STRESS-INDUCED INSULIN RESISTANCE: PREVENTING HISS-DEPENDENT INSULIN RESISTANCE SUBSEQUENT TO HEMORRHAGE

BY

Larissa Seredycz

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

MASTER OF SCIENCE

Department of Pharmacology and Therapeutics
Faculty of Medicine
University of Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Stress-Induced Insulin Resistance:

Preventing HISS-Dependent Insulin Resistance Subsequent to Hemorrhage

BY

Larissa Seredycz

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

of

MASTER OF SCIENCE

This thesis is dedicated to my late father, Boris Seredycz.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. W. Wayne Lautt for his guidance, direction and supervision. You made my first research experience a memorable one, and it was your support that gave me the confidence to pursue my goals. Your encouragement and valuable time have contributed extensively to this project and for this I thank you.

I would also like to thank my committee members Dr. Minuk and Dr. Sitar for their time and effort in making this thesis the best it could be.

Thank you to my fellow lab members, Parissa Sadri, Heather Prieditis, Cynthia Ritchie, Xie Ming, and Dallas Legare. Your advice and friendship has guided my path and has made coming to work a pleasure. A very special thank you to Maria Reid, who taught me all the methodology that appears in this thesis. Your patience and skill is something I aspire to.

To my wonderful mother and extended family, who have supported me through some of the most special and difficult times in my life. While I am sure there is much more I have yet to learn and conquer, I am comforted knowing that you will always be there for encouragement and support. Your love, and friendship is irreplaceable.

Most importantly, I would like to thank and dedicate all the work within my thesis to my father, Boris Seredycz. Words cannot adequately express the love and admiration I feel for you. Your grace, intelligence, passion and determination have impacted my life in the most profound and powerful way. It was your advice and council that made my bad days brighter and my good days better. Through example, you taught me the importance of education and resulting self-fulfillment. Your positive outlook, beautiful

courageous smile, and kind, encouraging words motivated me to pursue a career in the health sciences. Thank you for 24 glorious years. You are always in my thoughts and prayers.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

COMMONLY USED ABBREVIATIONS

AC – Adenylyl Cyclase

ACTH - Adrenocorticotrophic hormone

ADA – American Diabetes Association

ATP – Adenosine Triphosphate

Ca^{+} – Calcium ion

cAMP - Cyclic Adenosine Monophosphate

CCK - Cholecystokinin

CycloSS - Cyclosomatostatin

E – Epinephrine

FSH – Follicle-Stimulating Hormone

G_i1 – G Protein-Coupled Surface Receptor

G_o2 – G Protein-Coupled Surface Receptor

GH – Growth Hormone

HDIA - HISS-dependent Insulin Action

HDIR - HISS-dependent Insulin Resistance

HIEC - Hyperinsulinemic Euglycemic Clamp

HISS - Hepatic Insulin Sensitizing Substance

IDDM – Insulin Dependent Diabetes Mellitus

IP_3 – Inositol 1, 4, 5 Triphosphate

IPV - Intraportal Infusion

ITT - Insulin Tolerance Test

IV - Intravenous Infusion

LH – Luteinizing Hormone

NANC - Non-Adrenergic Non-Cholinergic

NE - Norepinephrine

NTS – Nucleus Tractus Solitarius

PKA – Protein Kinase A

PKCaM – Protein Kinase Calmodulin-dependent Pathway

PVN – Paraventricular Nucleus

R2 – Somatostatin Receptor type 2

R3 – Somatostatin Receptor type 3

RAAS - Renin-Angiotensin-Aldosterone System

RIA – Radioimmunoassay

RIST - Rapid Insulin Sensitivity Test

SMA – Superior Mesenteric Artery

SOM - Somatostatin

SS - Somatostatin

SSTRs - Somatostatin Receptors

TSH – Thyroid-Stimulating Hormone

UKPDS – United Kingdom Prospective Diabetes Study

VIP – Vasoactive Intestinal Polypeptide

WHO – World Health Organization

ABSTRACT

Hepatic Insulin Sensitizing Substance (HISS) accounts for 55% of the action of insulin. HISS blockade leads to a state of insulin resistance known as HISS-dependent insulin resistance (HDIR). The objective was to test the hypothesis that insulin resistance produced by hemorrhage was HDIR. Insulin sensitivity was measured using the rapid insulin sensitivity test (RIST). Hemorrhage was performed in anesthetized rats by removing blood from an arterial-venous shunt to reduce mean arterial pressure to 50 mmHg. Subsequent to blood removal, a RIST was performed. Complete HDIR was verified by administrating atropine, which blocks the release of HISS.

Hemorrhage caused complete HDIR. However, the post hemorrhage RIST was reduced by 34.3% and not the anticipated 55%. Insulin pharmacokinetics were determined during hemorrhage. It was discovered that the response to administered insulin was confounded by the impact of reduced hepatic blood flow on insulin metabolism that resulted in an increase in the HISS independent (direct) action of insulin against a background of complete HDIR. The effect on insulin kinetics is not relevant for endogenous insulin, since hemorrhage causes suppression of insulin release.

In an attempt to implicate somatostatin as an important mediator in HDIR subsequent to hemorrhage, I examined how exogenous administration would affection HISS action. Somatostatin induced HDIR following administration and its antagonist was shown to block the ability of somatostatin to cause HDIR.

Furthermore, I tested how HDIR was affected during adrenergic or somatostatic blockade subsequent to hemorrhage. Following a control RIST, either an α, β, or a somatostatin antagonist was administered prior to hemorrhage. A subsequent RIST was performed to

test the agent's ability to prevent HDIR. Atropine was administered in order to confirm the presence of HDIR.

To conclude, the sympathetic nerves were not a regulator of HDIR following hemorrhage. However, cyclosomatostatin prevented HDIR from occurring post hemorrhage, thereby indicating that the HDIR produced by hemorrhage was initiated by endogenous somatostatin. Systemic somatostatin levels were measured following acute hemorrhage and were not significantly increased. Therefore, the origin of somatostatin that induces HDIR following hemorrhage may be hepatic in nature, possibly pointing to the existence of somatonergic nerves.

Chapter 1 – Introduction to Type 2 Diabetes, the HISS Paradigm and the research methods used to detect insulin resistance

1.1 - Introduction

Diabetes remains the most commonly encountered endocrine-based disease, with the incidence of type 2 diabetes doubling in the past decade (Hibbert-Jones et al, 2004). The prevalence of diabetes is projected to continue to increase dramatically over the next several decades unless major public initiatives are undertaken.

The work presented in this thesis, as well as cited studies from the laboratory of Dr. W. Wayne Lautt are part of a new paradigm for type 2 diabetes. The paradigm under which we work is based on the depiction and classification of a hepatic putative hormone named Hepatic Insulin Sensitizing Substance (HISS). HISS accounts for 55% of insulin action in the fed state, and can thus lead to severe insulin resistance if its release is blocked via pharmacological, surgical or physiological means.

This thesis is divided into two major areas. Firstly, I will examine how acute blood loss affects HISS-dependent and independent insulin action by employing a test known as the Rapid Insulin Sensitivity Test (RIST). Secondly, I will investigate how certain neuroendocrine mediators induce HISS-dependent insulin resistance subsequent to hemorrhage. The administration of adrenergic antagonists, and a somatostatin antagonist will be used as tools to analyze their agonist's effects in inducing HDIR following acute hemorrhage.

1.2 - History of type 2 Diabetes

Although diabetes has been known since antiquity, and treatments were known since the middle ages, the elucidation of the pathogenesis of the disease occurred mainly in the twentieth century (Ferdinand and Clark, 2004). Diabetes is a Greek word meaning

"a siphon," which refers to the osmotic diuresis that occurs with uncontrolled diabetes. Mellitus comes from the Greek word "sweet," because of excess glucose in the urine. The Greeks named it this because the excessive amounts of urine that diabetics produced attracted flies and bees because of the glucose content. Diagnosis was later confirmed by testing the urine.

The discovery of the role of the pancreas in diabetes is generally credited to Joseph Von Mering and Oskar Minkowski, two European researchers who in 1889, found that when they removed the pancreas of a dog, the dog developed significant traits of diabetes and died shortly afterwards. In 1910, Sir Edward Albert Sharpey-Schafer suggested diabetics were deficient in a single chemical that was normally produced by the pancreas. He proposed calling this substance insulin (cited in McMichael, 1964). The endocrine role of the pancreas in metabolism and the existence of insulin was not fully clarified until 1921, when Frederick Grant Banting and Charles Herbert Best showed that they could reverse the induced diabetes in dogs by giving them an extract from the pancreatic islets of Langerhans of healthy dogs (Banting et al., 1922). For this, Banting and Best, received the Nobel Prize in Physiology and Medicine in 1923.

1.3 - History of Insulin Resistance

The term insulin resistance was first associated with diabetes in the early 1930s by a British physician, Dr. Harold Percival Himsworth (Himsworth, 1936). He coined the term insulin insensitivity to describe patients who were unable to effectively use injected insulin to lower blood glucose levels. Himsworth's work was the first to acknowledge the existence of two distinctly different types of diabetes, and his work was published in 1936 in The Lancet.

1.4 - Classes of Diabetes – Type 1, 2, 3, and 4

There are 4 distinct classes of diabetes. Type 1 diabetes, previously known as "insulin-dependent diabetes mellitus (IDDM) or "juvenile diabetes," is a life-long condition in which the pancreas stops producing endogenous insulin (Homann and Von Herrath, 2004). It occurs when pancreatic beta cells are attacked by the immune system that mistakes self for foreign antigens. People with type 1 diabetes must take daily injections or use an insulin pump to remain healthy and active. Onset may be quite fast (weeks or months), whereas type 2 has a very slow onset (often years).

Type 2 diabetes was formerly known by a variety of partially misleading names such as "adult-onset diabetes," "obesity-related diabetes," "insulin resistant diabetes," or "non-insulin-dependent diabetes" (NIDDM). The development of type 2 diabetes occurs when the peripheral tissues become insensitive to exogenous insulin, which leads to glucose intolerance.

While there are different types of diabetes mellitus, almost all other specific forms of diabetes, accounting for up to 5% of all diagnosed causes are termed type 3 (Deschamps et al., 1990). Members of this class develop this metabolic syndrome because of; genetic defects of beta cells (3A), genetically-related insulin resistance (3B), diseases of the pancreas (3C), abnormal hormonal effects (3D), or induction via chemicals and drugs (3E).

Type 4 diabetes or gestational diabetes mellitus, appears in about 2 – 5% of all pregnancies. It is temporary and treatable, but requires careful medical supervision during the pregnancy. In addition, about 20-50% of these women go on to develop type 2 diabetes (Linne, 2004).

1.5 - Type 2 diabetes – Pathology of the disease

Type 2 Diabetes mellitus is a metabolic disorder that is characterized by persistent hyperglycemia. It is preceded by a period of glucose intolerance, which may be undetectable and unrecognized for years before an official diagnosis is made (De Fronzo, 2004). In an effort to lower rising blood glucose levels, the beta cells in the pancreas secrete excess insulin, which results in a hyperinsulinemic state. When an individual can no longer produce enough insulin to compensate for the excess glucose, the pancreas is said to have "burnt out," and type 2 diabetes develops (De Fronzo et al., 1992). Although the pathology of diabetes is not fully understood, alterations in insulin action, binding, signal transduction and glycogen synthesis all contribute to hyperglycemia and insulin resistance seen in patients (DeFronzo et al., 1992).

All diabetics are asymptomatic for a variable time after onset, but share similar symptoms and complications at advanced stages. Symptoms of type 2 diabetes are polyuria, polydipsia, weight loss, increased appetite and fatigue (Ferdinand and Clark, 2004). In the diabetic, the kidneys excrete excess glucose, and the lost blood volume is replaced from intracellular fluid causing dehydration, thereby inducing polydipsia.

1.6 - Criteria for diagnosis

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia and is diagnosed by demonstrating any of the following:

1. 2 fasting plasma glucose levels above 7 mmol/l on different days
2. Plasma glucose above 11 mmol/l two hours after a 75 g glucose load
3. Symptoms of diabetes and a random glucose above 11 mmol/l

4. Elevated glucose bound to hemoglobin, HBA1c of 6% or higher (De Fronzo, 2004).

It has been reported that the concordance between the fasting glucose criterion of the American Diabetes Association (ADA) and the oral glucose tolerance test definition still used by the World Health Organization (WHO) is often low and that different subsets of subjects are diagnosed as diabetic by the two sets of criteria. The Diabetes Epidemiology Collaborative analysis of Diagnostic criteria in Europe study provides some insight into this problem. The DECODE report compared the prevalence of diabetes based on either the ADA criterion of a fasting plasma glucose level that is greater than 7 mmol/L or the World Health Organization criterion of a 2 hour postprandial plasma glucose level of 11.1 mmol/L. If fasting glucose alone was used as a diagnostic criterion, then 31% of diabetic subjects with a non-diabetic fasting glucose but a diabetic 2-hour glucose would not be diagnosed (DECODE study group, 1999).

1.7 - Long term complications

Among the major risks of the disorder are chronic problems affecting multiple organ systems, which eventually arise in patients with poor glycemic control. Damage to an organ usually incurs from injury to the blood vessel that is macro or microangiopathic in nature. Small vessel disease complications include; retinopathy, foot ulcers, necrosis, gangrene and nephropathy, whereas large vessel disease complications include; ischemic heart disease, stroke and vascular diseases (Newman et al., 2004).

Two large controlled studies, the Diabetes Control and Complications Trial (DCCT) (DCCT Research Group, 1993) and the U.K. Prospective Diabetes Study (UKPDS) (Stratton et al., 2000) have pointed to the importance of intensive blood

glucose control in reducing its associated morbidity. The UKPDS is the largest and longest trial ever conducted in patients with type 2 diabetes. Their findings illustrate that for each 1% reduction in hemoglobin A1C, there was a 21% decrease in any endpoint related to diabetes and in diabetes-related death, a 14% decrease in all cause mortality and myocardial infarction, a 43% decrease in amputation or death from peripheral disease and a 37% decreased risk for microvascular complications, each of which was statistically significant. Therefore, monitoring glycemic levels is extremely important in preventing long-term complications.

1.8 - Current Therapies

Diabetes is a chronic disease with no cure as of 2004, but it can be managed effectively in the initial stages. Diabetic management consists of a combination of diet, exercise and weight loss in any achievable combination, depending on the patient (Bell, 2004). Patients with diabetes play an integral role in any treatment strategy. Lifestyle modification, goal setting, self monitoring, preventing, detecting, treating acute complications and using medications correctly are all important components in achieving glycemic control (Mensing et al., 2004). This makes patient education and understanding crucial, particularly when it comes to dispelling myths about insulin therapy. In addition, the relationship between health care provider and patient is crucial to compliance. The regimen itself is a factor because if it is costly, or has numerous side effects, the patient's compliance may diminish. Patients who continue to have poor diabetic control after lifestyle modifications are typically placed on oral hypoglycemics.

Major classes of medications that are currently used are thiazolidinediones, and biguanides, which sensitize the body to insulin and control hepatic glucose production

(Lebovitz and Banerji, 2004). The sulfonylureas and meglitinides stimulate the pancreas to excrete more insulin, and alpha-glucosidase inhibitors slow the absorption of starches in attempts to reduce the amount of glucose that enters the circulation at one time (Hermans and Buysschaert, 2004). Although sulfonylurea therapy has been the mainstay of treatment for type 2 diabetes for over 40 years, the UKPDS reported that over a 6 year period, approximately 53% of patients who were randomized to receive treatment with sulfonylureas needed additional insulin therapy, reinforcing the concept that hyperglycemia in type 2 diabetes is progressive. Thus, this should be reinforced when establishing a therapeutic regimen (Wright et al., 2002). Some to most diabetics eventually fail to respond to the oral hypoglycemic agents listed above, and must proceed to insulin therapy.

1.9 - Statistics

In 1999, according to the WHO more than 150 million people worldwide suffer from diabetes. Its incidence is increasing rapidly and it is estimated that by the year 2025 this number will double (World Health Organization, 1999). About 90-95% of all North American cases of diabetes are Type 2, and about 20% of the population over the age of 65 is a type 2 diabetic (Lebovitz and Banerji, 2004).

1.10 – The need for advancement in the fight against type 2 diabetes

Currently, major therapies that are used for type 2 diabetics delay the onset of illness, but do not directly target the pathology of the disease. Our paradigm adds to the existing knowledge of the pathology of the disease, and may allow for earlier detection, whereas diagnosing a patient with type 2 diabetes today occurs only after symptoms of the illness have surfaced. Conventional theory does not focus on a mechanism but

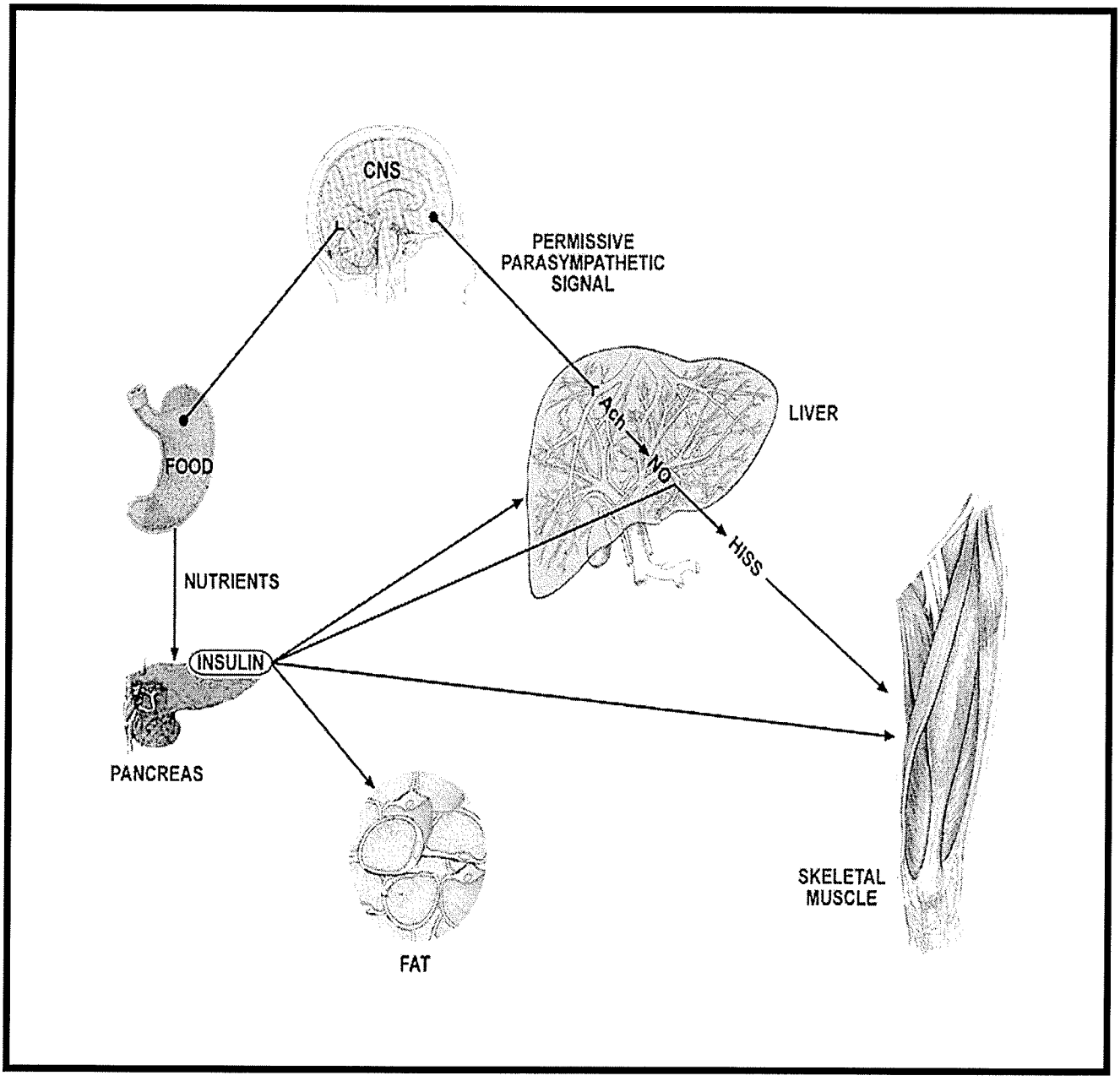
characterized events in the disease. I believe strongly that this new paradigm will contribute to assisting patients with this ever growing epidemic.

1.11 - A new paradigm for Type 2 Diabetes: The HISS Hypothesis

The conventional type 2 diabetic paradigm that currently exists insists that blood glucose fluctuations following a meal are managed solely by insulin (Henquin, 1994). Considering that human physiology can exhibit many redundant control systems, it is difficult to believe that insulin acts alone in storing glucose post-prandially.

Our laboratory has described a novel mechanism of insulin resistance that is induced when a humoral factor, Hepatic Insulin Sensitizing Substance (HISS) is absent. HISS acts selectively on skeletal muscle to stimulate glucose uptake following post-prandial insulin release (**Figure 1.1**). HISS-dependent insulin action accounts for approximately 55% of total insulin action in the fed state (Lautt et al., 2001).

Figure 1.1 – Hypothesis of HISS release (Lautt, 2004)



HISS action was first detected in 1993, when it was shown that hepatic anterior plexus denervation resulted in a reduction in insulin sensitivity (Xie et al., 1993). Further studies exemplified that glucose balance was dependent on intact parasympathetic nerves, as atropine administration into the liver mimicked the

insensitivity to insulin previously seen following denervation (Xie and Lautt 1995a). Shortly after it became evident, with the use of arterial-venous glucose gradients (Xie and Lautt, 1996b), that skeletal muscle was the peripheral site of insulin resistance. In order to demonstrate that the insulin resistance seen was induced by the liver, intraportal administration of acetylcholine (a muscarinic agonist) was shown to reverse the insulin resistance seen after surgical denervation (Xie and Lautt, 1996b). Therefore, it became evident that the hepatic parasympathetic nerves played a key role in insulin sensitivity. The existence of a post-prandial hormone that acted in accordance with insulin was shown by identifying a reduction in insulin sensitivity after fasting (Lautt et al., 1998). It was observed that placing food in a fasted rat's stomach increased insulin sensitivity (Lautt et al., 2001). Since this initial discovery, a state of insulin resistance that occurs following HISS blockade was termed HISS-dependent insulin resistance (HDIR).

HDIR has been shown to account for insulin resistance in the following situations including; fasting (Lautt et al., 2001), hepatic muscarinic cholinergic blockade with atropine (Xie and Lautt, 1995), hepatic nitric oxide synthase blockade with L-NMMA or L-NAME (Sadri and Lautt 1998 and 1999), hepatic cyclooxygenase inhibition (Sadri and Lautt, 2000), or surgical denervation of the liver (Xie and Lautt, 1994) (Latour and Lautt, 2002). HDIR has been suggested to account for postprandial hyperglycemia, hyperinsulinemia, and hyperlipidemia (Lautt, 2004).

