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Perioperative Stress in Dogs

Different Aspects of Manifestation and Characteristics with Medetomidine and Acepromazine Preanaesthetic Medication

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Academic Dissertation

To be presented, with the permission of the Faculty of Veterinary Medicine, University of Helsinki, for public criticism in the Auditorium XII, Fabianinkatu 33, Helsinki, Finland, on the 24th March, 2006, at noon

Helsinki 2006

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ISBN 952-91-9995-3 (paperback)
ISBN 952-10-2975-7 (pdf)
University Printing House
Helsinki, Finland, 2006
http://ethesis.helsinki.fi

"To all my friends,
Great and Small"

CONTENTS

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1. ABBREVIATIONS

aECG, ambulatory electrocardiogram
ACE, acepromazine maleate (0.05 mg kg^{-1}) and butorphanol tartrate (0.2 mg kg^{-1}) (IM)
ACTH, adrenocorticotrophic hormone
ADH, antidiuretic hormone (also called vasopressin)
ANS, autonomic nervous system
Apent, approximate entropy
AR, autoregressive (modelling)
AV block, atrioventricular block
CI, confidence interval
CL, chemiluminescence; emission of light by phagocytizing cells
CNS, central nervous system
CRF (CRH), corticotrophin releasing factor (hormone)
CRP, C-reactive protein; an acute-phase protein
DFA, detrended fluctuation analysis (of HRV)
DFA1, scaling exponent $\alpha 1$
DFA2, scaling exponent $\alpha 2$
ECG, electrocardiogram
FFT, fast Fourier transformation
fMLP, n-formyl-methionine-leucyl-phenylalanine
GH, growth hormone
HF-HRV, high frequency (HF) component of HRV; frequency ranges > 0.15 Hz.
HPA, hypothalamic-pituitary-adrenal (axis)
HR, heart rate
HRV, heart rate variability; variation in beat-to-beat intervals
IL, interleukin
IM, intramuscularly
IV, intravenously
LC, locus coeruleus; a dense concentration of noradrenergic cell bodies in the pons
LF-HRV, low-frequency (LF) component of HRV; frequency ranges 0.04-0.15 Hz.
LF:HF ratio, ratio between low-frequency (LF) and high-frequency (HF) -HRV
MAC, minimum alveolar concentration
MAP, mean arterial blood pressure
MED, medetomidine (0.02 mg kg^{-1}) and butorphanol tartrate (0.2 mg kg^{-1}) (IM)
MPO, myeloperoxidase
NADPH, nicotinamide adenine dinucleotide phosphate
NCount , blood neutrophil count
NSAID, nonsteroidal anti-inflammatory drug
OH, ovariohysterectomy
PD, pain / distress
PMA, phorbol myristate acetate
PMN, polymorphonuclear neutrophil; neutrophil
pNN50(%), (the percentage of) differences between successive RR intervals greater than 50 milliseconds
POMC, pro-opiomelanocortin
r, correlation coefficient (Pearson's correlation coefficient); *r-24h*, correlations calculated using values gathered during the entire study period; *r-Indiv*, correlations calculated at individual time points; *r-Time*, correlations calculated between individual time points

RLU, relative light unit
RMSSD, the square root of the mean squared differences of successive normal-to-normal RR intervals
ROS, reactive oxygen species
RR interval, the interval between R-wave peaks of normal QRS complexes
RSA, respiratory sinus arrhythmia
SA, sinoatrial
SD, standard deviation
SDNN, standard deviation of all normal to normal RR-intervals
SNS, sympathetic nervous system
ULF, ultra low frequency (-HRV)
VAS, visual analog scale
VLF, very low frequency (-HRV)
WhbCL, whole blood chemiluminescence
VPC, ventricular premature complex

2. SUMMARY

The series of investigations presented in this thesis examined different aspects of the manifestation of perioperative stress in client-owned dogs and compared the influences of two preanaesthetic medications. Data were obtained from 43 overtly healthy dogs that underwent ovariohysterectomy following preanaesthetic administration of medetomidine and butorphanol (MED) or acepromazine and butorphanol (ACE) (Study 1) and from 96 dogs that were recovering from day-case soft-tissue operations at home (Study 2; data obtained using owner-completed questionnaires).

The results indicated better abilities to attenuate perioperative neurohumoral arousal with MED preanaesthetic administration, but no significant differences were detected for the two treatment groups in the incidence of VPCs or in the inflammatory responses. The different physiological states were especially apparent during the early postoperative recovery, but were not reflected in the simultaneous behavioural observations. Overall, the correlations among the different hormonal measures and among plasma catecholamines and heart rates were weak, but the time-related associations increased in their strength during the early postoperative phase. The indices of efferent cardiac vagal activity showed consistent correlations with the heart rate data and the nonlinear measures of HRV seemed to complement the evaluation of beat-to-beat interval behaviour.

Among hospitalised dogs, signs indicative of preoperative emotional arousal were frequently encountered, but marked differences existed between individual animals with respect to the level of overall activity. Animal's preoperative physiological or behavioural states provided only minor indications of its characteristics at later time points of observation. As reported by animal owners, various aspects of behaviour were altered during the period of postsurgical recovery and the behavioural symptoms were influenced by the type of operation and owner-rated animal pain.

Altogether, the results underline the multi-faceted nature of perioperative stress responses in dogs and the value of using multiple observations for more complete evaluations. The clinical significance of the different effects on perioperative neurohumoral arousal with medetomidine and acepromazine preanaesthetic administration still needs to be determined and better indices to evaluate the characteristics of preoperative arousal are required. Owner-completed observations seem valuable in the assessment of the characteristics of later postsurgical recovery and could be used in future investigations when defining clinically-relevant milestones for postoperative recovery in client-owned dogs.

3. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications (investigations), referred to in the text by their Roman numerals (I-V).

I Väisänen M, Raekallio M, Kuusela E, Huttunen P, Leppäluoto J, Kirves P, Vainio O. Evaluation of the perioperative stress response in dogs administered medetomidine or acepromazine as part of the preanesthetic medication. Am J Vet Res 2002; 63: 969-75.

II Väisänen A-M, Vainio OM, Raekallio MR, Hietanen H, Huikuri HV. Results of 24-hour ambulatory electrocardiography in dogs undergoing ovariohysterectomy following premedication with medetomidine or acepromazine. J Am Vet Med Assoc 2005; 226: 738-45.

III Väisänen M, Lilius E-M, Mustonen L, Raekallio M, Kuusela E, Koivisto M, Vainio O. Effects of ovariohysterectomy on canine blood neutrophil respiratory burst: a chemiluminescence study. Vet Surg 2004; 33: 551-6.

IV Väisänen M A-M, Valros AE, Hakaoja E, Raekallio MR, Vainio OM. Preoperative stress in dogs - a preliminary investigation of behaviour and heart rate variability in healthy hospitalised dogs. Vet Anaesth Analg 2005; 32: 158-67.

V Väisänen M, Oksanen H, Vainio O. Postoperative signs in 96 dogs undergoing soft-tissue surgery. Vet Rec 2004; 155: 729-33.

4. INTRODUCTION

Perioperative stress refers to the alterations induced in the emotional states and in physiological activities of surgical patients. While partly representing perturbations induced to ensure survival, the perioperative stress responses can also adversely affect the host. Detailed knowledge of patients' perioperative statuses is of importance when evaluating the different interventions and when aiming at optimum patient care.

In dogs, the traditional approaches to documentation of surgery-related stress responses have involved intermittent determination of plasma hormone concentrations and heart rates, and analysis of postoperative behaviour of hospitalised patients. For instance, changes in the immunological functions and in beat-to-beat interval characteristics have seldom been used to characterise the nature of perioperative perturbations in physiology, and animals' emotional and behavioural states during the period of preoperative hospitalisation and in the later postsurgical phase have not been evaluated in detail. The effects of different preanaesthetic medications, administered to provide preoperative sedation and anxiolysis, have also seldom been compared. Medetomidine and acepromazine represent sedative and tranquilizing agents commonly administered to dogs prior to anaesthetic induction and possess different pharmacological features. Their use in surgical patients may result in differing stress-related characteristics.

The present thesis is aimed at addressing the aforementioned issues and expanding our knowledge on perioperative stress in client-owned dogs.

5. REVIEW OF THE LITERATURE

5.1. The Essence of Stress

The essence of stress was probably first introduced in the 19th century when Claude Bernard expressed his views on the existence of complex bodily activities that contributed to sustaining the stability of the internal environment. He defined the consistency of this "milieu interieur" as an essential part of "free and independent existence" (Bernard 1878). In the next century, the term "homeostasis" was introduced by Cannon (1935) to describe the steady states existing in the body and the coordinated physiological activities that control them. The concept of stress was further explained by other authors (Ewbank 1973, Selye 1973), as the terms "stressor" and "stress response" were introduced to characterise the stimuli capable of affecting the bodily equilibrium and the perturbations thus induced, respectively.

The physiological responses to threatening incidents were documented by Cannon (1914, 1928) as he examined the effects of physical and psychological influences on bodily functions. Cannon's work led to the discovery of the role of enhanced sympathetic nervous system (SNS) activities in contributing to the body's response when encountering adverse events. Based on his observations, a "flight or fight" response was described that was characterised by increased heart rates, blood pressure, secretion of catecholamines, and behavioural alterations, all of which enabled the organism to meet the demands of various challenges (Cannon 1935). The involvement of adrenocortical activities in the responses to physical influences were documented by Hans Selye (1936, 1973) as he reported secretion of adrenal corticosteroids and formation of gastric ulcers in individuals subjected to adverse circumstances.

Selye's writings emphasised the non-specific nature of stress responses, and various types of physical or psychological stimuli were considered to result in similar perturbations in physiological activities (Selye 1946). Mason (1971), however, pointed out that different types of influence could induce different physiological changes and that the psychological aspects of arousal played an important role in contributing to the quality of the response noted. Weiss (1972) conducted studies in rats that further documented the importance of perceiving the stressor as a determinator for the physiological characteristics of arousal.

Detailed knowledge currently exists on the qualitative and quantitative characteristics of stress-related responses, and the psychological components of bodily functions are viewed as important contributors to the overall physiological states (**Table 1**). The limbic system and amygdala are brain areas playing a role in emotional arousal, and have been shown to connect with various other brain regions influencing bodily activities, such as hypothalamus, brain stem and cortical areas (Sullivan *et al.* 1999). In addition to SNS-related arousal, alterations in efferent vagal activities have also been emphasised as potential markers of stress-related responses (Porges 2001) and the medullary sites of vagal projections receive innervation from higher brain areas, such as from amygdala (Henry 2002). Besides resulting in vagal withdrawal, as represented in depressed beat-to-beat interval variability (see also *Heart Rate Variability*), physical and psychological stimuli have also been described to involve enhanced vagal outflow, syncope and bradycardia (van Lieshout *et al.* 1991, Kinsella & Tuckey 2001). Higher brain activities are involved in the regulation of glucocorticoid secretion from the adrenal cortex through the synthesis and release of corticotrophin releasing factor (or hormone; CRF, CRH) from the hypothalamus and adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland (Kemppainen & Behrend 1997). This pathway is commonly referred to as the HPA axis (hypothalamic-pituitary-adrenal axis). Along with ACTH, endogenous opioids, such as beta-endorphin and beta-lipotropin, may also be released from the pituitary in response to threatening stimuli. These substances are derived from the same precursor molecule, i.e. pro-opiomelanocortin (POMC) (Guillemin *et al.* 1977). Negative feedback loops exist between the circulating glucocorticoid levels and the secretion of ACTH from the pituitary, and the HPA axis activities may also influence catecholamine synthesis in the adrenal medulla (Axelrod & Reisine 1984).

Various alterations with psychological and physical influences have been documented for immunological activities. Kiecolt-Glaser *et al.* (1995) demonstrated that women caring for demented relatives displayed slowed healing times for experimentally induced skin incisions, as compared to a group of age-matched individuals, and altered splenic lymphocyte mitogen responses were also documented in mice subjected to chronic auditory stimuli (Monjan & Collector 1977). The stress-related hormones are viewed as contributors to immunological perturbations and, for instance, lymphocytes and neutrophils possess adrenergic and opioid receptors (Khansari *et al.* 1990).

Despite the detailed knowledge existing on the characteristics of various stress-related states, controversies are still met in the scientific literature with respect to the proper description of terms used in stress-related research. The potentials of the altered bodily activities to negatively affect the individual have also not been clearly

defined. Moberg (1987) has described a model in which he suggests the development of a prepathological state due to altered biological functions that can further result in pathology in an individual responding to various stimuli. Broom and Johnson, in their book (2000), associate the potentials for reduced welfare with attempts to cope with psychological or physical influences, and further define the occurrence of "a state of stress" as a product of failed adaptation. However, the need for adaptive mechanisms to exist has also been recognised and, according to some authors, perturbations induced in bodily homeostasis (i.e., "moderate stress") may even be necessary to "optimize vigilance" (Wiepkema & Koolhaas 1993). In attempts to clarify the positive or negative effects that stress responses may elicit in an individual, some authors have suggested the utilisation of terms such as understress or overstress to describe the positive or potentially negative influences, respectively (Ewbank 1973). However, the confusion implicit in such terminology has also been emphasised by other authors (Broom & Johnson 2000), and some have even proposed the removal of the word "stress" from scientific publications (Rushen 1986). In the Oxford English dictionary (Hornby 2000), "stressed" is described as "too anxious and tired to be able to relax", whereas "stress" is explained as "mental or physical pressure put on an individual or on an object". Broom and Johnson (2000) define stress as "an effect, so that it is quite clear that the organism is changed by some outside variable", whereas Moberg (2001) considers stress "as the biological response elicited when an individual perceives a threat to its homeostasis". The latter definition applies to the purpose and discussions of this thesis.

Table 1. Schematic representation of stress-related pathways in the body. Refs: Chappell *et al.* (1986), Porro & Carli (1988), Sullivan *et al*. (1999), Bear *et al*. (2000), Lariviere & Melzack (2000), Moberg (2001).

Brain
Noradrenergic, dopaminergic and serotoninergic tracts, CRF:
altered secretion of neurotransmitters and changes in the levels of their metabolites
Amygdala and limbic system, cortical association areas:
generation of emotions, conscious perception
effects on hypothalamic and brain stem activities
Hypothalamus:
changes in the secretion of regulating hormones into local circulation
effects on anterior pituitary hormone secretion
changes in the secretion of hormones into general circulation (via posterior pituitary; ADH (vasopressin) and oxytocin)
effects on sympathetic and vagal efferent outflow
effects on organ function and secretion of catecholamines from adrenal medulla
Pituitary gland:
secretion of hormones into general circulation (eg, endogenous opioids, ACTH)
effects on metabolic rate, sexual functions and corticosteroid secretion from adrenal cortex
Periphery
Reflex responses
Behavioural responses
Altered organ function, metabolic state and immune system:
Tachycardia (or bradycardia)
Increased blood pressure
Increased circulating concentrations of adrenaline and noradrenaline, cortisol and glucose
Altered metabolism
Neutrophilia, alterations in immune functions

5.2. Perioperative Stress

Perioperative stress arises from the effects of physical and psychological stimuli encountered by a patient undergoing anaesthesia and surgical intervention. The widespread alterations induced in physiological activities and in behavioural and emotional states have collectively been referred to as stress responses to surgery (Desborough 2000, Hall *et al.* 2001). A number of studies, mostly performed on humans, have investigated their qualitative characteristics, temporal occurrence and the effects of different anaesthetic interventions.

5.2.1. Overview of Perioperative Stress Responses

The effects of anaesthesia and tissue trauma on bodily activities have long been recognised. At the beginning of the 20th century, anaesthetic exposure was speculated to affect the immune system (Gaylord 1916) and in 1956, Lassen *et al.* reported a depression of bone marrow function in humans anaesthetised for long periods of time as part of the treatment of tetanus. As early as the 1930s, Cuthbertson (1932) documented metabolic changes associated with bodily injuries, and in the 1950s, hypertensive responses were reported in humans in relation to endotracheal intubation (King *et al.* 1951). The psychological aspects of perioperative stress, as reflected in an individual's emotional states, have been emphasised in human medicine since the middle of the 20th century (Corman *et al.* 1958, Moss & Duncan 1958).

The **neuroendocrine** responses following surgical interventions are characterised by changes in hormonal secretion and autonomic nervous system (ANS) activity (**Table 2)**. Afferent nerve impulses arriving from the site of physical trauma (Egdahl 1959) and hypothalamic activities (Hume & Egdahl 1959) have been demonstrated to be responsible for the neurohumoral perturbations, and during the past decades cytokines secreted by the immune cells have also been viewed as important contributors to surgery-related endocrine and metabolic changes (Sheeran & Hall 1997, Farrar & Hall 1998). The alterations in hormonal secretion and in the ANS further affect various target organs, among them the cardiovascular system, and together with the immunological changes they act as contributors to the metabolic alterations in traumatised patients.

Perioperative neurohumoral responses typically arise after the start of surgical intervention, but in humans increases in heart rates, blood pressure and plasma catecholamine and cortisol concentrations have also been recorded in relation to the induction of anaesthesia, before skin incision (Russell *et al.* 1981, Lehtinen *et al.* 1984,

Aho *et al.* 1991). Increases in circulating cortisol have been reported in humans during the preoperative phase (Bursten & Russ 1965), and Robertson *et al.* (2001) detected increases in plasma vasopressin concentrations in dogs after preanaesthetic administration of acepromazine, before the induction of anaesthesia.

In small animals (cats and dogs), short-term alterations in plasma cortisol, ACTH and catecholamine concentrations have been recorded for individuals undergoing general anaesthesia alone, but the changes observed have been of a lesser degree than those occurring when surgical stimuli have been applied (Smith *et al.* 1996, Benson *et al.* 1991, 2000). Hardie *et al*. (1997) and Kyles *et al.* (1998) recorded stable and lower plasma cortisol concentrations for dogs undergoing general anaesthesia with or without concurrent opioid administration, when compared with the values obtained from animals that were operated on and underwent ovariohysterectomy (OH). Fox *et al.* (1998) found no effects of general anaesthesia with halothane on plasma cortisol concentrations in dogs, but recorded short-term increases in circulating cortisol after anaesthesia and concurrent butorphanol administration. Increased circulating cortisol concentrations were also detected in dogs after a visit to the anaesthesia induction room (Fox *et al.* 1998).

To some extent, the magnitude of neurohumoral changes is related to the type of the operation (Chernow *et al.* 1987). In humans major surgery, such as cardiac (Tonnesen *et al.* 1987) or major orthopaedic (Hall *et al.* 2001) intervention, can result in increased plasma cortisol concentrations for up to 6 days. With minor operations, such as OH in dogs, plasma cortisol has returned to its preoperative level within 12 to 24 hours (Kyles *et al.* 1998, Benson *et al.* 2000, Lemke *et al.* 2002). Byrne *et al.* (2000) reported a duration of some 24 hours for plasma cortisol responses in dogs after experimental aortofemoral grafting. In humans, in addition to alterations in plasma hormonal concentrations, perioperative changes in the ANS activity and in cardiac characteristics have also been documented to persist for several days (Marsch *et al.* 1994, Laitio *et al.* 2000), as apparent in continuous electrocardiogram (ECG) recordings and in the analysis of heart rate variability (HRV). Laparoscopic surgeries that result in minor tissue trauma as compared with open approaches are generally not considered to greatly modify the neuroendocrine responses (Kehlet 1999).

Table 2. Perturbations in physiology, emotions and behaviour described for surgical patients and with accidental trauma. Data derived from the human literature. Refs: Corman *et al.* (1958), Christensen & Kehlet (1993), Liu *et al.* (1995), Rosenberg-Adamsen *et al.* (1996), Sheeran & Hall (1997), Farrar & Hall (1998), Desborough (2000), Kindler *et al.* (2000), Laitio *et al.* (2000), Johnson *et al.* (2002), Kehlet & Wilmore (2002).

Neuroendocrine	
	Increases in circulating concentrations of
	catecholamines
	ACTH
	cortisol
	ADH (vasopressin)
	GH
	aldosterone
	Decreases in circulating concentrations of insulin
Metabolic	
	Increased glucogenolysis, gluconeogenesis, hyperglycemia
	Increased lipolysis
	Protein loss (negative nitrogen balance)
	Altered water and electrolyte balance
Immunological /Inflammatory	
	Leukocytosis, changes in lymphocyte subpopulations
	Cytokine production (pro- and anti-inflammatory)
	Production of acute-phase proteins
Emotional /Behavioural	
	Preoperative fear and anxiety
	Postoperative fatigue, cognitive dysfunction, sleep disturbances
	Pain
Changes in organ function /Other phenomena	
	Increases in heart rates, blood pressure
	Altered beat-to-beat interval variability
	Pulmonary and gastrointestinal dysfunction
	Altered coagulation

Perioperative perturbations in the **immunological** status are reflected in alterations induced in circulating leukocyte numbers, in the plasma concentrations of inflammatory mediators, and in the production of acute-phase proteins (**Table 2**). Increased white cell counts are mainly due to neutrophilia, whereas the numbers of circulating lymphocytes and eosinophils generally decrease (Salo 1992). Alterations in plasma cytokine levels and leukocyte counts can occur within hours of the start of an operation (Chambrier *et al.* 1996, Byrne *et al.* 2000), whereas increased production of acute-phase proteins, such as C-reactive protein (CRP), is observed later (after 12 to 24 hours) (Kristiansson *et al.* 1999, Hall *et al.* 2001). The immunological changes can persist for several days, over 7 days after major operations (Hall *et al.* 2001), and are related to the extent of injury. Significant attenuation of increases in plasma contents of proinflammatory cytokines and CRP occurs with laparoscopic techniques (Vittimberga *et al.* 1998, Kristiansson *et al.* 1999). With respect to OH in dogs, increased blood neutrophil counts have been documented at 4 hours following the operation with peak leukocyte counts occurring on the first and second postoperative days (Schmidt & Booker 1982).

Besides the quantitative changes in immunological characteristics, anaesthesia and surgery also affect their qualitative properties. In humans, surgical interventions have lowered blood lymphocyte proliferative responses and natural killer cell activities and affected various neutrophil functions, including chemotaxis responses, bactericidal activities and oxygen radical generation (Tonnesen *et al.* 1987, Salo 1992, Sheeran & Hall 1997). Some surgeries, however, may also result in enhanced cellular functions, such as the neutrophil activation described after cardiac operations (Schwartz *et al.* 1998). Ben-Eliyahu *et al.* (1999) reported depression of natural killer cell activity in rats after anaesthetic exposure and this was exaggerated with hypothermia and attenuated by administration of a β-adrenoceptor antagonist. In dogs, depressed blood lymphocyte blastogenic responses have been documented after minor surgeries (Felsburg *et al.* 1986).

Although not all the contributors to the perioperative immunological perturbations are completely known, both the effects of tissue trauma and the neurohumoral responses are viewed as factors influencing it (Salo 1992). Anaesthetic agents are also thought to play a role, and various perianaesthetic medications have been demonstrated *in vitro* to affect cellular functions. Among such agents have been halothane, isoflurane, propofol and midazolam that have, for instance, attenuated the phagocytosis, chemotaxis and oxidative activities of blood neutrophils (Nakagawara *et al.* 1986, Mikawa *et al.* 1998, Nishina *et al.* 1998).

The **metabolic** effects of surgical interventions include alterations in protein, glucose and fat metabolism (**Table 2**). Protein breakdown, hyperglycaemia, lipolysis and changes in water and electrolyte balance are all features of metabolic changes induced by accidental or surgical trauma, and an increase also occurs in the overall metabolic rate (Weissman 1990, Hill & Hill 1998, Desborough 2000). Several neurohumoral and inflammatory mediators are viewed as contributors to the perioperative alterations in metabolic activities, with the main hormonal components being cortisol, catecholamines, insulin and growth hormone (GH). In addition to the effects of catecholamines on blood glucose levels, insulin secretion is reduced and peripheral resistance to its actions occurs (Halter & Pflug 1980ab). The effects of cortisol are viewed as the major contributor to tissue protein breakdown and the inflammatory mediators further modulate hepatic metabolism to favour the production of acute phase proteins (Biffl *et al.* 1996, Gabay & Kushner 1999). Enhanced SNS efferent activities and increased secretion of aldosterone and ADH influence water and electrolyte balance, resulting in sodium and water retention (Desborough 2000). For dogs undergoing ovariohysterectomy (Benson *et al.* 2000) and aortic reconstruction (Byrne *et al.* 2000), increased blood glucose levels have been documented, and with thoracotomies, these responses have been attenuated with minimally invasive approaches (Walsh *et al.* 1999).

In addition to the above-described physiological changes, anaesthesia and surgical interventions are accompanied by a variety of **emotional and behavioural** responses (**Table 2**). In humans, the emotional aspects of perioperative stress have been documented during the preoperative phase, with up to 70-80% of individuals reportedly experiencing fear or anxiety while waiting for the surgical intervention (Corman *et al.* 1958, Ramsay 1972). In a recent study by Kindler *et al.* (2000) the waiting period preceeding surgery was among the commonest worries indicated by humans about to undergo surgery. During the postsurgical recovery, feelings of malaise or fatigue are among the subjective changes induced and have been documented for up to 30% of adults after major abdominal operations (Christensen & Kehlet 1993). In humans, postoperative sleep disturbances (Rosenberg-Adamsen *et al.* 1996) and impairments in cognitive function (Moller *et al.* 1998, Johnson *et al.* 2002) have also been documented and have been observed in 19 to 50% of adults after major noncardiac interventions. The duration of changes has ranged from days to up to 3 months, and higher incidences have been detected in the elderly. In children, hospitalisation and surgical intervention have resulted in a variety of behavioural alterations, some of which have still existed 14 to 30 days after the patient's discharge from the hospital (Kain *et al.* 1996, 1999a, Kotiniemi *et al.* 1997). In a study by Kotiniemi *et al.* (1997), parents reported postsurgical behavioural changes for up to 80% of children recovering from day-case operations at home; these included

alterations in sleeping patterns, social behaviour and crying. Some of the changes were still documented in 20% of individuals after two weeks had elapsed from the operation.

