82,93] diminishment of free radical generation[61,81,94-96] and alteration of K_{ATP} channel activity[80,97,98]. Additionally, adenosine action as a potent inhibitor of neutrophils[94], platelets[68] and mononuclear leukocytes[99] further demonstrates it "broad-spectrum" or pluripotent effects. Adenosine exerts it actions in a number of cellular areas and is directed at a number of effectors involved in ischemic and reperfusion injury.

Over the last several years it has been demonstrated that the "cardioprotective" effects of adenosine extend well into the reperfusion phase following transient ventricular ischemia[81,100-102]. The apparent duration of the physiological actions attributed to adenosine extends well beyond its plasma half-life[78], making adenosine a potentially clinically useful agent.

Adenosine has been shown to exert important "long-term" neuromodulatory effects in the central nervous system[38,103-106] as well as the gastrointestinal system[107]. Adenosine also exerts neuromodulatory effects on cardiac sympathetic efferent neurons[108-110] as well as on cardiac afferent neurons in nodose[4,48 111] and dorsal root ganglia[48]. Adenosine can also influence the activity generated by intrinsic cardiac neurons *in vitro*[112] or *in vivo*[43] in normally perfused hearts. Adenosine modulatory effects, however, have not been examined in porcine ventricular intrinsic cardiac neurons. In addition, it is

currently not known how reperfusion following bouts of focal ventricular ischemia affects porcine ventricular intrinsic cardiac neurons, or whether the resultant alterations in intrinsic cardiac neuronal activity can be modified by adenosine as seen in other neuronal systems.

1.5 Chronic Myocardial Ischemia and Therapeutic Modalities

1.5.1 Transmural myocardial infarction

It has been proposed that nerves coursing over a transmural ventricular infarction are rendered non-functional by eliminating the local arterial blood supply to the epicardial afferent and efferent axons overlying the infarction[113-115]. These data, however, were based on indirect analysis of cardiac neuronal function obtained by studying alterations in ventricular regional electrical repolarization[113,114]or chemical content[115].

Cardiac nerves, however, possess their own rich blood supply, much of which arises from extracardiac arteries[116]. For that reason, the blood supply of nerves coursing over a ventricular infarction is not affected when underlying myocardial tissue becomes ischemic. In accordance with this concept and in contradistinction to above findings, direct assessment of intrinsic cardiac neurons reveals that major nerve bundles continue to transmit action potentials over a transmural ventricular infarction of the canine heart *in situ*[116].

1.5.2 Spinal cord stimulation

Electrical stimulation of the dorsal columns in the posterior spinal cord called spinal cord stimulation (SCS) is increasingly used in the treatment of various chronic pain syndromes. This technique was initially utilized to treat chronic neuropathic pain syndromes such as "phantom-limb" pain following extremity amputation and "failed back surgery" syndrome. More recently, SCS has been applied to chronic pain syndromes involving tissue ischemia, both the in periphery[117] and within the cardiac system[118].

1.5.2.1 Gate-control theory of pain sensation modulation

SCS has been shown to modulate information processing within the central nervous system[119,120] by inhibiting the activity of spinal neurons. These anti-nociceptive effects were attributed to the "Gate Control Theory"[121]. This theory postulates that stimulation of the dorsal columns of the spinal cord affects segmental neurotransmission of low threshold, large diameter (Aβ) fibres. It is believed that anti-dromic stimulation of these large primary afferents that are concentrated and ascend in the dorsal columns subsequently inhibit afferent information that is transmitted via slower conducting, nociceptive Aδ and c-fibres. Spinal cord stimulation takes advantage of these large diameter nerve fibre inputs to modulate cells in lamina IV-V that respond to nociceptive inputs from the periphery[122]. This inhibitory effect was presumed to be secondary to activation of modulatory interneurons in the substantia gelatinosa[122,123](see Linderoth, 1999 for review[124]).

In addition to neurophysiological modulation in the manner described above, concurrent neurochemical mechanisms have been proposed as well. Nociceptive inputs to the spinal cord, via afferent nerves containing excitatory amino acids (EAAs), such as aspartate or glutamate, are normally tonally controlled by the γ-amino butyric acid (GABA) containing interneurons. GABA, in the normal setting, exerts both pre- and post-synaptic inhibition on primary afferents and the release of EAAs[125]. In the neuropathic state (i.e., peripheral nerve injury), GABA release from the modulatory interneurons is decreased and the release of EAAs from nociceptive afferents are increased, resulting in a hyperexcitable state in the spinothalamic tract cells with clinical sequalae of hyperalgesia[125,126]. Experimental evidence indicates SCS results in anti-dromic activation of dorsal column fibres that produces a pre-synaptic inhibition of excessive nociceptive afferent inputs from the injured peripheral neurons[117,120,127]. It has also been shown that SCS results in an increased release of GABA in the dorsal horn of the spinal cord[128]. It is believed that SCS has induced a "forced GABA release" re-establishing control of EAA release[125].

1.5.2.2 Spinal cord stimulation in refractory cardiac angina

SCS is a therapeutic modality that is increasingly utilized in the treatment of patients with therapeutically refractory angina pectoris. These are patients that suffer from symptomatic anginal pain at rest or with minimal exertion (Canadian Cardiovascular Society (CCS) anginal class III or IV) despite maximal medical therapy and are not amenable to conventional revascularization

strategies. This subpopulation of patients with ischemic heart disease represent those with end-stage coronary artery disease and typically have a poor quality of life secondary to debilitating, incessant anginal pain.

Similar to treatments performed for neuropathic pain syndromes, electrical stimulation is administered via an electrode placed in the epidural space and is positioned in the upper thoracic spinal cord (T1-T2 vertebral levels). Clinical studies show that SCS is a safe adjunct therapy for cardiac patients, producing anti-anginal as well as anti-ischemic effects[118,129-135] without masking symptomology associated with acute coronary syndromes[136]. The mechanisms whereby SCS produces its long-term effects in the setting of cardiac angina, however, remain unknown.

SCS has demonstrated clinically important benefits that extend well beyond those that can be attributed to the increased GABA neuronal inhibition of afferent pain signals that cause the increased activity of the spinothalamic tract cells. The therapeutic effects of spinal cord stimulation (SCS) can indeed persist for hours after its termination[135]. This includes improved exercise tolerance and prolonged relief well after the stimulating device has been terminated.

SCS has been shown to influence peripheral blood flow[137-141]. SCS effects have therefore been attributed to improved myocardial perfusion and/or alterations in the oxygen demand and supply ratio, reflected by a reduction in the ST segment elevation and improved myocardial lactate metabolism in the setting of myocardial stress[129,130]. In contradistinction to these findings however, it has

subsequently been shown that SCS does not alter blood flow distribution within either the normal or ischemic canine myocardium[142].

The intrinsic cardiac nervous system, in intact preparations, continually receives and integrates spinal cord neuronal inputs to assist in its regulation of regional cardiac function[12,25]. As stated above, transient ventricular ischemia is capable of altering intrinsic cardiac neuronal activity[35]. Preliminary evidence indicates that anti-anginal effects that SCS affords patients may be via short-term modification of the intrinsic cardiac nervous system[45]. Little information is known, however, about the prolonged effects of SCS during a clinically relevant focal ventricular ischemic event. Specifically, is SCS capable of modifying the intrinsic cardiac nervous system for a prolonged period?

1.5.3 Transmyocardial laser revascularization (TMLR)

Transmyocardial laser revascularization (TMLR) is another of the emerging alternative therapies for the treatment of end-stage coronary artery disease and is utilized in patients whose disease is not amenable to conventional medical or surgical therapy[143-149]. TMLR involves the creation of multiple, transmural ventricular canals by means of a laser device. Several prospective, randomized, multi-centre trials have documented the efficacy of TMLR in the relief of refractory angina of cardiac origin[144,145,150,151]. The mechanism whereby TMLR provides symptomatic relief, however, remains unclear. Three mechanisms have been proposed to account for the therapeutic benefits that TMLR provides to patients with myocardial ischemia. First, multiple channels

produced in the wall of the ischemic ventricle by TMLR has been proposed to permit blood from the chamber to reach underperfused cardiomyocytes by forming direct communications between the chamber and ventricular blood vessels[152,153]. Although some investigators have reported that transmyocardial channels remain patent for months[154], others have challenged this theory by demonstrating only transient patency[155,156]. Second, angiogenesis may be induced by TMLR[146,157-160]. Although it has been reported that angiogenesis does not occur following TMLR in animal models[154,156], accumulating evidence suggests this to be the primary mechanism for its clinically observed therapeutic benefits[161-163]. Third, it has been proposed that laser-induced myocardial injury is non-specific and that the damage created by the laser involves local neuronal tissue[164-167]. These results however, have been predicated upon indirect assessment of neuronal innervation[164-166,168]. As there are over 20 000 neurons on the human heart[169] and the fact that both afferent and efferent axons within the ventricular wall are both very thin, numerous and possess several orders of branching[170], it is unlikely that 10 – 40 transmural holes of less than 1 mm in diameter produced in a ventricular wall is sufficient to alter the function of these regional axons in a detectable fashion.

1.5.3.1 The effect of TMLR on the ICN in an acute setting

To adequately assess cardiac neuronal function, one needs to examine the intrinsic cardiac nervous system in a direct, functional manner. When recording from the intrinsic cardiac nervous system *in situ* acutely following

TMLR, the integrity of ventricular afferent and efferent axons in a ventricular region after performing TMLR, was found to be unaffected by this procedure (Fig. 1.1, 1.2)[171]. The clinical effectiveness of anginal relief, however, typically does not occur until several weeks or months after the procedure. A remaining question to be determined is whether TMLR alters the intrinsic cardiac nervous system in a chronic setting.

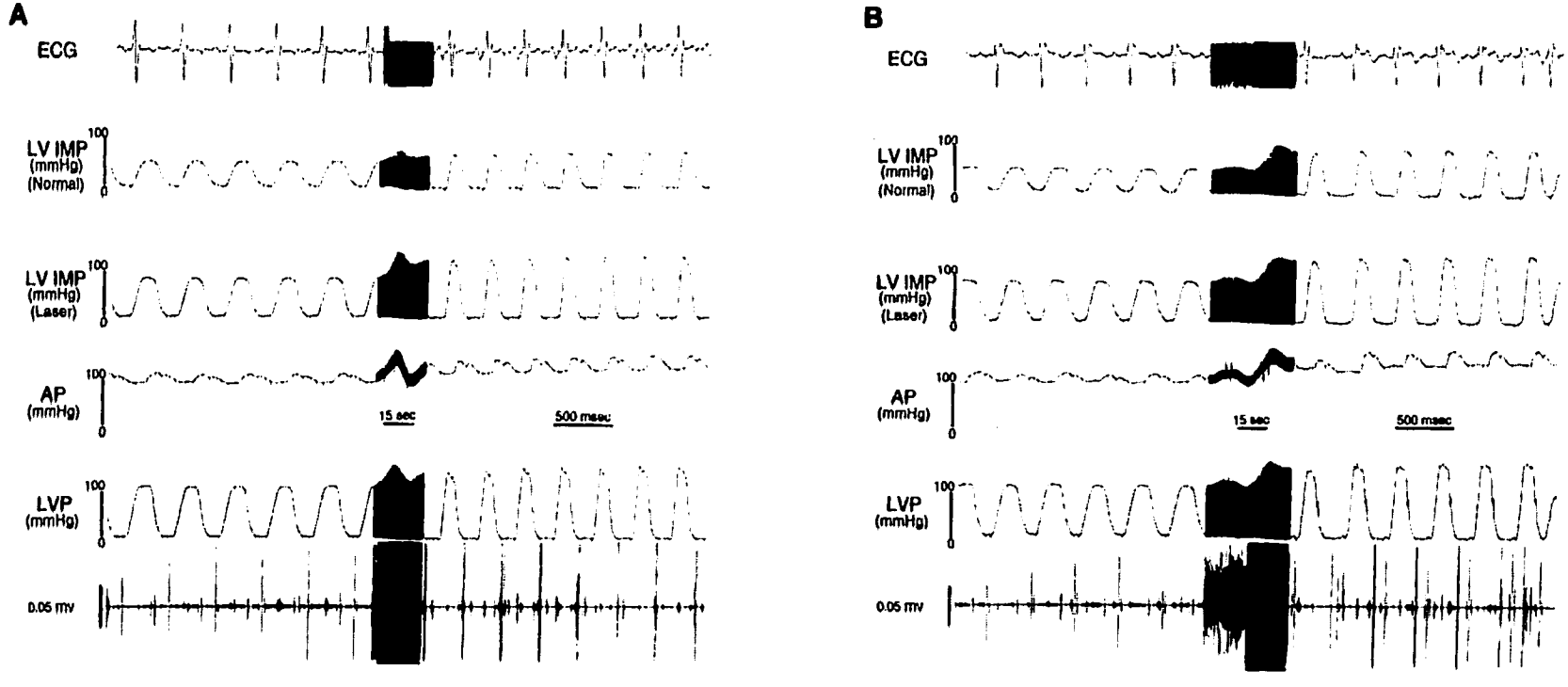


Figure 1.1 *Efferent Cardiac Neuronal Activity Acutely after TMLR.*

Cardiac and neuronal effects elicited by stimulation of the left stellate ganglion of one animal before (A) and acutely after (B) local application of TMLR. ECG = electrocardiogram; LV IMP (Normal) represents intramyocardial pressure in the unaffected region of the left ventricular ventral wall; LV IMP (Laser) represents intramyocardial pressure in the region of the left ventricular ventral wall subjected to TMLR; AP = aortic pressure; LVP = left ventricular chamber pressure. The lowest trace representing intrinsic right atrial neuronal activity, elicited by left stellate ganglion stimulation, is similar before and after TMLR.

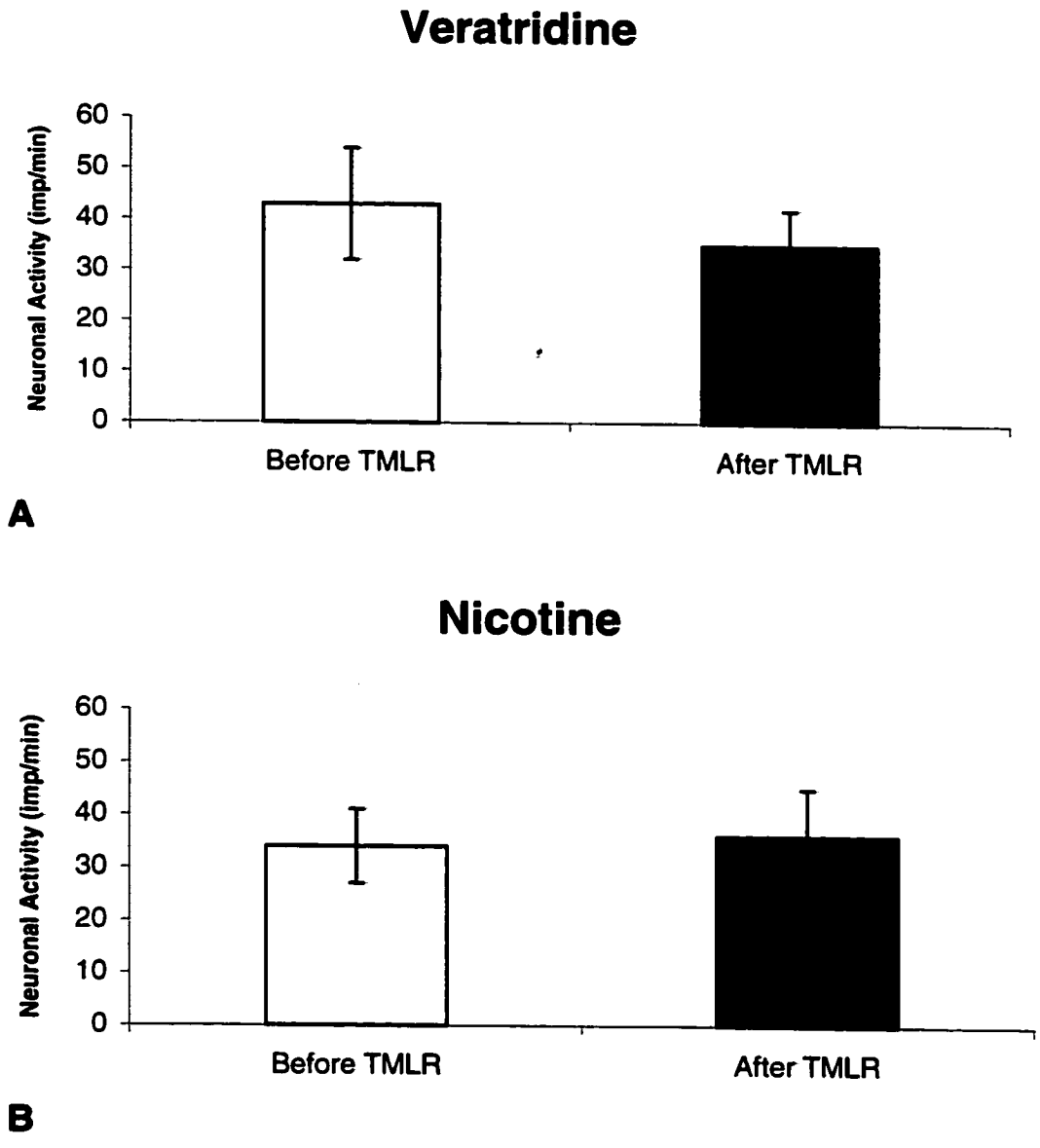


Figure 1.2 *Effect of veratridine and nicotine before and acutely after TMLR in the canine.*

Neuronal effects elicited above baseline levels: (A) by local application of veratridine to the epicardium overlying the region of the left ventricular wall that received TMLR treatment and (B) systemic administration of nicotine (5μg/kg). Note similar enhancement of intrinsic cardiac neuronal activity before and acute after TMLR in both cases (p = n.s.).

1.6 Purpose of Thesis

Ischemic heart disease accounts for the greatest single cause of morbidity and mortality in the North American populace. Current therapies that have been devised to treat ischemic heart disease have, thus far, focused on the cardiomyocyte. With increasing knowledge of the importance of the intrinsic cardiac nervous system in regulating cardiac function, targeting this neuronal system represents an important, as yet clinically unexplored therapeutic approach. In order for the intrinsic cardiac nervous system to be a clinically appropriate target it must have the ability to be manipulated in a wide variety of clinical scenarios. With regard to ischemic heart disease, to be effective, modification of the intrinsic cardiac nervous system must be possible in the immediate term (e.g., myocardial reperfusion), the short-term (e.g., acute anginal relief, such as SCS affords) or the long-term (e.g., persistent anginal relief that occurs after TMLR). Demonstration of the utility of the intrinsic cardiac nervous system must also been shown to be applicable in the human experience. The objectives of this thesis are to determine 1) the effects of ventricular ischemia and 2) therapeutic modalities to treat chronic ischemia on the intrinsic cardiac nervous system. Specifically, is the intrinsic cardiac nervous system capable of modification or alteration on a short and long-term basis?

Neurophysiological experimentation on cardiovascular regulation utilizing the porcine model is increasingly common[172-176]. There are several advantages to this model. Pigs have few native coronary artery collaterals[177-180] and do not

contain detectable levels of xanthine oxidase in their myocardium[109,181]. Both of these factors improve the analogy to the human experience[180,182]. The topographical organization of the porcine intrinsic cardiac nervous system, however, has not yet been elucidated in detail. In order to facilitate functional studies utilizing this animal model, the distribution of porcine intrinsic cardiac neurons to generate descriptive terminology of their major cardiac locations will be undertaken.

Once the model has been adequately characterized, examination of the effects acute focal ventricular ischemia on porcine ventricular neurons will be performed. As stated above, a consequence of myocardial reperfusion is the induction potentially lethal ventricular dysrhythmias. It has also been shown that excitation of specific populations of intrinsic cardiac neurons, as occurs during ventricular ischemia can also result in the induction of ventricular arrhythmias[41,183,184]. Currently, it is not known how reperfusion following bouts of focal ventricular ischemia affects porcine ventricular intrinsic cardiac neurons, or whether the resultant alterations in intrinsic cardiac neuronal activity can be modified pharmacologically.

Adenosine elicits several effects during reperfusion and has been implicated by some to possess an anti-arrhythmic effect[57,185]. To determine the ability of adenosine to modify the intrinsic cardiac nervous system during ischemia and reperfusion in an immediate or "real-time" fashion, a simple method of accessing the local coronary system of a ventricular ganglionated plexus was developed. Utilizing this technique, it will subsequently be determined if either

adenosine A_1 or A_2 receptors were involved in adenosine's actions on the intrinsic cardiac nervous system.

Patients with refractory angina represent a challenging group of patients to treat satisfactorily. These patients typically have anginal symptomology either at rest or with minimal exertion despite maximal medical therapy (Canadian Cardiovascular Society Class III or IV). In addition, these patients are typically not amenable to conventional revascularization procedures such as percutaneous transluminal coronary angioplasty (PTCA) and stenting or coronary artery bypass grafting (CABG) due to the extensiveness of their atherosclerotic disease. In response to this difficult management dilemma, novel therapies have been developed in order to reduce anginal symptoms and improve quality of life in these patients. Two of the more widely utilized newer modalities are neurostimulation (spinal cord stimulation or SCS) and transmyocardial laser revascularization (TMLR). To investigate the effects of these two treatment modalities, the canine model will be utilized for experimentation. While the porcine model has certain important advantages over the canine model (listed above), they are often less robust than dogs when examining longer ischemic episodes or chronic procedures[186]. The canine model will therefore be employed for the two studies examining therapeutic modality for refractory angina.

With respect to SCS in the treatment of ischemic heart disease, it is not known whether the effects that SCS imparts to the intrinsic cardiac nervous system can be maintained not only throughout its application, but also after it is terminated. It furthermore remains to be established whether SCS can overcome

the excitatory effects that focal ventricular ischemia exerts on the intrinsic cardiac nervous system thereby stabilizing neuronal control of regional cardiac function. The present experiments were devised to evaluate the effects of prolonged (17 minutes) SCS on the intrinsic cardiac nervous system in normally perfused and ischemic hearts. These experiments were also designed to evaluate whether the neurohumoral effects that SCS imparts on the intrinsic cardiac nervous system persist not only throughout its application but also for a prolonged period thereafter.

In order to determine whether TMLR has delayed effects on the function of the cardiac nervous system, the effects of TMLR were studied in a chronic, non-ischemic canine model. The non-ischemic model was investigated in order to avoid the confounding effects that ischemia has on the intrinsic cardiac nervous system[39,45,169]. Physiological analysis will be performed one month after the TMLR procedure or sham operation. In this manner, the chronic effects that TMLR exerts on not only local ventricular cardiomyocyte function, but also on the cardiac nervous system will be assessed.

Lastly, an intraoperative study of human intrinsic cardiac neurons *in situ* in patients with known ischemic cardiac disease will be undertaken. It is expected that this assessment will provide insight into the applicability of animal experimentation to the human experience and represents the first step of clinical implementation in exploiting the intrinsic cardiac nervous system for therapeutic benefit.

Chapter 2 Topography of intrinsic cardiac neurons in the porcine model

Data presented in this chapter has been, in part, submitted to the Anatomical Record.

2.1 Introduction

Intrinsic cardiac neurons, first identified on the human heart by Scarpa in 1794[187], have been described in a variety of mammalian species[25,169,170,188-197]. Intrinsic cardiac neurons are concentrated in collections of ganglia and interconnecting nerves that form identifiable ganglionated plexuses within epicardial fat[169,170,194-197]. Although they are typically found in epicardial fat on the base of the atria, in the interatrial septum and on the cranial aspects of the ventricles, there is considerable inter-species variation of the gross topographical (anatomical) distributions of the somata of intrinsic cardiac neurons on the heart. Intrinsic cardiac ganglia contain morphologically distinct neurons, including unipolar, bipolar and multipolar types[14,21,169,170,189,190,192,194,198]. Such anatomical complexity is consistent with physiological studies demonstrating that intrinsic cardiac neurons are functionally diverse and not simply cholinergic relay stations for preganglionic parasympathetic efferent neurons[12]. Functionally, intrinsic cardiac ganglia also contain postganglionic sympathetic efferent [22,25-27] and afferent[15,21,26,199] neurons. It has also been postulated that local circuit neurons in intrinsic cardiac ganglia interconnect afferent and efferent neurons, thereby forming functional regulatory networks on the heart[12,25-27,33,46,200].

Physiological studies increasingly utilize the porcine model to study the ontogeny of cardiovascular regulation[172], functional innervation of the neonatal heart [169,173,174,201]or the cardiodynamic effects of clinically relevant therapy[175,176]. However, the topographical organization of the porcine intrinsic cardiac nervous

system has yet to be elucidated in detail. The objectives of the present anatomical study were to determine the distribution, anatomic relationships and morphology of porcine intrinsic cardiac neurons and to propose a descriptive terminology of their major cardiac locations that will facilitate functional studies.

2.2 Methods and Materials

All experiments were performed in accordance with the guidelines for animal experimentation described in the "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-23, revised 1996) and the Canadian Council on Animal Care "Guide to the Care and Use of Experimental Animals" (Vol. 1, 2nd Ed., 1993).

2.2.1 Experimental model

Eight pigs (*Sus Scrofa*) of either gender, weighing 18-30kg, were sedated with a combination of ketamine (80mg/kg i.v.) and sodium pentothal (20 mg/kg i.v). After the endotracheal intubatation was performed, positive pressure ventilation was initiated with 0.95 FiO_2 and 0.05 $FiCO_2$ using a Bird Mark 7A ventilator (Palm Springs, CA). Anesthesia was maintained with sodium pentothal (10-15 mg/kg i.v. every 5 min) during surgical preparation. Thereafter, anesthesia was continued using α-chloralose (75 mg/kg, i.v. bolus, with repeat doses of 12.5 mg/kg i.v. as required). The adequacy of anesthesia was assessed at regular intervals by applying noxious stimuli to a limb to assess reflex withdrawal responses of a limb as well as monitoring jaw tone. Heart rate

was monitored throughout the physiological experiments with a lead II ECG. Systemic arterial pressure was monitored via a #6 Cordis catheter placed in the descending aorta and left ventricular pressure via a #7 Cordis catheter inserted through the femoral arteries. Core body temperature was maintained at 37-38°C through out the physiological experiments by means of a heating pad. At the completion of physiological experiments, hearts were collected for anatomical analysis.

2.2.2 Gross anatomy

Hearts were removed rapidly from 8 anesthetized pigs after completing functional studies. They were washed in normal saline to remove any remaining blood. The epicardial fat and underlying cardiac tissue were removed en bloc from the right and left atria, interatrial septum, superior and inferior vena cavae, left superior vena cava (which is present in the pig), the base of the ventricles and along major coronary arteries. In addition, the tissue adjacent to the right and left main coronary arteries, at the origins of the ventral interventricular coronary artery (VIV) in the ventral interventricular groove (analogous to the human left anterior descending artery in humans), the circumflex coronary artery (CCA), the right main coronary artery (RCA), the dorsal interventricular coronary artery (DIV) coronary artery (analogous to the human posterior descending artery) and the right marginal (RM) and left marginal (LM) coronary arteries were removed for analysis.

Tissues were washed in room-temperature physiological saline and stored in 1 mM phosphate buffered 4% paraformaldehyde at 4°C for later analysis. In order to identify nerves and ganglia among other tissues, a 1% solution of methylene blue in phosphate-buffered saline was dripped directly on the fixed tissue in a dissecting dish containing phosphate buffer. With the aid of a Zeiss dissecting microscope, tissues were gently teased apart to identify ganglionated plexuses. The number of ganglia identified in each atrial and ventricular ganglionated plexus was counted. Ganglia varied in size from those containing 2-3 neurons to ganglia with over 100 neurons. In smaller ganglia, visualization of all cells was possible so that total neuronal numbers could be determined with reasonable precision. In contrast, adequate visualization of all the neurons in larger ganglia was not possible. Therefore, larger ganglia were estimated to contain at least 100 neurons because that number represented the maximum number of somata that could be counted reliably in these ganglia. As a result, the number of neurons counted in the eight hearts studied, therefore represents an underestimation of the actual total. Drawings and photographs were made to demonstrate the anatomical arrangements of the ganglionated plexuses with respect to the heart and its major vessels.

2.3 Results

The gross topographical arrangement of the major clusters of atrial and ventricular ganglia that comprise the porcine intrinsic cardiac nervous system is shown in Figures 2.1 and 2.2. Interconnecting nerves, some of which had

relatively large diameters (up to ~0.5 mm; as measured with a camera lucida), were identified coursing between the various ganglia within each ganglionated plexus and between ganglionated plexuses.