1.12 - Detecting HISS action – The Rapid Insulin Sensitivity Test (RIST)

In order to evaluate HISS action in response to various interventions, the methodology employed must be shown capable of detecting HISS-dependent and HISS-independent insulin action. When HISS release is blocked, the glucose disposal effect of

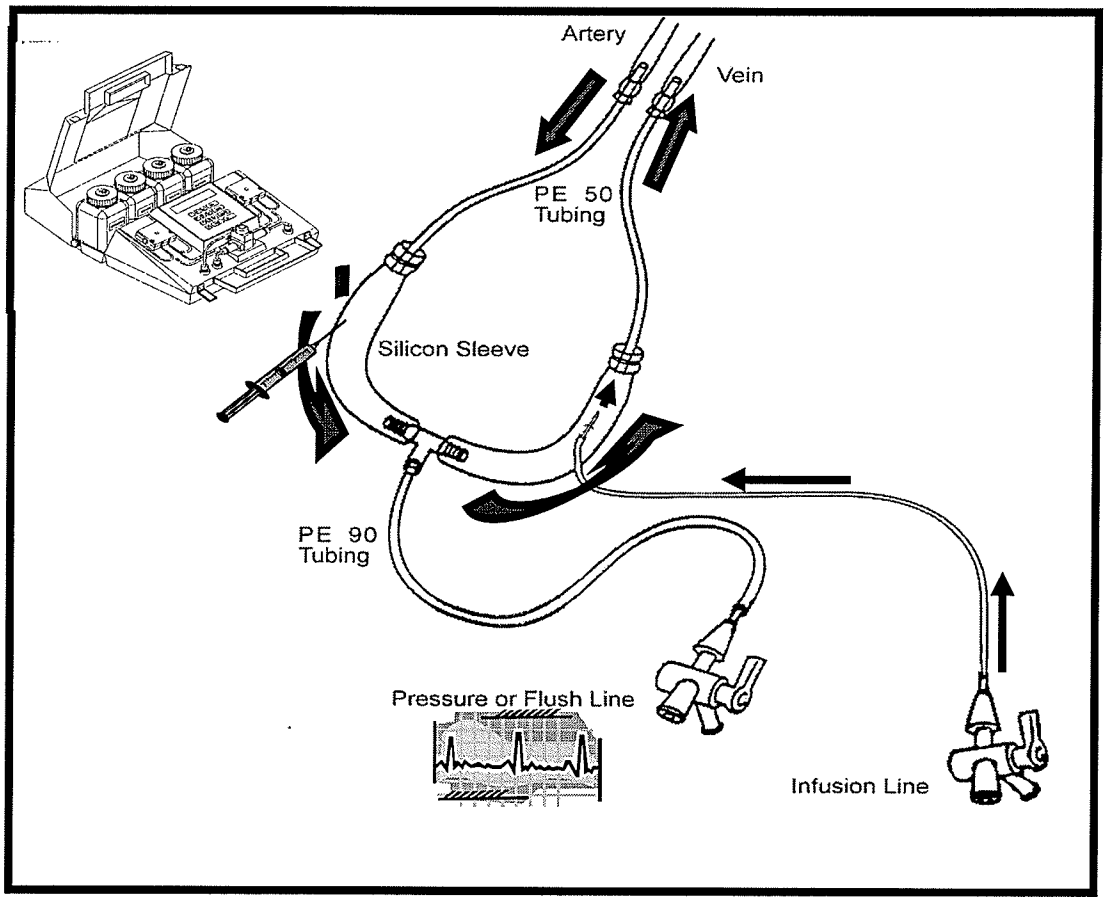
a bolus of insulin is reduced by approximately 55% (Lautt et al., 2001) and a state of HISS-dependent insulin resistance occurs. To ensure HISS detection when measuring insulin sensitivity, a comparison study was done between the Rapid Insulin Sensitivity Test (RIST), the insulin tolerance test (ITT) and the hyperinsulinemic euglycemic clamp (HIEC) (Reid et al., 2002). The insulin tolerance test was able to detect HISS action, but had an underlying complication of interference by counter-regulatory mechanisms to restore euglycemia. This prevented the observation of dynamic HISS action over the period of the test. The hyperinsulinemic euglycemic clamp was only able to detect HISS action over the first few minutes of insulin infusion, but eventually produced blockade of HISS action. Currently, the only test shown capable of measuring HISS action is the RIST. The RIST is highly reproducible and is unaffected by pentobarbital anesthesia.

1.13 – Standard surgical preparation

Male Sprague Dawley rats (Charles River, St.Constant, Quebec, Canada) were housed in climate-controlled conditions for at least 6 days prior to surgical preparation. The animals were fasted for 8 hours, and then allowed ad lib access to standard rat chow for 2 hours prior to surgical preparation. This re-feeding protocol was used to ensure that all rats had recently eaten as HISS release occurs maximally in the early postprandial state and decreases progressively with fasting duration (Lautt et al., 2001). The animals were weighed and anesthetized by an intraperitoneal injection of pentobarbital sodium (65 mg/ml, Somnotol, Biomeda-MTC Animal Health Inc., Cambridge Canada). Temperature was monitored by a rectal thermometer and kept constant at 37.5 ± 0.5 °C by a heated surgical table and an overhead lamp. The right femoral artery and vein were cannulated with catheters (polyethylene tubing PE60) that were connected with a silicon

segment (Masterflex – silicon tubing (Platinum) L/S 14), allowing blood to flow uninterrupted from the artery to the vein (Lautt et al., 1998). The blood flow contained within the silicon tubing is known as an arterial-venous shunt (**Figure 1.2**). Blood samples were taken by puncturing the arterial side of the shunt. The infusion of pharmacological agents was done by puncturing and inserting an infusion line into the silicon sleeve of the venous side of the shunt. Arterial pressure was measured by clamping the silicon sleeve on the venous side of the shunt, and measuring pressure from a side branch. Animals were heparinized (100IU/kg) to prevent clots from accumulating. A tracheotomy was performed (Micro-Renathane Tubing – Braintree Scientific Inc.) The left jugular vein was then cannulated with polyethylene (PE 60) tubing in order to allow for continuous supplemental anesthetic (sodium pentobarbital, 2.16 mg/ml saline) infusion at a rate of 0.5 ml/hr/100g of body weight. A variable glucose infusion line was inserted into a silicon sleeve on the jugular supplement line and was controlled by a variable infusion pump (Genie, by Kent Scientific Corporation, Litchfield, MA). The cannulation of the femoral artery and vein, the tracheotomy and the jugular vein cannulation is the standard surgical preparation and will be referred to as such throughout the thesis. In scenarios where superior mesenteric artery occlusions and portal infusions (IPV) into the liver were necessary, a laparotomy was performed. The portal vein was isolated and cannulated with an 18-guage I.V. catheter (Johnson and Johnson Medical), which was advanced with the introducer needle into the vessel and capped to prevent blood loss.

Figure 1.2 - The Arterial–Venous Shunt. The shunt allows for blood to flow unhindered, from the artery to the vein. Pharmacological agents can be infused into the venous side of the shunt, and blood samples can be taken from the arterial side of the shunt. Using the shunt minimizes the volume of blood lost during sampling (Lautt et al., 1998).



1.14 - Hemorrhage methodology

If a hemorrhage intervention was required within any series of this thesis, it was performed in the following manner:

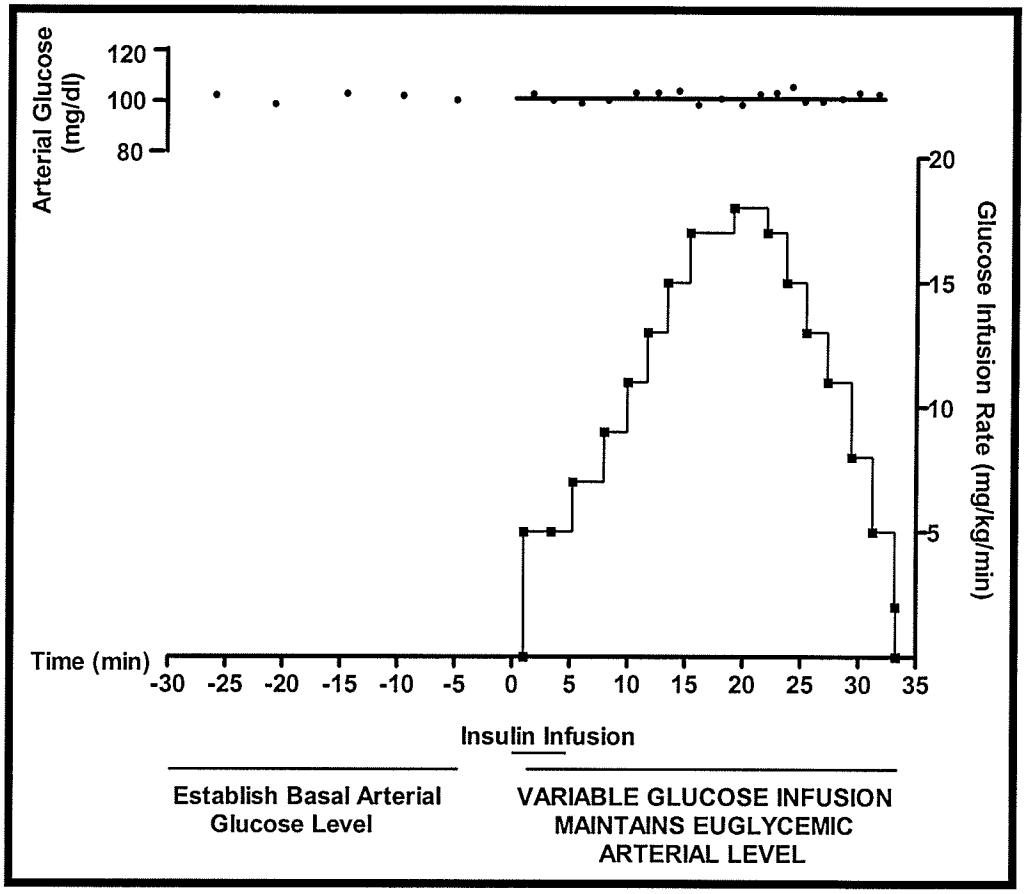
The anesthetized animal was hemorrhaged from the arterial side of the shunt (**Figure 1.2**), using a 23-gauge needle attached to a syringe, at a rate of 0.5 ml/min.

Arterial blood pressure was measured from the vascular shunt during brief occlusion of the venous side of the shunt. The animal was hemorrhaged to an arterial blood pressure of 50 mmHg and held at this pressure for a 10 minute interval, by withdrawing additional blood as needed. Following the 10 minute interval, the animal was restabilized by; taking blood samples to ensure a stable glycemic baseline, and by monitoring mean arterial pressure before attempting further interventions.

1.15 - RIST methodology

Arterial blood samples (25 μl) are analyzed for blood glucose concentration using a glucose oxidase analyzer (Yellow Springs Instruments, Yellow Springs, Ohio). Samples are analyzed at five-minute intervals until 3 successive stable measurements are obtained. The mean of these 3 samples is used as the basal glucose level. After the basal glucose level is established, a bolus of insulin (50 mU/kg, Humulin R) is infused intravenously into the animal over 5 minutes at a rate of 0.1 ml/min. At 45 seconds, a glucose infusion is initiated and adjusted to maintain euglycemia as determined by the basal glucose level. An arterial sample is taken every 2 minutes during the RIST, and the glucose infusion is increased or decreased accordingly (**Figure 1.3**). The RIST is complete when no further glucose is required, indicating the end of insulin and HISS action. The amount of glucose infused over the RIST (mg glucose/kg of body weight) is known as the RIST index (Lautt et al., 1998).

Figure 1.3 – The Rapid Insulin Sensitivity Test time line. Following intravenous insulin infusion, and the first arterial glucose sample, a variable glucose infusion is adjusted to maintain euglycemia based on arterial samples taken at 2-minute intervals throughout the test period. The RIST index is the total amount of glucose infused to maintain euglycemia over the test period (Lautt et al., 1998)



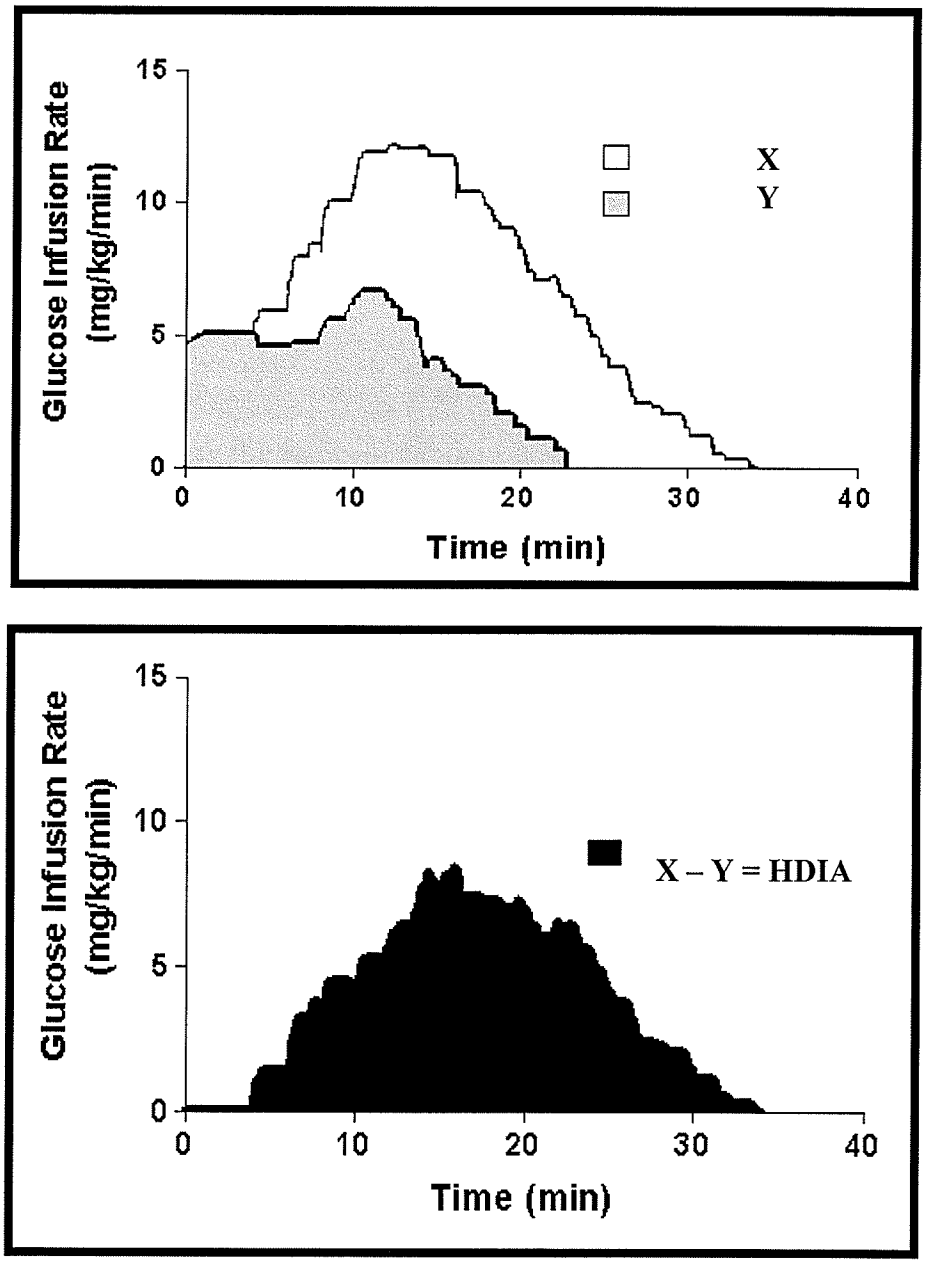
1.16 – Deciphering between HISS-dependent vs. HISS-independent insulin action

The RIST index is used to calculate HISS action. An animal that is sensitive to exogenous insulin during the RIST will result in a control RIST index with value X. Following the control RIST, an intervention can be employed to block HISS release. When the RIST is repeated, a new RIST index value (Y) will result. Value Y is known as

HISS-independent insulin action because only insulin action was measured, given that HISS release was blocked. HISS-dependent insulin action (HDIA) can be calculated by subtracting value X from Y (**Figure 1.4**)(Lautt et al., 1998).

Typically the value of a control RIST index in rats is approximately 200 mg/kg of glucose and the RIST with an intervention to block HISS release amounts to approximately 90 mg/kg of glucose. Given these approximations, we could say that the HISS-independent insulin action component amounted to 90 mg/kg of glucose, while the HISS-dependent insulin action component was 110 mg/kg of glucose.

Figure 1.4 - Deciphering the HISS-dependent insulin action (HDIA) component from the RIST index. X represents the action of insulin and HISS. Y only represents insulin action. HDIA is calculated by subtracting X from Y (Lautt et al., 1998).



Chapter 2 – Stress-Induced Insulin Resistance

2.1 - Introduction

Elective surgery is often associated with insulin resistance. The magnitude of the operation, the duration of surgery, and perioperative blood loss all affect the degree of insulin resistance produced, with reductions in insulin sensitivity reaching magnitudes of 50% or greater following uncomplicated elective surgeries such as a cholecystectomy (Thorell et al., 1999). It is not clear which mediators are most crucial for the development of insulin resistance following surgery. However, marked insulin resistance can develop without concomitant elevations in cortisol, catecholamines or glucagon, and the main sites for insulin resistance appear to be skeletal muscle (Andersson et al., 1982). Accumulating evidence illustrates that intensive glycemic monitoring and the administration of insulin infusions help to achieve tight glycemic control, which is strongly correlated with an improvement in perioperative mortality and morbidity (Robertshaw et al., 2004).

The objective of these studies was to determine if insulin resistance occurred following acute hemorrhage and if the confirmed resistance was attributable to HISS-dependent or HISS-independent insulin action.

2.1.1 - The concept of stress

Stress is a medical term for a wide range of strong external stimuli, both physiological and psychological, which can cause a physiological response. There are several stress theories, from authors such as Bernard, Cannon, Mason, Hennesy and Levine, Krantz and Lazar, Munck and Guyre, Levine and Ursine. However, Hans Selye

deserves much of the credit for introducing the term "stress" and for popularizing the concept of stress in the scientific and medical literature of the 20th century.

The general adaptation syndrome, first recognized in 1946 by Hans Selye is defined as the sum of all non-specific, systemic reactions of the body, which ensue upon long continued exposure to stress (Selye, 1946). Selye described the general adaptation syndrome as having three primary stages; an alarm reaction, where the body detects the external stimulus, adaptation, where the body engages defensive countermeasure against the stressor, and exhaustion, where the body begins to run out of defenses. Selye was able to separate the physical effects of stress from other physical symptoms suffered by patients through his research. He observed that patients suffered physical effects not caused directly by their disease or their current medical condition.

2.1.2 - Classification of stressful stimuli

A "Stressor," a term coined by Selye, is defined as a stimulus that disrupts homeostasis. Cannon, (Cannon, 1929) introduced homeostasis as "the coordinated physiological processes, which maintain most of the steady states in the organism." The maintenance of homeostasis requires precise coordination of autonomic, neuroendocrine and behavioral responses to content with the internal and external environment.

A stressor can be divided into four main categories: 1) physical stressors; 2) psychological stressors; 3) social stressors; and 4) stressors that challenge cardiovascular and metabolic homeostasis (McCarty et al., 1989). In terms of duration, stressors may then be sub-divided further into acute (intermittent) and chronic (prolonged exposure) stresses. Coping with stress and stressors successfully is highly dependent on efficient activation of a system, as well as prompt termination of the signal once the situation has

been addressed (McEwan, 1997). Thus, following activation, should a neuroendocrine system not be properly turned off, elevated hormone levels could become dangerous and may result in various stress related diseases such as hypertension, diabetes, stroke and other inflammatory disorders (McEwan, 1997). The extent to which an individual can cope with these stressful situations is highly variable and depends heavily on genetics, experiences, social support and current health (Delongis and Preece, 2000).

2.1.3 - Adaptive responses to acute stressors

Adaptive responses that are elicited in response to an acute stressor include major physiological processes that are essential to reestablish homeostasis. During an acute response to hemorrhage, physiological processes are activated in order to redirect energy utilization among life sustaining organs, such as the heart and brain (Robinson and Rapoport, 1986), and to selectively inhibit or stimulate organs to mobilize their energy reserves. Thus, upon exposure to metabolic stressors, certain tissues tend to reduce their consumption of energy while others selectively receive nutrients to function properly. The increased supply of energy to crucial organs is achieved preferentially by the release of catecholamines and glucocorticoids that increase glycogenolysis, inhibit glucose uptake, and enhance proteolysis and lipolysis (Robinson and Rapoport, 1986).

2.1.4 - Fight vs. flight – Sympathetic activation

The fight or flight response, also called the "acute stress response" was first described by Walter Cannon in the 1920s. It states that animals react to threats with a general discharge of the sympathetic nervous system. The response was later recognized as the first stage of Selye's general adaptation syndrome.

The activation of the sympathetic division of the autonomic nervous system is initiated primarily by the neurons of the locus ceruleus within the brain. Following a threatening stimulus such as acute hemorrhage, a prolonged discharge of the locus ceruleus sends a signal to the thalamus located in the brain stem. This signal causes the preganglionic sympathetic nerves to release acetylcholine. The flood of acetylcholine at the synaptic cleft activates postganglionic fibers to release norepinephrine or adrenaline (Wurtman et al., 1972;Unger and Phillips, 1983). Effector organs such as the heart and liver, that contain adrenergic receptors then respond accordingly to sympathetic innervation by initiating an appropriate compensatory response to the initial stressor.

Hemorrhage has been shown to cause sympathetic activation in: the hypothalamus (paraventricular nucleus (PVN), supraoptic nucleus, medial preoptic nucleus, supramimillary nucleus), thalamus (midline nuclei), limbic system (cinculate cortex, piriform cortex), midbrain (central grey), pons (parabrachial nuclei, barrington nucleus), medulla oblongata (Nucleus Tractus Solitarius (NTS), area postrema, lateral reticular nucleus), catecholaminergic cell groups (A1, A2, A6), and the spinal cord (PVN microdialysis). This activation was determined by measuring arterial plasma norepinephrine (NE), epinephrine (E), and ACTH concentrations in Sprague Dawley rats after exposure to hemorrhage of 25% blood loss (Pacak and Palkovits, 2001).

Some of the crucial responses and compensatory mechanisms that are initiated by the sympathetic nervous system in response to hemorrhage are the release of endogenous vasoconstrictor substances (catecholamines primarily) from the adrenal medulla (Wurtman et al., 1972), and the renal conservation of salt and water and eventual reabsorption of tissue fluids into the circulation in an attempt to increase cardiac output.

However, should the initial stressor prove too stressful, a number of decompensatory mechanisms occur, such as cardiac failure, acidosis, central nervous system depression, and aberrations of blood coagulation. Thus, survival depends strictly on the balance between compensatory and decompensatory mechanisms activated by the fight or flight response.

"The sympathetic nervous system and the adrenal medulla respond to different stress induced challenges by selective recruitment of subsystems as determined by site of release and receptor availability – all 88 keys on a piano are not struck at once, rather chords appropriate to the musical piece are played (Tepperman, 1968)."

2.1.5 – The endocrine system – A hormonal response to stress

The foundations of the endocrine system are the hormones and glands. As the body's chemical messengers, hormones transfer information and instructions from one set of cells to another. The hypothalamus is the major link between the endocrine and nervous systems; therefore when the sympathetic system is activated, components of the endocrine system respond accordingly.

Most scientists view and define stress as a condition when the internal stress-processing mechanism activates the hypothalamus-pituitary-adrenal system (Pacak et al., 1998). This system governs the amount and type of response the body produces in order to efficiently combat a stressing agent or state. Simplified, once a stressor has been inflicted, the hypothalamus sends a chemical message to the pituitary gland to release adrenocorticotrophic hormone (ACTH) into the blood stream. This signal prompts the adrenal cortex to release catecholamines (E and NE), which travel within the body and activate adrenergic receptors on various effector organs to facilitate physical reactions by

eliciting increases in heart rate and breathing, activating glycogenolysis, and constricting blood vessels in an attempt to increase cardiac output (Thase and Howland, 1995). E comes almost exclusively from the adrenal medulla, whereas NE is derived from both the medulla and the peripheral sympathetic nerve endings that innervate organs.

In addition to the activation of the hypothalamus-pituitary-adrenal system, some scientists suggest that the activation of other systems with or without concomitant elevation in ACTH levels reflect stress-induced homeostasis (Pacak et al., 1998). Hypotensive hemorrhage is characterized by intravascular volume depletion, renal sodium retention, the activation of the renin-angiotensin-aldosterone system (RAAS), and the release of adrenal medullary hormones (which was previously discussed).

The RAAS is triggered primarily via sympathetic stimulation (acting via β_1 adrenoreceptors), renal artery hypotension, and decreased sodium delivery to the distal tubules (Coevoet et al., 1982). All of these factors trigger the release of renin from the kidney. Following a cascade of cleavages, the octapeptide angiotensin II is formed. Angiotensin II has several crucial functions following blood loss such as:

1) Constriction of resistance vessels, which increases systemic vascular resistance and arterial pressure.
2) Release of aldosterone from the adrenal cortex, which increases sodium and fluid retention.
3) Release of vasopressin which acts upon the kidneys to increase fluid retention.
4) NE release from the sympathetic nerve endings and inhibition of NE re-uptake by nerve endings, thereby enhancing sympathetic adrenergic function.
5) Stimulation of cardiac hypertrophy (Coevoet et al., 1982).

Given all of the possible targets of angiotensin II, therapeutic manipulation of this pathway has been crucial in treating hypertension and heart failure.

2.1.6 - Insulin resistance and Hemorrhage – A poor prognosis

Elective surgery and perioperative blood loss evoke numerous stress responses. Activation of catabolic reactions are employed within the body to assist with energy requirements during stressful periods. This is often reflected by changes in circulating levels and the turnover of different substrates, hormones and mediators. It has been established that resistance to the effects of insulin on glucose metabolism develops after injury (Cutherbertson, 1942) as findings of increased rates of lipid oxidation and circulating levels of free fatty acids and glycerol after injury indicate a reduced anti-lipotic effect of insulin. In addition, insulin resistance has been shown to develop in close association with increasing nitrogen losses after injury (Frayn, 1986).