In cats and dogs, the behavioural aspects of perioperative stress responses have been documented in several studies, as apparent in the scoring of postoperative pain in hospitalised animals. In these observations, postoperative behavioural characteristics in dogs have included alterations in locomotive activities, postures, vocalisation, and in interactive behaviours. Few studies have utilised detailed video analysis to explore animals' perioperative behaviour, and in the studies of Hardie *et al*. (1997) and Kyles *et al*. (1998), dogs that had undergone OH spent more time sleeping and showed diminished interactive behaviour when compared with individuals that had only been anaesthetised ± treated with opioids. Firth and Haldane (1999), however, could not demonstrate significant differences in the level of overall activity between dogs that had undergone OH (and general anaesthesia) and those that had only been anaesthetised. All the animals in this study received acepromazine for preanaesthetic medication. In rats, postoperative video recordings have revealed multiple behavioural changes following laparotomy that have been attenuated by analgesic administration (Roughan & Flecknell 2001) and based on video observations and comparisons with exposure to anaesthesia alone, Fox *et al*. (2000) concluded that 166 behaviours could be potential indicators for postoperative pain following OH in dogs. Most of these behaviours, however, occurred quite infrequently.

5.2.2. Effects of Anaesthetic Interventions

In addition to the effects related to the extent of an operation, several studies have explored the influences of different anaesthetic interventions on surgical stress responses. In humans, opioid anaesthetic regimens, regional anaesthetic techniques and perioperative medications aimed at attenuating sympathetic nervous system activities have been among the commonest approaches investigated; in dogs, studies have concentrated on evaluation of the effects of different analgesic regimens, such as nonsteroidal anti-inflammatory drug (NSAID) or opioid administration, on plasma cortisol concentrations, intermittent heart rates and postoperative behavioural pain / distress scores (**Table 3**).

In **adult humans** and **in infants /children**, opioid anaesthetic regimens have resulted in the attenuation of neurohumoral and metabolic responses with major cardiac or abdominal operations, as apparent in plasma catecholamine, cortisol, and glucose concentrations (Anand & Hickey 1992, Aho *et al.* 1992, Duncan *et al.* 2000).

Table 3. Features of selected publications documenting perioperative stress responses in dogs. (GA, general anaesthesia)

Author	Type of study	Number (and source) of animals	Interventions, investigations	Main findings
Church *et al.* (1994)	Randomized	5 (client-owned) 14 (random source)	GA + Orthopaedic operation (for client-owned dogs) GA ± Laparotomy or ± Thoracotomy (for random source dogs) GA = halothane (+nitrous oxide)	Cortisol: surgery > GA alone Cortisol: no significant differences between surgery groups
Stobie *et al.* (1995)	Randomized Blinded	18 (random source)	GA + Thoracotomy (lateral) GA = isoflurane Groups: postop. morphine (IM), or or bupivacaine or morphine (interpleural)	Pain scores: no sign. diff. between groups Cortisol: bupivacaine > morphine No correlations between pain scores and physiologic data
Hansen *et al.* (1997) Hardie *et al.* (1997)	Randomized Blinded Placebo-controlled	22 (client-owned 22 (random source)	GA alone (for random source dogs) GA + OH (for client-owned dogs) GA = isoflurane Groups: pre+postop. oxymorphone or saline	Pain-sedation scores, times spent sleeping : OH > GA alone Pain scores: no sign. diff. between OH+oxymorphone and OH+saline With OH+oxymorphone sleeping pattern more similar to GA alone HR: no sign. diff. between OH+oxymorphone and GA+oxymorphone Cortisol: OH > GA alone; OH+saline > OH+oxymorphone Evaluations based on video recordings of postop. behaviour
Lascelles *et al.* (1997)	Randomized Blinded Placebo-controlled	40 (client-owned)	GA + OH GA = halothane (+nitrous oxide) Premed. = acepromazine Groups: saline or pre- or postop. pethidine	Pain scores: saline > pethidine Pain scores (later recovery): postop. pethidine > preop. pethidine Best attenuation of allodynia and wound hyperalgesia with preop. pethidine
Fox *at al.* (1998)	Randomized	client-owned and purpose-bred n tot. = 44	GA alone (+/- butorphanol) GA + OH (+/- butorphanol) Butorphanol alone, Handling alone OH for client-owned dogs GA = halothane	Cortisol: greatest increases with OH (+/- butorphanol) Cortisol: OH - butorphanol > OH + (postop.) butorphanol Increases in cortisol also with butorphanol alone and with handling alone
Kyles *et al.* (1998)	Randomized Blinded	20 (client-owned) 10 (random source)	GA alone (for random source dogs) GA + OH (for client-owned dogs) GA = isoflurane Groups: OH: oxymorphone or transderm. fentanyl; GA: transderm fentanyl (all dogs)	Pain-sedation scores, times spent sleeping: OH (+fentanyl) > GA (+fentanyl) HR: no sign. diff. between OH (+fentanyl) and GA (+fentanyl), or between OH (+fentanyl) and OH (+oxymorphone) Cortisol: OH (+fentanyl) > GA (+fentanyl) alone Cortisol: no sign. diff. between OH (+fentanyl) and OH (+oxymorphone) Evaluations based on video recordings of postop. behaviour
Firth & Haldane (1999)	Randomized Blinded Placebo-controlled	48 (client-owned)	GA + radiographs (n = 12) GA + OH (n = 36) GA = halothane Premed. = acepromazine (+/- butorphanol) Groups: postop. butorphanol or carprofen or no analgesics (surgery dogs only)	Activity scores: no signicant differences between groups Pain scores: all OH dogs > GA alone Evaluations based on video recordings of postop. behaviour

Table 3. *(continued)*

Author	Type of study	Number (and source) of animals	Interventions, investigations	Main findings
Grisneaux *et al.* (1999)	Randomized Blinded Placebo-controlled	93 (client-owned) 15 (other dogs)	GA + radiographs (for other dogs) GA + hind-leg orthop. operation (for client-owned dogs) GA = isoflurane Premed. = acepromazine Groups: preop. ketoprofen or carpro-fen or saline (oper. dogs only)	Analgesic requirements: placebo or carprofen > ketoprofen Pain scores: no sign. differences between ketoprofen and carprofen Cortisol: surgery > GA alone Cortisol: no sign. diff. between carprofen and ketoprofen
Walsh *et al.* (1999)	Blinded	14 (random source)	GA + Thoracoscopic pericardectomy GA + Open thoracotomy and pericardectomy GA = halothane Premed. = acepromazine (+ morphine) Postop. morphine for all dogs	Pain scores: Open > Thoracoscopic Cortisol: Open > Thoracoscopic Glucose: Open > Thoracoscopic Evaluations based on video recordings of postop. behaviour
Benson *et al.* (2000)	Randomized Placebo-controlled	12 (purpose-bred)	GA and GA+OH for each dog GA = isoflurane Groups: premed. with saline or medetomidine	Cortisol , ACTH, adrenaline, noradrenaline, and insulin: saline > medetomidine Glucose: only increased with OH, no significant differences between saline and medetomidine
Ko *et al.* (2000)	Randomized Blinded Placebo-controlled	12 (Walker-type hound dogs)	GA + OH GA = halothane Groups: premed. with saline or medetomidine	Pain scores: saline > medetomidine HR: saline > medetomidine Cortisol: saline > medetomidine MAP (noninvasive): more stable with medetomidine
Lemke *et al.* (2002)	Randomized Blinded Placebo-controlled	22 (client-owned)	GA + OH GA = isoflurane Premed. = acepromazine (+butorphanol) Groups: preop. ketoprofen or saline. Postop. butorphanol for all dogs	Activity levels: ketoprofen > saline (at 4 and 6 hours postop.) Pain scores: no sign. diff. between groups HR: no sign. diff. between groups Cortisol: no sign. diff. between groups Glucose: no sign. diff. between groups Evaluations based on video recordings of postop. behaviour
Carpenter *et al.* (2004)	Randomized Blinded Placebo-controlled	30 dogs (client-owned and random source)	GA + OH GA = isoflurane Premed. = acepromazine (+ butorphanol) Groups: intraperitoneal and incisional lidocaine or bupivacaine or saline	Pain scores (VAS): saline > bupivacaine
Wenger *et al.* (2005)	Randomized Blinded Placebo-controlled	20 dogs	GA + front-leg orthopaedic operation GA = isoflurane Premed. = acepromazine Groups: brachial plexus block (lidocaine-bupivacaine or saline)	Greater amounts of intra- and postop opioids for saline group

Intravenous opioid administration has also modulated the cardiovascular responses associated with tracheal intubation (Aho *et al.* 1991), and epidurally administered morphine has attenuated the increases in plasma catecholamines and blood pressures in humans undergoing major vascular surgeries (Breslow *et al.* 1989).

In humans, regional anaesthetic techniques, i.e. epidural or intrathecal administration of local anaesthetic agents (with or without concomitant opioids), have suppressed several aspects of the metabolic-neuroendocrine responses, including cortisol, catecholamine, and hyperglycaemic responses, and also modulated postoperative protein catabolism (Liu *et al.* 1995, Kehlet 2000). The effects on perioperative stress responses have been most prominent with lower abdominal or orthopaedic surgeries, whereas with higher abdominal or thoracic operations similar characteristics have not always been observed. Significant effects of regional anaesthetic techniques have been found in comparison with general anaesthesia and parenteral opioid administration, and also with respect to patient statuses when using epidural opioids without local anaesthetics. The regional anaesthetic techniques have not been reported to affect the incidence of postoperative cognitive dysfunction in humans (Kehlet & Holte 2001), but an incidental finding in a study by Johnson *et al.* (2002) was a positive association between the use of epidural anaesthesia and the postoperative occurrence of cognitive impairment (odds ratio 2.47; 95% CI 1.43-4.27). In humans undergoing thoracic surgery, combined epidural (thoracic) administration of bupivacaine and fentanyl was reported to result in greater preservation of beat-to-beat interval variability, when compared with intraoperative fentanyl and patient-controlled postoperative administration of morphine (Licker *et al.* 2003).

In humans undergoing cardiac, abdominal or vascular operations, preoperative administration of an α_2 adrenergic agent, such as clonidine or dexmedetomidine, has provided cardiovascular stability and resulted in lower plasma catecholamine, cortisol and beta-endorphin concentrations (Flacke *et al.* 1987, Aho *et al.* 1992, Talke *et al.* 2000). The effects of α_2 adrenergic agents have been apparent when compared with preoperative opioid or saline administration, although Aho *et al.* (1992) observed similar intraoperative cortisol concentrations for patients treated with opioids or dexmedetomidine. Beta-adrenergic blockade, as achieved with atenolol, has been reported to reduce the incidence of postoperative myocardial ischemia and mortality in high-risk human patients (Wallace *et al.* 1998). Loick *et al.* (1999) reported that in humans undergoing cardiac operations, high thoracic epidural anaesthesia better attenuated the release of cardiac troponin (an indicator of myocardial damage) and increases in plasma adrenaline, when compared with IV clonidine. In humans,

postoperative infusion of dexmedetomidine was found to result in reduced plasma interleukin (IL) 6 levels, when compared with propofol (Venn *et al.* 2001).

In adults, the choice of general anaesthetic alone has not been confirmed to affect the magnitude of perioperative stress responses (Crozier *et al.* 1992), but in children, the postoperative behavioural symptoms were found to be affected by the choice of preanaesthetic medication (Kain *et al.* 1999b).

In studies on **dogs**, preoperative administration of oxymorphone, as compared with placebo, attenuated the increases in plasma cortisol in association with OH (Hansen *et al.* 1997) and similar effects have been reported for medetomidine (Benson *et al.* 2000, Ko *et al.* 2000). Ängeby *et al*. (2001) studied dogs undergoing OH as part of the treatment of metritis / pyometra and reported lower rate-pressure products (heart rate x systolic arterial blood pressure) for premedication with medetomidine (0.008 mg kg^{-1}, IM) and buprenorphine (0.012 mg kg^{-1}, IM), when compared with acepromazine (0.05 mg kg^{-1}, IM) and buprenorphine, or saline. In laboratory Beagles, postoperative administration of medetomidine (0.02 mg kg^{-1}, IM) resulted in lower concentrations of plasma adrenaline and noradrenaline during a 6-hour period after thoracotomy, when compared with buprenorphine (Vainio & Ojala 1994). Interpleural or IM administration of morphine was found to attenuate the increases in serum cortisol after thoracotomy in dogs, compared with interpleural bupivacaine (Stobie *et al.* 1995). Torske *et al.* (1998) reported lower analgesic requirements for dogs recovering from orthopaedic operations with postoperative administration of epidural oxymorphone and bupivacaine, compared with (epidural) bupivacaine or (IV) oxymorphone alone. In dogs undergoing OH or limb amputation, IV administration of ketamine (± fentanyl) resulted in lower postoperative pain scores and degrees of wound hyperalgesia (Slingsby & Waterman-Pearson 2000, Wagner *et al.* 2002a) as well as in increased levels of overall activity, as evaluated by the animal owners at home (Wagner *et al.* 2002a).

In cats, postoperative administration of intramuscular morphine (0.1 mg kg^{-1}) and xylazine (1.0 mg kg^{-1}) have decreased the circulating catecholamine concentrations after onychectomy, and the most profound effects were achieved with the α_2 agonist regimen (Benson *et al.* 1991). In cats, plasma cortisol levels have also been decreased after postoperative butorphanol administration (Smith *et al.* 1996).

5.2.3. Perioperative Stress Responses and Postoperative Outcome

The relationships between perioperative stress responses and postsurgical outcome have been of interest among physicians for several decades. In the 1980s, positive associations were reported for intraoperative plasma catecholamine concentrations and postoperative organ dysfunction in humans undergoing major vascular operations (Roizen *et al.* 1987), and in infants, attenuation of perioperative neurohumoral arousal by opioid administration was found to result in improved recovery and survival (Anand & Hickey 1992).

Potentials to augment postsurgical recovery by attenuation of sympathetic nervous system activities have been discussed in the human anaesthesia literature, with emphasis on positive effects in high-risk patients (Roizen 1988). The true benefits and potential harms of such interventions, however, have also been emphasised (Howell *et al.* 2001). In 1991 Kehlet advocated "the concept of stress free anaesthesia and surgery" as "an important instrument" in improving "surgical outcome", but perioperative cortisol responses are also considered essential for survival (Salem *et al.* 1994). Recent analyses on randomized studies examining the effects of regional anaesthetic techniques on postoperative morbidity and mortality in humans (Rodgers *et al.* 2000, Liu *et al.* 2004) concluded that there were positive effects on pulmonary, cardiac and tromboembolic complications and overall mortality. In humans, the epidural analgesic techniques are viewed as the most effective methods for providing postoperative pain relief after major procedures (Kehlet & Holte 2001).

Significant associations have been described for intraoperative tachycardia or hypertension and postsurgical morbidity and mortality in humans (Reich *et al.* 2002), and a study on rats found that perioperative administration of morphine would attenuate increases in plasma cortisol, incidence of postsurgical behavioural symptoms and the spread of metastasis (Page *et al.* 1998).

The current view on the role of perioperative stress responses in contributing to postsurgical outcome emphasises the multifactorial nature of the recovery process and underlines the importance of controlling several factors when aiming at an optimum end result (Kehlet & Wilmore 2002). In addition to providing pain relief, preventing hypothermia, and ensuring adequate nutrition, the attenuation of surgical stress, in general, is among the items mentioned. The potential for improved control over perioperative events has also been postulated to be achieved by preoperative patient education and by controlling overall emotional states (Kehlet & Wilmore 2002). In their editorial, Carli and Mayo (2001) further emphasize the need for the development of validated outcome measures for humans to assess the

qualities of postsurgical recovery, and Wu and Fleisher (2000) imply the need for qualitative studies in future assessments of outcomes in regional anaesthesia. In humans, the role of preoperative patient assessments, as directed towards pain responses (Granot *et al.* 2003, Werner *et al.* 2004), levels of fatigue (Hall & Salmon 2002), presence of anxiety (Caumo *et al.* 2002) or genotypic characteristics (Iohom *et al.* 2004), have also been implicated as potential future tools when aiming at improved perioperative care.

5.3. Heart Rate Variability (HRV)

5.3.1. Physiological Background to HRV

Heart rate variability (HRV) refers to the oscillations existing in the heart period data that arise due to complex physiological processes. As early as in the 19th century, Ludwig (1847) described respiratory-related fluctuations in the cardiovascular variables, and with the improvements in analysis techniques, the heart period data have further been documented to undergo a series of oscillations.

High frequency (HF) fluctuations peak at respiratory frequencies and correspond to respiratory sinus arrhythmia (RSA; Task Force 1996). In humans and in dogs, at least 2 other periodic cycles have also been identified that display periodicities of less than one minute and have their frequencies centred around 0.12 and 0.04 Hz (low frequency (LF) and very low frequency (VLF) HRV, respectively) (Axelrod 1981, Task Force 1996). In 24-hour ambulatory electrocardiogram (aECG) recordings, fluctuations at frequencies of less than 0.003 Hz have also been described (ultra low frequency; ULF HRV) (Task Force 1996). In addition to heart rate data, the LF and HF oscillations have been found in the spectra of systemic arterial blood pressure variability and of sympathetic and vagal efferent nerve discharges (Lombardi *et al.* 1990, Malliani *et al.* 1991).

The periodic changes in beat-to-beat interval characteristics are thought to be induced by homeostatic mechanisms operating within the cardiovascular system aimed at maintaining optimum circulation in the face of various physiological perturbations. The long term fluctuations are thought to represent changes in the cardiac cycle induced due to alterations in vasomotor tone (Saul 1990) and in association with thermoregulation or with renin-angiotensin system activity (Axelrod 1981). The baroreceptor-mediated control of blood pressure affects the HRV at frequencies of around 0.1 Hz (Pagani *et al.* 1986). The genesis of the respiratory-related beat-to-beat interval changes has been explained as a reflex response of cardiac activities to the alterations induced in intrathoracic pressure, venous return

and lung volumes due to the physical effects associated with breathing (Moise 1998, Stauss 2003). In addition, the interplay between the respiratory and cardiovascular centres directly at the central nervous system (CNS) level has also been implicated (Task Force 1996, Moise 1998), and a respiratory pattern has been documented in cardiac vagal motoneurons in the nucleus ambiguus (Rentero *et al.* 2002). Central command, such as that affected by emotions, has been cited as a general contributor to the beat-to-beat interval characteristics and can manifest its effects with various frequency ranges (Saul 1990).

Interactions between sympathetic and parasympathetic efferent activities, circulatory neurohormones, and pacemaker properties determine the instantaneous heart rate. The influences of efferent sympathetic and vagal activities are transferred to cardiac function through the secretion of acetylcholine and norepinephrine from vagal and sympathetic nerve terminals, respectively. In studies using pharmacological interventions and direct nerve stimulation, the two branches of the ANS have been demonstrated to possess different abilities to modulate the interbeat interval characteristics (Parati *et al.* 1995). In humans (Pomeranz *et al.* 1985) and in dogs (Axelrod 1981, Rishniw *et al.* 1999), parasympathetic blockade using atropine or glycopyrrolate results in decreased HRV and nearly abolishes fluctuations occurring within the high frequency ranges (> 0.15 Hz). The same is true if the vagal nerve is severed (Chess *et al.* 1975).

In experimental studies on humans and on dogs, the physiological events related to increased sympathetic activities, such as those occurring with exercise (Rimoldi 1990a), postural changes (Pagani *et al.* 1986) or hypotension (Rimoldi *et al.* 1990b), have been found to increase the low-frequency component of HRV (frequency ranges between 0.04-0.15 Hz). Administration of atenolol, a beta-adrenergic blocker, attenuates the increases in LF oscillations during exercise (Rimoldi *et al.* 1990a) and bilateral stellectomy that results in cardiac sympathetic denervation produces similar effects during hypotension (Pagani *et al.* 1986). The combined administration of propranolol and a parasympatholytic agent (glycopyrrolate) nearly abolishes all frequency-specific heart rate fluctuations in dogs (Axelrod 1981).

The differences in heart rate behaviour in association with vagal or sympathetic influences have also been documented at the sinoatrial (SA) level. The atrial rate response to manipulations of efferent cardiac vagal activities has been found to be rapid, whereas effects of sympathetic nerve stimulation are associated with a time delay (Berger *et al.* 1989).

The above-described results have been taken to implicate the different effects of either vagal or sympathetic influences on HRV, and cardiac efferent vagal activity is generally considered the main mediator initiating short-term variations in the interbeat intervals, such as those occurring between successive beats or at high frequency ranges (Task Force 1996, Calvert 1998). Sympathetic modulation is mainly manifested at lower frequencies. The ratio between the LF and HF oscillations (the LF:HF ratio) has been implicated to characterise the balance between sympathetic and parasympathetic activities, with the increased values representing the presence of sympathetic dominance due either to enhanced sympathetic modulation or decreases in cardiac efferent vagal influences, or both (Pagani *et al.* 1986, Task Force 1996). This view is based on the existence of reciprocal changes in efferent autonomic nervous system activities, which do not, however, always occur in physiological systems (Eckberg 1997, Kinsella & Tuckey 2001). The frequency centres for the sympathetic and parasympathetic cardiac influences can vary, and the rate and depth of respiration also affects HRV (Parati *et al.* 1995). In conditions where the heart rate varies, the use of normalized units of spectral indexes of HRV or the LF:HF ratios have been stated as preferable methods for the estimation of the effects of autonomic nervous system influences on the sinoatrial node (Tulppo *et al.* 2005).

5.3.2. Clinical Implications of HRV

Over the past few decades, the measurement of heart rate variability has been used to explore cardiac autonomic regulation and overall perturbations in the ANS. The clinical relevance of HRV was appreciated as early as in the 1960s in humans in the assessment of foetal cardiac characteristics (Hon & Lee 1965), where a disruption of normal heart period behaviour was found to indicate the need for emergency intervention. In the late 1970s, Wolf *et al.* (1978) reported associations in human cardiac patients between depressed HRV and mortality after myocardial infarction, and several subsequent investigations have demonstrated a relationship between altered HRV and the risk of postinfarction mortality or acute death in human cardiac patients (Lombardi *et al.* 2001).

In humans, HRV measurements have been used as alternative tests in neuropathic states, such as those related to diabetes mellitus (Ewing *et al.* 1991), and in relation to brain injury, depressions in heart period fluctuations have reflected the clinical course of events (Rapenne *et al.* 2000). Studies on human surgical patients have recorded perturbations in HRV in relation to anaesthesia and surgical stimuli that have involved overall depression of LF and HF oscillatory components and alterations in adjacent beat-to-beat intervals, as measured using the time domain indexes (see below, *Methods used in Measurements*) (Kato *et al.* 1992, Marsch *et al.* 1994,

Amar *et al.* 1998, Laitio *et al.* 2000). In association with surgical stimuli, increases in the LF:HF ratios have also been reported for humans (Schubert *et al.* 1997) and in 1965, McGrady *et al.* documented decreased RSA for anesthetized dogs. Depressed HRV in dogs has further been reported with isoflurane anaesthesia (Picker *et al.* 2001) and with abdominal surgery (Shafford & Dodam 2003).

In stress-related research, a decreased HRV has been found in association with various physical or psychological stimuli (humans: Lindh *et al.* 1999, rats: Sgoifo *et al.* 1999, dogs: Pagani *et al.* 1991), but some investigators have also detected increased values, such as those observed in rodents during restraint (Sgoifo *et al.* 1997). Friedman and Thayer (1998) have proposed a low HRV to reflect overall personality characteristics, including shyness and decreased flexibility.

5.3.3. Methods Used in Measurement

Several methods can be used to describe the qualitative and quantitative aspects of HRV. Traditional techniques involve mathematical estimations of interbeat interval behaviour, as apparent in so-called time domain analysis, and frequency domain methods that quantify the spectral components existing in the heart period data, such as the LF and HF oscillations described above. Over the past few years, new analysis techniques have also evolved that explore the qualitative properties of heart period fluctuations and assess its characteristics based on nonlinear dynamics. These measures have shown promise as indicators of adverse events in the human cardiac population (Mäkikallio *et al.* 2002) and have also predicted the incidence of postoperative myocardial ischemia (Laitio *et al.* 2004) and hospital stay (Laitio *et al.* 2000).

Time domain analysis is possibly the simplest method to assess HRV (Kleiger *et al.* 1992, Task Force 1996, Calvert 1998) and provides descriptive statistics about the interbeat interval behaviour within selected periods of time. Among the common indices calculated are the standard deviation of all normal to normal RR-intervals (SDNN; N refers to the R-wave peak of normal QRS complexes), the square root of the mean squared differences of successive normal-to-normal RR intervals (RMSSD), and the percentage of differences between successive RR intervals greater than 50 milliseconds (pNN50%). The latter two characterise alterations between adjacent beats and short-term heart rate regulation, and are thus considered to mainly reflect cardiac vagal modulation; the SDNN provides an overall estimate of HRV.

