Surgical Stress in Rats

The Impact of Buprenorphine on Postoperative Recovery

RENÉE SUNDBOM
Abstract


During surgery, both anesthesia and tissue damage cause physiological stress responses in the body. The hypothalamic-pituitary-adrenal (HPA) axis is activated with increased levels of glucocorticoids. After surgical procedures the stress response may be a cause of postoperative morbidity and pre-emptive analgesic treatment can attenuate the stress response during the postoperative period. In laboratory animals, buprenorphine is a commonly used analgesic. Subcutaneous (s.c.) administration of buprenorphine is most common, but oral administration would be preferable in many cases, enabling administration without any handling of the rat.

In this thesis we studied the surgical stress response in laboratory rats during surgery and in the postoperative period, and its modulation by s.c. injection and oral voluntary ingestion (VI) of buprenorphine. Corticosterone levels and the clinical parameters body weight, water intake and behavior were observed. The concentration of buprenorphine in plasma was measured as well as stock-related differences in postoperative recovery.

During surgery and anesthesia there was a higher corticosterone release during a more severe surgery and corticosterone levels were reduced more effectively after buprenorphine treatment than after lidocaine treatment.

Buprenorphine treatment, independent of the route of administration, led to better postoperative recovery in body weight and water intake compared to local anesthetics. VI of buprenorphine resulted in a suppression of plasma corticosterone levels compared to s.c. buprenorphine treatment and treatment with local anesthetics during the first day after surgical catheterization. The corticosterone levels of all buprenorphine treated groups had, by the second postoperative day, reverted to the normal diurnal rhythm of corticosterone secretion. Buprenorphine treatment increased locomotor activity in non-operated rats only. The effect of buprenorphine in operated rats could not be detected via the monitoring of locomotor activity or the time spent resting in the present study.

Treatment with buprenorphine by VI has similar effects on postoperative plasma corticosterone levels in both Wistar and Sprague-Dawley rats. VI of buprenorphine resulted in a buprenorphine concentration in plasma at least as high as by s.c. treatment.

Thus, administration by VI of buprenorphine appears to be an effective stress-reducing method for administering postoperative analgesia to laboratory rats.

Keywords: Surgical stress, Corticosterone, Buprenorphine, Analgesia, Rats.

Renée Sundbom, Uppsala University, Department of Neuroscience, Comparative Medicine, Box 572, SE-751 23 Uppsala, Sweden.

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<th>Full Form</th>
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>CBG</td>
<td>Corticosteroid-binding globulin</td>
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<td>COX</td>
<td>Cyclooxygenase</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>GLM</td>
<td>General linear model</td>
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<td>GR</td>
<td>Glucocorticoid receptor</td>
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<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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<td>IASP</td>
<td>The International Association for the Study of Pain</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>MAP</td>
<td>Mean arterial blood pressure</td>
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<td>MR</td>
<td>Mineralocorticoid receptor</td>
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<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
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<td>s.c.</td>
<td>Subcutaneous</td>
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<td>SEM</td>
<td>Standard error of the mean</td>
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<td>TNF-α</td>
<td>Tumor necrosis factor-alpha</td>
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<td>VI</td>
<td>Voluntary ingestion</td>
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Introduction

Stress and glucocorticoids
A physiological stress response is induced in the body after exposure to a stressor. The sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis are activated, and hormones and cytokines are secreted to facilitate the ability of the body to cope with the stressor. Blood glucose levels and arterial blood pressure are increased, whereas the immune system is suppressed. Mechanisms for water and sodium retention, and potassium loss are also activated to maintain fluid volume. The blood concentration of the catabolic hormones, glucocorticoids and catecholamines are increased, but the anabolic hormones, insulin and testosterone, are instead decreased (Sayers 1950; Traynor and Hall 1981; Desborough 2000).

Physiological responses to stress are essential for survival and enable the body to maintain homeostasis and adapt to new situations (Selye 1946). It is however important to terminate this response and obtain normal hormone levels when the stressor is gone. A persistent response due to repeated stress or the inability to turn off the response results in an overproduction of stress hormones with pathophysiological consequences (McEwen 1998).

HPA activation
The HPA axis is one of the main components of the stress-response system. This activation leads to secretion of corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus, which induces secretion of adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary, which in turn stimulates secretion of glucocorticoids from the adrenal cortex (Fig. 1). Vasopressin, released from the posterior lobe of the pituitary, also plays a role in ACTH induction by acting synergistically with CRH. Glucocorticoids are distributed throughout the entire body (Smith and Vale 2006; Stoelting and Hillier 2006).

Negative feedback
Glucocorticoid secretion is regulated by a negative-feedback mechanism (Sayers and Sayers 1947) in which glucocorticoids suppress the release of both CRH and ACTH (Fig. 1). Thus, high levels of glucocorticoids will
cause a lower release of CRH and ACTH, and the levels of glucocorticoids will consequently decrease (Barrett 2003).

There appears to be at least two different mechanisms of feedback. In the delayed feedback system, glucocorticoids act by binding to glucocorticoid receptors in the brain and regulate the transcription of CRH and ACTH. The second mechanism is a fast, non-genomic feedback system. This system is sensitive to the rate of glucocorticoid secretion, but the exact mechanism is not clear (Keller-Wood and Dallman 1984; Smith and Vale 2006).

![Hypothalamic-pituitary-adrenal (HPA) axis diagram](image)

**Figure 1. The hypothalamic-pituitary-adrenal (HPA) axis.** Corticotropin-releasing hormone (CRH) induces secretion of adrenocorticotropic hormone (ACTH) which stimulates secretion of glucocorticoids. Glucocorticoids suppress the release of both CRH and ACTH.

**Glucocorticoids**

Glucocorticoids are steroid hormones that are synthesized in the adrenal cortex. They are lipophilic, and poorly soluble in plasma, and bound to the carrier molecule corticosteroid-binding globulin (CBG) when transported in the blood. Because of this binding, the hormone is protected from enzymatic degradation resulting in a half-life (60–90 minutes) longer than peptide hor-
mones. There is always a small fraction of unbound hormone in the blood, which easily diffuses through the cell membrane. Inside the cell, glucocorticoids bind to intracellular receptors (Fig. 2) (Silverthorn 2001).

![Diagram of glucocorticoid binding and transcription](image)

**Figure 2.** Glucocorticoids (GCs) are transported by corticosteroid-binding globulin (CBG). Unbound GCs diffuse into the target cell and bind to the glucocorticoid (GR) and/or mineralocorticoid receptor (MR). The GC-receptor complex translocates into the nucleus and regulates gene transcription.

Glucocorticoids bind to two types of intracellular receptors: glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). The affinity is higher for MRs and during normal conditions glucocorticoids mostly binds to MRs. During stress, the glucocorticoid levels are elevated and they bind both to GRs and to MRs (Smith and Vale 2006).

After binding to the receptor, the hormone receptor complex translocates into the nucleus where it binds to DNA. In this way, glucocorticoids regulate protein synthesis (Smith and Vale 2006), and the specific effect of the regulation is dependent on cell type. The lag time between binding of the receptor and synthesis of new proteins may be up to 90 min. Thus, the effect of glucocorticoids is not mediated through fast reflexes (Silverthorn 2001).

In humans, the main active glucocorticoid is cortisol and in rats and mice, it is corticosterone (Woodman 1997; Smith and Vale 2006).
**Glucocorticoid pulsatility**

The normal level of glucocorticoids varies during the day. HPA hormones are secreted in both a circadian rhythm and an ultradian rhythm. The circadian rhythm has a cycle of 24 hours and varies, with higher levels in the beginning of the active period and lower levels in the more passive period (Atkinson et al. 2006). Humans show the highest levels of cortisol in the mornings (Silverthorn 2001). Rats are nocturnal animals and thus, the highest levels of corticosterone are seen during the onset of the dark period and the lowest levels during the onset of the light period.

The secretory pulses of the ultradian rhythm are observed approximately every hour in the rat, during the whole 24-hour cycle. There is no change in frequency, but the amplitude of the pulses differs during the day (Lightman et al. 2008). Pulsatility is regulated by short periods of active HPA secretion followed by a short period of HPA inhibition by negative feedback (Sarabdjitsingh et al. 2010).

The rhythm of secretion of HPA hormones seems to affect the response to stressors. Rats have been shown to adapt to noise stress in the morning but not in the evening (Atkinson et al. 2006). Only stressors that coincide with a rising phase of the ultradian rhythm cause an HPA response (Windle et al. 1998). Many stressors, however, will persist over a longer time than the short period of inhibition. Noise stress during the rising phase has been shown to result in increased activity of rats compared to stress during the falling phase (Sarabdjitsingh et al. 2010).

**Effects of glucocorticoids**

Glucocorticoids have an overall catabolic effect on the body to mobilize substrates needed to cope with the stressor (Silverthorn 2001). GRs have been found in generally every cell type, with diverse effects (Munck et al. 1984). Blood glucose levels increase after stimulation of gluconeogenesis in the liver. Glucocorticoids also have a synergistic effect with glucagon and catecholamines in raising blood glucose levels (Munck et al. 1984). Increased protein breakdown, induced by glucocorticoids, provide substrates for gluconeogenesis, but decrease protein stores, especially in skeletal muscle cells, causing loss of body weight and muscle weakness (Traynor and Hall 1981; Desborough 2000; Stoelting and Hillier 2006). Lipolysis is also stimulated, and fat stored as triglycerides are converted into fatty acids and glycerol. The latter is used as a substrate in gluconeogenesis (Desborough 2000). Large amounts of glucocorticoids also cause a deposition of fat in the neck and trunk regions of humans (Silverthorn 2001; Stoelting and Hillier 2006).

Glucocorticoids possess anti-inflammatory effects that are used in the treatment of inflammatory diseases. Cortisol treatment is commonly used to
suppress allergic reactions and inflammation. Their long-term use is however associated with side effects due to their catabolic actions (Silverthorn 2001).