Metabolic changes observed during major surgery (such as a gastrectomy) were small; however there was a marked increase in insulin requirements during surgery. In all accounts, Raucoules-Aime has suggested that the increase in sympathetic activity during blood loss and surgery may have been implicated in the increase in insulin requirements (Raucoules-Aime et al., 1995).

An epidemiological study has reported that high blood glucose levels seen following elective surgery are associated with a significant risk for the development of cardiovascular diseases, as well as a grave prognosis for these patients during acute coronary events (Van Den Berghe et al., 2001). Two other studies suggested that hospital fatality rates are improved in patients with optimal glucose control, as judged by blood glucose levels measured before infarct (Rytter et al., 1985;Harrower and Clarke, 1976).

The hormonal and metabolic responses to stress conditions inflicted following major surgery and myocardial infarction have been examined by several groups. Their interpretations suggest that the only safeguard to hyperglycemia following surgical trauma is insulin; however with insulin deficiency or resistance, such as in a type 2 diabetic, there is further deterioration of glycemic control and an escalation in hyperglycemia (Halter et al., 1977).

2.1.7 - The use of hemorrhage as an acute stress

Hemorrhage is a useful model of acute stress because it can be quantified in anaesthetized animals and results in a wide range of metabolic consequences including hyperglycemia and insulin resistance (Ma et al., 2002). Therefore hemorrhage was used as an acute stressor, as it challenged both cardiovascular and neuroendocrine homeostatic responses.

2.2 - Methods

2.2.1 - Hemorrhage and HISS-dependent insulin resistance

Following the standard surgical procedure (**see section 1.13 for Standard surgical preparation**), a control RIST (**see section 1.15 for RIST methodology**) was performed. Following the hemorrhage (**see section 1.14 for Hemorrhage methodology**), arterial blood glucose levels were measured until they became stable for at least 15 minutes and another RIST was performed. After stabilization following the RIST, atropine (1 mg/kg – IV) was administered through the venous side of the shunt at a rate of 0.1 ml/min for 5 minutes. This dose of atropine results in a complete block of HISS release (Takayama et al., 2000). Following stabilization, a post atropine RIST was performed to quantify the RIST index during the complete absence of HISS action.

2.2.2 - Hemorrhage and ligation: Reductions in hepatic portal blood flow

The Superior Mesenteric Artery (SMA) ligation was performed with the notion that it would reduce portal blood flow to a similar extent as hemorrhage. A standard surgical preparation was performed, followed by a laparotomy and the isolation of the SMA (**see section 1.13**). A snare was inserted around the artery, so that it could be occluded and released accordingly. An ultrasonic perivascular V-type flow probe was placed around the portal vein (size, 3 mm) to allow for the measurement of portal blood flow with a small animal flowmeter (T206, Transonic Systems Inc., Ithaca, NY). Following a 30 minute stabilization period, the snare encompassing the SMA was constricted for 5 minutes. Portal blood flow, arterial pressure and glycemia were measured when the SMA was occluded. The snare was released, and following restabilization, a hemorrhage was performed as above. Portal blood flow, arterial pressure and glycemia were measured when arterial pressure was reduced to 50 mmHg and held for 10 minutes.

2.2.3 - Hemorrhage: Insulin pharmacokinetic studies

For these pharmacokinetic studies, insulin was administered for the RIST at the standard dose (50 mU/kg) but over a 30 second interval. Following the hemorrhage, another RIST was performed. Blood samples (80 μL) were taken from the arterial side of the shunt at 5 minutes before beginning the RIST, and at 1, 2, 3, and 5 minutes after insulin administration. The samples were centrifuged and the collected plasma was frozen immediately on dry ice, and later stored at –80 °C. These samples were analyzed in duplicate on a 1-2-3 Ultra Sensitive Rat Insulin ELISA (American Laboratory Products Company, Windham, NH).

2.2.4 - Insulin kinetics and SMA occlusion

A standard surgical preparation was performed, followed by a laparotomy and the isolation of the SMA. A snare was inserted around the artery, so that it could be occluded. The animal was allowed to rest for a 30 minute interval. Atropine was then administered (1 mg/kg – IV) followed by a 15 minute stabilization period. A post atropine RIST was then performed as described above for insulin kinetic determination. The SMA was then occluded; the animal was restabilized, and a RIST was repeated to determine insulin pharmacokinetics. The samples collected were analyzed using the above methods for insulin pharmacokinetic determination.

2.2.5 - Confirming HDIR with pre-treatment of atropine

A control RIST was performed after a 30 minute stabilization period. This was followed by administration of atropine (1 mg/kg – IV). A post atropine RIST was done, followed by hemorrhage to 50 mmHg (see above). A post hemorrhage RIST was then done as soon as a stable glycemic baseline had been established.

2.2.6 - Drugs

Human insulin (Humulin R) was purchased from Eli Lilly (Toronto, Canada). Atropine and D-glucose were purchased from Sigma Chemical Co. (St. Louis, Mo.). All drugs were dissolved in saline.

2.2.7 - Data analysis

Data were analyzed using a paired or unpaired t-test, and/or repeated-measures and one-way ANOVA, followed by the Tukey-Kramer multiple comparison test in each group. Insulin pharmacokinetic data were analyzed using WinNonlin Professional Version 4.0.1 (Pharsight corporation, Mountain View, CA), and the best fit was

calculated by a one compartment model, with zero order infusion and first order elimination. The data were expressed as means ± SE throughout. Differences were accepted as statistically significant at $P<0.05$. Animals were treated according to the guidelines of the Canadian Council on Animal Care, and the Protocol Management Review Committee at the University of Manitoba approved all protocols.

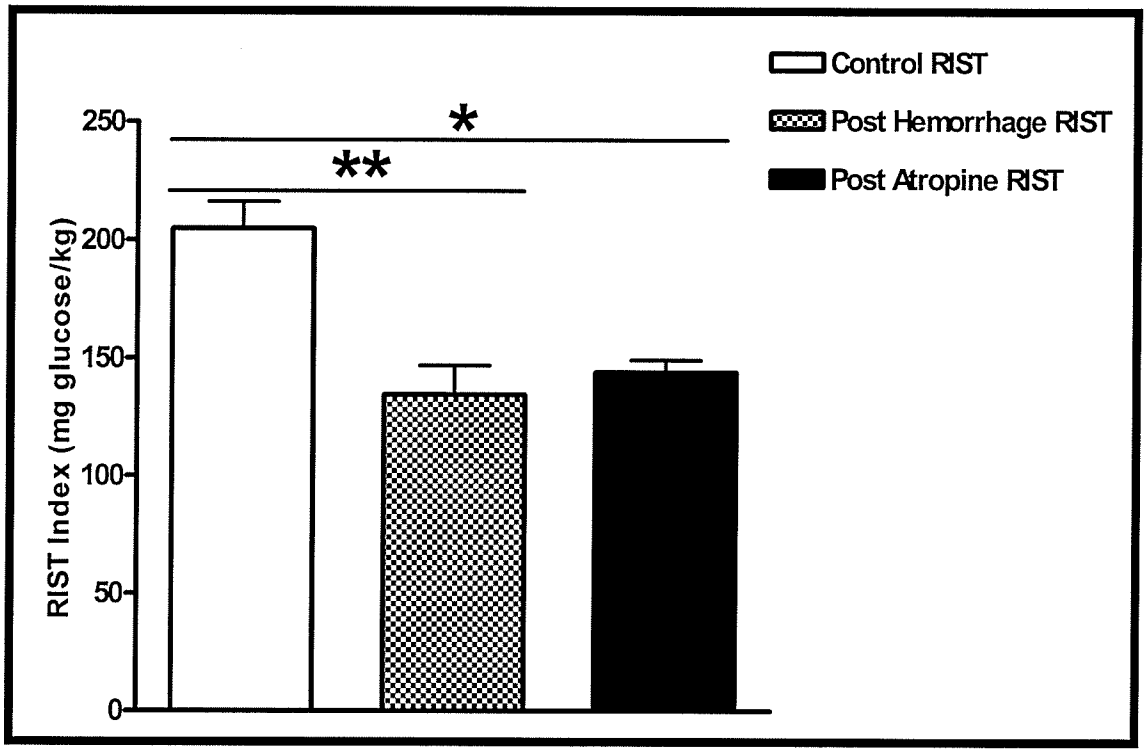
2.3 - Results

2.3.1 - Acute hemorrhage causes HDIR

Animals (n=5; 247.6 ± 6.7 grams) in this series had an initial RIST index of 204.8 ± 11.3 mg of glucose/kg, a post hemorrhage RIST index of 134.5 ± 12.3 and a post atropine RIST index of 144.0 ± 5.3 (**Figure 2.1**). The decrease (34.3 ± 4.8%) in insulin sensitivity between the control and the post hemorrhage RIST is significant ($P = 0.001$). There was no statistical significance between the post hemorrhage and post atropine RIST. The amount of time taken to remove the volume of blood (3.3 ± 0.7 ml) required to reduce mean arterial pressure to 50 mmHg was 4.1 ± 0.9 minutes. Hyperglycemia was immediately present, as basal glucose levels increased from a baseline of 109.5 ± 5.0 to 127.3 ± 6.3 mg/dl within 5 minutes of onset of hemorrhage. A partial restoration (50 ± 0 to 63 ± 4 mmHg) of mean arterial pressure was noted following hemorrhage. The suppression of HISS action after hemorrhage was complete, as shown by the inability of atropine to produce a further reduction. However, since full blockade of HISS action normally results in a decrease of approximately 55% of insulin action, and hemorrhage only resulted in a 34.3% reduction, we sought other complicating factors.

Figure 2.1 - Hemorrhage results in HISS-dependent insulin resistance (HDIR)

The post hemorrhage RIST index decreased and was not decreased further by the administration of atropine thereby indicating that hemorrhage caused full HDIR (P = 0.001, *P = 0.01).**



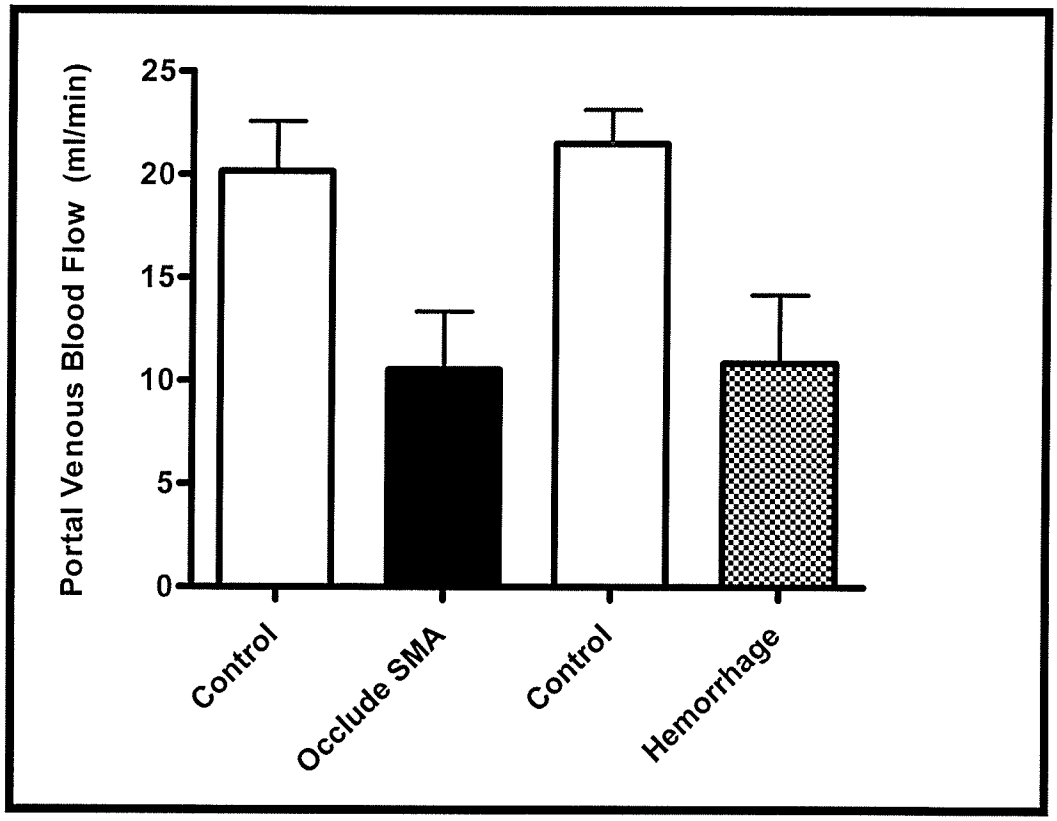
2.3.2 - Hemorrhage and SMA occlusion reduce portal blood flow to similar extents

Animals in this series (n=3; 277.6 ± 9.9 grams) were used to determine portal blood flow rates following SMA occlusion and hemorrhage. The amount of time taken to remove the volume of blood (3.5 ± 1.1 ml) required to reduce mean arterial pressure to 50 mmHg was 4.9 ± 0.8 minutes. Hyperglycemia was immediately present as basal glucose levels increased from a baseline of 106.6 ± 5.2 to 129.0 ± 4.4 mg/dl within 5 minutes of onset of hemorrhage. There were no significant differences between the pooled flow rates in the control state following SMA occlusion and hemorrhage (**Figure 2.2**). There

was a 51.0 ± 1.2% reduction in portal blood flow after SMA occlusion, and a 49.7 ± 2.4% reduction in flow after hemorrhage, with mean occlusion flow rates being 10.5 ± 2.8 and mean post hemorrhage flow rates equaling 10.8 ± 3.3 ml/min. Therefore, hemorrhage significantly reduced portal blood flow to the same extent as SMA occlusion in the fed state.

Figure 2.2 - The effect of Superior Mesenteric Artery (SMA) occlusion and hemorrhage on

portal blood flow. Portal blood flow during the control, during occlusion of the SMA, after the snare had been released to provide a second control flow, and after the animal was hemorrhaged to 50 mmHg and held at that pressure for a 10 minute interval. Occlusion of the SMA and hemorrhage significantly reduced portal blood flow to similar extents.



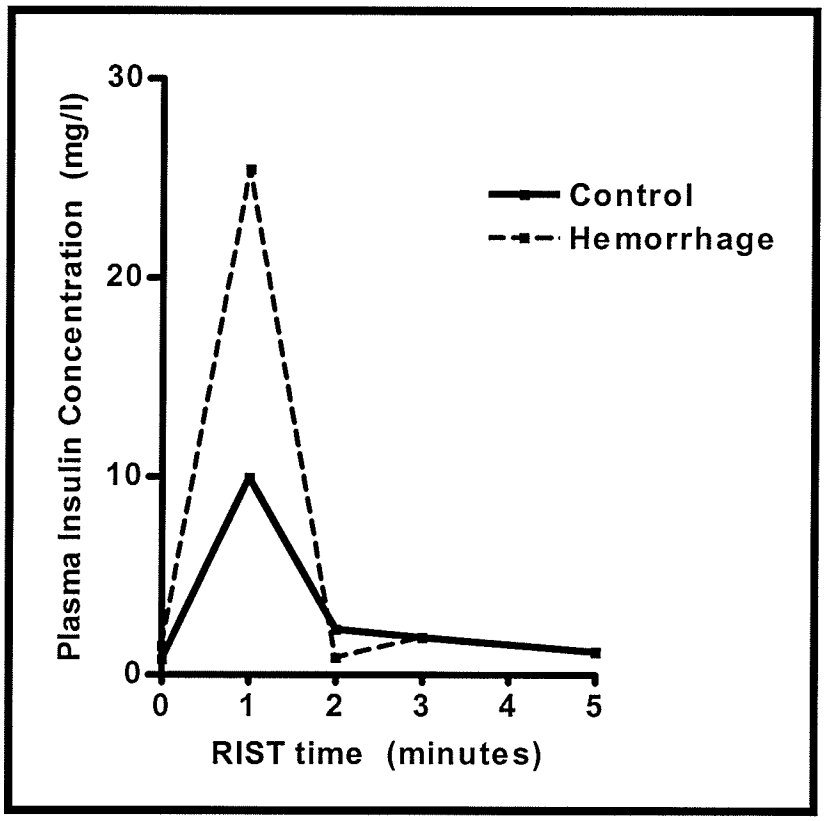
2.3.3 - An increase in insulin concentration during the RIST following hemorrhage

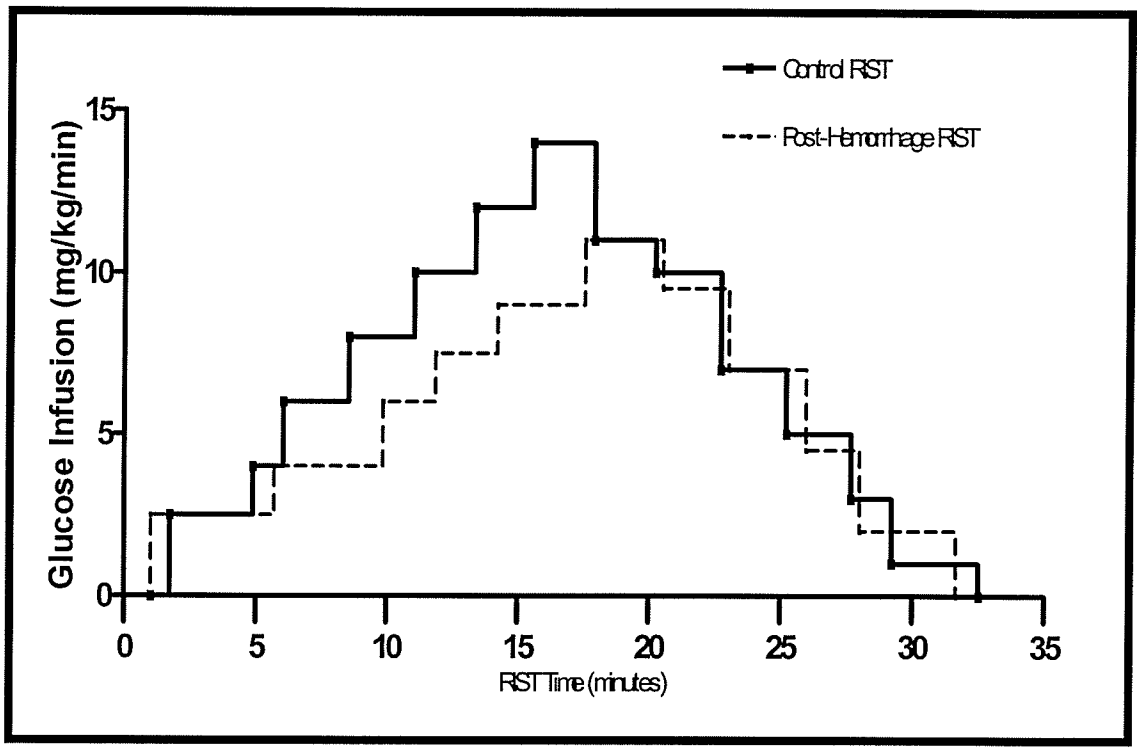
Animals (n=4; 265.8 ± 1.9 grams) in this series were used to analyze insulin kinetics during the RIST following hemorrhage. The control RIST index was 211.8 ± 15.8 and the post hemorrhage RIST index was 141.8 ± 9.6 mg of glucose/kg. The decrease (33.1 ± 4.4%) in insulin sensitivity between the control and the post hemorrhage RIST is significant (P = 0.002). The amount of time taken to remove the volume of blood (3.0 ± 0.9 ml) required to reduce mean arterial pressure to 50 mmHg was 4.3 ± 0.6 minutes. Hyperglycemia was immediately present as basal glucose levels increased from a mean baseline of 110.5 ± 4.1 to 126.9 ± 5.4 mg/dl within 5 minutes of onset of hemorrhage. A partial restoration (50 ± 0 to 60 ± 2 mmHg) of mean arterial pressure was noted following hemorrhage. Hemorrhage caused a significant increase in insulin concentration (27.5 ± 3.8 μg/l) at the 1 minute mark of the RIST, as compared to the control state (17.5 ± 1.8 μg/l) (P = 0.03). However, there was no statistical difference between the insulin plasma concentrations taken 5 minutes prior to the commencement of the control and post-hemorrhage RISTs. The area under the curve for insulin concentration was increased by 35.0 ± 2.1% secondary to reduced volume of distribution and clearance (**Table 2.1**).

Therefore, during the RIST, more insulin is available to interact with tissues following hemorrhage (**Figure 2.3**).

Figure 2.3 - The impact of hemorrhage after administration of a 30 second bolus of 50mU/kg of insulin on insulin kinetics and the dynamic curve of the RIST index in one typical experiment.

Insulin decreased to baseline after 5 minutes in both the control and hemorrhage RISTs. The hemodynamic alteration by hemorrhage caused an increase in insulin concentration following hemorrhage, and the area under the curve for insulin concentration was increased secondary to reduced apparent volume of distribution and clearance (n=4, see table 2.1 and text for mean data). Despite the increased availability of insulin to act on tissues, the response to insulin decreased because of the absence of HISS action.





2.3.4 - SMA occlusion and kinetics

Animals (n=7; 290.0 ± 10.0 grams) in this series were used to determine insulin kinetics and insulin sensitivity during SMA occlusion. Atropine (1 mg/kg – IV) was administered prior to a control RIST in order to eliminate HISS so that alterations in insulin kinetics and the RIST index could be interpreted more clearly. The SMA occlusion caused a significant increase (24.4 ± 2.6%) in insulin sensitivity when pre-treated with atropine, as the atropine RIST index was 77.0 ± 1.7, and the occlusion RIST index was 101.9 ± 11.3 mg of glucose/kg (P = 0.001, **Figure 2.4**). In addition, the SMA occlusion altered insulin kinetics thereby increasing the area under the insulin concentration curve (55.2 ± 3.2%) secondary to reduced volume of distribution and clearance (**Figure 2.4, Table 2.1**). This allowed for a higher insulin concentration to interact with tissues leading to an increase in the occlusion RIST index.

Figure 2.4 - The impact of Superior Mesenteric Artery (SMA) ligation after administration of a 30 second bolus of 50mU/kg of insulin on insulin kinetics and the dynamic curve of the RIST index.

Atropine was administered prior to the control RIST to completely block HISS release, so that only the impact of insulin action was measured. The post ligation RIST is significantly higher (*P = 0.001) than the control post atropine RIST, consistent with the altered insulin kinetics (Figure 2.3, Table 2.1). The area under the insulin concentration curve increased by 55.2% secondary to reduced volume of distribution and clearance (*P = 0.05).

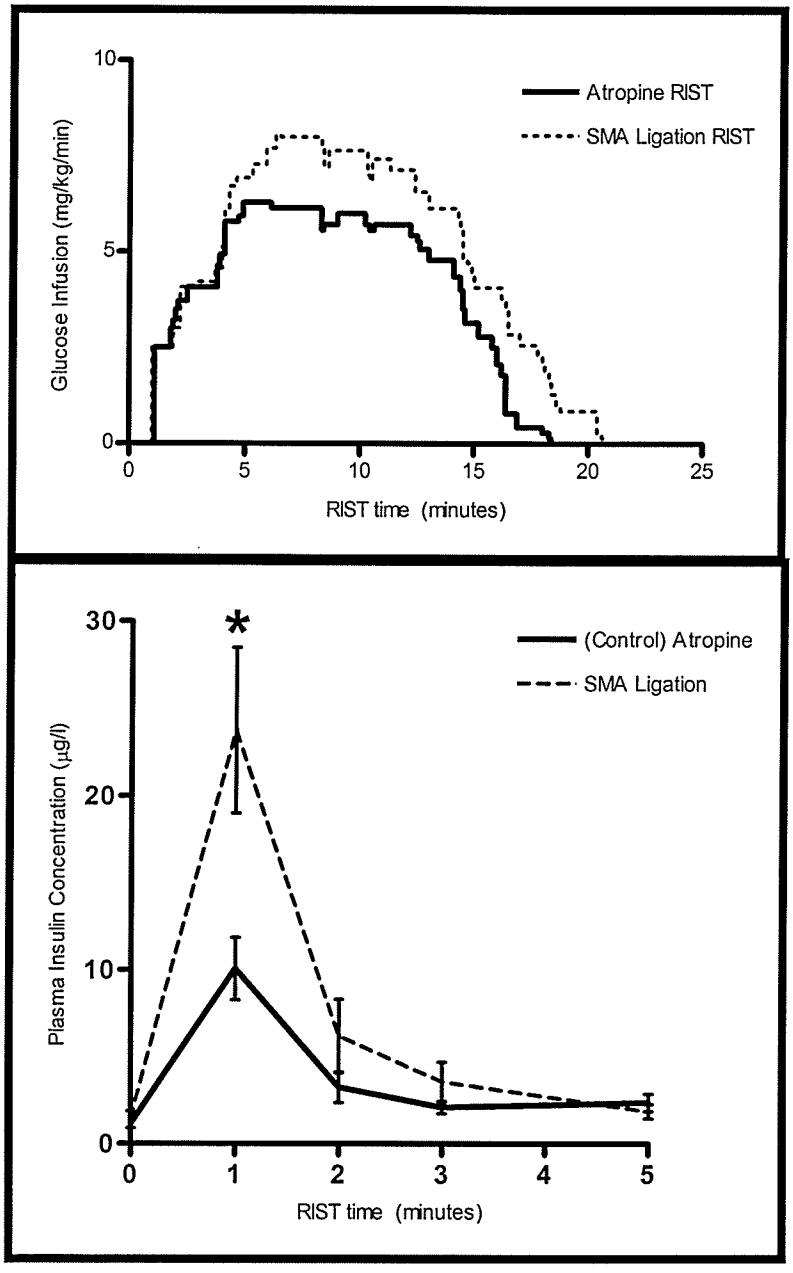


TABLE 2.1: Insulin Kinetics from the hemorrhage and Superior Mesenteric Artery (SMA) ligation series.