Power spectral analysis was introduced in the late 20th century to qualify and quantify the frequency-specific properties of HRV (Chess *et al.* 1975). Unlike the time domain measures, the spectral analysis method provides information about the frequency-related nature of heart rate oscillations and about the distribution of power (variance) among the spectral components of HRV (**Fig. 1**). Various algorithms can be used to compute the spectral properties of RR interval data, among them autoregressive (AR) modelling and fast Fourier transformation (FFT; Parati *et al.* 1995). The latter technique requires prior selection of the number and frequency ranges of the bands of interest, whereas AR modelling automatically provides the number, centre frequencies and the associated power of oscillatory components existing in the RR interval series data. Spectral powers can be presented using absolute (ms^2) or in normalised units (nu), the latter representing the relative value of each power component as a proportion of the variability existing at frequencies higher than 0.04 Hz (Task Force 1996).

The recommended frequencies to be used in the spectral analysis of human HRV (short-term recordings) are VLF, LF and HF (frequency ranges of <0.04 Hz, 0.04-0.15 Hz and 0.15-0.40 Hz, respectively) (Task Force 1996) and in studies done on dogs, frequency ranges of 0.01 to 0.15 and 0.10 to 1.0 (Hz) have been used to characterise the LF and HF bands, respectively (Palazzolo *et al.* 1998, Rishniw *et al.* 1999, Fuji & Wakao 2003). Matsunaga *et al.* (2001) reported the peaks of the HF fluctuations centered between 0.1 and 0.4 Hz in 24-hour ambulatory ECGs obtained from laboratory Beagles.

Among the newer techniques used to assess the qualitative properties of heart period fluctuations are estimations of entropy and Poincaré plot analysis (Pincus & Goldberger 1994, Huikuri *et al.* 2003). **Poincaré plot analysis** explores the shape and dimensions of a scattergram derived from plotting the RR-interval data as a function of successive beats. Visual inspection can be used to assess the shape of this figure, but its dimensions can also be mathematically explored (Tulppo *et al.* 1996). In dogs, the graphical display of Poincaré plots typically reveals a fan-shaped pattern due to the presence of marked respiratory sinus arrhythmia (Calvert 1998, Moise 1998).

Approximate entropy (Apent) and Kolmogorow entropy K represent HRV analysis techniques that assess the complexity of time series heart rate data (Huikuri *et al.* 2003). Higher values are representative of the presence of greater randomness, whereas lower values indicate more regular heart beat behaviour. In dogs, changes in the entropy measures of HRV have been correlated with changes in time domain (SDNN) and frequency domain (HF-HRV) indices (Palazzolo *et al.* 1998).

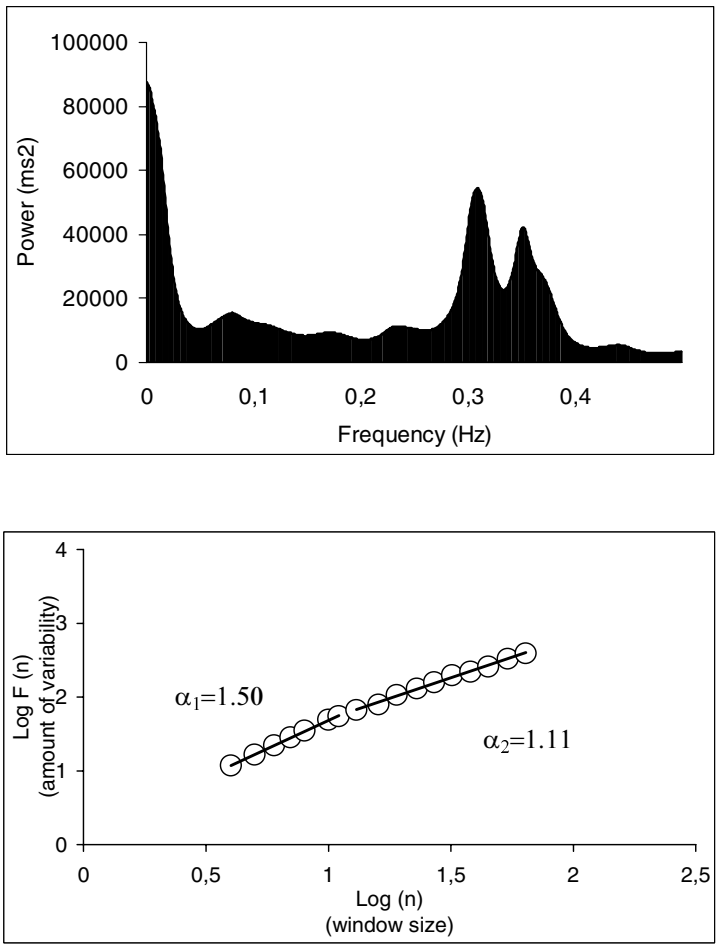

Fig. 1 Examples of results derived from frequency domain analysis (upper figure) and deterended fluctuation analysis (DFA; lower figure) of beat-to-beat interval data in a dog (unpublished observations). The $\alpha 1$ and $\alpha 2$ in the lower figure are the scaling exponents $\alpha 1$ and $\alpha 2$ obtained from detrended fluctuation analysis.

Detrended fluctuation analysis (DFA) of HRV uses an algorithm that explores the correlation properties existing in the RR interval data based on its nonlinear dynamics (Peng *et al.* 1995). The integrated and detrended interbeat interval time series data is examined over a series of selected time windows (time scales; boxes of equal length of n beats) in which the RR interval behaviour is characterised by estimating the fluctuation existing within the selected window. Typically, fluctuation increases with increasing window (box) size, and a scaling exponent α is used to characterise this relationship as it describes the slope of the line relating log fluctuation to log box size (**Fig. 1**). A value of 1 for scaling exponent α corresponds to 1/f noise and indicates the existence of long-range correlations in the RR series data, that is, the presence of partial dependence of the values at every time point on the values at all previous time points (Peng *et al.* 1995, Iyengar *et al.* 1996). This type of behaviour is characteristic for a fractal-like feature and is considered to represent nonlinear dynamic processes occurring in nature in which fluctuations are generated on different time scales (Goldberger 1996). In humans, window sizes of ≤11 and >11 beats have been used in the DFA analysis, and a scaling exponent of 1.0 has been

documented for normal HR dynamics (Peng *et al.* 1995, Laitio *et al.* 2000). In humans, the physiological bases of the nonlinear measures of HRV are currently under investigation.

5.4. Neutrophil Chemiluminescence (CL)

Neutrophil (polymorphonuclear neutrophil; PMN) chemiluminescence (CL) refers to the emission of light that occurs in relation to cell activation and generation of reactive oxygen species (ROS) (Allen *et al.* 2000). The ROS react with nearby atoms to form electrically excited states and when these excited products return to ground level, the energy sustained is released in the form of photons, i.e., light. Neutrophil CL was first described by Allen *et al.* (1972) after detecting light emission from human neutrophils phagocytizing bacteria.

The reactions involved in the neutrophil CL consist of multiple mechanisms that include the activation of cell-membrane receptors and several enzymatic pathways, among them NADPH (nicotinamide adenine dinucleotide phosphate) oxidase and myeloperoxidase (MPO) systems (Allen *et al.* 2000). In relation to these events, oxygen consumption is increased in the cell, and the process has also been referred to as neutrophil respiratory burst. Among the ROS generated are singlet oxygen, superoxide anion (O_2-), hydroxyl radical (OH-, OH.) and hydrogen peroxide (H_2O_2), which form an important part of neutrophil's bacterial killing abilities (Babior 1978). The generation of superoxide anion is dependent on NADPH oxidase activity, whereas hydrogen peroxide is formed due to MPO-related processes (Lilius & Marnila 1992, Allen *et al.* 2000). Individuals with deficiencies in these metabolic pathways, whose neutrophils do not generate CL, are susceptible to bacterial infections; increased neutrophil oxidative activities can also adversely affect the host (Weiss 1989).

In experimental animals, enhancements in blood neutrophil CL responses have been reported in association with organ rejection (Cale *et al.* 1993) and in clinical settings in humans, the blood PMN CL has been linked to the extent of injury and length of hospital stay (Brown *et al.* 1999), as well as to the severity of infectious states (Holzer *et al.* 2001). Wakefield *et al.* (1993) described the relationships between enhanced PMN oxidative activities and the occurrence of postoperative infections in humans; Perttilä *et al.* (1986), however, reported depressed blood neutrophil CL for patients after major surgical interventions. In humans, anaesthesia with propofol or isoflurane and minor surgical intervention has been documented to decrease blood neutrophil respiratory burst by 20% (Heine *et al.* 2000). Few studies have examined the perioperative blood neutrophil CL in dogs; Kosaka *et al.* (1996) reported

depressed neutrophil CL after halothane anaesthesia and minor surgical intervention, as evaluated from very mildly diluted (1:2) whole blood samples.

To document neutrophil CL and the accompanying oxidative activities, several analysis techniques can be utilised. Some methods involve the separation of circulating PMNs from the blood, whereas, in others, so-called whole blood analysis techniques, CL is determined by dividing the amount of light emitted by the number of neutrophils present in the diluted whole blood sample. Using whole blood for the assessment of neutrophil CL is possible, since neutrophils are the main phagocytes responsible for generating CL in circulating blood. Monocytes and eosinophils can also emit light, but the amount is less than that of neutrophils (Allen *et al.* 2000) and their circulating numbers, when compared with neutrophils, are considerably lower in individuals with normal leukocyte differential counts. The dilution of blood, however, is necessary since plasma proteins and erythrocytes absorb the light emitted, resulting in nonlinear relationships between the CL measured and the number of neutrophils in the specimen (Lilius & Waris 1984). When compared with the CL measurements obtained from separated cells, the whole blood analysis technique is considered a faster and easier method that produces results reflecting the *ex-vivo* state of PMNs (Lilius & Marnila 1992, Allen *et al.* 2000). For the assessment of human neutrophil CL with the whole blood analysis technique, dilutions of up to 1:5 000 have been recommended (Redl *et al.* 1983, Lilius & Waris 1984, Allen *et al.* 2000).

When documenting neutrophil CL, light emission is often induced using stimulants such as zymosan, a yeast cell wall preparation, PMA (phorbol myristate acetate) or fMLP (n-formyl-methionine-leucyl-phenylalanine). Zymosan is opsonized by complement and immunoglobulins and is recognised by neutrophils through surface receptors resulting in the process of phagocytosis, phagosome formation, and respiratory burst activity (Lilius & Marnila 1992). The CL arising from neutrophils stimulated by PMA or fMLP is due to direct activation of the cell membrane enzyme NADPH oxidase, and the generation of ROS does not involve opsonin-receptor ligation and the phagocytic process.

In addition to the stimulants added to the reaction mixture, when documenting the CL responses, chemiluminescent substances such as luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) and lucigen (10,10'-dimethyl-9,9'-biacridinium dinitrate) are used to increase the quantity of photons generated (Allen *et al.* 2000). This enhances the sensitivity of the assay, since native CL is weak and would require large numbers of phagocytes to obtain measurable responses. Lucigenin has a high specificity for superoxide anion and thus reflects NADPH activity, whereas the CL derived from

luminol-enhanced responses is closely dependent on the MPO content of the cells and on the production of hydrogen peroxide (Dahlgren & Stendahl 1983) The light emitted can then be measured using a luminometer (**Fig. 2**). In addition to humans, blood neutrophil CL responses induced by zymosan and enhanced by luminol have been described for rats (Lojek *et al.* 1997) and dogs (Angle & Klesius 1983).

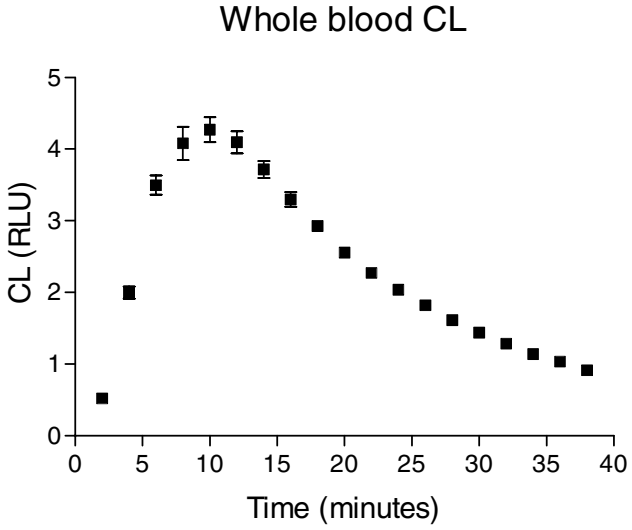

Fig. 2 Example of a chemiluminescense (CL) response curve documented from a diluted whole blood sample treated with opsonized zymosan and luminol. Values are means (SD) of triplicate measurements at individual time points. Data are derived from a dog (unpublished observations).

5.5. Features of Medetomidine and Acepromazine With Respect to Canine Anaesthesia

Medetomidine and acepromazine are sedative / tranquilizing agents licensed for use in dogs in Europe and in the United States. Medetomidine is an α_2 adrenoceptor agonist (α_2 agonist) and a racemic mixture of dex- and l-isomers, the former being considered as the active ingredient (Virtanen 1989, Kuusela *et al.* 2000). The α_1:α_2 receptor selectivity ratio of medetomidine is 1:1620 (Virtanen 1989) and the α_2 agonist properties are viewed as responsible for the drug's actions, although the imidazoline structure may also play a role (Kamibayashi *et al.* 1995, McDonald & Scheinin 1995, Khan *et al.* 1999).

The sedative effects of α_2 adrenergic agents are manifested through stimulation of both pre- and postsynaptic adrenoceptors in the CNS, and a dense concentration of α_2 adrenoceptors is located in the locus coeruleus (LC), a brain area involved in the control of attentive behaviour and vigilance (Segal *et al.* 1988, Stenberg 1989, Correa-Sales *et al.* 1992, Khan *et al.* 1999). Alpha$_2$ adrenoreceptors can also be found in the nociceptive pathways in the spinal cord, in various other areas in the brain, and in peripheral sites such as in platelets and adipose tissue (Scheinin & McDonald 1989). Medetomidine administration in dogs results in dose-dependent sedation, analgesia and muscle relaxation (Vainio *et al.* 1989a).

Acepromazine is classified as a phenothiazine tranquilizer and possesses antidopaminergic and α_1 antagonist activities (Gross 2001). The antidopaminergic actions are viewed as responsible for the tranquilizing properties of the drug, and blocking the α_1 adrenoceptors contributes to vascular effects causing vasodilation (Gross 2001). Acepromazine does not provide analgesia (Barnhart *et al.* 2000) and, to a certain extent, increasing the dose is considered to only lengthen the sedation but not to enhance its level (Brock 1994, Gross 2001). The tranquilizing effects of acepromazine are described to last for 4 to 6 hours (Brock 1994).

In clinical practice, both medetomidine and acepromazine are often combined with opioids to enhance the sedative and analgesic effects. Dyson & Atilola (1992) reported that 0.05 mg kg^{-1} of acepromazine and 0.2 mg kg^{-1} of butorphanol administered intravenously (IV) resulted in acceptable sedation in client-owned dogs that underwent radiological examinations. Bartram *et al.* (1994) found that medetomidine (10 µg kg^{-1}) and butorphanol (0.1 mg kg^{-1}) (IM) provided suitable sedative effects for dogs in association with various diagnostic or clinical procedures. In laboratory Beagles, when examining nociception by use of heat stimulation, the combined administration of acepromazine and oxymorphone (0.2 mg kg^{-1}, IM, each) was found to result in similar analgesic effects when compared with medetomidine (20 µg kg^{-1}, IM) (Barnhart *et al.* 2000).

Both medetomidine and acepromazine decrease the requirements of propofol and inhalant anaesthetics in dogs (Webb & O'Brien 1988, Watney & Pablo 1992, Ewing *et al.* 1993). The mean total dosage of propofol required to induce anaesthesia after acepromazine (0.05 mg kg $^{-1}$, IM) in dogs was reported to be 2.8 mg kg $^{-1}$ (Watney & Pablo 1992) and medetomidine (0.030 mg kg $^{-1}$, IV) was found to decrease isoflurane requirements (minimum alveolar concentration; MAC) by 47% (Ewing *et al.* 1993).

Bradycardia (heart rate < 60 beats per minute) commonly occurs after medetomidine administration, and has been demonstrated with doses as low as 5 µg kg^{-1} (IV) in dogs (Pypendop & Verstegen 1998). Accompanying the decrease in heart rates, atrio-ventricular (AV) blocks and sinus pauses can be seen (Vainio & Palmu 1989b, Kuusela *et al.* 2002). In dogs, medetomidine administration also results in decreased cardiac output and increased systemic vascular resistance (Pypendop & Verstegen 1998). Intravenous administration of medetomidine transiently increases the mean arterial blood pressure (MAP), after which the values decline to pre-administration levels, or slightly lower (Vainio & Palmu 1989b, Pypendop & Verstegen 1998).

At 80 minutes following administration, heart rates were found to be higher in Beagle dogs administered acepromazine (0.05 mg kg^{-1}, IM) and butorphanol (0.2 mg kg^{-1}, IM), compared with medetomidine (20 µg kg^{-1}, IM) and midazolam (0.3 mg kg^{-1}, IM) (77 *vs* 44 bpm) (Kojima *et al.* 1999). In a non-placebo-controlled study in Beagles (Stepien *et al.* 1995), 0.1 mg kg^{-1} acepromazine (IV) decreased MAP by 20% and cardiac output by 25%. When acepromazine (0.05-0.25 mg kg^{-1} IV) was administered to anaesthetised dogs, higher doses of phenylephrine were required to increase the MAP, possibly reflecting the peripheral α-adrenergic blockad achieved with acepromazine administration (Ludders *et al.* 1983). In halothane anaesthetised dogs, acepromazine administration was found to attenuate the occurrence of epinephrine-induced ventricular dysrhythmias (Muir *et al.* 1975); similar effects were not demonstrated for medetomidine (Lemke *et al.* 1993). In general, when administered as sole agents, respiratory function is not greatly altered with either medetomidine or acepromazine, although decreases in respiratory rates commonly occur (Vainio & Palmu 1989b, Jacobson *et al.* 1994, Stepien *et al.* 1995, Cullen 1996, Gross 2001).

6. AIMS OF THE STUDY

The overall goal of the series of investigations presented in this thesis was to expand our knowledge of perioperative stress in client-owned dogs. Emphasis was placed on the examination of different aspects of manifestation of stress at various stages of the perioperative period and on comparing the influences of two preanaesthetic medications. The specific aims were:

1. To evaluate the manifestation of preoperative stress in dogs, as apparent in the behavioural and cardiac characteristics of hospitalised patients. To investigate the relationships between an animal's preoperative behavioural and physiological states and its characteristics at later time points of (perioperative) evaluations.

2. To investigate the quality of postsurgical behavioural symptoms following anaesthesia and elective operations in dogs, as assessed by animal owners.

3. To compare the influences of medetomidine or acepromazine preanaesthetic administration on perioperative stress responses in dogs.

4. To examine the relationships between different perioperative stress-related measures in dogs undergoing ovariohysterectomy with medetomidine or acepromazine preanaesthetic medication.

7. MATERIALS AND METHODS

7.1. Animals, Recruitment and Operations

In Study 1 (I-IV), the animals were dogs (n = 43) that underwent OH at Helsinki University Small Animal Hospital with either MED or ACE administered as for preanaesthetic medication (**Tables 4, 5**). Inclusion criteria were: good body condition, weight between 15 and 40 kg, age between 2 and 7 years, not receiving concurrent medications, and at least 3 months elapsed from the last oestrus. The dogs were determined to be in good health on the basis of the results of a complete physical examination, anamnestic history, CBC count, and serum biochemical analysis. The study was advertised at the facilities of the Helsinki University Small Animal Hospital and in the magazine of the Finnish Kennel Club. The animals were recruited by the author of this thesis (MV). Written and informed owner consent was obtained and the protocol was approved by the local Ethical Committee.

In Study 2 (V), the animals were dogs (n = 96) that underwent day-case soft tissue operations at Helsinki University Small Animal Hospital (53%) or at 5 other veterinary practices in the Helsinki area (**Tables 4, 5**). The veterinary practices were selected on the basis of their similarities in patient management. Dogs suffering from preoperative symptoms, including possible preoperative pain, as well as dogs with chronic illnesses were not eligible. Operations involving head or perianal area were excluded. Forty-five percent of the animals represented small or medium breeds, 48% large breeds and 7% giant breeds. The animals were enrolled when being presented for the operation. Written and informed owner consent was obtained.

7.2. Patient Management and Perianaesthetic Medications

In Study I (I-IV), the dogs were hospitalised for 24 hours. The period of preoperative hospitalisation lasted approx. 60 minutes and the dogs were discharged approx. 20 hours after surgery. During their stay, the dogs were handled only by the members of the investigation team and were accommodated in quiet surroundings, isolated from the sight and sounds of other dogs. The ovariohysterectomy was performed in the early afternoon, using the ventral approach, and by one of the 3 experienced surgeons. The mean durations of the periods of preoperative sedation, anaesthesia and surgery, and the time intervals from the end of anaesthesia to extubation were not significantly different between the two preanaesthetic groups.

Dogs were randomly assigned to receive either MED or ACE for preanaesthetic medication (**Table 5**). Each dog was assigned a number in accordance with the sequence of enrolment and randomization was completed by using sealed envelopes. One investigator (MV), who was unaware of the type of the preanaesthetic medication but aware of the two possible protocols, administered all the sedative and anaesthetic agents and monitored the anaesthesias and recoveries. During the period of preoperative sedation, catheters were placed into cephalic and jugular veins (for the administration of medications and for data collection, respectively) and into femoral artery (for direct measurement of blood pressure). Local infiltration of lidocaine was used to facilitate catheter placement. During the period of preoperative sedation, the dogs were lying laterally or dorsally with minimum or no restraint.

Table 4. Summary of the animals, interventions and investigations included in this thesis.

	Study 1	Study 2
Animals		
n*, gender	43, all female	96, 61% female
age** (median, range)	5 (2-7)	6 (1-13)
breed	various breeds	various breeds
Study sites	1 University Hospital	1 University Hospital 5 private practices
Study period	Jan-Aug 2000	10 weeks (spring 2002)
Interventions	OH MED or ACE premedication	Soft-tissue surgeries***
Follow-up period	24 perioperative hours	3 postop. days
Main investigations (published in)	Plasma hormone conc. (I) HR, HRV, MAP, Arrhythmias (I, II) Postoperative behaviour (I, II) Neutrophil CL (III) Preoperative characteristics (IV)****	Postoperative behaviour Owner-rated animal pain Impact of demogr. factors (V)
Study design	Randomised, blinded clinical trial Observational study (IV)	Survey

*n, dogs that received the allocated intervention (Study 1) or for which completed owner-filled questionnaires were obtained (Study 2). **age, years. ***, minor surgery (skin tumor removal; approx. 13% of dogs), moderate or intermediate surgery (castration, mammectomy; 42%), major surgery (abdominal surgery; 45%). ****, the preoperative characteristics included measurements on behaviour and HRV.

Anaesthesia was induced with intravenous propofol and continued with isoflurane in oxygen. At least 10 minutes were allowed to elapse from the induction of anaesthesia until the beginning of surgery. Lack of the palpebral reflex, ventromedial rotation of the eye, and relaxation of the jaw tone were regarded as indices of adequate level of surgical anaesthesia. During surgery isoflurane administration was increased in both groups due to changes detected in response to surgical stimuli (sudden increases in heart rate, respiratory rate, or blood pressure). During anaesthesia, end-tidal (ET) CO_2 was maintained at < 60 mmHg by use of intermittent manual ventilation and arterial haemoglobin saturation (SpO_2%) stayed between 99 and 100% in all dogs. Additional boluses of fluids were used to maintain MAP ≥ 60 mmHg in the ACE group. Rectal temperature was kept at > 37 °C by use of a heating blanket. Dogs were allowed to recover from anaesthesia in their cages. At 6 hours after surgery, the dogs were taken for a walk and water and food were offered. Recovery was documented until suture removal, i.e., until approximately 10 to 12 days after surgery.

In Study 2, the patients were treated according to the routine at each veterinary practice. During the time when the dog was hospitalised, the staff were asked to keep a record of the anaesthetic and analgesic agents administered and of any adverse events, such as extreme excitement. The animals were most commonly discharged in the afternoon on the day of the operation. Isoflurane was used as general anaesthetic (**Table 5**).

7.3. Collection and Analysis of Study Data

7.3.1. Plasma Hormone Concentrations (I)

Blood samples for plasma cortisol, beta-endorphin and catecholamine measurements were taken on 8 occasions from the jugular vein into chilled tubes containing EDTA (**Table 6**). The first sample was collected by the use of a needle; the subsequent samples were collected via an indwelling catheter. Plasma was separated by refrigerated centrifugation, divided and frozen (-80 °C) within 30 minutes after collection. Catecholamines (adrenaline and noradrenaline) were analysed by means of high-performance liquid chromatography, and a sample that had a catecholamine concentration less than the limit of detection was assigned a value equal to the limit of detection (0.05 nmol l^{-1}). Cortisol and beta-endorphin were measured using a radioimmunoassay method. Analysis was completed within 14 months of sampling. Detailed descriptions of the analysis of the hormonal samples can be found in Publication I.

Table 5. Medications administered in Study 1 and Study 2.