Ganglia were concentrated in discrete areas of the epicardial fat associated with both atria as well as the cranial aspects of the ventricles. Thus, neuronal ganglia were not distributed ubiquitously throughout cardiac epicardial fat. Some ganglia lay adjacent to or among underlying cardiac muscle fascicles. In rare instances, somata of isolated neurons were found embedded in myocardial tissue. These were found at the base of the ventricles, in the cranial interventricular septum, as well as adjacent to the middle and distal portions of the ventral interventricular coronary artery. The number and relative size of identified ganglia are summarized in Table 2.1 according to their locations on the porcine heart. While the precise anatomical organization of identified ganglionated plexuses in a given cardiac region varied between animals, there was an overall consistency in the locations of the plexuses and the total numbers of ganglia identified in each plexus. The total number of neurons per heart was estimated to be over 23,000. The estimates of neuronal somata numbers via this methodology revealed that relatively equal numbers of neurons were present in atrial versus ventricular ganglionated plexuses (Table 2.1).

Neurons per Ganglia	1-10	10-50	50-100	>100	Number of ganglia	Number of neurons
Atrial ganglionated plexuses						
Ventral RA	9±3	22±8	3±1	2±1	218	5189
RVC-RA	6±1	1±1	9±0.3	1	112	2145
Dorsal A	6±3	11±4	2±1	2	95	2371
IAS	10±6	11±5	2±1	0	165	2863
LSVC-LA	4±2	4±1	1±0.3	0	50	756
				Subtotal:	**640**	**13324**
Ventricular ganglionated plexuses						
RCA	2±1	5±1	2±0.5	2±1	61	3345
RMA	2	1	1	0	4	129
DIV	10	6±4	3	1	25	593
VCM	5±3	11±5	2±0.5	0	102	1864
VIV	5±1	9±1	1±0.3	2±1	112	2698
CCA	3±1	6±2	1±0.3	2±1	62	1560
				Subtotal:	**366**	**10189**
TOTAL					**1 006**	**23 513**

Table 2.1 *Locations of intrinsic cardiac ganglia on the porcine heart.*
Number of ganglia identified, classified according to their estimated neuronal complement and locations (n = 8 pigs).

2.3.1 Atrial ganglionated plexuses

Over 600 ganglia of various sizes, containing an average of over 13,000 neurons, were identified in the five ganglionated plexuses located in the fatty tissue on the atria and vena cavae (Table 2.1). 1) The ventral right atrial ganglionated plexus (VRA GP) on the ventral aspect of the heart (Fig. 2.1) was located in fat cranial to the atrioventricular groove on the ventral and lateral surfaces of the right atrium. It extended in a craniomedial direction towards the junction of the right superior vena cava to the right atrium. This ganglionated plexus contained the largest number of neurons identified on porcine hearts examined (Table 2.1). 2) The right superior vena cava - right atrial ganglionated plexus (RVC-RA GP) was situated on the lateral border of the right atrium and caudal aspect of the right-sided superior vena cava (Fig. 2.1 - lateral view). 3) The dorsal atrial ganglionated plexus (Dorsal A GP) was located in fat lying on the dorsal surfaces of the two atria, medial to the roots of the pulmonary veins (Fig 2.2). This plexus extended ventrally in the fat lying between the two atria to form the 4) interatrial septal ganglionated plexus (IAS GP). 5) The left superior vena cava - left atrial ganglionated plexus (LSVC-LA GP) was a relatively long, narrow collection of ganglia and nerves originating on the ventrolateral aspect of the left atrium. This ganglionated plexus then extended to the dorsal aspect of the left atrium, medial to the origin of the left superior vena and subsequently projected cranially into the fat between the pulmonary artery and aorta (Fig. 2.2).

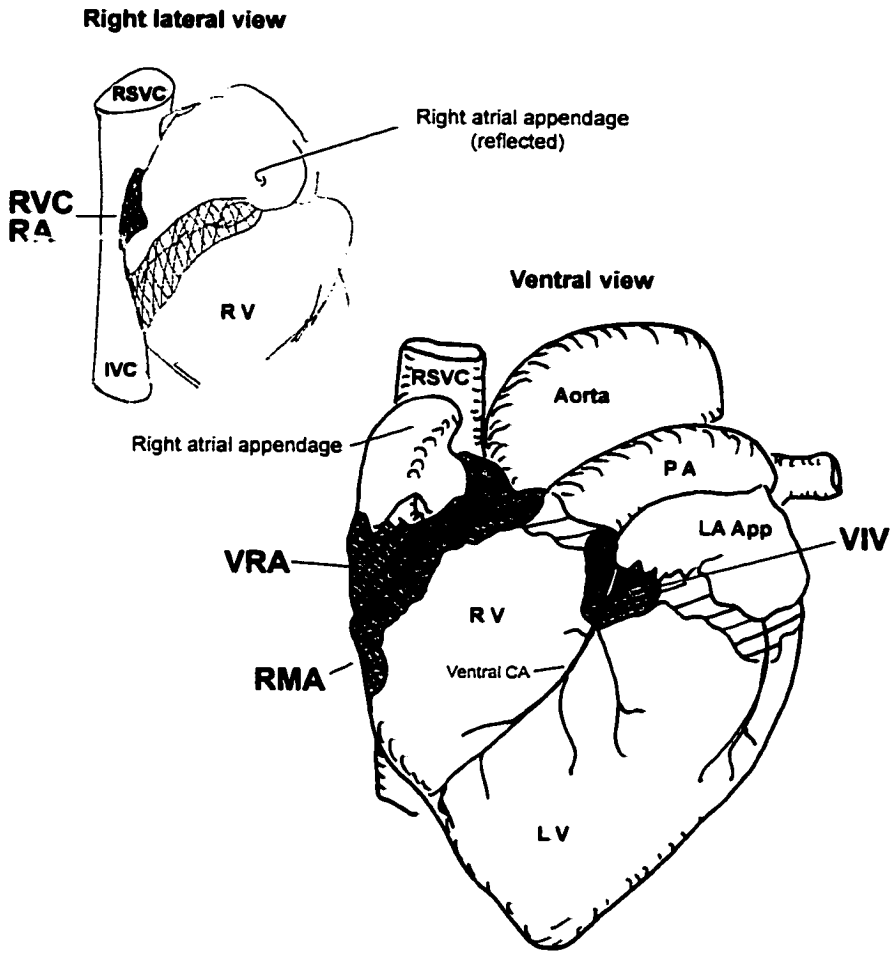


Figure 2.1 *Ventral and lateral view of the topographical arrangements of intrinsic ganglionated plexuses on the porcine heart.*

Cross-hatched areas indicate the epicardial fad pads. The cross-hatched areas with grey-shaded indicate locations of ganglionated plexuses within the epicardial fat (same for Fig. 2.2). The upper left diagram (with the right atrial appendage retracted) illustrates the location of the right vena cava - right atrial ganglionated plexus (RVC-RA GP) in the groove between the two right vena cavae and the ventral right atrial free wall. The right marginal artery ganglionated plexus (RMA GP) surrounds the first marginal artery. Abbreviations: Right superior vena cava (RSVC), inferior vena cava (IVC), right atrium (RA), left atrial appendage (LA app), right ventricle (RV), left ventricle (LV), pulmonary artery (PA).

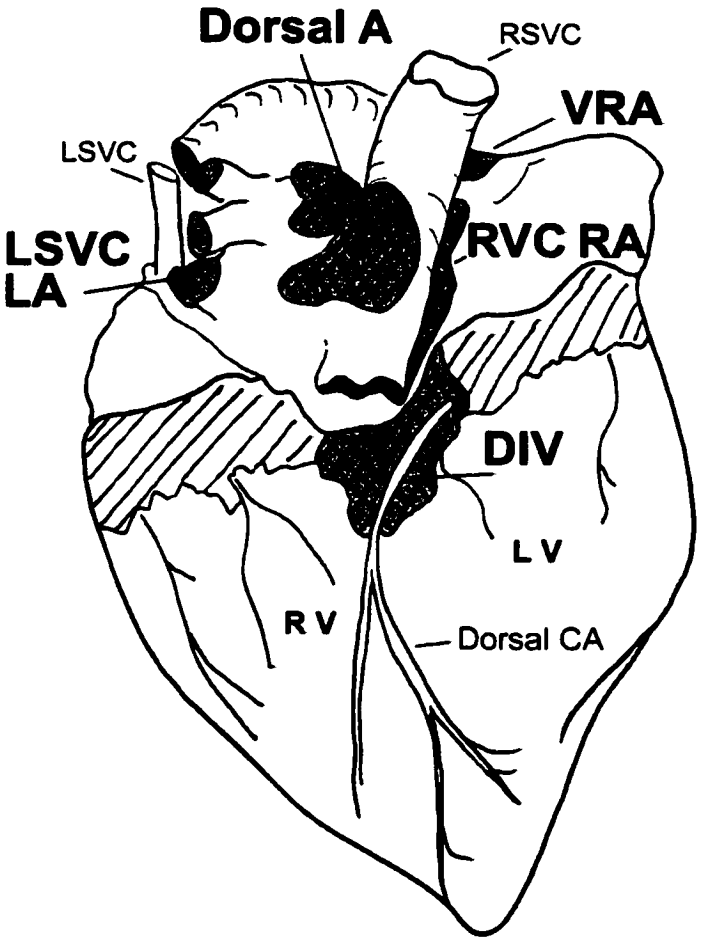


Figure 2.2 *Dorsal view of the topographical arrangements of intrinsic ganglionated plexuses on the porcine heart.*

The cranial extension of the ventral right atrial ganglionated plexus (VRA GP) is illustrated. In this view, the right vena cava – right atrial ganglionated plexus (RVC RA GP) can also be seen along the right-sided vena cavae. The dorsal atrial ganglionated plexus (Dorsal A GP) lies over the atrial septum. The left superior vena cava – left atrial ganglionated plexus (LSVC-LA GP) is located medial to the origin of the left superior vena cava; the dorsal interventricular ganglionated plexus (DIV GP) overlies the cranial aspect of the dorsal interventricular groove. Abbreviations: Dorsal interventricular coronary artery (Dorsal CA). Other abbreviations are the same as in figure 2.1.

2.3.2 Ventricular ganglionated plexuses

Over 350 ganglia of various sizes that contained over 10,000 neurons were identified in six ganglionated plexuses located in the fatty tissue on the cranial aspect of the ventricles (Table 2.1). With respect to the right ventricle, the 1) right coronary artery ganglionated plexus (RCA GP) extended around the first centimetre or more of the right main coronary artery; it was contiguous with the ventral right atrial ganglionated plexus. 2) The right marginal artery ganglionated plexus (RMA GP) was smaller and less consistent in location. It surrounded the origin of the first right marginal coronary artery. 3) The dorsal interventricular ganglionated plexus (DIV GP), which was located around the first centimetre of the dorsal interventricular coronary artery, contained fewer ganglia (Table 2.1).

At the origin of the left coronary artery and contained in fat surrounding the root of the aorta and pulmonary artery was the 4) ventral cranial medial ventricular ganglionated plexus (VCM GP). It extended along the left coronary artery. At the bifurcation of the left coronary artery, a relatively dense collection of neurons was identified that extended along the first two centimeters of the ventral interventricular and circumflex coronary arteries. These have been designated the 5) ventral interventricular artery ganglionated plexus (VIV GP) and the 6) circumflex coronary artery ganglionated plexus (CCA GP), respectively (Fig. 2.1). The latter included ganglia and nerves in the adjacent fat overlying the caudal and lateral borders of the left atrium. It was contiguous with the ganglia and nerves associated with the origin of the left superior vena cava that formed the LSVC-LA GP. A few scattered neurons were identified

throughout the interventricular septum, primarily in its cranial aspect; these did not form distinct ganglionated plexuses.

2.4 Discussion

The results of the present study demonstrate that porcine heart intrinsic cardiac neurons are localized primarily in discrete areas within atrial and ventricular epicardial fatty tissue as well as the interatrial septum. As demonstrated in other mammals[14,15,25,169,170,190,191,194,202], the porcine intrinsic cardiac nervous system contained a large number of neurons. These neurons, arranged ganglionated plexuses, were located in specific regions of the heart. In contrast to the canine model, the numbers of neurons identified were divided relatively evenly among atrial and ventricular ganglionated plexuses (Table 2.1)[194].

The largest collection of neurons identified was that found in the ventral right atrium (Table 2.1). As in the dog[194], fewer were found in the dorsal atrium and interatrial septum. In further distinction from the canine model, the pig has a relatively small diameter left superior vena cava entering into the coronary sinus in the dorsal aspect of the heart. Associated with the base of this vein was the left atrial-left superior vena cava ganglionated plexus (Fig. 2.2). Major ventricular ganglionated plexuses were located at the bifurcation of the left main, circumflex and ventral descending coronary arteries (Fig. 2.1; Table 2.1). In anesthetized animals, this area is readily accessible to functional studies such as have been conducted in the canine model[26,27].

Porcine intrinsic cardiac ganglia of sizes that ranged from a few neurons to more than 100 neurons. Many of the medium to large-sized ganglia that were identified had their neurons located primary in the periphery of the ganglia, with their dendritic processes being directed towards the interior of the ganglia where numerous axodendritic synapses were observed. In previous models employing this technique[194] revealed this methodology limited assessment of neuronal somata numbers to those situated primarily in the outer layers of a ganglion adjacent to the capsule. As such, estimation of the number of somata in larger ganglia based on their external appearance was inherently inaccurate. Consequently, the estimation of the total numbers of neurons listed in Table 2.1, was based upon more precise counts of neurons in smaller ganglia and neurons derived from the surface layers of larger ganglia (with a maximum of 100) likely represented an underestimation of the total numbers of neurons found within the porcine intrinsic cardiac nervous system.

2.4.1 Summary

In summary, this study has identified 11 major atrial and ventricular ganglionated plexuses associated with the porcine heart. The total number of neurons estimated in porcine intrinsic cardiac ganglia, although an underestimation of the whole population was roughly double the number of neurons identified in the canine model employing similar methodologies. In particular, porcine ventricular ganglionated plexuses possess more neurons than canine ventricular ganglionated plexuses. These data indicate that the porcine

model is suitable for the study of the functional interactions within the mammalian intrinsic cardiac nervous system, particularly with respect to its ventricular components.

Chapter 3 – Intrinsic cardiac neuronal adenosine A_1 receptors are involved in myocardial reperfusion

This work, in part, has been submitted to the journal Cardiovascular Research

3.1 Introduction

A consequence of myocardial reperfusion, following the re-establishment of coronary blood flow to ischemic myocardium, is the induction of dysrhythmias that range from ventricular premature beats to ventricular fibrillation[52,55,57,58,203]. These potentially lethal ventricular arrhythmias can occur within seconds of the onset of re-flow following coronary artery occlusion[59-61]. They have been observed in patients who undergo cardiac surgery[204-206], percutaneous transluminal angioplasty with stenting[207] or thrombolytic therapy[208-210]. Reperfusion ventricular dysrhythmias have also been implicated as a possible cause of sudden cardiac death following the spontaneous relief of coronary artery spasm[63-66,211,212]. Additionally, it has been reported that potentially malignant ventricular arrhythmias are more common after short, rather than long episodes of myocardial ischemia[213-215]. The precise mechanisms involved in the generation of reperfusion-induced alterations in cardiac function remain elusive.

Adenosine, a by-product of catabolism within the ischemic cell, is capable of modifying the cardiac nervous system. Adenosine has also been implicated in playing a role in improving electrical stability of the heart during reperfusion[216,217]. In animal studies, adenosine has been shown to exert neuromodulatory effects on cardiac sympathetic efferent neurons[108,110,218], as well as cardiac afferent neurons[40,67,219]. Adenosine also modifies the activity generated by populations of intrinsic cardiac neurons *in vitro*[220] or *in vivo*[43]. Chemical excitation of specific

populations of intrinsic cardiac neurons can result in the induction of ventricular arrhythmias[47,183], including ventricular fibrillation[51].

The neuromodulatory effects of adenosine on porcine ventricular intrinsic cardiac neurons, thus far, have not been determined. Additionally, it is not known how myocardial reperfusion following focal ventricular ischemia affects porcine ventricular neurons, nor it is known whether the alterations in intrinsic cardiac neuronal activity so induced are amenable to pharmacological therapy.

3.2 Methods and Materials

All experiments were performed in accordance with the guidelines for animal experimentation described in the "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-23, revised 1996) and the Canadian Council on Animal Care "Guide to the Care and Use of Experimental Animals" (Vol. 1, 2nd Ed., 1993).

3.2.1 Experimental model

Forty-four Hamshire-Duvoc pigs of either gender, weighing 20-30kg, were studied. The animals, placed in a supine position, underwent a bilateral thoracotomy through the fifth intercostal space. The animals were then gently rolled into a right lateral decubitus position and underwent a ventrolateral pericardotomy to expose the collection of neurons in the ventral cranial medial (VCM), ventral interventricular coronary artery (VIV) and circumflex coronary artery (CCA) ganglionated plexuses (GP). These ganglionated plexuses are located at the along the length of the left main coronary artery and along the first

2 centimetres of the ventral interventricular (analogous to the left anterior descending coronary artery) and circumflex coronary arteries. Neurons in these ganglionated plexuses are representative of those found throughout the intrinsic cardiac nervous system[12,169,194]. This area was chosen for study because of the relative density of its neurons and the fact that its arterial blood supply is spared when the left anterior descending coronary artery is occluded distal to first diagonal branch as the arteries supplying arterial blood to this collection of neurons originate cranial to that location[35].

A lead II ECG, left ventricular (LV) chamber pressure and aortic pressure were monitored continuously throughout the experiments. Left ventricular chamber pressure was monitored via a Cordis (Miami, FL) #6 French pigtail catheter that was inserted into the outflow tract of the left ventricular chamber via one femoral artery. Systemic arterial pressure was monitored via a Cordis #5 French catheter that was placed in the descending aorta via the other femoral artery. These catheters were attached to Bentley (Irvine, CA) Trantec model 800 transducers. Intrinsic neuronal activity, the ECG and LV pressure were recorded concomitantly on an Astromed MT9500 8 channel rectilinear chart recorder.

3.2.2 Neuronal activity recording

The activity generated by ventral ventricular neurons was recorded by means of a tungsten microelectrode (Frederick Haer 25-10-3, Brunswick, ME) that had a 250 μm shank diameter and an exposed tip of 5 μm (impedance of 9-11 MΩ at 1,000 Hz), as has been described elsewhere[25]. To minimize epicardial

motion during each cardiac cycle, a circular ring of heavy-gauge wire was gently placed around the epicardial fat on the cranioventral surface of the interventricular groove. Care was taken not to comprise the underlying coronary artery blood flow with this device. The tungsten microelectrode, mounted on a Marzhauser micromanipulator (model 25033-10, Fine Scientific Tools Inc., North Vancouver, BC), was used to explore the fat at varying depths ranging from the surface of the fat to regions adjacent to cardiac musculature. Proximity to cardiac musculature was indicated by increases in the amplitude of the ECG artifact. The reference electrode was attached to the adjacent pericardium. A grounding electrode was attached to the heavy-gauge wire-stabilizing ring. Signals generated by ventricular neurons were differentially amplified by a Princeton Applied Research (Princeton, NJ) model 113 amplifiers that had bandpass filters set at 300 Hz to 10 kHz and an amplification range of 100-500X. The output of this device, further amplified (50-200X) and filtered (bandwidth 100 Hz-2 kHz) by means of an optically isolated amplifier (Applied Microelectronics Institute, Halifax, N.S., Canada), was led to a Nicolet (Madison, WI) model 207 oscilloscope and to a Grass (Quincy, MA) AM8 Audio Monitor.

Loci in epicardial fat were identified in which action potentials with signal-to-noise ratios greater than 3:1 could be identified. Individual units were identified by the amplitude and configuration of their action potentials. Using these techniques and criteria, the microelectrode does not record action potentials generated by axons of passage, but rather records action potentials generated by somata and/or dendrites[26]. Periodic motion at the recording site

occurred due to cardiac and respiratory dynamics, thereby inducing minor fluctuations in the amplitude of individual action potentials generated by a given unit over time. Fluctuations in the amplitude of action potentials were found to vary by less than 10 μV over several minutes, retaining their same configurations over time. Thus, action potentials recorded in a given locus with the same configuration and amplitude (± 10 μV) were considered to be generated by a single unit.

3.2.3 Interventions

Ventricular mechanosensory inputs to identified neurons were tested by gentle manipulation of the epicardial surface of the right and left ventricle via a saline soaked cotton applicator or by a 5-second period of occlusion of the inferior vena cava. Chemical agents (~2.0 ml) were then applied for 60-120 seconds individually via 2 cm x 2 cm gauze squares placed on epicardial surface of the outflow tract of the right ventricle, as well as the ventral surface of the left ventricle. The chemical transduction properties of ventricular sensory neurites associated with identified neurons were tested via topical application of the Na^+-channel modifier veratridine (7.5 μM)[46] and adenosine (100 μM)[67] to identified sensory fields. Sensory fields were washed for 30 seconds with normal saline (~2 ml/sec) after each chemical was removed, at least 5-10 minutes being allowed to elapse before the next intervention. The application of chemicals to epicardial loci were repeated to be assured that spurious results due to tachyphylaxis did not occur. Gauze squares soaked with room-temperature normal saline were also applied to identified epicardial sensory fields to

determine whether neuronal responses elicited by epicardial chemical application were due to vehicle effects or the mechanical effects elicited by gauze squares.

3.2.4 Administration of chemicals into the local arterial blood supply of investigated neurons

In order to administer various chemicals to identified neurons, a 24-French catheter was inserted in the VIV coronary artery at the level of its first diagonal branch. The cannula was threaded proximally (retrograde to blood flow) so that its tip was positioned just cranial of the origin of the arteries that supplied blood to the VIV and CCA ganglionated plexuses. This was confirmed by gentle palpation of the cannula tip through the artery wall. The cannula was then fixed to the arterial wall with 2-3ml of adhesive. PE-15 tubing was inserted into this cannula, with a stopcock at its other end, to permit the administration of chemicals into the local arterial blood supply of the VCMGP. Monitored hemodynamic indices were unaffected by the placement of this cannula. Post-mortem examination of appropriate catheter placement was confirmed by injecting methylene blue dye through this catheter into the coronary artery.

The following pharmacological agents were infused at rates of approximately 0.05 cc/second into the local arterial blood supply of identified neurons via this catheter: adenosine (100 μM; 0.1cc), the selective adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1 mM, 1.0 cc) and the selective adenosine A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX; 1 mM, 1.0 cc). Administration of chemicals by this method affects tissues downstream, such as ventricular sensory neurites and

regional cardiomyocytes in addition to the somata of investigated neurons. The doses of pharmacological agents studied were chosen such that they did not alter monitored ventricular indices in preliminary test animals. Subsequently, the effectiveness of adenosine receptor blockade was confirmed by administering adenosine (100 μM; 0.5 cc) into their local arterial blood supply 5 and 60 minutes after local application of DPCPX and DMPX.

3.2.5 Myocardial ischemia

A 3-0 silk ligature passed around the VIV coronary artery just distal to its first diagonal branch was led through a snare in order to occlude this artery later in the experiments. Since investigated neurons derived their arterial blood supply from arteries proximal to this ligature[35], their arterial blood supply was not interrupted by during the transient coronary artery occlusions. A 30 second period of time was chosen for each coronary artery occlusion to create ischemic episodes. A minimum of 10 minutes was allowed to elapse between each coronary artery occlusion to allow neuronal and cardiovascular variables to return to pre-occlusion values. To ensure reproducibility of the ischemic/reperfusion responses, multiple occlusions were performed during the course of each experiment.

Short periods of occlusion (30 seconds) were chosen for two reasons. Firstly, multiple occlusions performed during the course of each experimental protocol could have elicited a "preconditioning" effect. The preconditioning effect refers to a form of myocardial adaptation in which the heart develops an increased tolerance to effects of prolonged periods of ischemia following

previous exposure to short periods of ischemic stress[203,221,222]. It has been demonstrated that myocardial preconditioning can be avoided if the periods of transient coronary artery occlusions are less than one to five minutes in duration[56,57,223]. Secondly, in preliminary experiments in which coronary artery occlusions of various durations (15 sec, 30 sec, 1 min and 2 min occlusion times) were tested, it was found that 30-second periods of occlusion elicited consistent neuronal responses. Occlusions longer than 30 seconds did not produce any further modification of neuronal activity, whereas occlusions shorter than 30-seconds produced inconsistent neuronal responses. Thus, 30-second occlusion times were employed for the remainder of the experiments.

Adenosine (100 μM, 0.1 cc) was administered into the arterial blood supply of investigated neurons immediately after the onset of myocardial ischemia or subsequent reperfusion. The administration of the chemical lasted was approximately 2-5 seconds in duration. In a preliminary set of experiments, adenosine administered locally to identified neurons during the ischemic periods, failed to exert a detectable effect on ischemic-induced neuronal responses (see below). Similarly, administration of either DPCPX or DMPX (1 mM; 1 cc) prior to the ischemic episodes did not alter the responsiveness of identified neurons to coronary artery occlusion. In light of these results, the effects of adenosine or the A_1 and A_2 adenosine receptor antagonists were not studied during the ischemic periods in subsequent experimentation.

To determine if adenosine A_1 or A_2 receptor antagonism modified the effects of adenosine on ventricular neurons during reperfusion, subsequent

experimentation in this study also focused on the effects of either DPCPX or DMPX (1 mM; 1 cc) pre-administration (in random order) on investigated neurons prior to infusion of adenosine.

3.2.6 Cardiac dysrhythmias

The number of dysrhythmic beats, as defined as QRS complex morphologically different from baseline, was determined during the five minutes of reperfusion following each transient VIV occlusion. The numbers of dysrhythmic beats induced were then compared to the numbers of abnormal beats that occurred during reperfusion after administration of adenosine, as well as following one or both adenosine receptor antagonists. These reperfusion arrhythmias were quantified during using a modification of the Lambeth Convention (scoring protocol B)[224,225], as reported by others[226].

3.2.7 Data analysis

Heart rate and LV chamber systolic pressure were measured for 1-minute periods of time before and during the peak response elicited by each intervention. Similarly, the activity generated by identified intrinsic cardiac neurons was analyzed for 1-minute periods before and during each intervention. Data obtained before and during each intervention are presented as means ± standard error of the mean (SEM). Repeated measures ANOVA or paired t-tests with Bonferroni correction were used for statistical analysis of the effects elicited by each intervention, where appropriate. Each chemical tested elicited neuronal responses in each animal. As intrinsic cardiac neuronal activity either increased

or decreased during each of the interventions, depending on the population of neurons investigated, change in neuronal activity from baseline was assessed during each intervention (absolute value of the delta change in neuronal activity)[42]. A significance value of $p < 0.05$ was ascribed for each determination.

3.3 Results

3.3.1 Ventricular neuronal mechanosensory and chemosensory inputs

Gentle touch applied to the epicardial surface of the right or left ventricle altered neuronal activity (Table 3.1). Epicardial application of veratridine also altered neuronal activity. Interestingly, epicardial application of adenosine, at the dose applied, did not alter activity of investigated neurons in the VCMGP. Transient occlusion of the superior vena cava decreased LV chamber systolic pressure and induced a concomitant change in intrinsic cardiac neuronal activity.

Intervention	*n*	HR (bpm)		LVP (mmHg)		Delta Neurons (imp/min)
		Pre	Post	Pre	Post	
LV Touch	9	135.1±10.7	131.3±10.6	113.1±4.1	110.9±3.6	24±6*
RV Conus Touch	9	133.9±11.8	133.3±11.6	112.8±4.3	109.8±4.4	14±6*
RV Sinus Touch	9	133.9±10.4	136.3±11.2	112.9±4.5	110.2±4.8	17±5*
Veratridine (topical)	11	131.3±9.6	140.0±12.6	109.5±2.1	105.4±3.9	38±13*
Adenosine (topical)	5	141.0±10.5	145.0±10.2	104.4±3.4	103.4±3.2	8±4
IVC X	6	122.5±12.7	127.5±14.5	114.7±4.9	99.7±4.1*	45±15*

Table 3.1 *Response of ventral neurons to ventricular to mechanical and chemical stimuli.*

n = number of animals studied. HR = heart rate (bpm = beats per minute); LVP = left ventricular chamber pressure; LV and RV = left ventricle and right ventricle respectively; IVC X = inferior vena cave occlusion. Neuronal activity presented as absolute delta change from baseline (control values) (imps/min = impulses per minute). * = $p<0.05$.

3.3.2 Effects of ischemic on intrinsic cardiac neuronal activity

Thirty-second periods of coronary artery occlusion and the subsequent reperfusion periods altered intrinsic cardiac neuronal activity by 112±14 impulses per minute (ipm) and 168±34 ipm, respectively ($p<0.01$ compared to control values). This intervention increased neuronal activity in 10 animals (Fig. 3.1) and decreased neuronal activity in 5 animals. Therefore, the absolute change of neuronal activity from baseline values ($|\Delta|$ change) was analyzed. HR was unchanged throughout the periods of ischemia and reperfusion (control, 154±11 bpm; ischemia, 156±12 bpm; reperfusion, 155±11bpm). During ischemia, left ventricular systolic pressure fell from 113±4 to 101±4 mmHg ($p<0.01$). This index returned to baseline values immediately upon reperfusion (114±4 mmHg).

