Post-operative Corticosterone Levels in Plasma and Feces of Mice Subjected to Permanent Catheterization and Automated Blood Sampling

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Abstract. This study investigated the effects of surgical placement of permanent arterial catheters on plasma corticosterone levels, fecal corticosterone excretion and body weight in male BALB/c/Sca mice. In addition, the effects of voluntarily ingested buprenorphine in doses of 0.5 and 1.0 mg/kg body weight on these parameters were studied. A catheter was placed in the carotid artery during isoflurane anesthesia. Immediately after surgery, the mice were connected to an AccuSampler® μ and blood samples for plasma corticosterone quantification were collected automatically during the first 24 h postoperatively. All fecal boli produced 24 h before and 24 h after surgery were collected for fecal corticosterone excretion measures and the pre- and post-operative body weights were registered. Plasma corticosterone levels were in the range of 150-300 ng/ml after the surgical procedure and the body weight was significantly lower 24 h after surgery compared to its pre-operative value. Contrary to what was expected, the total fecal corticosterone excretion was significantly reduced 24 h after surgery, as was the defecation. Buprenorphine treatment significantly lowered the plasma corticosterone levels, but had no effect on fecal corticosterone excretion or body weight change. It was concluded that surgical placement of an arterial catheter induces a significant stress response, as judged by its effect on plasma corticosterone and body weight. Voluntary ingestion of buprenorphine improved postoperative recovery by lowering plasma corticosterone concentrations. Neither fecal corticosterone excretion nor body weight change seems suitable for postoperative stress assessment in mice in the present experimental setup.

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neither causes stress to the animals in connection to sampling nor involves any human interaction with the animals during the period of interest, since there is a delay between the adrenal secretion of corticosteroids into the blood and the subsequent excretion in feces. Measuring the total amount of corticosteroids excreted per time unit also results in a measure of corticosterone that represents a true integrated amount of the steroid over time, compared to blood samples where an instantaneous concentration is analyzed (13, 14, 21-27). One of the main complicating factors related to the use of fecal corticosterone is the uncertainty in the delay between corticosteroid changes in the blood and the manifestation of these changes in feces. Hence, in order to establish corticosterone quantification as a reliable method for stress assessment, it is important to investigate the changes in blood and feces concentrations in various species as well as in various experimental situations. In mice, the corticosterone levels in the blood and feces after surgery and during the subsequent recovery phase have not been previously investigated.

Previous investigations in rats have demonstrated beneficial effects on postoperative recovery, when the animals were allowed to voluntarily ingest the opioid analgesic buprenorphine mixed in a sticky nut-and-chocolate paste. The treated animals had lower levels of circulating corticosterone and maintained body weight better, compared to non-treated animals or animals treated with subcutaneous injections of the same drug (9, 11, 12).

The aim of the present study was to evaluate the effects of surgical placement of permanent arterial catheters followed by automated blood sampling in male mice on postoperative plasma corticosterone levels, fecal corticosterone excretion and changes in body weight. In addition, the study investigated whether these parameters would be affected by pre-emptive treatment of buprenorphine, administered through voluntary ingestion. It was hypothesized that the surgery would result in elevated levels of circulating corticosterone and fecal corticosterone excretion and a loss of body weight during the first 24 h after surgery and that the analgesic treatment would counteract these negative effects.

**Materials and Methods**

**Animals.** All animal experiments were approved by the Uppsala Animal Ethics Committee, Uppsala, Sweden. Twelve male BALB/c/Sca mice from Scanbur B&K (Sollentuna, Sweden) weighing 25±1 g (mean ± standard deviation) were used. Female mice were not included, to avoid the estrus cycle hormonal fluctuations as a confounding variable.

**Housing conditions.** Before the experiments, the mice were housed in pairs in Macrolone type III cages (800-cm² floor area, 15-cm high) for at least one week in animal rooms with standard animal house conditions: diurnal rhythm was regulated with a 12-h light/12-h dark cycle with lights on from 6 am to 6 pm; temperature was kept at 20±2°C, relative humidity was 30-60%, the air was changed approximately 15 times per hour and clean cages were provided once a week. Aspen chips (Scanbur B&K) were used as bedding material. The animals had free access to food pellets (R36; Lantmännens, Stockholm, Sweden) and tap water at all times. Food pellets were placed on the bedding to improve accessibility after surgery. Two days before surgery, the mice were transferred to single housing in Macrolone type III cages and moved to a designated laboratory with similar environmental conditions to the animal holding rooms, where the experiments were conducted.