Repeated stress may cause habituation to a stressor with a decrease in the response to the stressor, but life-threatening stressors have been shown to not cause habituation (Selye 1946; Dallman 2007). Presentation of a new stressor in chronically stressed individuals may cause a sensitized HPA response (Pecoraro et al. 2006). A persistent response due to repeated stress or the inability to terminate the response results in chronically high levels of glucocorticoids with adverse effects. For example, normal levels of glucocorticoids in the hippocampus are essential for synaptic transmission and neuronal viability, but chronically high levels are instead a cause of suppressed hippocampal neurogenesis or neurodegeneration (Heuser and Lammers 2003). Glucocorticoids are also required to maintain cardiovascular homeostasis (Udelsman et al. 1986), but chronically high levels result in cardiovascular problems, for example, hypertension (Scheuer 2009). Chronic stress is associated with depression, high cortisol levels, and impaired negative feedback, resulting in excess CRH in the central nervous system (CNS) (Heuser and Lammers 2003).

Surgical stress

During surgery, both anesthesia and tissue damage cause physiological stress responses in the body with increased glucocorticoid levels (Sandberg et al. 1954). The HPA axis is activated by neural stimulation from the surgical wound and by several humoral factors released due to tissue damage. For example, certain cytokines, such as interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α), are involved in activation (Naito et al. 1992; DeKeyser et al. 2000) as well as nitric oxide and arachidonic acid metabolites (Kehlet 1997).

While the stress response is essential for the survival of an injured animal in the wild, after surgical procedures under controlled conditions, the effect may instead be a cause of postoperative morbidity (Hall 1985; Kehlet 2000). The feedback inhibition of glucocorticoids appears to be attenuated after surgery, where high levels of both ACTH and glucocorticoids are maintained in the circulation (Hall 1985; Desborough 2000). Postoperative recovery is affected by the stress response, but also by pain and decreased gastrointestinal motility, common side effects of surgery (Kehlet 1997).

Immunosuppression caused by glucocorticoids may lead to infections and a slower healing rate of surgical wounds (Glaser et al. 1987; Christian et al. 2006). Many cells of the immune system are inhibited in such cases; for example, leukocyte migration to a damaged area is reduced (Stoelting and Hillier 2006). The activity of natural killer cells has also been shown to be
suppressed, which may cause increased susceptibility to viral infections and tumors (Shakhar and Blumenfeld 2003).

To some extent, glucocorticoids are vital to prevent life-threatening cardiovascular complications during surgery. In a study where different doses of glucocorticoids were given to adrenalectomized monkeys during surgery, the necessity for glucocorticoids was studied. A sub-physiological dose of glucocorticoids (one-tenth the normal physiological level) caused overall hemodynamic instability, whereas a physiological dose (unstressed) was sufficient to maintain cardiovascular homeostasis (Udelsman et al. 1986). Patients receiving chronic glucocorticoid therapy have routinely been treated with stress doses of exogenous glucocorticoids during surgery so as not to develop hypotension. However, such a high-dose treatment has been questioned due to the disadvantages of glucocorticoids, such as reduced tissue repair and increased susceptibility to infection. Instead, treatment with physiological levels of glucocorticoids is enough (de Lange and Kars 2008; Marik and Varon 2008).

It is important to reduce the postoperative stress response, and preemptive analgesic treatment can attenuate the stress response during the postoperative period (Desborough 2000). For example, morphine treatment attenuates plasma corticosterone levels 5 hours after surgery in rats (Page et al. 1998), and epidural blockade with lidocaine reduces cortisol levels during surgery in humans (Gordon et al. 1973).

In experimental medicine, surgical procedures on laboratory animals are common. Since stress alters normal physiology, the stress response may cause large variations between individual animals, with the consequence that a larger group size in such studies is required (Öbrink and Waller 1996). Stress may also cause suffering in laboratory animals. Thus, to reduce both the suffering and the number of animals used, it is essential to reduce the stress response in connection with experimental surgery.

**Studying stress**

To estimate a stress response in rodents, clinical signs like body weight change, water consumption, and food intake (Martini et al. 2000; Roughan and Flecknell 2001; Shavit et al. 2005) are often used. With these methods, the stress response is studied with minimal interference with the animal.

Behavioral changes during the postoperative period are an additional commonly used parameter to assess postoperative recovery (Giamberardino al. 1995; Gonzalez et al. 2000; Roughan and Flecknell 2000). Behavioral changes may indicate inadequate pain relief and slow postoperative recovery and it is possible to perform such studies with minimal animal intervention. It is, however, a method associated with many problems. For instance, observations are time-consuming and extensive training of the observer is necessary and results may differ between observers due to different interpreta-
tions. Studying frequently occurring behaviors reduces the time needed for each observation and the existence of well-defined behaviors makes an assessment as objectively quantifiable as possible (Roughan and Flecknell 2003). Rats are prey animals that instinctively hide their pain, thus, pain-related behaviors are not always obvious (Roughan and Flecknell 2001). At present, very few adequate behavioral parameters have been developed for rats and mice. Large variations in behaviors, due to naturally occurring individual differences, may result in large variations of results and complicate their interpretation. In addition, analgesic treatment may in itself cause behavioral changes.

Quantification of biochemical substances may provide more detailed information about the stress response and the molecular mechanisms of the response. Since the HPA axis is a central part of the stress response, HPA-related hormones are commonly measured (Naito, et al. 1992; Uetsuki et al. 2005). Catecholamines released after sympathetic activation and immunological factors such as IL-6 (Kato et al. 1997) and IgA (Guhad and Hau 1996) are also used as stress markers.

Glucocorticoids are secreted rapidly after stimuli from a stressor (Gordon et al. 1973) and are often used as markers of stress (Desborough 2000; Siswanto et al. 2008). Low corticosterone levels may also be a result of negative feedback.

When studying stress related to a particular treatment, it is important to reduce other potential stressors from the environment and standardize the procedure. The normal circadian rhythm must also be taken into consideration when planning the experimental set-up and analysis of the data. The sampling method may in itself be stressful for the animal and thereby inflict bias on the hormonal secretion of interest. Quantification of corticosterone excreted in feces can be performed without interventions and is useful to determine the cumulative secretion of corticosterone during a certain time interval (Whitten et al. 1998). An increase in glucocorticoid levels is, however, delayed in rats (~8 hours) (Siswanto et al. 2008). The delay may be even longer after surgery. Surgery may also decrease feces production and confound corticosterone measurements (Royo et al. 2004), which have also been observed in mice (Sundbom et al. 2011; Teilmann et al. 2012).

Blood sampling is a better method for detection of small and immediate changes in corticosterone levels during rather short periods, but the measured amount of corticosteroids only displays the level at the moment of sampling. Blood sampling implies interference with the rats that may affect the results. Blood sampling methods include manual and automatic methods. With manual sampling, the rat is disturbed during every sampling occasion, which may cause a stress response in itself (van Hooff et al. 1993). Automated blood sampling using an AccuSampler enables sampling without disturbing the rat but requires a surgical procedure to insert a catheter into a vessel. Earlier studies have concluded that blood sampling through a surgi-
cally inserted catheter does not affect corticosterone levels (Royo et al. 2004; Abelson et al. 2005; Vahl et al. 2005), and rats adapt fast to the new environment in the AccuSampler (Siswanto et al. 2008).

Pain

The International Association for the Study of Pain (IASP) adopted the generally accepted definition of pain in 1979. Their definition is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain is always subjective. (…)” (IASP 1979).

Nociception

Nociception is the transmission of noxious information from the periphery to the brain via three orders of neurons. The first-order neurons originate in peripheral tissue and terminate in the spinal cord. The noxious stimulus activates nociceptors, which are free nerve cell endings of the first-order neurons, found in most peripheral tissues such as skin, muscle, joints, and viscera (Stoelting and Hillier 2006).

There are different types of nociceptors. Although the classification of nociceptors differs slightly depending on in which tissue they are located and the literature being studied, a common way of classifying nociceptors is by distinguishing between unimodal (mechanical, chemical, or thermal) and polymodal nociceptors. Mechanical nociceptors are activated by strong pressure, whereas chemical nociceptors are activated by, for example, kalium, extreme pH, and substances like bradykinin and histamine. Thermal nociceptors are activated by noxious temperatures, and polymodal nociceptors are activated by a combination of mechanical, thermal, and chemical stimuli (Connors 2003).

Noxious stimuli open ion channels and trigger action potentials along first-order afferents. There are two types of afferent nociceptive nerve fibers, small myelinated Aδ fibers and unmyelinated C fibers. Stimulation of Aδ fibers causes an early sharp pain, whereas C fiber stimulation causes a longer lasting, dull, burning pain (Livingston and Chambers 2000).

The first-order neurons, which have their cell bodies in dorsal root ganglia, project to the dorsal horn in the spinal cord where the synaptic transmission to the second-order neurons takes place. There are several pathways, such as the spinothalamic pathway, through which the second-order neurons transmit information to the brain. The pathways terminate mainly in the ventral and medial regions of the thalamus, but other areas like the hypothalamus and the midbrain can be involved (Rang et al. 1999; Stoelting and Hillier 2006).
Third-order neurons transmit information from the thalamus to several areas in the cerebral cortex, such as the primary somatosensory cortex, where localization and characterization of the painful stimuli take place (Rang et al. 1999; Stoelting and Hillier 2006).

Activation of the hypothalamus by nociceptive input results in stress response activation. Thus, surgical tissue injury and the subsequent nociception cause not only pain but also activate the HPA axis, resulting in physiological changes in the body (Desborough 2000).

Modulation of pain
Nociception is modulated in several ways. Inflammatory mediators, like prostaglandins, histamine, and bradykinin, from an injured site cause sensitization by lowering the threshold needed for the stimulus (hyperalgesia) (Stoelting and Hillier 2006). Substance P is released from the nerve endings after activation and contributes to inflammation by activating nociceptors (Purves et al. 2001; Connors 2003).

The spinal cord is an important site of regulation of transmission of pain. In a mechanism referred to as gate control, the signal to the brain is a summation of excitatory and inhibitory signals. Interneurons in the spinal cord can inhibit nociceptive transmission. They are, for example, activated by aβ fibers with low-threshold mechanoreceptors in the periphery, and light pressure or blowing on the hurt area reduces pain through this pathway (Silverthorn 2001). There is also a descending control mechanism from the brain, inhibiting transmission from the spinal cord. During such modulation, several neurotransmitters, such as noradrenaline, serotonin, GABA (Livingston and Chambers 2000; Connors 2003), and acetylcholine (Abelson et al. 2006), as well as endogenous opioids, enkephalins, endorphins, and dynorphins, are important signals (Livingston and Chambers 2000).