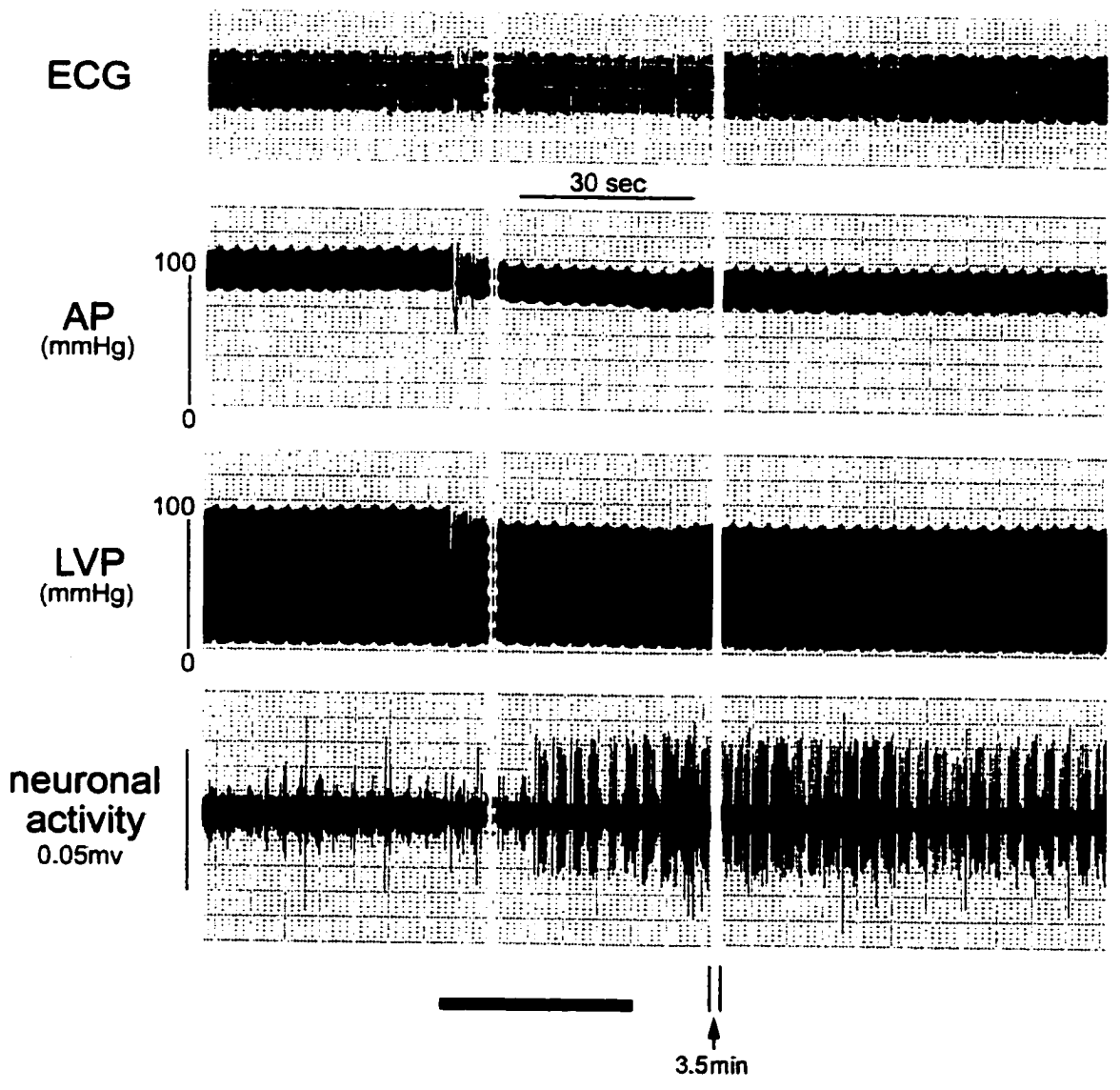


Figure 3.1 *The effect of ischemia on intrinsic cardiac neuronal activity.*
The black bar indicates the 30-second period of coronary occlusion with the ensuing reperfusion response of the intrinsic cardiac neurons afterwards. A break in the diagram signifies a 3.5-minute time elapse. Note that the reperfusion response of identified neurons firing persisted for a number of minutes in this example. ECG = lead II electrocardiogram; AP = aortic pressure; LVP = left ventricular pressure. These labels are common throughout the remaining figures.

3.3.3 Effect of multiple coronary artery occlusions

The effects of repeated coronary artery occlusions (30-seconds in duration) were studied in 4 animals in order to determine whether "preconditioning" effects were induced by multiple occlusions. Each occlusion produced similar hemodynamic and associated physical changes (i.e., myocardial cyanosis and dyskinesia) that resolved upon reperfusion. The absolute change in neuronal activity from baseline values that occurred during the 6 episodes of regional ventricular ischemia were similar and ranged from 71±8 to 105±25 ipm, with no significant differences found between 1st and 6th occlusion.

3.3.4 Adenosine and adenosine receptor antagonists

In normally perfused hearts of 15 pigs, administration of adenosine into the local coronary blood supply of identified neurons altered their activity by 37±11 impulses per minute (|Δ| ipm) ($p<0.01$). No change in cardiovascular variables was identified (HR: 138±14 to 141±16 bpm; LVP systolic: 116.9±4.3 to 116.7±3.7 mmHg). Local administration of DPCPX and DMPX (n=15) exerted minor effect on neuronal activity (|Δ| 12±2 ipm, $p<0.01$); no hemodynamic alterations (LVP: 113.5±3.1 to 113.9±2.6; HR 135±11 to 136±11, p = n.s.) were induced by these interventions. The dose of DPCPX and DMPX employed produced effective adenosine receptor blockade throughout the rest of

experiments since repeat administration of adenosine failed to elicit neuronal responses ($|\Delta|$ 8±3 ipm compared to baseline values; p = n.s.) in their presence.

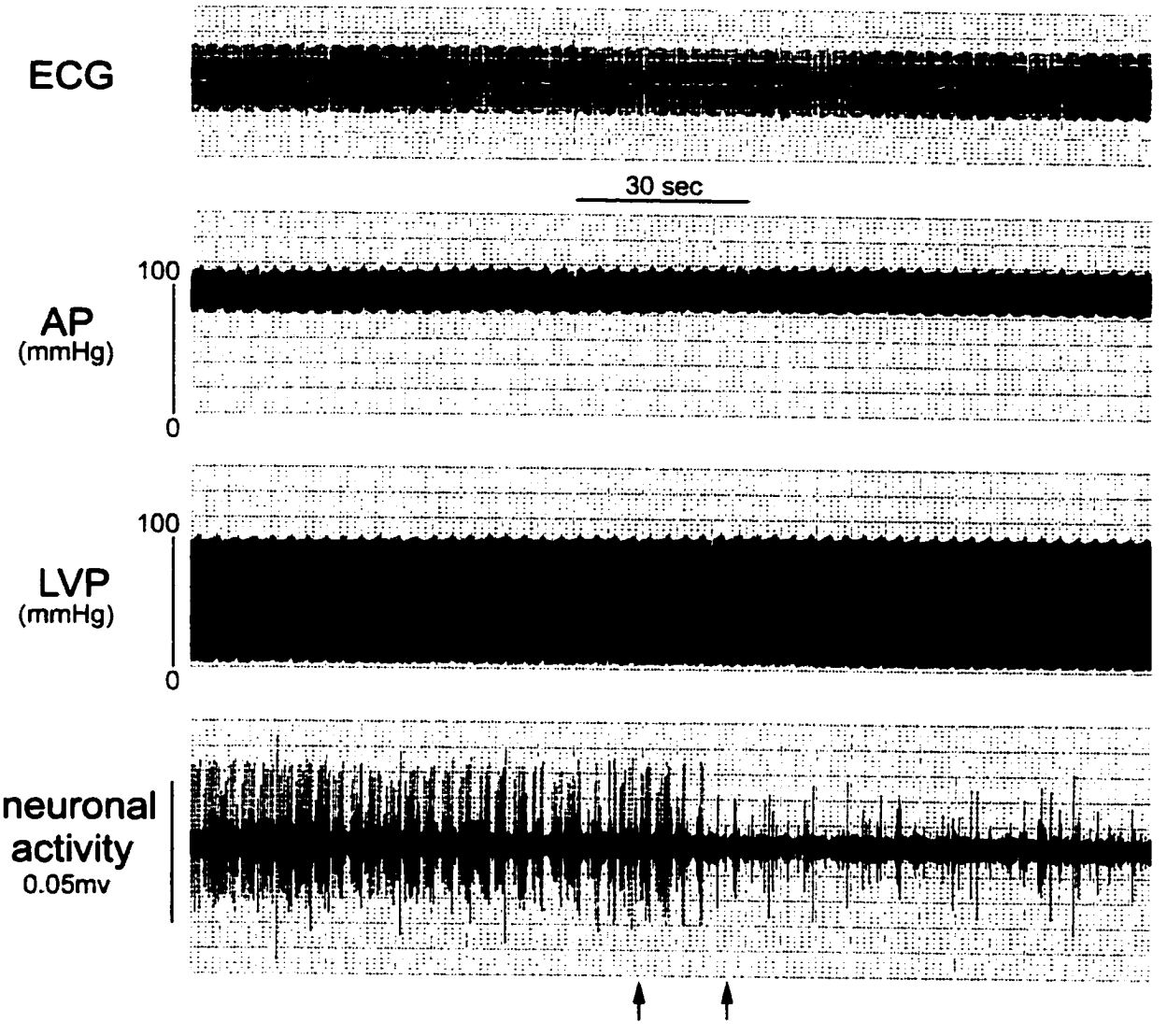


Figure 3.2 *The effect of adenosine administered during reperfusion*
Neuronal activity was altered by local administration of adenosine during reperfusion (between the two black arrows below). Note the reduction in the neuronal activity towards baseline level (compare baseline (pre-ischemic) neuronal activity in Fig. 3.1).

3.3.5 Adenosine and ischemia/reperfusion

In a preliminary group of animals, adenosine (n=5) or DPCPX plus DMPX (n=6) were administered locally during coronary artery occlusion. Neuronal responses elicited during ischemia were not affected by local infusion of either adenosine (92±52 vs. 127±41 |Δ| ipm; p = n.s.) or by DPCPX and DMPX (51±18 vs. 63±24 |Δ| ipm; p =n.s.). Therefore, for the rest of the experimental protocol we focused upon neuronal responses elicited during the reperfusion phase.

Infusion of adenosine into the local arterial blood supply of investigated neurons during reperfusion (n=15) modified their activity from baseline values by 75% (167.7±34.2 to 41.9±10.7 |Δ| ipm; $p<0.01$ compared to reperfusion in the presence of adenosine) (Figs. 3.2 & 3.3). Overall, adenosine returned intrinsic cardiac neuronal activity to baseline (pre-ischemic) values. That is, in the 13 animals in which an increase in neuronal activity was identified during reperfusion, during reperfusion adenosine suppressed neuronal activity (Figs. 3.2 & 3.3). Conversely, in two animals in which neuronal activity was diminished during reperfusion, adenosine increased neuronal activity during reperfusion.

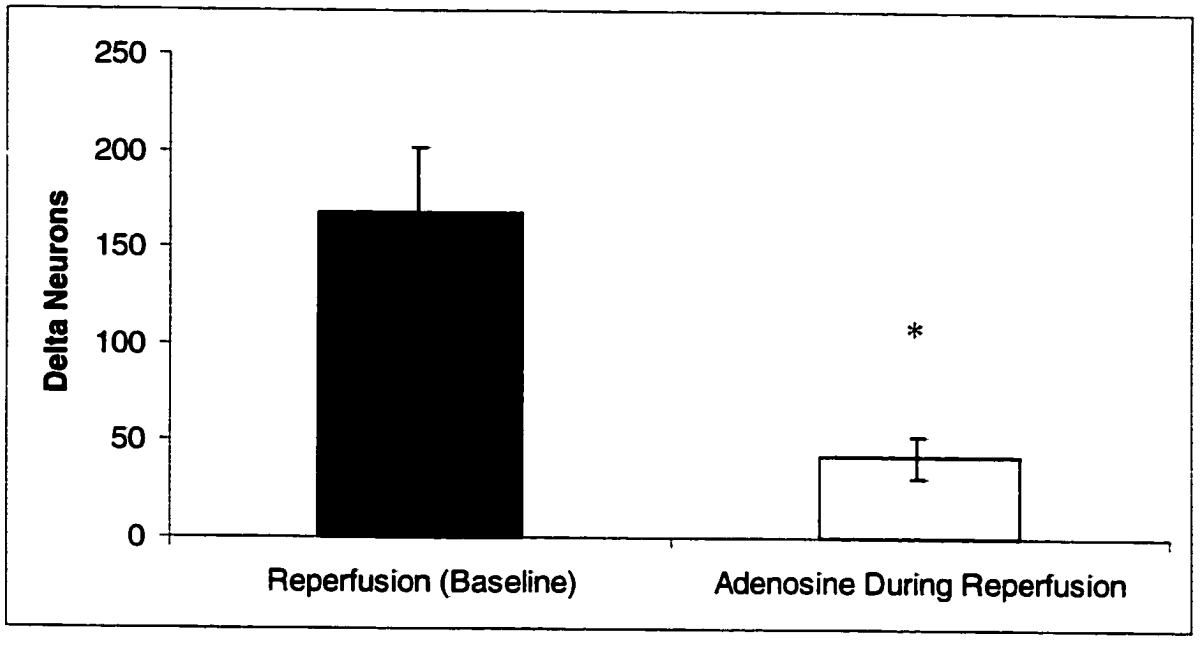


Figure 3.3 *Effect of adenosine on the reperfusion response.*
Reperfusion enhanced neuronal activity (measured as absolute delta change from control values) (imps/min = impulses per minute). When adenosine was administered during reperfusion following a 30-second coronary occlusion, neuronal activity was significantly reduced, approaching control (pre-ischemic level). * = $p < 0.01$ from control reperfusion response; p = ns of adenosine on reperfusion and control levels. (n = 15).

3.3.6 Adenosine antagonism and reperfusion

Adenosine was tested during reperfusion in the presence of the A_1 receptor antagonist DPCPX in 7 other animals. With the pre-administration of DPCPX, exogenous adenosine failed to exert any effects during reperfusion (230.3±63.8 vs. 216.6±94.7 |Δ| ipm; p = ns, comparing control reperfusion to reperfusion with DPCPX and adenosine) (Figs. 3.4 & 3.5). Conversely, pre-administration of the A_2 receptor antagonist DMPX in 7 different animals did not diminish the capacity of adenosine to modify neuronal activity during reperfusion (230.9±63.6 ipm before and 72.3±41.3 ipm; $p<0.02$ comparing control reperfusion to reperfusion with DMPX + adenosine) (Fig. 3.4). The presence of either adenosine receptor antagonist did not affect HR or LVP.

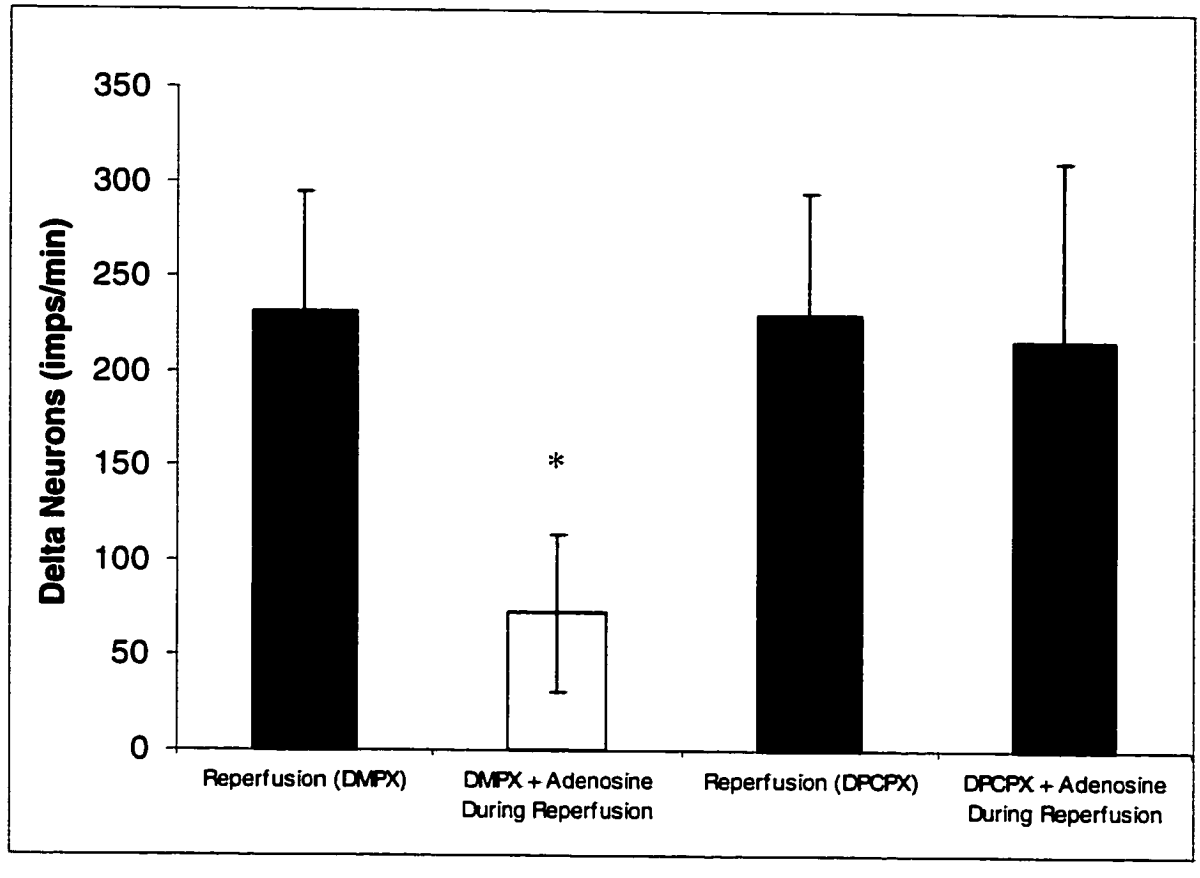


Figure 3.4 *The effect of exogenously administered adenosine during reperfusion on intrinsic cardiac neuronal activity in the presence or absence or A_1 and A_2 adenosine receptor antagonism.*

Neuronal activity measured as absolute delta change from baseline values (imps/min = impulses per minute). Reperfusion (DMPX) and Reperfusion (DPCPX) refers to reperfusion response of identified neurons with pre-administration of DMPX or DCPCX, respectively (1mM). Pre-administration of an A_2 antagonist, DMPX (bar labelled "DMPX + Adenosine During Reperfusion") did not alter adenosine suppression effect on the modified neuronal activity during reperfusion. Conversely, pre-administration of DPCPX, an A_1 receptor antagonist (bar label "DPCPX + Adenosine During Reperfusion"), blocked adenosine's suppressing effects. * = $p < 0.02$.

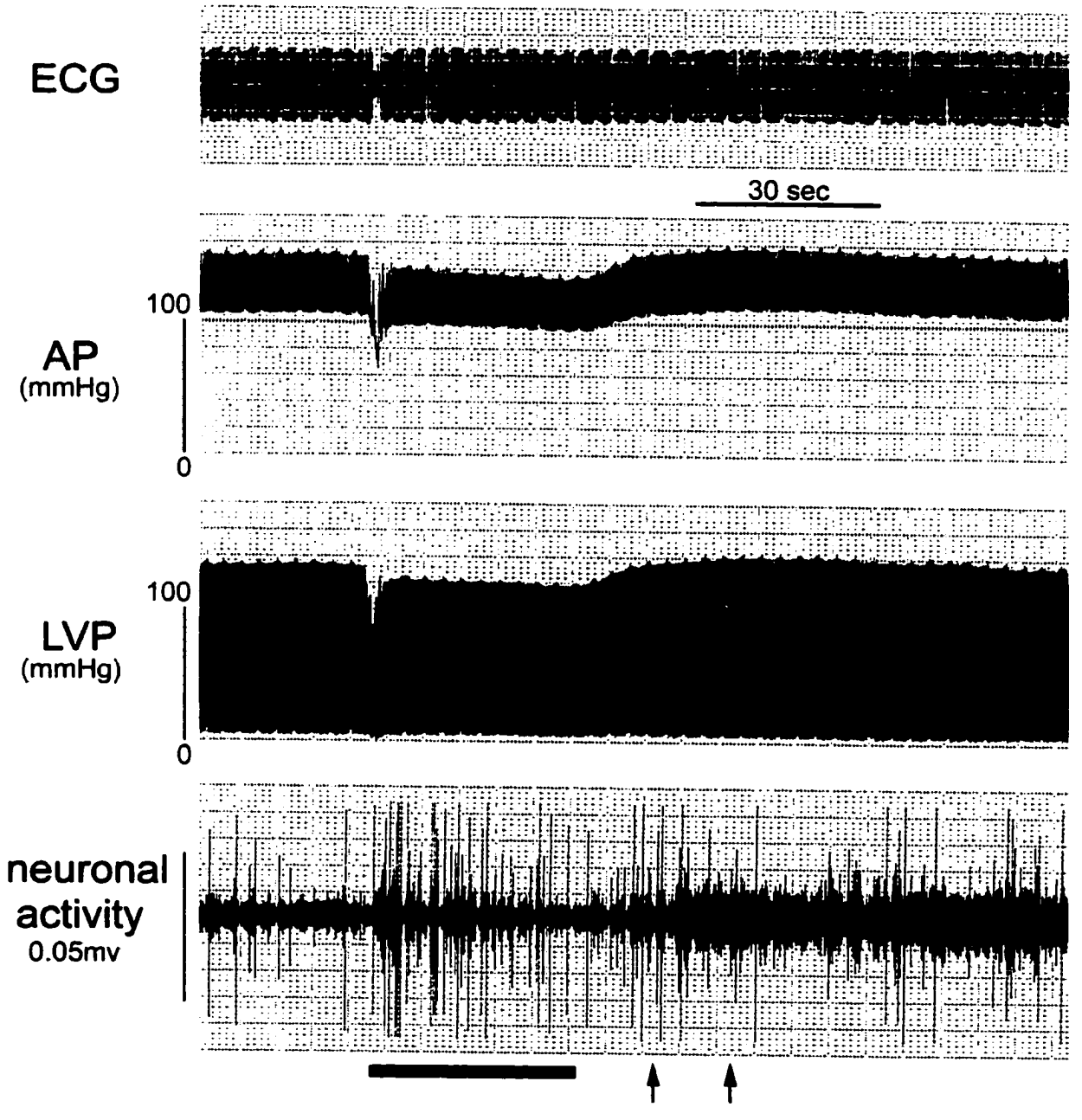


Figure 3.5 *Adenosine administration during reperfusion following pre-administration of adenosine A_1 receptor blockade.*

Neuronal activity and monitored hemodynamics are displayed during a 30-second coronary occlusion after the pre-administration of DPCPX (black bar). Arrows indicate the administration of adenosine into the local blood supply of identified neurons during reperfusion. Note that the neuronal activity remained enhanced during exogenous adenosine administration in the presence of A_1 receptor blockade.

3.3.7 Cardiac dysrhythmias

In most instances, cardiac dysrhythmias were not elicited during the 30-second periods of coronary artery occlusion. However, in 13 of the 44 animals tested, cardiac dysrhythmias did occur during reperfusion at a rate of 8±2 disturbances/minute. In 4 of these animals, administration of adenosine into the local arterial blood supply of investigated neurons reduced the number of abnormal beats elicited during reperfusion by 80% (10±3 to 2±0.6 ectopic beats/minute). This resulted in conversion to sinus rhythm in three of the four animals tested (Fig. 3.6A). Conversely, in 6 animals in which electrical disturbances were generated during the reperfusion phase, the number of dysrhythmic beats increased by 266% (9±3 to 24±9 disturbances/min) when tested in the presence of DPCPX. This included the induction of runs of ventricular tachycardia and one episode of ventricular fibrillation that required electrical defibrillation (Fig. 3.6B). The anti-arrhythmic effects of adenosine were no longer evident when adenosine was re-administered in the presence of the adenosine A_1 receptor antagonist DPCPX (Fig. 3.6C). DMPX, administered alone, did not affect the number of dysrhythmic beats elicited during reperfusion; nor did it affect the anti-arrhythmic effects of exogenously administered adenosine. Quantification of arrhythmias, using a modification of the Lambeth convention [226], revealed that arrhythmias elicited during reperfusion before therapy received a score 0.3±0.1. Conversely, application of adenosine during reperfusion reduced the arrhythmia score to 0. On the other hand, the increase

in ventricular arrhythmia formation in the presence of DPCPX reached a score of 1.6±0.5.

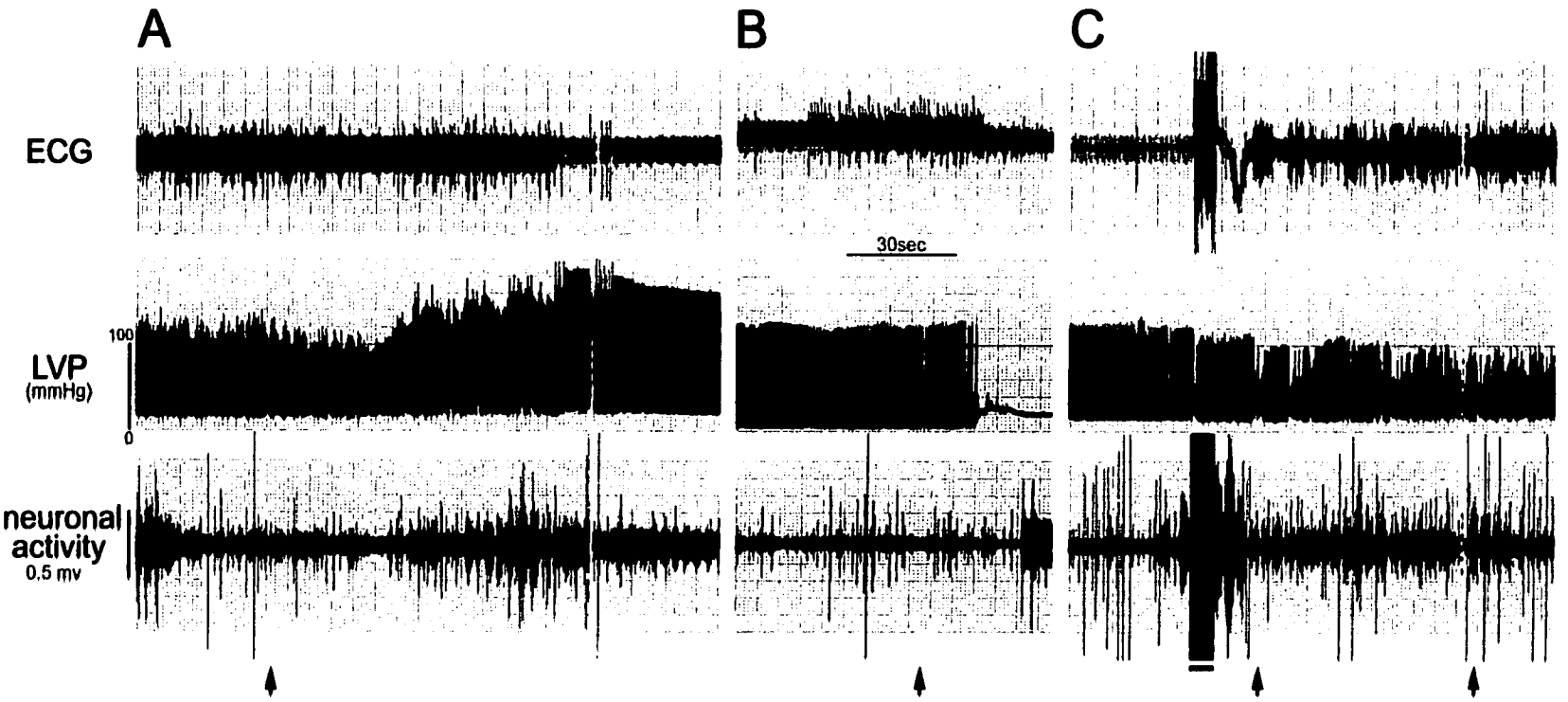


Figure 3.6 *The anti-arrhythmic effect of locally applied adenosine.*

A: Administration of adenosine (0.2cc of 100μM solution, black arrow) into the local blood supply of investigated neurons during an episode of atrial arrhythmia. Note the increase in LVP and eventual return to a sinus rhythm. B: Local administration of DPCPX (0.5cc of 1mM solution, black arrow) during an episode ventricular arrhythmia induced ventricular fibrillation rhythm requiring electric defibrillation. C: Electrically induced atrial arrhythmias (black bar below) following pre-administration of DPCPX. Despite the local administration of two doses of adenosine (0.5cc of 100μM solution, black arrows below), the animal did not revert to a sinus rhythm.

3.4 Discussion

Data derived from this study demonstrate that porcine ventricular neurons are affected by distal myocardial ischemic that does not directly involve their arterial blood supply. While intrinsic cardiac neuronal activity was not affected by exogenously administered adenosine during regional ventricular ischemia, adenosine did modify neuronal activity during the subsequent reperfusion phase. It did so by returning neuronal activity towards baseline (pre-ischemic) values. This effect was accompanied by a reduction in the propensity to ventricular arrhythmia formation during reperfusion. The stabilizing effects that adenosine imparted to the intrinsic cardiac nervous system were blocked by pre-existing A_1 receptor, but not by A_2 adenosine receptor anatagonism.

