INTRODUCTION

The use of laboratory animals in experiments often requires surgical operations. Post-operative conditions are most often associated with some pain and discomfort in the animals, and use of an adequate analgesic treatment is essential. This period is also associated with stress, due to tissue injury, anesthetic agents, and of course the post-operative pain (Martini et al. 2000; Shavit et al. 2005).

Stress in laboratory animals is an obstructive circumstance in most experimental conditions, since natural response of an animal to stressors include significant alteration of the normal physiology and metabolism, and thereby increase of variation within and between individual animals. This makes stress a major source of experimental error (Hau et al. 2001; Morton & Hau 2002). Persistent stress is accompanied by several adverse effects on most homeostatic mechanisms of the body, including the immune system, the endocrine system, and the reproductive system. These effects are caused by excessive blood levels of glucocorticoids, reduced growth rate and/or body weight loss, and reduced fecundity (Glaser et al. 1987; Klein et al. 1992; Moberg & Mench 2000).

In order to assess and recognize stress in laboratory animals, evaluation of various clinical signs and behavioral parameters can be undertaken, such as body weight gain, food and water consumption, urination, defection, activity, posture, vocalization etc. (Martini et al. 2000; Morton & Hau 2002). To complement behavioral studies, stress can also be assessed by quantifying different endogenous stress markers, of which the most commonly investigated are corticosteroids. A stressful stimulus results in an activation of the hypothalamic-pituitary-adrenal (HPA) axis. This triggers a release of adrenocorticotropic hormone (ACTH), which in turn results in a release of corticosteroids from the adrenal cortex (Desborough 2000; Morton & Hau 2002; Whitten et al. 1998). The biologically active corticosteroids are generally cortisol or corticosterone depending on species, the latter being the predominant corticosteroid in rats and mice (Woodman 1997).

The level of corticosteroids can be investigated by quantifying corticosteroid metabolites (CM) excreted in feces. This method has been shown to be useful to assess preceding stress and also diurnal rhythm in rats (Cavigelli et al. 2005; Lepschy et al. 2007; Thanos et al. 2008). Siswanto et al. (2008) showed an eight hour delay in post-surgical fecal CM levels compared to serum corticosterone, and high and long-lasting serum corticosterone levels was required to induce detectable levels of CM in Sprague-Dawley rats. The study by Siswanto et al. suggested that fecal corticosterone is not reliable for detecting minor stress (for example human interaction), but after significant stress such as surgically induced stress. Corticosteroids can also be quantified directly from blood samples, which can be obtained via either manual or automated sampling. Manual sampling requires no surgical procedure in order to obtain blood, but is associated with direct interaction with the animal and causes a stress response in itself (Vachon & Moreau 2001). In this context, automated blood sampling is preferable, since this method enables blood sampling without any interference with the animal during sampling. This method requires minor surgery to connect the animal to the automated blood sampling device. However, with an adequate post-operative treatment, several studies have shown that automated sampling has been found to be a useful tool for stress marker measurements (Abelson et al. 2005; Vahl et al. 2005; Royo et al. 2004).

Buprenorphine, a potent lipophilic opioid with μ and κ agonist-antagonist profile, is a very common analgesic for rats. A previous study has shown that oral administration by voluntary ingestion of Buprenorphine prior to surgery, suppresses the post-surgical corticosterone levels in male Sprague-Dawley rats compared to subcutaneous administration (Goldkuhl et al. 2008). However, very little is known about the impact of single housing and connection of the animal to the AccuSampler system, post-surgical corticosterone levels, and the influence on these by Buprenorphine treatment in strains other than Sprague-Dawley. It has been reported before that single housing can cause stress in rats (Boggiano et al. 2008). A strain of particular interest is the inbred strain Fischer 344 (F344), since this strain has been found to be highly responsive in stress and corticosterone release, due to surgery or environmental stressor such as single housing or novel environment, compared to other strains, which also has been found important for the analgesic effect of morphine in these animals (Baumann et al. 2000; Dhabhar et al. 1993; Potenza et al. 2004; Woolfolk & Holtzman 1995).
The hypothesis of the present study is that pre-operative treatment of Buprenorphine administered by oral voluntary ingestion can reduce stress and suppresses the post-operative serum corticosterone levels in F344 rats kept single-housed and connected to an automated blood sampling device. The study investigates both immediate effects during the first 24 hours and later effects two and three days after surgery. The study also aims to validate the oral treatment as a sufficient post-operative treatment by investigating its impact on clinical signs such as body weight gain and water consumption. The research significance is to give information about pain and stress response in laboratory rats and to serve as important guidance for better analgesic strategy and increased animal welfare.