Nociceptive signaling may cause central sensitization, which involves changes in the spinal cord and brain, resulting in increased excitability of secondary neurons. Prolonged central sensitization may lead to central neuroplasticity, which in turn may cause persistent pain. Central sensitization can be blocked or delayed by treatment with pre-emptive analgesia. The afferent neurons are then blocked before the initiation of tissue injury (Livingston and Chambers 2000; Stoelting and Hillier 2006).

Analgesia
Analgesic drugs are administered to modulate nociception and reduce pain sensation. Analgesics are categorized based on their route of action; here follows an overview of opioids, non-steroidal anti-inflammatory drugs (NSAIDs), and local anesthetics (Fig. 3).
Figure 3. Pain modulation by analgesic drugs. Opioids act by binding to opioid receptors in the periphery, spinal cord, and brain, and suppress nociception. NSAIDs inhibit prostaglandin synthesis and prevent activation of the nociceptive fibers. Local anesthetics (LA) block the transmission of the noxious information via the nerve.

**Opioids**

Opioids exert their effect by binding to opioid receptors. Opioid drugs mimic endogenous opioids and thereby inhibit the transmission of noxious stimuli. There are three subtypes of opioid receptors: µ, κ, and δ, where µ is considered the most important in anti-nociception (Stoelting and Hillier 2006). All µ-agonists are analgesics, but the potency and maximal effect differ between compounds. Opioid receptors are distributed in brain, spinal cord, and the periphery. Morphine is the reference opioid compound and is commonly used to treat moderate to severe pain.

Common side effects of opioids are euphoria, respiratory depression, depression of the cough reflex, nausea, reduced motility in the gastrointestinal tract with delayed gastric emptying, bronchoconstriction, and hypotension. Dependence and tolerance are common problems with opioids (Rang et al. 1999; Nolan 2000). The degree of side effects differs between opioid compounds and their dosing. Opioids are commonly used during surgery to reduce both postoperative pain and the amount of anesthetic agent required (Stoelting and Hillier 2006).

**Buprenorphine**

Buprenorphine is a potent analgesic and is a partial agonist of µ-receptors and an antagonist of κ-receptors. Buprenorphine is able to antagonize the
effect of other opioids (Rang et al. 1999). Due to its partial agonistic properties, its maximal effect is less than morphine, since partial agonists reach a ceiling effect (there is no upper limit when using pure µ-agonists). Its duration of action is long due to a slow dissociation from the receptor (Gueye et al. 2001). The main side effects of buprenorphine are less pronounced than morphine, and buprenorphine is less likely to cause dependence (Rang et al. 1999). Buprenorphine is used for the treatment of postoperative pain, chronic pain and opioid dependence in humans (LIF 2012).

In rodents, buprenorphine is a commonly used analgesic that has in analgesiometric tests been shown to be effective in pain relief of both acute and chronic pain (Cowan et al. 1977; Christoph et al. 2005). Postoperative pain is reduced by buprenorphine treatment when studying clinical parameters like body weight, and food and water intake (Liles and Flecknell 1993; Jablonski et al. 2001), and changes in pain-related behaviors (Liles et al. 1998). Buprenorphine has also been shown to prevent negative effects on the immune system, such as natural killer cell activity and tumor metastasis (Franchi, Panerai et al. 2007).

Subcutaneous (s.c.) administration of buprenorphine is most common, but oral administration would be preferable in many cases, enabling administration without any handling of the rat. Oral administration of buprenorphine mixed in flavored gelatin has been used for treatment of postoperative pain and was found to prevent postoperative body weight loss (Flecknell et al. 1999). Buprenorphine has also been mixed in Nutella® (Royo et al. 2004), although its analgesic effect was not evaluated in that investigation. The effect of buprenorphine treatment on perioperative corticosterone levels has been very sparsely investigated in previous studies.

**Local anesthetics**

Local anesthetics act by blocking sodium channels in nerve cells. Such blockade results in the prevention of axonal conduction and transmission of noxious information to the brain (Rang et al. 1999; Stoelting and Hillier 2006). Both myelinated Aδ fibers and unmyelinated C fibers are blocked by a similar concentration of local anesthetics and are blocked before larger myelinated nerve fibers. Thus, the sensation of pain can be blocked while pressure is still sensed (Nolan 2000; Stoelting and Hillier 2006). There are several routes of administration of local anesthesia, for example, topical addition, local infiltration, intravenous infusion, or epidural and spinal administration (Stoelting and Hillier 2006).

Unwanted effects of local anesthetics mainly affect the cardiovascular system and CNS. Local anesthetics may cause vasodilatation and myocardial depression, which lead to hypotension. CNS effects include restlessness and tremor, and in worst-case scenarios, convulsion and CNS depression. These unwanted effects are most adverse in combination with systemic administration. However, some systemic effects may occur by accident via injection.
into veins or absorption into the systemic circulation, even during local infiltration (Rang et al. 1999; LIF 2012).

Lidocaine is a commonly used local anesthetic with a rapid onset of action but short duration (~1 hour) (Nolan 2000). Lidocaine is found in several formulations and has numerous uses in humans, both alone and in combination with other local anesthetics. It can be infiltrated during short surgical procedures or added topically to facilitate blood sampling in children (LIF 2012).

**Non-steroidal anti-inflammatory drugs**

Non-steroidal anti-inflammatory drugs (NSAIDs) possess anti-inflammatory, analgesic, and antipyretic effects. They are mainly effective against pain associated with inflammation or tissue damage (Rang et al. 1999).

NSAIDs act by inhibiting the enzyme cyclooxygenase (COX), which is involved in the production of prostaglandins and thromboxanes. Prostaglandins sensitize nociceptors to inflammatory mediators and stimulate nociception at the spinal level. Since NSAIDs block prostaglandin production, nociception and thus pain are reduced (Nolan 2000). Furthermore, NSAIDs reduce vasodilatation and edema, causing reduced inflammation and associated pain. Prostaglandins in the hypothalamus mediate fever, and an inhibition of prostaglandins lowers body temperature. NSAIDs are also used in combination with opioids to reduce the amount of opioids needed (Rang et al. 1999).

Thromboxanes are involved in clotting mechanisms, and NSAIDs have antithrombotic effects as well. At the therapeutic doses used for most NSAIDs, this effect is of little importance. However, aspirin causes an irreversible inhibition of COX, blocking platelet aggregation at therapeutic dose and is thus used as an antithrombotic drug (Rang et al. 1999; Nolan 2000).

There are two main categories of COX enzymes, COX-1 and COX-2. Prostaglandins produced by COX-2 are associated with inflammation, pain, and fever, whereas COX-1 prostaglandins are important for gastrointestinal and renal function (Rang et al. 1999; Nolan 2000). The most common side effects of NSAIDs are gastrointestinal, since prostaglandins are important for the protection of the gastric mucosa. Dyspepsia, diarrhea, nausea, and ulcer formation are negative effects associated with NSAID use. Since prostaglandins are associated with renal function, an adverse but uncommon side effect is acute renal insufficiency. This may occur under conditions of reduced renal blood flow or chronic NSAID consumption (Rang et al. 1999; Nolan 2000).

Ibuprofen is a commonly used painkiller with a low incidence of unwanted effects (Rang et al. 1999).
Analgesia and stress

Tissue injury during surgery and pain in the postoperative period causes a stimulation of nociceptors and transmission of noxious information to the brain. As mentioned above, the hypothalamus is activated, resulting in stress response activation (Desborough 2000). By reducing nociception, activation of the HPA axis is also reduced.

The timing of analgesic administration is important, since noxious information must be prevented as early as possible before stress activation and central sensitization begin. Therefore, pre-emptive analgesia is administered before the start of the surgery. Analgesic treatment in the postoperative period may still be needed, but the pain will be more easily controlled (Dobromylskyj et al. 2000). Thus, pre-emptive analgesia is important to prevent both central sensitization and HPA activation.

The stress response can be modified by blocking neural afferents, and thereby, noxious information. A nerve blockade using epidural local anesthetics have been shown to inhibit cortisol levels in humans during lower body procedures (Kehlet 2000). Opioids reduce pain and the stress response by inhibition of hypothalamic function (Traynor and Hall 1981). For instance, morphine has been shown to reduce postoperative corticosterone levels in rats 5 hours after surgery (Page et al. 1998). Treatment with NSAIDs alone seems to have only a slight or no effect on the HPA response (Kehlet 2000). However, ibuprofen administered to human patients before cholecystectomy reduces ACTH and cortisol levels 1 hour after surgery (Chambrier et al. 1996). Regarding the use of pre-emptive analgesia for attenuating stress in laboratory rodents, much is yet to discover.

The laboratory rat

The laboratory rat belongs to the species *Rattus norvegicus*, the brown rat. Rats are commonly used in experimental medicine, since they are well defined, easily maintained, and relatively healthy (Harkness and Wagner 1983).

Animals have been used for research for many centuries. The first documentation of such use is from approximately 400 B.C. in Greece, and the development is parallel to the development of medical science (van Zutphen et al. 1993). In the 16th century, domestic rats were bred and used for combat with terriers. In the 18th century, these rats were also used for scientific research (Harkness and Wagner 1983). The Wistar Institute in Philadelphia developed the first laboratory rat—the Wistar rat—bred for biomedical research. Many other strains originated from this rat (Havenaar et al. 1993).

The rat is an omnivore and has powerful jaw muscles with two incisors and six molars in each half of the jaw. The incisors are continuously erupt-
The rat has no gall bladder, a divided stomach (Harkness and Wagner 1983), and a large cecum used for fermentation (Öbrink and Waller 1996).

The Sprague-Dawley and Wistar rats are outbred albino rats that are commonly used in medical research. Sprague-Dawley rats have a more narrow face and longer tail than Wistar rats (Harkness and Wagner 1983). An outbred stock is a closed group of genetically heterogeneous animals. Gene assembly in the population is unchanged, but each individual is genetically unique. Breeding in the stock must be random with no new animals introduced. In inbred strains, however, the individuals are nearly identical genetically (Öbrink and Waller 1996; Festing 2003).
The general aim was to study the surgical stress response in rats during surgery and the postoperative period, and its modulation by analgesic treatment. The specific aims were as follows:

- To study how plasma corticosterone levels during surgery are affected by the severity of the surgery and analgesic treatment with buprenorphine or lidocaine (Paper II).

- To study the effect of pre-emptive buprenorphine treatment by either subcutaneous injection or voluntary ingestion on postoperative corticosterone levels as well as clinical parameters in conscious rats (Papers I, III, and IV).