Adenosine has been shown to exert neuromodulatory effects on neurons in the central nervous system [103-106] and peripheral autonomic ganglia. The latter include those associated with the gastrointestinal system [107] or heart [43,108,220,227]. Adenosine [228,229] is liberated by the ischemic myocardium along with peptides such as substance P [230] and bradykinin [231,232]. These chemicals arise from many cell types, including cardiomyocytes [89,233] and possibly intrinsic cardiac neurons [220].

Adenosine is known to affect cardiac sensory neurites associated with afferent neuronal somata in dorsal root [40,43] and nodose [42,67] ganglia. In this series of experiments, topical application of adenosine to ventricular sensory neurites did not affect the activity generated by identified ventricular neurons (Table 3.1). Such data imply that ventricular afferent neuronal inputs to

investigated neurons were not responsive to adenosine. However, identified neurons were affected by ventricular chemosensory inputs sensitive to topically applied veratridine (Table 3.1). These data indicate that the ventricular sensory neurites associated with the afferent neuronal inputs to identified ventricular neurons studied did not transduce adenosine stimulatory effects. In light of the fact that the doses of adenosine administered into the local arterial blood supply of investigated neurons did not affect monitored cardiovascular indices, it appears that the neuronal effects induced by adenosine were primarily due to modification of ventricular somata rather than being secondary to altered cardiodynamics. Thus, the fact that no neuronal responses were elicited following epicardial application of adenosine combined with an absence of hemodynamic perturbations following locally administered adenosine indicates that the effects that adenosine exerted on the intrinsic cardiac nervous system were primarily due to alterations in local circuit (interneurons) rather than afferent or efferent neurons.

As is found in the canine model[35,45,234], regional myocardial ischemia and the subsequent reperfusion phase affected porcine intrinsic cardiac neuronal activity (Fig. 3.1). Presumably, the type of neuronal response (excitatory or inhibitory) so induced was due to the summation of inputs from multiple ventricular sensory neurites associated with intrinsic cardiac afferent neuronal somata, as well as indirect effects on local circuit neurons[35,42].

Myocardial release of adenosine, a by-product of catabolism within the ischemic cell, increases several-fold during myocardial ischemia [74,89-91].

Exogenously administered adenosine or, for that matter, A_1 or A_2 antagonists, did not alter ventricular neuronal activity during transient ventricular ischemia. Presumably that was due, in part, to the fact that mechanosensory inputs to the intrinsic cardiac nervous system affected by ischemia-induced local dyskinesia were not affected by adenosine [39]. In addition, chemicals other than adenosine are known to be liberated by the ischemic myocardium[42,67] such that they may also affect sensory inputs to the intrinsic cardiac nervous system. Thus, an enhancement of multiple sensory inputs to the intrinsic cardiac nervous system in such a state presumably overwhelmed any demonstrable effect secondary to neuronal adenosine receptor modification.

Conversely, demonstrable changes in neuronal activity were elicited when adenosine was administered locally during the reperfusion phase (Figs. 3.3 & 3.4). During reperfusion, adenosine acted to return neuronal activity to baseline (pre-ischemic) values. Administration of DPCPX, but not DMPX, eliminated the neuronal modulatory effect of adenosine (Figs. 3.5 & 3.6). These data indicate that intrinsic cardiac neuronal adenosine A_1 receptors are involved in the reperfusion response. As mentioned above, the modulator role of adenosine on the intrinsic cardiac nervous system in the presence of reperfusion post-ischemia involves, in part, alterations in local circuit neuronal responses to myocardial ischemia induced sensory inputs.

The genesis of reperfusion arrhythmias remains enigmatic. Ventricular dysrhythmias are more common after short periods of coronary artery occlusion rather than prolonged episodes [213-215]. Additionally, the generation of ventricular

dysrhythmias upon reperfusion is not inconsequential, having the potential to exert negative metabolic effects on the myocardium [235]. Therapeutic interventions such as thrombolysis and percutaneous coronary angioplasty employed to treat acute coronary artery syndromes or the increasing utilization of "off-pump" techniques for coronary artery bypass grafting surgery are clinically relevant scenarios that involve transient myocardial ischemic episodes in which arrhythmia may occur during reperfusion.

Adenosine has received considerable attention in this regard, given the evidence demonstrating its ability to exert modulator effects on a number of cellular mechanisms associated with reperfusion injury, including arrhythmia formation. These actions include interaction with α-adrenergic receptors [69,92], alterations in intracellular cyclic AMP [71,82], diminishment of free radical generation [61,81,94,96] and alteration of K_{ATP} channel activity [80,97,236]. Arrhythmias were induced during reperfusion in 13 of 44 animals studied, being limited to ventricular premature beats (Lambeth score of 0.3±0.1). The attenuation of neuronal responses elicited by exogenously administered adenosine during the reperfusion phase was associated in a number of instances with an improvement in electrical stability (Fig. 3.6A). In contrast, adenosine A_1 receptor antagonism accentuated arrhythmia formation, even resulting in the induction of ventricular fibrillation in one animal (Fig. 3.6B).

3.4.1 Summary

The data derived from this study indicate that adenosine obtunds the effects of reperfusion injury on the intrinsic cardiac nervous system, but not that induced by regional myocardial ischemia. These effects appear to involve intrinsic cardiac neuronal interactions that utilize adenosine A_1 receptors. It is concluded that therapy that modulates adenosine A_1 receptors may act to stabilize the processing of cardiac afferent information via the intrinsic cardiac nervous system during reperfusion injury. Further studies are required to determine the exact role of the various neuronal components of the intrinsic cardiac nervous system that are involved in reperfusion injury in order to establish the possible clinical utility of adenosine receptor modification in such circumstances.

Chapter 4 Long-term modulation of the intrinsic cardiac nervous system by spinal cord neurons in normal and ischemic hearts

This work has been published, in part, in: Autonomic Neuroscience: Basic and Clinical 2001; 95:71-79.

4.1 Introduction

High frequency, low intensity electrical stimulation of the dorsal aspect of the T1-T2 spinal cord alleviates angina pectoris in patients suffering from ischemic heart disease[129-131,133]. The therapeutic effects of spinal cord stimulation (SCS) can persist for hours after its termination[135]. Accumulating evidence demonstrates that SCS is a safe anti-anginal treatment modality that does not result in increased frequency of arrhythmia formation[132-134,237]. However, the mechanisms whereby SCS produces its long-term effects remain unknown.

Clinical studies have led to the hypothesis that SCS exerts its anti-anginal effects principally by altering ventricular oxygen supply: demand ratio[129,130]. Mannheimer suggested that SCS reduces cardiac metabolism, thereby reducing oxygen demand and, as a consequence, myocardial lactate production within the ischemic myocardium[129]. In this regard, Hautvast proposed that SCS redistributes myocardial blood flow from normal to ischemic regions of the heart[134]. However, in the canine model SCS does not alter cardiac chronotropism or inotropism suggesting that oxygen demand is minimally affected by such an intervention[45]. Furthermore, SCS does not alter blood flow distribution within either the normal or ischemic canine myocardium[142].

The therapeutic effects of SCS may also reflect changes within the CNS and/or changes in neurohumoral control of the heart. SCS modulates impulse transmission within the spinothalamic tracts of the spinal cord without blocking

afferent neuronal signals arising from the ischemic myocardium[119]. It also alters intrinsic cardiac neuronal function[45]. The intrinsic cardiac nervous system represents the final common regulator of regional cardiac function [8,12]. Its neurons are under the constant influence of central neurons, including those in the spinal cord[25]. Regional myocardial ischemia results in the heterogeneous activation of the intrinsic cardiac nervous system[35,46]. When sub-populations of intrinsic cardiac neurons become excessively activated, the cardiac electrophysiological consequences, such as the occurrence of ventricular tachycardia or ventricular fibrillation, may be devastating[12,13,20,23,40,51,238]. Stabilization of the intrathoracic intrinsic cardiac nervous system, especially in the presence of myocardial ischemia, would therefore be expected to ameliorate the potential for cardiac electrical instability.

Short duration SCS (4 min) transiently suppresses the activity generated by intrinsic cardiac neurons[45]. In a clinical setting, the anti-anginal effects of SCS persist long after its termination[135]. It is not known whether the effects that SCS imparts to the intrinsic cardiac nervous system can be maintained not only throughout its application, but also after it is terminated. Furthermore, it remains to be established whether SCS can overcome the excitatory effects that focal ventricular ischemia exerts on the intrinsic cardiac nervous system, thereby stabilizing neuronal control of regional cardiac function.

The present experiments were devised to evaluate the effects of prolonged (17 minutes) SCS on the intrinsic cardiac nervous system in normally perfused and ischemic hearts. These experiments were also designed to evaluate

whether the neurohumoral effects that SCS imparts on the intrinsic cardiac nervous system persist not only throughout its application, but also for a time thereafter.

4.1 Methods and Materials

All experiments were performed in accordance with the guidelines for animal experimentation described in the "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-23, revised 1996) and the Canadian Council on Animal Care "Guide to the Care and Use of Experimental Animals" (Vol. 1, 2nd Ed., 1993).

4.2.1 Animal preparation

Ten adult mongrel dogs weighing between 12.5 - 26 kg (mean: 19.6 kg), were used for this study. The animals were anesthetized in a standard manner by first administering a bolus dose of sodium thiopental (20 mg.kg^{-1}, iv). Anesthesia was maintained throughout the surgery period by means of bolus doses of thiopental (5 mg.kg^{-1}, iv) administered to effect every 5-10 minutes. Animals were intubated and then artificially ventilated using a Bird Mark VII respirator with 100% O_2. After completing the surgery, anesthesia was changed to α-chloralose by first administering a dose of alpha chloralose (75 mg.kg^{-1}, iv). Thereafter, repeat doses of α-chloralose (20 mg.kg^{-1}, iv) were administered, as required, during the remainder of the experiments. The level of anesthesia was checked throughout each experiment by observing pupil reaction as well as monitoring jaw tension, heart rate and blood pressure and by periodically

checking for the withdrawal reflex by squeezing a paw. Since each bolus of α-chloralose suppressed neuronal activity for a few minutes after its administration, these doses were administered between the interventions performed in each protocol. This anesthetic regimen produces adequate anesthesia without inordinately suppressing peripheral autonomic neural activity. Electrodes were inserted in the forelimbs and the left hind limb and connected to an Astro-Med, Inc. (West Warwick, R.I.) model MT 9500 eight channel rectilinear recorder to monitor a Lead II electrocardiogram throughout the experiments. In addition, a 12 lead electrocardiogram (ECG) strip-chart recorder (Nihon Ohden Cardiofax V model BME 7707) was employed to obtain standard lead electrocardiograms during control states and at 5-minute intervals during each intervention. Heart rate were analyzed during control states as well as 1, 5, 10 and 15 minutes after occlusions began in the absence or presence of SCS. In addition, alterations in the morphology of ST-T segments and arrhythmia formation were assessed.

4.2.2 Implantation of spinal cord stimulation electrodes

After induction of anesthesia, animals were placed in the prone position. The epidural space of the mid-thoracic spinal column was penetrated percutaneously with a Toughy needle (15 F). A Toughy needle has a slight angle at its tip to ease penetration between vertebral processes. Using the loss-of-resistance technique as is routinely done in a clinical setting, the tip of the Toughy needle was slowly advanced until it entered the epidural space, as visualized via A-P fluoroscopy. Once the inner cannula was removed from the

Toughy needle, a four-pole catheter electrode (Medtronic QUAD Plus Model 3888; Medtronic Inc., Minneapolis, MN) was introduced through the needle such that its tip could be advanced to the T1 level of the spinal column, as determined by fluoroscopy. The tip of this electrode was positioned slightly to the left of the midline, as is done in a clinical setting. The rostral and caudal poles of the stimulating electrode chosen for subsequent use (inter-electrode distance of 1.5 cm) were located at the levels of the T1 and T4 vertebrae. Correct placement of the stimulating electrodes was confirmed by delivering electrical current to induce motor responses using the rostral or caudal poles as cathodes, respectively.

The rostral cathode (T1 level) and caudal anode (T4 level) of the quadripolar electrode were connected to a Grass S88 stimulator via a constant current stimulus isolation unit (Grass model CCU1 and Grass SIU5). Stimuli, delivered at 50 Hz and 0.2 ms duration, were monitored on an oscilloscope to determine the amount of current delivered. Rostral stimulation above motor threshold resulted in proximal forepaw and/or shoulder muscle fasciculations, while caudal electrode stimulation induced contractions in the thoracic trunk. When the appropriate electrode position was confirmed, the electrode lead was covered by a Teflon protective sleeve and fixed to adjacent interspinous ligaments with a suture. Extension wires attached to the electrode leads were connected to the Grass constant current stimulator (see above). Motor responses were rechecked after the animal had been placed in the supine position to ensure that the electrodes had not moved during that manoeuvre.

4.2.3 Cardiac instrumentation

After placing the animal on its back, a bilateral thoracotomy was made in the fifth intercostal space. The ventral pericardium was incised and retracted laterally to expose the heart and the ventral right atrial deposit of fat containing the ventral component of the right atrial ganglionated plexus. We investigated the activity generated by neurons in the right atrial ganglionated plexus because not only are they representative of those found in other atrial and in ventricular ganglionated plexuses[12,23,25,26], but they do not receive their arterial blood supply from the left ventral descending coronary artery[35,36]. The regional arterial blood supply of these neurons and other cardiac tissues is unaffected by spinal cord stimulation[142]. Thus the blood supply of identified neurons was not affected in a significant manner by the procedures described below.

Left ventricular chamber pressure was monitored via a Cordis (Miami, FL) #7 French pigtail catheter that was inserted into the chamber via one femoral artery. Systemic arterial pressure was measured using a Cordis #6 French catheter placed in the descending aorta via the other femoral artery. These catheters were attached to Bentley (Irvine, CA) Trantec model 800 transducers.

4.2.4 Neuronal recording

To minimize epicardial motion during each cardiac beat, a circular ring of stiff wire was placed gently on the fatty epicardial tissue overlying the ventral surface of the right atrium containing the right atrial ganglionated plexus[25]. A tungsten microelectrode (10 μm shank diameter; exposed tip of 1 μm;

impedance of 9-11 MOhms at 1000 Hz) mounted on a mounted on a Marzhauser micromanipulator (model 25033-10, Fine Scientific Tools Inc., North Vancouver, BC), was lowered into this fat using a microdrive. The indifferent electrode was attached to mediastinal connective tissue adjacent to the heart. The electrode tip explored this tissue at depths ranging from the surface of the fat to regions adjacent to cardiac musculature. Proximity to the atrial musculature was indicated by increases in the amplitude of the ECG artifact. Signals generated by the somata and/or proximal dendrites of intrinsic atrial neurons were differentially amplified by a Princeton Applied Research model 113 amplifier with bandpass filters set at 300 Hz to 10 kHz and an amplification range of 100-500X. The output of this amplifier, further amplified (50-200X) and filtered (band width 100 Hz-2 kHz) by means of optically isolated amplifiers (Applied Microelectronics Institute, Halifax, N.S., Canada), was led to a Nicolet model 207 oscilloscope and to a Grass AM8 Audio Monitor. Signals were displayed on an Astro-Med, Inc., (West Warwick, RI) MT 9500 8 channel rectilinear recorder along with the cardiovascular variables described above. All data were stored via a Vetter (Rebesburg, Penn) M3000A digital tape system for later analysis. Action potentials generated by neurons in a site in the right atrial ganglionated plexus were recorded, individual units being identified by their amplitudes and configurations. The amplitudes of the identified action potentials varied by less than 10 - 50 μV over several hours; individual action potentials retained the same configuration over time. Individual action potentials so identified are generated by somata and/or dendrites rather than axons of

passage. Action potentials recorded at a given locus that displayed the same configuration and amplitude were considered to be generated by a single unit. When multiple action potentials were identified at an active site, action potentials generate by individual units were discriminated by means of a window discriminator (Hartley Instrumentation Development Laboratories, Baylor College of Medicine, Houston, TX).

4.2.5 Coronary artery occlusion

A silk (3-0) ligature was placed around the left anterior descending (LAD) coronary artery approximately 1.5 cm from its origin, distal to its first diagonal branch. If a relatively large number of collateral arterial branches from the apex or lateral wall were evident, ligatures were also placed around these vessels. These ligatures were led through short segments of polyethylene tubing in order to occlude these arteries later in the experiments. Since the arterial blood supply of investigated right atrial neurons arises from major branches of the right and distal circumflex coronary arteries, their blood supply remained patent during these coronary artery occlusions.

4.2.6 Spinal cord stimulation (SCS)

With the animal placed in the supine position, the intensity of the current delivered via the bipolar electrode was increased until a detectable skeletal muscle motor response was evident, as described above. This current intensity corresponds to the threshold for motor activity induction (MT). An intensity of

90% of MT was used for all subsequent stimuli as it recruits A-delta fibres and other axon populations[124]. This stimulus intensity corresponds to parameters used clinically to stimulate the thoracic spinal cord[124]. The stimulus intensity at 90% MT varied between 0.09 and 0.63 mA (mean 0.32 mA) among animals studied. Presumably the variation in current intensity at 90% MT among animals reflected slight differences in electrode position with respect to the dorsal surface of the thoracic cord. The MT was checked periodically and found to remain constant over time in individual animals.

4.2.7 Protocols

Two separate protocols were applied to each of 5 animals, the order of their application being randomized among the 10 animals. These were devised to evaluate the long-term effects of successive 15-minute periods of coronary artery occlusion performed with or without concurrent SCS. Electrical stimuli were delivered to the dorsal aspect of the thoracic spinal cord for 17-minute periods. Protocol #1 began with two 15-minute periods of coronary artery occlusion, with a 1.5-hour interval elapsing between occlusions (Fig. 4.1, top panels). The coronary artery occlusion was repeated in these 5 animals in order to determine the reproducibility of ischemia induced changes in ECG morphology and intrinsic cardiac neuronal activity. After an additional 1.5 hr recovery phase, 17 minutes of SCS (90% MT) was performed during which time a 15-minute period of coronary artery occlusion was instigated 1 minute after SCS began. This was followed by a one-hour period during which time neuronal

activity was quantified. Thereafter, veratridine was applied to epicardial loci (see below).

Protocol #2 was employed in the other 5 animals. In protocol #2, the effects of 17 minutes of SCS combined with 15 minutes of coronary artery occlusion were studied first. The coronary occlusion was initiated one minute after beginning SCS (Fig 4.1, bottom panels). After waiting for 1.5 hours, a 15-minute period of coronary artery occlusion was performed alone. After waiting another 1.5 hours, the combined SCS and coronary artery occlusion was performed again. Protocol #2 was performed to verify the reproducibility of effects induced by SCS in the presence of ventricular ischemia. This protocol was followed by a one-hour recovery period after which time veratridine was applied to epicardial loci.

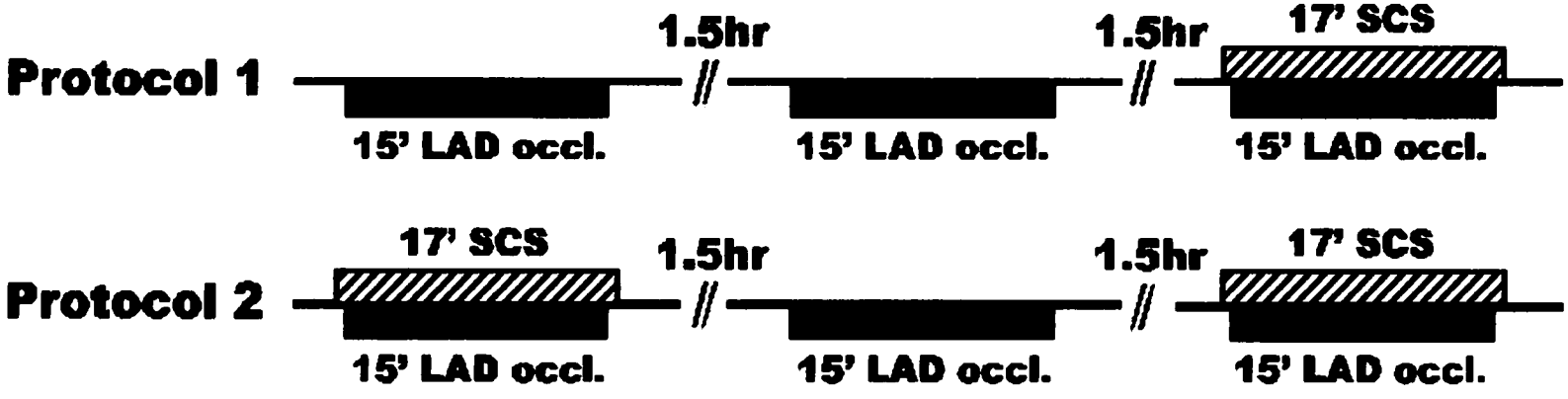


Figure 4.1 *Graphic representation of the two protocols employed in each group of 5 dogs.*

Note that a 1.5-hour period of time was allowed to elapse between each intervention in either protocol.

4.2.8 Epicardial application of veratridine

Veratridine is a selective modifier of Na^+ channels that excites sensory neurites associated with cardiac afferent neurons without inducing tachyphylaxis (Thompson et al, 2000). This agent, obtained from Sigma Chemical Co. (St. Louis, MO, USA), was dissolved in physiological Tyrode's solution to make a 7.5 μM solution. Gauze squares (1 cm x 1 cm) soaked with veratridine (0.5 ml) were applied for 60-100 seconds to discrete epicardial loci on the right ventricular conus and the ventral surface of the left ventricle at the end of each experiment (n = 10 dogs). In four animals, the effects that epicardial application of veratridine exerted on the intrinsic cardiac nervous system were also tested before the protocols described above had been performed. After removing the applied gauze, the epicardial region was flushed with normal saline for at least 30 seconds. Gauze squares soaked with room-temperature normal saline were also applied to identified epicardial sensory fields in order to determine whether neuronal responses elicited by chemical application were due to vehicle effects or the mechanical effects elicited by gauze squares.

4.2.9 Data analysis

Individual action potentials generated by the somata and/or dendrites of neurons within the right atrial ganglionated plexus were averaged over 30-second periods of time prior to and during each intervention. Average heart rate, left ventricular chamber systolic pressure and aortic pressure were determined concomitantly. Changes in ECG morphology induced by the protocols were

assessed. When the coronary artery occlusion was performed alone, data were assessed during baseline conditions and 14 minutes after the occlusion began (occlusion period), as well as starting 15 sec after reperfusion began (reperfusion period). When the occlusions were performed in the presence of SCS, cardiac indices and neuronal activity were assessed at 5 time points: 1) control period; 2) 30 seconds after SCS began; 3) 12 minutes after coronary artery occlusion began, in the presence of SCS; 4) after terminating the occlusion while the SCS persisted; and 5) within 30-60 seconds of terminating the SCS. Data are expressed as means ± SEM. Repeated measures ANOVA and paired t-test, with Bonferroni correction for multiple tests, were employed to examine grouped responses elicited during occlusion of a coronary artery alone (first occlusion) or when SCS and occlusions were performed in each protocol. Values of $p < 0.05$ were used to determine significance.

4.2 Results

4.3.1 Identification of active sites

Action potentials with signal-to-noise ratios greater than 3:1 were identified in 2-3 loci within the ventral right atrial ganglionated plexus of each animal. Based on the different amplitudes and configurations of action potentials recorded at one site per animal, an average of 3.2±0.5 (range 2-6) neurons generated spontaneous activity at investigated sites during control states. Neuronal activity during basal states was usually sporadic in nature. During basal states, a few spontaneously active neurons were identified in active loci of

most animals (Fig. 4.2) while in a few animals a number of neurons generated spontaneous activity (Fig. 4.3D). The neuron aggregates identified in one active locus in each of the 10 investigated dogs generate a range of 34.1±3.4 - 48.2±6.5 impulses per minute (Table 4.1).

Intervention	H R	LVP	AP	Neuronal activity
(n = 10 dogs)	(beats/min)	(mm Hg)	(mm Hg)	(impulses/minute)
Control	134±2	134±5	138±5/99±5	34.1±3.4
CAO	134±2	136±5	140±5/99±5	62.2±9.5*
Reperfusion	134±2	136±5	138±5/99±5	66.0±13.3*
Control	130±3	137±4	141±5/99±5	48.2±6.5
SCS	130±3	137±4	141±5/99±6	15.1±3.1*
SCS + CAO	128±3	139±4	141±5/98±6	13.5±2.4*
SCS + reperfusion	130±3	137±4	141±5/99±5	15.2±3.3*
Control	131±4	134±5	141±5/99±5	46.8±10.2

Table 4.1 *The effects of myocardial ischemia in the presence or absence of SCS on cardiodynamics and intrinsic cardiac neuronal activity.*

* denotes $p \leq 0.05$.

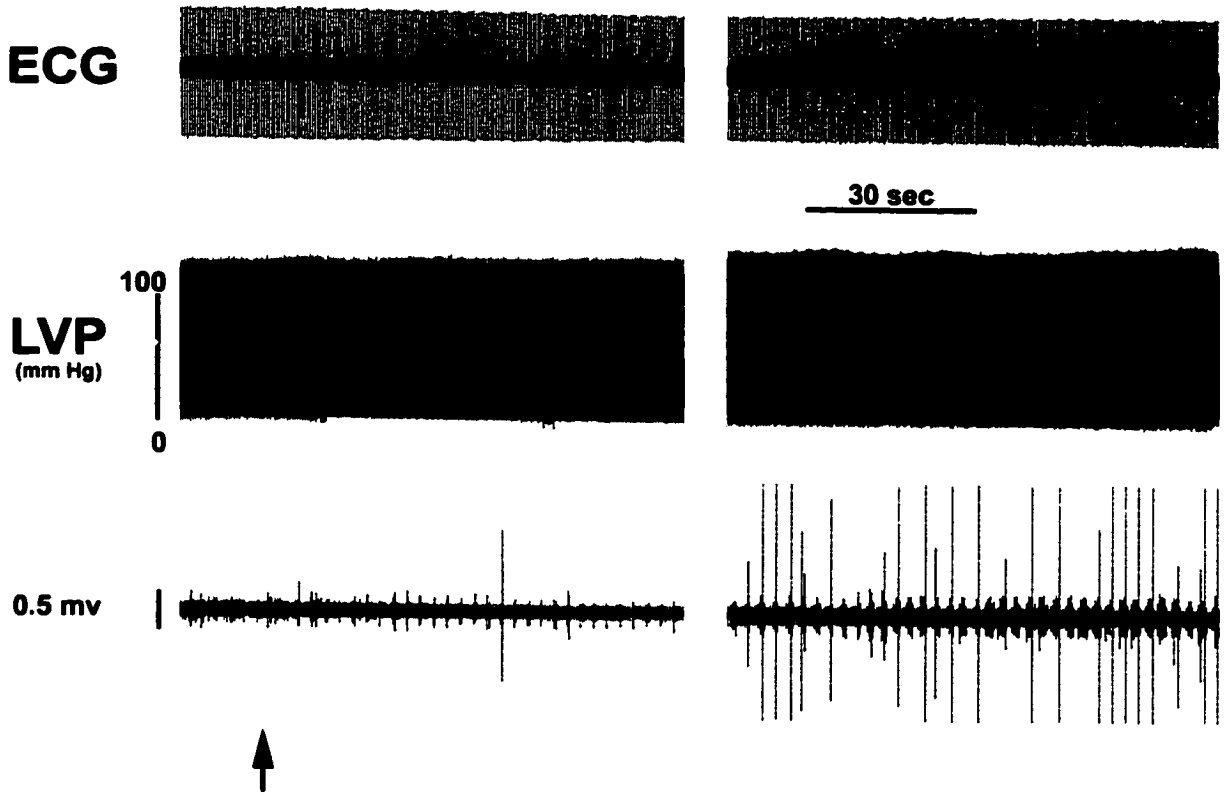


Figure 4.2 *Effects of coronary artery occlusion on the activity generated by intrinsic cardiac neurons in one animal.*

Following occlusion of the left anterior descending coronary artery (beginning at arrow below), the activity generated by right atrial neurons (lowest line) increased (right hand panel). Heart rate was unaffected by this intervention, while left ventricular chamber systolic pressure (LVP) increased a little. The time between panels represents 1.5 minutes.