Study 1

Purpose	Drug (route)	Dose (mg kg^{-1})	Group
Premedication	medetomidine and butorphanol (IM)	0.02 and 0.2	MED
	acepromazine and butorphanol (IM)	0.05 and 0.2	ACE
Induction of anaesth.	propofol (IV)	1.3 (0.2)*; 3.6 (0.7)*	MED; ACE
Maint. of anaesth.	isoflurane	1.4 (0.3)*†; 1.6 (0.2)*†	MED; ACE
Other (analgesics)	carprofen (IV)	4.0 (at induction)	MED and ACE
	buprenorphine (IV)	0.01 (end of anaesth.)	MED and ACE
		0.01 (at 6 hours postop)	MED (5 dogs) ACE (2 dogs)
Other	cephalotin (IV)	30 (at induction)	MED and ACE
	lactated Ringer's solution (IV)	10 ml kg^{-1} $hour^{-1}$ (during anaesth.)	MED and ACE
		addit. boluses (5-10 ml kg^{-1})	ACE (11 dogs)

Study 2

Purpose	Drug	Nro of dogs (%)
Premedication	medetomidine (or xylazine) and butorphanol (or l-methadone)	82 %
	medetomidine or xylazine alone	17 %
	acepromazine	1 %
Induction of anaesth.	propofol	100 %
Maint. of anaesth.	isoflurane	100 %
Other (analgesics)	buprenorphine (end of anaesth.)	77 %
	fentanyl (end of anaesth.)	2 %
	carprofen or meloxicam	
	end of anaesth., dosed for a 24-hour effect	98 %
	at home (Day 2)	73 %
	at home (Day 3)	62 %

*, values are means (SD). †, %end-tidal. Lactated Ringer's solution was used as intra-anaesthetic fluid and additional boluses were administered to maintain MAP ≥ 60 mmHg. Day 2 refers to the first day following the day of surgery; Day 3 to the second day following the day of surgery.

7.3.2. Cardiovascular Variables (I, II, IV)

The 24-hour continuous aECG recordings (II, IV) were obtained using two transthoracic leads and a Holter recorder carried on the back of the dog in a specifically-designed jacket. Separate measurements of heart rate (I) were also made together with the recording of the MAP (I) and, by palpation of the pulse, before the administration of the preanaesthetic medications and during the postoperative period, together with the collection of the hormone samples (**Table 6**). The MAP was recorded by use of a catheter placed in a femoral artery and a pressure transducer connected to an oscilloscope.

Manual editing was applied during the analysis of aECG recordings, and the full disclosure printouts were checked by a veterinarian and verified by a veterinary cardiologist to ascertain the correct labelling of arrhythmic events. For cardiac conduction disturbances, second- and third-degree AV blocks, sinus pauses (over 2.0 seconds) and ventricular premature complexes (VPCs) were recorded. The SDNN, RMSSD and pNN50 were calculated as time domain indices of HRV. Areas under two frequency bands were measured to document the power spectra of HRV (LF (0.04 to 0.15 Hz) and HF (0.15 to 0.40 Hz) frequency bands). Hence the "free-running" conditions of the current study, only the LF:HF ratio was used to examine the perioperative differences in sympathovagal balance between the two preanaesthetic groups. For the nonlinear HRV measures, scaling exponents $\alpha 1$ (DFA1) and $\alpha 2$ (DFA2) were documented using observation windows of ≤ 11 beats and >11 beats, respectively. All the HRV analyses were performed in segments of 30 minutes and average values were determined. Detailed descriptions of the analysis of aECGs can be found in Publication II.

For the preoperative heart rate characteristics (IV), the aECG recordings were analysed for mean heart rates, heart rates at one-minute time intervals, and time domain HRV indices. Simultaneous recordings were made of the dog's behaviour (see *Behavioural Observations*).

7.3.3. Immunological Measurements (III)

Samples for blood neutrophil counts and neutrophil CL responses were drawn from the jugular vein into 1-ml EDTA tubes on 3 occasions (**Table 6**). Blood neutrophil counts were automatically determined within 4 hours of sampling and were verified by manual inspection. For the assessment of neutrophil functional characteristics, luminol-enhanced and zymosan-induced whole blood CL responses were documented.

Table 6. Time points for study events and data collection in Study 1.

Study Event	Hormones	aECG	MAP	Ncounts and CL responses	PD and Sedation scores	Video-recorded behaviour
Arrival (at noon)	X	X		X		
Period of preop. hospitalisation (60 min)*					X	↓
Period of preop. sedation (85 min)*	X		X			
Induction of Anaesthesia						
Anaesthesia and Surgery (anaesthesia 74; surgery 39 min)*			X, X			
	X		X, X			
	X		X, X	X		
Extubation						
1h postop	X				X	
3h postop	X				X	
6h postop	X				X	
Following night		↓				
Next day (24 hours after arrival)	X			X	X	↓

*, times are average values for all dogs. At arrival, the blood sample was taken within 2 minutes of the owner's departure; during surgery, a blood sample was taken within 2 minutes after removal of the 2nd ovary and at the end of surgery. The measurements during the period of preoperative sedation were taken immediately prior to the induction of anaesthesia. Animals were extubated approx. 12 minutes following the end of anaesthesia.

The protocol used has been described in detail elsewhere (Grönlund *et al.* 1999), and for this thesis the optimum concentrations of the reagents to be utilised were determined in preliminary investigations (unpublished data). A final blood dilution of 1:5 000 was used. Results (RLU; relative light units) were read at peak activity. Both total (blood) CL responses (total CL activity in the diluted blood sample) and specific (neutrophil) CL responses (total CL activity related to the number of neutrophils present in the sample) were determined. Detailed descriptions of the CL assays can be found in Publication III.

7.3.4. Behavioural Observations (I, II, IV, V)

Data on animals' preoperative behaviour (IV), postoperative characteristics related to MED or ACE preanaesthetic administration (I, II) and late postsurgical condition (V) were obtained using video recording (II, IV), sedation and pain-distress scores (I) and owner-based observations (V).

For preoperative behavioural characteristics (IV), the video recordings were analysed for the first 30 minutes the dog spent undisturbed in the hospital cage. In addition dogs' responses to a person first entering the cage were noted. Detailed descriptions of the aspects of behaviour documented during the period of preoperative hospitalisation can be found in Publication IV.

The postsurgical behavioural characteristics related to MED or ACE preanaesthetic administration (I, II) were determined from the video recordings as times spent in an upright position (sitting or standing) during a period of 6 postoperative hours. In addition, for each animal, sedation and pain / distress (I) were scored by one investigator (MV), once before the administration of the preanaesthetic medication and 4 times during the postoperative period, just after the hormonal samples had been collected (**Table 6**). Please refer to Publication I (Appendix) for detailed descriptions of the scoring of sedation and pain / distress.

Questionnaires completed by animal owners were used to document dogs' characteristics when recovering from day-case operations at home (V). The owner filled in the questionnaire on 3 successive days after surgery: the first day (Day 1) started after the animal had arrived at home from the veterinary clinic and continued until the next morning; Days 2 and 3 lasted 24 hours each. Several behavioural characteristics were covered in the questionnaire and the owners were also asked to document the magnitude of postoperative pain, as assessed once-a-day using a visual analog scale (VAS). The individual behaviours covered in the questionnaire are presented in Figures 1 and 2 [V]. Data was also collected for selected demographic variables (outlined in Table 1 [V]) and for additional postoperative data (the duration of time (per day) that the dog wore an Elizabethan collar, the duration of time (per day) that the dog was left alone at home, and dog's interest in the wound, as reported by the owner).

Table 7. Patient flow and completion of measurements in Study 1 and Study 2.

Study 1

	Dogs included: n = 44		
	Randomization using sealed envelopes		
	Received intervention as allocated: n = 43:		
	MED, n = 21		ACE, n = 22
		Completed (n):	
Hormone meas.	21		21
aECG recordings	21		20
Analysis of time and freq. domain HRV	21		20
Analysis of DFA1	21		20
Analysis of DFA2	14		19
Immunological meas.	19		18
Preoperative behaviour	20		21

Study 2

Owners approached: n = 103
Informed consent obtained: n = 102
Questionnaires returned: n = 97
Questionnaires used in analysis: n = 96

7.4. Statistical Analyses

Sample size was calculated for Study 1 and was based on the assumption that 18 dogs in each treatment group would allow detection (with a power of 80% and a level of confidence of 5%) of a difference of 0.3 nmol l^{-1} in plasma catecholamine concentrations with respect to time and between the two study groups. One hundred animals were estimated as required for the investigation of postoperative behavioural changes (Study 2).

Effects of time and treatment on plasma hormone concentrations and on the cardiovascular and immunological variables were examined by use of repeated measures ANOVA. Linear mixed effects in S-plus (S-plus 2000, Mathsoft Inc, Seattle, Wash) were used to study the similarity of the response curves following MED or ACE preanaesthetic medication (I, II, III), and the values of the later measurements were contrasted with the first measurements and with the values obtained during sedation. Due to the skewed distribution of the original data, plasma hormonal

concentrations and the frequency domain HRV indices were analysed after logarithmic transformation. Since the treatment did not significantly affect the immunological indices, the two treatment groups were combined for the evaluation of the effects of time (III).

Fischer's exact test, Wilcoxon rank sum test and χ^2 test were used to study the incidence of arrhythmias (II) and the pain / distress and sedation scores (I). For the period of preoperative hospitalisation, the minute-to-minute heart rate behaviour of an individual dog was characterised by regression (IV). For all the measures, the level of significance was designated at $P < 0.05$, and 95% confidence intervals (CI) were calculated on the basis of data obtained for each measurement point in question (I, II).

Correlation coefficients (Pearson's correlation coefficients) were calculated for selected variables measured in Study 1. The correlation coefficients were determined using values obtained during the entire study period (*r-24h*) and also documented for individual time points (*r-Indiv*) up to 3 hours after surgery. The values recorded from unpremedicated animals and those registered during the period of preoperative sedation as well as during anaesthesia and surgery were contrasted with the data gathered at later time points, up to 3 hours after surgery (*r-Time*). For each measurement, scattergrams were plotted to examine the shape of the association and the effect of removal of a single data point, considered to represent a high-leverage observation, on correlation was investigated. With all the values, the MED and ACE groups were treated separately. Individual scattergrams were used to examine the relationships between the preoperative behavioural data (the 3 behavioural groups) and the hormonal concentrations and heart rates measured from awake dogs, during sedation, during surgery and at 1h and 3h after surgery. The documentation of the correlations is a feature of this thesis only and the results have not previously been published.

In Study 2, kappa coefficients were calculated for the postoperative behavioural measures (individual behaviours) and stepwise linear models were used to explain the behavioural changes (the sum of behaviours) and pain VAS scores. The explanatory variables studied were the observation day, the items included in the demographic variables (background information; Table 1 [V]), the type of the surgical operation (minor, intermediate (or moderate), major; Table 1 [V]), and the variables gathered as additional postoperative data. The level of significance was set at $P < 0.05$ and kappa coefficients ≥ 0.75 were considered to indicate excellent agreement, while values < 0.40 were taken to reflect poor agreement (Woodward 1999).

8. RESULTS

8.1. Plasma Hormone Concentrations (I)

Compared with ACE, MED preanaesthetic administration resulted in lower concentrations of circulating catecholamines and cortisol (Fig. 1 [I]; **Fig. 3**). The difference between the two preanaesthetic groups was already apparent during the period of preoperative sedation, but was also clearly evident during early recovery. With both MED and ACE-treated dogs, plasma cortisol and beta-endorphin increased during anaesthesia and surgery. In each of the preanaesthetic groups, plasma adrenaline or noradrenaline concentrations less than the limit of detection (0.05 nmol l^{-1}) were documented on 5 occasions. At individual time points, the maximum number of these observations was ≤ 3.

When using the data gathered during the entire study period (*r-24h*; **Table 8**), the correlation coefficients for the different hormonal measures and for plasma catecholamines and heart rates were not high. The strongest correlation (r = 0.57) was registered for plasma catecholamines in the MED group (**Fig. 4**).

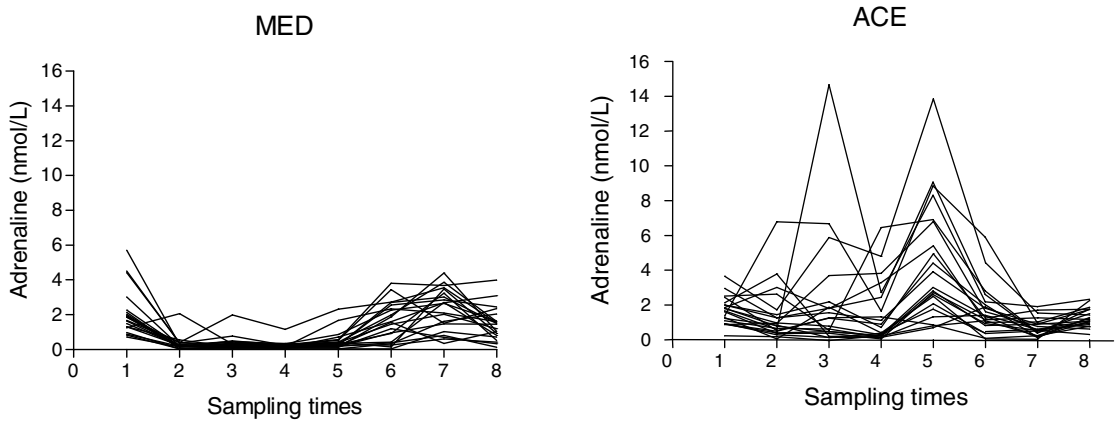

Fig. 3 Plasma adrenaline concentrations in individual dogs that underwent OH following preanaesthetic administration of MED or ACE (Study 1). Sampling time 1 represents data collected from awake dogs; s-time 2 data collected during the period of preoperative sedation; s-times 3 and 4 data collected during surgery; s-times 5, 6, and 7 data collected at 1, 3 and 6 hours after surgery, respectively; and s-time 8 data collected at 20 hours after surgery. The concentrations of plasma adrenaline were lower in the MED group.

Table 8. Correlation coefficients (*r-24h*) calculated for selected variables of Study 1.

Comparison	MED			ACE		
	r-24h	95%CI	*P*	*r-24h*	95%CI	*P*
Adrenaline vs Noradrenaline	0.57	0.46 – 0.67	*< 0.0001*	0.35	0.21 – 0.48	*< 0.0001*
Cortisol vs Beta-endorphin	0.28	0.14 – 0.47	*0.0002*	0.33	0.19 – 0.46	*< 0.0001*
Heart rate vs Adrenaline	0.47	0.34 – 0.58	*< 0.0001*	0.35	0.21 – 0.49	*< 0.0001*
Heart rate vs Noradrenaline	0.46	0.34 – 0.58	*< 0.0001*	0.09	-0.07 – 0.24	*0.2660*
Heart rate vs RMSSD	-0.86	-0.89 – -0.81	*< 0.0001*	-0.79	-0.85 – -0.73	*< 0.0001*
Heart rate vs LF:HF ratio	0.64	0.55 – 0.72	*< 0.0001*	0.61	0.51 – 0.70	*< 0.0001*
Heart rate vs DFA2	0.47	0.31 – 0.61	*< 0.0001*	0.15	-0.01 – 0.31	*0.0571*
DFA1 vs RMSSD	-0.71	-0.79 – -0.60	*< 0.0001*	-0.80	-0.85 – -0.74	*< 0.0001*
DFA1 vs LF:HF ratio	0.89	0.84 – 0.92	*< 0.0001*	0.94	0.92 – 0.96	*< 0.0001*
DFA2 vs RMSSD	-0.56	-0.68 – -0.42	*< 0.0001*	-0.14	-0.29 – 0.02	*0.0853*
DFA2 vs LF:HF ratio	0.26	0.08 – 0.43	*0.0057*	-0.27	-0.41 – -0.11	*0.0008*
DFA2 vs DFA1	0.44	0.27 – 0.58	*< 0.0001*	-0.16	-0.32 – -0.01	*0.0430*
RMSSD vs HF-HRV	0.98	0.97 – 0.98	*< 0.0001*	0.99	0.98 – 0.99	*< 0.0001*
RMSSD vs LF-HRV	0.59	0.47 – 0.68	*< 0.0001*	0.30	0.14 – 0.44	*0.0003*
RMSSD vs LF:HF ratio	-0.76	-0.81 – -0.69	*< 0.0001*	-0.74	-0.80 – -0.66	*< 0.0001*
LF-HRV vs HF-HRV	0.29	0.13 – 0.43	*< 0.0001*	0.21	0.05 – 0.36	0.0120
WhbCL vs Ncount	0.92	0.88 – 0.96	*< 0.0001*	0.91	0.84 – 0.95	*< 0.0001*

r, Pearson's correlation coefficient; *r-24h*, correlations calculated using values gathered during the entire study period (24 hours, 8 measurement points).

At individual time points (*r-Indiv*; **Table 9**) and as contrasted over time (*r-Time*; **Table 10**) the correlation coefficients were generally higher in the ACE –treated dogs, and in both groups the strongest correlations were recorded at the end of the operation and during the early recovery (**Fig. 5**). Removal of a single data point, however, affected these relations (**Table 11**).

Except for plasma beta-endorphin, most of the hormonal data obtained from awake dogs was not significantly correlated with the values obtained at later evaluations (**Table 10**). The extent of decrease in plasma noradrenaline (with both preanaesthetic groups) and in plasma adrenaline and cortisol (in the MED group) during the period of preoperative sedation was inversely related to the values obtained before preanaesthetic administration (**Fig. 6**).

8.2. Perioperative Cardiovascular Activities (I, II)

Heart rates were lower, the incidence of bradyarrhythmias higher and increases in the time domain HRV greater in the MED-treated dogs (Figs. 2 [I], 1 and 3 [II]; Table 1 [II]). With both preanaesthetic groups, the time domain HRV increased during the period of preoperative sedation and decreased during anaesthesia and surgery (**Fig. 7**). In the ACE group, the intraoperative LF:HF ratios were higher and MAP increased (Fig. 3 [I, II]). No significant differences were detected for the two preanaesthetic protocols in the incidence of VPCs (Table 1 [II]) or for the DFA2 (Fig. 3 [II]). The highest number of VPCs (198 / 24 hours) was recorded in a *Golden Retriever* (MED). After approximately 6 postoperative hours, statistical analysis revealed no systematic differences in the heart rate data between the two treatment groups. Results for the preoperative cardiac characteristics are presented with the behavioural data (see *Behavioural Observations*).

Heart rates recorded from unpremedicated dogs were not significantly correlated with the values obtained at later evaluations (**Table 10**). With both preanaesthetic groups, the extent of decrease in heart rates during the period of preoperative sedation was inversely related to the values obtained before preanaesthetic administration (**Fig. 8**).

Table 9. Correlation coefficients (*r-Indiv*) calculated for selected variables of Study 1.

		MED			ACE		
Time	Comparison	*r-Indiv*	95%CI	*P*	*r-Indiv*	95%CI	*P*
Sedation							
	Adrenaline vs Noradrenaline	0.16	-0.30 – 0.55	*0.50*	0.53	0.13 – 0.80	*0.01*
	Cortisol vs Beta-endorphin	-0.06	-0.48 – 0.38	*0.78*	0.41	-0.02 – 0.72	*0.06*
	Heart rate vs Adrenaline	0.30	-0.15 – 0.64	*0.19*	0.48	0.06 – 0.74	*0.03*
	Heart rate vs Noradrenaline	0.05	-0.47 – 0.39	*0.22*	0.49	0.07 – 0.76	*0.03*
Endop							
	Adrenaline vs Noradrenaline	0.08	-0.50 – 0.36	*0.72*	0.72	0.42 – 0.88	*< 0.001*
	Cortisol vs Beta-endorphin	0.29	-0.16 – 0.64	*0.20*	0.12	-0.33 – 0.53	*0.60*
	Heart rate vs Adrenaline	0.20	-0.25 – 0.58	*0.37*	0.49	0.07 – 0.76	*0.02*
	Heart rate vs Noradrenaline	0.24	-0.21 – 0.61	*0.28*	0.69	0.38 – 0.87	*< 0.001*
1h postop							
	Adrenaline vs Noradrenaline	-0.09	-0.51 – 0.36	*0.71*	0.58	0.20 – 0.81	*< 0.01*
	Cortisol vs Beta-endorphin	0.57	0.18 – 0.80	*< 0.01*	0.33	-0.13 – 0.67	*0.16*
	Heart rate vs Adrenaline	0.12	-0.34 – 0.53	*0.61*	0.60	0.22 – 0.82	*< 0.01*
	Heart rate vs Noradrenaline	-0.05	-0.48 – 0.40	*0.84*	0.67	0.33 – 0.85	*< 0.01*
3h postop							
	Adrenaline vs Noradrenaline	0.66	0.33 – 0.55	*< 0.001*	0.58	0.19 – 0.81	*< 0.01*
	Cortisol vs Beta-endorphin	0.07	-0.37 – 0.49	*0.75*	0.50	0.09 – 0.77	*0.02*
	Heart rate vs Adrenaline	0.55	0.15 – 0.79	0.01	0.19	-0.29 – 0.59	*0.43*
	Heart rate vs Noradrenaline	0.28	-0.17 – 0.63	*0.21*	0.26	-0.22 – 0.64	*0.28*

		MED			ACE		
Time	Comparison	*r-Indiv*	95%CI	*P*	*r-Indiv*	95%CI	*P*
Sedation							
	Heart rate vs RMSSD	-0.72	-0.91 – -0.54	*< 0.0001*	-0.69	-0.87 – -0.35	*< 0.001*
	Heart rate vs DFA2	0.07	-0.48 – 0.58	*0.81*	-0.21	-0.61 – 0.29	*0.41*
	DFA1 vs LH:HF ratio	0.89	0.70 – 0.97	*< 0.0001*	0.96	0.87 – 0.98	*< 0.0001*
Endop							
	Heart rate vs RMSSD	-0.81	-0.92 – -0.56	*< 0.0001*	-0.64	-0.85 – -0.27	*< 0.01*
	Heart rate vs DFA2	0.44	-0.11 – 0.79	*0.11*	0.37	-0.11 – 0.70	*0.13*
	DFA1 vs LH:HF ratio	0.86	0.61 – 0.96	*< 0.0001*	0.91	0.79 – 0.96	*< 0.0001*
1h postop							
	Heart rate vs RMSSD	-0.82	-0.92 – -0.58	*< 0.0001*	-0.88	-0.95 – -0.72	*< 0.0001*
	Heart rate vs DFA2	0.53	-0.00 – 0.83	*0.05*	0.41	-0.05 – 0.73	*0.08*
	DFA1 vs LH:HF ratio	0.93	0.77 – 0.98	*< 0.0001*	0.94	0.84 – 0.98	*< 0.0001*
3h postop							
	Heart rate vs RMSSD	-0.79	-0.91 – -0.55	*< 0.0001*	-0.89	-0.95 – -0.73	*< 0.0001*
	Heart rate vs DFA2	0.54	0.00 – 0.83	*0.05*	0.40	-0.06 – 0.73	*0.09*
	DFA1 vs LH:HF ratio	0.97	0.91 – 0.99	*< 0.0001*	0.83	0.60 – 0.93	*< 0.0001*

r, Pearson's correlation coefficient; *r-Indiv*, correlations calculated at individual time points: Sedation, period of preoperative sedation; Endop, end of surgery; 1h (and 3h) postop, 1 (and 3) hours after surgery.

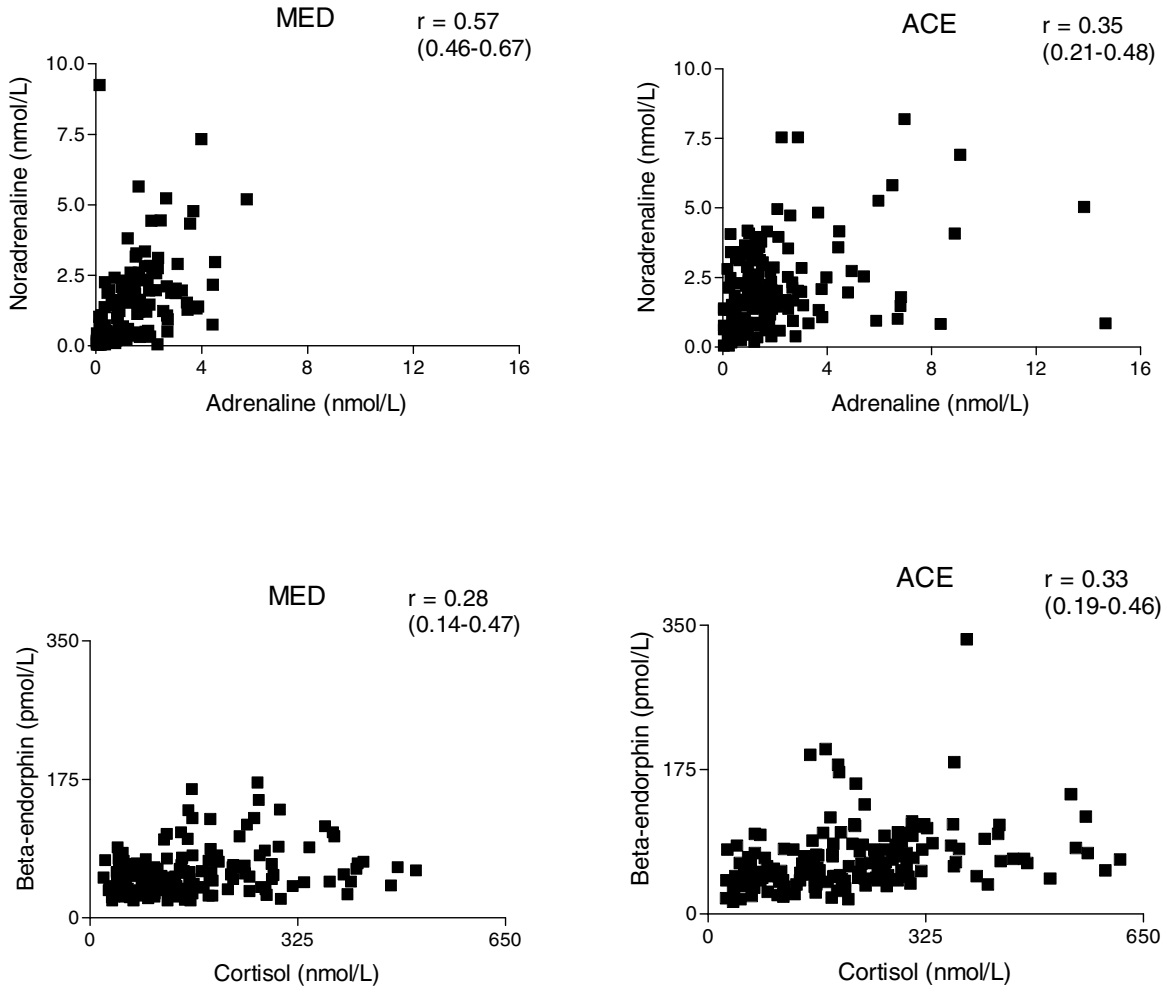

Fig. 4 Scattergrams showing the associations between selected hormonal measures obtained from dogs that underwent OH following preanaesthetic administration of MED or ACE (Study 1). The correlation coefficients (*r-24*, 95%CI) were calculated using data gathered during the entire study period (24 hours; 8 measurement points). The strongest correlations were determined for plasma catecholamines in the MED group. See **Table 8** for further results.

