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To my girls

Contents

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I Peter Hager, Beth Hagman, Ann-Charlotte Wikström, Lisa Strömmer.
CRF-Receptor 1 Blockade Attenuates Acute Posttraumatic Hyperglycemia in Rats
Journal of Surgical Research ***119,*** *72–79 (2004)*

II Peter Hager, Johan Permert, Ann-Charlotte Wikström, Margery K. Herrington, Claes-Göran Östenson, Lisa Strömmer
Preoperative glucocorticoid administration attenuates the systemic stress response and hyperglycemia after surgical trauma in the rat
Submitted to *Metabolism Clinical and Experimental*

III Peter Hager, Johan Permert, Lisa Strömmer
An experimental model of intestinal resection and compensated nonhypotensive blood loss
In press, *Journal of Surgical Research*

IV Peter Hager, Johan Permert, Claes-Göran Östenson, Lars Hållström, Lisa Strömmer
Systemic stress response during and after major gastrointestinal surgery
Submitted to *Annals of Surgery*

Abstract

Systemic stress response and hyperglycemia after abdominal surgery in rat and man

Surgical trauma results in a complex neuroendocrine and metabolic response known as the systemic stress response, which is initiated by neuronal and humoral signals from the site of the injury. These signals converge at central sites and result in the activation of the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic nervous system and an inflammatory response. The systemic stress response is crucial for survival and results in metabolic changes in order to provide fuel and retain fluids until the damage is healed. An exaggerated and prolonged stress response however, results in depletion of energy stores with deleterious consequences on wound healing and convalescence. The implementation of new anesthesiological treatments regimes, such as epidural anesthesia and fluid restriction necessitates the administration of vasopressors such as noradrenaline in order to maintain adequate tissue perfusion. Peri-operative insulin therapy has also been recently introduced aiming to reduce postoperative morbidity. However, insulin can act as a metabolic stressor and the effect of this pluri-potent hormone in different surgical populations needs to be further explored.

The general aim of this thesis work was to investigate the systemic stress response and hyperglycemia after abdominal surgery in rat and man.

For this purpose, studies were performed in experimental models as well as after major abdominal surgery in humans. We investigated whether preoperative suppression of the HPA-axis by corticotropin releasing factor antagonism or suppression of the inflammatory response by the administration of glucocorticoids affects the stress response and glucose metabolism after intestinal resection in the rat. Furthermore, the effect of glucocorticoids in a concentration similar to that after surgical trauma in the rat on the metabolic and hormonal stress response was investigated. We used a new experimental model including intestinal resection and compensated blood loss, aiming to reflect major abdominal surgery in man. In humans, the metabolic and hormonal responses after major gastrointestinal surgery were characterized and related to the intra- and post-operative clinical course in order to identify changes of special importance for outcome.

Corticotropin releasing factor antagonism decreased postoperative hyperglycemia and hypercorticosteronemia without affecting insulin transduction or glucose uptake in skeletal muscle. Preoperative administration of glucocorticoids attenuated the postoperative stress response by reducing plasma concentrations of interleukin-6 and catecholamines. Plasma glucocorticoids administered in concentrations similar to levels observed after intestinal resection did not cause hyperglycemia in the rat. Compensated blood loss and intestinal resection potentiated the hormonal response and induced persistent volume-dependent hyperglycemia which was independent on changes in plasma corticosterone and, in the early postoperative phase, mean arterial blood pressure and catecholamine levels. During pancreatic surgery in humans, we observed hyperglycemia, increased lipolysis and high levels of exogenous noradrenaline. Intraoperative lipolysis and plasma concentrations of interleukin-6 were correlated to the incidence of postoperative complications and length of hospital stay.

In conclusion, our results showed that inhibition of the stress response's efferent pathways results in compensatory mechanisms in order to maintain the glucocorticoid response and hyperglycemia after surgery. New anesthesiological and surgical techniques attenuated the postoperative systemic stress response in humans. However, marked metabolic changes induced by infusions of vasopressors were observed during surgery.

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Abbreviations

ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
ATP	Adenosine-5'-triphosphate
AUC	Area under the curve
BMI	Body mass index
BSA	Bovine serum albumin
bw	Body weight
cAMP	Cyclic adenosine monophosphate
CORT	Corticosterone
CRF	Corticotropin releasing factor
DHBA	3,4-dihydroxybenzylamine
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immuno sorbent assay
GC	Glucocorticoid
GT	Glucose tolerance
GLUT-4	Insulin-regulated glucose transporter
GTP	Guanosine-5'-triphosphate
HPA-axis	Hypothalamic-pituitary-adrenal-axis
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	High pressure liquid chromatography
IL-6	Interleukin-6
IL-10	Interleukin-10
IRS-1	Insulin receptor substrate 1
MAP	Mean arterial blood pressure
OGTT	Oral glucose tolerance test
PI 3-kinase	Phosphoinositide 3-kinase
PNMT	Phenylethanolamine N-methyltransferase
qRT-PCR	Quantitative real time polymerase chain reaction
RIA	Radioimmunoassay
RNA	Ribonucleic acid
TRIS	Tris(hydroxymethyl)aminomethane
SEM	Standard error of the mean
SNS	Sympathetic nervous system
WHO	The World Health Organization

Background

What is Surgery?

Surgical trauma is the most common type of trauma in the western world. Nine percent of Sweden's inhabitants undergo surgery every year [1].

Historically, surgery was considered to be "the branch of medicine that deals with the diagnosis and treatment of injury, deformity and disease by manual and instrumental means" [2]. As time went on, surgical as well as anesthesiological technique and the instrumental repertoire improved, which had beneficial effects on survival after surgery. Today, more extensive surgical procedures are performed on older and more morbid patients, and the demands for an optimal outcome have increased. Nowadays, from a metabolic point of view, surgery is considered to be the combination of multiple factors including anesthesia, medication, tissue trauma, blood loss, and temperature changes [3]. Each of these factors causes metabolic changes. Together, they produce the postoperative stress response.

What is the postoperative adaptive stress response?

Major body injury, surgical or accidental, evokes reproducible metabolic, hormonal, and hemodynamic responses [4].

Stress is defined as a state of threatened homeostasis. Homeostasis is reestablished by a complex repertoire of physiologic and behavioral responses of the organism. These changes are normally adaptive and improve the chances of the individual for survival. The organism also activates restraining forces during stress to prevent an over-response from both central and peripheral components of the stress system. The balance between these forces is essential for successful adaption. If the various elements of the stress response are not restrained, the adaptive changes may become excessive, prolonged, and

maladaptive and may contribute to the development of pathology. Alternately, the stress response may be inadequate for the reestablishment of homeostasis. In either case, a healthy steady state is not attained.

The pathways that are involved are central to the host's adaption to acute challenges. They function by mobilizing energy substrates, by ensuring cardiovascular and hemodynamic compensation, by increasing awareness, and by contributing to the host's immune function and tissue repair [5].

How is it mediated?

The afferent pathways:

The peripheral and central nervous system and local tissue factors, including products of the immunological system, seem to be involved [6].

Tissue damage results in local inflammation that involves changes in the microcirculation, with accumulation of both cellular and chemical mediators at the site of the insult leading to the classical signs of inflammation: increased procoagulant activity, blood flow, vascular permeability and vasodilatation [7]. The stress response is then activated by afferent nociceptive neuronal impulses [8] and by inflammatory mediators that are released from the site of injury into the systemic circulation and then act directly at central sites. The inflammatory mediators also act on afferent nerve fibers, resulting in an afferent neuronal signal [9]. As early as 1959, Hume and Egdahl demonstrated that sectioning the nerves to an extremity after third degree burns completely blocked the secretion of adrenocorticotropic hormone (ACTH) and cortisol that is associated with severe burns [10, 11].

The involvement of factors other than direct neural stimuli is shown by the inability of afferent neural blockade to inhibit some aspects of the systemic response, e.g. acute phase protein response, granulocytosis and hyperthermia. The importance of other factors is supported by the finding that severe injury to a denervated limb may still elicit an adrenocortical response [12].

The efferent pathways:

The stress response to surgery is characterized by increased secretion of pituitary hormones and activation of the sympathetic nervous system [13].

The hypothalamic-pituitary-adrenal axis

Afferent input appears to converge in the median eminence where corticotropin releasing factor (CRF) is secreted [14]. CRF is then carried by the vessels of the hypothalamic hypophysial portal system into the anterior pituitary, where it binds to CRF receptor type one on chromophobe cells and stimulates the release of ACTH [15]. The pituitary synthesizes ACTH as part of a larger precursor molecule, pro-opiomelanocortin. The precursor is metabolized within the pituitary into ACTH, ß-endorphin and an N-terminal fragment. ACTH secretion has a circadian rhythm, and the hormone acts on the cells of the adrenal *zona fasciculata* by activation of adenylate cyclase and cAMP production [16]. cAMP then activates protein kinases that facilitate the enzymatic conversion of cholesterol into pregnolone, the first and probably rate-limiting step of glucocorticoid biosynthesis. After a delay of 1-3 minutes [17], ACTH stimulation induces the secretion of cortisol in humans and corticosterone in rodents, respectively. Glucocorticoids interact with the brain and the pituitary gland, creating a closed-loop feedback system [18]. Usually, increased concentrations of circulating glucocorticoid inhibit further secretion of ACTH. However, this control mechanism appears to be ineffective after surgery, and concentrations of both ACTH and glucocorticoid remain high [19].