Data (mean ± SE) were collected during the hemorrhage and SMA occlusion protocols. Hemorrhage and SMA ligation interventions reduced hepatic portal blood flow to similar extents. The apparent volume of distribution and the clearance rates were not found to be statistically significantly different.

Intervention		Volume of Distribution (ml/kg)	Clearance (ml/kg/hr)
Hemorrhage	Control	56.0 ± 12.0	62.0 ± 9.0
	Hemorrhage	24.0 ± 7.0	34.0 ± 5.0
	Percent Change	57.1%	45.2%
SMA Ligation	Control	98.0 ± 14.0	87.0 ± 1.8
	SMA Ligation	30.0 ± 3.0	40.0 ± 1.0
	Percent Change	69.4%	54.0%

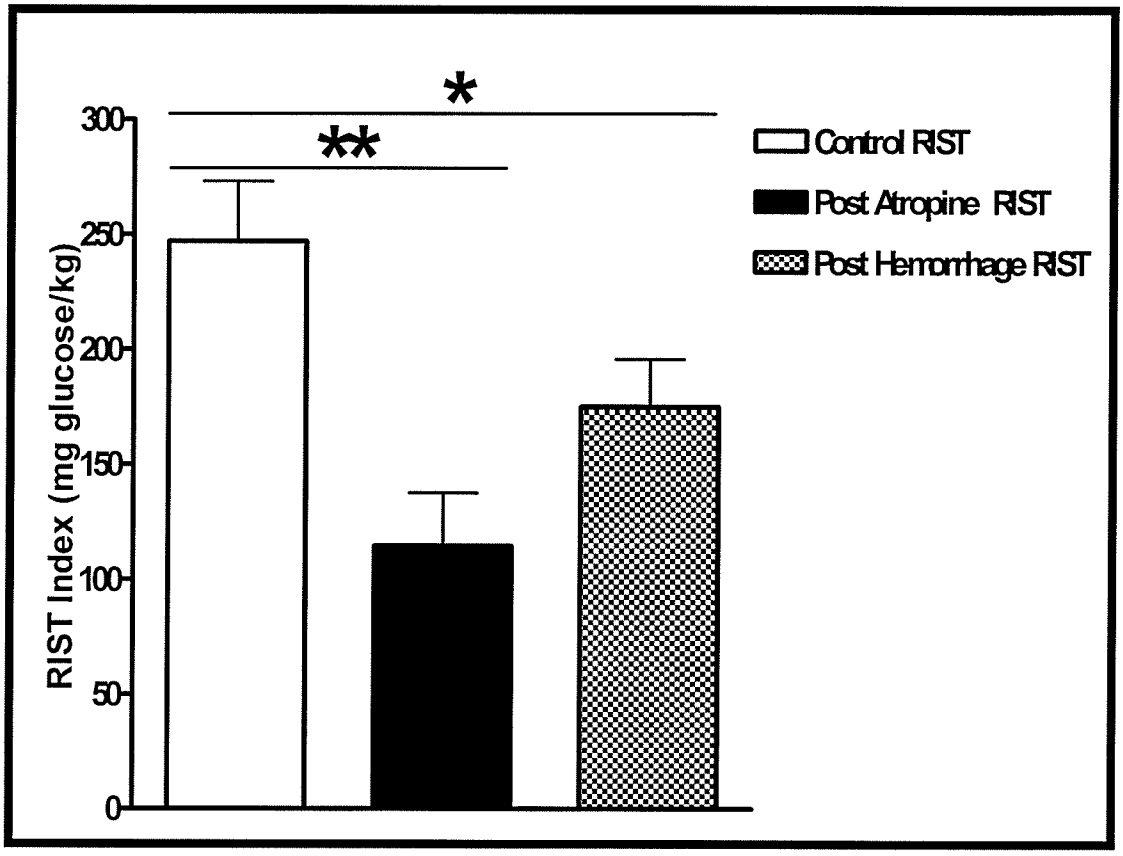
2.3.5 - Pre-treatment with atropine confirms HDIR in response to hemorrhage

To confirm that hemorrhage led to increased HISS-independent insulin action, we did a control RIST, blocked HISS production using atropine, and then performed another RIST. The animal was then hemorrhaged and a post-hemorrhage RIST was done. Animals (n=4; 253.1 ± 8.1 grams) in this series had an initial RIST index of 247.1 ± 26.1, a post atropine RIST index of 114.6 ± 23.1 and a post hemorrhage RIST index of 175.0 ± 20.7 mg of glucose/kg (**Figure 2.5**). The amount of time taken to remove the volume of blood (3.4 ± 0.5 ml) required to in reduce mean arterial pressure to 50 mmHg was 4.1 ± 0.9 minutes. Hyperglycemia was immediately present as basal glucose levels rose from a mean baseline of 108.8 ± 7.1 to 131.1 ± 3.3 mg/dl within 5 minutes of onset of hemorrhage. A partial restoration (50 ± 0 to 60 ± 2 mmHg) of mean arterial pressure was noted following hemorrhage. Subsequent to hemorrhage, the RIST index increased (29.1 ± 6.1%) in comparison to the post atropine RIST. The decrease (53.6 ± 3.0%) in insulin

sensitivity following atropine administration confirms full HISS blockade (P = 0.05) (**Figure 2.5**). The post atropine RIST index was due entirely to the direct action of insulin (HISS-independent component) and was significantly elevated following hemorrhage (P = 0.05).

Figure 2.5 - The effect of hemorrhage on HISS-dependent insulin resistance when pre-treated with atropine.

The post atropine RIST index was due entirely to the direct action of insulin (HISS-independent component) and was significantly elevated following hemorrhage because of altered insulin kinetics secondary to reduced apparent volume of distribution and clearance (, P = 0.05, *P = 0.05) (Table 2.1).**



2.4 - Discussion

The hemorrhage model demonstrated insulin resistance that was accountable by HDIR with the direct HISS-independent action being unsuppressed. The hemodynamic responses to hemorrhage led to a reduction in the apparent volume of distribution and clearance of insulin. The response to injected insulin was complicated by the occurrence of full HDIR against a background of reduced insulin clearance. Complete HDIR normally leads to a decrease in glucose disposal of 55% (Lautt et al., 2001); however in these studies the reduction in metabolism of injected insulin led to a final total decrease in RIST index of only 34.3 ± 4.8%.

2.4.1 - Hemorrhage induces HDIR: No effect on insulin action

Atropine administration (1 mg/kg – IV) results in the complete blockade of HISS and a 55% reduction in the RIST index (Xie and Lautt, 1994 and 1995). Given that the post hemorrhage RIST caused a decrease in insulin sensitivity that was not decreased further by the administration of atropine, it was concluded that hemorrhage caused the complete suppression of HISS action and led to HDIR (**Figure 2.1**). However, the degree of decrease in insulin sensitivity seen following hemorrhage (34.3%) was not compatible with the 55% reduction reported when atropine is used alone. This unexpected response was clarified by examining the effect of hemorrhage on the RIST index in the absence of HISS action produced by pre-treatment with atropine.

2.4.2 - Hemorrhage, ligation and blood flow

The reduction in hepatic portal blood flow caused by hemorrhage and SMA occlusion was used to investigate effects on insulin kinetics. The insulin kinetic studies

were analyzed using a one compartment model, with zero order infusion and first order elimination.

Insulin administered during the RIST is rapidly equilibrated and distributed among the tissues and body fluids. It is then cleared mainly by the liver and kidneys. The hepatic clearance of many compounds is blood-flow dependent. The SMA contributes a major component of portal blood flow. We hypothesized that hemorrhaging an animal to an arterial pressure of 50mmHg would closely mimic the hemodynamic effect of a SMA occlusion on hepatic portal flow. Hemorrhage did mimic the occlusion as both interventions reduced portal flow by approximately 50% in rats (**Figure 2.2**).

Although the RIST is usually done using a 5 minute infusion, there is no substantive difference in the RIST index or profile if the same dose is given over 30 seconds in rats (Lautt et al., 1998). In order to determine insulin kinetics we used the 30 second bolus administration. The RIST index (204.8 ± 11.3) using the 5 minute bolus and the RIST index (211.8 ± 15.8 mg of glucose/kg) for the 30 second bolus were not statistically different. Thus, the RIST index was analogous using the 5 minute or 30 second insulin infusion.

While the hemorrhage reduced portal blood flow to a level equivalent to the SMA occlusion (**Figure 2.2**), the ligation produced a large reduction (69.4 ± 2.2%) in the apparent volume of distribution primarily because of the static exclusion of a large portion of the gastrointestinal tract from the circulation (**Figure 2.4, Table 2.1**). The hemorrhage intervention caused a smaller reduction in the apparent volume of distribution (57.1 ± 4.0%) in comparison to the SMA ligation study (**Figure 2.3**). Although blood volume was physically removed from the anaesthetized animal during

the hemorrhage, the gastrointestinal circulation would have been reduced but not completely stagnant. The clearance of insulin from the plasma was decreased by 54.0 ± 1.9% in the ligation study and by 45.2 ± 3.8% in the hemorrhage intervention (**Table 2.1**). The discrepancy between the clearance rates may be accounted for by the fact that a time delay (35 ± 4.9 minutes) subsequent to the hemorrhage intervention allowed for the gradual increase of hepatic blood flow as compared to the SMA ligation. This partial restoration of hepatic blood flow was consistent with the increase in mean arterial pressure from 50 ± 0 to 62 ± 2 mmHg following hemorrhage. These data support the hypothesis that although full HDIR occurred in response to hemorrhage, the reduced hepatic blood flow led to reduced insulin clearance so that the direct effect of injected insulin was increased thus accounting for hemorrhage reducing the RIST index by only 34.3% versus the anticipated 55%.

The reduction in hepatic portal venous flow by 49.7 ± 2.4 % following hemorrhage was associated with a reduction in insulin clearance by 45.2 ± 3.8%. Most likely, the total reduction in clearance can be explained by the reduction in portal flow without meaningful compensatory changes to the extraction ratio. Insulin has a reported hepatic extraction ratio of 19% in the rat at 3 hours and 24 hours after feeding (Messerli et al., 1997). A reduction in portal flow would, according to clinical pharmacokinetic theory (Branch et al., 1973), be expected to lead to an increased extraction ratio and a modest reduction in clearance. Drugs or hormones that have high extraction ratios are expected to have blood flow dependent clearance, while compounds with low extraction ratios are expected to have little flow dependence for clearance. Discrepancy with this theory has previously been reported for lidocaine clearance in cats, where the extraction

ratio of 30% was not altered by reduced flow, thereby making clearance directly related to hepatic blood flow (Lautt and Skelton, 1977). Hormones which have hepatic flow dependent clearance include aldosterone, cortisol, and progesterone (Yates, 1965), to which insulin can be added.

2.4.3 - The hepatic arterial buffer response and acute hemorrhage

The hepatic arterial buffer response (Lautt, 1981) was defined in 1981, and proposes that the hepatic artery ensures steady hepatic blood flow in order to maintain the body's homeostatic needs rather than supplying nutrients or needed oxygen to the tissues. The mechanism of the arterial buffer response depends on the ability of portal blood flow to wash away local adenosine from the area of arterial resistance. If portal flow decreases significantly (as illustrated by the hemorrhage and SMA ligation interventions), less adenosine is washed away and the local concentration increases resulting in the dilation of the hepatic artery. It was previously demonstrated that following acute hemorrhage in cats, the hepatic artery did not constrict (Lautt and McQuaker, 1988). In order to establish the role of adenosine in inducing the protective arterial buffer response, an adenosine antagonist was administered. It was concluded that following antagonist administration, the hepatic artery constricted significantly in order to help to maintain blood pressure. Thus, in response to a significant decrease in hepatic portal flow, it would be expected that hepatic arterial blood flow would dramatically increase, thus affording the protection of the liver and valuable metabolic reactions during low flow states.

Because hepatic clearance of many hormones and compounds is blood flow dependent (see above), it is crucial that the rate of removal must not be allowed to

fluctuate following an alteration in hepatic portal flow in order to maintain hormone homeostasis. In order to maintain hormone level stability during times of acute blood loss, it is essential that the hepatic artery respond quickly to buffer changes in portal blood flow in order to prevent any significant changes in clearance rates of compounds or hormones that are blood flow dependent.

2.4.4 - The effect of hemorrhage on insulin resistance when pre-treated with atropine

Atropine (1 mg/kg –IV) was administered prior to the hemorrhage in order to induce HDIR, and as expected, the RIST index was reduced by 53.6 ± 3.0% (**Figure 2.5**). However, subsequent to hemorrhage, the RIST index increased by 29.1 ± 6.1% in comparison to the post atropine RIST. As the only contribution to the RIST index was insulin, this response suggested that hepatic clearance of the injected insulin had been reduced secondary to the decrease in hepatic blood flow that occurred in response to hemorrhage.

2.4.5 - Hemorrhage-induced hyperglycemia

The release of adrenal catecholamines and the activation of the hepatic sympathetic nerves play a role in the development of acute hyperglycemia following hemorrhage (Lautt et al., 1982). Glycogenolysis provides substrates that are required for plasma volume restoration and the mobilization of metabolic fuel for crucial cardiovascular and neural-operating systems (Jarhult, 1975). Our studies re-affirmed the presence of hyperglycemia following hemorrhage as basal glucose levels rose from a mean baseline of 109.5 ± 5.0 to 127.3 ± 6.3 mg/dl within 5 minutes of onset of hemorrhage.

Survival after hemorrhage is closely related to the capacity of the animal to develop hyperglycemia (Alibegovic and Ljungqvist, 1993). The administration of glucose intravenously to 24 hour fasted rats before an otherwise lethal hemorrhage (Nettelbladt et al., 1996) resulted in substantial hyperglycemia while glucose levels fell in saline treated animals. The saline treated rats developed irreversible shock and died within 3 hour of bleeding, while the glucose treated rats recovered and survived the seven-day observation period. Administrating carbohydrate before surgery can significantly reduce post-operative insulin resistance (Nygren et al., 1998).

Insulin levels are dramatically decreased during shock and trauma in several species, including man (Hiebert et al., 1972 and 1976), following stress-induced hyperglycemia. Reductions in insulin and HISS action following hemorrhage are physiologically advantageous as glucose can be utilized strictly for life-sustaining systems, and excess glucose in the plasma may assist osmotic-induced mobilization of extravascular fluid into the circulation (Yamaguchi, 1992).

Chapter 3 – The effect of exogenous somatostatin on HISS-dependent insulin resistance

3.1 - Introduction

The response to stress and surgical trauma has been described as a multi-hormonal reaction that is designed to act in a selective manner specific to the stimulus imposed upon it (Mason et al., 1975). Levels of somatostatin, a poorly understood hormone, have been shown to increase dramatically following hemorrhage (Lautt et al., 1982). Given that somatostatin is a potent inhibitor of insulin and glucagon, we hypothesized that it may play a key role in initiating HDIR, subsequent to hemorrhage. In an attempt to implicate somatostatin as an important mediator in HDIR following hemorrhage, we examined how exogenous administration would affect HISS action and release.

3.1.1 - The characteristics, secretion, and location of somatostatin stores in the body

The existence of somatostatin was first described in 1973 as a hypothalamic hormone that inhibits growth hormone (GH) secretion (Brazeau et al., 1973). Somatostatin is a peptide widely distributed in both the central nervous system and peripheral tissues. It is found as two bioactive peptides of 14 and 28 amino acids (Reichlin, 1983a).

Somatostatin secretion is stimulated by glucagon, insulin deficiency, α_2 agonists, several amino acids, acetylcholine and glucose (Reichlin, 1983a).

In the peripheral nervous system, somatostatin is produced by delta cells of the pancreas where it plays an important role in the control of both insulin and glucagon secretion (Koerker et al., 1974). Somatostatin is also produced in the gastro enteric tract, mainly in the antral portion of the stomach where it controls the release of

cholecystokinin (CCK), secretin and vasoactive intestinal peptide (VIP) (Reichlin, 1983b).

In the CNS, the highest somatostatin concentrations have been detected in the hypothalamus in the tuberinfundibular neurons where, acting as a neurohormone, the peptide regulates the hypothalamic-hypophyseal axis (Schettini, 1991). Somatostatin-carrying neurons are also present in many other areas of the brain such as the cerebral cortex, the limbic system and the nigro-striatal pathway (Schettini 1991). In these areas somatostatin acts as a true neurotransmitter or as a neuromodulator of release of neurotransmitters such as acetylcholine (Grey et al., 1990) and dopamine (Thal et al., 1986).

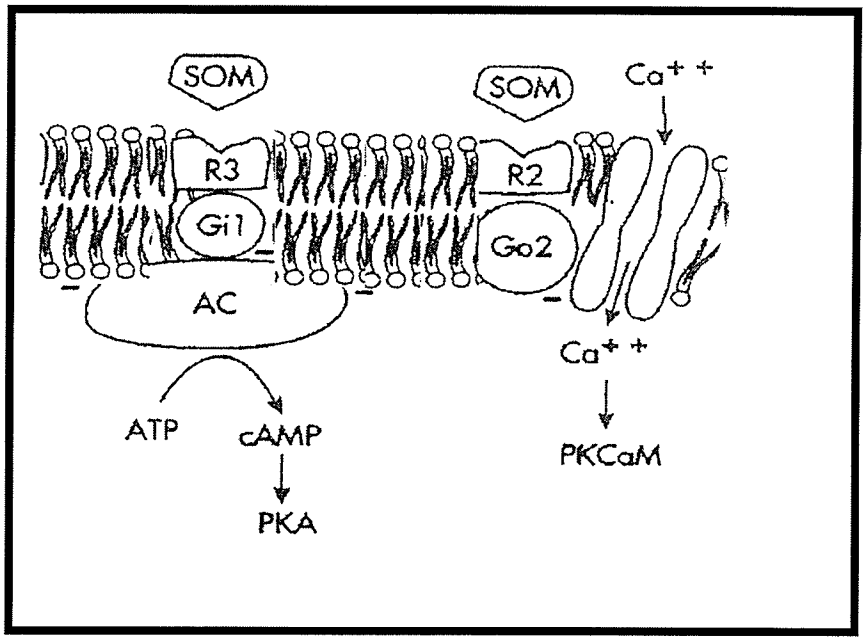
3.1.2 - Somatostatin receptors (SSTRs)

Somatostatin is capable of acting as a hormone, local chemical messenger or a neurotransmitter, and can emit a wide range of physiological actions that reflect both its widespread distribution and the existence of several receptor subtypes. There are 5 known somatostatin receptor subtypes SSTR-1 through 5 (Coy et al., 1993), that can be divided into two subgroups through sequence similarity and affinity for somatostatin analogs (Coy and Taylor, 1996). All 5 receptors are coupled to G proteins, and affect a number of distinct signaling transduction pathways (Fluori and Schettini, 1996). G protein mediated signal transduction is a complex signaling network with diverging and converging transduction steps at each coupling interface. These receptors belong to a superfamily of integral membrane proteins characterized by seven hydrophobic stretches of amino acids that span the plasma membrane. The N terminus of these receptors is located extracellularly and the C terminus extends into the cytoplasm. G proteins are

linked to adenylyl cyclase that dephosphorylates ATP to form cyclic AMP. Increased cAMP then activates protein kinase, which increases the influx of calcium, causing intracellular modifications. Extensive effort has been directed towards identification of the possible physiological roles and intracellular transducing mechanisms specifically coupled to each somatostatin receptor subtype.

Somatostatin receptor subtypes are widely distributed in tissues and organ systems, ranging from the CNS, pituitary gland, thyroid gland, pancreas, gastro enteric tract, kidney, lung and others. The tissue distribution of SSTRs has been studied in rats by Northern Blotting (Kaupmann et al., 1992;Kong et al., 1994). In the CNS, SSTR1 and 2 are widely expressed, with the highest levels in the cortex, hippocampus, amygdala, and hypothalamus. SSTR1 is expressed mainly on thyroid-stimulating hormone (TSH) secreting cells (Day et al., 1995), SSTR2 on both TSH and luteinizing hormone (LH) secreting cells, SSTR3 on prolactin and follicle-stimulating hormone (FSH) cells and SSTR4 and 5 mainly on GH secreting cells (O'Carroll and Krempels, 1995). In the periphery, all 5 receptors have been detected in the small intestine and spleen (Bruno et al., 1993) and other localizations were heart (1,3,4) liver (3) stomach (1,2,3,4), pancreas (2) kidney and lung (1,3,4) (Bruno et al., 1993) (**Figure 3.1**).

Figure 3.1 - Somatostatin receptors 3 and 5: The signal transduction pathways in the liver (3) and pancreas (2) (Modified from Bruno et al, 1993)



3.1.3 - The inhibitory effects of somatostatin

Somatostatin, which is released from the pancreatic delta cells is known to inhibit insulin, glucagon (Hellman and Lernmark, 1969), growth hormone (Brazeau et al., 1973), prolactin (Reichlin 1983a), and thyrotropin (Weeke et al., 1975). The inhibitory action of somatostatin on insulin and glucagon may be a paracrine response as a finding by Patel (Patel et al., 1982) has illustrated that the receptors for somatostatin are present on the A, B and D cells.

3.2 - Methods

3.2.1 – The effect and duration of action of somatostatin on the RIST

A standard surgical preparation, laparotomy and portal puncture was performed (**see section 1.13 for standard surgical preparation**). A control RIST (**see section 1.15 for RIST methodology**) was performed after a 30-minute stabilization period.

Subsequent to continuous somatostatin (0.16 μg/kg/min – IV) infusion at a rate of 0.025 ml/min, the animal was stabilized, and a RIST was completed. Initially, a somatostatin infusion rate of 0.8 μg/kg/min – IV was administered to the rat, as it was previously illustrated that this dose of somatostatin was capable of completely inhibiting endogenous insulin release in dogs (Moore et al., 2002). However, due to species differences and the size of the subject, the dose was considered to be super-maximal, and was reduced by one-fifth to 0.16 μg/kg/min. Immediately following the RIST, somatostatin administration was discontinued and the animal was allowed to restabilize for 50 minutes, after which a RIST was performed to determine the reversibility of HDIR caused by somatostatin. An additional RIST was not performed beyond 50 minutes as the intravenous half-life of exogenous somatostatin is 1.5 minutes (Shen et al., 1982). According to clinical pharmacokinetic theory, the rate of elimination is constant and is equivalent to five times the elimination half-life (Mycek et al., 1997), therefore at 50 minutes, administered somatostatin was completely eliminated from the circulation.

3.2.2 - Somatostatin and atropine series

A standard surgical preparation, laparotomy and portal puncture was performed. After a control RIST was completed, somatostatin (0.16 μg/kg/min – IV) was continuously administered as above and a somatostatin RIST was done. Immediately following the RIST, somatostatin administration was discontinued, and atropine was administered (1 mg/kg – IV). A post atropine RIST was then done to determine the extent of HDIR produced by somatostatin.