Table 10. Correlation coefficients (*r-Time*) calculated for selected variables of Study 1.

Comparison	Variable	MED			ACE		
		r-Time	95%CI	*P*	*r-Time*	95%CI	*P*
Awake vs Sedation							
	Adrenaline	-0.04	-0.47 – 0.40	*0.86*	-0.24	-0.21 – 0.61	*0.29*
	Noradrenaline	0.26	-0.19 – 0.62	*0.25*	0.16	-0.28 – 0.55	*0.47*
	Cortisol	0.42	-0.01 – 0.72	*0.06*	0.41	-0.02 – 0.71	*0.07*
	Beta-endorphin	0.83	0.63 – 0.93	*< 0.0001*	0.67	0.33 – 0.85	*< 0.001*
	Heart rate	0.33	-0.11 – 0.67	*0.13*	0.06	-0.38 – 0.49	*0.78*
Awake vs Endop							
	Adrenaline	0.11	-0.34 – 0.51	*0.64*	0.45	0.03 – 0.74	*0.04*
	Noradrenaline	-0.12	-0.52 – 0.32	*0.59*	0.35	-0.19 – 0.68	*0.12*
	Cortisol	0.12	-0.33 – 0.52	*0.60*	0.60	0.23 – 0.83	*< 0.01*
	Beta-endorphin	0.39	-0.04 – 0.70	*0.07*	0.59	0.22 – 0.82	*< 0.01*
	Heart rate	0.04	-0.39 – 0.46	*0.85*	0.31	-0.15 – 0.66	*0.18*
Awake vs 1h postop							
	Adrenaline	0.30	-0.15 – 0.65	*0.19*	0.58	0.20 – 0.81	*< 0.01*
	Noradrenaline	0.19	-0.26 – 0.58	*0.41*	0.47	0.05 – 0.75	*0.03*
	Cortisol	0.62	0.26 – 0.83	*< 0.01*	0.63	0.27 – 0.83	*< 0.01*
	Beta-endorphin	0.38	0.05 – 0.81	*< 0.01*	0.57	0.16 – 0.81	*< 0.01*
	Heart rate	0.15	-0.31 – 0.56	*0.59*	-0.07	-0.50 – 0.38	*0.74*
Sedation vs Endop							
	Adrenaline	0.18	-0.28 – 0.56	*0.45*	0.29	-0.16 – 0.64	*0.20*
	Noradrenaline	-0.10	-0.51 – 0.35	*0.67*	0.07	-0.38 – 0.49	*0.77*
	Cortisol	-0.13	-0.53 – 0.32	*0.59*	0.40	-0.04 – 0.71	*0.07*
	Beta-endorphin	0.56	0.17 – 0.80	*< 0.01*	0.52	0.11 – 0.78	*0.02*
	Heart rate	-0.02	-0.44 – 0.41	*0.93*	0.56	0.16 – 0.80	*< 0.01*
Sedation vs 1h postop							
	Adrenaline	0.06	-0.38 – 0.48	*0.79*	0.40	-0.04 – 0.71	*0.07*
	Noradrenaline	0.36	-0.08 – 0.68	*0.11*	0.20	-0.25 – 0.58	*0.37*
	Cortisol	0.04	-0.39 – 0.46	*0.86*	0.31	-0.14 – 0.65	*0.17*
	Beta-endorphin	0.50	0.09 – 0.76	*0.02*	0.54	0.13 – 0.79	*0.01*
	Heart rate	0.07	-0.39 – 0.49	*0.78*	0.53	0.12 – 0.79	*0.01*
Endop vs 1h postop							
	Adrenaline	0.76	0.50 – 0.90	*< 0.0001*	0.75	0.46 – 0.89	*< 0.0001*
	Noradrenaline	0.02	-0.41 – 0.45	*0.94*	0.71	0.41 – 0.87	*< 0.001*
	Cortisol	0.37	-0.08 – 0.69	*0.10*	0.73	0.44 – 0.88	*< 0.001*
	Beta-endorphin	0.73	0.45 – 0.89	*< 0.0001*	0.90	0.75 – 0.96	*< 0.0001*
	Heart rate	0.79	0.54 – 0.91	*< 0.0001*	0.60	0.21 – 0.83	*< 0.01*
1h vs 3h postop							
	Adrenaline	0.45	0.02 – 0.74	*0.04*	0.76	0.49 – 0.89	*< 0.0001*
	Noradrenaline	0.19	-0.25 – 0.58	*0.38*	0.67	0.33 – 0.86	*< 0.001*
	Cortisol	0.53	0.12 – 0.78	*0.01*	0.66	0.31 – 0.84	*< 0.001*
	Beta-endorphin	0.35	-0.09 – 0.68	*0.11*	0.89	0.73 – 0.95	*< 0.0001*
	Heart rate	0.68	0.34 – 0.86	*< 0.001*	0.63	0.25 – 0.84	*< 0.01*

r, Pearson's correlation coefficient; *r-Time*, correlations calculated between individual time points; Awake, before pranaesthetic administration; Sedation, period of preoperative sedation; Endop, end of surgery; 1h (and 3h) postop, 1 (and 3) hours after surgery.

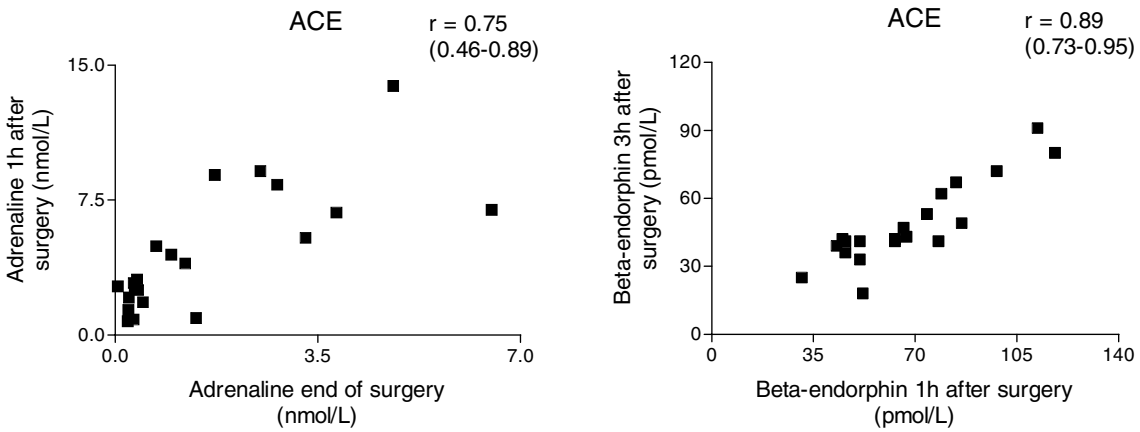

Fig. 5 Examples of time-related associations (*r-Time*, 95%CI) between concentrations of hormones measured during surgery and concentrations of hormones measured during the early recovery in individual dogs of Study 1. With both preanaesthetic groups (MED and ACE), the strongest correlations (*r-Time*) were recorded at the end of the operation and during the early recovery. See **Table 10** for further results.

Strong correlations existed between heart rates and the RMSSD, between the DFA1 and LF:HF ratios and between the RMSSD and HF-HRV (**Tables 8, 9**; **Fig. 9**). The time domain HRV indices also strongly correlated with each other (*r-24h* ≥ 0.84; *r-Indiv* ≥ 0.70). Especially in the ACE group, the correlations between the DFA2 and other cardiac indices were weak or nonsignicant (**Tables 8, 9; Fig. 9**).

For MAP and the cardiac data (heart rates, RMSSD, LF:HF ratios) and for MAP and plasma catecholamine concentrations, significant correlations were only detected twice. In the MED group, the MAP correlated with heart rates (r = 0.64; *P* = 0.002) and with the LF:HF ratios (r = 0.61; *P* = 0.003) during the period of preoperative sedation. These correlations, however, were markedly reduced by removal of a single data point (to r = 0.29 and to r = 0.17, respectively).

Table 11. Correlation coefficients (*r-Indiv, r-Time*) found to be affected by high-leverage data points. A change in $r > 0.10$ after removal of a single data point was noted (*r-before, r-after*).

Treatment	Comparison (r-Indiv)	Time	*r-before*	*r-after*
ACE	Adrenaline vs Noradrenaline	Endop	0.72	0.45
		3h postop	0.58	0.47
	Heart rate vs Adrenaline	Endop	0.49	0.21
		1h postop	0.60	0.47
	Heart rate vs Noradrenaline	Endop	0.69	0.50
		1h postop	0.67	0.51
MED	Cortisol vs Beta-endorphin	1h postop	0.57	0.41

Treatment	Comparison (r-Time)	Variable	*r-before*	*r-after*
ACE	Awake vs Endop	Cortisol	0.60	0.44
	Awake vs 1h postop	Adrenaline	0.58	0.37
		Noradrenaline	0.47	0.31
		Cortisol	0.63	0.49
	Awake vs 3h postop	Cortisol	0.62	0.36
	Sedation vs Endop	Noradrenaline	0.07	0.23
	Sedation vs 1h postop	Adrenaline	0.40	0.29
	Sedation vs 3h postop	Adrenaline	0.06	0.38
	Endop vs 1h postop	Adrenaline	0.75	0.83
		Noradrenaline	0.71	0.43
	Endop vs 3h postop	Cortisol	0.73	0.62
		Beta-endorphin	0.90	0.61
	1h vs 3h postop	Noradrenaline	0.67	0.41
MED	Endop vs 1h postop	Adrenaline	0.76	0.25

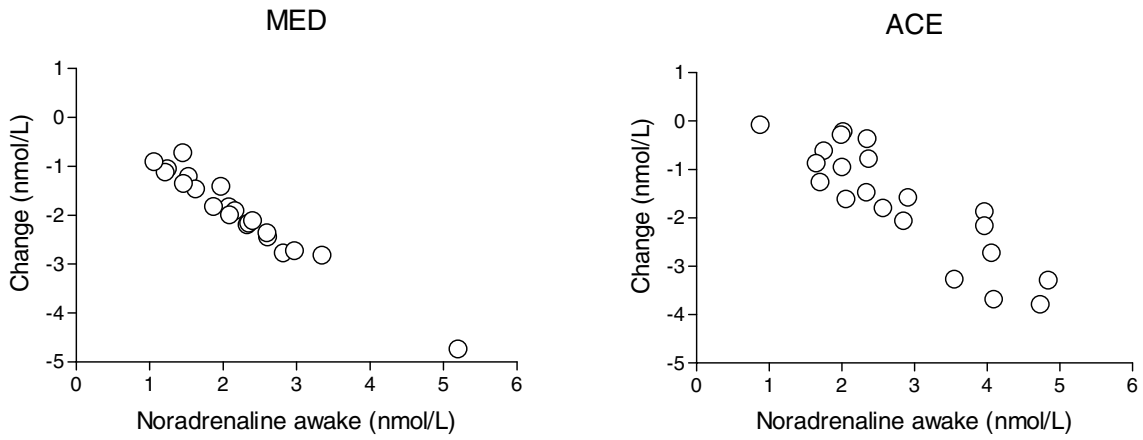

Fig. 6 Associations between the concentrations of circulating noradrenaline measured in awake dogs and the decrease noted in values during the period of preoperative sedation (Study 1). The extent of decrease was inversely related to the baseline values, i.e., to the values obtained before preanaesthetic administration. Dogs received either MED or ACE for preanaesthetic medication.

8.3. Immunological Measures (III)

On the day following surgery, blood neutrophil counts were elevated in all dogs. No significant alterations were detected for blood neutrophil CL responses (Figs 1 and 2 [III]). When compared with preoperative values, the greatest change in neutrophil CL was a 50% decrease recorded in a *Golden Retriever* (Fig. 2 [III], MED). The spontaneous respiratory burst activity (the CL measured without addition of zymosan) remained below the limits of detection throughout the study (background noise was 0.03 RLU). During postsurgical recovery, a wound infection was diagnosed in one dog; this was successfully treated with antibiotics.

Significant and strong correlations existed between the blood neutrophil counts and whole blood CL responses (**Table 8**; *r-Indiv* 0.52 to 0.76; $P \leq 0.02$). Significant correlations, however, were only twice detected between the immunological measures (blood neutrophil counts or neutrophil CL responses) and plasma hormone concentrations (adrenaline or cortisol). In the MED group, the blood neutrophil counts correlated with plasma cortisol on the day following the operation ($r = -0.64$; $P = 0.004$) and in the ACE group, the neutrophil CL responses correlated with plasma cortisol at the end of surgery ($r = -0.63$; $P = 0.005$).

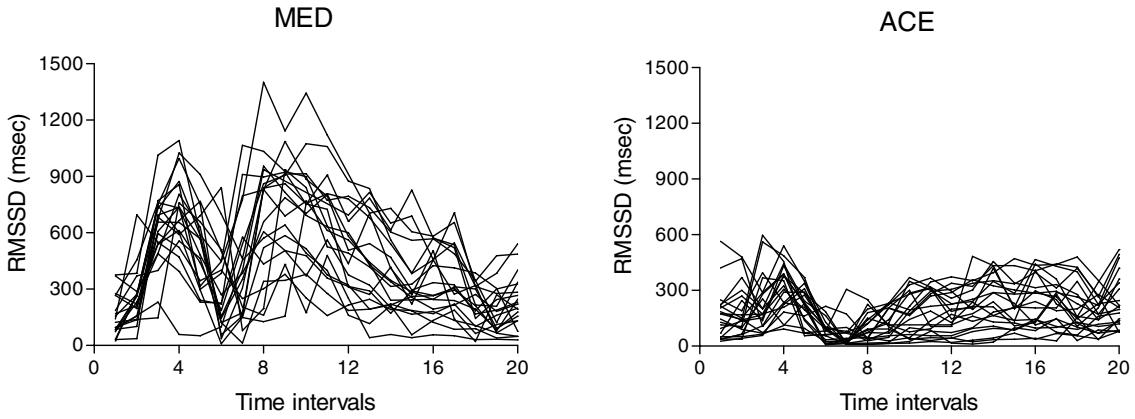

Fig. 7 Values for the RMSSD in individual dogs that received either MED or ACE for preanaesthetic medication and underwent OH under isoflurane anaesthesia (Study 1). The data are average values calculated for 30-minute time intervals. The 4th and 5th intervals are time periods related to the period of preoperative sedation; the 6th and 7th intervals are related to anaesthesia and surgery; data on the 20th interval represents the 6th hour after surgery. Increases in the RMSSD were greater in the MED-treated dogs.

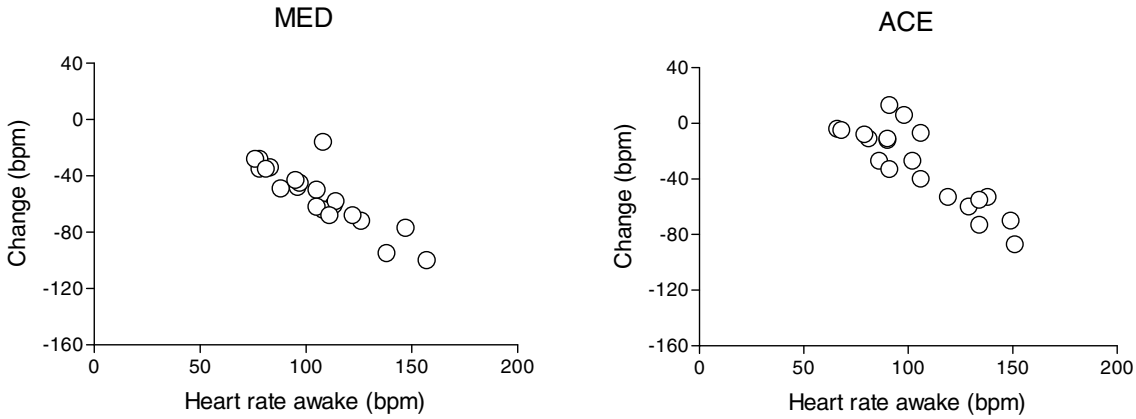

Fig. 8 Associations between heart rates measured in awake dogs and the decrease noted in values during the period of preoperative sedation (Study 1). The extent of decrease was inversely related to the baseline values, i.e., to the values obtained before preanaesthetic administration. Dogs received either MED or ACE for preanaesthetic medication.

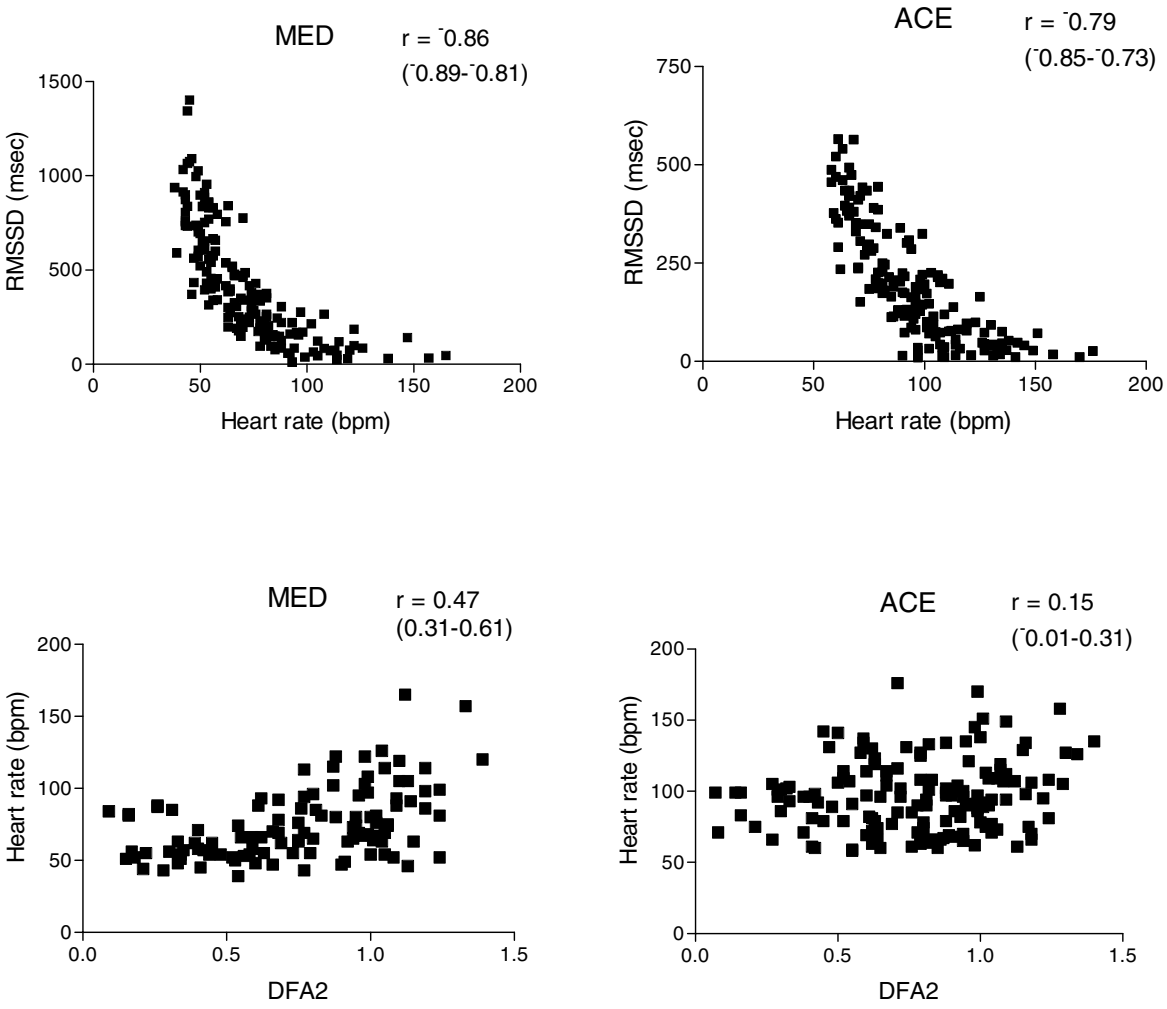

Fig. 9 Scattergrams showing the associations between heart rates and the RMSSD, and between heart rates and the DFA2 in dogs that underwent OH following preanaesthetic administration of MED or ACE (Study 1). The correlation coefficients (*r-24h*, 95%CI) were calculated using data gathered during the entire study period (24 hours; 8 measurement points). The correlations between heart rates and the RMSSD were strong, contrary to correlations between heart rates and the DFA2. Note the different scale used for the RMSSD in the ACE group. See **Table 8** for further results.

8.4. Behavioural Observations (I, II, IV, V)

8.4.1. Preoperative Patient Characteristics (IV)

On the basis of behavioural activities (frequencies of vocalisation, attempts to flee and manipulations of the environment), 3 groups of animals were defined: highly active (*high*; *n* = 13), intermediate (*int*; *n* = 15) and passive (*pass*; *n* = 13) dogs (**Fig. 10**; Table 1 [IV]). The highly active dogs vocalised and / or manipulated their environment in vigorous bursts that lasted at least 5 consecutive minutes. Panting and displacement behaviours (yawning and oral behaviours) were noted in nearly every animal (Fig. 1 [IV]). The panting and displacement behaviours always occurred during the first 5 minutes of observation and still existed in 80% of the animals after 25 minutes had elapsed from the moment the dog had been left alone in the hospital cage. When a person entered the cage, 12 dogs were not seen to approach her of their own accord (score 0; Table 2 [IV]); seven of these were passive dogs.

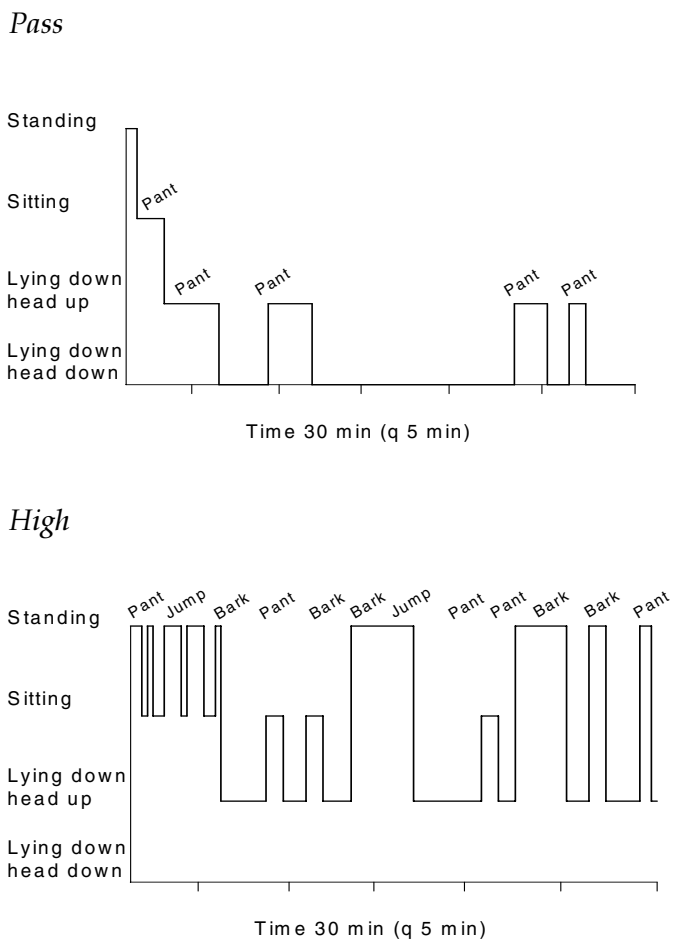

Fig. 10 Examples of behavioural activities displayed by dogs left alone in a hospital cage before administration of preanaesthetic medications and before elective OH (Study 1). *Pass*, dog regarded behaviourally as passive; *High*, dog regarded behaviourally as highly active. Bark, dog barking; Pant, dog panting; Jump, dog jumping against the cage wall.