**Preoperative and surgical procedures.** Two days before surgery (Day-2), 2.5 g/kg body weight Nutella hazelnut chocolate paste (Ferrero, Pino Torinese, Italy) was placed in the cages to habituate the mice to this novel food item. Body weight was recorded on Day-1 and Day 0 to ensure that all animals included in the study gained weight as expected before subjected to surgery. All fecal bolus produced during 24 h between Day-1 and Day 0 were collected in order to obtain preoperative values of fecal corticosterone excretion.

Prior to surgery, six of the mice were subjected to preemptive analgesia with buprenorphine (Temgesic®; Schering-Plough Europe, Brussels, Belgium) either 0.5 mg/kg body weight (BUPR 0.5) or 1.0 mg/kg (BUPR 1.0) mixed in Nutella® (2.5 g/kg body weight; 1 h before surgery) for oral administration through voluntary ingestion. The remaining six mice were given plain Nutella without buprenorphine according to the same schedule. All animals consumed all of the buprenorphine-mix or plain Nutella within a few minutes in every case. Analgesic doses were based on those recommended in the literature (28, 29) and on the Authors’ previous investigations in rats, where voluntary ingestion has been used (9, 13, 14).

The mice were placed in an induction chamber and anesthesia was induced with 5% isoflurane delivered in pure oxygen. Once the paw withdrawal reflex was absent, the mice were shaved at the incision sites and attached to an anesthetic face mask for spontaneous respiration. Isoflurane was maintained at approximately 2.5-3% to ensure adequate anesthesia and rectal body temperature was maintained at 37-38°C. The shaved body parts were washed with iodine (Jodopax vet®; Pharmaxin AB, Helsingborg, Sweden). An incision was made in the skin of the neck and the carotid artery was catheterized with an arterial catheter for mice (DiLab, Lund, Sweden), filled with heparinized saline. The catheter was placed with the tip close to the aortic arch, secured in the vessel by three sutures and led subcutaneously through an incision in the nape of the neck and kept in place by a harness. The catheter was led further through a metal spring and connected to an AccuSampler® μ (DiLab) for automated blood sampling. The whole procedure, from induction to recovery, was completed within 45-60 min. The mice were observed regularly during the recovery phase. To avoid bias in corticosterone levels by disturbing the animals during the experiment, the observations took place only immediately after blood withdrawal.

**Blood and fecal sampling.** All surgical procedures were completed before noon and blood sampling started immediately when the mice regained consciousness. Thereafter, blood was collected at 2 pm, 6 pm, 10 pm, 2 am and 6 am, i.e. 2, 6, 10, 14 and 18 h after completed surgery. Each sample volume was 20 μl. The AccuSampler μ requires approximately 5 μl waste volume for each sample, which means that the total amount of blood withdrawn from each mouse was 150 μl, corresponding to a blood withdrawal of approximately 0.6% of the animals’ body weight during the whole experiment.
After completion of blood sampling, all fecal boli produced during the first 24 h after surgery were collected, and the postoperative body weight was recorded. The experiments were terminated by an infusion of 100 mg/kg body weight pentobarbital (Apoteket, Uppsala, Sweden) into the blood circulation via the catheter.

Corticosterone quantification. The blood samples were collected in tubes and stored overnight at 4°C and, subsequently, centrifuged to remove blood cells and obtain plasma. Plasma was stored at −20°C until analysis. The plasma concentration of corticosterone was quantified with enzyme-linked immunosorbent assay (ELISA), using a commercial ELISA kit for corticosterone (Correlate-EIA; Assay-Designs Inc., Ann Arbor, MI, USA) according to the manufacturer’s instructions. The kit has been verified to have a cross-reactivity equivalent to 28.6% against deoxycorticosterone, 1.7% against progesterone, 0.13% against testosterone, 0.28% against tetrahydrocorticosterone, 0.18% against aldosterone and less than 0.05% against cortisol, pregnenolone, betaestradiol, cortisone and 11-dehydrocorticosterone acetate.