- To study stock-related differences in postoperative recovery after buprenorphine treatment with regard to corticosterone levels and clinical parameters (Paper IV).

- To study the concentration of buprenorphine in plasma after oral and subcutaneous treatment (Paper IV).
Materials and methods

Animals

In all studies, male Sprague-Dawley rats were used (Scanbur B&K, Sollentuna, Sweden) and in study IV, male Wistar rats were also included (Scanbur B&K, Sollentuna, Sweden). No females were included to avoid fluctuations in the corticosterone pattern due to hormonal changes of the estrus cycle.

The rats were housed in groups of 2–3 rats in Makrolon type IV cages in the laboratory animal facility for at least 1 week after arrival. The diurnal rhythm was regulated with 12-hour light (6.00–18.00) and 12-hour dark, and the rats had free access to pelleted food (R36, Lantmännen, Stockholm, Sweden) and tap water. At least 2 days before surgery, the rats were moved to the laboratory and singly housed in Makrolon type III cages. During the experiment when the rats were attached to the AccuSampler (described below), they were housed in the same cages as before. The catheter connected to the rat obstructs environmental enrichment with houses or tunnels. Instead, the cage was enriched with paper in which the animal could hide. The food pellets were placed on the floor to facilitate access.

The Animal Ethics Committee in Uppsala (Sweden) approved all the animal experiments in this study (C46/6, C185/6). The experiments were terminated by injection of an overdose of pentobarbitone (Pentobarbital, Apoteket, Sweden).

Analgesic treatment

Analgesics were given via different routes of administration. An overview of the analgesic treatments and dosing intervals is presented in Table 1. Buprenorphine (Temgesic®, Schering-Plough Europe, Brussels, Belgium) was administered both subcutaneously and orally. Subcutaneous buprenorphine (papers I, II, and IV) was administered at a dose of 0.05 or 0.1 mg/kg one-half hour or 1 hour before surgery, depending on experiment. The administration was then repeated 8 hours after surgery followed by injections every 12 hours.

The oral dose of buprenorphine (papers I, III, and IV) was 0.4 mg/kg administered 1 hour before surgery and then every 24 hours. Sublingual buprenorphine tablets were crushed into a powder, which was mixed with a
chocolate and hazelnut paste (Nutella). The mixture (2 g/kg body weight) was placed in a small plastic bowl in the cage, and the rats ingested it voluntarily within a few minutes (for detailed instructions, see Abelson et al. 2012). Pure Nutella was given to the rats 2 days before the treatment to habituate them to the taste. This route of administration is named oral or voluntary ingestion (VI) in the papers.

Lidocaine (Xylocain®, Astra Zeneca, Södertälje, Sweden) at a dose of 4 mg/kg in a solution of 0.01 mg/µl was administered to anesthetized rats by infiltration in the skin and muscles at each incision site before opening the skin (papers I–III). Lidocaine was administered to the control groups in order to avoid the use of completely non-pain relieved rats. Bupivacaine (Marcain®, Astra Zeneca, Sweden) at a dose of 1 mg/kg was administered in the same way as lidocaine (paper III). Lidocaine or bupivacaine was administered to all control rats that were allowed to regain consciousness after surgery.

Table 1. Overview of analgesic treatments and dosing intervals.

<table>
<thead>
<tr>
<th>Analgesia</th>
<th>Dose</th>
<th>Pre-op</th>
<th>Intra-op</th>
<th>Post-op</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS Bup s.c.</td>
<td>0.05</td>
<td>0.5 h</td>
<td>-</td>
<td>8 h, then every 12 h</td>
<td>I, IV</td>
</tr>
<tr>
<td>AS Bup VI</td>
<td>0.4</td>
<td>1 h</td>
<td>-</td>
<td>Every 24 h</td>
<td>I, III, IV</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>4</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>I, III</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>1</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>III</td>
</tr>
<tr>
<td>Acute Bup s.c.</td>
<td>0.05</td>
<td>1 h</td>
<td>-</td>
<td>-</td>
<td>II</td>
</tr>
<tr>
<td>Acute Bup s.c.</td>
<td>0.1</td>
<td>1 h</td>
<td>-</td>
<td>-</td>
<td>II</td>
</tr>
<tr>
<td>Acute Lidocaine</td>
<td>4</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>II</td>
</tr>
</tbody>
</table>

*AS = study where an AccuSampler was used. Acute = study where the rats were anesthetized during the entire study. Bup = buprenorphin.*

Surgical procedure

Isoflurane delivered in pure oxygen was used for both induction and maintenance of anesthesia. Body temperature was maintained at 37–38°C using a heated pad and rectal thermometer. The skin was shaved and washed with iodine before surgery. The surgical procedure consisted of the insertion of a catheter into a vessel to enable blood sampling. Either the vena jugularis (papers I and IV) or arteria carotis communis (papers II and III) was used (see Table 2). Catheterization of the vein is a somewhat simpler surgery with less risk of bleeding complications compared to arterial catheterization. However, vein catheterization is associated with more clotting of the tubing and blood sampling failure compared to arterial catheterization.
Catheterization began with the opening of the skin, and the muscles were then dissected to expose the vessel. A catheter filled with heparinized saline was inserted into the vessel with its tip close to the heart. To enable connection with the sampling equipment (papers I, III, and IV), the catheter was led subcutaneously to the back of the neck where a DiLab Dacron button was attached with two sutures in the nape muscles. The catheter was led through the Dacron button and a metal spring was attached to it. The skin was then sutured using silk.

In paper II, laparotomy was included as a more severe type of surgery. During laparotomy, a 2-cm midline incision through skin and muscle was made in the abdominal wall. The viscera was then gently touched with the index finger for 1 minute, as previously described by Roughan and Flecknell (2001). The muscle layer and skin were then sutured.

### Table 2. Overview of surgery and blood sampling.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Surgery</th>
<th>Blood sampling</th>
<th>Sampling time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Venous catheterization</td>
<td>AccuSampler</td>
<td>18/96</td>
</tr>
<tr>
<td>II</td>
<td>Arterial catheterization, laparotomy</td>
<td>Manual</td>
<td>6</td>
</tr>
<tr>
<td>III</td>
<td>Arterial catheterization</td>
<td>AccuSampler</td>
<td>18</td>
</tr>
<tr>
<td>IV</td>
<td>Venous catheterization</td>
<td>AccuSampler</td>
<td>96</td>
</tr>
</tbody>
</table>

Sampling time = number of hours of blood sampling after surgery.

### Blood sampling

During the experiments, blood was sampled through the inserted arterial or venous catheter. An overview of blood sampling is presented in Table 2. In papers I, III, and IV, the rats were conscious and sampling equipment (AccuSampler®, DiLab, Lund, Sweden) was used to sample blood for 18 or 96 hours depending on the experimental design. When using the AccuSampler, the rat can move freely in the cage (Makrolon type III) with the catheter protected by a metal spring and connected at the top of the cage (Fig. 4).

The catheter was automatically washed with heparinized saline repeatedly every 30 minutes to avoid blood clotting. The AccuSampler was programmed to sample blood at specified time points, and the blood volume was replaced by saline injection. The samples were stored in a cooling rack at 4°C and centrifuged to obtain plasma, which was stored at -20°C until analysis.

In paper II, the rats were anesthetized during the whole experiment and the blood was manually sampled from the catheter during surgery and during
anesthesia 6 hours following the completion of surgery. In addition to blood sampling, mean arterial blood pressure (MAP) was monitored in paper II.

**Figure 4. Experimental set-up. The rat was catheterized, and the catheter was led through a metal spring and connected to the AccuSampler, which was programmed to sample blood. The rat moved freely in the cage. The blood samples were stored in a cooled rack and later analyzed by ELISA.**

**Enzyme-linked immunosorbent assay**

The plasma concentrations of corticosterone (papers I–IV) and buprenorphine (paper IV) were quantified using commercially available competitive Enzyme-linked immunosorbent assays (ELISAs). In a competitive ELISA, the sample is added together with a competitor conjugated to a relevant substance that produces an alteration in absorbance upon manipulation with a substrate. The competitor binds with the same affinity as the sample and is detected using quantification of absorbance. The more sample added, the fewer competitors are bound and the lower is the signal (Fig. 5) (Crowther 1995).

Corticosterone was quantified using the Corticosterone EIA assay (Assay Designs Inc., Ann Arbor, USA). A 96-well plate was pre-coated with donkey anti-sheep antibody. The sample was added together with sheep anti-corticosterone antibody and alkaline phosphatase-conjugated corticosterone, followed by incubation. The plate was then washed and the substrate p-nitrophenyl phosphate was added. Alkaline phosphatase is an enzyme that converts p-nitrophenyl phosphate into p-nitrophenol, which generates a yellow color that was quantified at 405 nm using a Labsystems Multiskan RC.

Buprenorphine was detected by the Buprenorphine One-step ELISA (International Diagnostic Systems Corp.). The sample was added to wells pre-
coated with an anti-buprenorphine antibody (Fig. 5). An enzyme conjugate was added before incubation. After washing, substrate was added to generate a blue color, which was quantified using a Thermo Multiskan Ex microplate reader at 450 nm.

Corticosterone/buprenorphine and the conjugate bind to the antibody in a competitive manner and the less corticosterone/buprenorphine in the sample, the more conjugate will bind and the more intense will be the color. The color is inversely proportional to the concentration of corticosterone/buprenorphine in the sample.

![Corticosterone and Buprenorphine Diagram](image)

*Figure 5. Competitive ELISA. The sample (S) and the conjugate (C) bind to the antibody in a competitive manner. E represents the enzyme part of the conjugate. After adding a substrate, the enzyme is activated and generates a color (*), which can be measured.*

**Body weight and water intake (papers I, III, and IV)**

The animals were weighed before surgery and at the end of the experiment to obtain the change in body weight. The water bottles were also weighed every day to obtain daily water intake. These are commonly used and well-validated parameters for assessing postoperative recovery (Varma et al. 1999; Martini et al. 2000; Shavit et al. 2005).

**Behavior (paper III)**

The behavior of the rats was monitored during two occasions in the postoperative period. The rats were filmed for 30 minutes at 25 minutes and 5 hours after they regained consciousness. The first five recorded minutes were excluded from the analysis, because the presence of the operator starting the camera may have disturbed the rats. Non-operated control rats were recorded at the same time of day (approximately at 12.30 and 17.00).
The same person analyzed all the films. To estimate the level of locomotor activity, the frequencies of the following behaviors were scored: exploration, walking, digging, and rearing (Table 3). The sum of these behaviors was used to determine differences in activity between groups. The time the rats spent resting was registered and the percentage of resting time was calculated.