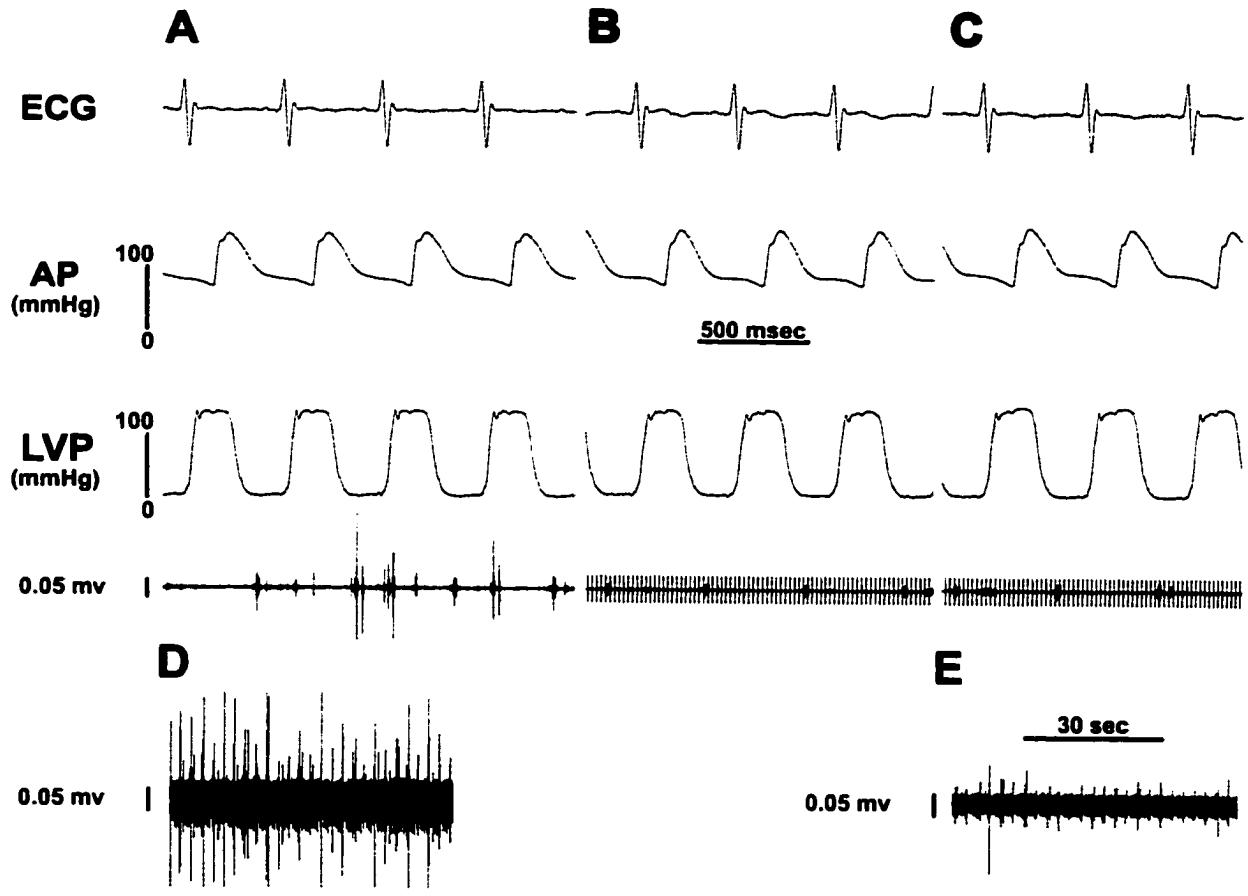


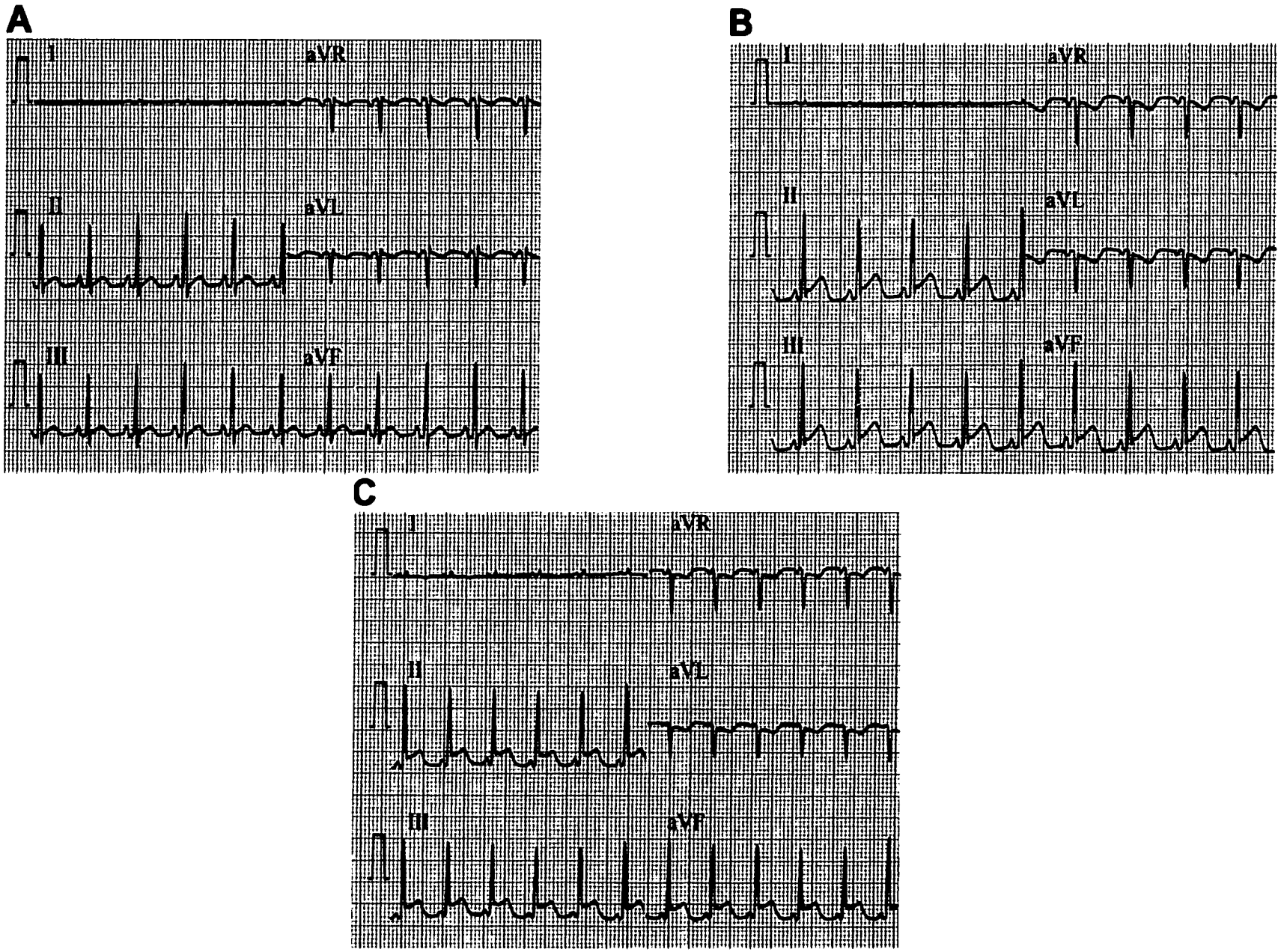
Figure 4.3 *The effects of spinal cord stimulation on the intrinsic cardiac nervous system.* The activity generated by intrinsic cardiac neurons in one animal during control states (panel A, lowest line) decreased when the dorsal aspect of the spinal cord was stimulated (panel B). The suppressor effects of SCS persisted during coronary artery occlusion (panel C). The electrical stimuli delivered during SCS are represented in panels B and C by regular, low signal-to-noise artefacts (note that atrial electrical artefact is recorded during each cardiac cycle as a low signal during the p wave of the ECG). The suppression of spontaneous activity generated by intrinsic cardiac neurons persisted after discontinuing SCS (panels D and E: panel D represents basal activity at same time scale obtained before commencing these interventions; panel E represents neuronal activity recorded 5 min post-SCS and 6 min post-LAD occlusion). ECG = electrocardiogram; AP = aortic pressure; LVP = left ventricular chamber pressure.

4.3.2 Effects of transient myocardial ischemia

Monitored cardiac indices did not change significantly overall during coronary artery occlusion or the reperfusion period, except when cardiac arrhythmias occurred. For instance, heart rate was 134±2 beats per minute (b/m) before occlusion and 130±3, 134±3, 132±2 and 134±2 b/m after 1, 5, 10 and 15 minutes of ischemia, respectively. ST segment alterations and terminal QRS slurring was evident in the ECG pattern of each animal during ischemic episodes (Fig. 4.4). The ST segments remained altered (elevated or depressed by 1.0±0.2 mm) during the first 2-5 minutes of reperfusion. ECG patterns returned to baseline values within 20 minutes of re-establishing coronary artery blood flow. Short bursts of ventricular arrhythmias occurred in most animals during coronary artery occlusion. In 2 animals, ventricular fibrillation developed during or immediately after the first coronary artery occlusion. In those instances, the hearts were successfully defibrillated and after one hour the protocol was continued. These animals did not exhibit any unusual alterations in monitored indices throughout during the rest of the protocols. The data obtained during these short bouts of arrhythmias or fibrillation was excluded from the study. Overall, these electrophysiological data are consistent with the substantial ischemia insult that was induced by 15 minutes periods of left anterior descending coronary artery (LAD) occlusion.

Figure 4.4 *ECG recordings in one animal in normal, myocardial ischemia and myocardial ischemia with concurrent SCS.*

Representative ECG records obtained from one animal during control states (A), as well as a few minutes after beginning coronary artery occlusion in the presence of spinal cord stimulation (B) and at the end of occlusion while SCS was maintained (C). Note that ST segment alterations occurred throughout the period of ischemia.


A
I
aVR
II
aVL
III
aVF
B
I
aVR
II
aVL
III
aVF
C
I
aVR
II
aVL
III
aVF

When the LAD was occluded in either protocol in the absence of SCS, the activity generated by right atrial neurons increased by 82% (Fig. 4.2; Table 4.1). Neuronal excitation persisted through the period of occlusion. During protocol #1, the two successive 15-minute periods of coronary artery occlusion separated by 1.5 hours of recovery induced similar neuronal excitation. Twelve minutes after initiating the first LAD occlusion, neuronal activity was 69% greater than identified in normally perfused states (31.7±6.9 to 53.5±10.2 impulses per minute; $p < 0.01$). During the second period of coronary artery occlusion, neuronal activity increased by 95% (28.3±4.1 to 55.1±8.9 impulses per minute; $p < 0.01$). Neuronal activity began to increase within 30 - 45 seconds after coronary artery occlusion began. This occurred despite the fact that coronary artery occlusion did not interfere with the arterial blood supply to identified right atrial neurons as it arose from the right and distal circumflex coronary arteries. Furthermore, neuronal activity remained elevated not only throughout the period of occlusion but during the early reperfusion period following re-establishing coronary artery flow. Five to ten minutes after reestablishment of coronary artery flow neuronal activity began to diminish, reaching steady state values within 15 minutes.

4.3.3 Effects of spinal cord stimulation in the presence of myocardial ischemia

During normal coronary artery perfusion, SCS did not alter the ECG or monitored cardiac indices (Table 4.1). The activity generated by identified right atrial neurons was reduced from 48.2±6.5 to 23±2.5 impulses per minute within

30 seconds of applying electrical current to the dorsal aspect of the rostral thoracic spinal cord in hearts with normal coronary arterial blood supply (Figs. 4.3B, 4.5). After the coronary artery occlusion had been maintained for 1 minute in the presence of SCS (2 minutes after beginning SCS), right atrial neuronal activity was reduced to 15.1±3.1 impulses per minute. Thus, SCS suppressed the activity generated by intrinsic cardiac neurons not only in normally perfused hearts (Fig. 4.3B), but also in the presence of regional ventricular ischemia (Fig. 4.3C). Furthermore, the neuronal suppressor effects of SCS persisted throughout the ischemic periods. Monitored cardiovascular indices did not change overall when SCS was applied during coronary artery occlusion. Ischemia-induced alterations in ECG patterns also remained throughout the period when SCS was applied concomitant with the occlusions. Neuronal activity gradually increased after discontinuing SCS such that by 20 to 25 minutes after terminating SCS neuronal activity was similar statistically to that recorded during basal conditions (Fig. 4.3E). It increased a little thereafter (Fig. 4.5).

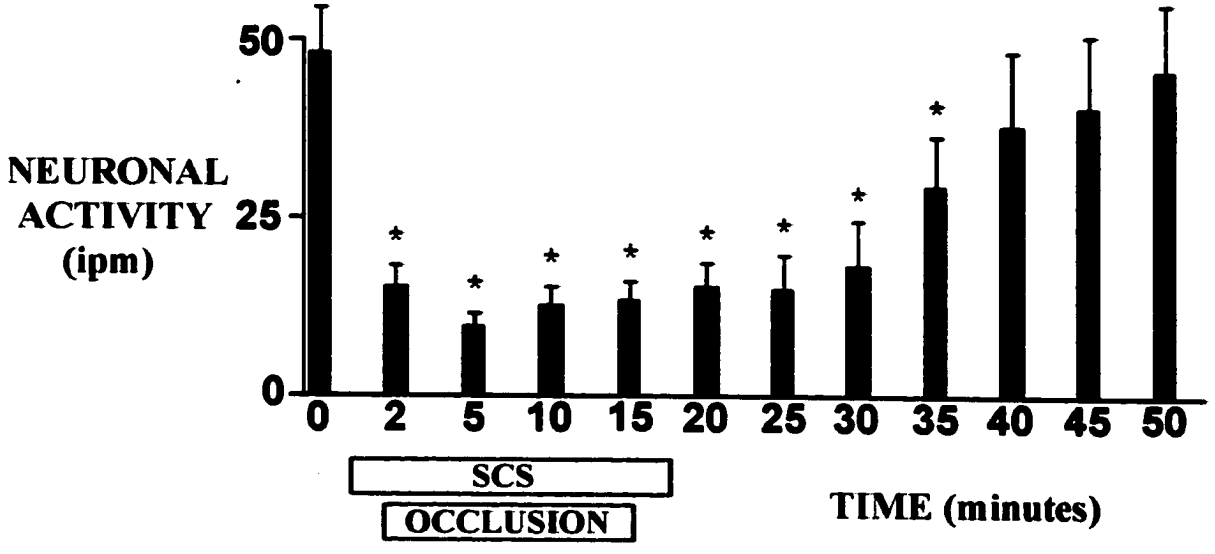


Figure 4.5 *Average neuronal activity recorded in all animals before, during and after dorsal spinal cord stimulation (SCS) delivered in the presence of coronary artery occlusion.*

Note that SCS reduced neuronal activity soon after its application began. SCS also prevented enhancement in intrinsic cardiac neuronal activity normally associated with coronary artery occlusion (see Table 4.1). Neuronal activity remained reduced for 17 minutes after terminating SCS despite the induction of myocardial ischemia. These data were collected during application of the first SCS in protocol 2.

4.3.4 Epicardial application of veratridine

The activity generated by right atrial neurons increased when veratridine was applied topically to their ventricular sensory inputs. Veratridine-induced excitation of intrinsic cardiac neurons was studied both before and after application of SCS in four animals. In those cases, veratridine enhanced intrinsic cardiac neuronal activity by 124% (40.5±26.7; $P < 0.05$) before application of SCS and by only 39% (11.8±2.5 to 16.5±4.6 impulses per minute; no significant difference) after its application. When veratridine was applied to their ventricular sensory inputs after completing the protocols in all 10 dogs (following SCS and regional ventricular ischemia), intrinsic cardiac neuronal activity increased by only 58% (25.6±5.7 to 40.6±12.5 impulses per minute; $P < 0.05$).

4.4 Discussion

The results obtained from the experiments conducted in the present study not only confirm that spinal cord neurons can modulate the intrinsic cardiac nervous system[45], but they also demonstrate that such modulation persists unabated throughout 17 minute periods of stimulating the dorsal thoracic spinal cord (Table 4.1). They also indicate that spinal cord neurons continue to exert their suppressor effects on the intrinsic cardiac nervous system long after their activation terminates (Fig. 4.5). Furthermore, these data indicate that spinal cord neurons reorganize information processing within the intrinsic cardiac nervous system arising from the ischemic myocardium, including during the reperfusion post-ischemic phase. Finally, as indicated by the neural responses

evoked by veratridine application to the ventricular epicardium, the stabilizing influence that SCS exerts on the intrinsic cardiac nervous system extends to intrinsic cardiac reflex responses evoked by activating cardiac sensory neurites associated with afferent neurons within the cardiac neuroaxis.

Detailed mechanisms and specific neuroanatomic pathways mediating spinal cord modulation of the intrinsic cardiac nervous system remain unknown. Given that bilateral transection of the ansae subclavia abolishes the neuro-suppressor effects that SCS imparts upon the intrinsic cardiac nervous system[45], it appears that the sympathetic nervous system is involved. Activation of spinal cord neurons may inhibit intrinsic cardiac local circuit neurons in a manner similar to that which occurs when they receive increasing inputs from sympathetic efferent preganglionic neurons[239,240]. Based on the results obtained during application of SCS to the lumbosacral spinal cord[124], sympathetic afferent as well as efferent axons may contribute to the suppressor effects that SCS exerts on the intrinsic cardiac nervous system.

Activation of sympathetic efferent preganglionic axons attenuates the activity generated by sub-populations of neurons within intrathoracic ganglia, including those on the heart[12]. Supramaximal stimulation of sympathetic efferent preganglionic neurons also leads to a rapid reduction in the capacity of intrathoracic sympathetic efferent neurons to influence cardiodynamics[31]. It has been proposed that such suppressor effects are most likely due to inhibitory synapses within intrathoracic ganglia, including those on the heart[8,12,23]. In

accord with that, spinal cord neurons, when activated, suppress the activity generated by intrinsic cardiac neurons.

It is known that the activity generated by many intrinsic cardiac neurons increases secondary to transient ventricular ischemia[35,46]. Right atrial neurons are supplied by arterial blood in the sinoatrial artery arising from the right coronary artery and distal branches of the circumflex coronary artery[35,36]. Since occlusion of the left anterior descending coronary artery does not compromise the arterial blood supply of investigated right atrial neurons[35], the effects that regional ventricular ischemia exerted on investigated neurons were primarily the result of ischemia induced enhancement of ventricular sensory neurite inputs to identified neurons rather than any direct effects of ischemia on identified somata and/or dendrites[12]. Intrinsic cardiac neuronal activity remained elevated throughout the 15-minute periods of regional ventricular ischemia when performed in the absence of SCS. That these regional coronary artery occlusions affected the ST segments of the ECG presumably is reflective of the underlying myocardial ischemia so induced (Fig. 4.4).

The processing of cardiac sensory information within the intrinsic cardiac nervous system was affected by SCS, as indicated by changed neuronal responsiveness to chemical (veratridine) activation of their ventricular sensory inputs. Given the fact that the capacity of veratridine to affect sensory neurites associated with cardiac afferent neuron exhibits no tachyphylaxis[42], the changed transduction properties of ventricular sensory inputs to the intrinsic cardiac nervous system may be due, in part, to remodelling of the intrinsic cardiac

nervous system subsequent to SCS. It should be noted that in clinical studies the sensory effects that SCS imparts persist long after the stimulation has stopped. Patients with refractory angina pectoris continue to experience decreased episodes of pain after terminating SCS[135]. Furthermore, the allodynia associated with neuropathic pain can be reduced for as long as one hour after terminating SCS[126].

Application of SCS immediately prior to onset of LAD occlusion did not blunt the evolution of ischemic-induced changes in the ECG. It is unlikely that the results obtained by SCS in a clinical setting can be ascribed to alterations in hemodynamics[124,134] or coronary artery blood flow[142]. Perhaps this is because SCS exerts its primary effects on the intrinsic cardiac nervous system that, in turn, may influence control over regional cardiac electrical or mechanical events.

4.4.1 Summary

These data indicate that activation of spinal cord neurons induces a conformational change in the intrinsic cardiac nervous system that persists for a considerable period of time after terminating such activation. This remodelling of the intrinsic cardiac nervous system can override excitatory inputs to it arising from the ischemic myocardium. This suggests that thoracic spinal cord neurons can act to stabilize the intrinsic cardiac nervous system in the presence of ventricular ischemia and during reperfusion. Thus, the prolonged salutary effects that SCS imparts to some patients long after it is discontinued may, in part, be due to remodelling of the cardiac nervous system.

Chapter 5 Transmyocardial laser revascularization remodels the intrinsic cardiac nervous system in a chronic setting

This work has, in part been published in: Circulation 2001; 104 [Suppl I]: I-115 - I-120.

5.1 Introduction

Patients with anatomically diffuse, end-stage coronary artery disease not amenable to standard revascularization procedures typically suffer from intractable angina (CCS functional class 3-4) despite maximal medical therapy. Several prospective studies have demonstrated that the creation of multiple transmural channels in the heart by means of a laser (transmyocardial laser revascularization; TMLR) provides symptomatic relief of refractory angina in such patients[144-147,151]. Channels produced by TMLR in the wall of an ischemic ventricle were initially thought to remain patent after the procedure, thereby permitting blood from the chamber to reach underperfused cardiomyocytes by forming direct communications between the chamber and ventricular blood vessels[154,155,241,242]. Recently, this theory has been challenged with the demonstration that such channels are only transiently patent[156,164,243]. An alternate hypothesis proposed is that non-specific laser-induced myocardial damage may involve local neuronal tissue[164,168]. It has been proposed that such injury damages local ventricular sensory neurites as well as autonomic efferent axons, thereby producing local cardiac denervation and thus anginal relief. However, we have shown that TMLR does not destroy cardiac afferent or efferent neuronal function in an acute setting[171].

TMLR imparts symptomatic relief of at least two CCS anginal classes in 25-76% of patients one-year post TMLR[144-146,244]. Such symptomatic relief usually is evidenced by reduction in nitrate usage[144-146], increased exercise

tolerance[145,146,244] and higher quality of life scores[145,245]. While TMLR does not modify the cardiac nervous system on an acute basis, it remains to be established whether TMLR induces delayed effects on this system. This is particularly relevant with respect to its effects on the intrinsic cardiac nervous system.

Intrinsic cardiac neurons are situated in ganglionated plexuses located in discrete atrial and ventricular regions[194]. In the canine and human model, these ganglionated plexuses contain afferent, efferent and local circuit neurons that allow for "fine tuning" of cardiac function on a beat-to-beat basis. As such, the intrinsic cardiac nervous system acts as the final common regulator of regional cardiac function[8,12,46].

In order to determine whether TMLR imparts delayed effects on the function of the cardiac nervous system, the effects of TMLR were studied in a chronic, non-ischemic canine model. The non-ischemic model was investigated in order to avoid confounding effects ischemia has on the intrinsic cardiac nervous system[45]. This was done by: 1) activating local sensory neurites associated with intrinsic cardiac afferent neurons with the topically applied ion modifying agent veratridine; 2) electrical activation of sympathetic and parasympathetic efferent neurons that modulate regional ventricular dynamics; 3) chemically activating sympathetic and parasympathetic efferent neurons via systemically administered nicotine; 4) chemically activating sympathetic efferent neurons via systemically administered angiotensin II and; 5) testing the capacity of laser-treated ventricular muscle to respond to an exogenously applied ß-

adrenergic receptor agonist. All interventions were performed one month after the TMLR procedure or sham operation. In this manner, we assessed the chronic effects that TMLR exerts on not only local ventricular cardiomyocyte function, but also on the cardiac nervous system.

5.2 Methods and Materials

All experiments were performed in accordance with the guidelines for animal experimentation described in the "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-23, revised 1996) and the Canadian Council on Animal Care "Guide to the care and use of experimental animals" (Vol. 1, 2nd Ed., 1993).

5.2.1 Animal preparation

Adult mongrel dogs (n = 14) of either sex, weighing between 25-30 kg, were used in this study. The dogs were sedated with a mixture of morphine (0.5 mg/kg i.m.), atropine (0.2 mg/kg i.m.) and acepromazine (0.1 mg/kg i.m.). Following endotracheal intubation, general anesthesia was maintained to effect with halothane. Four animals underwent a sham operation consisting of a thoracotomy and pericardotomy as described below.

5.2.2 Laser therapy

Using sterile surgical technique, 10 dogs had the pericardium exposed via a limited left-sided thoracotomy. Following a pericardial incision to expose the

left ventricle, 20 separate channels approximately 1 mm in diameter were created that penetrated through the ventral-lateral wall of the left ventricle in a 4 cm x 4 cm epicardial region (Fig. 5.1).

This was done with a holmium:yttrium-aluminium-garnet (Ho:YAG) laser (λ = 2.1 μm; pulse length = 250 μs) (Eclipse Surgical Technologies, Sunnyvale, CA; model TMR 2000). These channels were created in the distribution of the terminal left anterior descending and diagonal coronary arteries as in a previous acute study[171]. An average of 7-9 pulses over 2-4 seconds were required to traverse the left ventricular wall. Digital pressure was applied to the epicardial openings of transmural channels sites, when required, to achieve hemostasis. If ineffective, a figure-of-eight suture was placed around the epicardial opening. The creation of transmural channels were noted intra-operative by the change in the pitch of the laser as it enters the LV cavity and confirmed on examination of myocardial sections at the completion of the chronic physiologic experiments described below. Subsequently, the thoracotomy was closed, pneumothorax reduced and the animal was recovered. Appropriate post-operative pain management was achieved using a combination of long and short acting narcotics (morphine sulphate 0.5-1mg/kg and bupinorephine 0.02mg/kg) and non-steroidal anti-inflammatory medication (ketoprofen 1-2mg/kg). All animals received a dose of cefazolin pre and post-operatively (20mg/kg).

5.2.3 Physiological studies

Four to five weeks after creating these transmural channels, the animals underwent functional studies. The animals were sedated with sodium pentothal (15 - 20 mg/kg i.v.) and then anesthetized with sodium pentothal (~5 mg/kg i.v. to effect q 5-10 minutes). Following initiation of anesthesia, the animals were intubated and respiration was maintained with a Bird Mark 7 positive-pressure respirator using 100% O_2. After completion of surgery, anesthesia was maintained with an initial bolus infusion of α-chloralose (50 mg/kg i.v.). Thereafter, supplemental doses of α-chloralose were administered throughout the experiments every 30 minutes or less. The adequacy of anesthesia was assessed throughout the experiments by checking jaw tone and squeezing a paw while monitoring any change in heart rate.

A lead II ECG was recorded on an Astromed MT9500 8 channel rectilinear chart recorder. Left ventricular chamber and aortic pressures were measured using Bentley Trantec model 800 transducers connected to a Cordis #7 pig-tail catheter inserted into the left ventricular chamber and a Cordis # 6 catheter inserted into the ascending aorta via a femoral arteries. Two miniature solid-state pressure transducers (Konigsberg Instruments, Pasadena, Ca, model #P190; 5 mm diameter, 1.5 mm thick) were inserted in the left ventricular ventral wall to record regional intramyocardial pressures. One transducer was placed in the region that underwent the laser treatment and the other in an adjacent untreated region. All data, including neuronal activity, were recorded on an

Astro-Med, Inc. model MT 9500 eight channel rectilinear recorder. The outputs of the optical recorder were led to an A.R.Vetter Co. model 820 videocassette recorder.

A midline incision was made in the neck to expose the right and left cervical vagosympathetic trunks. The cervical vagi were divided so that the distal ends of each could be stimulated electrically without producing afferent axonal-induced cardiovascular reflexes. Following a bilateral thoracotomy to expose the heart, the ventral pericardium was incised to expose the ventral right atrial deposit of fat that contains the ventral component of the right atrial ganglionated plexus (RAGP). Neurons in the RAGP are representative of those found in the various intrinsic cardiac ganglionated plexuses [194]. To minimize epicardial motion during each cardiac cycle a circular ring of heavy-gauge wire was gently placed around the circumference of the epicardial fat on the ventral surface of the right atrium. The RAGP was explored with a single tungsten microelectrode mounted on a mounted on a Marzhauser micromanipulator (model 25033-10, Fine Scientific Tools Inc., North Vancouver, BC) and placed over the epicardial fat so that the assembly can be slowly advanced into the fat to search for neuronal activity at depths ranging from the surface of the fat to regions adjacent to cardiac musculature. The recording microelectrode (Frederick Haer 25-10-3, Brunswick, ME) had a 250 μm shank diameter, an exposed tip of 10 μm and impedances of 9-11 MW at 1,000 Hz. Proximity to cardiac musculature was indicated by increases in the amplitude of the ECG artifact. The indifferent electrode was attached to the adjacent mediastinum.

Signals generated by atrial neurons were differentially amplified by a Princeton Applied Research model 113 amplifier that had bandpass filters set at 300 Hz to 10 kHz and amplification ranges of 100-500X. The output of this device, further amplified (50-200X) and filtered (band width 100 Hz-2 kHz) by means of optically isolated amplifiers (Applied Microelectronics Institute, Halifax, N.S., Canada), was led to a Nicolet model 207 oscilloscope and to a Grass AM8 Audio Monitor. Activity generated by individual neurons was identified by the amplitude and shape of recorded action potentials. Separate loci were identified from which action potentials with signal to noise ratios greater than 3:1 were recorded, individual units being identified by the amplitude and configuration of their action potentials. Using these techniques and criteria, the microelectrode does not record action potentials generated by axons of passage, but rather records action potentials generated by cell bodies and/or dendrites[25-27].