Under normal circumstances, approximately 75% of circulating glucocorticoids are bound tightly to cortisol-binding globulin (which is an alpha-1 glycoprotein) and 15% are loosely bound to albumin. The remaining 10% circulate unbound in the plasma and are able to affect cells. Traditionally, glucocorticoids have been thought to exert their action solely via a slow genomic mechanism. After glucocorticoids pass freely through cell membranes, they bind to specific receptors in the cytosol of target cells, and the steroid-receptor complex migrates to the nucleus of the cell, where it interacts with DNA to modulate the transcription of messenger RNA. Through this cellular mechanism, glucocorticoids can have profound effects on the protein and enzyme content and activity

in specific organs. This mechanism also explains the delay of 1-2 hours seen in the effects of cortisol following acute administration. However, some rapid effects of the glucocorticoids are not inhibited by the use of transcription inhibitors, which indicates the existence of non-genomic mechanisms. It has been suggested that the lipophilic steroids physically dissolve into lipid membranes and modify physicochemical membrane properties; this in turn affects the activity of membrane-associated proteins [20]. Furthermore, there is evidence for the existence of glucocorticoid signaling mechanism that is receptor-bound but non-genomic [21].

Glucocorticoids have complex metabolic effects on the metabolism of carbohydrates, fat, and proteins [19, 22-25]. They promote protein breakdown [26] and gluconeogenesis [27]. Glucocorticoids also promote lipolysis [28, 29], which increases the production of gluconeogenic precursors from the breakdown of triglyceride into glycerol and fatty acids. Furthermore, glucocorticoids facilitate the effects of other hormones e.g. the catecholamines, resulting in so-called permissive effects, a phenomenon involved in the development in hypertension in states of pathological hyperglucorticoidemia [30].

Glucocorticoids are known to increase arterial blood pressure, heart rate, and cardiac output. However, the differentiation between pure glucocorticoid effects and the effects that are the result of an interaction between glucocorticoids and other hormones such as the catecholamines is a difficult task. Many glucocorticoid actions are permissive; basal concentrations of glucocorticoids allow catecholamines and other vasoconstrictors to exert their full actions [31].

Glucocorticoids even play a predominant role in the regulation of fluid balance after injury. By amplifying vasoconstrictor effects, they prevent over-secretion of vasoactive substances that would have deleterious effects on the organism [32]. Furthermore, postoperative vasodilatation is reduced by the anti-inflammatory effects of glucocorticoids on prostaglandin synthesis [30, 33].

The sympathetic nervous system

The sympathetic nervous system is controlled by the hypothalamus, the same area of the brain that is responsible for the secretion of releasing factors, e.g. corticotropin releasing factor (CRF) [34]. Hypothalamic activation of the sympathetic autonomic nervous system

results in increased secretion of catecholamines from the adrenal medulla and release of norepinephrine from presynaptic nerve terminals. After stimulation and a delay of only a few seconds [31], catecholamines are released by the adrenal medulla via exocytosis and by splanchnic nerves, and they exert their action in the immediate vicinity of their release [35].

The catecholamines:

Norepinephrine is primarily a neurotransmitter, but there is some spillover of norepinephrine released from nerve terminals into the circulation [13]. Plasma concentrations of norepinephrine are used as an index of sympathetic nervous system activity. Plasma concentrations of epinephrine reflect adrenomedullary secretion [36].

Catecholamines exert their functions via G-protein-coupled alpha or beta receptors, resulting in decreased (alpha) or increased (beta) intracellular cAMP levels. To a substantial degree, most catecholamines act on both receptor types. Increased sympathetic activity results in the well-known cardiovascular effects of tachycardia and hypertension [18], and in immunological effects [9], as well as in profound metabolic changes. High levels of catecholamines inhibit insulin release [37, 38] and enhance glycogenolysis [39], hepatic glucose production [40] and peripheral insulin resistance [41], producing hyperglycemia as the net effect on glucose metabolism.

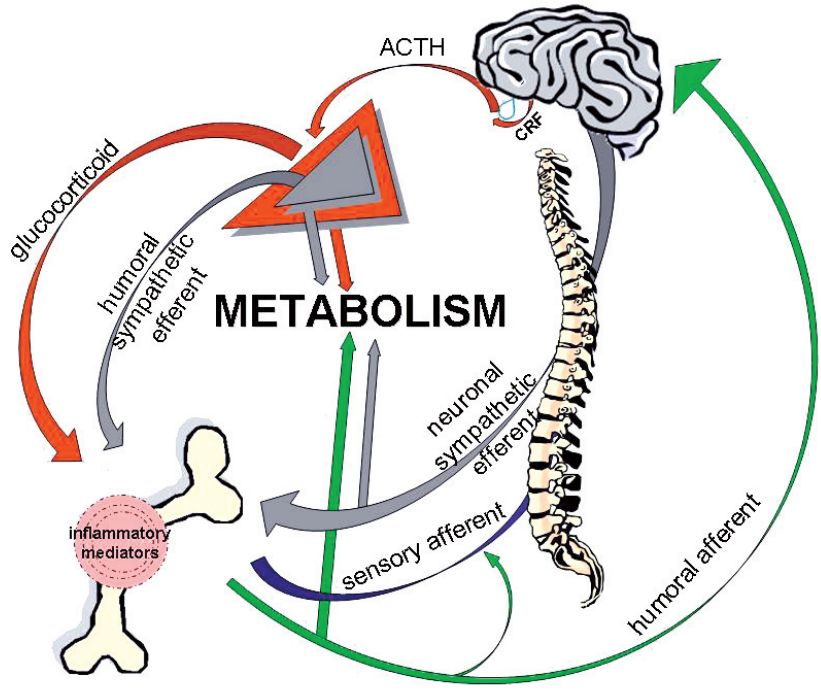
The immune system

Tissue damage results in the five typical signs of inflammation, which were described 2000 years ago by Celsus. The immune system not only has functions related to host defense and mediation of the stress response but also has profound effects on metabolism and fluid balance [9]. The cytokines are low-molecular-weight proteins that are one component of the humoral inflammatory response. This group of proteins includes the interleukins and interferons, which are produced as an early response to tissue injury by activated leucocytes, fibroblasts, and endothelial cells [42] and also by cells in neuroendocrine and endocrine tissues such as the hypothalamus [43], anterior pituitary [44-47] and adrenal cortex [48]. After major surgery, the main cytokines released are interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF) and interleukin-6 (IL-6). The initial reaction is the release of IL-1 and TNF-alpha from activated macrophages and

monocytes in the damaged tissues. TNF infusion produces many of the same alterations in the metabolism of glucose, fat, and protein that are commonly found in the septic state, suggesting that this cytokine has a major role in the metabolic response to acute infection. However, TNF administration in humans is associated with the appearance of other cytokines, one of which is IL-6. Therefore, the *in vivo* effects of TNF may be partially mediated by IL-6 [49, 50]. Furthermore, it has been previously shown in both experimental [51] and clinical [52] studies that IL-6 and TNF are regulated differentially. Surgical trauma without significant blood loss is known to increase IL-6 levels but not TNF levels.

Interleukin-6 is a 26 kDa protein produced by immune cells. It has a short half-life of less than one hour, suggesting that plasma IL-6 levels are the result of constant *de novo* synthesis [53]. Concentrations of circulating cytokines are normally low and may be undetectable. However, cytokine levels increase 30–60 min after the start of surgery, and significant increases are seen after 2–4 h [54]. Because cytokine production reflects the degree of tissue trauma [51, 54-56], cytokine release is lowest with the least invasive and traumatic procedures, e.g. laparoscopic surgery. The greatest increases in IL-6 occur after major procedures such as joint replacement, major vascular and abdominal surgery. Cytokine concentrations reach maximal levels after about 24 h after major operations, and they remain elevated for 48–72 h postoperatively [53].

IL-6 plays an important role in the regulation of fluid balance and blood pressure by stimulating the secretion of aldosterone and arginine vasopressin [57], and IL-6 has been suggested to be a biomarker of insulin resistance [58]. IL-6 was reported to have positive and negative actions on carbohydrate metabolism in liver tissue [59], adipose tissue [60] and skeletal muscle [61] but it does not affect insulin secretion [62]. Recent studies, however, have shown that IL-6 improves glucose metabolism in intact human skeletal muscle [63].



How do these systems interact?

Interactions between the sympathetic nervous system and the components of the hypothalamic-pituitary-adrenal (HPA)-axis

The central components of the HPA-axis and the sympathetic nervous system originate from co-localized areas in the hypothalamus [34]. Neural connections between the CRF neurons and the noradrenergic neurons within the central nervous system create excitatory and inhibitory feedback loops [5]. Intracerebroventricular administration of CRF results in increased plasma levels of noradrenaline and adrenaline in the rat [64-67]. This effect can be partially blocked by corticotrophin-releasing factor antagonists such as CP-154,526, indicating that CRF activates the sympathetic nervous system via the type 1

CRF receptor [68]. Glucocorticoids exert negative feedback on ACTH production and can also stimulate the adrenomedullary secretion of catecholamines. It was thought for several decades that circulating epinephrine controlled the release of ACTH by acting directly on the pituitary. Later, the converse was found to be true: glucocorticoids control epinephrine synthesis, and thereby affect the its secretion [69]. Intra-adrenal glucocorticoid is of crucial importance for the synthesis and secretion of adrenaline. Phenylethanolamine-N-methyltransferase (PNMT), the adrenal medullary enzyme that converts noradrenaline into adrenaline, is induced by the very high concentrations of glucocorticoid (100 times the plasma concentration) that the adrenal cortex delivers to the adrenal medulla via the adrenal gland's portal system [70].

Glucocorticoids act synergistically with epinephrine and other beta-agonists (an action employed in the treatment of asthma). This effect involves inhibition of catecholamine degradation [71], a blockade of catecholamine re-uptake [72, 73] and an increase in catecholamine sensitivity [30, 31].