During the period of preoperative hospitalisation, a tendency existed for higher heart rates among the highly active dogs, as compared to those regarded as behaviourally passive (Figs 2 and 3 [IV]). Lowest HRV, however, was recorded in two passive dogs (a Golden Retriever and an Airedale Terrier). These two animals were also the ones seen to stand for nearly (or over) half of the time monitored, and the Golden Retriever showed the highest number of oral behaviours (Fig 1 [IV]). During the 30-minute observation, no tendency (regression slope exceeding + 0.03) for the heart rate to decrease was detected in 17 dogs; four of these were passive, 7 intermediate and 7 highly active dogs. During surgery and in the early recovery, a tendency existed for higher heart rates (with both preanaesthetic groups) and higher circulating cortisol and noradrenaline (in the ACE group) for the dogs regarded preoperatively as behaviourally passive, compared with the highly active animals (**Fig. 11**).

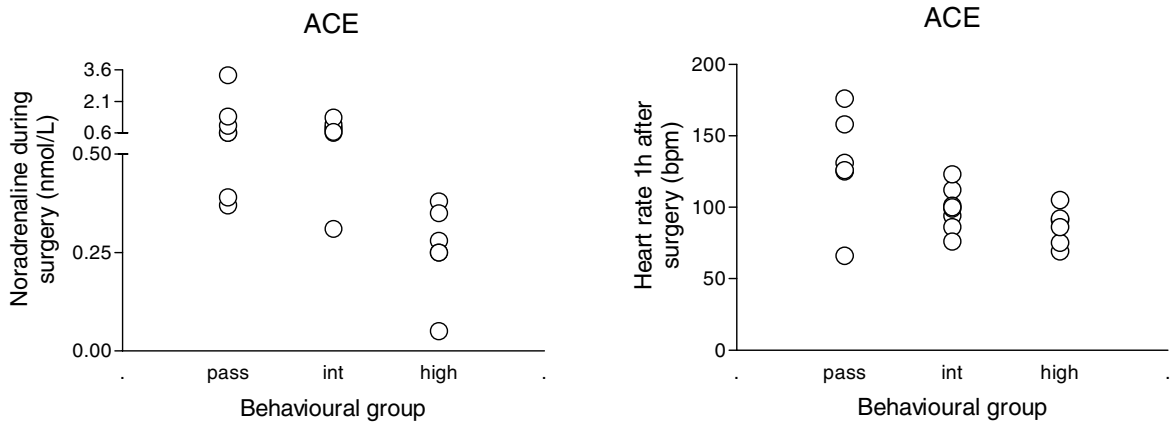

Fig. 11 Examples of distribution of hormonal data and heart rates during surgery and after surgery in individual dogs regarded preoperatively as behaviourally passive (pass), intermediately active (int), or highly active (high) (Study 1). The dogs received either ACE or MED for preanaesthetic medication and underwent OH under isoflurane anaesthesia. During surgery and after surgery, a tendency existed for higher concentrations of plasma noradrenaline and cortisol (ACE group) and for higher heart rates (MED and ACE groups) in dogs regarded preoperatively as behaviourally passive, compared with the so-called highly active animals.

8.4.2. Postoperative Behaviour Following MED and ACE Preanaesthetic Medication (I, II)

Dogs treated with MED scored lower in sedation at 3 and 6 hours after surgery. At 3 and 6 hours, the median [range] scores for sedation were 1 [1-2] and 0 [0-1] in the MED group and 2 [1-3] and 1 [0-3] in the ACE group, respectively. At 6 hours after surgery, the MED-treated dogs scored significantly higher in pain / distress (**Table 12**).

The times spent in an upright position during the 6 postoperative hours were similar between the two preanaesthetic groups. The median hourly minutes spent lying down during the first 5 postoperative hours ranged from 53 to 59 minutes in the MED group and from 57 to 59 minutes in the ACE group. During the 6th postoperative hour, the dogs were standing or sitting for 42 and 44 minutes with the MED and ACE groups, respectively.

Table 12. Pain/distress (PD) scores assigned at 1, 3 and 6 hours after surgery for dogs that underwent OH with MED or ACE preanaesthetic medication (Study 1). The data represent the numbers of dogs assigned a PD score of 0, 1, 2 or 3 (of the maximum 3). At 6 hours after surgery, the MED-treated dogs scored significantly higher in pain/distress. See Appendix [I] for detailed descriptions of scoring.

	PD score 0		PD score 1		PD score 2		PD score 3	
Time	MED	ACE	MED	ACE	MED	ACE	MED	ACE
1h	10	8	7	12	1	0	3	1
3h	7	10	10	10	1	1	3	0
6h	7	13	5	6	4	0	5	2

8.4.3. Owner-reported Behavioural Symptoms (V)

The most commonly-reported changes in behaviour in dogs recovering at home from day-case soft-tissue operations were alterations in the level of overall activity, playfulness, dependence (contact seeking), posture, movement and demeanour (Figs 1 and 2 [V]). The incidence of behavioural symptoms decreased over time and was higher in dogs after major surgeries (**Fig. 12**). Alterations in behaviour were also slightly, but significantly, more frequently reported for younger dogs (an increase in animal's age by 10 years added 2 symptoms) (**Fig. 13**). The incidence of behavioural symptoms increased with increasing pain VAS scores (an increase in VAS score by 10 mm added 2 symptoms).

On the basis of kappa coefficients, no pair of reported changes in behaviour reflected the same phenomenon. None of the kappa coefficients showed excellent agreement (value of > 0.75), and the majority of them (90%) indicated poor agreement (value of < 0.40). The majority of the behavioural signs that the owners described to indicate of the presence of postoperative pain in their dog were related to vocalization (46%), restlessness (26%) and differences in movement (24%). Excitement was the only reported adverse event during the period of hospitalisation and occurred in two dogs.

On Day 1, the owners indicated pain VAS scores of over 30 mm (on a 100-mm scale) for approximately 60% of the dogs (Fig. 3 [V]). The VAS scores on the following days were significantly lower (**Fig. 14**).

Owners' ratings for the level of stress they themselves experienced as their dog was facing an operation were not found to affect the reported incidence of behavioural symptoms. The ratings of owners for the level of stress were not affected by previous surgeries performed on the dog or by the type of operation the dog was now assigned to (**Fig. 15**).

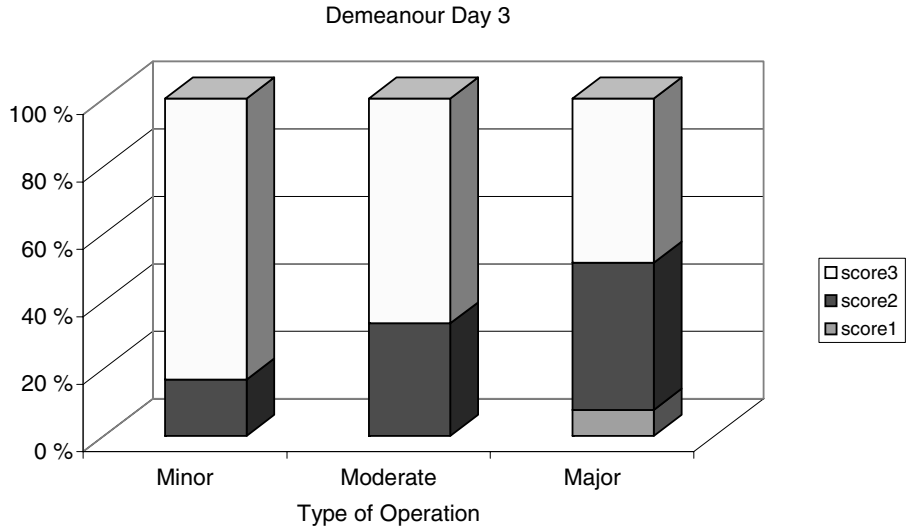

Fig. 12 The percentage of dogs (n = 96) recovering at home from day-case soft-tissue operations with owner-assigned scores to indicate markedly (score 1) or moderately (score 2) altered demeanour, or normal demeanour (score 3) (Study 2). Type of operation: major, abdominal surgery; moderate, castration, mammectomy; minor, skin tumor removal. Day 3 represents the 2nd day following the operation. Alterations in behaviour were more frequently reported for dogs recovering from major surgery.

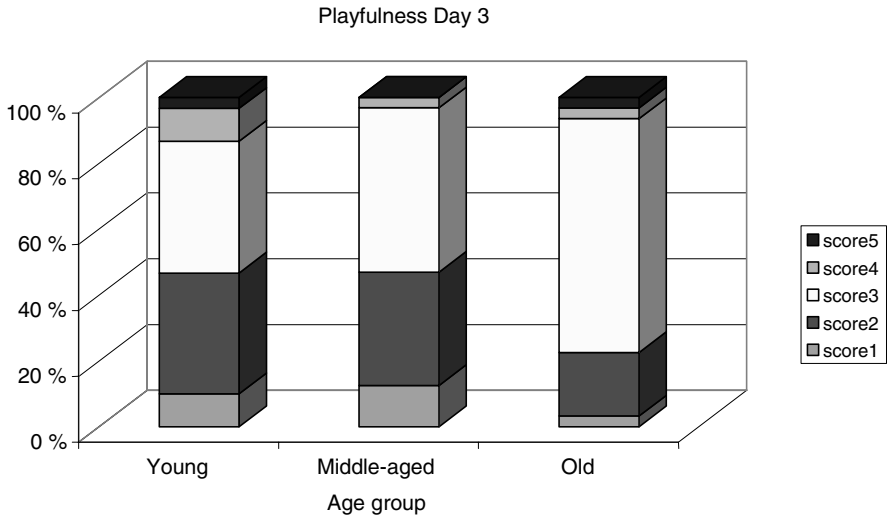

Fig. 13 The percentage of dogs (n = 96) recovering at home from day-case soft-tissue operations with owner-assigned scores to indicate decreased playfulness (scores 1 and 2), increased playfulness (scores 4 and 5), or normal level of playfulness (score 3) (Study 2). Age group: young, dogs aged 1 to 3 years (approx. 33% of the animals); middle-aged, dogs aged 4 to 8 years (33%); old, dogs aged 9 to 13 years (33%). Day 3 represents the 2nd day following the operation. Alterations in behaviour were more frequently reported for younger dogs.

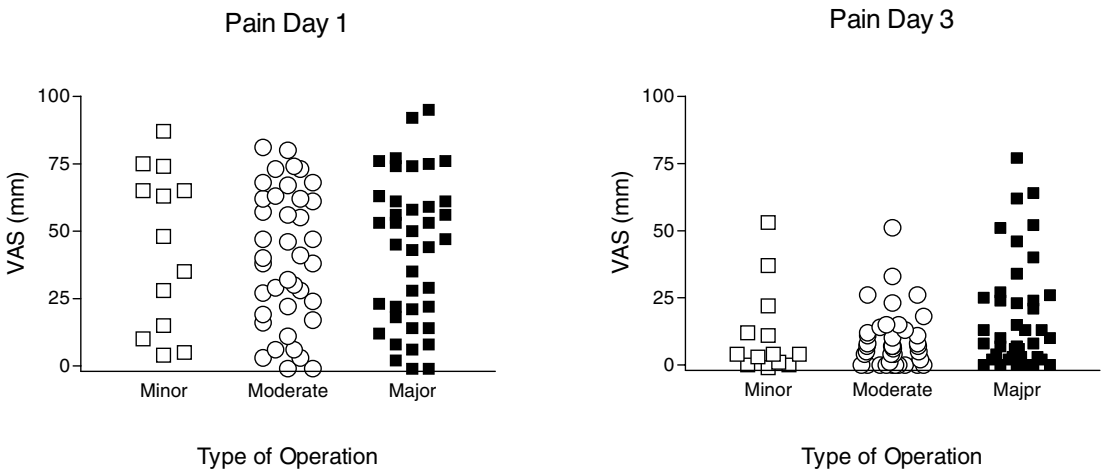

Fig. 14 The VAS pain scores assigned by owners for dogs recovering at home from day-case soft-tissue operations (Study 2). Type of operation: major, abdominal surgery; moderate, castration, mammectomy; minor, skin tumor remonal. Day 1 represents the day of operation; Day 3 represents the 2nd day following the operation. The owner-rated scores for pain decreased over time and were higher for dogs recovering from major surgery.

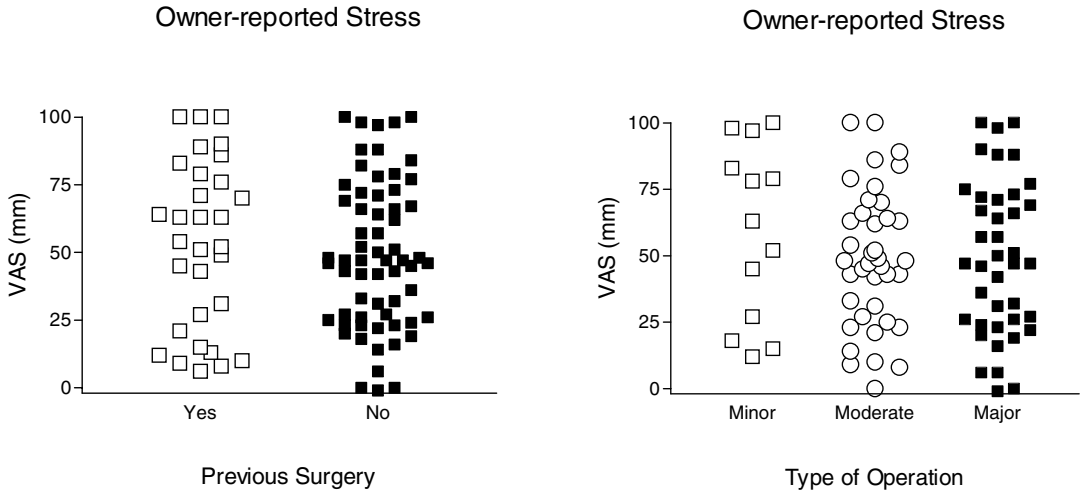

Fig. 15 The VAS scores for the level of stress the owners themselves experienced while their dog was facing a day-case soft-tissue surgical operation (Study 2). The scores for stress were not affected by previous surgeries performed on the dog or by the type of operation the dog was now assigned to. The owner-reported stress was not found to affect the reports on dog's postoperative behaviour.

9. DISCUSSION

9.1. Plasma Hormone Concentrations (I)

9.1.1. Differences between MED and ACE Preanaesthetic Administration

The plasma hormone measurements revealed lower perioperative concentrations of circulating catecholamines and cortisol for the medetomidine-treated dogs compared with acepromazine preanaesthetic administration (I). Intraoperative increases in plasma beta-endorphin and cortisol were recorded for both preanaesthetic groups.

The decrease in plasma noradrenaline and adrenaline noted in the animals treated with medetomidine reflects a common finding with α_2 agonist administration. Circulating catecholamines have been reported to decrease by up to 90% in response to α_2 agonist-induced sedation in humans (Kallio *et al.* 1989) and in dogs (Kuusela *et al.* 2001) and similar influences have been noted in surgical patients (Benson *et al.* 1991 (cats), Aho *et al.* 1992 (humans), Benson *et al.* 2000 (dogs)). In a study on laboratory Beagles (Ambrisko & Hikasa 2002), the sympatholytic effects of medetomidine (20 µg kg^{-1} IM) lasted up to 3 hours, and such actions could have accounted for the duration of the decrease noted here. However, medetomidine and acepromazine only formed part of the medication administered in the current investigation, which complicates the prediction of their pharmacokinetic profiles. Since no control group was included, the estimation of the extent of perturbations is also speculative, and 40% decreases in plasma adrenaline have been reported for Beagle dogs when left undisturbed (Kuusela *et al.* 2001).

The sympatholytic actions related to α_2 agonist administration are viewed to reflect the influences of these agents on the brain areas involved in the control of vigilance and sympathetic outflow, in addition to the effects on peripheral noradrenaline release (Langer 1981, Khan *et al.* 1999). The main target area of the sedative / hypnotic effects of α_2 agonist actions is presumed to be the locus coeruleus (LC) situated in the pons, where the stimulation of α_2 adrenoceptors decreases the firing rate of adrenergic neurons (Svensson *et al.* 1975, Callado & Stamford 2000). The LC projects to several other brain areas, such as hypothalamus, amygdala and cortical structures (Sullivan *et al.* 1999), and is considered to display a central role in the regulation of the level of arousal (Nelson *et al.* 2003). With acepromazine administration, the degree of CNS depression achieved is most likely of a different magnitude, although the blockade of dopamine receptors also affects various CNS sites including those involved in the generation of emotions and motor activity (Kaplan & Sadock 1998,

Gross 2001). With respect to circulating catecholamines, the pharmacological properties of antidopaminergic actions have been speculated to even enhance the SNS activities due to attenuation of the restrictive effects of dopaminergic stimulation on norepinephrine release (Langer 1981, Mannelli *et al.* 1988). In an *in vivo* canine preparation, Sumikawa *et al.* (1985) demonstrated increased efflux of adrenaline and noradrenaline from adrenomedullary tissues after local application of droperidol.

In the current study, no consistent potentials for marked increases in plasma catecholamine concentrations could be demonstrated for either of the preanaesthetic protocols, when the values obtained at the first measurement were used for comparison (Fig. 1 [I]). Up to 9-fold increases in circulating catecholamines have been documented in bitches undergoing OH under isoflurane anaesthesia with no concurrent use of sedative or analgesic medications (Benson *et al.* 2000). The first measurement point of the current trial, however, did not truly represent basal activity and the results must be reflected upon such settings. In addition, especially in the ACE-treated dogs and at 1 hour after surgery, the confidence intervals for the (mean) catecholamine concentrations were wide, reflecting potentials for marked differences between individual responses. During the period of preoperative sedation, anaesthesia and surgery, the circulating noradrenaline decreased even in the ACE-treated dogs, possibly due to the effects of anaesthetic administration on sympathetic firing (Seagard *et al.* 1984) or diminished locomotor activity, or both. Compared with adrenaline, some authors have suspected the circulating noradrenaline to be more readily affected by physical activity (Axelrod & Reisine 1984, Kaji *et al.* 1989).

Compared with their intraoperative levels, a sharp increase in plasma catecholamine concentrations was noted in the ACE-treated dogs during the early postoperative recovery, while plasma cortisol showed no concurrent decreasing tendencies. At this time the animals were considered physically inactive and in many individuals, the circulating concentrations of adrenaline were even higher than those observed at the first sampling (**Fig. 3**). Postanaesthetic delirium and possible pain are potential contributors to neurohumoral arousal and small concentrations of inhalation anaesthetics, likely to exist in the body during the early recovery, have also been suspected to possess anti-analgesic properties (Zhang *et al.* 2000). Preanaesthetic administration with an α_2 agonist has attenuated the increases in stress-related hormones, as well as the cardiovascular responses during early recovery (Proctor *et al.* 1991, Benson *et al.* 2000, Talke *et al.* 2000). The hormonal characteristics registered for the ACE group in the present investigation may reflect lesser abilities to affect patients' statuses during the early stages of the postoperative period; however, they

may also represent the ability to counteract the effects of anaesthetic administration. In the present investigation, acepromazine was combined with partial opioid agonists and NSAIDs to enhance sedation and analgesia, and different end-results might have been obtained if full opioid agonists had been utilised (Cornick & Hartsfield 1992, Wagner 2002b). In dogs, a combination of acepromazine and oxymorphone resulted in similar analgesic effects, compared with medetomidine alone (Barnhart *et al.* 2000), and in humans, preanaesthetic administration with dexmedetomidine or oxycodone was associated with similar intraoperative cortisol concentrations (Aho *et al.* 1992). In the current trial, the decision to administer buprenorphine only some 15 minutes prior to discontinuation of isoflurane administration might also have affected the characteristics of postsurgical recovery. Less than optimum effects on sedation and analgesia might have been achieved as the peak effects of the drug may not be reached until 60 minutes following administration (Wagner 2002b).

As evidenced in the increased plasma cortisol and beta-endorphin concentrations, neither of the anaesthetic regimens prevented enhancements in hypothalamic-pituitary-adrenal axis (HPA axis) activities (Fig. 1 [I]). With respect to α_2 agonist administration, despite their abilities to lower plasma catecholamines and despite some reports of decreased circulating cortisol following α_2 adrenergic agonist-induced sedation (Kallio *et al.* 1988), perioperative responses of plasma cortisol have been observed in several studies (Aho *et al.* 1992, Benson *et al.* 2000, Ko *et al.* 2000). Although some evidence further suggests that α_2 adrenergic agents may, to some extent, affect steroidogenesis (Maze *et al.* 1991) and depression of central noradrenergic activity can attenuate stress-related cortisol responses (Ziegler *et al.* 1999), α_2 agonist administration in humans in the clinical settings has not prevented plasma cortisol responses to ACTH administration (Venn *et al.* 2001). Such results, along with the observations of the present investigation, may demonstrate the ability to mount these types of responses even when α_2 agonists are utilized, contrary to some concerns existing with regard to capabilities for sympathoadrenal arousal. In a surgical patient, afferent nerve impulses originating from the site of trauma and the inflammatory mediators are among the known stimulants of adrenocortical activities.

With the ACE-treated dogs, the antidopaminergic actions could also have contributed to the higher cortisol concentrations, instead of these characteristics arising due to mere stress-related influences. A study on dogs reported increased plasma cortisol after haloperidol administration (Kemppainen & Sartin 1986) and dopaminergic pathways are presumed to inhibit the release of POMC -derived peptides in canines (Sharp *et al.* 1982). Overall, the plasma cortisol responses

documented for both anaesthetic regimens studied here resembled those observed in earlier investigations on dogs with various types of surgery and perianaesthetic medication (Church *et al.* 1994, Hansen *et al.* 1997, Grisneaux *et al.* 1999).

The peak in plasma beta-endorphin in the middle of the operation with both MED and ACE-treated dogs may represent a protective physiological response to tissue trauma and nociceptive stimuli. In humans, perioperative increases in circulating beta-endorphin have been observed at the time of surgical stimulation, both with and without α_2 agonist preanaesthetic administration (Cohen *et al.* 1981, Aho *et al.* 1992). These events have further been attenuated with local anaesthetic application (Gordon *et al.* 1997). In dogs, the perioperative behaviour of plasma beta-endorphin has rarely been documented, and its role as a marker of intraoperative responsiveness should be further evaluated. In horses, this hormonal index is considered as a "sensitive marker" of stress-related responses (Taylor *et al.* 2000), and in the data collected from laboratory Beagles (Kuusela *et al.* 2003), anaesthesia alone (following dexmedetomidine premedication) did not seem to affect plasma beta-endorphin concentrations. However, the exact role of circulating beta-endorphin in, for instance, analgesia is still somewhat controversial and the current study could not separate the effects of various physiological perturbations on plasma beta-endorphin concentrations.

9.1.2. Results from Correlation Coefficients

Correlation coefficients were calculated for selected hormonal indices and for plasma catecholamines and heart rates documented in Study 1. The calculations were executed in appreciation of the fact that the results would lack the abilities to reflect causal relationships and that the associations, as now examined, would be expected to display linear characteristics.

Overall, the correlation coefficients indicated modest or even weak relationships for the different hormonal measures (**Fig. 4**; **Tables 8, 9**), implicating the complex nature of perioperative nominators for stress-related statuses. With the MED-treated dogs, the overall association (*r-24h*) between plasma noradrenaline and adrenaline was stronger, compared with ACE, possibly reflecting the greater extent of decrease induced in values. When using repeated measurements to calculate correlation coefficients, artificially high *r*-values may be achieved due to clustering of data collected at different measurement points.

The dissimilar behaviour of circulating cortisol and beta-endorphin resembled earlier observations on humans (Cohen *et al.* 1981) and may reflect the role of neuronal

factors and the pars intermedia of the hypophysis in contributing to increases in endogenous plasma opioids, as compared with the CRF-induced concomitant release of beta-endorphin and ACTH from the anterior pituitary gland. Peak-like appearances, however, have also been described for perioperative ACTH secretion (Roth-Isigkeit & Schmucker 1997), and this hormone was not followed in the present investigation.

Besides differing in their perioperative behaviour, the two catecholamines also failed to strongly correlate with the heart rate data (**Tables 8, 9**). Similar findings have previously been made in clinical studies in humans (Daniel *et al.* 1998), and the state of generalised SNS activation may differ from that affecting the cardiovascular system in surgical patients. In humans, blood lymphocyte beta adrenoceptors have been noted to undergo down-regulation in association with surgical operations (Amar *et al.* 1998) and such finding may further underline the potentials for complex relationships between the extent of neuronal firing and end-organ responses. The use of plasma catecholamines as indices of ANS activity may also be obscured by their short half-lives and uptake by neuronal endings resulting in difficulties in obtaining representative samples.

With both preanaesthetic protocols, the strongest time-related correlations (*r-Time*) for the hormonal (and heart rate) data were recorded at the end of the operation and during the early recovery (**Fig. 5**; **Table 10**). During these times, the values measured at later time points were significantly correlated with the values obtained at earlier observations. Despite the correlation coefficients not adequately reflecting causal relationships, these results could have implications when aiming at improved perioperative care. Sustained nature of individual patient characteristics or intraoperative responsiveness could account for the extent of neurohumoral arousal following an operation and in clinical settings, the described associations could emphasise the importance of the qualities of intraoperative care and be of assistance when planning the management of individual animals during the postoperative phase. The moderately strong correlations (r^2 = 0.5 to 0.6) might also be needed to be notified when evaluating the results of clinical studies. Breslow *et al*. (1993) described similar associations for humans and reported significant relationships between intraoperative concentrations of circulating noradrenaline and the values obtained at 1 hour after surgery.