LITERATURE REVIEW

Pain, Stress and Corticosterone

The ability to experience pain is vital for the survival of all animals. Pain is a subjective experience and serves as a warning signal to make an escape or evasive action possible. The physiological and pharmacological activities that lead to a painful sensation are denominated nociception. The nociceptive pathway can be described as a three-neuron chain that transmits the nociceptive information from peripheral tissue to cerebral cortex.

The first order neurons are transferring the nociceptive signal from periphery tissue to the spinal cord. This is caused by degeneration of cell membranes that lead to accumulation of phospholipids in the tissue, which are converted to arachidonic acid, which in turn is converted to prostaglandins. The latter sensitize and activate the nociceptors and elicit a transmission of information to the spinal cord. From the spinal cord, the nociceptive information ascends via the second order neurons to the thalamus, hypothalamus, and other regions of the brain. (The second order neuron). This results in activation of most brain structures, including the Hypothalamus-Pituitary-Adrenal (HPA) axis which causes release of corticosterone. From the thalamus, the nociceptive information is transmitted to the cerebral cortex via the third order neurons (Abelson 2005).

Corticosterone is a steroid hormone that consists of 21 carbons and excreted by the glamorous zone and the fasciculate zone of adrenal gland. Corticosterone is a glucocorticoid hormone that regulates the conversion of amino acids to glucose (gluconeogenesis) and glycogen (glycogen synthesis) in the liver and also stimulates glycogen synthesis in other tissue (Turner & Bagnara 1976). This class of hormone (Figure 1) also regulates cardiovascular, immune, and behavioral processes. Glucocorticoids are steroid hormones that have the capability to perform a binding with glucocorticoid receptors (GR), a 94 kD cytosolic protein. This complex will bind to specific DNA motifs termed glucocorticoid response elements (GREs) in the promoter region of glucocorticoid responsive genes and regulate the expression of this hormone through interaction with transcription factors (Smith & Vale 2006).

Corticosterone has several important functions. One of them is increasing the rate of gluconeogenesis in liver. The gluconeogenesis pathway uses carbon from amino acids and glycerol (from fat catabolism) to make glucose. Corticosterone will increase the expression of enzymes that are involved in gluconeogenesis. Corticosterone also increase mobilization of amino acids to liver and this also related to the immunosuppressive effect of corticosterone. Corticosterone also inhibits glucose uptake in muscle and adipocyte (Schmidt-Nielsen 1966).

Corticosterone release is induced by brain response to stressful stimuli. In stress conditions, stressful stimuli can cause activation of the paraventricular nucleus (PVN) in the hypothalamus to release Corticosteroids Releasing Factors (CRF). CRF contains Corticosteroids Releasing Hormone (CRH), Vasopressin, and some unknown factors. CRF will in turn induce the release of Adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH will recognize its receptor (melanocortin type 2 receptor) on parenchymal cells of fasciculate zone of adrenal cortex and cause activation of adenylate cyclase. This results in the accumulation of cAMP inside the cell and activates the phosphorylase enzyme. This enzyme will catalyze glycogen catabolism and therefore increase in full (NADPH) concentration inside the cell. NADPH is the main substrate for steroid synthesis (glucocorticoid) by adrenal cortex. Specifically ACTH promotes the conversion of cholesterol into 5-5 pregnolone during the initial step of glucocorticoid biosynthesis (Moberg & Mench 2000; Gordon et al. 1982; Smith & Vale 2006; Cohen et al 2006). Once