Interactions between the immune system and the components of the hypothalamic-pituitary-adrenal axis

Glucocorticoids are potent immunosuppressive agents that affect cellular immunity as well as specific and non-specific humoral immunity [18]. Experimental studies have shown that many of the immunological events during the stress response can be inhibited or restrained by glucocorticoids. These events include cytokine secretion, granulocyte functions and arachidonic acid metabolization [6]. The function of the effects of glucocorticoids on the immune system in stress situations is not really clear, but it has been suggested that glucocorticoids counteract acute inflammatory effects on the microcirculation, thereby causing vasoconstriction, reducing edema, and decreasing the rate of leukocyte migration [74]. Glucocorticoids inhibit the accumulation of macrophages and neutrophils in areas of inflammation and can interfere with the synthesis of inflammatory mediators, particularly cytokines and prostaglandins [31, 75]. By inhibiting prostaglandin synthesis, glucocorticoids block vasodilatation [30, 33], thus

reducing the secretion of endogenous vasopressors and the need for administration of exogenous vasopressors [31].

The HPA-axis and the immune system have bidirectional effects. For example, cytokines such as IL-6 are potent stimulators of the HPA-axis and increase the secretion of CRF, ACTH, and glucocorticoids at their sites of production [76-78], which will then inhibit cytokine secretion.

Interactions between the sympathetic nervous system and the immune system

The neurotransmitters released by the sympathetic nervous system have well-known cardiovascular, hemodynamic and metabolic effects. These neurotransmitters also affect immune function through specific adrenergic receptor-mediated pathways, thus creating an important link between the immune system and the sympathetic nervous system [9]. Sympathectomy performed before hemorrhagic shock through the use of the neurotoxin 6-hydroxydopamine increases the TNF response [79]. This suggests that norepinephrine exerts anti-inflammatory effects and serves as a brake in the inflammatory cascade, controlling and regulating the magnitude and profile of cytokine responses.

In lymphoid tissues, lymphocytes and macrophages are located at sites adjacent to neuronal fibers. This exposes them to local release of neuropeptides and forms synaptic-like neuroimmune interactions [9]. The descending projections are capable of stimulating cells of the immune system directly through neurotransmitter release in the parenchyma of lymphoid organs [80] and indirectly through neurotransmitter and hormonal release into the circulation, thereby creating feedback loops and establishing further connections between the humoral, neuronal and immune system [9]. Therefore, the immune system not only plays a predominant role in the defense of the host against infections, it is even involved in the transduction, modulation, and execution of signals in the adaptive stress response after surgery.

Glucose metabolism

The major fuel source in normal humans is glucose. It enters the circulation either from endogenous sources (glycogenolysis and gluconeogenesis) or from external sources (via the digestive tract or intravenously). Glucose is then either metabolized to carbon dioxide, water, and energy and stored in ATP and GTP, or converted into glycogen and stored or converted into fat by lipogenesis.

Of the two insulin-sensitive tissues (skeletal muscle and adipose tissue) that are involved in peripheral glucose utilization, skeletal muscle accounts for up to 75% of the glucose disposal that occurs after a glucose load. Therefore, skeletal muscle is quantitatively the most important tissue involved in maintaining glucose homeostasis under insulin-stimulated conditions [81]. Furthermore, any change in the rate of glucose uptake by muscle will significantly affect the overall rate of glucose uptake because skeletal muscle constitutes approximately of 40 % of the body mass [82].

In response to increased plasma glucose concentrations, insulin is released from the pancreatic beta cells and binds to insulin receptors on cell surfaces. Insulin rapidly stimulates the tyrosine kinase activity of its own receptor, resulting in phosphorylation of the receptor's cytosolic substrate, insulin receptor substrate-1 (IRS-1). In turn, IRS-1 associates with phosphatidylinositol 3-kinase (PI 3-kinase), thus activating that enzyme [83]. Activation of PI 3-kinase is crucial for the translocation of GLUT-4 glucose transporters and subsequently the activation of insulin-mediated glucose uptake [84-86]. The steps located downstream from PI 3-kinase include the activation of protein kinase B, which has been suggested to be involved in the trafficking of GLUT-4-glucose transporters to the cell surface [87, 88]. GLUT-4 has been shown to be the predominant glucose transporter isoform in insulin-sensitive tissues such as skeletal muscle and adipose tissue [89-91]. In normal subjects, glucose transport across the plasma membrane of skeletal muscle is the rate-limiting step for glucose metabolism [92, 93].

Plasma glucose concentrations increase after surgery is begun. In fact, anesthesia itself results in hyperglycemia, and this increase in blood glucose is then further aggravated by the surgical procedure [94-96]. Plasma glucose concentrations after surgery are related to the magnitude of surgical injury [97]. The initial increase in plasma glucose after injury is due to mobilization of liver glycogen [98]. Because liver glycogen stores are limited [99], catecholamine-induced hyperglycemia is only initially the result of activation of glycogenolysis; at a later time, hepatic gluconeogenesis becomes the major factor in liver glucose release [100, 101]. The usual mechanisms that maintain glucose homeostasis are ineffective in the perioperative period, and hyperglycemia persists because catabolic hormones promote the production of glucose.

What effects do glucocorticoids have on glucose metabolism during conditions of stress?

Glucocorticoids alter extrahepatic as well as hepatic glucose metabolism [102].

After surgical trauma, plasma glucose levels remain high in spite of the presence of high levels of insulin, suggesting insulin resistance. Insulin resistance is a state in which a given concentration of insulin is associated with a subnormal glucose response [103]. Insulin resistance, which results in hyperglycemia, can be of either hepatic origin (increased glycogenolysis and gluconeogenesis) or peripheral origin (involving skeletal muscle). Glucocorticoid treatment is known to produce insulin resistance, but the exact molecular mechanism is unknown.

Interpretation of the pancreatic beta cell's response to glucocorticoids is difficult. Several studies have shown a mild inhibitory effect of glucocorticoids on insulin secretion [104-106]. However, even though glucocorticoids inhibit insulin secretion *in vitro,* data from *in vivo* studies show that this inhibitory effect on the beta cell is partially compensated for by the increase in insulin secretion that is induced by hyperglycemia [107]. Thus, the diabetogenic influence of elevated glucocorticoid concentrations is partly compensated for by increased insulin secretion.

Studies of the effects of glucocorticoids on insulin receptor binding have produced inconsistent data. Glucocorticoids have been reported to increase, decrease or have no effect on insulin binding in several cell types such as hepatocytes, adipocytes, fibroblasts, and blood cells. These inconsistent observations can in large part be explained by an interaction between the effects of glucocorticoids and hyperinsulinemia on insulin binding. The effect of glucocorticoids on skeletal muscle remains unclear [19]. Previous studies have shown that high levels of glucocorticoids have an inhibitory effect on steps of the insulin transduction cascade that are located downstream of the insulin receptor, e.g. insulin-stimulated PI 3-kinase activity [83]. However, elevated glucocorticoid concentrations do not seem to affect the levels of GLUT-4, which is the principal insulin-dependent glucose transporter across the cell membrane in skeletal muscle [108].

In summary, glucocorticoids act as typical insulin antagonistic hormones, and even short-term glucocorticoid administration results in hyperglycemia [24, 25, 109, 110]. Glucocorticoid levels are high after surgery. However, a large number of the actions of glucocorticoids differ in *in vitro* and *in vivo* settings, suggesting that glucocorticoids are only one part of a complicated, interconnected system that regulates glucose metabolism after surgical trauma.

What are the benefits of the adaptive stress response?

In evolutionary terms, it seems likely that the stress response developed as a survival mechanism that allowed injured animals to sustain themselves until their injuries were healed. By using stored body fuels and retaining salt and water, an animal had a chance to survive without food until healing and repair had taken place. The adaptive stress response provides energy for the local inflammatory reaction, since glucose is the major metabolic fuel in the wound [6].

The hypothesis that the adaptive stress response contributes to survival is supported by the fact that pathological states of glucocorticoid deficiency have been shown to be associated with increased morbidity and mortality after surgical trauma [32, 111, 112].

Why is the adaptive stress response potentially harmful?

In current surgical and anesthetic practice, it is questionable whether the stress response is necessary [13].

Some elements of the adaptive stress response appear to be harmful to the host. Hyperglycemia has been identified as an independent risk factor for increased morbidity and mortality after surgery [113]. Teleologically, it can be argued that insulin resistance was a way to ensure a supply of glucose to the brain (and other tissues dependent on glucose as a source of energy) when the supply of nutrients was limited. The overall effect achieved during insulin resistance is a diversion of glucose from skeletal muscle, which is one of the most glucose consuming tissues, to other sites such as injured tissue or the brain.

However, in modern medicine, it is rarely difficult to provide an exogenous supply of energy to the injured patient. Since insulin resistance and catabolism counteract the anabolism needed for recovery, it would seem reasonable to assume that any reduction in the posttraumatic stress response might be advantageous. Short-term activation of the stress response mechanisms is vital because it provides substrates needed to sustain increased metabolic demands. However, a prolonged, high-magnitude stress response has deleterious effects on metabolism [114], and immune function [115]. Furthermore, high levels of endogenous or exogenous glucocorticoids attenuate protein anabolism, wound healing, and the activity of the immune defense system after surgery. Activation of the sympathetic adrenal system or even the administration of exogenous catecholamines increases plasma glucose to levels that are known to be associated with increased postoperative morbidity and mortality [113].

Summary

Why are the glucocorticoids of particular interest? The glucocorticoids as friend or foe?

Glucocorticoids may improve the organism's defense mechanism by reducing an amplified or sustained release of potentially-harmful mediators [116].

Glucocorticoids are vital for the survival of the organism in stressful conditions. Previous studies have shown that the administration of glucocorticoids results in many of the metabolic changes seen after trauma [24, 117] and that the diabetogenic effect of the glucocorticoids can be prevented by the use of glucocorticoid receptor antagonists [118]. Glucocorticoids play a central role in the adaptive stress response after surgery and are of particular interest because of their duality of action.

On the one hand, the glucocorticoids appear to be important modulators of the stress response that prevent an overshoot of other efferent pathways such as the sympathetic nervous system and the immune system. The essential role played by glucocorticoids becomes obvious in states of glucocorticoid deficiency of iatrogenic or pathological nature. Adrenalectomized animals and patients with Addison´s syndrome fare poorly when stressed [32]. This was well demonstrated by the increased mortality rate observed following the use of etomidate to sedate critically ill patients. It was subsequently discovered that etomidate blocks adrenal steroidogenesis [119].