Fecal samples were stored at −20°C until analysis. Since corticosterone is a rather stable molecule, the corticosterone levels in the samples were considered to be similar from the time of excretion to analysis. Royo et al. (13) demonstrated that corticosterone levels in feces change by less than 10% even when the fecal samples are stored at room temperature for 24 h. Corticosterone was quantified according to a previously described method (30). All fecal boli from one sampling window were thawed, weighed and submerged in 96% ethanol (5 ml/g feces). Each sample was vigorously vortexed and incubated on a shaking table overnight (for at least 12 h). The homogenate was centrifuged at 2,000 × g in a Hermle Z 400 K (Hermle Labortechnik GmbH, Wehingen, Germany) for 20 min, the supernatant was decanted and the pellet discarded. A 1 ml aliquot of the supernatant was further centrifuged at 10,000 × g for 15 min in a tabletop centrifuge (Eppendorf 5415D; Eppendorf AG, Hamburg, Germany). A volume of 200 μl of the supernatant was recovered with a pipette, while carefully avoiding aspirating any pelleted material. The final sample was diluted in ethanol (final dilutions of 1:2 to 1:10 were used) and analyzed using the DRG-Diagnostics corticosterone (competitive) ELISA (EIA-4164; DRG Instruments GmbH, Maburg, Germany) according to the manufacturer’s instructions. The following cross-reactivities were reported for the assay: progesterone (7.4%), deoxycorticosterone (3.4%), 11-dehydrocorticosterone (1.6%), cortisol (0.3%), pregnenolone (0.3%) and other steroids (<0.1%). The analytical sensitivity was less than 1.6 nmol/l. The absorbencies were recorded at 450 nm (reference wave length, 650 nm) using a Thermo Multiscan Ex micro plate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA). Concentrations of corticosterone are expressed as either ng corticosterone/24 h or ng corticosterone/g feces.

Statistical analysis. Treatments were administered randomly. Statistical analysis of the experimental results was performed using SPSS Statistics version 17.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, CA, USA). A one-way analysis of variance (ANOVA) with Tukey’s post-hoc test was used to calculate differences in fecal corticosterone excretion, defecation and fecal corticosterone concentrations between the different groups. A one-sample Student’s t-test was used to determine whether postoperative changes in body weight for each group were different from zero and differences in mean body weight change between different groups were determined using one-way ANOVA with Tukey’s post-hoc test. The ANOVA results are presented as F(df1, df2) and a corresponding p-value, where df1 and df2 are degrees of freedom between and within groups, respectively. The GLM results are presented as F(df0) and a corresponding p-value. The Student’s t-test results are presented as t(df) and a corresponding p-value. Statistical significance was determined at p<0.05.

Results

The mean corticosterone levels in plasma after surgery were approximately 150-200 ng/ml in the control group during the entire 18-h period. In the BUPR 0.5 group, the levels were approximately 300 ng/ml immediately after awakening and declined to approximately 100 ng/ml after 18 h. (Figure 1). In the BUPR 1.0 group, the corticosterone level immediately after awakening was significantly lower than in both the control (F(1,29)=12.9, p=0.031) and BUPR 0.5 groups (F(1,29)=12.9, p=0.002), as determined by ANOVA with Tukey’s post-hoc test. ANOVA with Tukey’s post-hoc test also showed significant differences between the BUPR 0.5 and BUPR 1.0 groups at 2 h (F(1,29)=4.3, p=0.045) and between the control and both treatment groups at 14 h (F(2,29)=49.5, p=0.005). GLM with repeated measures showed that the control and BUPR 0.5 groups were significantly different at 14 h and 18 h after surgery (F(1)=16.3, p=0.027), and that the control and BUPR 1.0 groups were significantly different from each other during the entire 18-h period (F(1)=64.9, p<0.001).

Figure 2A shows the total corticosterone excretion during 24 h before and 24 h after surgery for each group. It was significantly lower in all groups during the postoperative period, compared to the preoperative period, as determined by a two-factor ANOVA (F(1,16)=23.8, p<0.001). Treatment had no effect on corticosterone excretion and there were no differences between the different treatment groups. Figure 2B shows the defecation before and after surgery for each group. Similar to corticosterone excretion, defecation was significantly lower in all groups during the postoperative period, compared to the preoperative period, as determined by a two-factor ANOVA (F(1,16)=66.5, p<0.001). Treatment had no effect on defecation and there were no differences between the treatment groups. Figure 2C shows the fecal corticosterone excretion expressed as ng/g feces during 24 h before and 24 h after surgery, respectively. A two-factor ANOVA showed that there were no differences with respect either to time or to treatment.

The changes in body weight 24 h after surgery, as compared to its preoperative values, are shown in Figure 3. The postoperative body weight was significantly reduced in
the control ($t_{(5)}=4.56$, $p<0.01$) and BUPR 1.0 groups ($t_{(2)}=8.51$, $p<0.05$). In the BUPR 0.5 group, all three individual mice lost body weight after surgery. However, due to large variations, the body weight changes were not significantly different from zero. There were no differences in postoperative body weight change between the different treatment groups.