**Table 3. Definitions of active behaviors**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploration</td>
<td>The rat explores the cage by moving around sniffing</td>
</tr>
<tr>
<td>Walking</td>
<td>The rat walks or runs</td>
</tr>
<tr>
<td>Digging</td>
<td>The rat digs in the bedding material</td>
</tr>
<tr>
<td>Rearing</td>
<td>The rat rears on his hind legs</td>
</tr>
</tbody>
</table>

**Statistical methods**

Statistical analysis was performed using GraphPad Prism and SPSS Statistics software packages. The animals were randomly grouped and P values less than 0.05 were considered significant.

In paper I, analysis of variance (ANOVA) with Tukey’s post-hoc test was used to calculate differences between corticosterone levels at different time points depending on treatment. A general linear model (GLM) with repeated measures was used to determine differences in corticosterone levels between groups during the entire sampling period. Differences in mean body weights and changes in mean water intake between different groups were determined using ANOVA with Tukey’s post-hoc test. One-sample t test was performed to determine whether the changes in body weight and water consumption were different from zero.

In paper II, ANOVA with Bonferroni’s post-test was performed to calculate differences in serum corticosterone levels between treatments and surgery groups at each time point. The MAP was compared between groups by ANOVA.

In paper III, GLM was used to determine significant differences in corticosterone levels. Changes in body weight and water intake were analyzed using t tests. The Kruskal-Wallis test was used to analyze the behavioral data, which were non-parametric.

In paper IV, a two-factor ANOVA was used to determine differences in body weights, and corticosterone and buprenorphine concentrations. Since there were no stock-related differences in corticosterone or buprenorphine concentrations, the stock data were pooled. These data were then further analyzed with GLM for the first 18 hours after surgery.
Results

Paper I

Sprague-Dawley rats were treated with buprenorphine before surgery either orally or subcutaneously. One control group without buprenorphine treatment was also included. This control group received lidocaine during surgery. The treatment groups are presented in Table 4. Plasma corticosterone levels were measured postoperatively as well as body weight and water intake.

Table 4. Treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Lidocaine</td>
<td>9</td>
</tr>
<tr>
<td>S.c.</td>
<td>Buprenorphine 0,5 h pre-op, 8 h post-op, then every 12 h</td>
<td>6</td>
</tr>
<tr>
<td>Oral</td>
<td>Saline injection Buprenorphine 1 hour pre-op*</td>
<td>6</td>
</tr>
<tr>
<td>Oral</td>
<td>No injection Buprenorphine 1 hour pre-op, every 24 hours</td>
<td>6</td>
</tr>
</tbody>
</table>

n = number of rats. *This group was terminated after 18 hours instead of 36 hours.

The initial mean corticosterone levels (Fig. 6A) were between 160 and 210 ng/ml, and began to decline immediately in all groups. At 10 hours, the levels had increased in control (125 ng/ml) and subcutaneous groups (120 ng/ml), and an ANOVA displayed a significant difference compared to the oral treatment (50 ng/ml). GLM analysis displayed a significant difference between the oral treatment and control group and between the oral and subcutaneous treatment groups during the entire period from 0 to 18 hours postoperatively (Fig. 6A). The oral group was divided in two subgroups to test whether the elevated corticosterone levels after subcutaneous treatment were directly affected by the injection. One-half of the rats were left undisturbed while the other half got a saline injection to mimic the treatment of the subcutaneous group. There was no significant difference between these two subgroups (Fig. 6B).

The corticosterone measurements continued for 96 hours after surgery to investigate the diurnal rhythm, except for saline injected part of the oral group which was terminated after 18 hours. After the first 24 hours the corticosterone levels were measured every 12-hour, and were low (~20 ng/ml) in
the light phase and high (~60 ng/ml) in the dark phase with no differences between groups (Fig. 7).

* Figure 6. Postoperative plasma corticosterone levels 18 hours after surgery [mean ± standard error of the mean (SEM)]. The gray area represents the dark period. * Significant difference from the control group (ANOVA). # Significant difference from the subcutaneous group (ANOVA). ** Significant difference between the oral and control groups (GLM). ## Significant difference between the oral and subcutaneous groups (GLM). A. All treatment groups. B. Subgroups of the oral group.
Body weight in the control group was reduced 1 day after surgery compared to preoperative values (Fig. 8). Buprenorphine-treated rats had no change in body weight 1 day after surgery and there was a difference between buprenorphine and control groups. There were no significant differences in body weight between the subcutaneous and oral groups.

Water intake in the control group was reduced 1 day after surgery compared to preoperative intake (Fig. 8). Water intake was less in the control group compared to the buprenorphine-treated groups. There was a significant difference between the oral and subcutaneous buprenorphine groups, with higher water intake in the subcutaneous group.
Sprague-Dawley rats received saline, lidocaine, or two different doses of buprenorphine (s.c.). They were subjected to either catheterization or the more severe surgery, catheterization and laparotomy (Table 5).

Table 5. Treatment groups

<table>
<thead>
<tr>
<th>Group name</th>
<th>Analgesic treatment</th>
<th>Surgery</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAL</td>
<td>Saline</td>
<td>CAT</td>
<td>6</td>
</tr>
<tr>
<td>SAL+LAP</td>
<td>Saline</td>
<td>CAT+LAP</td>
<td>6</td>
</tr>
<tr>
<td>LID</td>
<td>Saline</td>
<td>Lidocaine</td>
<td>CAT</td>
</tr>
<tr>
<td>LID+LAP</td>
<td>Saline</td>
<td>Lidocaine</td>
<td>CAT</td>
</tr>
<tr>
<td>BUP0.05</td>
<td>Buprenorphine 0.05 mg/kg</td>
<td>CAT</td>
<td>6</td>
</tr>
<tr>
<td>BUP0.05+LAP</td>
<td>Buprenorphine 0.05 mg/kg</td>
<td>CAT+LAP</td>
<td>6</td>
</tr>
<tr>
<td>BUP0.1</td>
<td>Buprenorphine 0.1 mg/kg</td>
<td>CAT</td>
<td>6</td>
</tr>
<tr>
<td>BUP0.1+LAP</td>
<td>Buprenorphine 0.1 mg/kg</td>
<td>CAT+LAP</td>
<td>6</td>
</tr>
</tbody>
</table>

$CAT = \text{catheterization, LAP = laparotomy}$

The corticosterone levels of the surgery groups, regardless of treatment, are presented in Figure 9.

Figure 9. Plasma corticosterone levels in the surgery groups (mean ± SEM). $^* p < 0.05$ significant difference between groups. $CAT = \text{catheterization, LAP = laparotomy}$.
Laparotomy resulted in higher plasma corticosterone levels than did catheterization, from immediately after surgery to 1 hour after surgery and at 3 hours after surgery.

There was a difference in corticosterone levels depending on treatment, just after catheterization (-0.3 hour) to 1 hour after surgery (Fig. 10).

Lidocaine reduced the corticosterone levels compared to saline only after the less severe surgery, catheterization, and only immediately after surgery was completed (0 hour). Thereafter, there was no significant difference between the saline and lidocaine treatment groups.

Figure 10. Plasma corticosterone levels from -0.3 to 1 hour after surgery (mean ± SEM). * p<0.05 significant difference from saline treatment, # p<0.05 significant difference from lidocaine treatment. SAL = saline, LID = lidocaine, BUP0.05 = buprenorphine 0.05 mg/kg, BUP0.1 = buprenorphine 0.1 mg/kg. A. Catheterization. B. Laparotomy.
Buprenorphine treatment resulted in significantly lower corticosterone levels compared to both the saline and lidocaine groups, during surgery and until 1 hour after surgery. Two different doses were used (0.05 and 0.1 mg/kg) and the reducing effect was seen earlier with the higher dose and this effect was more pronounced after laparotomy. However, the effect was not extended by the higher dose.

Hypotension is a common side effect of most opioids and thus, MAP was measured during the experiment. At the beginning of the surgery, MAP was lower in the buprenorphine group (buprenorphine data pooled) compared to the pooled data of the saline and lidocaine groups. There was no difference between saline and lidocaine groups in MAP. During the surgery, the animals were more deeply anesthetized compared to the period after completed surgery. Between one-half hour and 4 hours after surgery, MAP was higher in the buprenorphine group compared to the other groups (Fig. 11).

Figure 11. Mean arterial blood pressure (mean ± SEM). *p<0.05 significant difference between groups. SAL = saline, LID = lidocaine, BUP = buprenorphine.

Paper III

The stress response in Sprague-Dawley male rats was assessed for 18 hours after arterial catheterization. Plasma corticosterone levels and changes in body weight and water intake were measured as well as behavioral changes. The operated rats were divided into two treatment groups: oral buprenorphine and control that received the local anesthetics lidocaine (n=9) or bupivacaine (n=10). Two non-operated groups were also included for behavioral
monitoring, one buprenorphine-treated and one untreated group (see Table 6). The non-operated rats were filmed at the same time of the day as the operated rats.

Table 6. Experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Analgesia</th>
<th>Surgery</th>
<th>Rats (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>Buprenorphine</td>
<td>Catheterization</td>
<td>9</td>
</tr>
<tr>
<td>Control</td>
<td>Lidocaine/bupivacaine</td>
<td>Catheterization</td>
<td>19</td>
</tr>
<tr>
<td>Non-op buprenorphine</td>
<td>Buprenorphine</td>
<td>None</td>
<td>8</td>
</tr>
<tr>
<td>Non-op control</td>
<td>-</td>
<td>None</td>
<td>16</td>
</tr>
</tbody>
</table>

Plasma corticosterone levels were measured during surgery and 18 hours postoperatively. During surgery, there was no difference in plasma corticosterone levels between the treatment groups. However, during the first 18 hours after surgery, corticosterone levels were lower in the buprenorphine-treated group compared to controls, as determined by the GLM (Fig. 12).