On the other hand, even short-term glucocorticoid treatment induces or aggravates diabetes mellitus [24, 25, 109, 110], induces arterial hypertension [120, 121], and interferes with the normal function of the HPA-axis, thereby producing states that are potentially harmful to the patient. Even though it has been known for a long time that glucocorticoids are important for the organism in order to cope with stressors, attention has been focused on the negative effects of glucocorticoids on metabolism. During the last decades, however, the beneficial effects of glucocorticoids in modulating the stress response have became more apparent, making it worthwhile to reconsider the role of glucocorticoids in the stress response.

Aims

The general aim of this thesis work was to investigate the systemic stress response and hyperglycemia after abdominal surgery in rat and man. For this purpose, a series of studies was undertaken. These individual studies had specific aims as follows:

- To investigate whether preoperative
  suppression of the HPA-axis by corticotropin releasing factor antagonism (I) or
  suppression of the inflammatory response by the administration of glucocorticoids (II) affects the stress response and glucose metabolism after surgical trauma in the rat.
- To investigate if glucocorticoids in a concentration similar to that after surgical trauma affect glucose metabolism in the rat (I, II).
- To develop an experimental rat model for studies on the stress response and metabolic alterations seen after major gastrointestinal surgery by combining intestinal resection with compensated non-hypotensive blood loss (III).
- To characterize the metabolic and hormonal responses after major gastrointestinal surgery and to relate responses to the intra- and post-operative clinical course in order to identify changes of special importance for outcome (IV).

Methods

For more detailed descriptions, please read the individual papers (I-IV)

Experimental studies (I-III)

Animals

The Wistar rat has been extensively used for studies of trauma and metabolism. We used male Wistar rats (B&K Universal, Stockholm, Sweden). The animals were housed individually with a 12 h light / dark cycle and had free access to standard rat chow and water at all times. The rats were conditioned by daily handling for at least one week prior to the experiments in order to minimize anxiety and stress prior to the experiments. All experiments were started between 6 and 8 a.m. to avoid any circadian interference.

Anesthesia

In all papers, animals, even those in the control groups that did not undergo surgery, were anesthetized.

In paper I, the animals were anesthetized by an intraperitoneal injection of ketamine (Ketalar®, 70 mg/kg) and xylazine (Rompun®, 10 mg/kg) (70 mg/kg and 10 mg/kg respectively) 15 min prior to the surgical procedure. The duration of anesthesia was about one hour.

In papers II and III, we used inhalation anesthesia. For induction of anesthesia, rats were put in an airtight anesthetizing chamber containing 4 % isoflurane (Forene®). After the animals fell asleep, they were removed from the chamber and breathed 1.5-3 % isoflurane through a nose cone connected to a vaporizer (Univentor 400®, Agntho´s, Lidingö, Sweden). After the induction of anesthesia, buprenorphine hydrochloride (Temgesic®, 0.075-0.01 mg/kg) was given subcutaneously for postoperative pain relief. In all the experimental studies, animals were placed on heating pads during anesthesia to maintain body temperature.

Surgical trauma model

In the groups receiving surgery, a five-centimeter incision was made along the midline of the abdomen and a five centimeter long resection of the small intestine was performed five centimeters distal to the ligament of Treitz. The intestine was sutured with six interrupted non-absorbable sutures (Prolene®, 6/0), and the abdominal cavity was closed using interrupted absorbable sutures (Vicryl®, 4/0). The surgical procedure lasted about 15 minutes. After surgery, animals were returned to individual cages and had free access to water and food.

Catheterization of the jugular vein

Rats were given a preoperative intraperitoneal dose of cefazolin (Zinacef®, 50 mg/kg) at the time of anesthesia as prophylaxis against infection. A silicon catheter was placed in the right jugular vein and advanced to the right cardiac atrium through a small skin incision on the ventral side of the neck and was tunneled under the skin to the dorsal side of the neck of the rat. The process of fabrication and implantation of the jugular catheters has been described previously [122]. Rats were allowed to recover for a minimum of one week after the surgical catheterization procedure before the experiments were started. During the recovery period, the animals were handled daily and accustomed to blood-sampling procedures by flushing the catheter. Rats that failed to gain weight during the recovery period were excluded before the experiments.

Blood loss

Paper III included two groups in which the rats were bled after surgery by repetitive blood samples drawn every 15 minutes for one hour. Assuming a total blood volume of 58 ml/kg body weight [123], the total postoperative blood loss was 7 % of the total blood volume in one group and 16 % in the other. To prevent hypovolemia and hypotension during and after blood loss, twice the volume of the drawn blood was replaced with saline (NaCl 0.9 %) and hydroxyethyl starch (Voluven®, 60 mg/ml), in a 1:1 ratio.

Blood sampling procedures

No sedation or anesthesia was used during blood sampling from the jugular catheters. Blood samples for basal levels were drawn between 06:00 and 08:00 three days before the start of the experiments. Blood was collected in chilled tubes containing 10 µl EDTA (0.250 mol/l, pH 8.0) and kept on ice. After the tubes were centrifuged at 4^{o}C for 15 min

at 3000 rpm, the plasma was removed and stored at −70°C until analysis. Unless otherwise stated, the sampled blood was replaced with twice the volume of intravenous saline (0.9% NaCl) in order to avoid hypovolemia.

Blood pressure measurement

Animals in paper III were anesthetized as described above and kept anesthetized until the end of the 135 min observation period. After the induction of anesthesia, a silicon catheter was placed in the right jugular vein through a small incision in the skin on the ventral side of the neck and advanced into the right cardiac atrium for use in blood sampling and fluid replacement. Thereafter, a polyethylene catheter was placed in the left common carotid artery for determination of blood pressure. This catheter was connected to a transducer for continuous blood pressure data acquisition. Data were analyzed using a computerized program (Powerlab-Chart 5®, AD-Instruments Pty Ltd, Bella Vista, NSW, Australia). Blood pressure was calculated as mean arterial blood pressure (MAP) during the 13 min period after blood sampling. In order to avoid artifacts, the two minutes required for the withdrawal of blood from the venous catheter and the injection of the replacement fluid were excluded from the calculation of blood pressure.

Dexamethasone-suppression test

The dexamethasone-suppression test (DST) is a method for investigating whether the HPA-axis responds adequately by suppressing endogenous glucocorticoid secretion when exogenous glucocorticoids are administered [124]. Animals received an injection of dexamethasone (15 µg/kg, i.v. dissolved in 0.9 % NaCl) (Sigma-Aldrich Chemie, Steinheim, Germany) two hours after surgery or anesthesia, and blood was sampled one and two hours after dexamethasone injection to measure plasma corticosterone and ACTH. Finally, two hours after the administration of dexamethasone, we investigated the function of the adrenal cortex by injecting synthetic ACTH (Synacthen®, 0.15 µg/kg, Novartis, Täby, Sweden) [125]. Thirty minutes after the ACTH injection, a blood sample was drawn for plasma corticosterone analysis.

Clinical study (IV)

Oral glucose tolerance test

One to two weeks prior to surgery, an oral glucose tolerance test (OGT) was performed in all patients. Baseline plasma glucose was analyzed in venous blood using an automatic analyzer (Accu-Check, Roche Diagnostics Scandinavia, Bromma, Sweden). Another blood sample was taken 2 hours after patients received 75 g of glucose as a beverage. The WHO guidelines (World Health Organization. *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation*. Geneva, 1999. WHO/NCD/NCS 99.2) were used for interpretation of the results.

Drugs and anesthesia

Patients received paracetamol and oxycodone as premedication. Thereafter, all patients received thoracic epidural anesthesia (Th6-Th9; bupivacain 1 mg/ml, fentanyl 2 μg/ml and adrenaline 2 μg/ml; 10-15 ml/h) for intra- and post-operative anesthesia and analgesia. Somatostatin was given before and during surgery (300 μg/24h) to decrease pancreatic secretion. Antibiotic prophylaxis (intravenous cefuroxim and metronidazole, both 1.5 g) was administered before the induction of anesthesia. Propofol was used for induction of anesthesia and sevoflurane (~ 1.5 %) for maintenance. Noradrenaline infusion (40 μg/ml) was started after induction of anesthesia at a dose of 0.03-0.1 μg/kg/min to maintain a mean arterial blood pressure (MAP) above 70 mmHg. Atracurium besylate was used for muscle relaxation. Ringer-acetate (2 ml/kg/h), hydroxyethyl-starch (Voluven®, 60 mg/ml, Fresenius Kabi, Bad Homburg, Germany, 2 ml/kg/h), and glucose (2.5 %, 1 ml/kg/h) were given intravenously as intra-operative fluid replacement. Blood lost during surgery was replaced with crystalloids, colloids or blood products. At the end of the surgical procedure, glycopyrrolate and neostigmine were given for reversal of muscle relaxation. Intravenous insulin infusion (~ 4 U/h) was used to keep plasma glucose levels below 10 mmol/l during surgery. Post-operatively, insulin was administered by subcutaneous injection. Patients in whom post-operative pain control was not satisfactory received ketobemidon (1-5 mg) intravenously on demand. Total parenteral nutrition (Kabiven, 25-30 kcal/kg/d) was provided from post-operative day three until oral intake of nutrients reached an adequate level.

Surgical procedure

In paper IV, a standard pancreaticoduodenectomy *ad modum* Whipple was performed in all patients. Briefly, a long midline incision was made and then adequate tissue mobilization was achieved through *Kocher* mobilization and mobilization of the right colonic flexure. A hepatico-jejunostomy, a pancreatico-jejunostomy, and an antecolic gastro-entero-anastomosis were performed after removal of the pancreatic head, the tissue in the hepatoduodenal ligament, the duodenum, the first part of jejunum, and the distal third of the stomach. Two abdominal drainage tubes were then set in place, the abdominal wall was sutured, and the skin was stapled.