5.2.4 Electrical stimulation of autonomic efferent neurons

Stellate ganglia were decentralized and bipolar electrodes placed around them so that they could be stimulated electrically later in the experiments. Acutely decentralized right and left stellate ganglia were stimulated individually (10 Hz, 5 ms, 4 V) for 20 seconds. The distal ends of the sectioned right or left cervical vagi were stimulated individually for 10 seconds (20 Hz, 5 ms, 4 V). The bipolar stimulating electrodes (electrode tips 5 mm apart) were connected to a Grass (Quincy, MA) SD-9 square wave stimulator, the output of which was monitored on a Telequipment (Beaverton, OR) D-54 oscilloscope.

5.2.5 Administration of pharmacological agents

Veratridine (7.5 μM) was applied for 30 seconds to epicardial loci overlying the affected left ventricular TMLR zone using 2 cm x 2 cm gauze squares soaked with 2 ml of the chemical. After removal, the epicardial locus was washed with normal saline for 30 seconds (~2 ml/sec). At least 10 minutes was allowed to elapse before the next intervention. Gauze squares soaked with room-temperature normal saline were applied to the same epicardial sensory fields to determine whether neuronal responses elicited by epicardial chemical application were due to vehicle effects or the mechanical effects elicited by gauze squares.

The following three chemicals were then administered individually into the systemic circulation: 1) nicotine (5-20 μg/kg i.v.), a chemical that binds to cholinergic nicotinic receptors thereby activating sympathetic and parasympathetic efferent postganglionic neurons; 2) angiotensin II (AII) (0.01 - 5μg/kg i.v.), a chemical that binds to AT_1 receptors associated with intrinsic cardiac neurons; and 3) the β-adrenoceptor agonist isoproterenol (5 μg i.v.), that acts directly on the myocyte. These agents were then re-administered to ensure reproducibility of effects. At least 5 minutes was allowed to elapse between these interventions for cardiovascular indices to return to baseline values.

5.2.6 Echocardiographic analysis

Echocardiographic assessment of left ventricular wall motion was performed in 2 animals before and during stellate ganglion stimulation using an Ultramark 8 Ultrasound Systems echocardiographic apparatus (Model #UM-8-

OPO1; Advanced Technology Laboratory, Bellevue, WA). The cross-sectional mid-papillary view of the left ventricle was obtained via an Access C ultrasound probe (Bothell, WA) placed on the free wall of the left ventricle.

5.2.7 Data analysis

Heart rate, left ventricular chamber systolic pressure, as well as intramyocardial systolic pressure in the normal and TMLR treated zones of the left ventricular free wall, were monitored for 1 minute before and after each of the interventions described above. The activity generated by identified right atrial neurons was analyzed for 1-minute periods before and during each intervention. Cardiac, vascular and neuronal data obtained before and during each intervention are presented as means ± SEM. One-way ANOVA and paired t-test with Bonferroni correction for multiple tests were used for statistical analysis. Significance values of $p < 0.05$ or 0.01 were used for these determinations.

5.3 Results

5.3.1 Laser treated animals

Left ventricular dynamics responded in a normal fashion to systemically administered isoproterenol, including the zone that has undergone TMLR. Echocardiographic analysis performed in two of the animals demonstrated normal left ventricular transverse diameters in diastole and systole during control states. These dimensions were reduced during stellate ganglion stimulation in a manner consistent with that found in normal dogs[171,246]. The ventricular free wall

zone that had undergone TMLR did not display any detectable wall motion abnormality.

However, the heart did not appear to respond to stress in a normal fashion. Indeed, the LV systolic pressure progressively diminished throughout the experiment (during exposure of the heart and its instrumentation, LV systolic pressure fell from 132±6 to 96±8 mm Hg; $p<0.01$). Additionally, dysrhythmias developed in two animals. The heart of one dog fibrillated during the dissection of pericardial adhesions to expose the ventricular epicardium. After defibrillating this heart, baseline hemodynamic variables returned to control values. In another animal, atrial pacing was required due to the development of a third degree heart block. In this animal, only responses to stellate ganglion stimulation and systemic administration of the chemicals were studied.

The Na^+-channel modifier veratridine, when applied to the epicardium over the laser treated zone, modified the activity generated by identified right atrial neurons (increase in activity in 7 animals, decreased in 2 animals; delta neuronal change = 19.0 ± 3.1 impulses/min, $p< 0.01$). Re-application of veratridine to the same epicardial region induced similar neuronal responses.

Electrical stimulation of acutely decentralized stellate ganglia enhanced all monitored cardiac indices in a normal fashion (Table 5.1). Specifically, both the lasered and non-lasered regions responded in a similar fashion to this intervention (Table 5.1, Fig. 5.2). Electrical stimulation of parasympathetic efferent neurons suppressed intramyocardial systolic pressure in both regions of the left ventricle to similar degrees (Table 5.1).

Intervention	HR (bpm)		LVP (mmHg)		LVIMP Control (mmHg)		LVIMP Laser (mmHg)		Neurons (imps/min)	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
All 0.01	116.3±12.0	111.3±10.5	97.5±7.5	120.5±4.9	80.5±19.0	87.5±16.2	78.5±5.7	85.5±7.9	31.5±15.3	31.5±8.6
All 5	111.0±8.2	137.0±13.9	94.0±5.9	147.2±11.1*	73.2±17.2	104.4±16.5*	76.4±7.7	98.4±7.4*	29.4±13.0	38.2±17.0
Isoproterenol	110.5±5.0	164.0±7.3*	91.0±8.6	105.2±11.5*	57.6±10.0	137.6±22.5*	57.2±8.1	121.8±20.9*	27.2±8.8	42.8±13.5
RSG	117.5±7.1	210.0±11.3*	92.8±8.5	136.0±8.3*	59.1±6.8	181.5±31.3*	61.6±7.0	176.7±22.9*		
LSG	121.0±7.8	186.0±11.3*	93.4±9.0	142.8±11.7*	60.2±12.3	194.2±29.8*	61.2±8.1	166.6±23.2*		
RV	117.8±7.0	72.2±7.5*	97.7±10.1	73.3±6.0*	58.9±13.1	43.1±10.4*	54.4±5.2	42.4±4.9*		
LV	119.4±7.6	58.3±6.8*	95.3±10.6	73.6±5.9*	58.4±14.3	46±10.7*	56.2±5.1	41.6±3.8*		

Table 5.1 *Alterations in recorded variables in several interventions in the chronic TMLR model.*
Heart rate, intramyocardial pressures, left ventricular chamber systolic pressure (LVP) and intrinsic cardiac neuronal responses induced in 10 anesthetized dogs when the right (RSG) or left (LSG) stellate ganglia or right (RV) or left (LV) vagus were stimulated. Responses to systemically administered angiotensin II (either 0.01 or 5 μg/kg), nicotine (20 μg/kg) and isoproterenol (5μg/kg) as also shown. Neuronal activity could not be recorded during electrical stimulation of the stellate ganglia and vagi. HR = heart rate; LVIMP = left ventricular intramyocardial systolic pressure in either the lasered or control regions of the left ventricle; Neurons = the activity generated by right atrial neurons. * = $p<0.01$.

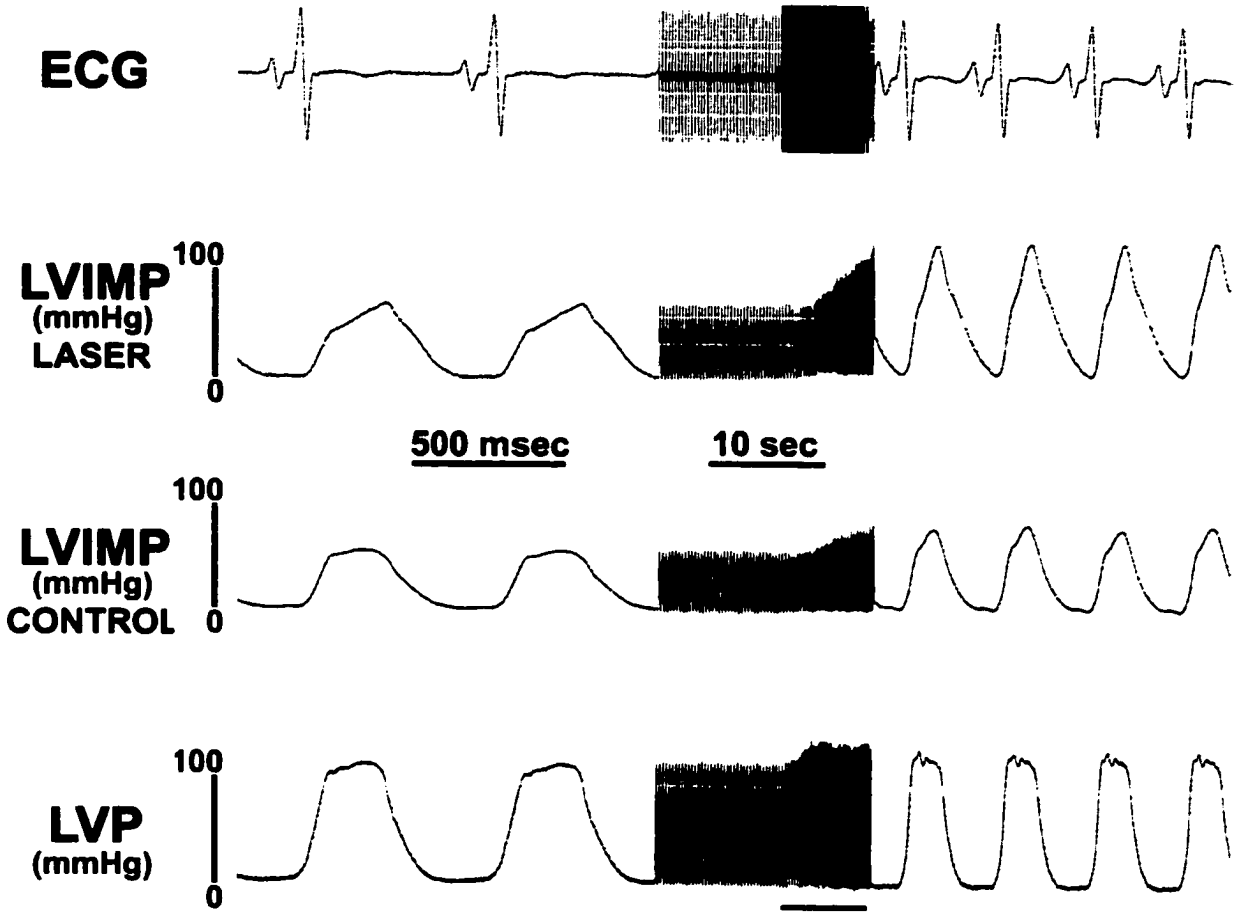


Figure 5.2 *The effect of right stellate stimulation on cardiac inotropy and chronotropy four weeks after TMLR in the canine.*

Cardiac effects elicited by stimulation of the right stellate ganglion (acutely decentralized) of an animal that had received TMLR in a region of the left ventricular free wall (laser) 4.5 weeks previously. ECG = electrocardiogram; LV IMP (laser) = intramyocardial pressure in the region of the left ventricular ventral wall previously subjected to TMLR; LVIMP (control) = intramyocardial pressure in the unaffected region of the left ventricular ventral wall; LVP = left ventricular chamber pressure (similar abbreviations in the other figures). Note the responses to stellate ganglion stimulation were similar in both regions of the left ventricular free wall examined.

On the other hand nicotine, which normally activates the intrinsic cardiac nervous system, failed to alter recorded neuronal activity (Fig 5.3). Neuronal activity was unaffected even when supra-normal doses of nicotine were administered (Fig 5.4). Minor alterations in recorded cardiovascular variables occurred only when the highest dose of nicotine (20 μg/kg i.v.) was studied. Angiotensin II, when administered in doses that normally induces intrinsic cardiac neuronal and cardiac responses in normal hearts (i.e., 0.01-5 μg/kg i.v.), failed to elicit neuronal responses overall. When the largest dose of angiotensin II was studied (5 μg/kg), cardiac indices increased a little (Table 5.1). Isoproterenol not only enhances both inotropy and chronotropy, but also increases intrinsic cardiac neuronal activity. In this chronic model, isoproterenol enhanced recorded cardiac indices, including left ventricular intramyocardial systolic pressures in the TMLR zone; it failed to enhance intrinsic cardiac neuronal activity (Table 5.1).

Phenol destroys epicardial nerves. In order to demonstrate that ventricular myocyte responses elicited by autonomic efferent neurons can be eliminated, in 2 animals phenol was applied to the epicardium surrounding the intramyocardial pressure sensor located in the treated (lasered) ventricular zone. After epicardial application of phenol, stimulation of either stellate ganglion failed to produce regional inotropic responses that were previously inducible (Fig. 5.5A, laser). The unaffected ventricular region (not surrounded by epicardial phenol) responded to stellate ganglion stimulation in a fashion that was similar to that induced before phenol application (Fig. 5.5A, control). To test myocyte viability independent of the intrinsic cardiac nervous system, isoproterenol was

exogenously administered. Both the phenol treated and untreated ventricular regions responded similarly to this intervention (Fig. 5.5B).

5.3.2 Sham treated animals

Unlike the laser treated animals, the performance of the thoracotomy did not alter hemodynamics in the 4 sham animals. Inotropic and chronotropic changes elicited during stellate ganglia stimulation were similar to those in the chronic TMLR animals (RSG: LVIMP Control vs. Stimulation – 50 ± 11.3 vs. 134 ± 22.5; HR Control vs. Stimulation – 140 ± 7.1 vs. 163.7 ± 6.6; expressed at mean ± SEM). In contrast to laser treated animals, neuronal responses elicited with nicotine (5μg/kg i.v.: delta neuronal change = 67.5 ± 35.0) and angiotensin II (0.01μg/kg i.v.: delta neuronal change = 27.5 ± 11.1) were similar to previously reported normal controls [45,171,247,248].

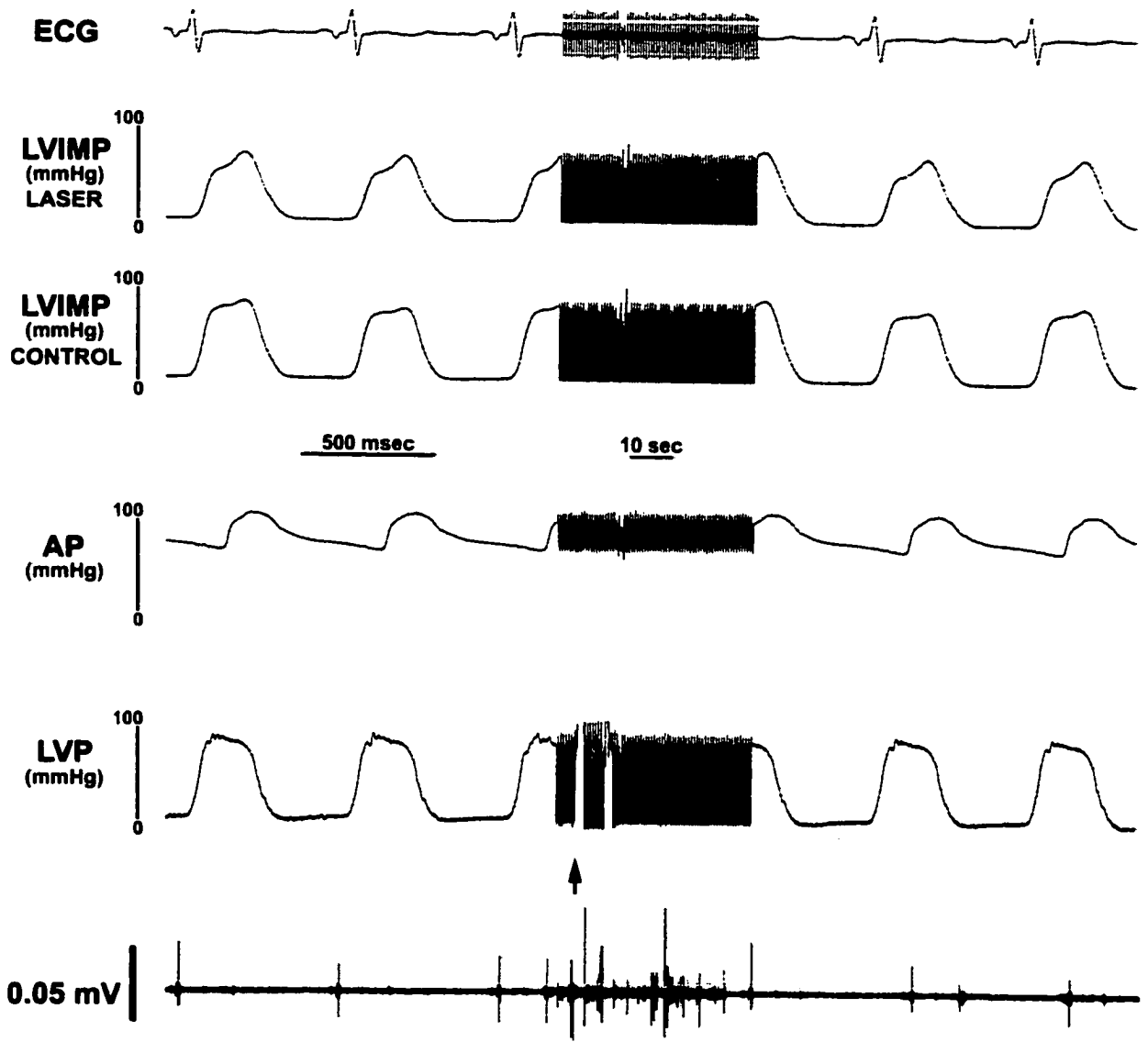


Figure 5.3 *The effect of system nicotine on the intrinsic cardiac nervous system four weeks after TMLR in the canine model.*

Cardiac effects elicited by systemic administration of nicotine (20 μg/kg iv, black arrow). This large dose of nicotine elicited no cardiovascular or neuronal responses after chronic TMLR. The lowest trace represents extracellular activity generated by right atrial neurons.

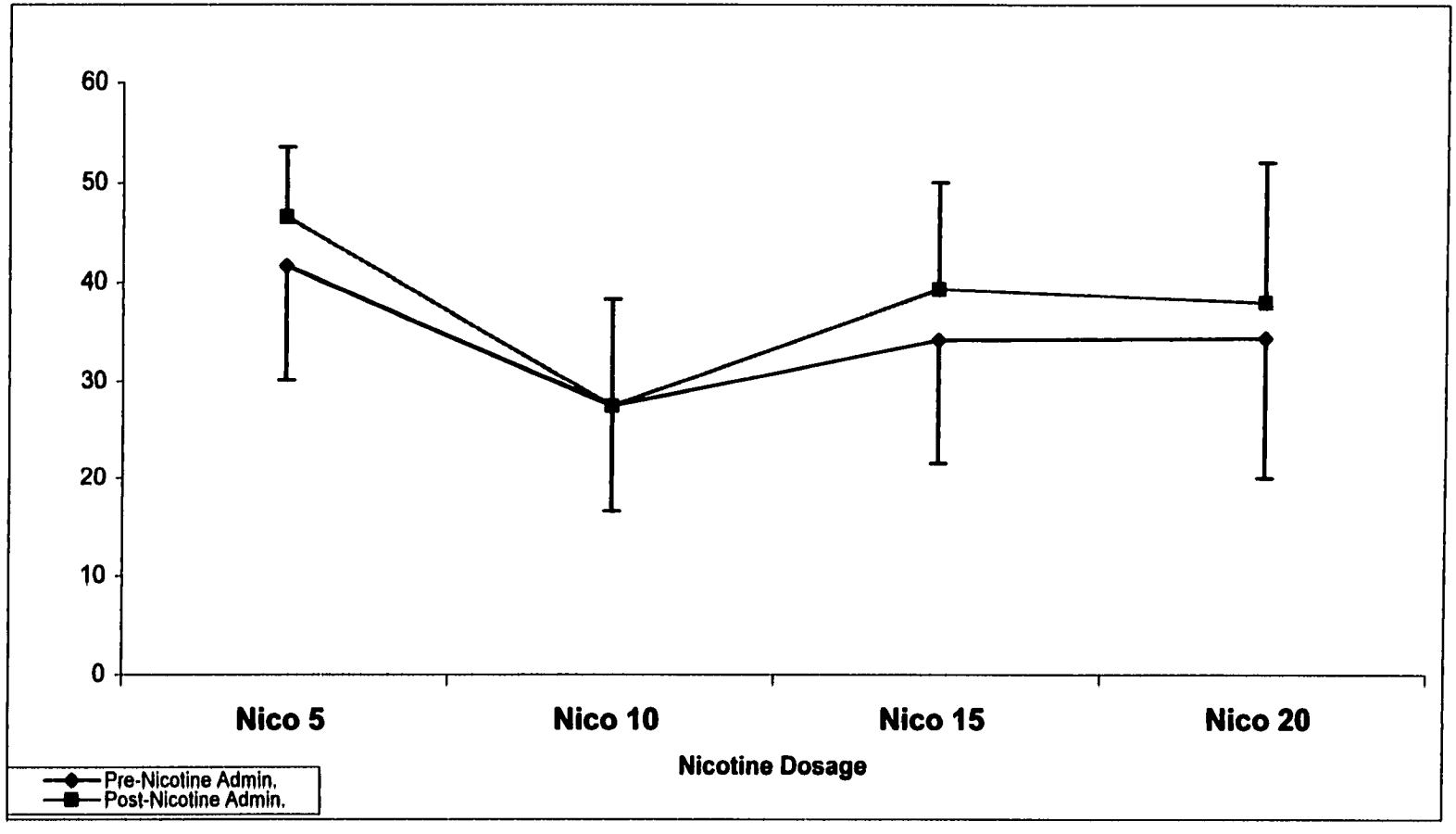


Figure 5.4 *A dose response curve of the effect of increasing dose of nicotine on the activity generated by the right atrial neurons*

Nicotine failed to elicit a change in atrial neuronal activity at any dose tested. Nico 5-20 = nicotine at 5- 20µg/kg. (p = n.s. at all data points).

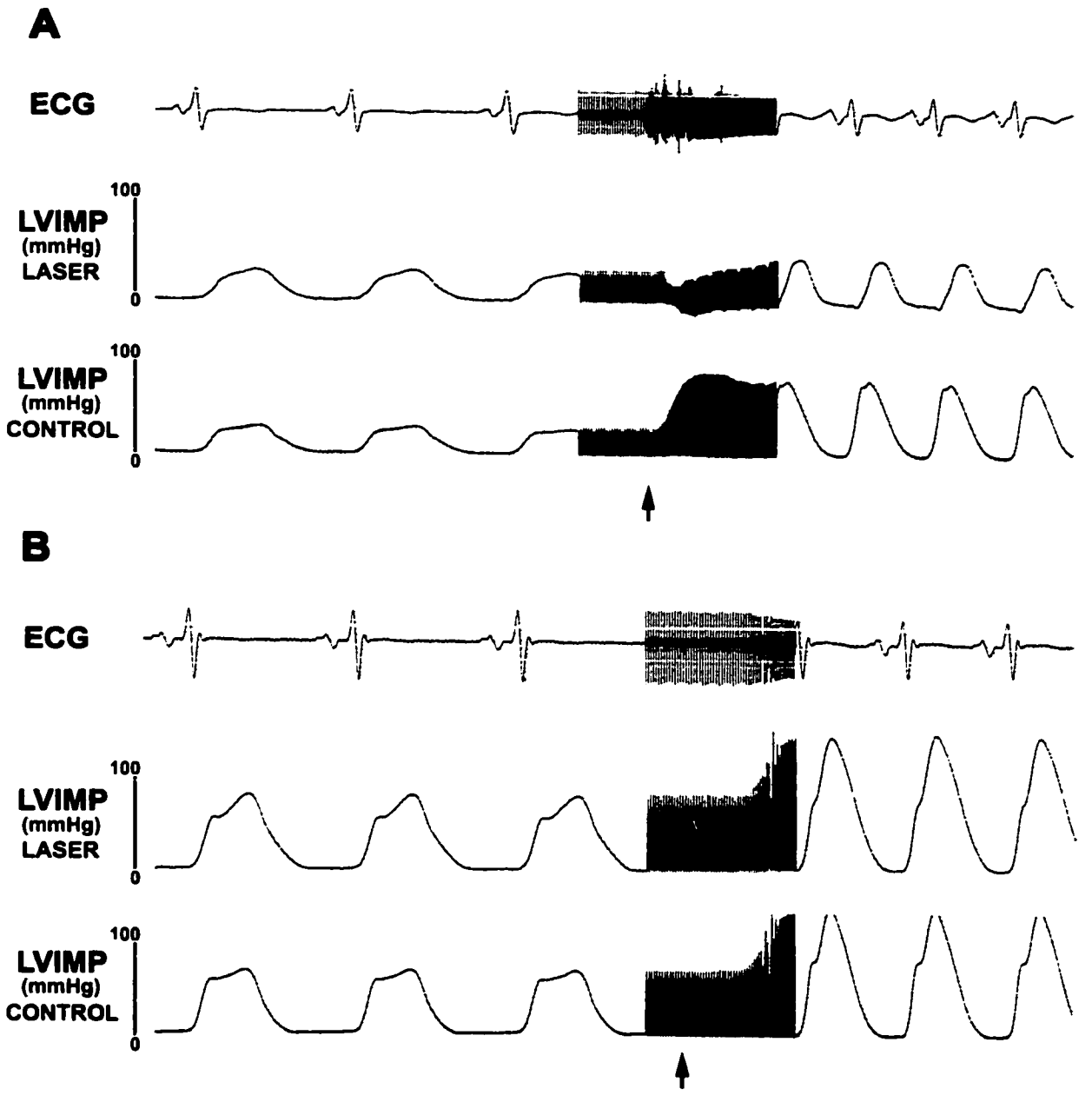


Figure 5.5 *True denervation achieved with epicardial phenol application.* A. Cardiac augmentation induced by right stellate ganglion stimulation (arrow) after the topical application of phenol around the lasered region of the left ventricle. Note that intramyocardial systolic pressure increased in the control region but not the region surrounded by epicardial phenol (laser). Both these regions responded similarly to stellate ganglia stimulation, as before phenol application (Fig 5.2). B. Thereafter, isoproterenol (after phenol application in the same animal) induced normal responses in both regions of the left ventricle.

5.4 Discussion

The results of the present study indicate that TMLR remodels the intrinsic cardiac nervous system over time, thereby affecting its capacity to influence regional cardiac function. This occurs despite the fact that TMLR does not affect the function of cardiac sensory neurons; nor does it alter the capacity of sympathetic and parasympathetic efferent neurons to modulate regional ventricular dynamics.

Prospective studies indicate that TMLR relieves angina of cardiac origin[144-146]. The mechanisms whereby this occurs remain unknown. While up 85% of patients receive significant symptomatic improvement after TMLR, peak anginal relief is usually delayed 3-6 months after TMLR, regardless of the type of laser employed[144-146,249,250]. It has been suggested that cardiac denervation may be one mechanism whereby TMLR exerts its salutary effects[164]. Kwong et al[164], by applying high doses of bradykinin to the ventricular epicardium before and after TMLR, concluded that TMLR renders sensory neurites in the affected myocardium non-functional. However, direct assessment of cardiac afferent neuronal function before and after acute TMLR indicated that such is not the case[171]. The cellular sodium-channel modifier veratridine modifies ventricular sensory neurites associated with cardiac afferent neurons in a consistent fashion[42]. The effects of veratridine on ventricular sensory inputs to intrinsic cardiac neurons following chronic TMLR was similar to those induced in normal

preparations[46]. These data indicate that the functional integrity of intrinsic cardiac afferent neurons is preserved in this model of chronic TMLR.

It has been suggested that myocardial tyrosine hydroxylase immunoreactivity, an indirect measure of sympathetic efferent postganglionic axonal density, becomes reduced in ventricular regions subjected to TMLR equates with functional sympathetic efferent neuronal denervation[168]. However, direct assessment of the function of cardiac sympathetic efferent neurons demonstrated that TMLR does not affect their capacity to influence ventricular tissues in an acute, non-ischemic canine model [171]. Neither did TMLR modify the capacity of cardiac adrenergic efferent neurons to enhance regional ventricular dynamics in a chronic setting, when activated electrically (Fig. 5.2, Table 5.1). Similarly, electrical stimulation of parasympathetic efferent preganglionic axons suppressed regional intramyocardial systolic pressures in treated and untreated regions of the left ventricular free wall to comparable degrees (Table 5.1). These data demonstrate that TMLR does not obtund the influence of extra-cardiac autonomic efferent neurons (with intracardiac axonal projections) on ventricular function in a chronic setting.

In contrast, regional denervation occurs following the topical application of phenol, which negates the ability of electrical activation of sympathetic efferent postganglionic neurons to enhance regional ventricular contractility. In confirmation of that, ventricular regions treated with phenol failed to respond to electrical activation of sympathetic efferent neurons even though adjacent untreated ventricular regions elicited normal responds to the stellate ganglion

stimulation (Fig. 5.5A). On the other hand, systemic administration of isoproterenol enhanced intramyocardial systolic pressure in both regions similarly (Fig. 5.5B). These data indicate that one can functionally destroy the sympathetic efferent innervation to a region of the heart without causing detectable cardiomyocyte injury.