![Figure 12. Postoperative corticosterone levels (mean ± SEM). * p<0.05 significant difference between groups (GLM). The gray area represents the dark period.](image)

Body weight and water intake in the control group were reduced 1 day after surgery compared to preoperative values and there was a difference compared to buprenorphine-treated rats. The buprenorphine-treated rats had no change in body weight or water intake 1 day after surgery (Fig. 13).
Locomotor activity and time spent resting were recorded for 25 minutes at one-half hour and 5 hours after surgery (Fig. 14). Differences in behavior between groups were only seen during the first period. During this period, the non-operated buprenorphine-treated rats were more active and spent less time resting than untreated non-operated rats. Buprenorphine-treated operated rats rested for a longer period than non-operated buprenorphine-treated rats. No behavioral differences were seen between the operated rats.
Figure 14. The observed behavior at 25 minutes and 5 hours after surgery (mean ± SEM) A. Percentage time spent resting. B. Number of active behaviors.

Paper IV

Sprague-Dawley and Wistar rats were treated with buprenorphine preoperatively. Corticosterone and buprenorphine levels in plasma were measured during the postoperative period. Both rat stocks were divided into two treatment groups; VI and subcutaneous buprenorphine treatment. Two non-operated control groups (Sprague-Dawley and Wistar rats) were also included for body weight comparisons (see Table 7).
Table 7. Experimental groups

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Treatment</th>
<th>Surgery</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague-Dawley</td>
<td>VI</td>
<td>Catheterization</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>Catheterization</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>None-op</td>
<td>6</td>
</tr>
<tr>
<td>Wistar</td>
<td>VI</td>
<td>Catheterization</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>Catheterization</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>None-op</td>
<td>6</td>
</tr>
</tbody>
</table>

Plasma corticosterone levels were measured for 96 hours. A two-factor ANOVA displayed differences between subcutaneously and orally treated rats at 2 and 10 hours after surgery. No differences of the stocks were observed, and thus, the two stocks were pooled for further analyses. A GLM analysis during the first 18 hours showed higher levels of corticosterone in the subcutaneous group (Fig. 15).

![Graph](image)

Figure 15. Postoperative corticosterone levels. Data from Wistar and Sprague-Dawley rats were pooled (mean ± SEM). The gray area represents the dark period. ## Significant difference between groups (p<0.01) with GLM. Ψ p<0.05, ΨΨ p<0.01 significant difference between treatment groups with t-test.

There was no difference in body weight between the groups before surgery (Fig. 16). A two-factor ANOVA showed that changes in body weight were
dependent on both stock and treatment. The non-operated control rats in both stocks gained more weight than rats subjected to treatment and surgery. There was a stock-related difference in body weight between subcutaneous and VI treatment in Wistar rats but not in Sprague-Dawley rats, where the subcutaneously treated Wistar rats reduced their weight more than the VI rats.

![Figure 16. Percentage body weight change (mean ± SEM). Hatched brackets represent statistical significance between groups at p<0.01 and continuous brackets at p<0.001. SD = Sprague-Dawley.](image)

Plasma buprenorphine levels during the first 18 hours were quantified (see Fig. 17). There were no differences between the stocks and thus, the data were pooled. In subcutaneously treated rats, the concentration of buprenorphine was 1.5 ng/ml immediately after surgery (0 hour). After 2 hours, the concentration had declined to 0.1 ng/ml and was 0.03 ng/ml after 6 hours. A re-injection was done at 8 hours, and at 10 hours, the concentration in plasma was high again, 2 ng/ml. Once again, a fast decline was seen but the levels were not as low as before the second injection (0.4 ng/ml at 18 hours). The buprenorphine concentration in orally treated rats declined very slowly over this period and was between 1.2 and 0.4 ng/ml. At 2 and 6 hours after surgery, the buprenorphine concentration was higher in the VI than in the subcutaneous group. At 10 hours, the buprenorphine concentration was higher in the subcutaneous group.
Figure 17. Plasma concentration of buprenorphine. $\Psi\Psi p<0.01$, $\Psi\Psi\Psi p<0.001$
Significant difference between groups.
Discussion

Surgery induces a physiological stress response with neural and endocrine alterations. During this response, the HPA axis is activated and glucocorticoids are secreted from the adrenal gland. Such a response is essential to adapt to new situation, but during surgery, persistent stress is a cause of postoperative complications (Desborough 2000).

Laboratory rats are often subjected to surgery in biomedical science. It is important to reduce the stress and pain associated with the surgery to reduce the suffering of the animal and alterations of its normal physiology. Physiological alterations may cause large individual variations in the results, since individual rats respond differently to stress responses (Öbrink and Waller 1996). Analgesic treatment with buprenorphine reduces pain (Roughan and Flecknell 2002), but has also been shown to reduce corticosterone levels in rats (Franchi et al. 2007). The use of analgesic treatments in rats is however not widespread, and more research is needed to optimize techniques (Stokes et al. 2009).

In this thesis, the surgery-induced stress response in rats was studied, and the impact of orally and subcutaneously administered buprenorphine on postoperative recovery. Intra- and/or postoperative corticosterone levels were quantified and surgery-induced corticosterone release was observed as well as changes in water consumption, body weight and behavior.

Stress assessment

It is problematic to measure plasma corticosterone levels without disturbing the animals, as mentioned above. We used an automated blood sampling system (AccuSampler) to collect blood from conscious rats during the postoperative period (papers I, III, and IV). According to earlier studies, this is a convenient method and the rats seem to rapidly adapt to the new situation, as indicated by a return to basal corticosterone levels and normal diurnal fluctuations (Royo et al. 2004; Siswanto et al. 2008). Since stress is associated with a disturbance in the diurnal rhythm of corticosterone secretion (Barriga et al. 2001; Dispersyn et al. 2008), a return to a normal rhythmic pattern indicates stress reduction. A normal diurnal rhythm was observed on postoperative day 2 in papers I and IV, supporting the findings by Royo et al. (2004).
Rats must be singly housed when connected to the blood-sampling device. Single housing may itself be a source of stress and bias the interpretation of the results. Thus, we let the animals acclimatize to the single housing for 2 days before surgery. In a study performed in our lab, no effect of transferring Sprague-Dawley rats from group housing to single housing was observed on fecal corticosterone metabolites or body weight gain (Dahlin et al. 2009). The rats in this thesis also gained weight during the two pre-surgical days, indicating a rapid acclimatization to their new environment. The rats were housed in the same cage after surgery to minimize stress due to environmental changes.

Repeated blood sampling may be a source of stress and frequent blood sampling (200 µl every 15 minutes for 6 hours; nearly 5 ml in total) causes elevated corticosterone levels as shown by Abelson et al. (2005). The sample volume in the papers of this thesis, was 100–150 µl and a maximum of 10 samples over 24 hours, with 13 samples per study, were sampled (total volume of approximately 2 ml).

Postoperative changes in body weight and water intake are common parameters in pain assessment (Martini et al. 2000; Roughan and Flecknell 2001). Water intake may be affected by saline infusions. Saline infusions (1.8 ml) in Wistar rats over 24 hours following halothane anesthesia have been shown to reduce water intake by 3 ml (Liles and Flecknell 1992). The AccuSampler replaces the obtained blood volume with saline, and the catheter is washed with 20 µl saline every 30 minutes. This infusion is the same for all treatment groups and should thus not be a source of bias.

Behavioral observations are commonly used to assess postoperative recovery (Roughan and Flecknell 2001; Roughan and Flecknell 2003; Wright-Williams et al. 2007). In paper III, locomotor activity, as well as time resting were analyzed. No differences in locomotor activity or resting time were observed between treatment groups among the operated animals despite differences in corticosterone levels, body weight, and water intake. These findings may be due to the large variation of data within a treatment group. Thus, the behavioral observations applied in the present study were not sufficiently sensitive to assess postoperative recovery after the surgical procedure and analgesic treatment.

**Plasma corticosterone levels**

Surgery in all papers caused an increase in plasma corticosterone levels. According to the results from paper II, the corticosterone response is correlated with the severity of the surgery. More severe abdominal surgery combined with arterial catheterization resulted in higher corticosterone levels compared to catheterization alone. This result is consistent with earlier findings in humans, where open surgery caused a higher response than laparo-
scopic surgery (Karayiannakis et al. 1997; Marana et al. 2000) and intra-abdominal surgery caused a higher response compared to superficial surgery (Clarke et al. 1970).

Plasma corticosterone levels increase within a few minutes after ACTH stimulation, as described by Siswanto et al. (2008). Thus, the observed corticosterone levels are likely a result of an ongoing stress response. Measured corticosterone levels only represent the corticosterone level at the moment of measurement and tell nothing about the period between two sampling events. Since corticosterone is secreted in an ultradian rhythm, there may be large variations between individuals in a group, especially during the dark period, as some samples are at peak level and some are at nadir. However, blood sampling causes relatively small variations between individuals in comparison with feces measurements.

Corticosterone during surgery
Corticosterone is essential for maintenance of cardiovascular homeostasis during surgery. The level of glucocorticoids needed is not clear but, as described above, unstressed levels of glucocorticoids seem to be enough (de Lange and Kars 2008; Marik and Varon 2008; Udelsman et al. 1986). Hence, substantially increased corticosterone levels both during and after surgery are probably not beneficial to the organism, especially not if corticosterone remains at high levels over a longer period of time.

The corticosterone levels during surgery were measured in papers II and III. The corticosterone levels were measured in blood collected from the tail artery just after induction of anesthesia (-1-hour sample) in paper II. The individual rats responded differently to handling and induction of anesthesia, and corticosterone levels ranged from 51 ng/ml to 275 ng/ml at this time point. There were, however, no differences between groups, and the mean value (± SEM) was 124 ± 7.7 ng/ml.

After catheterization (-0.3 hours), corticosterone release differed between groups in paper II. Levels in the buprenorphine-treated groups were lower than in the saline control and lidocaine treatment groups. The 0.1-mg/kg buprenorphine treatment caused a clear difference but did not differ significantly from the 0.05-mg/kg dose. A small but statistically significant difference was displayed after catheterization between low dose-treated rats compared to lidocaine and control populations within the laparotomized group but not in the catheterization group. Since the blood sample is taken before laparotomy, there should be no differences in the results between the surgery groups at this time. The reason for this discrepancy is unclear but could be due to buprenorphine acting more rapidly in some individual animals in the laparotomy group. Although the buprenorphine treatment reduced the corticosterone levels the levels were not completely suppressed.
In paper III, corticosterone levels were quantified after catheterization but before the rats regained consciousness. There was no difference between oral buprenorphine-treated rats and lidocaine- and bupivacaine-treated control rats. Since buprenorphine is orally administered in paper III and subcutaneously administered in paper II, the results are not fully comparable. However, subcutaneous administration is known to induce a more rapid effect, indicating that buprenorphine after oral administration has not reached a serum level as high as that after subcutaneous injection in paper II. This is supported by the findings in paper IV, as well as by a study by Kalliomaki et al. (2011).