Hormones and metabolites

Plasma glucose

Plasma glucose levels were determined either enzymatically (papers I and IV, Glucose analyzer YSI 2000 system®, Kebo, Stockholm, Sweden) or by the use of an automatic analyzer (papers II and III, Accu-Check®, Roche Diagnostics Scandinavia).

Plasma insulin

In paper I, plasma insulin was analyzed with a ^{125}I radioimmunoassay kit (Linco Research Inc., St. Charles, USA). In papers II, III and IV, immunoreactive insulin in plasma was measured by RIA using ^{125}I-labeled porcine insulin as the tracer, rat insulin (papers II and III) and human insulin (Novo Nordisk, Denmark) (paper IV) as the standards, and antibodies against porcine insulin [126].

Plasma ACTH

Plasma ACTH levels were analyzed using a ^{125}I radioimmunoassay kit (DiaSorin, Stillwater, Minnesota, USA)

Plasma corticosterone

Plasma corticosterone was analyzed using a radioimmunoassay kit from ICN, Biomedicals (Costa Mesa, CA, USA).

Plasma Cortisol

Plasma cortisol was analyzed using an electrochemoluminiscence immunoassay (Roche Diagnostic GmbH, Mannheim, Germany)

Plasma Free fatty acids

Non-esterified (free) fatty acids in plasma were determined by enzymatic colorimetric methodology using a kit from Wako (Wako Chemicals GmbH, Neuss, Germany).

Plasma Glycerol

Plasma glycerol was analyzed using an ultrasensitive automatic bioluminescence method as described by Hellmer [127].

Plasma IL-6 & IL-10

IL-6 and IL-10 analyses were performed using the quantitative sandwich enzyme immunoassay technique (R&D Systems, Inc. Minneapolis, USA).

Plasma adrenaline & noradrenaline

Plasma noradrenaline and adrenaline concentrations were determined by high-performance liquid chromatography with electrochemical detection. For extraction, 120µL aliquots of the plasma sample, 0.22 ng of the internal standard DHBA and 1.5 M Tris-buffer (pH 8.6, containing 10 nM EDTA-2Na) were applied to the cartridges, which were then washed with 120µL of 2% acetic acid containing 100µM EDTA-2Na. The collected samples were transferred onto a TopTip C18 pipette tip cartridge and 20 µL of each eluate was injected onto the HPLC column. The mobile phase was a mixture of methanol and 0.1 M phosphate buffer (pH5.7) (89:11, v/v) containing 2.8 mM octanesulfonic acid sodium salt and 0.13 mM EDTA-2Na [128, 129]. The chromatograms were recorded and integrated by use of the computerized data acquisition system Clarity® (Data Apex, Czech Republic).

Basal and insulin-stimulated PI 3-kinase activity in skeletal muscle *in vivo*

Two hours after surgical trauma, rats were re-anesthetized, and a heparinized catheter was inserted into the right external jugular vein. A bolus injection of 1 ml NaCl (0.9 %) was administered intravenously (i.v.) over a one-minute period, and a muscle biopsy was obtained from the right gastrocnemius muscle. Thereafter, insulin (10 U/kg insulin in 1 ml NaCl 0.9 % and 0.01 % bovine serum albumin) was administered intravenously during another one-minute period. Four minutes after the insulin injection, a second

biopsy was obtained from the left gastrocnemius muscle. Muscle biopsies were immediately frozen in liquid nitrogen. Thereafter, the muscle biopsies were ground to a fine powder with a mortar and a pestle in liquid nitrogen and immediately homogenized in ice-cold buffer as previously described [130]. Homogenates were solubilized by gentle mixing for 30 min at 4°C. After centrifugation (35,000 g for 45 min at 4°C), the supernant was removed, protein was determined (BCA protein assay kit, Pierce, Rockford, IL), and aliquots were stored at –70°C. Equal amounts of protein (1 mg) were immunoprecipitated overnight at 4°C with anti-insulin receptor substrate 1 (IRS-1) antibody coupled to protein A-sepharose. The immune complexes were washed as previously described [131] and resuspended in 40 µl of buffer (20 mM HEPES, pH 7.5, 180 mM NaCl). PI 3-kinase activity was assessed directly on the protein A-sepharose beads as previously reported [132]. The bands corresponding to PI 3-phosphate were quantified using a phosphorimager (Fujix 2000®, Fuji Photo Film, Fuji, Japan) and calculated as a percentage of the intensity of the whole blot.

Basal and insulin-stimulated glucose transport in skeletal muscle *in vitro*

Incubation medium was prepared from oxygenated (95 % O_2 / 5 % CO_2) Krebs-Henseleit buffer supplemented with 5 mM HEPES, 0.1 % bovine serum albumin, 2 mM pyruvate and 18 mM mannitol. Rats were re-anesthetized and the soleus muscles were removed bilaterally. Muscles specimens were divided in two equal portions (~30 mg each) that were placed in individual sealed vials containing KHB medium and then incubated for 15 min at 35°C. The vials were continuously oxygenated with 95 % O_2 / 5 % CO_2 and maintained at 35°C during all incubations. Following the 15 min equilibration period, the muscles were incubated in the presence or absence of insulin for 30 min in KHB medium containing 2 mM pyruvate and 18 mM mannitol. Glucose transport was assessed under basal conditions (0 µU insulin/ml) or insulin-stimulated conditions (100, 200, or 1000 µU insulin/ml) using 8 mM [^{3}H]-3-*O*-methylglucose (2.5 µCI/mmol) and 12 mM [^{14}C]-mannitol (26.3 µU/mmol) (ICN Biomedical Costa Mesa, CA). The total insulin exposure time was 40 min. The muscle specimens were processed as previously described [133],

and sample aliquots were counted in a liquid scintillation counter with channels preset for simultaneous registration of [^{3}H] and [^{14}C]. The amounts of each isotope present in the samples were determined and utilized to calculate the extracellular space and the intracellular concentration of 3-*O*-methylglucose. The intracellular water content of the muscle specimens was determined by subtracting the measured extracellular water space from the total muscle water. The rate of 3-*O*-metylglucose transport was expressed as µmol per milliliter of intracellular water per hour and calculated as the increase of glucose uptake over the corresponding basal value in µmol/ml/h.

Statistical analyses

Data are presented as mean ± SEM. In paper I, blood glucose, plasma hormone levels, PI 3-kinase activity and glucose transport data were analyzed by ANOVA. The Tukey-Kramer´s test for multiple comparisons was used after ANOVA had determined that there were significant differences among the groups. In papers II and III, plasma concentration data were analyzed by two-way analysis of variance (ANOVA), with the Bonferroni correction for multiple comparisons. In paper IV, analysis of pre- and intra-operative data was performed by analysis of variance (ANOVA) and Student's T-test. The area under the curve (AUC) was calculated for intra- and postoperative hormonal and metabolic data, and comparisons were performed by the use of Student's T-test or Wilcoxon signed rank test. Spearman's Rank Order was used for correlation analysis of intra- and post-operative AUC values as well as pre-, intra-, and post-operative clinical data. $P<0.05$ was considered to be statistically significant. Individual comparisons of plasma metabolites and hormone changes in each patient were performed in LabPilot® (CMA Microdialysis, Solna, Sweden).

Ethical approvals

All procedures were conducted with approval from the local ethics committee.

Results and Discussion

This section summarizes and discusses the hormonal and metabolic stress response after surgery. Particular attention is directed toward the effects of:

- CRF antagonism by the use of CP-154,526 (I)
- Glucocorticoid-induced inhibition of the inflammatory response (II)
- Magnification of the surgical trauma by the addition of blood loss (III)
- Pancreatic surgery in humans (IV)

Effects on the HPA-axis (I-IV)

Preoperative administration of CP-154,526 did not completely abolish the hypothalamic-pituitary-adrenal response induced by surgical trauma. CRF is a primary mediator for ACTH secretion, but it is not the only one. Other mediators such as AVP or the catecholamines as well as inflammatory mediators such as interleukin-6 (IL-6) [57, 134] could have compensated for relative CRF deficiency. Therefore, the results of paper I indicate that the hypothalamic stress response after surgical stress is dependent on other factors apart from CRF. In contrast to the small but significant effect on plasma corticosterone seen in CP-154,526 treated rats after trauma, plasma ACTH did not seem to be attenuated by the drug. Considering the short half-life of ACTH (3.5 minutes) [135] it is possible that changes in ACTH levels were missed because of too few sampling points. An alternative explanation is that the increase seen in plasma corticosterone after surgical stress was induced by another factor than ACTH, for instance IL-6, which is able to directly stimulate glucocorticoid production from rat adrenocortical cells [48].

As shown in paper II, preoperative administration of corticosterone to rats at 8 mg/kg body weight resulted in plasma concentrations similar to those seen after intestinal

resection. This dose, however, did not affect endogenous glucocorticoid secretion after trauma or disturb the physiological diurnal rhythm of glucocorticoid secretion.

In paper III, intestinal resection in the rat in combination with non-hypotensive blood loss resulted in plasma corticosterone levels that were comparable to those reported in studies on hypotensive hemorrhage [32, 136]. The threshold for increased ACTH secretion and subsequent corticosterone release in the rat has been shown to be at a blood loss of 8 ml/kg [137], equivalent to loss of 15% of the total blood volume. According to this previous observation, the 16% blood loss used in paper III should have resulted in an additional stimulus of corticosterone secretion in rats after intestinal resection. However, in contrast to previous studies, we used intensive fluid replacement after blood loss and we suggest that this regime efficiently counteracted volume changes after blood loss and subsequent activation of the HPA-axis. Alternatively, surgical trauma produced a maximal glucocorticoid secretion and additional stimulus could not further increase glucocorticoid release. In paper IV, during pancreatic surgery in humans, intraoperative plasma cortisol levels did not correlate to patient (age, BMI) or intraoperative factors (operation time and blood loss) or postoperative complications. This is in line with our previous observation that the glucocorticoid response is not amplified by the addition of blood loss in our experimental model of surgical trauma (Paper III, Hager, in press J Surg Res). Plasma cortisol levels were low compared to those in previous studies investigating the hormonal stress response after major upper abdominal surgery [138]. This finding, together with the low plasma adrenaline levels, indicates that the anesthetic protocol used in paper IV markedly attenuated the hormonal response during pancreatic surgery.