3.2.3 - Cyclosomatostatin and somatostatin series

A standard surgical preparation, laparotomy and portal puncture was performed. After a control RIST was completed, cyclosomatostatin (20 μg/kg/min – IPV) infusion was initiated. 10 minutes after the initiation of cyclosomatostatin infusion, somatostatin (0.16 μg/kg/min- IV) was continuously administered. Glycemia was monitored until a stable baseline was established, and a RIST was performed during the continuous infusion of both cyclosomatostatin and somatostatin. Following completion of the RIST, the cyclosomatostatin infusion was terminated, and the somatostatin infusion continued. A RIST was repeated. Atropine (1 mg/kg –IV) was then administered, and a post atropine RIST was performed to determine the extent of HDIR produced by somatostatin. Pilot studies indicated that a dose of 10 μg/kg/min of cyclosomatostatin did not produce a full blockade of somatostatin induced HDIR **(Figure 3.4)**.

3.2.4 - Drugs

Human insulin (Humulin R) was purchased from Eli Lilly (Toronto, Canada). Atropine, D-glucose, cyclosomatostatin and somatostatin were purchased from Sigma Chemical Co. (St. Louis, Mo.). All drugs with the exception of cyclosomatostatin were dissolved in saline. Cyclosomatostatin was initially dissolved in twice-distilled water and then diluted in saline.

3.2.5 - Data analysis

Data were analyzed using repeated-measures and one-way ANOVA, followed by the Tukey-Kramer multiple comparison test in each group. The data were expressed as means ± SE throughout. Differences were accepted as statistically significant at $P<0.05$. Animals were treated according to the guidelines of the Canadian Council on Animal

Care, and the Protocol Management and Review Committee at the University of Manitoba approved all protocols.

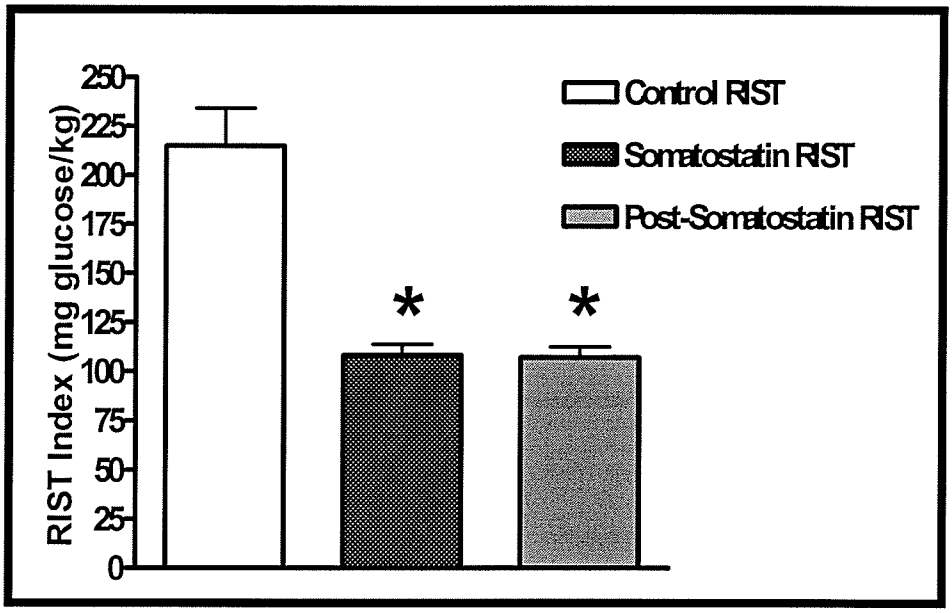
3.3 - Results

3.3.1 - Somatostatin causes a reduction in the RIST index

Animals (n=4; 278.0 ± 13.9 grams) in this series were used to analyze the effects of somatostatin (0.16 μg/kg/min – IPV) on the RIST index (**Figure 3.2**). The somatostatin infusion had no effect on glycemic levels during its continuous infusion as the glycemic baseline for the control (108.0 ± 3.5 mg/dl), somatostatin (106.2 ± 4.1 mg/dl), and post-somatostatin (109.3 ± 2.4 mg/dl) RISTs were not significantly different. The control RIST index was 215.0 ± 19.2 and the somatostatin RIST index was 108.4 ± 5.6 mg of glucose/kg. The decrease (49.6 ± 5.8%) in insulin sensitivity between the control and somatostatin RIST is significant (*P = 0.001). The post-somatostatin RIST index (107.3 ± 5.5 mg of glucose/kg) was not significantly different from the somatostatin RIST index, which occurred 50 minutes following the discontinuation of somatostatin infusion thereby illustrating that the insulin resistance produced by somatostatin was well maintained.

Figure 3.2 - The effect of somatostatin on the RIST index following administration and subsequent to ceasing infusion.

The RIST index with the somatostatin infusion significantly (*P = 0.001) decreased by 49.6%. The somatostatin infusion (0.16 µg/kg/min – IPV) was turned off and the animal was restabilized for 50 minutes. The resulting post-somatostatin RIST index was not significantly different from the somatostatin RIST index.

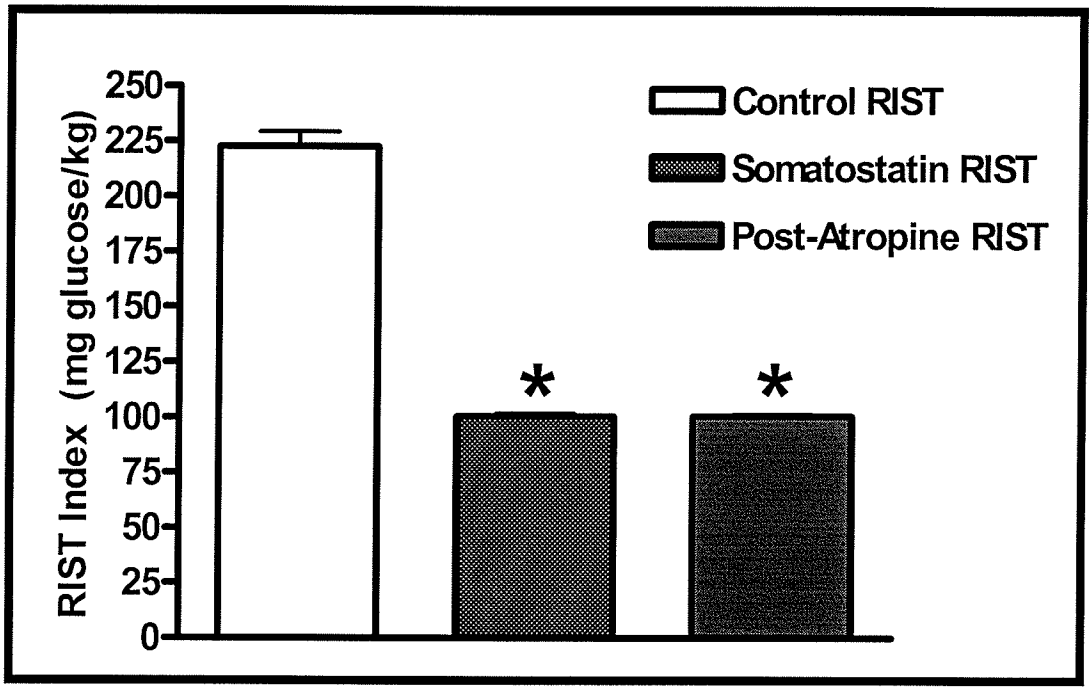


3.3.2 - Somatostatin causes HDIR

Animals in this series (n=3; 269.5 ± 2.5 grams) were used to illustrate that somatostatin (0.16 µg/kg/min – IPV) administration causes a reduction (54.9 ± 1.2%) in the RIST index that was not reduced further by atropine administration (1 mg/kg – IV) (**Figure 3.3**). The control RIST index was 222.7 ± 6.5, the somatostatin RIST index was 100.5 ± 1.3 and the post atropine RIST index was 100.5 ± 0.9 mg of glucose/kg. The 54.9% reduction in the RIST index in both the somatostatin and atropine interventions confirms full HISS blockade (*P = 0.001), thus demonstrating the presence of insulin action only.

Figure 3.3 - Somatostatin resulted in HISS-dependent insulin resistance as confirmed by a post-atropine test.

The RIST index with the somatostatin infusion (0.16 μg/kg/min – IPV) decreased (*, P = 0.001) by 54.9% and was not decreased further by the administration of atropine (1 mg/kg – IV). Somatostatin caused complete HISS-dependent insulin resistance.

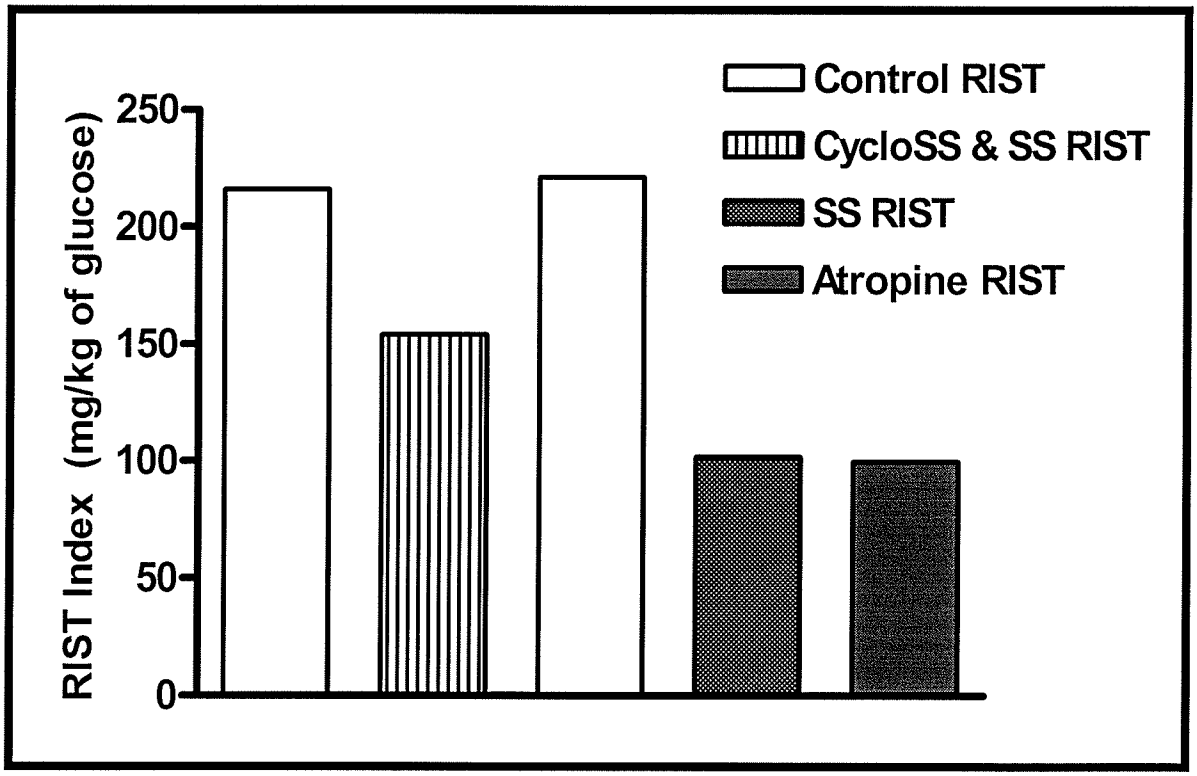


3.3.3 - Determining a blocking dose of Cyclosomatostatin

One animal (mass = 272.0 g) was used in this pilot experiment in order to establish a full blocking dose of cyclosomatostatin (**Figure 3.4**). Initially, 10 μg/kg/min of cyclosomatostatin (IPV) was administered. Following the control RIST (216.18) the cyclosomatostatin and somatostatin RIST (154.32 mg/kg of glucose) still showed a reduction in the RIST index as compared to the control. This was demonstrated by turning off both infusions and repeating a control RIST (221.30). In order to establish

that somatostatin was capable of inducing HDIR, it was administered by itself, and another RIST (101.84) was attempted. In order to verify the presence of HDIR, an atropine intervention (RIST) was done (99.63 mg/kg of glucose).

Figure 3.4 – Cyclosomatostatin (10 µg/kg/min – IPV) was incapable of preventing HDIR subsequent to somatostatin administration. Only a partial prevention was detected following continuous somatostatin and cyclosomatostatin administration.



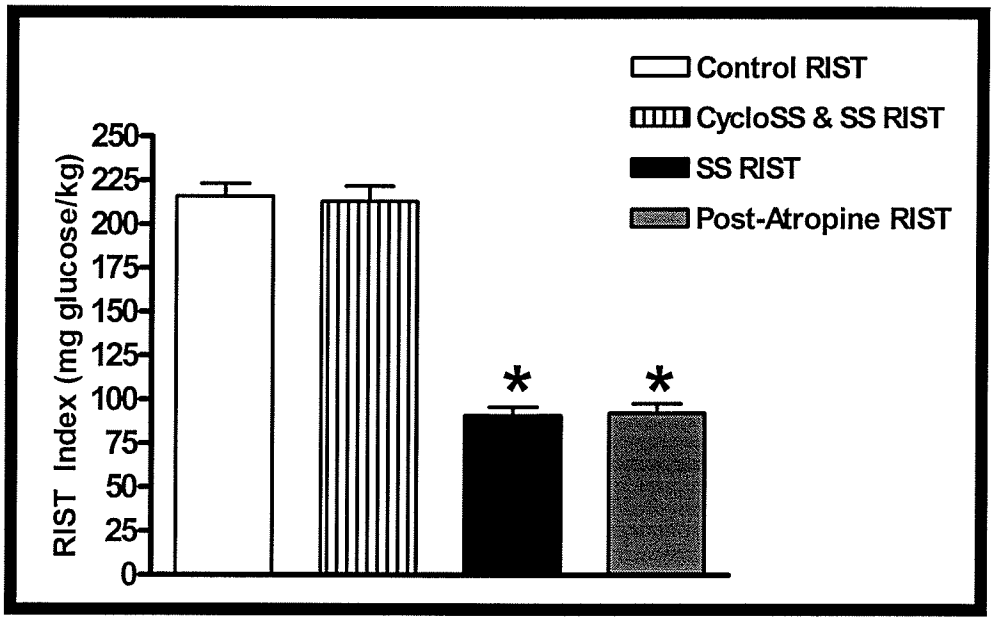
3.3.4 - Cyclosomatostatin can prevent HDIR following somatostatin administration

Animals in this series (n=3; 274.9 ± 4.8 grams) were used to illustrate that the continuous infusion of cyclosomatostatin (20 µg/kg/min – IPV) significantly prevented the occurrence of HDIR (P = 0.0001). The control RIST index was 215.9 ± 7.4, the cyclosomatostatin and somatostatin RIST index was 213.1 ± 8.6, the somatostatin RIST index was 91.1 ± 4.7, and the post atropine RIST index was 92.7 ± 5.2 mg of glucose/kg.

Cyclosomatostatin was continuously administered for 58.1 ± 7.6 minutes, and somatostatin was continuously infused for 87.8 ± 5.5 minutes, and neither drug had a significant effect on glycemic levels. HDIR was produced with both atropine (1 mg/kg) and somatostatin (0.16 μg/kg/min – IV) interventions (**Figure 3.5**), as shown by the inability of atropine to cause a further reduction in the RIST index. The 57.1 ± 1.4% reduction in the RIST index in both the somatostatin and atropine interventions confirms full HISS blockade (P = 0.0001), thus demonstrating the presence of insulin action only.

Figure 3.5 - Cyclosomatostatin (20 µg/kg/min – IPV) administered prior to somatostatin prevents the occurrence of HDIR.

There was no significant difference between the control and cyclosomatostatin (CycloSS) and somatostatin (SS) RIST index, thereby indicating that HDIR was prevented. The RIST index with the somatostatin infusion (0.16 µg/kg/min – IPV) decreased (*, P = 0.0001) by 57.1% and was not decreased further by the administration of atropine (1 mg/kg – IV).



3.4 - Discussion

3.4.1 – Exogenous somatostatin causes a reduction in insulin sensitivity that is HDIR.

Exogenous somatostatin (0.16 µg/kg/min) caused a reduction (49.6%) in insulin sensitivity that was identifiable using RIST methodology (**Figure 3.2**). Following the termination of a continuous somatostatin infusion, and after a 50 minute stabilization period, it was evident that the reduction in insulin sensitivity was not reversible, even though the half-life of somatostatin is approximated at 1.5 minutes (Shen et al., 1982).

In order to decipher if the reduction in insulin sensitivity seen during somatostatin administration was attributable to the absence of HISS, a subsequent study was performed with atropine, which is known to block HISS release (Takayama et al., 2000). Following the completion of this series, it was concluded that the reduction in insulin sensitivity seen during somatostatin infusion was complete HDIR, as the atropine intervention illustrated no further reduction in the RIST index (**Figure 3.3**). Therefore, somatostatin demonstrated insulin resistance that was accountable by HDIR with direct HISS-independent action being unsuppressed.

3.4.2 – Cyclosomatostatin prevents the occurrence of somatostatin-induced HDIR.

In order to determine if the blockade of HISS action following somatostatin-induced HDIR could be prevented, a somatostatin antagonist (cyclosomatostatin) was employed. Cyclosomatostatin (20 μg/kg/min) prevented the blockade of HISS (**Figure 3.5**) when it was administered concurrently with somatostatin (0.16 μg/kg/min). In order to demonstrate that HISS action could be blocked in this study, an atropine intervention confirmed complete HDIR as indicated by a 57.1% reduction in the RIST index. Therefore, the dose of 20 μg/kg/min of cyclosomatostatin prevented somatostatin-induced HDIR.

3.4.3 – Assessing insulin sensitivity – a speculated role for somatostatin

The glucose tolerance test is a commonly used method of assessing insulin sensitivity (De Fronzo et al., 1992). After an overnight fast and a blood glucose sample, patients are given a large bolus of glucose in order to evaluate the presence and action of their endogenous insulin. Two hours after administration, plasma glucose levels are

analyzed and if glucose levels are above a concentration of 11.1 mmol l^{-1}, the patient is diagnosed with diabetes mellitus (World Health Organization, 1999).

A study conducted by Reid has indicated that the glucose tolerance test is incapable of detecting HISS action (Reid et al, 2002). One possibility as to why this test cannot effectively measure HISS may be because the glucose administered may induce endogenous somatostatin release (Reichlin, 1983a). Given that somatostatin has been shown to block HISS, only insulin action would be detectable. Therefore, a postulation as to why the glucose did not produce HISS-dependent meal-induced insulin sensitization may be because the hyperglycemia blocked HISS release.

Chapter 4 – The role of the sympathetic nervous system in inducing HDIR following hemorrhage: α and β adrenergic blockade.

4.1 - Introduction

There are many regulators and mediators of the hemorrhagic response that may induce HDIR following acute blood loss. The adrenergic nervous system is one of many neural and hormonal factors that influence the secretion of insulin and glucagon (Smith et al., 1979), and ultimately the regulation of glucose metabolism in the body (Rizza et al., 1980). Pharmacological subdivision of these receptors into two groups (α and β) was first suggested by Ahlquist in 1948 (Ahlquist: Historical Perspective.,1980) based upon the effects of adrenaline at peripheral sympathetic sites. In order to determine if the hepatic sympathetic nerves were responsible for causing HDIR subsequent to hemorrhage, experimental blockade of both α and β adrenoreceptors was attempted.

4.1.1 - Defining agonists and antagonists.

A simplistic biochemical definition of an agonist is a drug or other chemical that can combine with a receptor on a cell to produce a physiologic reaction that is typical of a naturally occurring substance. The name agonist is derived from the Latin word, "agonista," meaning contender. A simplistic definition of an antagonist is a chemical substance or drug that interferes with the physiological action of another, especially by combining with and blocking its receptor.

Adrenergic antagonists bind to adrenoreceptors but do not trigger the usual receptor-mediated intracellular effects. These drugs act either reversibly or irreversibly attaching to the receptor, thus preventing its activation by endogenous catecholamines.

Like the agonists, the adrenergic antagonists are classified according to their relative affinity for α or β receptors in the peripheral nervous system.

4.1.2 – Adrenergic control of insulin release

Adrenergic receptor mechanisms involved in the regulation of the β-cells appear complex and may vary depending on the species. The beta cell within the islet of Langerhans possesses both types of alpha and beta adrenoreceptors, which can exert both a stimulatory and inhibitory action (Loubatieres-Mariani,1986). The α_2 adrenoreceptor may be responsible for the inhibition of glucose-induced insulin secretion, as shown by the ability of an epinephrine infusion to prevent insulin secretion and cause a rise in blood glucose levels (Smith et al., 1979). It has been shown that in conscious rats that a selective α_2 adrenoreceptor agonist (UK 14304) can reduce the insulin response (John et al., 1990). Therefore, it appears that insulin inhibition is mediated via an α_2 adrenoreceptor (Nakadate et al., 1980).

Adrenaline inhibits insulin secretion via an α-adrenergic action (Porte, 1967) so that the expected rise in plasma insulin concentration during hyperglycemia fails to occur. However, we have recently shown (Seredycz et al., submitted for publication in 2004) that the intervention of hemorrhage causes a significant reduction in hepatic portal flow, which directly affects insulin clearance, showing that insulin concentration increases when administered exogenously for the RIST.

β-adrenergic stimulation causes a release of insulin from the B-cells of the islet of Langerhans when agonists such as albuterol (a β_2 agonist) are administered and thus, the release of insulin is a β_2 response (Porte, 1967).

4.1.3 - Hyperglycemia may be mediated by α-adrenergic stimulation following hemorrhage in rats.

Claude Bernard was the first to note in 1877 that following acute hemorrhage in dogs, a marked increase in blood glucose levels occurred, and his findings in dogs were later confirmed in many species including man (Aub and Wu, 1920). It has been suggested that changes in blood glucose levels during an acute stress state, such as hemorrhage are mediated by multi-synaptic, hypothalamic-hepatic connections, whereas for long term stress, the regulation of blood glucose levels are maintained by hormonal factors like the adrenal medulla and the pancreas (Shimazu, 1981, 1983).

The adrenal catecholamines and the hepatic sympathetic nerves are the primary activators of hyperglycemia following acute blood loss, and are known to be a redundant control system (Lautt et al., 1982) (McLeod et al., 1986). The adrenal medulla and the hepatic sympathetic nerves act as a redundant control system because if one of the two responses is terminated, hyperglycemia will still occur. If both systems are inactivated there is a radical decrease in blood glucose levels subsequent to hemorrhage (Lautt et al., 1982).

The hepatic sympathetic nerves reach the liver via the anterior and posterior nerve plexus in the rat (Lautt, 1980), and following stimulation and release of norepinephrine onto α-adrenergic receptors, hepatic glucose stores are released. The particular receptor type that mediates adrenergic stimulation of glycogenolysis is still uncertain; however, neurally-induced glycogenolysis is likely to be mediated by α receptors, which results in an increase in intracellular inositol 1,4,5-triphosphate (IP_3) and Ca^{2+} concentration (Beckh et al., 1982). The glycogenolysis induced by blood-borne catecholamines is mediated by both α and β adrenoreceptors. Some animal studies have eluded that

glycogenolysis in the liver is an α_2-mediated effect as clonidine has been reported to increase hepatic glycogenolysis (Kobayshi and Kohei, 1972), whereas human studies have illustrated that β stimulation activates this process (Pogasta and Dubecz, 1979;Rizza et al, 1980).

Adrenergic β agonists and glucagon are linked to adenylcyclase causing an increase in cyclic AMP, which activates protein kinase, resulting in an increase in hepatic glycogenolysis. An increase in hepatic sympathetic activity may also cause the formation of prostaglandins, which stimulate glycogenolysis by their direct action on parenchymal cells (Yamaguchi, 1992).

Figure 4.1 further illustrates the adrenoreceptors that are involved in glucose production and **Figure 4.2** demonstrates the effects of activated adrenoreceptors.