Corticosterone levels 0–18 hours after surgery
Corticosterone is secreted in a diurnal rhythm with low levels in the morning and high levels in the early dark phase in unstressed rats (Atkinson et al. 2006). The corticosterone profile during the first 18 hours after surgery, is similar in papers I, III, and IV. When rats regained consciousness, the levels were high (~150–250 mg/ml). Surgery was always completed before noon and the normal unstressed levels for Sprague-Dawley male rats during this time of the day have been shown to be approximately 0–30 ng/ml (Atkinson et al. 2006; Siswanto et al. 2008). This indicates that the observed elevated corticosterone levels are a result of surgery.

At 2 hours, corticosterone levels had declined but at 6 and 10 hours after surgery, an increase was observed in some of the treatment groups. This increase may be influenced by the normal increase seen in the early dark phase. The levels in these groups are above 100 ng/ml, which is above previous reported levels obtained from unstressed Sprague-Dawley rats at this time of the day (~0–75 ng/ml) (Atkinson et al. 2006). Thus, the observed increase in these groups is most likely also affected by surgery. At 10 hours, a significant difference was observed (ANOVA or t test) between orally buprenorphine-treated (~60 ng/ml) and subcutaneously treated (~130 ng/ml) rats in papers I and IV. In the orally treated groups, there was no increase in corticosterone levels at this time point, compared to 6 hours. The levels are similar to those of unstressed rats at that time of day (Atkinson et al. 2006), indicating that oral treatment attenuates corticosterone release.

In paper III, the results were somewhat different from papers I and IV. The decrease in corticosterone levels was not as pronounced in the oral buprenorphine-treated group at the early dark phase (t = 10 hours) as seen in papers I and IV, and there were no significant differences at this single time point compared to the control group treated with local analgesic, when analyzed by ANOVA. The cause of the slightly different results is not clear, but could be due to seasonal variations or that the rats were subjected to arterial catheterization, which may cause more surgical stress than jugular catheterization, which was used in papers I and IV. However, in all papers (I, III, and
GLM analysis displayed a difference between the oral and subcutaneous buprenorphine-treated groups during the entire period, indicating that oral treatment with buprenorphine has a lowering effect on corticosterone release. The results of body weight and water intake changes are consistent between papers I and III, and support the beneficial effects of oral buprenorphine treatment.

Corticosterone levels 18–96 hours after surgery

Corticosterone levels were measured 96 hours after surgery in papers I and IV. There were no differences between treatment groups (VI and s.c. buprenorphine) at 18 hours and afterward. Corticosterone levels were low in the light phase (~20 ng/ml) and high (~60 ng/ml) in the dark phase as expected. This indicates a fast recovery from surgery and adaptation to the new situation with a normal corticosterone diurnal rhythm, independent of treatment. This is consistent with the results of clinical parameters, body weight and water intake changes. It should be pointed out that only one blood sample every 12 hours was taken, to avoid causing more blood loss than necessary. Thus, corticosterone levels between the sampling events remain unknown. However, the diurnal fluctuations observed are consistent with previous results, where corticosterone levels in plasma, as well as its cumulative secretion in feces, were measured in rats connected to an AccuSampler (Siswanto et al. 2008).

It is important with a rapid return to normal levels, since sustained, elevated levels have adverse effects (as mentioned above), for instance due to suppression of the immune system. It is also important with a return to a normal diurnal rhythm (distinct peak and nadir levels) as observed in our results. A disturbed diurnal rhythm with flattened cortisol levels (small difference between nadir and peak secretion) in humans is associated with depression (Leitch et al. 2003). A flattened corticosterone profile has been shown to cause a decrease in expression of structurally and functionally important mRNAs in the hippocampus (Gartside et al. 2003) and has an impact on serotonin receptors associated in the development of depression (Leitch et al. 2003).

Local anesthetics

Lidocaine was used in the control groups in papers I and III. A control without any analgesic treatment would have been more straightforward, but for ethical reasons, we decided to administer lidocaine during surgery. Lidocaine is commonly used during minor procedures and has a short duration of action (45–60 minutes) (Nolan 2000). Therefore, it is unlikely that lidocaine will have any considerable effects on plasma corticosterone levels during the
postoperative period. In paper II, it was concluded that the corticosterone-
reducing effect of lidocaine was seen only immediately after surgery and not
during the period after surgery when the rats were kept anaesthetized. This
effect was only observed after less severe surgery, catheterization, but not
after laparotomy.

In paper III, the local anesthetic bupivacaine was also included. Bupiva-
caine has a longer duration of action, varying from 2–6 hours (Nolan 2000).
There were, however, no differences between lidocaine and bupivacaine on
the parameters tested. Rats treated with local anesthetics were unable to
maintain body weight postoperatively, in contrast to buprenorphine-treated
rats (papers I and III), indicating that treatment with local analgesics is insuffi-
cient for postoperative pain treatment associated with the surgical proce-
dures used in the present thesis.

Our results are consistent with other studies. Hayes and co-workers
(1999) showed postoperative body weight loss after treatment with bupiva-
caine in rats. In a study where c-fos expression in the paraventricular nucleus
was used as a stress marker, infiltration of local anesthetics had no stress-
reducing effects, although spinal nociception was reduced (Stenberg et al.
2005). Thus, the effect of local infiltration of local anesthetics on stress re-
duction in rats is apparently transient and not detectable when studying ef-
fects over a long time. In a pig study, local anesthetics reduced cortisol lev-
els during surgery and 20 minutes after laparotomy. These effects, however,
had disappeared 50 minutes after surgery, indicating a rather short reducing
effect also in pigs (Lykkegaard et al. 2005).

Buprenorphine

The opioid buprenorphine is one of the most commonly used pain relievers
in rats (Roughan and Flecknell 2002; Stokes et al. 2009). In paper II, it was
shown that buprenorphine treatment led to lower corticosterone levels during
surgery and anesthesia compared to untreated animals and treatment with
local anesthetics. Buprenorphine also reduced postoperative plasma corti-
costerone levels compared to local anesthetics (papers I, III, and IV).

Changes in body weight and water intake support the findings from cor-
ticosterone measurements that postoperative recovery is improved after bu-
premorphine treatment. Both body weight and water intake were reduced in
control groups treated with local anesthetics in papers I and III but not in
buprenorphine-treated groups.

Interestingly, the effects observed during surgery and anesthesia in paper
II are rather short, lasting only 1 hour after surgery. In papers I and III, bu-
premorphine treatment showed an effect for 18 hours after surgery. It may be
explained by the fact that the animals in paper II were anesthetized during
the period after surgery, suggesting that anesthesia increases corticosterone
levels, and counteracts and masks the suppressing effects from buprenorphine over time. Paper IV shows that the buprenorphine concentration in blood after subcutaneous injection is very low 2 hours after surgery, and this may also explain the short effect.

Buprenorphine has been shown to reduce food consumption in non-operated rats after 24 hours (Liles and Flecknell 1992), but treatment after bile-duct ligation showed an increase in food consumption compared to saline controls (Liles and Flecknell 1993). In a study by Bomzon (2006), no effect was seen on body weight or food consumption in non-operated rats during a 7-day treatment with buprenorphine (s.c.). In laparotomized rats in the same study, buprenorphine treatment for 7 days delayed the time to restore presurgical body weight, although there were no changes in food consumption. Thus, buprenorphine may have negative effects on food consumption and body weight gain. However, the findings presented in this thesis indicate that buprenorphine treatment does not necessarily affect food consumption negatively, since buprenorphine treatment resulted in improved body weight gain compared to treatment with local anesthetics.

Hypotension is a common side effect of most opioids (Rang et al. 1999), and therefore, MAP was recorded in paper II. Anesthesia is also known to influence blood pressure, and isoflurane reduces MAP (Mutoh et al. 1997; Janssen et al. 2004). During surgery, MAP was lower in buprenorphine-treated rats than in rats receiving lidocaine or saline. When isoflurane levels decreased after surgery, MAP of buprenorphine-treated rats increased and was higher than in rats treated with saline or lidocaine. A previous study by Martinez et al. (1997) showed a slight decrease in blood pressure for approximately 2 hours in buprenorphine-treated isoflurane-anesthetized dogs. An increase in blood pressure 2 to 13 hours after buprenorphine treatment was seen in conscious rats (Ilbäck et al. 2008). Thus, it seems that buprenorphine does not exclusively cause hypotension in rats.

Previous studies have concluded that buprenorphine alters normal behavior with an increase in active as well as inactive behaviors in non-operated rats and in rats recovering from anesthesia (Liles et al. 1998; Roughan and Flecknell 2000). Ilbäck and co-workers (2008) observed a small increase in activity but only after a high dose of buprenorphine in non-operated rats. In paper III, we observed a difference in non-operated rats treated with buprenorphine compared to untreated controls. Less resting and more activity distinguished buprenorphine-treated rats from untreated rats. This effect was only observed during the first period of filming (25 minutes postoperatively), namely approximately 3 hours after administration (1 hour preoperatively), and not in the operated groups.

A side effect of buprenorphine is pica behavior, namely, when the rat eats bedding material or other non-food items. This phenomenon appears to be relatively rare and dependent on dose (Roughan and Flecknell 2002). In our
studies, we did not observe any rats eating bedding material, but a few buprenorphine-treated rats were observed gnawing at it. There are conflicting results on the effects of opioids on the HPA axis. Morphine has been shown to induce HPA activation with an increase in corticosterone levels and suppression of the immune system in rats (Hayes and Stewart 1985; Gomez-Flores and Weber 2000). In other studies, however, HPA activation is reduced by morphine in both humans (McDonald et al. 1959; George et al. 1974) and in rats (Page et al. 1998). Buprenorphine has been shown to reduce corticosterone levels in rats and not suppress the immune system after injection of buprenorphine (Gomez-Flores and Weber 2000; D’Elia et al. 2003) as well as after surgery (Franchi et al. 2007).