Contrary to the characteristics of the postoperative period, most of the hormonal measures recorded from awake dogs or during the period of preoperative sedation were not found to significantly correlate with the values obtained at later evaluations. With both preanaesthetic protocols, the extent of decrease in plasma

hormone concentrations (and heart rates) during the period preoperative sedation was inversely related to the values obtained before preanaesthetic administration (**Figs 6, 8**). Roizen *et al.* (1981) described similar behaviour for various physiological indices measured at the time of anaesthetic administration in humans, and further referred the induction of anaesthesia as a "great equalizer". These observations may imply the non-deterministic role of preoperative physiological arousal in contributing to an individual's characteristics at later evaluations. For horses, however, the plasma hormone concentrations in awake animals have been suggested to influence the physiological (and sedative) state after α_2 agonist administration (Raekallio *et al.* 1992), and further studies await the characterisation of this phenomenon in surgical patients. The present investigation did not use frequent sampling, did not investigate dose-dependent effects and did not specifically determine the quality of preoperative sedation, which indicates the clinical value of preanaesthetic agents. Interestingly, with both preanaesthetic groups, the plasma beta-endorphin was among the few indices to show frequent time-related associations (**Fig. 5**; **Table 10**) and possibly, this might reflect the individual characteristics described for endogenous opioid secretion (Lei Yu 2004). Whether such phenomenon would further transfer into differences between individual animals with respect to surgery-related pain and responses to analgesics might be worthy of future investigation.

9.1.3. Conclusions on Current Findings

In conclusion, the results from the hormonal measurements documented differences in perioperative neuroendocrine characteristics for the medetomidine and acepromazine preanaesthetic protocols and demonstrated different perioperative behaviour for individual hormones. Although the exact contributors to the data obtained are difficult to determine, differences in the overall state of CNS depression, in the abilities to attenuate the influences of both psychological and physical stimuli, and certain pharmacological features of medetomidine and acepromazine themselves most likely played a role in the characteristics recorded.

Including medetomidine as part of the preanaesthetic regimen seemed more effective in preventing perioperative neurohumoral arousal, but the clinical significance of this finding is yet to be determined. Overall, the optimum levels for neurohumoral responses are yet to be fully defined for surgical patients and the role of the hormonal responses as markers of exaggerated arousal, inadequate depth of anaesthesia or vital protective physiological functions remains to be documented. The current study failed to demonstrate differing effects on the incidence of ventricular dysrrhythmias or on the immunological responses for the two

preanaesthetic groups, despite the differing circulating hormone concentrations. Nevertheless, for instance, exaggerated SNS activities are without a doubt a phenomenon possessing potentials to produce adverse effects, especially with suspectable patients, and are best avoided.

Further studies may be warranted with acepromazine to examine in more detail the nature of perioperative responsiveness, especially regarding the events taking place in the early recovery and when using acepromazine in conjunction with other perianaesthetic medications. With medetomidine, defining its role as a perianaesthetic sedative or as an anaesthetic adjuvant in veterinary patients would probably benefit from future studies in which the effects of different dosing on the physiological states would be evaluated in order to optimise the beneficial influences without causing exaggerated effects. The narrow confidence intervals documented for plasma catecholamines in the MED-treated dogs in the current trial, however, would seem to indicate potent effects on these parameters in the general population.

9.2. Perioperative Cardiovascular Activities (I, II)

9.2.1. Changes in Heart Rates and MAP

The extent of bradycardia observed with the MED-treated dogs during the period of preoperative sedation was of similar magnitude as previously described for medetomidine alone, when administered to dogs at doses of (or above) 5 $\mu g\ kg^{-1}$ (IV, Pypendop & Verstegen 1998). Similar heart rates as those commonly observed with α_2 agonist administration have also been documented for (presumably) sleeping dogs (Moise 1999) and the fact that the minimum heart rates registered for both preanaesthetic protocols of the current trial during the postoperative night were higher might reflect the effects of anaesthesia, surgery and hospitalisation on postoperative physiological states. However, control aECG recordings would be needed to confirm these remarks. Interestingly, for both preanaesthetic protocols, the minimum heart rates recorded during the night were close to the “bradycardia tolerance” often applied to small animal patients, i.e., 60 beats per minute, and most of the maximum heart rates did not occur during the operation, but instead were noted in awake dogs and during the preoperative phase. These observations may characterise the extent of perioperative perturbations for both preanaesthetic regimens.

The bradycardia documented for the MED-treated dogs was not unexpected and was probably related to medetomidine’s sympatholytic effects as well as to the effects on cardiac efferent vagal modulation. $Alpha_2$ adrenoceptors are represented in the brain

areas involved in modulating efferent vagal outflow (Robertson & Leslie 1985) and the imidazoline structure of α_2 agonists has also been implied as a contributor to their vagally-mediated influences (Kamibayashi *et al.* 1995). In addition, diminished sympathetic activities *per se* may allow more complete cardiac vagal modulation due to attenuation of the restrictive interactions at the sinoatrial node (Levy 1971, Hall & Potter 1990). The present trial could further not differentiate between the receptor-specific effects and those arising from perturbations induced in circulation, such as changes in venous capacitance.

The ambulatory ECG recordings documented neither profound decreases in heart rates nor persistent tachycardic episodes for the ACE-treated dogs. Acepromazine has not been profiled as an agent causing exaggerated influences on the heart rate of an average dog, although, in theory, tachycardia may follow high doses and lowered blood pressures (Brock 1994, Stepien *et al.* 1995). In the current trial, the opioids administered most likely further contributed to the cardiovascular characteristics with both preanaesthetic protocols.

The more stable intraoperative blood pressures (MAP) registered for the MED-treated dogs reflected the findings of earlier studies concluding higher and more stable intraoperative blood pressures for dogs treated preoperatively with medetomidine, when compared with saline (Ko *et al.* 2000) or acepromazine (Ängeby *et al.* 2001) preanaesthetic administration. Especially in humans, the perioperative cardiovascular stability is among the issues specifically listed as advantageous with α_2 agonist agents, although potentials for profound bradycardia and hypotensive effects are also appreciated (Khan *et al.* 1999, Muntazar & Kumar 2004). The greater increases seen in the intraoperative MAP with the ACE preanaesthetic protocol could have resulted from lighter levels of anaesthesia provided, from greater abilities to mount these types of responses or from the additional boluses of fluids that were administered to these animals. The vasodilatory properties of acepromazine together with the greater dosing of anaesthetics probably contributed to the decreases in MAP observed after the induction of anaesthesia.

9.2.2. Indices of HRV

The indices of HRV were documented to expand the evaluation of perioperative ANS activities and cardiac autonomic modulation. Different analysis techniques were further used to examine the different aspects of beat-to-beat interval behaviour. Similarly to obtaining more detailed photographs of the characteristics of a given landscape, the analysis of interbeat intervals can also be considered to allow

characterisation of the more (and more) detailed nature of the heart rate behaviour embedded in the average heart rate itself.

9.2.2.1. Time and Frequency Domain Measures

Unlike the weak correlations existing between heart rates and plasma catecholamine concentrations, strong correlations were found between heart rates and the time domain indices of HRV (**Fig. 9; Tables 8, 9**). The RMSSD also strongly correlated with the HF-HRV corroborating with conclusions of earlier studies on humans and on dogs on the existing relationships between the time- and frequency domain measures (Kleiger *et al.* 1992, Calvert & Wall 2001, Picker *et al.* 2001). Both the RMSSD and the HF-HRV characterise short-term perturbations imbedded in the beat-to-beat interval behaviour and are considered strongly influenced by vagal modulation (Kleiger *et al.* 1992, Task Force 1996).

Since the decreased HRV also closely followed the intraoperative increases in heart rates with both preanaesthetic groups of the current trial, even in the presence of unaltered plasma catecholamine concentrations, it could further be stated that vagal withdrawal seemed to represent one of the surgery-related stress responses notable in current dogs. Decreased HRV has been a common finding in studies on human surgical patients whether using short-term (Schubert *et al.* 1997, Amar *et al.* 1998) or long-term (24 hours) (Marsch *et al.* 1994, Laitio *et al.* 2000) recordings, and in anaesthetised dogs (Picker *et al.* 2001). The decreases in HRV may represent the effects of lowered blood pressures, nociception or other anaesthesia-related influences on the heart rate characteristics. Increased values of the time domain indices noted with both preanaesthetic groups during the period of preoperative sedation would further implicate the occurrence of enhanced cardiac efferent vagal modulation, with markedly more pronounced effects achieved with MED preanaesthetic administration (**Fig. 7**; Fig. 3 [II]). The incidence of AV blocks was also higher with the MED-treated dogs (Table 1 [II]).

The lack of control of perioperative perturbations in respiration, however, confounds the detailed examination of the contributors to the beat-to-beat interval characteristics in the present trial. Respiration is known to modulate the nature of HRV and its influences are difficult to separate from those directly related to pharmacological interventions or those of other physiological entities. Respiration-related perturbations have been documented in the discharge of efferent cardiac vagal motoneurons (Porges 2001, Rentero *et al.* 2002), but it has also been speculated that the characteristics of respiration would affect the HRV independently of changes in efferent cardiac vagal activity. The ability of the sinoatrial node to respond to

changes in the concentrations of acetylcholine would be more complete with slow respiratory rates, whereas with higher respiratory frequencies (when the bursts of acetylcholine release would appear at accelerated rates) the effects of the neuromodulator would be less completely transferred into intracellular events (Eckberg 1997).

Respiration, however, is not the sole modulator of HRV and decreased heart rate variability during anaesthesia has also been documented in the (few) trials where ventilation has been controlled (Kato *et al.* 1992, Schubert *et al.* 1997, Picker *et al.* 2001). In addition, in the experimental studies on humans where the changes in respiration have been used as investigatory tools, not all the time domain indices have always been found similarly affected (Penttilä *et al.* 2001). In the current trial, at least the respiratory rates appeared similar between the two preananesthetic groups, as evaluated using the data derived from medical records, which might validate at least the comparisons made. For instance, the (mean, SD) respiratory rates during anaesthesia and within the first postoperative hour were 14 (12) and 15 (6) breaths per minute for the MED-treated dogs and 11 (8) and 12 (14) breaths per minute for the ACE-treated dogs.

In addition to the higher heart rates and lower time domain HRV documented for the ACE-treated dogs, these animals also displayed markedly elevated intraoperative LF:HF ratios (Fig. 3 [II]). This finding would suggest a change in cardiac autonomic balance towards a more pronounced sympathetic dominance, and worth noticing is the fact that such features were recorded without concurrent episodes of clinically significant tachycardia, which might have alarmed the clinician of the ongoing perturbations. Although not representing a perfect measure for cardiac autonomic modulation, the LF:HF ratio has been considered to provide rate-independent data on the characteristics of cardiac autonomic influences (Zaza & Lombardi 2001), and in the current trial the enhanced cardiovascular sympathetic modulation associated with surgical stimuli seemed also reflected in the increased MAP in the ACE-treated dogs. Unfortunately, however, validated measures for cardiac sympathetic modulation are lacking in the HRV indices (Eckberg 1997) and further studies are needed to confirm these remarks. For instance, slow breathing could have affected the LF:HF characteristics in the dogs of the current trial with a decrease in respiratory rates shifting the power embedded in the respiration-related oscillations of HRV from the HF-range more towards to the LF-range.

With the MED-treated dogs, no significant increases could be demonstrated for the intraoperative LF:HF ratios despite the data suggesting occurrence of vagal withdrawal and despite the strong correlations between the time domain indices and

the HF-HRV. In addition to a number of unidentified causes, this finding could also be explained by the contribution of vagal influences to the behaviour of the LF-HRV, and up to 90% decreases in the LF-component have been documented following atropine administration in dogs (Houle & Billman 1999). Toledo *et al*. (2003) have further speculated that a change in vagal activity without significant changes in sympathetic activity would result in parallel changes in the LF and HF peaks.

Despite the moderately strong correlations between heart rates and the time domain indices of HRV, this relationship also seemed to display nonlinear characteristics, as apparent in the scattergrams drawn for the heart rate and the RMSSD data with both MED and ACE –treated dogs (**Fig. 9**). In their editorial, Zaza and Lombardi (2001) discuss the properties of the dependence of the RR interval length on neural activity and conclude that the sinus cycle length (the RR interval) would be a nonlinear function of the neuromediator concentrations. At increased acetylcholine levels, the response of the pacemaker cells seems to be accelerated, resulting in a curve-like shape describing the associations between the extent of neural stimulation and the increases noted in sinus cycle length. The data presented in the article of Zaza and Lombardi (2001) markedly resembles current remarks and together with the earlier statements on the effects of respiration, these characteristics further underline the complexity embedded in the HRV data as an indirect measure of cardiac neural modulation.

9.2.2.2. Characteristics of Nonlinear Indices

The nonlinear indices were included in the analysis of HRV to characterise the heart rate behaviour using measures not profoundly restricted by pre-defined analysis limits (the frequency domain measures) and to expand our understanding of the qualitative nature of beat-to-beat interval behaviour not assessable by simple statistics (the time domain measures). Before preanaesthetic administration, animals in both preanaesthetic groups displayed a scaling exponent close to 1.0, which has been described for healthy humans and is considered to indicate fractal-like behaviour of beat-to-beat intervals (Peng *et al.* 1995, Iyengar *et al.* 1996, Laitio *et al.* 2000).

With both preanaesthetic groups, the DFA1 increased during anaesthesia and operation and was closely correlated with the LH:HF ratio. This association resembled earlier findings on humans (Tulppo *et al.* 2001a) and administration of an anticholinergic has been noted to increase the DFA1 in experimental studies in humans (Tulppo *et al.* 2001b). To a certain extent, the estimates of RR interval dynamics obtained from the DFA analysis are related to their frequency-specific

nature since the behaviour of individual beats is investigated using selected time windows. However, the DFA measures are also considered more robust against the effects of uncontrolled study settings (Huikuri *et al.* 2003).

The dissimilar behaviour of scaling exponent α2 (DFA2) compared with the other cardiac data is an interesting finding and may further underline the complex nature of the nominators for different estimates of beat-to-beat interval characteristics. In investigations performed on humans, different perioperative perturbations have been described for different features of heart rate variability, including its nonlinear indices (Laitio *et al.* 2000). With both of the anaesthetic regimens studied here, the DFA2, to some extent, followed the clinical course of events, as the values tended to decrease during sedation, anaesthesia and surgery. Some characteristics of HRV have been postulated to reflect CNS changes related to anaesthetic depth (Pomfrett 1999), and the fractal-like behaviour of the heart rate has further been suggested as a representative of a central organising principle responsible for physiological functions (Goldberger 1996). However, the exact mechanisms behind the perturbations detected in the present investigation remain unresolved, and the inability to obtain measures for every individual animal during the DFA analysis further indicates that future assessments are needed to more precisely characterise the heart period fluctuations in the canine RR interval data. With respect to current observations, the results may only apply to the acepromazine preanaesthetic protocol, since difficulties were met in the analysis of DFA especially with the medetomidine-treated dogs. However, it is also possible that the medetomidine administration could be associated with different relationships between various HRV measures, and whether such differences would indeed indicate the existence of different physiological states or merely different behaviour with respect to mathematics need further determination. Unfortunately, the DFA-values can also be affected by the pattern of respiration (Penttilä *et al.* 2003), which was not controlled in the current investigation.

9.2.3. Perioperative Arrhythmias

One of the purposes of the continuous ambulatory ECG recordings utilised in the current trial was to document, in detail, the occurrence of VPCs. Perturbations in the ANS, mainly those related to enhanced sympathetic activities or decreased cardiac vagal influences, have been considered as inducers of ventricular events (Malik & Camm 1994), and the sensitivity to these types of arrhythmias may also increase with mere psychological stressors (Lown *et al.* 1973).

The incidence of VPCs documented in the present trial resembled the findings of previous studies on overtly healthy dogs when utilising 24-hour aECG recordings to characterise the frequencies of ventricular arrhythmias during normal daily activities in client-owned dogs (Meurs *et al.* 2001), or when examining the incidence of VPCs in anaesthetised laboratory Beagles (Kuusela *et al.* 2002). As the animals studied here were clinically healthy and the operation of moderate intensity, the low incidence of VPCs was not completely unexpected. It may further underline the moderately benign nature of the perioperative perturbations induced in bodily homeostasis, with the two preanaesthetic protocols otherwise resulting in marked differences in the cardiovascular and neurohumoral statuses. Due to their seemingly infrequent nature in overtly healthy dogs and with elective operations, larger scale studies are necessary to examine the differences in perioperative VPCs between different perianaesthetic medications. In the present investigation, however, one individual animal (a *Golden Retriever*) was found to display frequent ventricular premature beats and, interestingly, this same dog was also the individual that showed distinctively different behavioural and immunological characteristics (see also *Preoperative Patient Characteristics*).

9.2.4. Conclusions on Current Findings

In conclusion, medetomidine and acepromazine preanaesthetic regimens resulted in different perioperative cardiovascular characteristics, and a higher incidence of bradyarrhythmias was detected for the medetomidine-treated dogs. Altered cardiac autonomic influences seemed to play a role in the intraoperative heart rate behaviour with both preanaesthetic protocols, also with the animals in the MED group, who otherwise showed marked stability in their plasma catecholamine concentrations (I). Although the exact contributors to the HRV characteristics are complex, including factors related to sinoatrial properties, reflex arch responses, and both higher (cortical) and lower (brainstem) brain activities, the data gained here indicate the value of utilising heart period measurements in canine surgical patients to improve the characterisation of perioperative physiological perturbations. Including nonlinear measures among the HRV analysis techniques may further expand the knowledge on perioperative cardiac function, although improvements in the study protocol and analysis paradigms must be made to obtain valid information.

9.3. Immunological Measurements (III)

Blood neutrophil counts and neutrophil CL responses were examined in dogs undergoing OH to investigate the quantitative as well as qualitative aspects of perioperative immunological perturbations. Whole blood CL method was used to

determine the light emitted by circulating neutrophils as this method is easy to use and is considered to provide measurements reflecting the *ex vivo* states of blood phagocytes (Lilius & Marnila 1992, Allen *et al.* 2000). The increased number of circulating neutrophils recorded for the MED and ACE -treated dogs confirmed the activation of the immunological branch of bodily systems. Neither of the preanaesthetic protocols, however, resulted in significant alterations in neutrophil functional characteristics, as determined by whole blood CL.

At least 3 potential explanations can be offered for the non-significant changes in blood neutrophil oxidative activities. Firstly, it is possible that the neutrophil function, as measured here, did not undergo major alterations despite the changes induced in their circulating numbers, and despite the hormonal stress responses. In humans, perioperative neutrophil oxidative activities have been reported as unaffected, although neutrophilia has occurred or markers of other types of immunological perturbations, such as increased levels of plasma cytokines, have been documented (Perttilä *et al.* 1987, Kowatari *et al.* 1999). Furthermore, for humans, depressions in perioperative granulocyte respiratory burst have often been registered with severe illnesses and in relation to major surgical interventions (Perttilä *et al.* 1986) and such demographic features were not applicable to the animals of the current investigation.

With respect to the effects of stress, *per se,* despite its abilities to modulate immunological statuses, for instance, studies on the effects of cortisol on neutrophil oxidative activities *in vitro* have not revealed conclusive results (Trowald-Wigh *et al.* 1998). The present series of investigations could also not demonstrate consistent relationships between the hormonal and immunological measures, and similar observations have been made on humans with respect to surgical operations (Tonnesen *et al.* 1987, Salo 1992). In the current investigation, the increased blood neutrophil counts were detected on the first postoperative day, when plasma hormone concentrations had already returned to their preoperative levels. For dogs undergoing ovariohysterectomy (Burton *et al.* 1994), peak occurrences of acute phase responses, as measured in plasma CRP concentrations, have also been recorded on the day following the intervention, possibly reflecting the peak occurrence of inflammatory responses following these types of operations. The similar neutrophil characteristics registered for the two preanaesthetic protocols in the current study could be explained by the effects of the extent of the operation on circulating neutrophil numbers and their oxidative activities (Isozaki *et al.* 1999).

Secondly, it is possible that the operation performed or the drugs administered had short-term effects on blood neutrophil oxidative characteristics that were not

detectable due to the sampling interval utilised. Although the clinical significance of such short-term changes is yet to be determined, the present investigation lacked adequate depth to truly assess their occurrence and also to truly explore the detailed nature of the potential differences existing between the two preanaesthetic protocols. For instance, acepromazine has been reported to attenuate the oxidative activities of equine neutrophils *in vitro* (Serteyn *et al.* 1999), whereas in humans no effects on ROS production have been found with separated neutrophils subjected to α_2 agonist agents (Nishina *et al.* 1999). The sampling protocol used in the present investigation was also not designed to follow any long-term changes. However, in many of the studies reporting altered perioperative neutrophil oxidative activities, the perturbations detected have been evident on the first postoperative day.

Thirdly, the lack of significant findings with respect to blood neutrophil functional characteristics may have been due to the presence of factors both enhancing and depressing neutrophil oxidative activities. For example, the anaesthetic agents propofol and isoflurane, which were administered to the dogs in the present study, have been found to depress activities related to the neutrophil respiratory burst and could also have had a similar effect in this investigation. However, the amounts used and the time of exposure were relatively minor in the animals followed here when compared, for instance, with the concentrations of isoflurane required to produce suppressive effects on human neutrophils *in vitro* (Nakagawara *et al.* 1986). On the other hand, contrary to dampening effects, the inflammatory mediators released due to tissue trauma could have acted as enhancers of neutrophil functional characteristics, including their oxidative activities. The exposure of PMNs to plasma cytokines can result in cellular activation and increased responses to succeeding stimuli (Condliffe *et al.* 1998), such as the zymosan particles used in the present study. The measurement method utilised here recorded cellular characteristics that could have been affected by both the drugs administered and the physiological state of the dogs. The nonsteroidal anti-inflammatory agents administered to every individual animal could further have modulated the perioperative immunological activities with the two preanaesthetic protocols. NSAIDs have been reported to attenuate perioperative increases in circulating cytokines in humans (Chambier *et al.* 1996) and have also been documented to affect PMN CL responses and ROS production *in vitro* (Angelis-Stoforidis *et al.* 1998).

Finally, a few methodological issues should be mentioned. Despite the measurement of whole blood CL being regarded as a useful tool when examining the functional characteristics of circulating neutrophils, it merely evaluates the end results of complex processes involved in PMN respiratory burst and oxidative activity. More detailed analysis techniques are therefore needed to more completely characterise

neutrophil function. In addition, the blood CL only reflects cellular characteristics in circulation and not at the periphery, where the phagocytes mount most of their actions and where changes due to anaesthesia and operation may occur. The overall difficulties in the assessment of neutrophil oxidative activities due to the presence of various confounding factors have also been previously pointed out, and no standardized measurement method for canines has generally been accepted. In the present investigation, a close correlation existed between the blood neutrophil counts and whole blood CL responses, indicating that neutrophil-related activities were, indeed, followed. The study protocol further aimed at controlling the potential influences of daytime dependence (Heberer *et al.* 1982) and gender (Ristola & Repo 1990) on neutrophil characteristics. The lack of sensitivity when documenting the spontaneous emission of light, however, does underline some of the pitfalls of the present investigation, and the results also emphasise the existence of marked individual variation in the CL values. In the study by Perttilä *et al.* (1986), the SD reported for neutrophil CL responses was only approximately 10% (of the mean; 0.3 units).

In conclusion, as measures of qualitative immunological changes, the whole blood CL responses indicated no significant alterations in neutrophil function in the animals of the present series of investigations. In addition, similar increases in blood neutrophil counts were documented for the two preanaesthetic groups that resulted in different perioperative neurohumoral arousal (I, II). The role of neutrophil CL as an index of perioperative stress needs further evaluation, as does its role in contributing to perioperative characteristics with different patient populations. Several methodological issues need to be clarified in future studies on perioperative CL in canines, and the extent of perturbations bearing any clinical significance also need to be determined.

9.4. Behavioural Observations (I, II, IV, V)

9.4.1. Preoperative Patient Characteristics (IV)

The behavioural characteristics of hospitalised dogs, as assessed before preanaesthetic administration, demonstrated the occurrence of preoperative arousal. Behavioural signs often recorded in dogs in association with psychological or physical stimuli, such as panting or oral activities (Beerda *et al.* 1997), were commonly noted. While the dogs observed here were probably not consciously aware of the impending operation, which may have represented an almost certain stressor for many humans, hospitalisation in itself certainly presents a battery of threatening stimuli, such as a novel environment, lack of control and social isolation.

Besides the signs of arousal, the preoperative characteristics revealed distinct differences in overall activities. One-third of the dogs were seen to bark, howl or manipulate the environment in a vigorous fashion (so-called highly active dogs), whereas in another third such activities did not occur (passive dogs) (Table 1 [IV]). Since the present study did not include the documentation of detailed demographic data, the reasons for the different behavioural characteristics can only be speculated on. Among them may have been breed-related factors, differences in overall reactivity, motivational states and earlier experiences. For various animal species it has further been suggested that the behavioural responses induced by a threatening incident would follow a certain main stream of actions and reflect so-called active or passive coping strategies (Koolhaas *et al.* 1999). Some individuals would thus exhibit behaviours directed to the removal of the stimulus, whereas others would show behavioural passiveness. In the dogs studied here, similar strategies, or behavioural styles, could have existed and contributed to the overall activities. Accordingly, the manipulation of the environment and the high level of vocalisation observed in some individuals, which were behaviours not observed in others, could have reflected the dogs' attempts to actively resist the situation, including the use of vocalisation as a tool to enforce social reunion. The existence of a true coping style, however, would require the behavioural type to be sustained over a wider range of stimuli, and such hypothesis was not tested in the animals followed here. The somewhat differing responses of the so-called high, intermediate and passive dogs to a person entering the cage, a stimulus of another kind (Table 2 [IV]), would further argue against the ability to explain the perioperative behavioural characteristics with the above-described grouping. Due to limitations in the study design, the dogs' behavioural characteristics were not closely followed throughout the postoperative phase, which would be of importance in future investigations.