Figure 4.1 - The Adrenoreceptors involved in glucose production. (Modified from Mills and Horn, 1985, pg. 247)

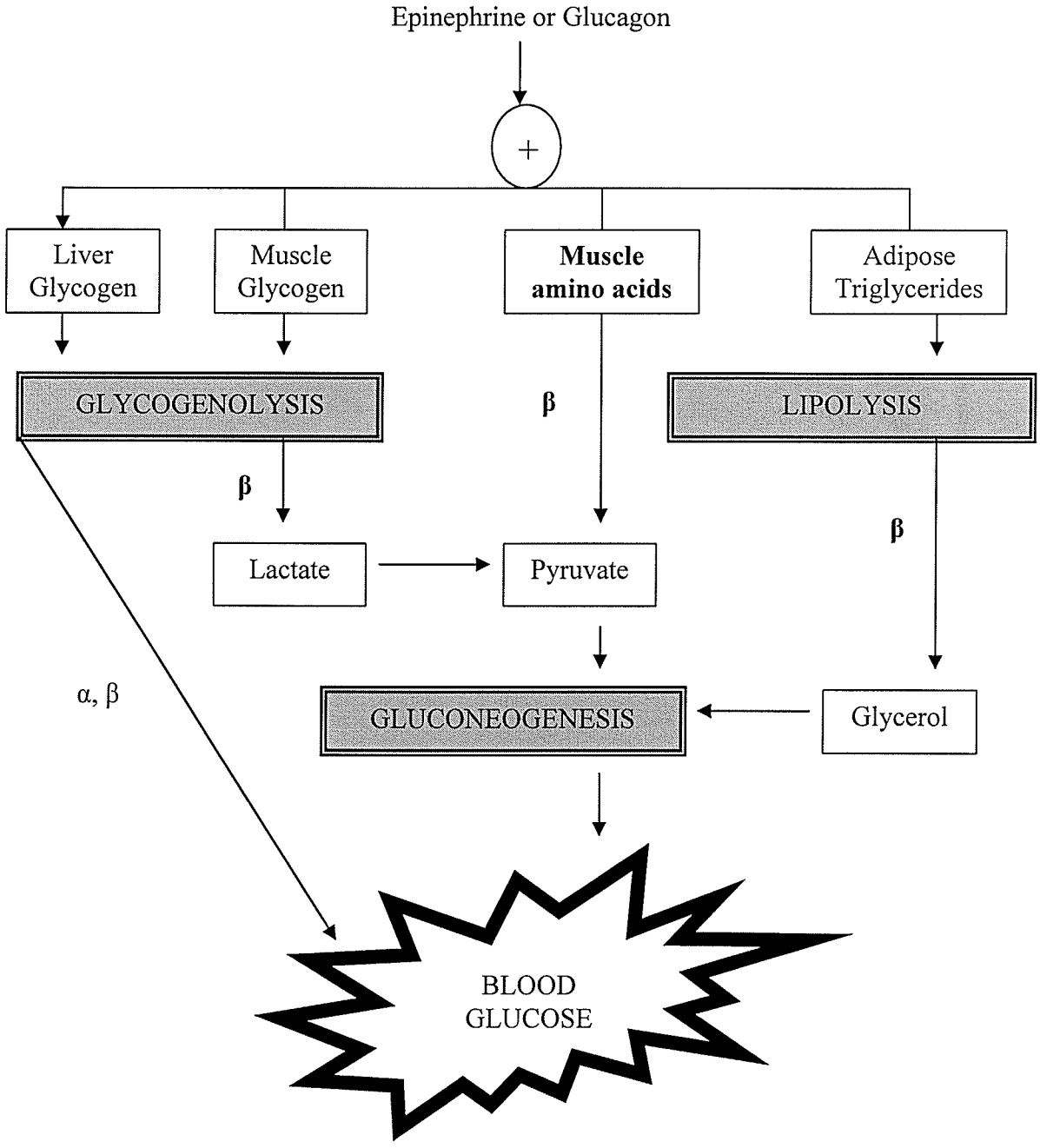
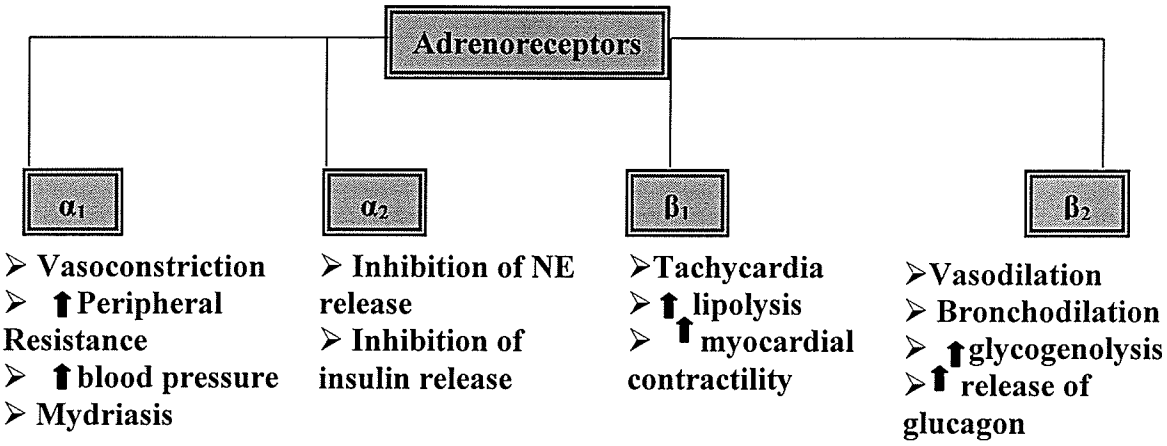


Figure 4.2 - Activation of adrenoreceptors by their respective agonists initiates the following effects. (modified from Mycek et al., 1997 pg.60)



4.1.4 - Phentolamine: a non-selective, competitive α-adrenergic antagonist

Phentolamine blocks the effects of circulating epinephrine and norepinephrine on α_1 and α_2 adrenoreceptors following sympathetic stimulation. Normal sympathetic control of the vasculature occurs in large part through agonists on α-adrenergic receptors; therefore blockade of these receptors causes a reduction in the sympathetic tone of the blood vessels, resulting in decreased peripheral resistance.

Phentolamine has little effect on the blood pressure of healthy individuals or patients with essential hypertension (Kirsten et al., 1998), and is known to have a half-life of 19 minutes following intravenous administration. The route of elimination is not completely known; however approximately 13% of a single intravenous dose is excreted in urine as unmetabolized drug (Kirsten et al., 1998).

Accepted therapeutic uses for phentolamine are for the treatment of hypertension, paraoxysmal hypertension prior to and during surgery for pheochromocytoma, and

dermal necrosis. Examples of typical adrenergic blockers are doxazosin, prazosin, terazosin (α1 competitive blockers), and phenoxybenzamine (α1 and α2 non-selective, non-competitive). In contrast to phenoxybenzamine, phentolamine produces a complete block of α1 and α2 receptors. Like phenoxybenzamine, it produces postural hypotension and causes epinephrine reversal.

4.1.5 - Propranolol: a non-selective β-adrenergic antagonist

Propranolol is a nonselective β adrenoreceptor antagonist. It was the first of its class to be clinically approved and the standard to which newer drugs have been compared. β-adrenoreceptor blockers are used primarily for their cardiovascular effects, as blockade of the β_1 receptor prevents an increase in cardiac contractile force, contraction, cardiac output and work that is initiated by the sympathetic nervous system (Mills and Horn, 1985). The release of renin from the kidney is also regulated by the β_1 receptor. By blocking renin secretion, there is a reduction in renin formation and hence the biological activity of angiotensin II, thereby decreasing blood pressure (Mills and Horn, 1985).

β-adrenoreceptor blocker use is relatively contraindicated for diabetics, because catecholamines utilize the β_2 receptor to promote glycogenolysis and glucose mobilization. Therefore, if an insulin-dependent diabetic is given propranolol, careful monitoring of blood glucose is essential, as this drug can attenuate the normal physiologic response to hypoglycemia (Tregaskis and McDevitt, 1990).
Some β-adrenoreceptor blockers, such as propranolol actually have a modest degree of agonist activity, or are known as partial agonists with low intrinsic activity. This is referred to as intrinsic sympathomimetic activity (Wagstaff et al., 1996). β-

adrenoreceptor blockers have a variety of side effects such as sedation, fatigue, and impairment of mental function.

4.2 - Methods

4.2.1 - Phentolamine and hemorrhage

Following a standard surgical preparation, a laparotomy and portal venous puncture was performed (**see section 1.13 for Standard surgical preparation**). After a 30 minute stabilization period, a control RIST was performed (**see section 1.15 for RIST methodology**). Following the RIST, phentolamine (400 μg/kg/min – IPV) was administered for 10 minutes at a rate of 0.025 ml/min. The effectiveness of the blocking dose of phentolamine was assured by the ability to block hyperglycemia induced by hemorrhage. The rat was restabilized and then hemorrhaged to an arterial blood pressure of 50 mmHg and held at this pressure for a ten-minute interval (**see section 1.14 for Hemorrhage methodology**). Glycemic levels were closely monitored during and after hemorrhage, until they became stable for at least 15 minutes. A post-hemorrhage RIST was performed.

4.2.2 - Identifying a blocking dose of propranolol

A standard surgical preparation, laparotomy and portal vein puncture were performed on an anesthetized rat. After a 30 minute stabilization period, in which both glucose and basal heart rate readings were stable, propranolol (1.5 mg/kg – IPV) was administered at a rate of 0.1 ml/min for 5 minutes. Heart rate and glucose levels were analyzed in the last 30 seconds of propranolol administration. (Heart rate was measured using a Grass AC/DC Strain gauge amplifier and National Instrument data acquisition software.) Following stabilization, isoproterenol (0.5 μg/kg/min – IV) was administered at a rate of

0.1 ml/min for 5 minutes. Heart rate and glucose levels were analyzed in the last 30 seconds of isoproterenol administration and 15 minutes subsequent to discontinuation of the isoproterenol infusion.

4.2.3 - Propranolol and hemorrhage

Following a standard surgical preparation, a laparotomy was performed. The portal vein was isolated and cannulated. After a 30 minute stabilization period, a control RIST was performed. Following the RIST, propranolol (1.5 mg/kg – IPV) was administered at a rate of 0.025 ml/min for 10 minutes. The rat was restabilized and then hemorrhaged to an arterial blood pressure of 50 mmHg and held at this pressure for a ten-minute interval. After a stable glycemic baseline had been reached, a post-hemorrhage RIST was performed.

4.2.4 - Drugs

Phentolamine, and isoproterenol were purchased from Sigma Chemical Co. (St. Louis, Mo.). Propranolol hydrochloride USP was purchased from Sabex (QC, Canada). All drugs were dissolved in saline.

4.2.5 - Data analysis

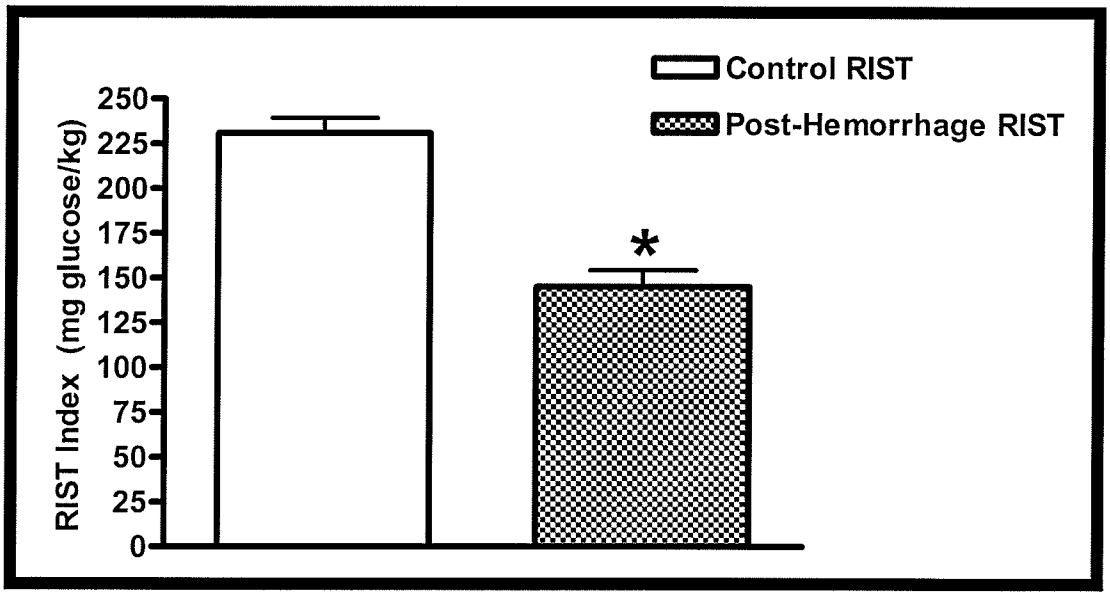
Data were analyzed using a paired t-test. The data were expressed as means ± SE throughout. Differences were accepted as statistically significant at $P<0.05$. Animals were treated according to the guidelines of the Canadian Council on Animal Care, and the Protocol Management and Review Committee at the University of Manitoba approved all protocols.

4.3 - Results

4.3.1 - Phentolamine is incapable of preventing HDIR subsequent to hemorrhage

Animals (n=7; 293.7 ± 5.0 grams) in this series were used to analyze the effects of phentolamine on HDIR (**Figure 4.3**). Phentolamine (400 μg/kg/min – IPV) was administered at 15.1 ± 2.2 minutes following the control RIST, prior to hemorrhage. The decrease (31.8 ± 5.5%) in insulin sensitivity between the control (213.1 ± 8.4) and post hemorrhage RIST (145.4 ± 9.2 mg of glucose/kg) is significant (*, P = 0.0001) and is similar to the HDIR produced in the absence of phentolamine (**see Section 2.3.1 – Acute hemorrhage causes HDIR**). It was previously shown that glycemia increased by 18.2 ± 4.2 % during hemorrhage (completed with no pharmaceutical agents – **see Section 2.3.1**), while phentolamine administered prior to hemorrhage diminished this effect significantly to 1.1 ± 0.9 % (P = 0.0001). The impediment of hyperglycemia was indicative of a complete block of hepatic α-adrenergic receptors given that phentolamine is known to block the α_2 mediated effect of glycogenolysis (Kobayshi and Kohei, 1972). The volume of blood removed (3.5 ± 0.8 ml) and the amount of time taken (4.3 ± 0.6 minutes) to reduce mean arterial pressure to 50 mmHg was not significantly different from the series where no pharmacological agents were administered (**see section 2.3.1 – Acute hemorrhage induces HDIR**). Therefore, it was concluded that phentolamine was unable to prevent HDIR from occurring subsequent to hemorrhage.

Figure 4.3 - HDIR induced by hemorrhage after α-adrenergic receptor blockade. The post-hemorrhage RIST was significantly different (*,P = 0.0001) from the control RIST. The decline in the RIST index (31.8%) is similar to the reduction seen when atropine induced full HDIR. Phentolamine (400 μg/kg – IPV) was unable to prevent the reduction in the RIST index from occurring post hemorrhage.



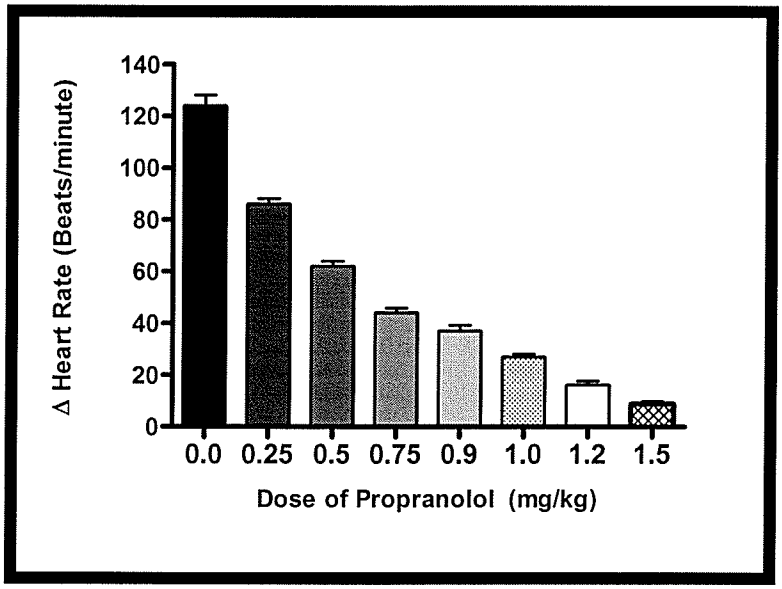
4.3.2 – The blocking dose of propranolol

Animals (n=3, mass = 277.2 ± 9.5 grams) in this series were used to identify a blocking dose of propranolol that would completely block hepatic adrenergic receptors following hemorrhage. Isoproterenol caused an increase in basal heart rate equivalent to 125.2 ± 9.8 beats/min while propranolol (1.5 mg/kg – IPV) caused a reduction in basal heart rate by 65.3 ± 11.2 beats/min. When isoproterenol was administered (0.5 μg/kg/min – IV) subsequent to propranolol (1.5 mg/kg), the difference in heart rate (8.9 ± 3.3 beats/min) was significantly reduced (**Figure 4.4**). The dose of 1.5 mg/kg of

propranolol was shown to block the acceleration in heart rate caused by isoproterenol and thus ensured the complete blockade of hepatic β receptors.

Figure 4.4 - A dose response curve: The ability of propranolol to inhibit heart rate following 0.5 μg/kg/min – IV of isoproterenol.

Administration of isoproterenol at 0.1 ml/min and subsequent administration of varying doses of propranolol illustrates that 1.5 mg/kg of propranolol is required to fully inhibit the acceleration in heart rate caused by isoproterenol.



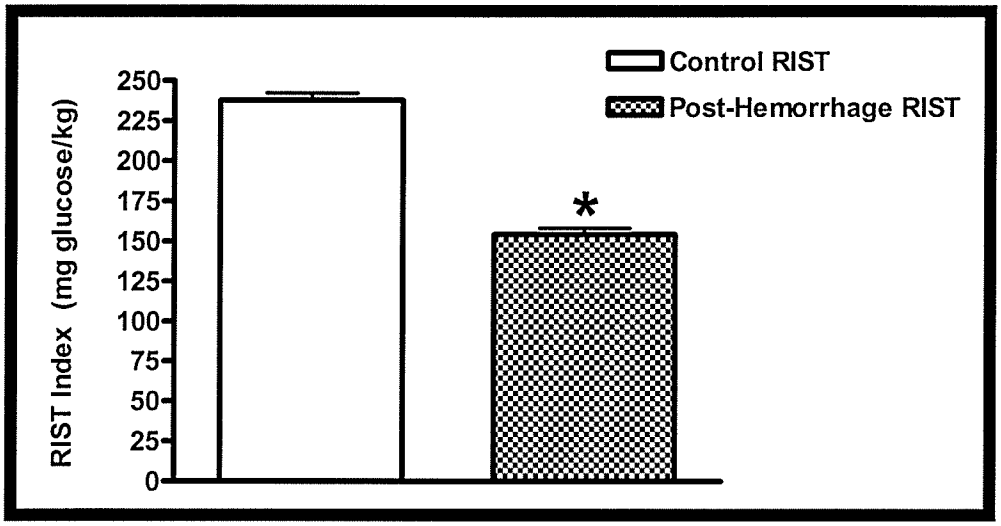
4.3.3 - Propranolol is incapable of preventing HDIR subsequent to hemorrhage

Animals (n=5; 265.4 ± 5.8 grams) in this series were used to analyze the effects of propranolol on HDIR. (**Figure 4.5**) To ensure complete blockade of hepatic β receptors, a previously established blocking dose of propranolol (1.5 mg/kg – IPV) was administered 8.2 ± 5.4 minutes following the control RIST, prior to hemorrhage. The decrease (35.1 ± 2.7%) in insulin sensitivity between the control (237.8 ± 4.3) and post hemorrhage RIST (154.4 ± 3.9 mg of glucose/kg) is significant (*, P = 0.0001) and is similar to the HDIR produced in the absence of propranolol (**see section 2.3.1 – Acute**

hemorrhage causes HDIR). The volume of blood removed (3.4 ± 0.8 ml) and the amount of time taken (4.4 ± 1.0 minutes) to reduce mean arterial pressure to 50 mmHg was not significantly different from the series where no pharmacological agents were administered (**see section 2.3.1 – Acute hemorrhage induces HDIR**).

Surprisingly, following hemorrhage, glycemia increased by 17.4 ± 3.3%; thereby implying that propranolol did not alter glycemic levels significantly after hemorrhage. Propranolol was unable to prevent HDIR from occurring subsequent to hemorrhage.

Figure 4.5 - HDIR induced by hemorrhage after β receptor blockade.
The post-hemorrhage RIST was significantly different (*, P = 0.0001) from the control RIST. The decline in the RIST index (35.1%) was similar to the reduction seen when atropine induced full HDIR. Propranolol (1.5 mg/kg – IPV) was unable to prevent a reduction in the RIST index from occurring after hemorrhage.



4.4 - Discussion

4.4.1 – Phentolamine (400 μg/kg/min) causes complete α-adrenergic receptor blockade during acute hemorrhage.

Phentolamine is a non-selective α_1 and α_2 adrenergic blocker, and can prevent α-adrenoreceptor activation during sympathetic innervation. Given that the hyperglycemia exhibited during acute blood loss is mediated primarily through the activation of α_2 adrenergic hepatic receptors (Kobayshi and Kohei, 1972), a sufficient dose of an α-adrenergic antagonist would terminate the response. When phentolamine (400 μg/kg/min) was administered prior to hemorrhage, the hyperglycemic response that was observed after 5 minutes was virtually abolished (only a 1.1% increase in glycemic levels). Therefore, the dose of phentolamine administered through the hepatic vein was sufficient to block hepatic α-adrenergic receptors.

4.4.2 – α-Adrenergic blockade with phentolamine (400 μg/kg/min) does not prevent HDIR following hemorrhage

Although phentolamine blocked hepatic α-adrenergic receptors, the hemorrhage intervention still produced a significant reduction (31.8%) in insulin sensitivity as measured by the RIST methodology (**Figure 4.3**). This reduction in insulin sensitivity is similar, and not statistically different from the reduction in insulin sensitivity seen following hemorrhage alone (34.3%). As stated previously, hemorrhage results in complete HDIR, which was shown by the inability of atropine to cause a further reduction in the RIST index (**see section 2.3.1**). Therefore, it is possible to conclude that HDIR is still present during complete α-adrenergic blockade, and that α-adrenergic stimulation via the sympathetic nervous system is not directly responsible for HISS blockade following hemorrhage.

4.4.3 – Propranolol (1.5 mg/kg) blocks hepatic β-adrenergic receptors completely during hemorrhage.

Propranolol is a non-selective β_1 and β_2 adrenergic blocker, and can prevent β-adrenoreceptor activation during sympathetic innervation when sufficiently administered. In order to ensure complete β-adrenergic blockade, heart rate was used as an indicator to assess the efficacy of the dose. Propranolol (1.5 mg/kg – IPV) completely blocked β-adrenergic receptors, as concurrent isoproterenol administration was unable to cause an acceleration in heart rate. In addition, propranolol was administered intraportally; therefore the concentration of propranolol at the hepatic receptors was substantially higher than the concentration of propranolol at the β-adrenergic receptors in the heart. The time taken to complete the hemorrhage intervention and the subsequent RIST (49.1 ± 8.0 minutes) was a short enough time period to ensure that the hepatic beta adrenergic receptors were still blocked given that the plasma half life of propranolol is 2 hours (Tregaskis and McDevitt, 1990).

4.4.4 - β adrenergic blockade does not prevent HDIR following hemorrhage

Propranolol was administered in order to block hepatic β-adrenergic receptors. Although hepatic β-adrenergic receptors were blocked, the hemorrhage intervention still produced a significant reduction (35.1%) in insulin sensitivity as measured by the RIST methodology (**Figure 4.5**). This reduction in insulin sensitivity is similar, and not statistically different from the reduction in insulin sensitivity seen following hemorrhage alone (34.3%). Following propranolol administration (1.5 mg/kg – IPV) and the hemorrhage intervention, basal glucose levels rose by 17.4%. It was concluded that β-adrenergic stimulation in the rat does not assist in glycogenolysis during the hemorrhage

intervention, as glycemia increased to a value (17.4 ± 3.3%) that was not significantly different from the hyperglycemia exhibited during hemorrhage alone (18.2 ± 4.2%). Therefore, it is possible to conclude that HDIR is still present during complete β-adrenergic blockade, and that β-adrenergic stimulation via the sympathetic nervous system is not directly responsible for HISS blockade following hemorrhage.

4.4.5 - α and β adrenergic blockade does not prevent HDIR following hemorrhage

A reduction in insulin sensitivity still occurs following hemorrhage despite complete α and β adrenergic blockade. Therefore, it is possible to conclude that adrenergic sympathetic activation in response to acute blood loss is not a major regulator of HISS-dependent insulin resistance.