Despite the normalcy of cardiac afferent and extra-cardiac efferent neurons, intrinsic cardiac neuronal function was not normal following chronic TMLR. As previously stated, the intrinsic cardiac nervous system is an important regulator of regional cardiac function. In extensive experience with the canine model in our laboratory, the canine preparation employed is a robust model, capable of tolerating manipulation without preparation compromise. However, 4-5 weeks following the TMLR procedure it was apparent that the animals did not tolerate surgical stress in the usual fashion. Two animals suffered significant dysrhythmias on exposing the heart. No dysrhythmias or hemodynamic instability was seen in the sham animals. Additionally, the intrinsic cardiac nervous system proved to be non-responsive to the potent chemical agonists nicotine and angiotensin II.

Nicotine activates both parasympathetic and sympathetic efferent postganglionic neurons (cell bodies) of the intrinsic cardiac nervous system, thereby inducing bradycardia (parasympathetic efferent neuronal effects on atrial tissues) followed by enhancement of cardiac indices (via activating sympathetic efferent neurons). In other words, both populations of autonomic efferent neurons on the heart become activated following systemic administration of

nicotine[20]. Nicotine continues to enhance cardiac indices immediately after performing TMLR in a canine model[171] and in sham operated animals in this study. In the chronic TMLR model, however, nicotine not only failed to enhance intramyocardial pressures in the treated zone, but it also failed to alter the other monitored cardiac indices monitored (Fig. 5.3). This occurred despite the fact that the maximum dose of nicotine studied was four times that required to elicit a maximal response in normal preparations[20,171]. Angiotensin II actives AT_1 receptors associated with the ICNS. Normally, angiotensin II activates this neuronal system to enhance cardiac indices[247,248]. There was also a lack of effect when angiotensin II was administered to this chronic TMLR model (Table 5.1). Taken together, these data indicate that the global functioning of intrinsic cardiac efferent neurons becomes obtunded within a month following TMLR. This correlated with the animals' inability to tolerate the usual manipulation required during the course of experimentation.

On the other hand, TMLR did not affect the capacity of ventricular myocytes to respond to the exogenously administered β-adrenergic agonist isoproterenol. This agent enhanced intramyocardial systolic pressure by 47% in the treated region and by 42% in the untreated area (Table 5.1). That the treated zone retained its normal contractile responsiveness to such a challenge was confirmed in two animals by echocardiography (data not shown).

5.4.1 Summary

In conclusion, data derived from this study indicate that the function of the intrinsic cardiac nervous system is substantively altered within a few weeks of performing TMLR. TMLR does not denervate cardiac afferent or extracardiac efferent neuronal function in ventricular regions undergoing the procedure in a chronic setting. This is in accordance with the lack of TMLR effects on an acute basis [171]. Thus the beneficial effects that transmyocardial laser treatment affords patients with ischemic heart disease cannot be ascribed to local ventricular denervation. Rather, alteration or "remodelling" of the intrinsic cardiac nervous, the final common regulator of regional cardiac function, may occur within a month of TMLR. Remodelling of the ICNS has been shown to occur in auto-transplanted dogs[251], chronically decentralized dogs [15] and dogs undergoing spinal cord stimulation [45]. It has subsequently been clinically shown that aggravation of myocardial ischemia[252] and a reduced left ventricular function with echocardiographic evidence of resting left ventricular wall motion abnormalities occurs in patients who have undergone the TMLR procedure[250,253,254]. It therefore appears that some surgical procedures and therapeutic interventions have the ability to alter the functioning of this important neuronal system. As such, remodelling observed chronically after TMLR may represent a novel mechanism whereby such therapy imparts symptomatic relief. However, remodelling the intrinsic cardiac nervous system in this manner may exert deleterious effects on cardiac function.

Chapter 6 Function of human intrinsic cardiac neurons *in situ*

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6.1 Introduction

Multiple neuronal subtypes have been identified within the mammalian intrinsic cardiac nervous system, both anatomically[14,169,190,191,199,202] and functionally[12,13]. It has been proposed that the intrinsic cardiac intrinsic nervous system is important for the maintenance of adequate cardiac output[200], particularly in disease states such as myocardial ischemia[12,23]. Canine intrinsic cardiac neurons display complex behavioural patterns that rely, to a considerable degree, on their cardiovascular sensory inputs. These inputs, in turn, are dependent upon cardiovascular status[8,13,33,39]. It is known that the human cardiac efferent nervous system displays functional characteristics similar to those found in animals[255,256]. It remains to be established whether neurons on the human heart behave in a manner similar to that identified in animal models. Furthermore, we do not know what effects cardiopulmonary bypass (CPB) and cardioplegia have on the human intrinsic cardiac nervous system.

The present experiments were designed to determine whether human intrinsic cardiac neurons generate spontaneous activity and, if so, how they respond to altered cardiovascular status. Additionally, we sought to determine whether cardiac afferent inputs to the human intrinsic cardiac nervous system alter its behaviour. Finally, we sought to determine whether CPB and cardioplegia exert deleterious effects on human intrinsic cardiac neuronal function.

6.2 Methods and Materials

6.2.1 General methods

Ethical approval for human experimentation at our institution is reached through a clinical and layperson peer-reviewed process at the Queen Elizabeth II Hospital, Halifax, Nova Scotia, Canada. Once full ethical approval was achieved, patients were approached for entry into the study. Qualification of entry into this study consisted of any patient with angiographic evidence of a lesion in at least one major coronary artery that required the coronary artery bypass grafting (CABG) procedure (Table 6.1). Patients were excluded if they had depressed left ventricular function, if they required another procedure combined with the CABG procedure or refused to consent to the recording procedure. Ten patients scheduled for coronary artery bypass procedures entered the study after signing the appropriate consent form, thereby providing permission to perform these investigations. Basic demographic data and co-morbid variables were collected from each patient upon entry into the study. Left ventricular ejection fractions (EF) were determined in each patient by means of transthoracic or transesophageal echocardiography, radionucleotide imaging or left ventriculography performed at time of selective coronary catheterization. Operative variables relating to pump and aortic cross-clamp times, times to extubation post-procedure, as well as ICU and total length of stay were assessed for each patient.

6.2.2 Operative procedures

Following instigation of general anesthesia, a midline sternotomy was performed to expose the heart and the appropriate bypass conduit was harvested in the usual fashion for coronary artery bypass grafting. Once the pericardotomy was completed, baseline systemic and pulmonary artery pressures were recorded along with a two lead (leads II and V_5) electrocardiogram. Patients were heparinized and underwent aorto- right atriocaval cannulation in case cardiopulmonary bypass compromised hemodynamics so that cardiopulmonary bypass could be instigated immediately.

6.2.3 Recording neuronal activity

Fatty tissue on the lateral surface of the right atrium that contains the right atrial ganglionated plexus (RAGP)[169,196] was exposed. A tungsten recording microelectrode was employed to record the extracellular activity generated by right atrial neurons[25]. The microelectrode had a shank diameter of 100 μm, an exposed tip of 5 μm and an impedance of 9-11 MΩ at 1,000 Hz. The electrode was held in place by a Marzhauser micromanipulator (model 25033-10, Fine Scientific Tools Inc., North Vancouver, BC) which was then attached to a variable extension arm (Octopus 1; Medtronic, Minneapolis, Minn.) to stabilize its motion.

The fat on the lateral surface of the right atrium was explored with this microelectrode from its epicardial surface deeper to adjacent atrial tissue. An indifferent electrode was attached to the adjacent mediastinum. The RAGP was

chosen for investigation because it contains one of the largest collections of neurons without an associated large coronary artery[169]. Thus it could be explored with an electrode without potentially injuring a major coronary artery. The midline sternotomy permitted easy access to this plexus and thereby minimized stimulation of mechanosensory nerve endings located in the epicardium that could confound neuronal activity results.

The activity generated by right atrial neurons so recorded was amplified differentially by means of two Princeton Applied Research model 113 amplifiers which had bandpass filters set at 300 Hz to 10 kHz and amplification ranges of 100-500X placed in series. The output of these battery-driven amplifiers was led to an audio monitor as well as an Astromed MT9500 8-channel rectilinear chart recorder. Action potentials generated by individual neurons with signal-to-noise ratios greater than 3:1 were studied, individual neural units being identified by the amplitude and configuration of their action potentials. Using these techniques and criteria, the microelectrode does not record action potentials generated by axons of passage, but rather records action potentials generated by neuronal somata (cell bodies) and/or dendrites.

6.2.4 Intraoperative procedures

Once an active site was identified, various loci on the exposed epicardial surfaces of the right and left ventricles were touched gently. The epicardial mechanical stimuli so applied were insufficient to distort the heart and thus alter the position of the recording electrode tip. This procedure was performed in

order to determine if identified RAGP neurons received mechanosensory inputs from such epicardial regions. Then systemic arterial pressure was reduced or increased by approximately 30% following administration of nitroglycerine (50-100 μg i.v. boluses) or phenylephrine (50-100 μg i.v. boluses), respectively.

Neuronal activity and cardiovascular variables were also monitored during the following interventions: i) just prior to instigating total cardiopulmonary bypass (CPB); ii) when the patient was on full cardiopulmonary bypass prior to applying the aortic cross-clamp; iii) during cardioplegia as ECG quiescence occurred, and iv) at the end of the initial cardioplegia infusion prior to starting the first distal coronary artery anastomosis. A combination of cold blood and crystalloid (4:1 ratio) is employed for cardioplegia at our institution during CPB. Once the coronary revascularization procedures had been completed, neuronal activity and cardiovascular variables were monitored as bypass was discontinued. Lastly, variables were monitored during the subsequent administration of protamine hydrochloride in 0.9 sodium chloride (150-250 mg i.v.). This agent is routinely employed following completion of the coronary artery bypass procedure in order to reverse the effects of the previously administered heparin. Protamine was administered over a 10-15 minute period. Monitored hemodynamic variables were unaffected by this intervention.

6.2.5 Data analysis

Cardiac variables were analyzed over 30 second periods of time before and during peak responses elicited by each of the interventions described

above. Action potentials with signal-to-noise ratios greater than 3:1 generated within the RAGP were counted for 30-second periods of time in order to establish average activity immediately prior to and during maximal responses elicited by each intervention. Fluctuations in the amplitude of action potentials generated by individual neurons varied by less than 25 μV over several minutes. Thus, action potentials so generated retained their same configurations over time. Action potentials recorded in a given locus with the same configuration and amplitude (± 25 μV) were considered to be generated by the somata and/or dendrites of a single neuron. The means (± SEM) of data recorded during control states as well as during each intervention were calculated. ANOVA and paired t-test with Bonferroni correction for multiple tests were employed for statistical analysis. A significance value of $p < 0.01$ was used for these determinations.

6.3 Results

6.3.1 Patient and operative variables

Patient demographics, co-morbid variables and medications are listed in Table 6.1. The average age of patients was 62 years, with 80% of the patients being male. All patients had preserved left ventricular function. All but one patient received at least two grafts: a left internal mammary artery (LIMA) to the left anterior descending artery (LAD) and either a radial artery (RA) and/or a reversed saphenous vein graft (rSVG) to the remaining diseased territories. Two patients were classified as "in-house" urgent cases, having been in the coronary

care unit awaiting the CABG procedure. There were no complications arising as a consequence of the intraoperative recording procedure. No in-hospital deaths occurred in this cohort of patients. The majority of patients were extubated within 4-7 hours post-operatively. Both "in-house" urgent patients had prolonged hospital stays (17 and 18 days). One of these urgent patients developed left lower lobe pneumonia post-operatively. The other patient had low cardiac output postoperatively requiring low doses of dopamine and epinephrine for 24 hours. The rest of the patients had uncomplicated recoveries.

Patient Demographics (± SEM)	
Age	61.7 ± 2.8 (range: 49-74)
Sex (M/F)	8/2
Number of Disease Vessel Territories (median ± IQR)	3 (2-3)
Pts. with Proximal RCA Lesion	6
Pts. with Cardiac Risk Factors:	
Current Smoker	1
NIDDM	4
HTN	2
Hyperlipemia	7
Medications:	
β-Blockers	10
CCB	4
ACE-I	3
Nitrates	8
EF (%)	61.0 ± 2.9
Pump Time (min)	110.6 ± 12.0
X-Clamp Time (min)	84.2 ± 11.3
Time to Extubation (hrs)	7.2 ± 1.3
Length of Stay (days) (median ± IQR)	8 (5-9)

Table 6.1 *Tabulated data concerning patients' age, sex, diseased vessel territories and risk factors.*

Medications provided to patients are listed along with their left ventricular ejection fractions, pump time, aortic cross-clamp time (X-clamp), time to extubation and length of hospital stay.

6.3.2 Neuronal activity

Two to three intrinsic cardiac neurons in a RAGP locus in each investigated patient generated spontaneous activity during control states before cardiopulmonary bypass was instigated. The activity so identified was sporadic in nature and, for the most part, not related to a specific phase of the cardiac cycle (Fig. 6.1). Immediately before instigating cardiopulmonary bypass, the activity generated by right atrial neurons in anesthetized patients averaged 59±11 impulses per minute (imp). At that time these patients' systemic arterial pressure was, on average, 90/56 mm Hg (Table 6.2).

The activity generated by identified spontaneously active neurons increased in 7 of the 10 patients when limited loci on the exposed anterior epicardium of the right or left ventricles were touched gently (Table 6.2). No responses were elicited in the other 3 patients when the exposed surfaces of the two ventricles were touched. In 6 of these 7 patients, additional neurons were recruited when mechanical stimuli were applied to the epicardium, as determined by the varied configurations of the action potentials identified (Fig. 6.2).

Interventions	HR (beats/min)	PAP (mm Hg)	AP (mm Hg)	Neuronal activity (ipm)
Before (n = 10)	71±3	24±1/12±1	90±5/56±2	59±11
Following phenylephrine administration	69±3	27±2/14±2	126±7/69±3	167±18*
Before (n = 10)	68±2	26±2/13±1	111±5/66±3	144±27
Following nitroglycerine administration	68±3	23±2/12±1	95±4/57±2	45±10*
Before touching epicardium (n = 7)	72±3	20±2/10±1	100±5/58±2	66±17
Touching epicardial loci	70±3	20±2/10±2	104±6/66±4	202±46*
Prior to CPB and cardioplegia (n = 9)	73±3	22±2/11±1	100±6/60±2	83±19
After CPB and cardioplegia	75±3	23±3/12±1	89±10/69±5	114±46

Table 6.2 *Intrinsic cardiac neuronal and cardiodynamic changes with various interventions during the course of the procedure.*

Alterations heart rate (HR), pulmonary artery pressure (PAP) and aortic pressure (AP) as well as the activity generated by right atrial neurons (ipm = impulses/minute) with during various interventions. Neuronal activity increased when aortic pressure increased following phenylephrine administration. Neuronal activity decreasing as systemic pressure fell following systemic administration of nitroglycerine. Touching ventricular epicardial loci activated neurons in 7 patients. Neuronal activity was similar before and after cardiopulmonary bypass (pump) and cardioplegia. * $p < 0.01$ control versus intervention.

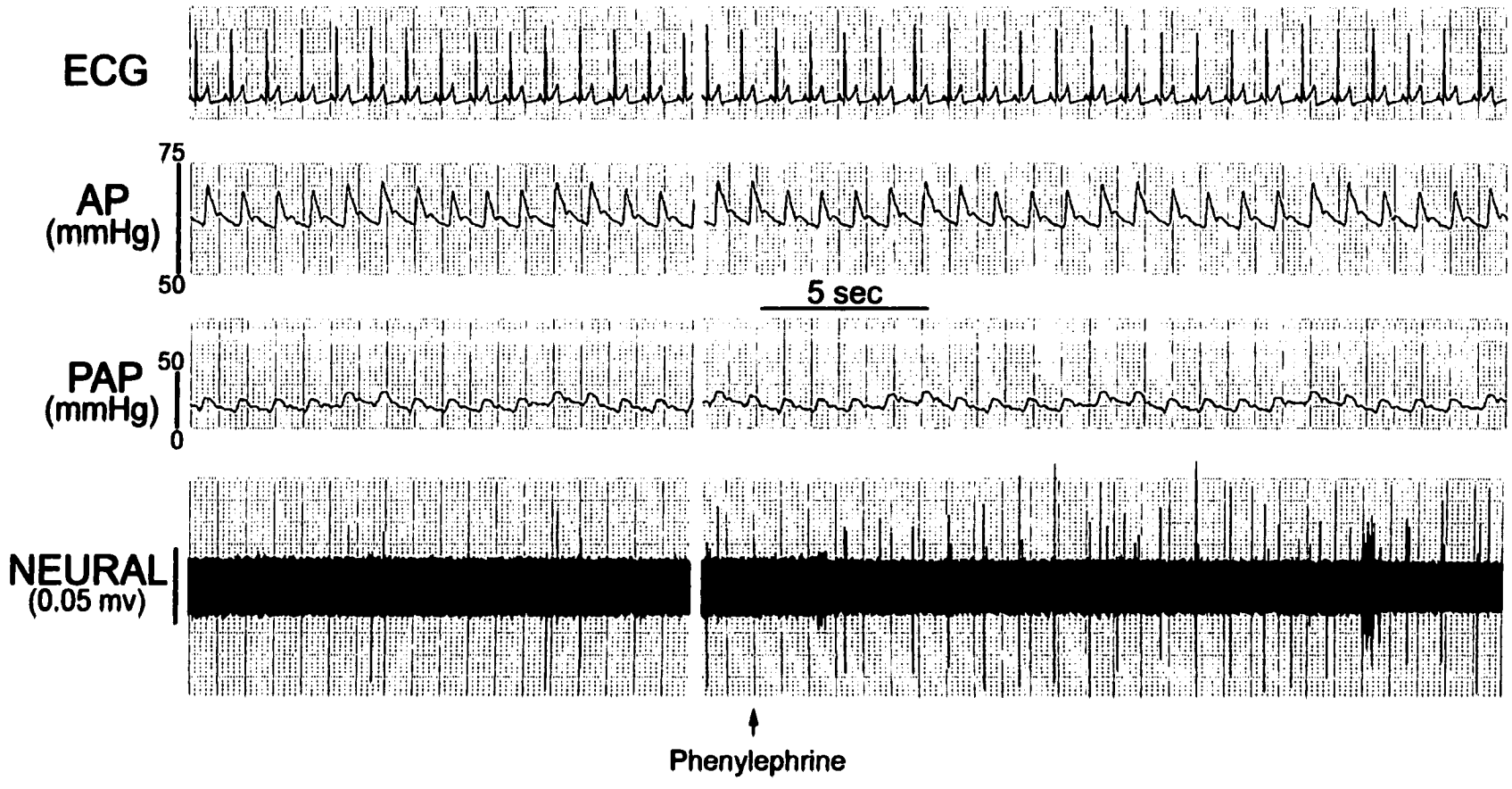

Figure 6.1 *The effect of phenylephrine on the human intrinsic cardiac nervous system.*

Continuous recording of the activity generated by right atrial neurons and cardiovascular indices obtained before and immediately after administering a bolus dose of phenylephrine into the circulation (at arrow below). Neuronal activity increased soon after this occurred before any change in recorded variables occurred (note that a paper fold prevented recording part way through the record).

When systemic arterial pressure increased following systemic administration of the alpha-adrenoceptor agonist phenylephrine, intrinsic cardiac neuronal activity increased (Table 6.2). This included the recruitment of new units. In some instances, the activity generated by right atrial neurons increased before any detectable changes in monitored cardiovascular variables became evident (Fig. 6.1). In contrast, when systemic arterial pressure was reduced by the systemic administration of nitroglycerine, intrinsic cardiac neuronal activity decreased (Table 6.2).

The activity generated by intrinsic cardiac neurons remained at control levels (57±18 impulses per minute) upon instigation of CPB prior to the application of the aortic cross-clamp and infusion of cardioplegia. Intrinsic cardiac neurons continued to generate activity (41±29 imp) during infusion of the cardioplegia solution (Fig. 6.3B). Neuronal activity persisted when the ECG became quiescent, but at a reduced level. For instance, after a period of prolonged cardiac standstill after the initial cardioplegia period right atrial neurons generated 21±12 imp ($p<0.01$, compared to control values) (Fig. 6.3C). At the completion of the coronary artery bypass grafts and weaning the patient from cardiopulmonary bypass, neurons generated activity levels that were similar to those recorded before these interventions were instigated (Table 6.2). At the end of the procedure, protamine hydrochloride was administered into the systemic circulation to reverse the effects of previously administered heparin. When neuronal activity was monitored in 4 patients, administration of this peptide enhanced the activity generated by some neurons while activating

previously quiescent neurons in other instances (Fig. 6.4). This occurred without any alterations in monitored cardiovascular variables being detected. No untoward reactions were elicited following protamine administration.

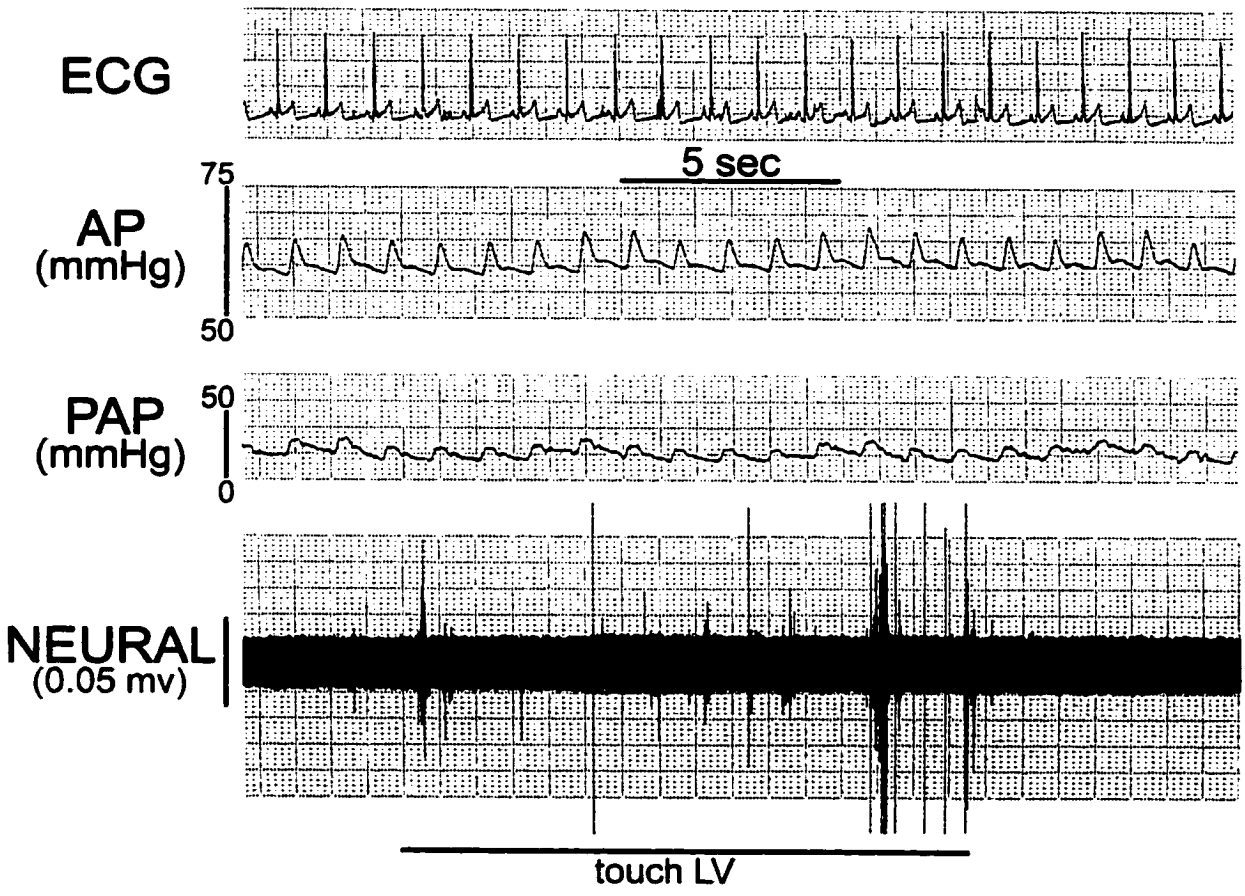

Figure 6.2 *The effect of epicardial touch on human intrinsic cardiac neuronal activity.* Human intrinsic cardiac neuronal response elicited by gently touching a locus on the left ventricular ventral epicardium (horizontal line below). A burst of activity was generated by right atrial neurons (neural: lowest panel) during application of this stimulus. Monitored cardiovascular variables remained unchanged. ECG = electrocardiogram; AP = aortic pressure; PAP = pulmonary pressure (the same abbreviations are used in the other figures).

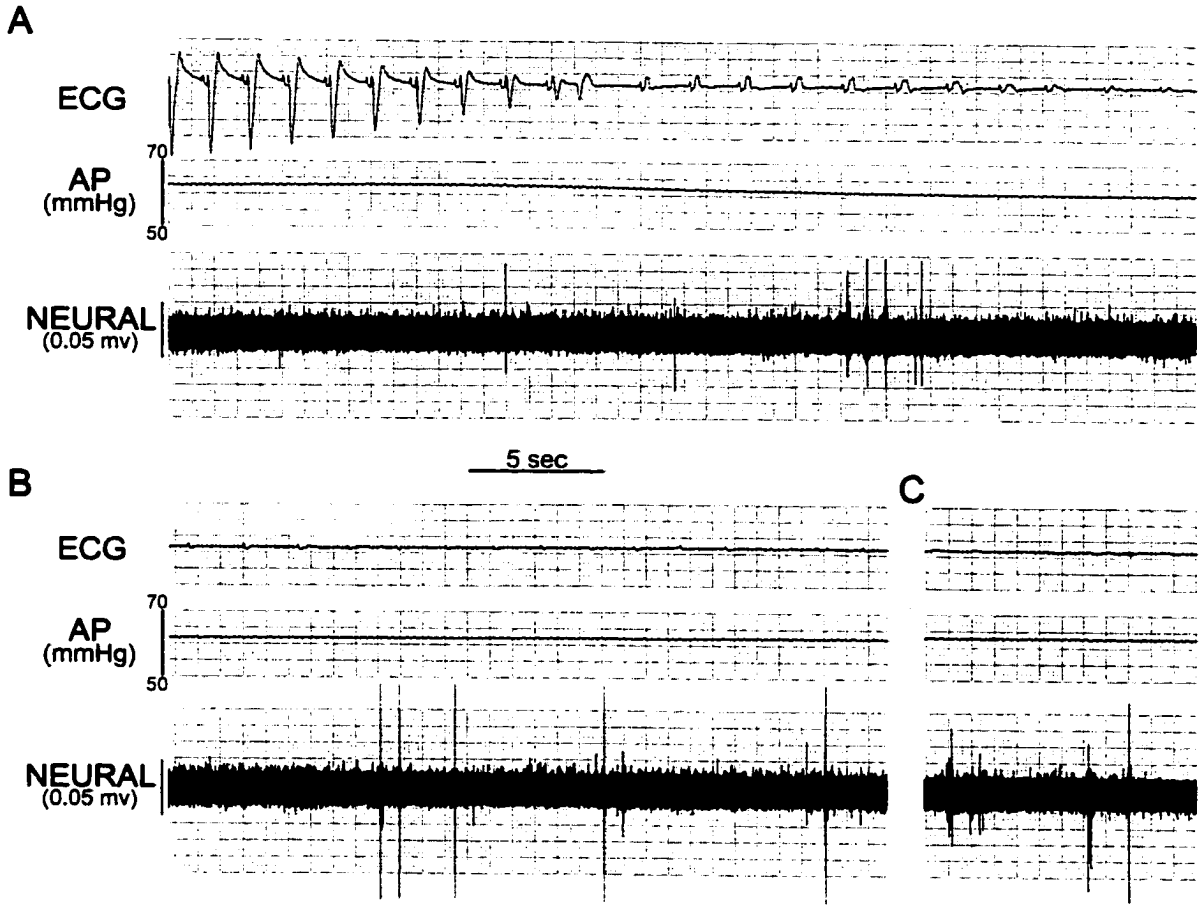

Figure 6.3 *Human intrinsic cardiac neuronal activity during the infusion of cardioplegia.* A and B represent a continuous record of the ECG, aortic pressure and right atrial neuronal activity obtained from the beginning of infusing cardioplegia solution until the point of ECG quiescence. Note that neuronal activity persisted in the presence of suppressed cardiac electrical activity (panel B). Panel C represents data obtained after cardioplegia infusion has been instituted for some time, just prior to performing the first distal anastomosis.