The reducing effect of buprenorphine on postoperative corticosterone levels observed in this thesis is likely caused by a reduction of pain in the postoperative period, leading to inhibited nociception-induced stress, but could also be a direct effect on the HPA axis at the hypothalamic level (Franchi et al. 2007). The different effects of morphine and buprenorphine may be due to their binding to different opioid receptors. Morphine is a μ2 and kappa receptor agonist, while buprenorphine is a μ1 agonist and kappa antagonist (Gomez-Flores and Weber 2000).

Oral buprenorphine treatment

The traditional route of administration of buprenorphine is subcutaneous injection every 8–12 hours (Dobromylskyj et al. 2000). An oral route of administration enables dosing without disturbing the animal, and the frequency of administration required is seemingly lower with an oral agent (every 24 hours), since the regimen described in this thesis was sufficient to suppress the surgical stress response for at least 18 hours, as shown in papers I, III, and IV. In papers I and IV, buprenorphine was injected every 12 hours. This frequency was chosen as it was considered a relevant time interval for the relatively minor surgical procedure to which the rats were subjected, according to recommendations in the literature (Hedenqvist and Hellebrekers 2003; Kohn et al. 2006).

The effect of oral buprenorphine treatment in rats has been questioned and conflicting result have been presented. There are studies (Liles et al. 1998; Flecknell, Roughan et al. 1999) showing that oral buprenorphine at a dose of 0.4–0.5 mg/kg in gelatin results in better recovery in body weight and water consumption after surgery compared to control. Buprenorphine has also been administered in drinking water, where the subsequent analgesiometric tests display an increase in paw withdrawal latency time compared to subcutaneous treatment (Jessen et al. 2007). However, results from analgesiometric tests suggest that oral treatment at a dose of 0.5 mg/kg is insufficient for pain relief (Martin et al. 2001; Thompson et al. 2004). These conflicting results may be due to the different parameters studied—
analgesiometric tests and clinical parameters. Analgesiometric testing may not always be comparable to postoperative pain, since postoperative pain is a complex condition, where clinical signs and other parameters during the postoperative period may be more accurate (Roughan and Flecknell 2002; Cooper et al. 2005).

In this thesis, plasma levels of corticosterone as well as clinical signs after oral buprenorphine treatment were studied. Buprenorphine was mixed in Nutella, which was offered to the rats in their cages. The method for applying this regimen in practice has recently been described in detail (Abelson et al. 2012). We name this to treatment by voluntary ingestion, since the rats readily ingested the buprenorphine mix voluntarily. Rats habituated with pure Nutella prior to treatment ingested all the Nutella-buprenorphine mix within a few minutes when left undisturbed. Thus, the ingested amount of buprenorphine was always known.

VI treatment (0.4 mg/kg) was compared to controls receiving local anesthetics (papers I and III) and to subcutaneous treatment (0.05 mg/kg; papers I and IV). VI treatment resulted in lower serum corticosterone levels compared to controls and s.c. treatment for 18 hours postoperatively. After 10 hours, an increase in corticosterone levels was observed in the subcutaneous group in both papers I and IV. These rats were re-injected after 8 hours and the stress associated with the injection may have caused this increase. This is however unlikely, since there were no differences seen between VI-treated rats injected with saline and rats not subjected to injection at this time point (paper I). Although buprenorphine-treated rats should not feel any pain from the injection, stress from the restraint should be the same. Thus, the higher levels seen after subcutaneous treatment was probably not due to stress caused by the injection, but a result of insufficient pain reduction by the subcutaneous treatment. There was no difference in changes in body weight or water intake between oral and subcutaneous treatment. Our results have been confirmed in a recent study in mice, where oral buprenorphine treatment also displayed lower plasma corticosterone levels during the first hours postoperatively (Sundbom et al. 2011).

Plasma buprenorphine concentrations, measured in paper IV, also indicate that oral buprenorphine treatment is at least as effective as subcutaneous treatment. Buprenorphine plasma concentrations in VI-treated animals were as high as (except directly after injection) or higher than in subcutaneously treated rats. In subcutaneously treated rats, there was a fast decline, whereas in VI-treated animals, buprenorphine levels were more stable with a slow decline over the entire period (18 hours). Thus, one oral dose was as least as effective as two subcutaneous doses. Studies in mice also revealed a high serum concentration of buprenorphine after oral treatment and a longer duration compared to subcutaneous treatment (Kalliokoski et al. 2011).

Taking these results together, buprenorphine treatment by VI appears to provide adequate analgesia in the present experimental design. VI every 24
hours seems to be at least as effective in reducing postoperative stress as subcutaneous treatment. This may prove to be very useful in postoperative analgesic treatment of laboratory rats, since VI of buprenorphine diminishes the amount of animal handling and reduces the number of drug administrations. The positive aspects of VI of buprenorphine are further confirmed in a study by Kalliokoski et al. (2010) where rats subjected to surgically induced global cerebral ischemia were treated with buprenorphine in Nutella. Buprenorphine treatment resulted in a decrease in corticosterone levels but also reduced between-animal variation. Furthermore, the treatment seemed to have little or no effect on the ischemia model.

**Stock-related differences**

There are differences in corticosterone levels and susceptibility to stress between different stocks and strains of rats. Differences between the commonly used Sprague-Dawley and Wistar rats are known, specifically in terms of growth rate and endocrine function (Kuhn et al. 1983). In paper IV, postoperative plasma corticosterone levels were measured in both Sprague-Dawley and Wistar rats after different treatments. The only stock difference found in paper IV was a more pronounced reduction in postoperative body weight in subcutaneously treated Wistar rats compared to Sprague-Dawley rats. There were, however, no differences observed in postoperative plasma corticosterone levels. Thus, VI treatment of buprenorphine seems to be effective in both Sprague-Dawley and Wistar rats.
Conclusions

Paper I

Oral buprenorphine treatment resulted in a suppression of plasma corticosterone levels compared to subcutaneous buprenorphine and lidocaine treatments during the first day after surgical catheterization of the jugular vein. Buprenorphine treatment, independent of the route of administration, led to better recovery in body weight and water intake. The corticosterone levels of all groups had, by the second postoperative day, reverted to the normal diurnal rhythm of corticosterone secretion. Thus, oral administration of buprenorphine appears to be an effective stress-reducing method for administering postoperative analgesia to rats.

Paper II

The severity of the surgical procedure influenced the corticosterone response during surgery and anesthesia, with higher corticosterone release during more severe surgery. The stress response was also affected by the type of analgesic treatment. Corticosterone levels were reduced more effectively after buprenorphine treatment than after lidocaine treatment. Thus, both the surgical procedure and the analgesic treatment should be taken into consideration when planning an experiment. Since corticosterone levels are high during surgery, acute experiments may be affected by physiological changes due to a stress response.

Paper III

Oral buprenorphine treatment reduced plasma corticosterone levels in rats effectively after arterial catheterization when compared to control groups receiving local anesthetics. Buprenorphine treatment also improved recovery when measuring body weight and water intake. Buprenorphine treatment increased locomotor activity in non-operated rats only. The effect of buprenorphine in operated rats could not be detected via the monitoring of locomotor activity or the time spent resting in the present study.
Paper IV

Oral buprenorphine had similar effects on postoperative plasma corticosterone levels and changes in body weight in both Wistar and Sprague-Dawley rats. Oral buprenorphine treatment resulted in buprenorphine concentrations in plasma at least as high as by subcutaneous treatment.
Svensk populärvetenskaplig sammanfattning


I de ingående delarbetena undersökte vi stressresponsen hos råttor under och efter operation samt hur återhämtningen påverkades av subkutan och
oral smärtlindring med buprenorfin. Buprenorfin gavs oralt genom att Nutella blandat med buprenorfin sattes in till rättan som snabbt åt upp blandningen. Två rättstammar användes i studierna, Sprague Dawley och Wistar. För att kunna uppskatta återhämtningen mättes halten av kortikosteron i blodet, viktförändring, förändring av vattenintaget samt förändringar i beteendet. Dessutom mättes buprenorfinkoncentrationen i blodet.

I samtliga delarbete opererades en kärlkateter in i rättan och i delarbete II gjordes även en bukoperation på hälften av rättorna. I delarbete I, III och IV väcktes rättorna upp efter operationen och kärlkatetern kopplades till en automatisk blodprovstagare (AccuSampler) som programmerades att ta blodprov vid bestämda tidpunkter. Rättorna kunde då gå fritt omkring i buren medan proven togs utan att de blev störda. I delarbete II var rättorna sövda hela tiden och blod togs manuellt.

Delarbete I visade att förebyggande behandling med buprenorfin, såväl oralt som subkutan, gav bättre återhämtning i form av bibehållen kroppsvikt och vattenintag under det första dygnet efter operationen jämfört med kontrollgruppen som fick lokalbedövningsmedlet lidokain. Den orala behandlingen gav upphov till lägre kortikosteronnivåer efter operationen jämfört med den subkutan behandlingen. Ett dygn efter operationen hade kortikosteron nivåerna återgått till normala nivåer.

Delarbete II visade att en artärkateterisering i kombination med bukkirurgi gav högre kortikosteron nivåer än enbart en artärkateterisering. Vid artärkateteriserings resulterade behandlingen med lidokain i tillfälligt lägre kortikosteron nivåer direkt efter operation jämfört med den obehandlade kontrollgruppen. Vid den mer invasiva bukoperationen sågs ingen skillnad mellan lidokainbehandling och den obehandlade kontrollgruppen. Buprenorfin gav däremot sänkta kortikosteron nivåer efter både artärkateterisering och bukoperation och sänkningen höll i sig längre jämfört med lidokain. En högre buprenorfindos gav en tidigare och mer uttalad sänkning, särskilt efter bukkirurgi.


Delarbete IV visade att det inte var någon skillnad i kortikosteron nivåerna mellan Sprague Dawley och Wistarhanar efter operation. Buprenorfinkoncentrationen i plasma uppmättes och visade att oral administrering gav minst lika höga nivåer som subkutan administrering.
Sammanfattningsvis tyder resultaten på att förebyggande behandling med buprenorfin är bra för att förbättra återhämtningen hos råttor efter operation. Buprenorfin minskade kortikosteronnivåerna under de första 18 timmarna efter operation och medförde ingen minskning av kroppsvikt eller vattenintag. Oral behandling verkar dessutom vara ett effektivt och smidigt administrationssätt eftersom den gav sänkt kortikosteronfrisättning efter operationen och koncentrationen av buprenorfin i plasma blev minst lika hög vid oral behandling som vid subkutan, trots att behandlingen endast behöver ges en gång per dygn.
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