The data derived from examinations of associations between the preoperative behavioural state and the characteristics of physiological arousal at later time points of observation revealed interesting findings. Contrary to expectations, animals regarded preoperatively as behaviourally the most active, and in whom the preoperative heart rates also tended to be the highest (Figs 2, 3 [IV]), were not found to display the highest level of physiological arousal at later time points of evaluation. Instead, especially in the ACE-treated dogs, the physiological arousal during anaesthesia and operation and during the early recovery seemed higher among the so-called passive dogs, as compared with the highly-active animals (**Fig. 11**).

The behavioural passiveness determined here may have been unable to discriminate animals possessing the best abilities to cope with perioperative stressors, and despite these dogs not performing behaviours commonly associated with apprehension,

such as vocalisation or attempts to flee, potentials for preoperative arousal were evident. The passive dogs were seen to pant and perform displacement activities and although affected by individual variation and the lack of baseline measurements, two passive dogs were also found to display a low (the lowest) HRV, possibly implying the existence of stress as represented in vagal withdrawal (or increased cardiac sympathetic modulation). Another possibility, however, would be that in the highly active animals, some sort of down regulation occurred in the level of arousal that smoothed their characteristics at later evaluations. In experimental dogs, dampened heart rate responses to adrenergic stimulation have been demonstrated with chronically increased plasma catecholamine concentrations and the associated desensitization of beta-adrenergic signalling (Vatner *et. al*. 1989). It could be speculated whether such conditions might also exist with certain types of personalities and individual patient characteristics. Salmon & Kaufman (1990) reported that humans considered preoperatively as more anxious personalities would actually be the ones displaying lower concentrations of circulating catecholamines and cortisol during the postoperative period.

Altogether, these observations may underline the difficulties encountered when trying to estimate perioperative (physiological) responsiveness on the basis of preoperative behavioural characteristics, and with respect to the dogs of the current study, also the insensitive nature of vocalisation *per se* as a marker of stress-related states. The fact that the tendency for unsuspected associations between the level of preoperative behavioural activity and the state or physiological arousal at later time points of evaluation was more frequently observed with the ACE-treated dogs might further imply the differing abilities of the two preanaesthetic regimens to modulate animal's perioperative statuses. In veterinary research, descriptive data is rarely collected for individual animals, as compared to the examination of different interventions by use of randomised grouping, and obtaining more detailed evaluations of individual patient characteristics might help in defining the true existence of preoperative arousal in client-owned dogs. In the current trial, it was further one of the so-called passive animals, the *Golden Retriever*, who showed consistent signs of preoperative apprehension and was later found to display the highest number of perioperative VPCs and the greatest perturbations in neutrophil CL.

9.4.2. Postsurgical Behaviour Following MED and ACE Premedication (I, II)

Despite the differing levels of neurohumoral arousal, the behavioural observations during the early recovery did not indicate differing levels of overall behavioural activity or differing degrees of postoperative pain / distress for the MED and ACE-

treated dogs. This result corroborates earlier observations concluding non-significant or only weak correlations between the physiological states and postoperative pain scores in dogs (Stobie *et al.* 1995, Hendrix *et al.* 1996, Conzemius *et al.* 1997, Holton *et al.* 1998) and also observations on humans, in whom the differing levels of neurohumoral arousal have not always been found reflected in the differing degrees of postoperative analgesia (Rutberg *et al.* 1984, Pither *et al.* 1988). Pain *per se* is not the sole nominator for the surgery-related physiological responses and the separate influences of a drug on nociception, behaviour and the neurohumoral and mental states can all contribute to the observed characteristics of recovery.

In the current trial, the animals were regarded as fast-asleep during the first time of observation (at 1 hour following surgery), whereas differing degrees of sedation were recorded for the two preanaesthetic groups at later stages of recovery. Especially at the later time points and when compared with the ACE-treated dogs, the animals in the MED group were found less sedated and responding more promptly to external stimuli, although the (hourly) times spent in lying down did not differ between the two groups. With acepromazine, it could be argued that the drug's effects on behaviour affected the animal's ability to interact with the observers and influenced the estimation of pain / distress and that a state of behavioural quiet existed despite the fact that the physiological arousal, associated with pain, was noted to occur. Similar levels of overall activity may not adequately differentiate between the differing degrees of pain following general anaesthesia and ovariohysterectomy in a dog, as concluded by Hardie *et al.* (1997) when comparing the influences of oxymorphone and placebo analgesic regimens on postsurgical video-recorded behaviour, and by Firth & Haldane (1999) when examining the effects of general anaesthesia alone and OH carried out with various analgesic protocols. In the current trial the dogs treated with MED were considered to show greater degrees of pain / distress at 6 hours after surgery, when their plasma epinephrine levels were also higher as compared to those measured with the ACE-treated dogs. This could reflect the recovery characteristics observed with the wearing-off of medetomidine's actions.

Ovariohysterectomy *per se* is not considered to cause severe pain in the dog (Hellyer 2002) and the administration of analgesics to every animal of the current trial could have resulted in such degrees of postoperative pain where the detection of significant and treatment-related effects became difficult. The sensitivity and specificity of the evaluation method to differentiate between individual animals would thus have played a notable role in data collection. The composite score used to evaluate the presence of pain / distress in the current trial involved interaction with the dog, assessment of behaviour with movement and palpation of surgical site. It was not

based on validated measures, although it included items previously used by several researchers. While the animals showing signs indicative of considerable levels of pain or distress were most likely identified by the use of the current scoring system, it is especially the differences between and within the mild and moderate degrees of pain that may have been overlooked. For instance, a score of 1 could have been assigned to an animal displaying intermittent vocalisation or restlessness and a clear response to palpation of the surgical site, whereas a score 0 could have included dogs with none of these behaviours (Appendix [I]). However, it is also possible that the two preanaesthetic protocols indeed resulted in similar and acceptable levels of postoperative pain relief which was reflected in the behavioural observations, despite the differing neurohumoral states and despite the potentials for improved analgesia associated with the pre-emptive nature of medetomidine administration. The pre-emptive effects of medetomidine, on the other hand, might also have been apparent especially during the early stages of postsurgical recovery when the evaluation of analgesia was probably most affected.

Whether different end-results in the current trial would have been obtained by use of a different scoring system is a matter of debate. Compared with the numerical rating of pain, the VAS may be considered to provide more sensitive tools for the assessment of analgesia, especially with milder degrees of suspected pain, and undoubtedly allows the evaluator to choose from a continuum of options instead of making him select from pre-defined categories (Welsh *et al.* 1993). However, even the VAS has failed to show significant or strong correlations with the results obtained from mechanical pain threshold tests following surgery in a dog (Conzemius *et al.* 1997, Lascelles *et al.* 1997), implicating the inabilities to cover all aspects of postsurgical pain even with this assessment protocol. The VAS and a pain score based on numerical ratings have been found to significantly correlate with each other when used during the postoperative period in dogs (Conzemius *et al.* 1997); however, such results do not indicate that either of the measures has strictly reflected pain-related phenomena. The use of numerical ratings in the assessment of postsurgical pain *per se* may not be considered as an inappropriate method in a dog as these types of scales, with or without a specified composition, have successfully been used to demonstrate the effects of analgesics in placebo-controlled trials (Grisneaux *et al.* 1999). With a composite scale, however, the items included in the assessment protocol, the weight placed on separate factors as well as the overall range of scores allowed are all important contributors to valid results.

Currently, there are no universally accepted measures for the assessment of postsurgical analgesia in hospitalised animals and variation exists between the protocols used in different trials. Overall, the role of the physiological measurements

and the differing effects on behaviour associated with administration of different analgesics should be better defined. Future work also lies ahead in examining the potentially unknown phenomena that may be included in the animal's postsurgical recovery, as apparent in hospital settings, and may only be revealed by the use of detailed analysis of video-recordings (Hardie *et al.* 1997, Roughan & Flecknell 2001). Without doubt, the assessment and adequate treatment of postoperative pain remains one of the major items contributing to the quality of perioperative care.

9.4.3. Owner-reported Postsurgical Symptoms (V)

In Study II, the owners of dogs recovering at home from day-case soft-tissue operations were asked to document the qualities of later postsurgical recovery, as apparent in their animal's behavioural symptoms. Changes in behaviour after surgical interventions are not unexpected and can be viewed to reflect both the conscious (and unconscious) behaviours associated with the avoidance of pain and also the effects of the hormonal, metabolic and inflammatory components of perioperative stress responses that modulate the individual's mood, appetite and physical performance.

The extent of the behavioural symptoms noted in the current trial was affected by the extent of operation and followed the degrees of owner-rated postoperative pain, providing similar results to those previously documented for children recovering from day-case operations at home (Kotiniemi *et al.* 1997). Many of the aspects of behaviour found most affected in the current trial have also been described for children (Kain *et al.* 1996, 1999ab, Kotiniemi *et al.* 1997), and the data further resembled the findings of the few existing studies examining the owner-reported behavioural symptoms in a dog, as associated with illness-related states. In the study by Wiseman *et al*. (2001), over 70% of the 17 dog owners interviewed to determine the behavioural signs associated with chronic orthopaedic conditions reported alterations in the animal's activity, demeanour and playfulness. Activity, posture-mobility and dependence were further among the items included in the final matrix of behavioural domains generated (on the basis of owner-derived descriptions) to characterise the behaviour of a dog as associated with chronic pain or health-related qualities of life (Wiseman-Orr *et al.* 2004). Some of the postsurgical data derived from video-recordings made on dogs in hospital settings have also revealed that surgery (compared with general anaesthesia alone) may result in increased times spent sleeping and affect the animal's ability to respond during interactions with the observer (Hardie *et al.* 1997).

Certain types of behaviour thus seem associated with the illness and pain-related states in a dog and may also be notable in home settings after surgical interventions. The low kappa coefficients documented for the different aspects of behaviour investigated in the current trial may further indicate that by the use of multisided approaches, the different dimensions of an animal's behaviour can indeed be evaluated and that their documentation can be used to more completely characterise the nature of postsurgical recovery. The present results are, however, specific only to certain types of operations and perianaesthetic medications; thus, their generalisation to larger patient populations needs to be evaluated further.

Overall, owner-rated animal pain was found associated with the incidence of behavioural symptoms, but did not relate to the extent of the operation on Day 1. On Day 3, however, greater pain VAS scores were assigned to animals that had undergone more invasive procedures (**Fig. 14**). Vocalisation was among the most common behavioural signs that the owners indicated they had used to detect the presence of pain in their dog, and vocalisation also showed steep decreases towards normality during the 3-day period of observation. However, on Day 3, the demeanour of the dog, for instance, was still found altered in about some one third of the animals (Fig. 2 [V]; **Fig. 12**).

While the exact contributors to the above-described findings are difficult to conclude, it can be stated that after common day-case surgeries, and despite analgesic administration, owners regard their dog to suffer from postoperative pain and that the pain is greater with more invasive procedures, at least at later stages of recovery. However, although a significant association exists between the postoperative behavioural symptoms and the degree of owner-reported postoperative pain, the dependency of the pain scores on a specific type of behaviour needs further clarification. Ratings of animal owners with respect to the existence of pain in their pet animal may be viewed as opinions of assessors with the greatest knowledge of the normal characteristics of the individual animal, but also as opinions of evaluators possibly unfamiliar with the circumstances associated with the current condition. Along with the effects of administered medications, the metabolic and inflammatory responses, irrespective of pain, also contribute to the animal's characteristics during postsurgical recovery and may lead, for instance, to lethargy and decreased appetite. In dogs, peak concentrations in circulating CRP have been measured on the first day following OH (Burton *et al.* 1994).

Scarce information is currently available on the opinions of pet animal owners with respect to the behavioural signs notable in pet animals recovering from surgical operations, the extent of pain possibly following certain types of surgeries and the

suspected pain relief associated with analgesic administration. The role of the animal owner as a caretaker, however, is of importance in contributing to the qualities of postoperative recovery and their views might need to be evaluated further in order to optimise the administration of postoperative analgesics and also in order to better define clinically relevant measures of the postsurgical outcome. The lack of validated measures for postsurgical recovery has recently also been re-emphasised in the human literature (Carli & Mayo 2001).

In addition to examining the broad effects related to the extent of the operation and the day of observation, the current study also investigated the influences of certain demographic features thought to play a role in owner-reported animal behaviour. No effects could be demonstrated for dogs wearing an Elizabethan collar or for the ratings the owners provided for the extent of stress they themselves experienced when facing an operation on their dog. Interestingly, these latter ratings were also found similar between the groups of owners whose dog had or had not previously undergone a surgical intervention and irrespective of the type of operation the dog was now assigned to (**Fig. 15**). The ratings of the animal owners in the current trial were also higher than some of the (VAS) scores previously obtained from humans with respect to fears related to anaesthesia (Kindler *et al.* 2000). The concerns and fears of animal owners may be viewed as representing an additional aspect of stress responses associated with surgeries on pet animals and are not well evaluated. More detailed knowledge of their characteristics might be indicated to improve owner-veterinarian communication as well as owner satisfaction.

The current trial did find a significant effect of the animal's age on the incidence of postoperative behavioural symptoms, and the behavioural changes were more frequently reported for younger dogs (**Fig. 13**). The result might reflect the overall differences in the behavioural characteristics of dogs of different ages and, for instance, if playfulness was an infrequent activity in a dog preceding an operation, a notable effect of surgery on its incidence would be difficult to detect. However, in the current study, it was the younger animals that more frequently underwent a major operation, as compared with the older dogs, and this might also explain the finding. In dogs aged 1 to 3 years, abdominal procedures were performed on approximately 65% of the individuals, whereas only some 20% of the dogs aged 9 to 13 years underwent this type of procedure.

9.4.4. Conclusions on Current Findings

The behavioural observations on hospitalised dogs about to undergo an elective operation revealed marked differences between individual animals with respect to

the level of overall activity, but similarities in the incidence of behavioural signs indicative of emotional arousal. Vocalisation *per se* does not seem to represent a sensitive marker for stress-related states in a hospitalised and awake dog and it may be especially the so-called passive animals that require further attention with respect to defining the existence of preoperative arousal and the physiological responses notable at later stages of the perioperative period.

The behavioural characteristics documented for the MED and ACE treated dogs during the early stages of postoperative recovery did not reflect the findings of hormonal measurements. Since the associations between the degree of physiological arousal and the degree of postoperative pain are yet to be completely resolved, these results may implicate difficulties encountered when evaluating the qualities of postsurgical recovery in clinical settings. When compared with medetomidine, inclusion of acepromazine as part of the preanaesthetic medication results in a longer duration of effects on behaviour, as determined by the use of a sedation score, and this may complicate the assessment of animal's emotional states and also the degree of postoperative pain. However, the current study could not conclude which of the preanaesthetic protocols resulted in better postoperative analgesia, and preoperative administration of acepromazine, compared with medetomidine, may also smoothen the animal's characteristics at later stages of recovery.

The postsurgical behavioural signs reported by animal owners for dogs recovering from day-case operations at home emphasise the value of using multi-sided approaches to more completely characterise the nature of later postoperative recovery. The use of animal owners as assessors of animals' states could further be utilised in order to optimise the administration of postoperative analgesics and to define clinically-relevant measures of the postsurgical outcome. However, further research is indicated in several areas related to surgical interventions performed on pet animals. These may include the assessment of the opinions of caretakers with respect to the behavioural signs indicative of postoperative pain and the fears and the concerns that the owners themselves may have regarding operations performed on their pet animal.

9.5. Further Comments on Experimental Designs

As the current investigations were based on studies on clinical patients, their value as true evaluators of physiological incidents must be treated with caution. No control groups were included, which affects the estimations. The inability to control or to closely monitor the perioperative perturbations in respiration especially affects the evaluation of HRV, but was not considered possible since the study was conducted

in clinical settings and covered periods both preceding and following general anaesthesia. The sample size in Study I was based on calculations using catecholamines only, which influences the conclusions on all non-significant findings.

Most of the data was gathered from female individuals, and as both the behavioural (Wells & Hepper 1999) and physiological (Kemppainen & Sartin 1984) stress-related characteristics may differ between sexes, the results may be representative of responses in bitches only. The data comparing the influences of MED and ACE preanaesthetic protocols was derived from only one type of surgery that further restricts the generalisation of findings. However, these features of the study probably also improved the validity of the observations by restricting the number of confounding factors. With respect to the study settings, the behaviours detected during the period of preoperative hospitalisation (IV) may also be specific to the current investigation only, as certain types of dogs were investigated and the surroundings were probably more isolated than those in typical clinical settings. These circumstances, however, were considered necessary for the examination of individual differences.

The long period of preoperative sedation in the current trial (Study I) was different than commonly applied in every-day practice and may have represented an additional stressor to the dogs. The effects of the quality of preoperative sedation on an animal's characteristics at later stages of the perioperative period have previously been speculated (Reese *et al.* 2000), but no strong indications (as evaluated through correlations) were apparent in the current trial. With the ACE preanaesthetic regimen, however, a tendency existed for higher correlation coefficients with various stress-related measures at different stages of the perioperative period, which may indicate the different nature of perioperative patient characteristics induced with this preanaesthetic regimen, compared with MED. Whether this would also indicate the existence of more generalised responsiveness or merely the preservation of individualism needs further clarification. The study conditions probably affected the animals' characteristics and could have contributed to the above-described findings. A decision to use blinding may also have affected the quality of patient care, especially with respect to anaesthetic administration. Furthermore, no efforts other than clinical evaluations were made to ensure similar levels of anaesthetic depth.

A final remark may be made on the ability of the recruitment procedure to provide subjects that were representative of the general canine population. In Study 1 the owners were informed about the detailed nature of the investigations to be performed on their animals and about the fact that the dog would spend 24 hours at

the clinic, which in Finland and with elective operations is not a commonly applied practice. This could have resulted in an over-representation of animals with owners who regarded their dog as a suitable candidate either because the dog was considered overly active, and thus potentially difficult to manage after the operation, or as overly well-tempered, and thus easy to leave alone in a the hospital setting for a lengthy period of time. In addition, the owners of Study 1 were themselves responsible for making the contact if interested in the study and no records were available on the demographic features of owners refusing to participate.

10. CONCLUSIONS AND FUTURE IMPLICATIONS

1. The manifestation of preoperative stress in overtly healthy dogs seems characterised by frequent occurrence of panting and oral behaviours, yet marked differences exist between individual animals in the level of overall activity. Higher heart rates and lower HRV may be recorded among dogs regarded behaviourally as most active, but this is not a consistent finding. Animal's preoperative physiological or behavioural states seem to provide only minor indications of its characteristics at later time points of observation, and the preoperatively behaviourally passive dogs may not represent the group of individuals with the lowest level of physiological arousal during surgery or in the early recovery. The data gained in this preliminary investigation emphasises the existence of emotional arousal in dogs during the period of preoperative hospitalisation and the difficulties encountered when estimating its true nature. Future investigations are needed to develop validated measures for preoperative apprehension in dogs and to evaluate the needs for specific interventions.

2. Behavioural changes are multisided in their nature in dogs recovering from day-case soft-tissue operations at home, and include alterations in overall activity, playfulness and social behaviours. The extent of operation affects the incidence of postsurgical behavioural symptoms and the behavioural changes decrease together with the decreasing owner-reported pain scores. The data of the current trial emphasises the multi-faceted nature of postoperative behavioural recovery in client-owned dogs and outlines entities that could be of value in future studies when defining clinically-relevant measures of the postsurgical outcome.

3. When administered as part of the preanaesthetic medication in dogs undergoing ovariohysterectomy, medetomidine, compared with acepromazine, more potentially attenuates the perioperative neurohumoral arousal, and results in lower concentrations of circulating catecholamines and cortisol and lower heart rates. The differing physiological characteristics already appear during the period of preoperative sedation, and seem especially apparent in the early postoperative phase. The differences in physiology, however, are not easily appreciated in the evaluations of animal's postoperative behaviour. The differences between the two preanaesthetic protocols with respect to inflammatory responses seem to be minor. Further studies are needed to determine the clinical significance of the described findings.

4. The hormonal indices indicating sympathoadrenal and HPA-axis activities display different perioperative behaviour in dogs undergoing OH with medetomidine or

acepromazine preanaesthetic medication. The correlations between heart rates and indices of cardiac efferent vagal activity, however, are stronger and with both preanaesthetic groups the time-related associations among different physiologic measures increase in their strength during the early recovery. The nonlinear indices of HRV seem to complement the evaluation of perioperative perturbations in beat-to-beat interval behaviour, but the physiological background for these characteristics needs to be determined. The differing physiological states not being readily appreciated in the evaluations of animal's postoperative behaviour may complicate the estimation of postoperative arousal in clinical settings. Overall, the results underline the complexicity of perioperative stress responses in client-owned dogs and the value of using multi-sided observations for more complete evaluations.

11. ACKNOWLEDGEMENTS

The studies included in this thesis were carried out at the Helsinki University Small Animal Hospital, Dept of Clinical Veterinary Sciences, Faculty of Veterinary Medicine, and at 5 private veterinary practices in the Helsinki area. Laboratory analyses were also carried out at the University of Oulu and at Deaconess Institute, Helsinki. Preliminary laboratory work was done at the Dept of Biochemistry, University of Turku.

My sincere gratitude goes to the Head of the Dept of Clinical Veterinary Sciences, Professor Riitta-Mari Tulamo, for her supportive interest in this thesis and to my supervisors, Professor Outi Vainio and Docent Marja Raekallio, for their encouragement and help in this work. I would also like to express my deepest and most sincere thanks to my dear friend and an extraordinary statistician, Ms. Hanna Oksanen (now deceased), for her work with this thesis and for her support as a friend. Pioneers and those standing on the opposite side of the river are always needed.

I greatly acknowledge the opportunity to attend the postgraduate training sessions at the Helsinki University Central Hospital, as offered by Professor Per Rosenberg, which were always enjoyable. I am indebted to the help I got from Dr. Katri Hamunen, MD, PhD, in the planning of Study 2. I wish to express my sincere gratitude to Professor Heikki Huikuri and his research team at the University of Oulu for their invaluable help in conducting the analyses of heart rate variability for this thesis and for their most unselfish scientific contribution. My warmest thanks to the team of Docent Esa-Matti Lilus at the University of Turku for their help in planning and execution of the CL assays and for scientific advice; working with you was always enjoyable! I am further grateful for the time, effort and knowledge that Dr. Heikki Hietanen, MD, put into this thesis with the analysis of ECG recordings, and for the expertise that Professor Juhani Leppäluoto and Docent Pirkko Huttunen provided. I further wish to express my gratitude to Professors John Benson and Sydney Moise for their interest in this thesis and for personal help.

I am grateful to Dr. Tuomas Kärkkäinen, former Head of the Helsinki University Small Animal Hospital, and for Professor Elias Westermarck for their help in carrying out and financing Study 1. Many, many thanks to those veterinary students, technicians, veterinarians and owners of veterinary practices who were involved in the execution of the clinical phases of this thesis, and who helped in the analysis of raw data. Ansku, Maarit, Petra, Tanja, Elisa and Tinttu –your work was extraordinary; without you, mine would not have been possible! I wish to express

my gratitude also to a dear colleague, Dr. Leena Mustonen, for her valuable help in carrying out Study 1 and for her enjoyable company during our trips to Turku. My most sincere thanks to the owners of dogs who participated in the studies of this thesis, and thus, made them possible.

My special thanks to the staff of Depts of Biochemistry, Pharmacology and Physiology at the Faculty of Veterinary Medicine, University of Helsinki, for their help in laboratory analyses and in executing the clinical phase of Study 1. Many thanks to the technical personnel at the Faculty of Veterinary Medicine for their brilliant co-operation with the preparations for Study 1. My deepest gratitude goes to Mr. Matti Järvinen and Mr. Kristian Lindqvist for their excellent assistance with technical issues. Professor Anna-Kaisa Järvinen is warmly acknowledged for sharing her expertise in ambulatory ECG recordings in dogs. Dr. Erja Kuusela, DVM, PhD, is gratefully acknowledged for introducing me into this field of veterinary medicine and for her valuable help in the execution of Study 1. I am deeply grateful for the help I got from Professor Anna Valros with the analysis of behavioural data.

My sincere gratitude goes to Professor Peter Pascoe and to Professor Jacky Reid for their excellent job as official reviewers of this thesis and for their constructive and valuable comments. I also want to express my thanks to Mr. Roy Siddall, BSc, PhD, for his contribution in checking the language of this thesis. I wish to expand my gratitude to the staff at the library of Faculty of Veterinary Medicine for their excellent assistance.

The studies in this thesis were supported by grants from Finnish Veterinary Foundation, Helvi Knuuttila Foundation, Juliana von Wendt Foundation, Dept of Clinical Veterinary Sciences, Helsinki University Research Funds, Finnish Kennel Club and OrionPharma Corporation. The grants are gratefully acknowledged.

Finally, I own my deepest gratitude to my parents, Seppo and Marja-Liisa Väisänen, for their support and love and for understanding the emotional hardship involved in the making of this thesis. My warmest gratitude goes to my dear colleague and friend, Merja Rantala, for her sparkling personality and for those exciting and fruitful academic discussions we so many times shared. My special thanks to my dearest friends, Piia, Sari and Evi, for their gracious patience and support. I also want to thank my dear dog, Mopsu, for her (unfailing) enthusiasm in showing me the true joys in life –eating mice excluded.

Helsinki, February 2006

Misse Väisänen

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