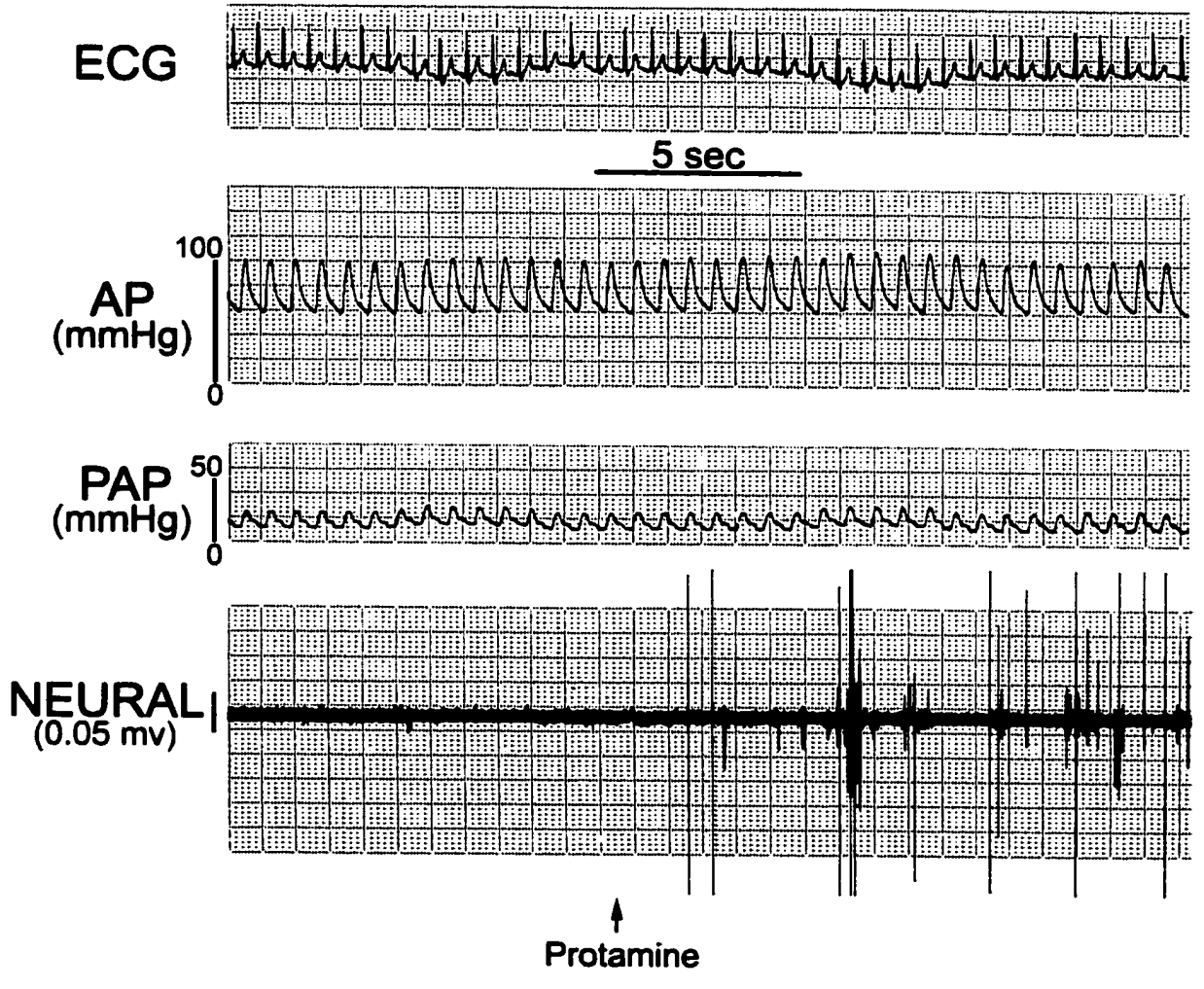

Figure 6.4 *The effect of intravenous infusion of a peptide agent on the human intrinsic cardiac neuronal activity.*

A burst of activity was generated by right atrial neurons soon after commencing the administering of protamine into the circulation (arrow below). Recorded cardiovascular indices remained unchanged.

6.4 Discussion

The results obtained in these experiments demonstrate that populations of human intrinsic cardiac neurons generate spontaneous activity in patients undergoing cardiac surgery following induction of anesthesia and performing a midline sternotomy. These data support those derived from the canine model[23,25-27] in as much as the activity generated by right atrial neurons was for the most part sporadic in nature and thus unrelated to the cardiac cycle (Fig. 6.1). As has been found in experimental animals, populations of human intrinsic cardiac neurons receive inputs from ventricular mechanosensory neurites. It was additionally found that CPB or cardioplegia do not appear to reduce the capacity of human intrinsic cardiac neurons to generate spontaneous activity after their discontinuance (Table 6.2). Lastly, the activity generated by human intrinsic cardiac neurons is dependent on cardiodynamic status.

Right atrial neurons were activated in most patients when limited loci on the ventral epicardial surfaces of either ventricle were touched briefly (Table 6.2). Presumably this occurred because not all investigated neurons received mechanosensory inputs from the epicardial areas investigated with local mechanical stimuli, as occurs in animal models[12,25]. The fact that human intrinsic cardiac neurons receive cardiac mechanosensory inputs (Fig. 6.2) may explain, in part, the fact that the activity generated by many identified right atrial neurons changed in concordance with alterations in the cardiovascular status (Table 6.2). For instance, when systemic arterial pressure increased as a

consequence of administering phenylepherine (representing an increase in afterload) neuronal activity increased. Likewise, when systemic arterial pressure decreased during administration of nitroglycerine (representing a reduction in afterload and preload), neuronal activity decreased. These data support those derived from the canine model in as much as animal intrinsic cardiac neurons are known to receive direct inputs from ventricular mechanosensory neurites and thus are sensitive to changing cardiodynamics[12,25,39]. In accord with that, most identified neurons became inactive when systemic arterial pressure fell below 60 mm Hg. Presumably that was primarily due to a relative reduction of cardiac mechanosensory inputs to the intrinsic cardiac nervous system. This agrees with the fact that neuronal activity was lowest during cardiac standstill when the ECG was quiescent.

Administering the alpha-adrenergic agonist phenylephrine increased neuronal activity concomitant with increases in systemic arterial pressure (Table 6.1). These neuronal responses accompanied changes in monitored cardiovascular indices presumably reflecting increased sensory inputs arising from central reflexes as a result of global changes in cardiovascular status. In some patients activation of right atrial neurons occurred even before any detectable changes in monitored cardiovascular indices became evident (Fig. 6.1). Populations of canine intrinsic cardiac neurons are known to be sensitive to exogenous applied alpha-adrenoceptor agonists[257]. Thus alpha-adrenoceptor agonists may directly affect some human intrinsic cardiac neurons. Human intrinsic cardiac neurons also proved to be sensitive to protamine (Fig. 6.4). The

fact that some atrial neurons were modified by this peptide is in accord with the fact that canine intrinsic cardiac neurons are sensitive to multiple chemicals, including peptides and amino acids[12,258].

Cardioplegia and CPB do not appear to adversely affect the capacity of human intrinsic cardiac neurons to generate spontaneous activity (Fig. 6.3). Intrinsic cardiac neuronal activity was restored to baseline values by the end of bypass (Table 6.2). We have proposed that proper functioning of the final common regulator of cardiac behaviour, the intrinsic cardiac nervous system, may be important in the maintenance of adequate cardiac output[200]. This aspect of the human intrinsic cardiac nervous system may be relevant with respect to modifying cardiac function in the perioperative period.

6.4.1 Summary

Human intrinsic cardiac neurons generate spontaneous activity, as is found in animal models. Furthermore, as in the canine model, human intrinsic cardiac neuronal activity is dependent upon cardiovascular status. Exogenously administered therapeutic agents can also modify their behaviour. These data provide a basis for the development of novel therapy targeting the human intrinsic cardiac nervous system in the perioperative period. The fact that intrinsic cardiac neurons retain their function post cardiopulmonary bypass implies that the human intrinsic cardiac nervous system can be manipulated to advantage in the post-operative period.

Chapter 7 Discussion

7.1 Overview

This work represents an initial venture of translating decades of "bench-side" data on the mammalian intrinsic cardiac nervous system to the "bedside" with the hopes of gaining an understanding of how the intrinsic cardiac nervous system functions in clinically relevant practice. Specifically, I sought to investigate how the intrinsic cardiac nervous system is altered in myocardial ischemia and in novel treatment modalities for ischemic heart disease. In addition, I sought to determine if any resultant neuronal alteration in these settings could be manipulated therapeutically. This section begins with an overview of the main findings covered in this thesis. This will then be followed by a discussion of the concept of "remodelling" as it pertains to the intrinsic cardiac nervous system and concludes with an examination of the directions in which this area of research may evolve.

7.2 Anatomical Study

Previous studies have examined the location and distribution of nerves on the porcine heart[173,174]. To utilize the adult porcine model in functional physiological analyses, determination of the topographic arrangement of the intrinsic cardiac nervous system was required. The porcine heart contained a large number of intrinsic cardiac neurons (~23 000) located in 11 discrete atrial and ventricular locales. These neurons were arranged in ganglia that varied in size from small ganglia containing a few neurons to larger collections with greater

than 100 neurons (the latter number being the arbitrary maximum set for the larger ganglia). The number of neurons identified in the porcine heart was approximately double the number of neurons identified in the canine and human models[169,194]. A distinct feature of the porcine model was the comparable distribution of neurons to both the atrium and ventricle. These data indicated that the porcine model was suitable for the study of physiological intrinsic cardiac neuronal interactions, particularly with respect to its ventricular components. This study, therefore, provided the anatomical substrate for functional analysis of the effects of ventricular ischemia on the intrinsic cardiac nervous system using the porcine model.

7.3 Physiological Studies

7.3.1 Manipulating intrinsic cardiac neuronal response during reperfusion

Timely reperfusion following myocardial ischemia is necessary for limiting the amount of myocardial necrosis associated with coronary occlusion. In certain conditions this "re-oxygenation" may not be without consequence and carries the potential to cause further myocardial injury [52,54,55,259]. This reperfusion injury has been implicated in the generation of ventricular arrhythmias following coronary thrombolysis, interventional revascularization and as a cause of death following spontaneous resolution in coronary spasm syndromes[55,63-66,259].

Physiologic investigation of porcine ventricular intrinsic cardiac neurons using an extracellular recording technique *in situ*[25] demonstrated that transient regional ventricular myocardial ischemia and subsequent reperfusion significantly

altered neuronal activity. It is important to reiterate that coronary artery occlusion employed did not directly involve the local arterial blood supply to the somata of investigated neurons. Exogenous adenosine administered into the local blood supply of investigated neurons was able to alter intrinsic cardiac neuronal activity associated with myocardial reperfusion but not during a preceding ischemic episode. This adenosine effect during reperfusion altered neuronal activity towards pre-ischemic levels. It was additionally determined that this attenuation effect of adenosine could be blocked with A_1 but not A_2 receptor antagonism. Lastly, adenosine administration during reperfusion was also associated with a trend of increased electrical stability and a reduction of ventricular dysrhythmias whereas A_1 receptor blockade had the opposite effect.

These data suggest therapeutic intervention that targets intrinsic cardiac neuronal adenosine A_1 receptors may act to stabilize the processing of cardiac sensory information within the intrinsic cardiac nervous system during myocardial reperfusion. The potential for clinical application of these findings could be utilized in a "real-time" manipulation of the intrinsic cardiac nervous system in the setting of PTCA or off-pump coronary artery bypass grafting procedures where the administration of therapeutic doses of adenosine could prevent the occurrence of lethal ventricular arrhythmias. Further studies are required to determine the role of the neuronal components within the intrinsic cardiac nervous system that are involved in reperfusion injury to confirm the clinical utility of adenosine receptor modification.

7.3.2 The effect of novel therapies utilized in the treatment of end-stage coronary artery disease on the intrinsic cardiac nervous system

The effect of myocardial ischemia on cardiac neurons demonstrates intrinsic cardiac nervous system is not an "idle bystander" during such pathophysiological events. Chemical manipulation of these neurons can improve the stability of the heart during reperfusion following acute, regional myocardial ischemia. The following section elaborates on this concept with discussion of the utility of electrical/mechanical manipulation to assist in the mitigation of potential neuronal imbalances created during chronic myocardial ischemia.

7.3.2.1 Spinal cord stimulation (SCS)

Functional studies of the neuronal effects of electrical stimulation of spinal cord neurons demonstrated their ability to modulate the intrinsic cardiac nervous system in normally perfused hearts as well as during periods of regional myocardial ischemia and reperfusion. Spinal cord stimulation continued to exert its modulatory effects on the intrinsic cardiac nervous system long after its activation had ceased.

Spinal cord stimulation stabilized intrinsic cardiac neuronal activity associated with myocardial ischemia and reperfusion. SCS altered information arising from the ischemic myocardium, attenuating activity generated by intrinsic cardiac neuronal somata. In contrast to adenosine, stimulation of spinal cord neurons reorganized information processing within the intrinsic cardiac nervous system arising from the ischemic myocardium, in addition to the reperfusion post-

ischemic phase. The neuronal-stabilizing influences of SCS also extend to modification of the cardiac sensory inputs to the intrinsic cardiac nervous system. Data generated from these experiments demonstrated a reduction in neuronal responses normally elicited by the application of topical veratridine to the ventricular epicardium. The sustained effect of SCS on the intrinsic cardiac nervous system has the clinical correlate of symptomatic anginal relief extending well after termination of the stimulation device. The prolonged salutary effects that SCS imparts to some patients long after it is discontinued may, in part, be due to remodelling of the intrinsic cardiac nervous system.

7.3.2.2 Transmyocardial laser revascularization (TMLR)

In an acute study that utilized TMLR, the creation of multiple channels with a Ho:YAG laser did not alter afferent or efferent neuronal activity in the canine heart[171]. As there are over 20 000 neurons on the human heart, it was deemed unlikely that the creation of 20-40 laser channels would have a significant impact on the functioning of the intrinsic cardiac nervous system.

Data derived from this study, however, indicate that the function of the intrinsic cardiac nervous system is substantially altered within four weeks of performing the TMLR procedure. While TMLR did not denervate cardiac afferent or extracardiac efferent neuronal function in ventricular regions in a chronic setting, the functioning of post-ganglionic sympathetic and parasympathetic efferent neurons were remodelled. This was demonstrated by the lack of response of the intrinsic cardiac nervous system despite the application of supra-

maximal doses of nicotine. This indicated a global change in the functioning of the intrinsic cardiac nervous system following the creation of multiple, small diameter transmural channels. Thus, the beneficial effects that TMLR affords patients with ischemic heart disease cannot be ascribed to local ventricular denervation as has been suggested in previous reports [164-166,168]. Instead, long-term alteration or "remodelling" of the intrinsic cardiac nervous system, the final common regulator of regional cardiac function occurs within a month following TMLR.

7.4 Remodelling of the Intrinsic Cardiac Nervous System

While the development of certain dysautonomias, like Shy-Drager Syndrome, can result in functional denervation of the heart, it is becoming increasingly apparent that in most cardiac pathologies the intrinsic cardiac nervous system continues to function, albeit in a reorganized (remodelled) manner. Remodelling, as defined by both the Merriam-Webster[260] and Oxford (Canadian Ed.)[261] dictionaries, is an "[alteration in] the structure or shape" of an object. Preliminary data derived from human specimens suggest that remodelling of the physical structure of intrinsic neurons may occur in ischemic heart disease [169,196]. In addition to this architectural re-arrangement of neuronal somata, a functional re-structuring of neuronal activity can occur following surgical or chemical manipulation of the system. An example of this functional alteration in intrinsic neuronal activity has been investigated following cardiac transplantation.

Traditionally, surgical interruption of the nerves innervating the heart, as occurs in cardiac transplantation, has been thought to result in degeneration of intrinsic cardiac neurons and thus the chronic loss of neuronal inputs to cardiomyocytes. Even if the heart "re-innervates" post-transplantation[262,263], the lack of extra-cardiac parasympathetic inputs to intrinsic cardiac neurons in the acute period following the procedure was felt to result in the degeneration of these intrinsic cardiac neurons due to their inactivation[264]. As a result, the maintenance of appropriate cardiac responses to stress/stimuli in a 'denervated' transplanted heart would therefore be relegated to circulating neurochemicals (such as epinephrine and norepinephrine) acting directly on the cardiomyocyte[265,266].

In contrast, functional assessment of intrinsic cardiac neuronal activity following cardiac auto-transplantation in the canine model demonstrated that cardiac transplantation induced a reorganization of the neurohumoral control mechanisms regulating regional cardiac function rather than denervation[16,251]. The transplanted heart retained a functional nervous system that was comprised of afferent, efferent (cholinergic and adrenergic), and local circuit neurons[16,251]. Upon examination of post-ganglionic efferent neuronal activity, however, the functioning of intrinsic cardiac neurons was modified. Similar to the findings in the chronic TMLR model, regional cardiac function was more dependent on angiotensin II than nicotine-sensitive intrinsic cardiac neurons.

Re-organization of the intrinsic cardiac nervous system has also been demonstrated in chronically decentralized preparations. In this model, extra-

cardiac neuronal inputs were surgically severed instead of direct manipulation of the heart itself[15]. In a chronic, canine model of decentralization, intrinsic cardiac neuronal activity could still be modulated by adrenergic and peptidergic agonists, amino acids and other neurotransmitters[15,20]. In this setting, remodelling of intrinsic cardiac neurons consisted of an alteration in responsiveness to cholinergic modulation. This was demonstrated by an increased sensitivity to muscarinic receptor activation as compared to nicotine receptor activation[267].

It would appear therefore, that a number of surgical procedures and therapeutic interventions have the capacity to alter the function of intrinsic cardiac neurons. The issue of neuronal remodelling assumes clinical relevance with respect to understanding the neurocardiological basis for the integrated cardiac response to myocardial ischemia and the effects of specific intrathoracic surgical interventions on cardiac control.

Exogenous adenosine administered to the local coronary blood supply of ventricular intrinsic cardiac neurons re-organized neuronal activity in a manner associated with increased neuronal stability and decreased arrhythmia formation in animals tested. In confirmation with these results, others have indirectly demonstrated the ability of adenosine to modulate sympathetic inputs in the post-ischemic phase based on norepinephrine effluent in isolated heart preparations[110]. While current anti-arrhythmia therapies have focused on cardiomyocytes, targeting the cardiac nervous system may serve as a viable alternative approach to such pathological conditions.

The activation of spinal cord neurons induced a conformational change in the intrinsic cardiac nervous system that persisted for an extended period of time (though not permanently) after terminating such activation. This remodelling of the intrinsic cardiac nervous system, unlike the results found following local administration of adenosine, can override excitatory inputs arising from the ischemic myocardium. The contribution of adenosine (if any) to this SCS response is not clear. Adenosine may play a role in the reperfusion phase of neuronal remodelling with SCS, however further examination is required to elucidate this matter.

A more persistent form of remodelling of the intrinsic cardiac nervous system occurred following TMLR. In contrast to the previous two examples described above, this remodelling may not be beneficial to the overall function of the heart. Indeed, while the canine preparation employed is usually a robust model capable of tolerating manipulation without hemodynamic compromise, animals that had undergone the TMLR procedure did not tolerate surgical stress in the usual fashion. The performance of a repeat thoracotomy for physiologic assessment 4 weeks following TMLR was accompanied by a notable decrease in systemic blood pressure and an episode of ventricular fibrillation in one animal. Hemodynamic compromise or dysrhythmia formation did not occur in animals that underwent a sham procedure. The intrinsic cardiac nervous system additionally proved to be non-responsive to the potent chemical agonists nicotine or angiotensin II. Taken together, these data indicate that the global functioning of intrinsic cardiac efferent neurons becomes obtunded within a month following

TMLR. A clinical correlate of this abnormal cardiac function observed in treated animals has now been examined in patients who have undergone the TMLR procedure. A reduction in left ventricular function (as determined by a reduced left ventricular ejection fraction and echocardiographic data demonstrating resting ventricular wall motion abnormalities have been observed several months after the TMLR procedure[250,253,254]. It would appear therefore, while the remodelling of the intrinsic cardiac nervous system observed chronically after TMLR may represent a novel mechanism whereby such therapy imparts symptomatic relief, remodelling in this manner may exert deleterious effects on cardiac function.

7.5 Does Remodelling of the Intrinsic Cardiac Nervous System Involve Memory?

The intrinsic cardiac nervous system is capable of remodelling for variable durations (short-term and long-term effects). Remodelling of the intrinsic cardiac nervous system may be beneficial to cardiac functioning, as in the use of exogenous adenosine or SCS. Conversely, remodelling following TMLR may possibly be maladaptive in maintaining adequate cardiac output. In effort to develop an "apt analogy" for the remodelling observed in this neuronal system, it is suggested that the functional re-organization of the functioning of the intrinsic cardiac nervous system may result in the induction of memory.

7.5.1 Conceptual framework for memory system development outside of the cerebral cortex

The Oxford dictionary defines memory as "the faculty by which things are recalled to or kept in mind"[261]. The fundamental principles of the development of memory include the following processes: 1) registration: the retrieval of information; 2) encoding: a process by which information is held "online" and prepared for storage; 3) consolidation: placing encoded information into storage for later recall; 4) retrieval: getting the information out when needed. In consideration of storage of information, memory can be broadly grouped into two general classes: short-term and long-term memory. The function of short-term memory (STM) (also referred to as primary memory[268] or working memory[269] is to register and retain incoming information for limited periods of time. This time limit is usually seconds in duration, but may persist beyond the physical duration of the stimulus[270,271]. Long-term memory (LTM), on the other hand, involves the permanent storage of memory that is accessible at a delayed point in time after the initial presentation of a stimulus[268,270]. LTM is associated with indefinite periods of storage, lasting from hours to days to the lifespan of an individual.

These definitions for memory systems have been traditionally applied only to neurocognitive behaviour within the cerebral cortex and not processes elsewhere in the body. Upon reflection of the fundamental processes required in the development of memory, however, memory can be aptly applied to other organ systems. The immune system is one such example.

Upon exposure to a particular immunogen (i.e., stimulus), a process begins whereby production of antigen-specific anti-bodies to combat the

offending agent occurs. At a later point in time, when qualitatively similar immunogen is re-introduced, a much more rapid response is initiated resulting in a more intensive and persistent anti-body production. It has been proposed that "memory" T and B lymphocyte cells generated during the primary response are responsible for the more vigorous and pronounced secondary responses[272]. This adaptation, or memory, of the immune system benefits the organism by reducing the circulation time of the offending immunogen. In certain circumstances, however, this memory produced by the primary exposure can result in an excessively energetic secondary response causing cardiorespiratory or possibly lethal complications (such as seen in a hypersensitivity reaction, i.e., anaphylaxis). In this second example it is apparent that "memory" can also be maladaptive.

A more applicable definition for the discussion of memory is put forth in the Merriam-Webster dictionary. It is defined as "...the store of things learned and retained from an organism's activity or experience as evidenced by modification of structure or behaviour or by recall and recognition". This more general definition is echoed by Tulving[273] in which he states that all different "...memory systems share a [common] feature – they enable the organism to make use of information acquired on an earlier occasion." He further states that "...a behavioural event registered in a more primitive system may carry minimal information about the past event, although sufficient information to determine or modify future behaviour". These more broad definitions of memory can also be appropriately applied to the peripheral nervous system.

7.5.2 Biological basis for memory in the intrinsic cardiac nervous system

A biological explanation for memory has been described using a model utilizing abdominal neuronal ganglia in invertebrate species. Kandel and co-workers have examined the defensive withdrawal reflex of the respiratory organ in large marine molluscs (*Aplysia Californica*)[270,274]. In this primitive invertebrate, stimulation of parts of this organ (the gill and siphon) causes the organ to vigorously contract, a reflex that is analogous to vertebrate autonomic reflexes[270,274,275]. The experimental presumption of Dr. Kandel and colleagues' work (for review see Kandel, 2000[276]) equate increased synaptic activity (or synaptic strength) for the anatomical basis for improved neuronal plasticity (i.e., memory)[270,274,277,278]. This model of the "processing and storage" of sensory information in abdominal ganglia has generated important molecular data on the differences between short- and long-term memory.

These two phases of memory differ not only in their duration but also in their molecular mechanisms. Short-term memory involved the modification of existing proteins (neurotransmitters), resulting in their increased quantal release and strengthening of pre-existing synaptic connections[270,278-280]. Conversely, long-term memory involved altered expression of certain genes and subsequent protein expression with the addition of new synaptic connections[274,281-283]. In addition to these findings, the changes associated with LTM can be manipulated. Long-term memory in the *Aplysia* model can be either enhanced or suppressed by manipulation of the genes involved in the expression of proteins associated with improved synaptic strength[282,284].

7.5.3 Memory in the intrinsic cardiac nervous system

Based on the notion that lower organisms are capable of establishing memory systems in neurons in abdominal ganglia[270], data presented here may suggest memory can also be extended to the mammalian intrinsic cardiac nervous system. Adenosine modulatory effects on reperfusion afferent inputs to the intrinsic cardiac nervous system were short-lived, akin to short-term memory effects. Evidence of this concept is further demonstrated by the observation of a diminution in neuronal activity in the animals that experienced increases in neuronal activity during reperfusion and an increase in activity in animals in which reperfusion resulted in a reduction of intrinsic neuronal activity. Adenosine administration altered the processing of sensory information (likely via local circuit neurons) during reperfusion and was associated with increased stability within the system. A similar parallel can be drawn with the data presented with SCS. The salutary effects of SCS on the intrinsic cardiac nervous system persisted well after the termination of the stimulation episode. This extended short-term memory-like effect is supported by research performed in the central nervous system demonstrating that high-frequency stimulation of olfactory afferent fibres can induce a prolonged adaptation of synaptic responses in the hippocampus[278,285].

In addition to the above examples of short-term memory, it would appear that the intrinsic cardiac nervous system is also capable of long-term memory. It was found that TMLR produced a long-term re-organization of the intrinsic cardiac nervous system within four weeks following the procedure. The reduced

capacity of the animals to respond appropriately to surgical stressors, however, questions if all memory effects (or perhaps lack of memory) are beneficial to the functioning of this nervous system.

7.5.4 Summary

The theoretical framework for neural networks establishing a memory system in the intrinsic cardiac nervous system is supported by preliminary data presented in this thesis. Whether it is possible to enhance the "memory" of the intrinsic cardiac nervous system in patients with ischemic heart disease, however, remains to be determined. It is interesting nonetheless, that "centrally" acting therapeutic agents used to improved memory in patients with Alzheimer's-type dementia also have been shown to have modulatory effects on the intrinsic cardiac nervous system[176,286]. As such, with further refinements, it is possible to envision the development of novel drug or gene therapeutics that target this important regulator of cardiac function.

7.6 Future Perspectives

For the intrinsic cardiac nervous system to be a clinically valuable therapeutic target it must have the capability of being manipulated in a wide variety of clinical scenarios. Evidence presented in this thesis demonstrates the intrinsic cardiac nervous system is capable of being modified or remodelled in "real-time" (e.g., myocardial reperfusion) as well as during the short-term (e.g., such as SCS affords) or long-term (e.g., persistent remodelling occurring chronically after TMLR). In addition to these laboratory-based studies,

examination of the intrinsic cardiac nervous system must also be performed in the human subjects.

Others have initiated pioneering work in this direction with intraoperative electrical mapping of ventricular signals associated with arrhythmia formation[287,288] and electrical stimulation of the intrinsic cardiac nervous system[256,289,290]. A preliminary *in vivo* analysis of human intrinsic cardiac neuronal activity has been presented in this thesis. Human intrinsic cardiac neurons generate spontaneous activity, as is found in animal models. An additional important finding is that human intrinsic cardiac neurons are capable of being manipulated in the perioperative period by commonly used therapeutic agents. One could therefore envision targeting ventricular intrinsic cardiac neurons with local administration of adenosine either via a catheter (e.g., such as with PTCA) or a coronary bypass graft intraoperatively, to modify their activity for therapeutic benefit. Another possible role of the intrinsic cardiac nervous system may be to assist the cardiac surgeon in maintaining adequate cardioprotection during the course of an operative procedure requiring the use of cardiopulmonary bypass (the heart-lung machine). For example, insufficient cardioprotection of the right ventricle is often a concern when using a retrograde cardioplegic delivery technique via the coronary sinus (a technique that may be employed in valvular or re-operative surgery). It may be possible to use the intrinsic cardiac nervous system (recording from the RAGP) as a "real-time" intraoperative monitoring tool for the effectiveness of cardioprotection. Alternatively, monitoring this important regulator of cardiac function in the postoperative period (i.e., in an

intensive care setting) may provide an early indication of inadequate cardiac output or, conversely to assess the adequacy of therapies already instituted.

In order for the intrinsic cardiac nervous system to be used in this manner, further refinement of recording techniques and the complete functional assessment of each of the components of the system is required in order to understand how the whole cardiac nervous system functions in specific cardiac disease states.

7.7 Conclusion

The complex role that the cardiac nervous system plays in coordinating regional cardiac function in the normal as well as diseased hearts is just becoming appreciated. I have had the good fortune of entering this field at an exciting time of increasing interest and growth in neurocardiology and its potential clinical implications. The overall objective of this dissertation is to further the process of bridging the void between the bench-side and bedside in the hopes of developing strategies that may be utilized in future treatment strategies for ischemic heart disease. In conclusion, it is my thesis that the intrinsic cardiac nervous system represents an important and so far unexploited frontier of cardiovascular therapy that has the potential of playing a significant role in the treatment of cardiac disease processes.

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