However, the adrenocortical response to stress is complex and influenced by metabolism, adrenal sensitivity, binding to plasma proteins, sampling time etc [139]. The effect of a given dose of glucocorticoid varies among patients, which necessitates taking each individual's glucocorticoid sensitivity into account [140].

Effects on the sympathetic nervous system (I- IV)

The sympathetic nervous system and the hypothalamic-pituitary-adrenal axis originate from locations adjacent to each other in the hypothalamus. Intracerebroventricular administration of CRF is known to increase sympathetic tone [65, 67] whereas CP-154,526 decreases it [68]. The locus coeruleus gives rise to noradrenergic pathways that are widely distributed to the brain [141]. The observation that CRF stimulates locus coeruleus cell firing has served as evidence for a role of this peptide in modulating noradrenergic tone during periods of stress [66, 67, 142]. Therefore, in paper I, CP-154,526 may have attenuated noradrenergic tone in the brain and thereby reduced the centrally mediated stimulation of hepatic glucose production after surgery. In paper II, preoperative corticosterone administration attenuated the surgery-induced rise in plasma catecholamines so effectively that the catecholamine levels were similar to those in non-operated, anesthetized control animals. This further emphasizes the tight interconnection between the sympathetic nervous system and the hypothalamic-pituitary-adrenal system in actions on the stress response.

The immune system is also tightly connected to the sympathetic nervous system and the HPA-axis. This connection is called the inflammatory reflex [143] and involves afferent and efferent nerve fibers from lymphoid tissue to and from the central nervous system, in particular the paraventricular nucleus of the hypothalamus and the locus coeruleus [9]. Against this background, the lack of increase of plasma catecholamines noted in paper II might be explained as follows: corticosterone given preoperatively could have reduced surgery-induced cytokine release in lymphoid tissues, which in turn attenuated the afferent input from injured tissues into brain regions important for sympathetic output, directly via afferent neurons and indirectly via reduced release of cytokines into the circulation. This would attenuate activation of the sympathetic nervous system and consequently reduce catecholamine release. It is also possible that high preoperative corticosterone concentrations reduced postoperative plasma catecholamines via negative feedback on ACTH release [144] by causing a sustained inhibition of the adrenal medullary enzyme phenylethanolamine-N-methyltransferase (PNMT). PNMT is induced

by very high local concentrations of glucocorticoid delivered by the adrenal's portal vascular system and it favors the conversion of noradrenaline to adrenaline [70]. Even though the exposure time to exogenous corticosterone was short, the results in paper II cannot rule out that high preoperative concentrations of exogenous corticosterone increased vascular reactivity [145] and, as a consequence, attenuated vasopressor responses (i.e. catecholamine release) to surgery.

In paper III, low plasma adrenaline levels during blood loss in the rat indicate that the fluid replacement regime in this animal model prevented a reduction in circulatory volume and subsequent sympathetic nervous system activation. This is confirmed by comparison with a study performed by Ljungqvist et al. who showed ten-fold higher plasma adrenaline levels after hypotensive hemorrhage [146]. In partial contrast to adrenaline, noradrenaline levels showed a tendency toward higher values during blood loss but the difference was not significant compared to basal. At two hours after surgery combined with blood loss, both plasma adrenaline and noradrenaline levels were increased compared to surgery alone, indicating a later activation of the sympathetic nervous system.

During pancreatic surgery in humans (paper IV), intraoperative plasma noradrenaline levels were very high compared to those reported in previous studies with [96] or without epidural anesthesia [147]. It is therefore reasonable to assume that the high intraoperative noradrenaline levels were the result of exogenous administration and did not reflect sympathetic activation induced by surgical stress. The large variation in plasma noradrenaline levels may be explained by several factors such as individualized noradrenaline infusion regimens, the sampling time-points in relation to changes in infusion rate, as well as the short half-life of noradrenaline ~ 15 seconds [148]. No statistically significant correlation was found between plasma noradrenaline and metabolites, possibly because of the large variations in plasma noradrenaline concentrations. Alternatively, the lack of correlation between plasma noradrenaline levels and hormones and metabolites measured in paper IV was due to marked individual differences in the sensitivity to the metabolic effects of noradrenaline.

Effects on the immune system (II, IV)

Interleukin-6 (IL-6) is one of the primary mediators of the acute stress response to injury [52] and stimulates the release of stress hormones [57, 62, 149, 150]. In paper II, we showed that preoperative glucocorticoid administration reduced postoperative plasma IL-6 levels in the rat. Interestingly, this marked effect was observed after just a short preoperative exposure (two hours) to physiological concentrations of the hormone. Similar rapid effects on the inflammatory response have been observed in mice given glucocorticoids two hours before asthma induction [151]. In patients given a single preoperative dose of methylprednisolone (10 mg/kg) thirty minutes before esophageal surgery, plasma IL-6 and IL-10 concentrations were reduced and increased, respectively [152]. We hypothesize that, in paper II, glucocorticoids attenuated the inflammatory response in injured tissues, thereby reducing afferent input into brain areas that regulate the neuroendocrine stress response.

In humans, in paper IV, the positive correlation between plasma IL-6 levels and markers of the scale of the surgical procedure (operating time, blood loss and volume of intravenous fluids given intraoperatively) is in line with previous observations [52, 54]. Interestingly, after pancreatic surgery in humans, plasma IL-6 levels reached mean levels of 891 pg/ml which is high compared to previous observations of 450 pg/ml after duodeno-pancreatectomy [138] and 500 pg/ml after elective aortic surgery [153]. The highest levels of IL-6 (2900 and 2300 pg/ml) on the first day after surgery were seen in two patients who later developed pancreatic leakage. It remains to be determined whether noradrenaline infusion and its strong intraoperative lipolytic effects in patients with complications contributed to the increase in plasma IL-6 levels [154] or whether the high IL-6 levels reflect a stronger overall stress stimulus due to a complicated surgical procedure.

Effects on glucose levels (I-IV)

In paper I, preoperative administration of CP-154,526 in the rat reduced postoperative hyperglycemia. This finding could have been mediated via mechanisms regulating either glucose uptake or glucose production. The CRF-receptor type 1 has never been shown to be present in the major tissues involved in glucose metabolism, i.e. skeletal muscle and liver. It is therefore likely that CP-154,526 antagonized CRF action on central binding sites. Acute hyperglycemia is mediated by the action of circulating adrenaline and glucagon as well as the direct noradrenergic innervation of the liver. The result is hepatic glycogen breakdown and glucose release into the systemic circulation [155, 156]. Previous studies have shown that even short-term glucocorticoid administration results in hyperglycemia [24, 25, 109, 110]. However, the increases in glucagon and adrenaline after intestinal resection in this model [157] were small compared to those elicited by other forms of stress in the rat, such as hypovolemic stress [146]. Furthermore, plasma corticosterone levels were only moderately decreased by CP-154,526, making the glucocorticoids unlikely to be the acute mediator of post-stress hyperglycemia. This indicates that in addition to circulating factors, other mechanisms are involved in mediating the hyperglycemic response to surgery. Central neuronal mechanisms are of importance for the regulation of hepatic glucose metabolism [158]. Intracerebroventricular administration of CP-154,526 is known to decrease noradrenergic tone [68]. Therefore, CP-154,526 may have attenuated trauma-induced hyperglycemia by reducing noradrenergic tone and consequently reducing the centrally-mediated stimulus of hepatic glucose production. We hypothesize that CRF action within the central nervous system can regulate the hyperglycemic response to surgical stress via mechanisms other than the pituitary-adrenal axis.

In paper II, corticosterone in a concentration comparable to that seen after surgical trauma in the rat did not affect plasma glucose levels in the non-stressed rat. This strengthens the conclusion from paper I that acute increments in plasma glucocorticoids do not result in hyperglycemia. In contrast, preoperative corticosterone administration resulted in a significant, but transient, attenuation of early postoperative hyperglycemia.

Therefore, as discussed above, the reduced hyperglycemic response in the corticosterone treated group was most likely due to a reduction in sympathetic stimulus on hepatic glucose output.

In paper III, the addition of blood loss to surgery in the rat resulted in a more pronounced and sustained hyperglycemia than surgery alone. The volume-dependent increase in plasma glucose levels in paper III is in accordance with previous studies showing that blood loss rapidly induces hyperglycemia [159, 160]. Even though plasma noradrenaline showed a trend towards an increase in paper III, postoperative plasma catecholamine levels were not increased when plasma glucose levels were high after blood loss. Pancreatic beta cell function was not affected by the addition of blood loss in paper III, as shown by the similar plasma insulin levels in all animals that underwent surgical trauma. Therefore, plasma insulin levels could not explain the differences in plasma glucose levels. These data indicate that during compensated (non-hypotensive) blood loss, other factors than catecholamines mediate early hyperglycemia and inhibition of insulin release after surgery.

However, the results from paper III do not exclude metabolic effects of catecholamines up to two hours after surgery and blood loss. Darlington et al. have previously shown that after hemorrhage, high circulating concentrations of glucocorticoids potentiate the effect of vasoactive hormones i.e. vasopressin and noradrenaline [32]. It is therefore possible that high levels of plasma corticosterone after blood loss potentiated the metabolic effects of plasma noradrenaline to increase hepatic glucose output. Furthermore, noradrenaline is primarily a neurotransmitter and increased noradrenergic activity is not necessarily reflected by increased plasma noradrenaline in the systemic circulation. At two hours after surgery, however, plasma catecholamines were significantly increased and are likely to have contributed to both postoperative hyperglycemia and inhibition of plasma insulin release.