Chapter 5 – The preventative role of cyclosomatostatin in HDIR

5.1 - Introduction

As demonstrated in Chapter 3, somatostatin (0.16 μg/kg/min- IV) is capable of inducing HDIR, and its antagonist (cyclosomatostatin) administered concomitantly (20 μg/kg/min – IPV) can prevent its occurrence. Given that somatostatin levels escalate dramatically by 232% following hemorrhage (Lautt et al., 1982) in the cat, I hypothesize that somatostatin induces HDIR subsequent to hemorrhage in the rat, and that cyclosomatostatin (a somatostatin antagonist) can prevent the occurrence of HDIR.

5.2 - Methods

5.2.1 - Dose concentration curve for somatostatin

Following standard surgical preparation (**see section 1.13 for Standard surgical preparation**), and 30 minutes of stabilization, saline was administered intravenously at a rate of 0.025 ml/min for 10 minutes. Subsequent to initial saline administration, somatostatin was consecutively administered IV at doses of: 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 μg/kg/min at a rate of 0.025 ml/min for 10 minutes each. Following the maximal dose of somatostatin, saline was administered in order to establish a second baseline. Blood samples (120 μL) were collected every 10 minutes from the arterial side of the shunt during each consecutive saline or somatostatin infusion. The samples were centrifuged and the plasma was frozen immediately on dry ice, and later stored at –80 °C. These samples were analyzed for somatostatin in duplicate on a peptide enzyme immunoassay for unextracted rat plasma (Peninsula Laboratories, INC. San Carlos, CA).

5.2.2 - Somatostatin is measured systemically following acute hemorrhage

Following standard surgical preparation, and 30 minute recovery time, the first baseline blood sample (120 μL) was taken and treated as above. Following the initial sample, the animal was hemorrhaged to 50 mmHg (**see section 1.14 for Hemorrhage methodology**). The second blood sample was taken at the instant that arterial pressure had been reduced to 50 mmHg. The third sample was taken 10 minutes later. The animal was then stabilized for 100 minutes, with 2 more samples being taken 50 minutes apart during this period. The volume of blood taken from the animal (120μL x 3 = 360 μL) prior and subsequent to hemorrhage was counted towards the total volume required to reduce the animal's arterial blood pressure to 50 mmHg.

5.2.3 - Identifying a dose of cyclosomatostatin

The dose of cyclosomatostatin (20 μg/kg/min – IPV) that was used to block exogenously administered somatostatin (0.16 μg/kg/min) was employed again (**see section 3.3.4**) to assess if it would prevent endogenous somatostatin from initiating HDIR subsequent to hemorrhage.

5.2.4 - Cyclosomatostatin and hemorrhage

Following the standard surgical preparation, a laparotomy was performed. The portal vein was isolated and cannulated (**see section 1.13 for Standard surgical preparation**). After a 30 minute stabilization period, a control RIST was performed (**see section 1.15 for RIST methodology**). Following the RIST, cyclosomatostatin (20 μg/kg/min - IPV) was continuously administered at a rate of 0.025 ml/min. The animal was then hemorrhaged to an arterial blood pressure of 50 mmHg and held at this pressure for a ten-minute interval (**see section 1.14 for Hemorrhage methodology**). Glycemic

levels were closely monitored during cyclosomatostatin administration and after hemorrhage. A post-hemorrhage RIST was performed. Following the RIST, cyclosomatostatin administration was ceased, and atropine was administered (1 mg/kg – IV). The animal was stabilized and a post atropine RIST was performed.

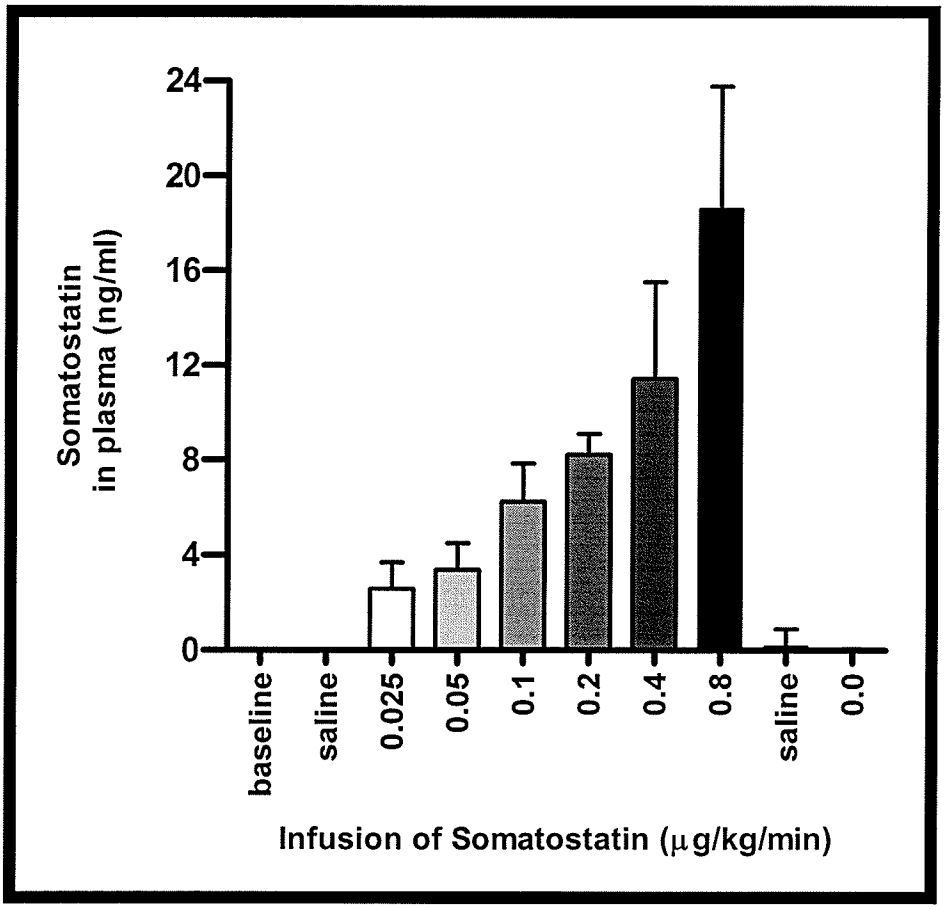
5.3 - Results

5.3.1 - Somatostatin can be measured with an RIA in the rat

Animals (n=3; 287.1 ± 6.0 grams) were used to create a dose concentration curve. The infusions of somatostatin (saline, 0.025, 0.05, 0.1, 0.2, 0.8 μg/kg/min) were administered for 10 minutes to ensure that steady state had been reached (**Figure 5.1**).

Figure 5.1 - Plasma somatostatin concentration in the rat following differing administered doses of somatostatin.

Somatostatin was detected in the plasma by a peptide enzyme immunoassay. The assay was able to sensitively distinguish the increasing concentration of the somatostatin doses administered.



5.3.2 - The presence of systemic somatostatin subsequent to hemorrhage

Given that the peptide enzyme immunoassay was previously assessed and deemed capable of measuring somatostatin in the plasma, this assay was employed again to measure endogenous somatostatin released following acute hemorrhage (**Table 5.1**: n =

5, 286.2 ± 5.9 grams). The time points selected were able to detect somatostatin but only small increments, and in sporadic patterns that were not statistically significant.

Table 5.1 - Somatostatin levels that were detected in rat plasma by the peptide enzyme immunoassay.

Prior to hemorrhage, a blood sample was collected and assisted in establishing a baseline somatostatin concentration. After the rat's arterial pressure reached 50 mmHg, another sample was collected, followed by an additional sample 10 minutes later. In order to mimic the timings that 2 consecutive RISTs would take, 2 other samples were taken spaced 50 minutes apart.

Baseline	MAP = 50 mmHg	10 minutes later	60 minutes later	110 minutes later
0.006	0.014	0.017	0.009	0.023
0.022	0.017	0.021	0.006	0.044
0.003	0.002	0.016	0.007	0.003
0.001	0.013	0.02	0.014	0.053
0.027	0.028	0.027	0.003	0.018
0.013	0.019	0.021	0.008	0.012
0.006	0.026	0.033	0.015	0.019
0.011 ± 0.004	0.017 ± 0.009	0.022 ± 0.002	0.009 ± 0.002	0.025 ± 0.007

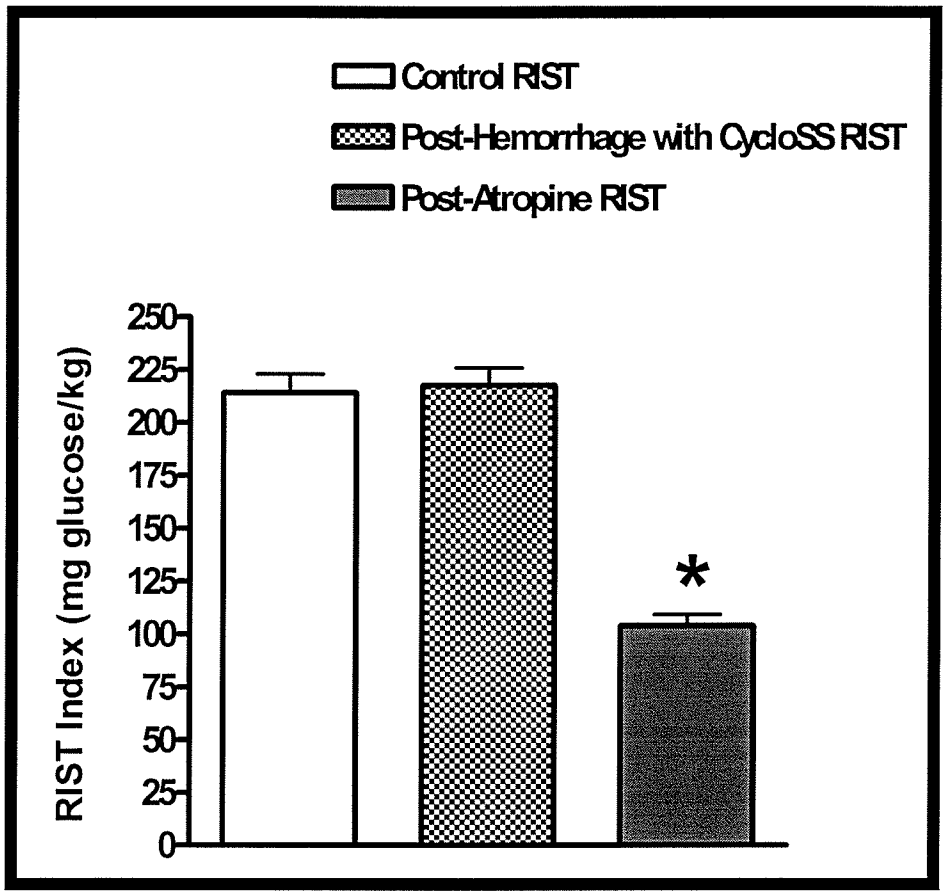
5.3.3 - Cyclosomatostatin prevents HDIR subsequent to hemorrhage

Animals (n=5; 276.9 ± 3.6 grams) in this series had an initial RIST index of 214.3 ± 8.8, a post hemorrhage RIST index of 217.5 ± 8.5 and a post atropine RIST index of 104.0 ± 5.6 mg of glucose/kg (**Figure 5.2**). The decrease (52.2 ± 5.1%) in insulin sensitivity between the post hemorrhage RIST and the post atropine RIST is significant (P = 0.0001). The volume of blood removed (3.3 ± 1.2 ml) and the amount of time taken (4.4 ± 0.8 minutes) to reduce mean arterial pressure to 50 mmHg was not significantly

different from the series where no pharmacological agents were administered (**see section 2.3.1 – Acute hemorrhage induces HDIR**). Glycemia increased by 19.9 ± 2.0 %, thereby implying that cyclosomatostatin did not alter the hyperglycemia seen typically after hemorrhage. The post atropine test confirmed complete HISS blockade in the cyclosomatostatin-hemorrhage series, as its RIST index was significantly reduced by 52.2%.

The dose of cyclosomatostatin (20 μg/kg/min) was found to restore HISS-dependent insulin action, as there was no significant difference between the control RIST and the post-hemorrhage RIST (**Figure 5.2**). Cyclosomatostatin was allowed to reach steady state by infusing for 10 minutes prior to hemorrhage commencement, after which it was continuously administered for 67.2 ± 4.8 minutes. There was no significant difference between the control and post hemorrhage RIST, indicating that cyclosomatostatin administration prevented the occurrence of HDIR following hemorrhage.

Figure 5.2 - Cyclosomatostatin administered prior to hemorrhage prevented the occurrence of HDIR. The administration of cyclosomatostatin (20 μg/kg/min – IPV) prior to hemorrhage is preventative as the post hemorrhage RIST was not significantly different from the control RIST (*, P = 0.0001). The post atropine RIST was decreased by 52.2% illustrating complete HDIR.



5.4 - Discussion

5.4.1 – Cyclosomatostatin prevents HDIR subsequent to acute blood loss

As stated in chapters 2 and 3, both acute hemorrhage and exogenous somatostatin (0.16 μg/kg/min) cause insulin resistance that is accountable by HDIR with the direct HISS-independent action being unsuppressed. Cyclosomatostatin (20 μg/kg/min) administered concurrently with somatostatin was able to prevent the occurrence of

somatostatin-induced HDIR. Therefore, I hypothesized that endogenous somatostatin, which is released in copious amounts in the cat following hemorrhage (Lautt et al., 1982) was a major mediator of HDIR subsequent to hemorrhage. This postulation proved correct as cyclosomatostatin (20 μg/kg/min) was able to prevent the blockade of HISS following hemorrhage, which was shown by the absence of significant differences between the control and post-hemorrhage RIST indexes (**Figure 5.2**).

5.4.2 – HISS and somatostatin

Very few studies have been conducted that have direct relevance to our new paradigm for type 2 diabetes. Specifically, the relationships between somatostatin and HISS, and species differences have not been addressed.

Studies conducted by Cherrington's group have illustrated the presence of HISS-dependent insulin action (Moore et al., 2002), even though these studies were carried out in 18 hour, fasted, conscious dogs with a hyperinsulinemic euglycemic clamp. While a 24 hour fast is sufficient to prevent significant HISS release in rats, 18 hour fasted rats (Lautt et al., 2001), cats (Xie et al., 1993) and dogs (Moore et al., 2002) still have 25-35% of insulin action accounted for by HISS release. The unexpected result of this study is that HISS action was not completely deleted using the HIEC in dogs, whereas the clamp cannot detect HISS action in rats. In addition, this study used somatostatin in order to suppress endogenous insulin and glucagon release, and as demonstrated in this thesis, the administration of somatostatin would block HISS release in rats. Thus, this may reflect a species difference in responses dependent on vastly different methods.

A study done to illustrate the role of hepatoportal glucose sensors on glucose utilization obtained unexpected results following concurrent somatostatin and glucose

administration (Burcelin et al., 2000). Glucose (administered at a rate equivalent to the net endogenous glucose production during the postabsorptive state) infused into the portal vein caused hypoglycemia in mice. However, when glucose and somatostatin (1 μg/kg/min) were infused simultaneously into the portal vein, there was no alteration of the insulin profile and a hyperglycemic state resulted (Burcelin et al., 2000). In a concluding statement, it was acknowledged that the activation of the glucose sensor and its resulting signal to the target tissues was inhibited and blocked by somatostatin (Burcelin et al., 2000). I speculate that the administration of intraportal glucose caused hypoglycemia because of rapid glucose uptake by skeletal muscle through HISS action, while concurrent administration of somatostatin and glucose caused blockade of HISS release, leading to insensitivity by the peripheral tissues to glucose and ensuing hyperglycemia. Given that the insulin profile was not significantly altered by somatostatin administration, which it should by principle (Koerker et al., 1974), I postulate that the hyperglycemic state exhibited was due to the inhibition of HISS release. Preliminary studies conducted by this group had previously shown that the dose of somatostatin (1 μg/kg/min) could dramatically reduce hyperinsulinemia during a hyperglycemic clamp. In my own studies; somatostatin altered the glycemic profile resulting in a reduction of glucose usage by 54.9%, somatostatin did not impair insulin action, and atropine administration confirmed the blockade of HISS by somatostatin (**Figure 3.3**).

A study conducted by Porsazsz purports that HISS can be identified as somatostatin (Porszasz et al., 2003). However, the methods and techniques used by this group ultimately allowed for the discredit of their hypothesis. Firstly, they used the

hyperinsulinemic euglycemic glucose clamp to identify HISS and insulin sensitivity in rats. Reid et al. (2002) had previously shown that HISS release was inhibited by the continuous infusion of insulin. Secondly, each animal was fasted for 24 hours before the clamp was administered. We have shown that a 24 hour fast completely eliminates HISS release, and results in full HDIR (Lautt et al., 1998). Lastly, the work within this thesis clearly demonstrates that somatostatin induces complete HISS blockade resulting in a 54.9% reduction in insulin sensitivity following administration and that cyclosomatostatin, a somatostatin antagonist prevented somatostatin-induced HDIR.

5.4.3 - The presence of endogenous somatostatin in the rat following hemorrhage

As stated previously, the rapid release of somatostatin following hemorrhage has been observed in cats (Lautt et al., 1982). Insulin levels are dramatically decreased during shock and trauma in several species including man (Hiebert et al., 1972 and 1976) following stress-induced hyperglycemia. Therefore, the release of excess somatostatin during a period of traumatic stress is beneficial, as reductions in insulin and HISS levels allow for the utilization of glucose for life-sustaining systems and for the osmotic pressure-induced mobilization of extravascular fluid into the circulation (Yamaguchi, 1992). However, there appears to be significant species differences between the cat and rat, as systemic endogenous somatostatin levels were not elevated and were only present in small, sporadic amounts following hemorrhage in the rat (**Table 5.2**). Given that HDIR subsequent to hemorrhage is mediated by endogenous somatostatin, the lack of increase in circulating plasma somatostatin levels suggests that the somatostatin of relevance to HDIR is released from within the liver and that circulating systemic levels of somatostatin does not play a role in the rat.

5.4.4 - The release of endogenous somatostatin in the rat – a postulation for the existence of somatonergic nerves

The existence of sympathetic neurons that release somatostatin and norepinephrine have been identified in the submucosal neural plexus of the gut (Hökfelt et al, 1977). Additionally, the presence of somatostatin has been demonstrated in sympathetic nerves, mucosal cells, myenteric nerves of the gastrointestinal tract, salivary glands and in some parafollicular cells of the thyroid. By means of the indirect immunofluorescence, somatostatin-like immunoreactivity has been demonstrated in principal ganglion cells of some sympathetic ganglia. In guinea pigs, the somatostatin immunoreactive material was found in almost two-thirds of all principal ganglion cells of the celiac-superior mesenteric ganglion (Hökfelt et al, 1977). The presence of somatostatin within these ganglia may represent the storage of a small peptide in a neuron, and thus may provide further support for the existence of somatonergic nerves. Furthermore, the detection of somatostatin receptors with terminal nodular swellings located beneath the endothelium of the portal vein supplements this possibility (Hevener et al., 1997; Nakabayaski et al., 1986).

5.4.5 - A postulation – Hepatic somatonergic nerves are a class of NANC nerves

The existence of Non-Adrenergic and Non-Cholinergic (NANC) Nerves and their presence in the gut have been studied by Burnstock (Burnstock, 1976). The examination of the stomach and its innervation lead to the discovery of these nerves. It was believed that contraction of the gut was regulated by the cholinergic nervous system, releasing acetylcholine as neurotransmitter, whereas relaxation of the gut mainly resulted from adrenergic neural input, via the release of noradrenaline (Furness et al., 1992). Following electrical stimulation of the cholinergic vagal nerve it was noted that the stomach relaxed

by releasing a neurotransmitter different from noradrenaline. Furthermore, nerve stimulation of the gut resulted in relaxation resistant to adrenergic antagonists (Lefebvre, 1993). These experiments led to the introduction of the concept of NANC innervation, consisting of nerves releasing a neurotransmitter different from the classical candidates acetylcholine and noradrenaline (Burnstock, 1976). Several substances were proposed as the inhibitory NANC neurotransmitter, such as adenosine 5'-triphosphate (ATP) (Burnstock, 1976), vasoactive intestinal polypeptide (VIP) and nitric oxide (Li and Rand, 1990). The term "nitrergic nerves" was adopted to describe the subset of NANC nerves from which NO is released, and the term "purinergic nerves" was coined to describe the division of NANC nerves from which ATP is released.

Given that endogenous somatostatin mediates HDIR subsequent to hemorrhage, and that large circulating levels of somatostatin were not detected in the rat, the existence of hepatic somatonergic nerves that are not cholinergic or adrenergic in nature may be a possibility with somatostatin being its inhibitory NANC neurotransmitter.

Chapter 6 – Final Remarks

6.1 - Conclusions

6.1.1 - Hemorrhage induces HDIR

Hemorrhage caused complete HDIR and did not induce resistance to the direct action of insulin. However, the response to administered insulin was confounded by the impact of reduced hepatic blood flow on insulin metabolism that resulted in an increase in the HISS independent (direct) action of insulin. This complication is of concern only for the specific experimental protocol where exogenous insulin was administered and is not of consequence in the physiological response to hemorrhage since part of the neuroendocrine response includes the suppression of insulin secretion. In this way, the glycogenolysis, inhibition of insulin release and suppression of HISS action serve to maintain hyperglycemia following hemorrhage.

6.1.2 - The adrenergic sympathetic nervous system does not directly causes HDIR subsequent to hemorrhage

Surprisingly, the data have illustrated that the adrenergic sympathetic response to hemorrhage does not appear to induce HDIR, as blockade of α and β hepatic adrenergic receptors still resulted in complete HDIR following hemorrhage. Activation of glycogenolysis in rats appears to be an α-receptor mediated effect as the administration of phentolamine terminated the hyperglycemic response to hemorrhage, and propranolol had no effect.

6.1.3 - Somatostatin causes HDIR

Administered somatostatin (0.16 μg/kg/min) caused complete HDIR that was not reversible following discontinuation of infusion. The post-atropine RIST illustrated that

the reduction in sensitivity following somatostatin administration was due solely to HISS-dependent insulin action. Concurrent infusion with cyclosomatostatin (20 μg/kg/min) prevented the blockade of HISS action by somatostatin and therefore ultimately prevented the occurrence of somatostatin-induced HDIR.

6.1.4 - Cyclosomatostatin prevents HDIR subsequent to hemorrhage intervention

The blockade of HISS subsequent to hemorrhage was linked directly to somatostatin, a poorly understood hormone. Following continuous cyclosomatostatin administration and hemorrhage, the presence of normal HISS-dependent and independent insulin action was detected by the RIST. The ability to prevent HDIR subsequent to hemorrhage with a somatostatin antagonist illustrated the important role of somatostatin as a mediator of HDIR during trauma. However, evaluation of systemic levels of somatostatin following hemorrhage in the rat illustrated a species difference from the cat, and allowed for the speculation of hepatic somatostatin release via somatonergic nerves.

A defined role of somatostatin in the pathogenesis of insulin resistance is not clear; however cyclosomatostatin may have some use in the management of the disorder. Further studies are required to confirm the existence of hepatic somatonergic nerves so that potential targets can be employed as therapeutic interventions.

6.2 - Future Implications

Clearly, this new paradigm for type 2 diabetes is an interesting, exciting and progressive area of research. The identification of the physical structure of HISS and its receptor is the next major step required in order to create new therapeutic interventions for the millions of individuals with type 2 diabetes. The identification and full characterization of this compound would allow many pathways and mechanisms to be

explored and would assist in creating new therapeutic interventions for the treatment of type 2 diabetes. However, because we are still able to detect HISS action through the use of the RIST methodology, other important questions can be postulated in the meantime.

Somatostatin, a poorly understood hormone in respect to the pathology of insulin resistance and trauma should be further examined for therapeutic possibility. The use of its antagonist has been able to prevent stress-induced insulin resistance, and therefore may have some therapeutic use in the management of this disorder.

These experiments have clearly indicated a major role for somatostatin in HDIR induced subsequent to hemorrhage and are indicative of new scenarios where HDIR can be shown to account for insulin resistance. HDIR can now be demonstrated in the fasted state, (Lautt et al., 2001), during hepatic muscarinic cholinergic blockade with atropine (Xie and Lautt, 1995), during hepatic nitric oxide synthase blockade (Sadri and Lautt, 1998 and 1999), during hepatic cyclooxygenase inhibition (Sadri and Lautt, 2000), during surgical denervation of the liver (Xie and Lautt, 1994) (Latour and Lautt, 2002), and most recently following acute hemorrhage (Seredycz et al., submitted for publication in 2004) and somatostatin administration (Seredycz and Lautt, submitted for publication in 2004).