**Discussion**

The present study investigated the postoperative effects of surgical placement of a permanent arterial catheter in male BALB/c mice on corticosterone levels in blood and feces and on body weight. In serum, the mean corticosterone levels in the control group were not less than 150 ng/ml during the entire 18-h period. These are considered as elevated, non-physiological levels, since the levels during the dark period are expected to be below 50 ng/ml (31). Thus, the high corticosterone levels in the circulation and the significant drop in body weight suggest that the surgical procedure caused stress in the animals (14). The stress response is essential for the survival of an injured animal by maintaining circulatory functions and mobilizing energy stores (3). However, after surgical procedures under controlled conditions, the stress response and the catabolic effect may be a cause of postoperative morbidity (32, 33), since the negative feedback mechanism of glucocorticoid release appears to be suppressed after surgery (32, 34). Thus, it is desirable to minimize the surgical stress response and an adequate analgesic treatment is an important part of this reduction. Buprenorphine has been shown to possess analgesic effects in mice when administered parenterally (35-37), but little is known about the effects of oral buprenorphine in mice. Two recent studies on both rats and mice showed that the serum concentrations of buprenorphine after voluntary ingestion are higher and remain at higher levels for a longer period than after parenteral administration (12, 38). The beneficial effects of voluntarily ingested buprenorphine for preemptive analgesia, in connection to permanent catheterization, have been previously validated in rats (9, 11, 12) and, thus, it was hypothesized that this treatment would be beneficial also for mice subjected to the same type of surgery. According to the results from circulating corticosterone, there is nothing that falsifies the hypothesis. Measuring corticosterone in the circulation is a sensitive and reliable method for assessment of an acute

Figure 1. The plasma corticosterone levels 18 h after surgical catheterization in non-treated and buprenorphine-treated BALB/c/Sca mice. At each time point, significant differences between groups, as determined by ANOVA with Tukey’s post-hoc test, are indicated by *($p<0.05$) and **($p<0.01$) and the respective groups are connected with vertical solid or dotted lines. Significant difference between the control and BUPR 0.5 groups, as determined by GLM with repeated measures, is indicated by a dotted bracket ($p<0.05$). Significant difference between the control and BUPR 1.0 groups, as determined by GLM with repeated measures, is indicated by a solid bracket ($***p<0.001$).
stress response, especially if the blood is obtained through automated blood sampling, where no stress response related to handling or restraint may bias the measures (7, 9, 14, 20, 21, 39). The present study demonstrated that the corticosterone levels in plasma were significantly lower after buprenorphine treatment, especially after the higher dose. Hence, it seems that the oral buprenorphine treatment has a beneficial effect on the mice with respect to this parameter. However, further studies are needed to thoroughly validate the benefits from the treatment in mice.

Measuring fecal corticosterone excretion has been shown to be an adequate method for assessing preceding elevated levels of corticosterone in the blood (14, 26) and is often a useful method for noninvasive stress assessment. However, the method is associated with several complicating factors that limit its applicability. Among them, the delay from the increase in blood to the detection in feces is prominent, since the delay may vary between 4 and 18 h (14, 21, 24, 26) or even longer (13). In addition, the variation in the corticosterone amount between individual samples may be substantial, limiting the sensitivity of the method (14, 21, 40). In the present study, the corticosterone excretion was significantly lower in the fecal samples after surgery than before, when studying the total amount of corticosterone excreted (ng/24 h). Meanwhile, the defecation rate after surgery was substantially reduced. Thus, the decrease in the total amount of excreted corticosterone in feces seen after surgery most likely does not represent a decreased stress level.
The body weight of the animals was reduced after surgery, regardless of analgesic treatment. This finding was contrary to the initial hypothesis. Measuring changes in body weight is commonly used for the assessment of postoperative recovery in laboratory rodents (47, 48) and previous studies in rats have shown that the animals maintain their body weight after surgery, when treated with oral buprenorphine (9, 28, 49). However, opioids such as buprenorphine are known to cause nausea and reduce appetite in most animals and studies have shown that buprenorphine does not always counteract the postoperative body weight loss in rats (30, 50). The lack of an effect on body weight loss from the buprenorphine treatment in the present study is, therefore, not necessarily due to the lack of an analgesic or stress-reducing effect, but may be due to reduced appetite, as a consequence of the opioid treatment.

In conclusion, it was evident from the present study that the surgical placement of an indwelling arterial catheter induces a significant stress response in BALB/c mice, as judged by the corticosterone response in the circulation and the reduced body weight. The data indicated that buprenorphine treatment through oral voluntary ingestion in doses of 0.5-1.0 mg/kg body weight improves postoperative recovery by attenuating the plasma corticosterone response. It was evident that 24-h post-surgical fecal corticosterone excretion is not suitable for postoperative stress assessment in mice in the present experimental setup, since the measures are biased by reduced defecation. In addition, unlike many reports on rats, body weight did not seem to be a sufficiently sensitive parameter to distinguish between groups of untreated mice and mice treated with buprenorphine.

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**References**


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