In the clinical setting, during pancreatic surgery, intravenous insulin infusion was used in order to maintain plasma glucose levels below 10 mmol/l. The amount of insulin needed to maintain intraoperative plasma glucose levels within the target range increased during surgery, which indicates that insulin resistance gradually develops during surgery, as found previously [161]. Even though we did not find any significant positive correlation

between the plasma concentrations of glucose and the amount of noradrenaline administered during surgery, plasma noradrenaline and glucose concentrations increased in parallel early during surgery. It could therefore not be ruled out that noradrenaline induced insulin resistance in the liver and/or skeletal muscle and thereby caused hyperglycemia. Furthermore, these patients had high levels of free fatty acids because of noradrenaline induced lipolysis (as will be discussed below): the free fatty acids could have reduced glucose uptake in skeletal muscle [162-164].

Effects on insulin levels (I-IV)

CRF-receptor type 1 (CRF-R1) antagonism in the rat resulted in decreased plasma insulin levels. It has been demonstrated that CRF-R1 is present in rat pancreatic beta cells and that CRF is capable of stimulating insulin secretion *in vitro* [165]. Thus, it is possible that the decrease in plasma insulin was caused by a direct inhibitory effect of CP-154,526 on the pancreatic beta cell.

In paper II, corticosterone in a concentration comparable to that seen after surgical trauma in the rat did not affect preoperative plasma insulin levels in the non-stressed rat. Previous *in vitro* studies reported an inhibitory effect of glucocorticoids on the beta cell [104, 106]. *In vivo* studies as well as the results in paper II showed that in a physiological setting, the potential inhibitory effect of the glucocorticoids on insulin secretion is compensated for by an increased glucose-induced stimulation, thereby leaving insulin and glucose levels unchanged [107].

In paper III, the addition of blood loss to surgical trauma in the rat did not further attenuate pancreatic beta cell function as shown by the similarly reduced plasma insulin levels in all groups that underwent surgical trauma (with or without blood loss). The results are in line with the transient inhibition of glucose-stimulated insulin secretion observed after intestinal resection without blood loss [166]. However, hyperglycemia could not be explained by differences in pancreatic beta cell function.

Plasma insulin levels during pancreatic surgery in humans were high. In paper IV, patients received exogenous insulin to maintain plasma glucose levels within the target

range. The methodology used to determine plasma insulin levels in paper IV did not distinguish between endogenous and exogenous insulin. However, the high noradrenaline levels during surgery are likely to have decreased insulin secretion from the pancreatic beta cells. We therefore suggest that plasma insulin levels in paper IV resulted from administration of exogenous insulin rather than pancreatic beta cell secretion.

Effects on skeletal muscle (I)

Several intracellular signaling proteins have been suggested to be involved in insulin resistance. Attention has focused on the intracellular enzyme PI 3-kinase because of its central role in insulin-stimulated glucose transport [93]. Reduced insulin-stimulated PI 3-kinase activity in skeletal muscle has been associated with insulin resistance in severely obese and diabetic patients [131, 167] as well as in animal models of obesity and diabetes [168]. We have previously demonstrated reduced insulin-stimulated glucose transport and an increase in PI 3-kinase activity in skeletal muscle two hours after intestinal resection in the rat [169, 170] and those results were confirmed in paper I [171]. Our findings indicate that this change in PI 3-kinase activity, which is paradoxical compared to other insulin-resistant states, is specifically associated with surgical stress. Increased insulin stimulated PI 3-kinase activity in combination with decreased glucose uptake in skeletal muscle suggests a defect in insulin transduction downstream of PI 3-kinase and can be interpreted as a compensatory mechanism in order to increase glucose uptake.

As previously demonstrated, in this model of intestinal resection in the rat [169, 170], intestinal resection resulted in decreased insulin-stimulated glucose transport in skeletal muscle. This surgery-induced effect occurred independently of CP-154,526 and showed that increased glucose uptake was not the mechanism responsible for lowering blood glucose levels in CP-154,526-treated rats after surgery.

Effects on lipid metabolism (IV)

During lipolysis, triglycerides are hydrolyzed to free fatty acids (FFA) and glycerol, which are then released from the fat cell. Catecholamines and insulin are the primary mediators of fat metabolism and have opposite effects on lipolysis and FFA re-esterification [172]. In previous studies investigating lipolysis during abdominal surgery without noradrenaline infusion and epidural anesthesia, glycerol and FFA levels increased to at most 1.5-fold compared to basal levels [147, 173]. During surgery with epidural anesthesia, both FFA and glycerol levels were unchanged [96] or even reduced [174]. In humans (paper IV), concentrations of FFA and glycerol were increased up to three-fold basal levels in parallel with plasma noradrenaline concentrations early during surgery. The individual high FFA levels during surgery seen in the patients who developed complications were associated with both very high (up to 20-30 nmol/l) and "normal" post-stress levels (2-4 nmol/l) of noradrenaline in plasma, suggesting that sensitivity to the lipolytic effect of noradrenaline differs between patients.

Effects on hemodynamics (III)

Loss of 7 or 16 % of total blood volume in the rat (paper III) is considered moderate and within the range seen after abdominal surgery in man [123, 175-178]. Mean arterial blood pressure decreased in all groups, probably as a result of isoflurane induced vasodilatation [179]. However, hypotension as a result of blood loss was avoided by the use of fluid replacement. In accordance with the clinical routine at our department, we used hydroxyethyl-starch and saline in a ratio of 1:1 as volume replacement in the rat. Hematocrit values in all groups remained within the physiological range for rats [180] but decreased over time in the trauma groups. This reduction was most pronounced after surgical trauma in combination with blood loss and occurred during the later part of the experiment. Decreased hematocrit was observed also in the non-bled but operated rats. Surgical trauma induces a redistribution of body fluids in order to preserve water and salt.

Therefore, the hemodilution in paper III could have been the result of increased postoperative levels of antidiuretic hormone (ADH), aldosterone and subsequent activation of the renin-angiotensin II system [13, 181].

Effects of the metabolic stress response on the postoperative clinical course (IV)

In paper IV, pancreatic surgery was associated with high postoperative morbidity and three out of seventeen patients developed pancreatic leakage. The incidence of pancreatic anastomotic leakage following pancreaticoduodenectomy is known to be high [182]. In paper IV, after pancreatic surgery in humans, plasma IL-6 levels remained elevated longer in patients who developed postoperative complications. Furthermore, the increase in plasma IL-6 levels preceded the clinical onset of the complications by several days. Similar to the observations in paper IV, IL-6 has also been shown to correlate to the incidence of posttraumatic complications [183] and to be a predictor for poor outcome after sepsis [184]. The levels of plasma glucose and FFA during pancreatic surgery in humans were positively correlated to the length of in hospital stay and intraoperative plasma FFA levels were positively correlated to the number of postoperative complications. This indicates that a stronger metabolic response during surgery has a negative impact on postoperative clinical course. Analysis of each individual hormone and metabolic profile revealed that the stimulation of lipolysis was stronger in patients with than those without complications, as shown by higher FFA levels (>1.5 mmol/L) and glycerol levels (>500 μmol/L), and was maintained throughout the surgical procedure. If the vasoconstrictor effects of noradrenaline were similarly increased in patients with complications, especially effects on visceral macro- and micro-circulation, this could have potential implications for the development of postoperative complications such as anastomotic leakage. This hypothesis is supported by a previous study showing that transfusion of four or more units of blood during surgery (which implies a need for intraoperative fluids and vasopressor support) increases the incidence of anastomotic leakage [185].

Summary

In this thesis work, we have shown that intestinal resection in the rat as well as major abdominal surgery in humans activates the efferent pathways of the adaptive stress response, namely the sympathetic nervous system, the hypothalamic-pituitary-adrenal axis and the immune system.

The hormonal result of this response was high intra- and postoperative levels of glucocorticoids (I-IV), an altered negative feedback system within the HPA-axis (I), increased levels of catecholamines (II-IV) and high levels of interleukin-6 (II, IV).

The metabolic response in the rat (I-III) was characterized by increased intra- and postoperative levels of plasma glucose, impaired insulin signaling and impaired glucose transport in skeletal muscle. During pancreatic surgery in humans (IV), we observed hyperglycemia, increased lipolysis and supraphysiological plasma levels of noradrenaline during major gastrointestinal surgery. In patients who later developed postoperative complications, the intra-operative lipolytic response was stronger and the postoperative IL-6 levels higher compared to patients without complications. Our results also indicate that the sensitivity to the lipolytic effects of noradrenaline show inter-individual variation during conditions of stress.

Conclusions

- Corticotropin releasing factor antagonism moderately decreased postoperative hyperglycemia and hypercorticosteronemia without affecting insulin transduction or glucose uptake in skeletal muscle.
- Preoperative administration of glucocorticoids attenuated the postoperative stress response by reducing the activity of the inflammatory response without altering plasma glucocorticoids.
- Glucocorticoids play an important regulating role in the stress response after surgery by decreasing plasma catecholamine levels which suggests the existence of a tight interconnection between the sympathetic-adrenal efferent and the hypothalamic-pituitary-adrenal system of the stress response.
- Plasma glucocorticoids in concentrations seen after surgical trauma do not increase plasma glucose levels.
- The addition of blood loss to surgical trauma in the rat potentiated the hormonal response and induced persistent volume-dependent hyperglycemia that was independent on changes in plasma corticosterone and, in the early postoperative phase, MAP and catecholamines.
- Intra-operative metabolic and hormonal changes are strongly influenced by the peri-operative anesthesiological treatment regimens including epidural anesthesia as well as insulin- and noradrenaline infusions.