Future studies can be designed to determine if the mechanism of HDIR in any of the above situations and circumstances is attributable to a mechanism that employs HISS blockade with somatostatin.

REFERENCES

Ahlquist, R.P. Historical perspective. Classification of adrenoreceptors. J. Auton. Pharmacol. Nov 1(1);101-6, 1980.

Alibegovic, A., Ljungqvist, O. Pretreatment with glucose infusion prevents fatal outcome after hemorrhage in food-deprived rats. Circ Shock. Jan 39(1):1-6,1993.

Andersson, P.O., Farnebo, L.O., Fredholm, B.B., Hamberger, B., Holst, J and Jarhult, J. Metabolic and hormonal adjustments during hemorrhage in cats after interference with the sympathy-adrenal system. Acta Physiol Scand. 114:111-119, 1982.

Aub J.C., and H. Wu. Studies in experimental traumatic shock. Chemical changes in the blood. Amer. J. Physiol. 54:416-424, 1920.

Banting, F.G., Best, C.H., Collip, J.B., Campbell, W.R., Fletcher, A.A. Pancreatic extracts in the treatment of diabetes mellitus. Can Med Assoc J. 12;141-146, 1922.

Beckh, K., Balks, H.J., Jungermann, K. Activation of glycogenolysis and norepinephrine overflow in the perfused rat liver during repetitive perivascular nerve stimulation. FEBS Lett. Nov 29;149(2): 261-5, 1982.

Bell, D.S. Type 2 diabetes mellitus: what is the optimal treatment regimen? Am J Med. Mar 8;116 Suppl 5A:23S-29S, 2004.

Branch, R.A., Shand, D.G., Wilkinson, G.R., and Nies, A.S. The reduction of lidocaine clearance by dl-propranolol: an example of hemodynamic drug interaction. J. Pharmacol. Exp. Ther. 184;515-519, 1973.

Brazeau, P., Vale, W., Burgus, R. Hypothalamic peptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science. 179:77-9, 1973.

Bruno, J.F., Xu, Y., Song, J., Berelowitz, M. Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat. Endocrinology. 133:2561-2567, 1993.

Burcelin, R., Dolci, W., Thorens, B. Portal glucose infusion in the mouse induces hypoglycemia: evidence that the hepatoportal glucose sensor stimulates glucose utilization. Diabetes. Oct;49(10):1635-42, 2000.

Burnstock, G. Purine nucleotides. Adv Biochem Psychopharmacol.15;225-35,1976.

Cannon, W.B. Organization for physiological homeostasis. Physiol. Rev. 9;399-431, 1929.

Coevoet, B., Fievet, P., Comoy, E., Legrand, F., Makdassi, R., Verhoest, P., Boulanger, J.C., Fournier, A. Renin angiotensin aldosterone system and the adrenergic system. Clin Exp Hypertens B.1(4):479-91, 1982.

Coy, D.H., Murphy, W.A., Raynor, K., Reisine, T. The new pharmacology of somatostatin and its multiple receptors. J. Pediatr. Endocrinol. 6;205-209, 1993.

Coy, D.H., Taylor, J.E. Receptor-specific somatostatin analogs: correlations with biological activity. Metabolism. 45;21-23, 1996.

Cutherbertson, D.P. Post-shock metabolic response. Lancet. 1;433-437,1942.

Day, R., Dong, W., Panetta, R., Kraicer, J., Grenwood , M.T., Patel, Y.C. Expression of mRNA for somatostatin receptor types 2 and 5 in individual pituitary cells. A double labeling in situ hybridization analysis. Endocrinology. 136;5232-5235, 1995.

DCCT Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N. Engl. J. Med. 320;977-986, 1993.

De Fronzo, R.A., Tobin, D.J., Andres, R. Pathogenesis of NIDDM. Diabetes Care. 15;318-368, 1992.

DeFronzo, R.A. Pathogenesis of type 2 diabetes mellitus. Med. Clin. North. Am. Jul;88(4);787-835, ix. 2004.

DECODE study group. European Diabetes Epidemiology Group. Glucose tolerance and mortaility:comparison of WHO and ADA diagnostic criteria. Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe. Lancet. 354;617-21, 1999.

Delongis, A., Preece, M. Coping skills. In: Fink G, ed Encyclopedia of Stress, Vol 1. New York: Academic Press;532-541, 2000.

Deschamps, I., Robert, J.J., Hors, J. Genetic counseling of diabetics Diab. Metab.16(2 Pt 2):160-71, 1990.

Ferdinand, K.C., Clark, L.T. The epidemic of diabetes mellitus and the metabolic syndrome
Rev Cardiovasc Med. 5 Suppl 3:S28-33, 2004.

Fluori, T., Schettini, G. Multiple intracellular effectors modulate physiological functions of the cloned somatostatin receptors. J. Mol Endocrinol. 17;89-100, 1996.

Frayn, K.N. Hormonal control of metabolism in trauma and sepsis. Clin Endocrinol. 24;577-599, 1986.

Furness, J.B., Bornstien, G.C., Murphy, R., Pompolo, S. Roles of peptides in the transmission in the enteric nervous system. Trends Neurosci. 15;361-372, 1992.

Grey, D.B., Zelazny, D., Manthay, D., Pilar, G. Endogenous modulation of Ach release by somatostatin and differential role of calcium channels. Journal of Neuroscience. 10;2687-2698, 1990.

Halter, J.B., Peflug, A., Porte Jr, D. Mechanism of plasma catecholamines increases during surgical stress in man J. Clin. Endocrinol. Meta. 45;936-944, 1977.

Harrower, A.D., Clarke, B.F. Experience of coronary care in diabetes. Br. Med. J. 1;126-8, 1976.

Hellman, B., Lernmark, A. Inhibition of the *in vitro* secretion of insulin by an extract of pancreatic α_1 cells. Endocrinology. 84;1484-7, 1969.

Henquin, J.C. Cell Biology of insulin secretion. In Joslin's Diabetes Mellitus. Edited by C.R. Kahn and G.C. Weir. Lea & Febiger, Philadelphia. P56, 1994.

Hermans, M.P., Buysschaert, M. Pharmacological treatment of type 2 diabetes. Acta. Clin. Belg. Mar-Apr;59(2):59-66, 2004.

Hevener, A.L., Bergman, R.N., Donovan, C.M. Novel glucosensor for hypoglycemic detection localized to the portal vein. Diabetes. 46;1521-1525, 1997.

Hibbert-Jones, E., Regan, G., Bramwell, J. What do we know about...diabetes and obesity in adults and children? J. Fam. Health Care.14(4);95-8, 2004.

Hiebert, J. M., J. M. McCormick, R. H. Egdahl. Direct measurement of insulin secretory rate: studies in shocked primates and postoperative patients. Ann. Surg. 176;296-304, 1972.

Hiebert, J. M., C. Kieler, J. SSS. Soldner, R.H Egdahl. Species differences in insulin secretory response during hemorrhagic shock. Surgery. 79;451-455, 1976.

Himsworth, H.P. Diabetes mellitus: its differentiation into insulin sensitive and insulin-insensitive types. Lancet. 1 I;127-130, 1936.

Hökfelt, L. G. Elfvin, R. Elde, M. Schultzberg, Goldstein, M., Luft, R. Occurrence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons. Neuroscience. 1(2);131-6, 1977.

Homann, D., Von Herrath, M. Regulatory T cells and type 1 diabetes. Clin Immunol. Sep;112(3);202-9, 2004.

Jarhult, J. Role of the sympatho-adrenal system in hemorrhagic hyperglycemia. Acta. Physiol. Scand. 93;25-33, 1975.

John, G.W., Doxy, J.C., Walter, D.S., and Reid, J.L., The role of alpha and beta adrenoreceptor subtypes in mediating the effects of catecholamines on fasting glucose and insulin concentrations in the rat. Br. J. Pharmacol. 100;699-704, 1990.

Kaupmann, K., Burns, C., Lubbert, H. Distribution and second messenger coupling of four somatostatin receptor subtypes expressed in brain. FEBS Letters. 331;53-59, 1992.

Kirsten, R., Nelson, K., Kirsten, D., Heintz, B. Clinical pharmacokinetics of vasodilators. Part II. Clin. Pharma. Jul;35(1);9-36, 1998.

Kobayashi, S., Kohei, H. Some metabolic actions of imidazoline sympathomimetics: hyperglycemia and lipid mobilization J. Pharmacol. 22(Suppl);66, 1972.

Koerker, D., Ruch, W., Chickadel, I., Palmer, J., Goodner, C., Ensink, J., Gale, C. Somatostatin:hypothalamic inhibitor of the endocrine pancreas, Science. 184;482-484, 1974.

Kong, H., DePaoli, A.M., Breder, C.D., Yasuda, K., Bell, G.I., Reisine, T. Differential expression of somatostatin receptor subtypes SSTR1, SSTR2, SSTR3 in adult rat brain, pituitary and adrenal gland. Neuroscience. 59;175-184, 1994.

Latour, M.G., Lautt, W.W. The hepatic vagus nerve in the control of insulin sensitivity in the rat. Autonomic Neurosci. 80;8-12, 2002.

Lautt, W.W. Hepatic nerves: a review of their functions and effects. Can. J. Physiol. Pharmacol. Feb;58(2);105-23, 1980.

Lautt, W.W. Relationship between hepatic blood flow and overall metabolism: The hepatic arterial buffer response. Fed. Proc. 42:1662-1666, 1981.

Lautt, W.W., McQuaker, J.E. Maintenance of hepatic arterial blood flow during hemorrhage is mediated by adenosine. Can. J. Physiol. Pharmacol. 67:1023,1028, 1988.

Lautt, W.W. A new paradigm for diabetes and obesity: The hepatic insulin sensitizing substance (HISS) hypothesis. J. Pharmacol. Sci. 95;9-17, 2004.

Lautt, W.W., Skelton, F.S. Effected of SKF-525A and of altered hepatic blood flow on lidocaine metabolism. Can. J.Physiol. Pharmacol. 55;7-12, 1977.

Lautt, W.W., Dwan, P.D., Singh, R.R. Control of the hyperglycemic response to hemorrhage to cats. Can. J. Physiol. Pharmacol. 60;1618-1623, 1982.

Lautt, W.W., Wang, X., Sadri, P., Legare, D.J., and Macedo, M.P. Rapid insulin sensitivity test (RIST). Can. J. Physiol. Pharmacol. 76;1080-1086, 1998.

Lautt, W.W., Macedo, M.P., Sadri, P., Takayama, S., Ramos, F.D., Legare, D.J. Hepatic parasympathetic nerve-dependent control of peripheral insulin sensitivity is determined by feeding and fasting: dynamic control of HISS-dependent insulin action. Am. J. Physiol. Gastrointest. Liver Physiol. 281;G29-G36, 2001.

Lebovitz, H.E., Banerji, M.A. Treatment of insulin resistance in diabetes mellitus. Eur. J. Pharmacol. Apr 19;490(1-3);135-46, 2004.

Lefebvre, T. Electric nerve stimulation and its association to NANC nerves. Rev. Hist. Pharm. 40(297);225-31, 1993.

Li, C.G., Rand, M.F. Nitric oxide and vasoactive intestinal polypeptide mediate non-adrenergic non-cholinergic inhibitory transmission to stomach muscle of rat gastric fundus. Eur. J. Pharmacol. 191;303-309, 1990.

Linne, Y. Effects of obesity on women's reproduction and complications during pregnancy. Obes. Rev. Aug (3);137-43, 2004.

Loubatieres-Mariani, M.M. Insulin secretion and it's regulation. J. Pharmacol. 17 (Supp II); 83-103, 1986.

Ma, Y., Want, P., Kuebler, J.F., Chaudry, I. H., Messina, J.L. Hemorrhage induced the rapid development of hepatic insulin resistance. Am. J. Physiol. Gastrointest. Liver Physiol. 284; G107-G115, 2002.

Mason, J.W., Wherry, F.E., Pennington, L.L., Spector, N.H. A historical view of the stress field. J. Human Stress. Mar;1(1);6-12, 1975.

McCarty, R., Kirby, R.F., Callahan, M.F., Johnson, A.K. Cardiovascular and sympathetic nervous system responses to an acute stressor in borderline hypertensive rats (BHR). Physiol. Behav. Aug;46(2);309-13, 1989.

McEwan, B.S. Stress, brain and behavior: life long effects upon health and disease. In: Kinney JM, Tucker HN, eds Physiology, stress and malnutrition. Functional correlates of nutritional intervention. Philadelphia: Lippincott-Raven;113-130, 1997.

McLeod, M.K., Carlson, D.E., Gann, D.S. Hormonal responses associated with early hyperglycemia after graded hemorrhage in dogs. Am. J. Physiol. 251;E597-E603, 1986.

McMichael, J. EDWARD PETER SHARPEY-SCHAFER. Br. Heart. J. May 26;430-2, 1964.

Mensing, C., Boucher, J., Cypress, M., Weinger, K., Mulcahy, K., Barta, P., Hosey, G., Kopher, W., Lasichak, A., Lamb, B., Mangan, M., Norman, J., Tanja, J., Yauk,

L., Wisdom, K., Adams, C. National standards for diabetes self-management education. Diabetes Care. Jan;27 Suppl 1;S143-50, 2004.

Messerli, F.H., Nowaczynski, W., Honda, M., Genest, J., Boucher, R., Kuthel, O., Rojo-Ortega, J.M. Effects of angiotensin II on steroid metabolism and hepatic blood flow in man. Circ. Res. 40;204-207, 1997.

Mills, G.A, Horn, J.R. Beta-blockers and glucose control. Drug Intelligence and Clinical Pharmacology. 19;246-251, 1985.

Moore, M.C., Satake, S., Baranowski, B., Hsieh, P.S., Neal, D.W., Cherrington, A.D. Effect of hepatic denervation on peripheral insulin sensitivity in conscious dogs. Am. J. Physiol. Endocrinol. Metab. 282;E286-E296, 2002.

Mycek, M.J., Harvey, R.A., Champe, P.C. Lippincott's Illustrated Reviews: Pharmacology 2nd Edition. Lippincott-Raven Publishers, Philadelphia, New York. P60. 1997.

Nakabayaski, H., Niijima, A., Kurata, Y., Usukura, N., Takeda, R. Somatostatin-senstive neural system in the liver. Neuroscience Lett. 67;78-81, 1986.

Nakadate, T. Nakaki T., and Kato, R. Adrenergic regulation of blood glucose levels: possible involvement of postsynaptic alpha 2 type adrenergic receptors regulating insulin release. J. Pharmacol. Exp. Ther. 215:226-30, 1980.

Nettelbladt, C.G., Aligobevic, A., Ljungqvist, O. Pre-stress carbohydrate solution prevents fatal outcome after hemorrhage in 24 hour food deprived rats. Nutrition. 12:10;696-699, 1996.

Newman, S., Steed, L., Mulligan, K. Self-management interventions for chronic illness. Lancet. Oct 23:364(9444);1523-37, 2004.

Nygren, J., Soop, M., Thorell, A., Efendic, S., Nair, S., Ljungqvist, O. Preoperative oral carbohydrate administration reduces postoperative insulin resistance. Clin. Nutr. 17;65-71, 1998.

O'Carroll, A.M., Krempels, K. Widespread distribution of somatostatin receptor messenger ribonucleic acid in rat pituitary. Endocrinology. 136;5224-5227, 1995.

Pacak, K., Palkovits, M., Yadid, G., Kvetnansky, R., Kopin, I.J., Goldstein, D.S. Heterogeneous neurochemical responses to different stressors: a test of Selye's doctrine of nonspecificity. Am. J. Physiol. 275;R1247-R1255, 1998.

Pacak, K., Palkovits, M. Stressor Specificity of Central Neuroendocrine Responses: Implications for Stress-Related Disorders. Endocrine Reviews. 22(4);502-548, 2001.

Patel, Y.C., Amherdt, M., Orci, L. Quantitative electronmicroscope autoradiography of insulin, glucagon and somatostatin binding sites on islets. Diabetes Care. 8;230-4,1982.

Pogasta, G., Dubecz, E. Effect of propranolol on drug-induced and physiological glycogenolysis in the liver. J. of Pharmacol. 29;1104-7, 1979.

Porszasz, R., Legvari, G., Pataki, T., Szilvassy, J., Nemeth, J., Kovacs, P., Paragh, G., Szolcsanyi, J., Szilvassy, Z. Hepatic insulin sensitizing substance: a novel 'sensocrine' mechanism to increase insulin sensitivity in anaesthetized rats. Br. J. of Pharmacol. 139;1171-79, 2003.

Porte, D. Beta adrenergic stimulation of insulin release in man. Diabetes. 16;150-5, 1967.

Raptis, S. Effects of cardioselective and non-selective beta adrenoreceptor blockade on glycogenolysis, lipolysis and growth hormone secretion. J. Clin Pharmacol. 13;419-20, 1982.

Raucoules-Aime, M., Roussel, L.J.,Rossi, D., Gastaud, P., Dolisi, P., Grimaud, B. J. Effect of severity of surgery on metabolic control and insulin requirements in insulin-dependent diabetic patients. N. Engl. J. Med. 74;231-233, 1995.

Reichlin, S. Somatostatin. N. Engl. J. Med. 309;1495-1501, 1983a.

Reichlin, S. Somatostatin. N. Engl. J. Med. 309;1556-1563, 1983b.

Reid, M.A.G., Latour, M.G., Legare D.J., Rong, W., and Lautt, W.W. Comparison of the rapid insulin sensitivity test (RIST), the insulin tolerance test (ITT), and the hyperinsulinemic euglycemic clamp (HIEC) to measure insulin action in rats. Can. J. Physiol. Pharmacol. 80;811-818, 2002.

Rizza, R.A., Creyer, P.E., Haymond, M.W., Gerich, J.E. Adrenergic mechanisms for the effect of epinephrine on glucose production and clearance in man. J. Clin. Invest. 65;682-9, 1980.

Rizza, R.A., Gerich, J.E. Hypoglycemia: physiologic and pathophysiologic consideration. In: Service FJ, ed. Hypoglycemic disorders, pathogenesis, diagnosis, and treatment. Boston: GK Hall,1-34, 1983.

Robertshaw, H.J., McAnulty, G.R., Hall, G.H. Best Pract. Res. Clin. Anaesthesiol. Dec 18, (4);631-43, 2004.

Robinson, P.J., Rapoport, S.I. Glucose transport and metabolism in the brain. Am. J. Physiol. 250;R127-R136, 1986.

Rytter L, Troelsen S, Beck-Nielsen H. Prevalence and mortality of acute myocardial infarction in patients with diabetes. Diabetes Care. May-Jun;8(3);230-4, 1985.

Sadri, P., Lautt, W.W. Blockade of nitric oxide production in the liver causes insulin resistance. Proc. West. Pharmacol. Soc. 41;37-38, 1998.

Sadri, P., Lautt, W.W. Blockade of hepatic nitric oxide synthase causes insulin resistance. Am. J. Physiol. 277;G101-G108, 1999.

Sadri, P., Lautt, W.W. Insulin resistance caused by hepatic COX inhibition. Diabetes. 49(Suppl 1);A245, 2000.

Schettini, G. Brain somatostatin: receptor coupled transducing mechanisms and role in cognitive functions. Pharm. Res. 23;203-215, 1991.

Selye, H. The general adaptation syndrome and the diseases of adaptation. J. of Clin. Endo. 6;117-231, 1946.

Seredycz, L., Lautt, W.W. The role of Somatostatin in preventing HISS-dependent insulin resistance subsequent to hemorrhage. Submitted to the American Journal of Physiology in 2004.

Seredycz, L. Xie, H., Lautt, W.W. Acute Hemorrhage causes HISS-dependent insulin resistance. Submitted to the American Journal of Physiology in 2004.

Shen, L.P., Pictet, R.L,, Rutter, W.J. Human somatostatin I: Sequence of the cDNA. Proc. Natl. Acad. Sci. 79;4575-9, 1982.

Shimazu, T. Central Nervous System regulation of liver and adipose tissue metabolism. Diabetologia. 20;343, 1981.

Shimazu, T. in: A.J Szabo (ed): Advances in Metabolic Disorders, Vol.10 (CNS Regulation of Carbohydrate Metabolism) Academic, New York, 1983.

Smith, P.H., Porte, D., Robertson, R.P. Neural regulation of the endocrine pancreas. In:Pierlussi J, ed. International symposium on the endocrine pancreas and diabetes. Amsterdam: Excerpta. Medica. 64-95, 1979.

Stratton, I.M., Adler, A.I,, Neil, H.A.W., Mathews, D.R., Manley, S.E., Cull, C.A., Hadden, D., Turner, R.C., Holman, R.R. Association of glycemia with macrovascular and microvascular complications of type 2 diabetes: prospective observational study. Br. Med. J. 321;405-412, 2000.

Takayama, W., Legare, D.J., Lautt, W.W. Dose-related atropine-induced insulin resistance:comparing intraportal versus intravenous administration. Proc. West. Pharamcol. Soc. 43;33-34, 2000.

Tepperman, J. Metabolic and Endocrine Physiology, An Introductory Text. 2nd ad. Chicago: Year Book Medical Publishers, Inc, 1968.

Thal, L.J., Laing, K., Horowitz, S.G., Makman, M.H. Dopamine stimulates rat cortical somatostain release. Brain Research. 372;205-209, 1986.

Thase, M.E., Howland, R.H. Increased responsiveness of the hypothalamus-pituitary-adrenal (HPA) axis to stress. Neuroendocrinology.. Jul:18(3);705-11, 1995.

Thorell, A., Nygren, J., Ljungqvist, O. Insulin resistance: a marker of surgical stress. Curr. Opin. Clin. Nutr. Metab. Care. 2;69-78, 1999.

Tregaskis, B.F., McDevitt, D.G. Beta-adrenoceptor-blocking drugs. J. Cardiovasc. Pharmacol. 16:Suppl 5;S25-8, 1990.

Unger, A., Phillips, J.H. Regulation of the adrenal medulla. Physiol. Rev. 56;778-826, 1983.

Van Den Berghe, G., Wouters, P., Weekers, F. Intensive insulin therapy in critically ill patients. N. Engl. J. Med. 345;1359-67, 2001.

Wagstaff, A.J., Davis, R., McTavish, D. Propranolol : a reappraisal of its pharmacology and therapeutic efficacy in essential hypertension. Drugs. May:51(5);777-91, 1996.

Weeke, J., Hansen, A.P., Lundaek, K. Inhibition by somatostatin of basal levels of serum thyrotropin in normal men. J. Clin. Endocrinol. Metab. 41;168-71, 1975.

World Health Organization. Definition, diagnosis, and classification of diabetes mellitus and its complications [online]. World Health Organization Department of Noncommunicable Diseases Surveillance, Geneva. © 1999. Available from http://whqlibdoc.who.int/hq/1999/WHO_NCD_NCS_99.2.pdf [cited in November 2004].

Wright, A., Burden, A.C.F., Paisey, R.B., Cull, C.A., Holman, R.R. for the UK Prospective Diabetes Study Group: Sulphonylurea inadequacy – efficacy of addition of insulin over 6 years in patients with type 2 diabetes in the UK. Diabetes Care. 25;330-336, 2002.

Wurtman, R.J., Pohorecky, L.A., Baliga, B.S. Adrenocortical control of the biosynthesis of epinephrine and proteins in the adrenal medulla. Pharmacol. Rev. 24;411-426., 1972.

Xie, H., Lautt, W.W. Insulin resistance produced by hepatic denervation or muscarinic cholinergic blockade. Proc. West. Pharmacol. Soc. 37;39-40, 1994.

Xie, H., Lautt, W.W. Induction of insulin resistance by cholinergic blockade with atropine in the cat. J. Auton. Pharmacol. 15;361-369, 1995.

Xie, H., Lautt, W.W. Insulin resistance caused by hepatic cholinergic interruption and reversed by acetylcholine administration. Am. J. Physiol. 271;E587-E592, 1996.

Xie, H., Tsybenko, V.A., Johnson, M.V., Lautt, W.W. Insulin resistance of glucose response produced by hepatic denervations. Can. J. Physiol. Pharmacol. 71;175-178, 1993.

Yamaguchi, N. Sympathoadrenal system in neuroendocrine control of glucose: mechanisms involved in the liver, pancreas, and adrenal gland under hemorrhagic and hypoglycemic stress. Can. J. Physiol. Pharmacol. 70(2);167-206, 1992.

Yates, F.E. Contributions of the liver to steady-state performance and transient responses of the adrenal cortical system. Federation Proc. 723-730, 1965.