Future Aims

Glucocorticoid responses to surgery in experimental models and humans

During the past decade, there has been a major shift in how we take our patients through the perioperative phase. We can now partially eliminate the stress response by using improved general anesthesia, epidural anesthesia and analgesia [39, 186-191]. At the same time, the potent drugs, such as vasopressors and insulin, we administer during and after the operation affect hormonal responses and strongly influence metabolism and visceral blood flow. Preoperative glucocorticoid administration has been shown to reduce postoperative inflammation although its clinical impact needs to be elucidated [152, 192, 193].

The glucocorticoid response and its effects on inflammation and circulation following surgery is not fully understood and careful investigation as to which type of surgery would constitute an indication for prophylactic administration of glucocorticoids is warranted.

Many of our elderly patients with obesity or diabetes type 2 are likely to show weak or abnormal glucocorticoid responses to stress [194-196] and could represent a potential target group for preoperative glucocorticoid treatment. These patient categories are common in our patient population and have an increased frequency of severe complications after major abdominal surgery [197, 198]. Furthermore, inter-individual variation in glucocorticoid-receptor sensitivity is likely to be an important factor for the diverging postoperative events [140]. In order to further characterize glucocorticoid sensitivity before and after surgery in experimental models and humans, we aim to perform the following studies:

Experimental

Method: Plasma glucocorticoid levels and glucocorticoid receptor quantity in peripheral white blood cells will be determined in different populations of rats by the use of flow

cytometry. The glucocorticoid sensitivity will be determined by measurement of glucocorticoid-regulated genes by qRT-PCR after *in vitro* glucocorticoid incubation in RNA from peripheral white blood cells from normal, aged (24-28 months) and obese rats (induced by high fat diet) subjected to intestinal resection in combination with blood loss and in anesthetized control animals.

Human

Method: Salivary cortisol and quantification of glucocorticoid receptors with flow cytometry of peripheral white blood cells isolated from pre-and postoperative blood samples in patients subjected to major upper gastrointestinal surgery. The glucocorticoid sensitivity will be determined by measurement of glucocorticoid-regulated genes by qRT-PCR after *in vitro* glucocorticoid incubation in RNA from peripheral white blood cells from the same patients and time-points. Results will be correlated to age, BMI, and postoperative morbidity.

Effects of vasopressors on visceral and systemic metabolism and postoperative clinical course

The introduction of potent epidural anesthesia and analgesia and restrictive fluid regimens has resulted in the need for perioperative vasopressor support (in our department, this means continuous noradrenaline infusion) in order to counteract peripheral vasodilatation during and after major abdominal surgery. Even though, there are indications that noradrenaline is superior to other vasopressors in terms of gut circulation [199], its effects on delicate anastomoses in the upper gastrointestinal tract and its effects on metabolism and postoperative morbidity have to be further explored. By causing splanchnic vasoconstriction, perioperative noradrenaline infusions could, in theory, compromise blood flow to organs and intestines being operated on and thereby have a negative effect on microcirculation and healing of anastomoses and organs. In paper IV, our results firstly indicate that the sensitivity to the lipolytic effects of intra-operative noradrenaline differ among patients. Secondly, we observed that the patients

who developed postoperative complications also showed a strong lipolytic response. Furthermore, we have preliminary unpublished data supporting a correlation between intraoperative intra-abdominal glycerol release (measured by microdialysis) and anastomotic leakage. Thus, it is possible that a high sensitivity to noradrenaline´s metabolic and/or cardiovascular effects may affect postoperative clinical course. Therefore, the sensitivity to noradrenaline should be investigated before surgery in order to individualize perioperative vasopressor treatment.

The effects of vasopressors on visceral and systemic metabolism, cardiovascular reactivity and postoperative clinical course will be investigated in patients subjected to major gastrointestinal surgery in the following planned studies:

Human

Method: Twenty patients planned to undergo pancreaticoduodenectomy will be investigated two weeks before surgery. During continuous observation of ECG, heart rate and blood pressure, patients are infused with noradrenaline (40 μg/ml) in doses 0.01-0.1 μg/kg/min during four hours. Repeated sampling for immediate determination of glycerol, glucose, lactate and pyruvate will be taken from an intravenously placed microdialysis catheter (every 15 min). Sampling for noradrenaline and adrenaline will be performed at later time by HPLC in microdialysis samples. Inflammatory markers and cortisol will be measured once per hour. During surgery, identical sampling will be performed from an intravenously placed microdialysis catheter and from an intraabdominally placed microdialysis catheter placed in between loops of small intestines. The postoperative clinical course will be registered.

Method: Patients undergoing major abdominal surgery that requires perioperative noradrenaline infusion (major upper gastrointestinal surgery) or do not require perioperative noradrenaline infusion (colorectal surgery) will be included in the study. At the end of surgery, intra-abdominal microdialysis catheters will be placed in between loops of small intestines and a subcutaneous catheter will be placed as a reference.

Primary outcome measures will be the following: local tissue metabolism markers (lactate/pyruvate ratio, glycerol and glucose) determined during and after the operation; plasma hormone levels (catecholamines, cortisol, and glucagon) and metabolic responses (plasma glucose, free fatty acids, glycerol, and lactate). The incidence and severity of postoperative complications will be determined as secondary outcome measure.

Preoperative modulation of the adaptive responses to stress

Glucocorticoids can modulate metabolic and circulatory effects via their permissive effect on adrenoreceptors. Preoperative exogenous corticosteroids could therefore increase vascular reactivity [145] and, as a consequence, attenuate vasopressor responses to surgery (e.g. catecholamine release) as suggested by our results in Paper II, thus being an indirect approach to vasopressor therapy. In line with this reasoning, increased vascular reactivity induced by glucocorticoids could be one way to reduce the need for peri- and postoperative exogenous vasopressor support and thereby attenuate possible adverse effects of the catecholamines on visceral circulation and intra-abdominal metabolism. The mechanisms by which preoperative glucocorticoids can modulate the adaptive responses to surgical stress and the effects on circulation will be investigated in the following study:

Experimental

Method: Corticosterone will be administered two hours prior to blood loss (7 or 16% of total blood volume) and/or intestinal resection in rats and in anesthetized control rats. The effects on postoperative parameters of glucose metabolism (plasma glucose and insulin), lipid metabolism (plasma FFA and glycerol), the inflammatory response (plasma IL-6), activity of the sympathetic nervous system (plasma adrenaline and noradrenaline). The effect on hemodynamic parameters (mean arterial blood pressure, heart rate, hematocrit), will be investigated in an identical parallel study.

Summary in Swedish

Populärvetenskaplig sammanfattning

Stressvaret i samband med bukkirurgi och dess betydelse för ämnesomsättningen.

Ett kirurgiskt ingrepp är en typ av vävnadskada som sker under noggrann kontroll av kroppens funktioner. Ett så kallat stressvar utlöses genom att den skadade vävnaden skickar signaler via nervbanor och via blodbanan till hjärnan. Detta leder till en ökad insöndring av stresshormoner (framförallt kortisol, noradrenalin och adrenalin) och en inflammatorisk reaktion som i sin tur påverkar ämnesomsättningen. Stressvaret är betydelsefullt för kroppens läkning och återhämtning efter skada. Ett för starkt eller förlängt stressvar kan däremot leda till att kroppens energireserver förbrukas, läkningen försämras och att återhämtningen går långsammare. Vi kan idag med förbättrad kirurgisk teknik, mer skonsamma sövningsmetoder och bättre smärtlindring minska stressvaret under och efter kirurgi. Samtidigt behandlar vi patienten med olika läkemedel under operationen som i sig kan öka stressvaret. Syftet med denna avhandling var att studera stressvaret och dess effekter på ämnesomsättningen under och efter kirurgi. Vi har använt oss av en experimentell modell av kirurgisk trauma på råtta (tarmoperation) och studerat patienter under och efter stor bukkirurgi. I våra studier har vi använt ett antal substanser i syfte att hämma stress svaret på kirurgi i hjärnan och/eller i det skadade området. Vi har även studerat om stress svaret påverkas om man adderar ytterligare ett trauma som till exempel blodförlust vilket vanligen förekommer i samband med operationer på människa.

Kortisolsvaret efter kirurgi kunde enbart delvis slås ut trots en blockerande behandling av reglerande områden i hjärnan. Om inflammations hämmande behandling gavs innan kirurgi så minskade både nivåer av adrenalin och noradrenlin. Likaså minskade den tidiga blodsockerstegringen med denna behandling, men effekten var kortvarig. Om blodförlust adderades till tarmkirurgi resulterade detta i ökade nivåer under en längre tid av både stresshormoner och blodsocker jämfört med enbart tarmkirurgi. Däremot var blodsockernivåerna mer beroende av hur mycket blod som djuret förlorat än av stresshormonnivåer. Delar av stressvaret under och efter stor bukkirurgi hos människor hämmas kraftfullt av en effektiv sövning och ryggbedövning vilket bidrar till att blodtrycket sjunker i samband med kirurgi. Därför måste läkemedel som till exempel noradrenalin, vilka reglerar blodcirkulationen och ökar blodtrycket, ges under och ibland efter stor kirurgi. Noradrenalin har även kraftfulla effekter på ämnesomsättningen bland annat genom att öka nedbrytningen av fett (lipolys) och att höja blodsockernivåerna under kirurgi. Hos patienter som genomgår stor bukkirurgi fann vi att de patienter som hade en markant ökad lipolys under operationen i större utsträckning utvecklade komplikationer efter kirurgi. Känsligheten för noradrenlins effekter på ämnesomsättningen skilde sig stort mellan olika patienter.

Sammanfattningsvis visar våra studier på råtta att om vissa delar av stress svaret slås ut träder andra kompletterande mekanismer in i syfte att bibehålla ett kortisolsvar och en blodsockeröknin under och efter kirurgi. Våra studier på människa visar att kroppens stressvar kraftigt minskar med modern sövning och kirurgi, men att tillförsel av noradrenalin under operationen delvis återskapar stressvarets effekter på ämnesomsättningen.

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