

BRIEF RESEARCH REPORT

Urinary Corticosterone Levels in Mice in Response to Intraperitoneal Injections With Saline

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The concept of refinement is an important issue in the field of laboratory animal science. Refinement-based research aims to improve animal welfare, to increase the reliability of experimental outcome, and to diminish variation. In search of refinement of experimental techniques, this study investigated whether urinary corticosterone can be used as a noninvasive measure of acute stress in mice.

In mice, corticosterone (CORT) usually is measured in blood or urine samples. In urine, CORT is bound to corticosteroid-binding globulin. In the blood, CORT mostly is unbound. After an animal is aroused, CORT is released quickly in the blood and usually can be demonstrated after about 2 min. Together with its relatively short half-life, this makes plasma or serum CORT a frequently used parameter for acute stress. However, because of the quick release, blood samples

must be drawn within 2 min after the onset of the procedure to avoid confounding effects of the procedure (Broom & Johnson, 1993). The release of corticosteroid-binding globulin-bound CORT in urine is much slower, facilitating the sampling procedure. In addition, the collection of urine is considered less invasive than is blood sampling; the latter requires restraint and puncture or incision of blood vessels, which potentially causes pain and sometimes results in pathologies (see Balcombe, Barnard, & Sandusky, 2004). Furthermore, we know from our own experience that the amount of blood that can be collected through, for example, tail incision decreases each time a sample is drawn.

Therefore, within the framework of our refinement-based research, we were interested in finding if the effects of an acute, mild stressor would be visible in urinary corticosterone (uCORT) samples and, if so, how long after the induced stress this would occur. Analogous to a preceding study (Meijer et al., in press), a routine experimental procedure—an intraperitoneal (IP) injection with saline—was used as the acute, mild stressor. In addition, injecting saline was likely to increase the volume of urine voided, which would be an advantage for the biological analysis.

METHOD

The Animal Ethics Committee of the Faculty of Veterinary Medicine of Utrecht University approved all procedures. Ten female, experimentally naïve mice of two different inbred strains ($n = 6$ BALB/cByJlco; $n = 4$ C57BL/6Jlco; Charles River, Maastricht, The Netherlands), control animals from a previous experiment, were housed in a conventional animal room (temperature 18° to 24° C; 12:12 hr light:dark cycle with lights on at 700 hr, light intensity at shelf level about 100 lux). At the time of testing, the mice were about 13 months of age and had a mean body weight of 28.6 g \pm 1.2 g (BALB/c) and 34.1 g \pm 0.7 g (C57BL/6). They were housed socially in groups of two in elongated Makrolon® II cages (floor area: 530 cm²) and provided with sawdust bedding (Lignocel® ¾; Rettenmaier & Sohne, Ellwangen-Holzmühle, Germany). For experimental reasons, cage enrichment had not been provided in the preceding experiment. This situation was maintained during the current study to avoid confounding effects. Food pellets (Rat and Mouse Chow [CRM], Special Diet Services, Witham Essex, United Kingdom) and tap water were provided *ad libitum*.

The mice received five IP injections with 0.5 ml of sterile saline (0.9%, Brain Melsungen AG; Melzungen, Germany) at room temperature, gradually injected—using a 26-gauge needle under an approximately 10° angle—in the lower quadrant of the abdomen off midline. Urine collection followed the injection, with a time interval varying between 1 hr to 5 hr. To obtain a baseline uCORT value, urine also was collected once without a preceding injection. Urine always was col-

lected at 1700 hr to control for the circadian rhythm of uCORT. Therefore, injections were administered at different times of the day, in the following randomized order: 1200 hr, 1400 hr, 1300 hr, no injection (baseline uCORT), 1600 hr, and 1500 hr. There always were 2 or 3 days between procedures.

To collect urine, the mice were placed individually into plastic buckets (1.1-L volume; Emergo, Landsmeer, The Netherlands) and provided with a plastic salad dish (250 cc, Depa®; Veriplast BV, Apeldoorn, The Netherlands). The mice who did not urinate spontaneously were picked up and restrained by the scruff and the base of the tail. If necessary, the bladder was gently massaged until the mouse urinated. Using this method, it was possible to collect urine of all mice within 15 min (modified method of Dahlborn, Van Gils, Van de Weerd, Van Dijk, & Baumans, 1996, and Van Loo, Mol, Koolhaas, Van Zutphen, & Baumans, 2001).

Urine was collected with a 1-ml syringe and stored in polypropylene tubes at -20° C. Corticosterone levels were measured using a solid-phase ^{125}I radioimmunoassay (CAC® Rat Corticosterone TKRC1; Diagnostic Products Corporation; Los Angeles, California). Creatinine concentrations, indicative for the dilution of urine, were determined with the use of a commercial test combination (ABX Diagnostics; Montpellier, France) on a COBAS-MIRA-S auto-analyzer (ABX; Montpellier, France).

UrinaryCORT:creatinine ratio values were analyzed using a linear mixed effects model with strain as the between-subjects factor and time (the interval between injections and urine collection), as the within-subject factor. Overall differences were assessed; in addition, all time intervals were compared individually to baseline. Bonferroni correction was applied where appropriate. The analyses were performed in S-plus 2000 Professional Release 2© (1988–1999, MathSoft, Inc., Delft, The Netherlands). Data are presented as mean values \pm standard error of the mean (*SEM*).

RESULTS AND DISCUSSION

The results are presented in Figure 1. Overall, a difference between the five time intervals and the baseline uCORT/creatinine ratio was detected ($p = .0008$). When comparing each of the time intervals with the baseline value, a significant increase compared to baseline was found for the 1-hr ($p = .0045$) and the 2-hr interval ($p = .0005$). No statistically significant differences were found between the two strains.

The mice in this study were 13 months of age, meaning that basal uCORT/creatinine ratio levels were rather high (Van de Weerd, Van Loo, Van Zutphen, Koolhaas, & Baumans, 1997). This can be explained by the increase with aging of glucocorticoids because of the impaired feedback inhibition of the hypothalamic–pituitary–adrenal axis (Pedersen, Wan, & Mattson, 2001).

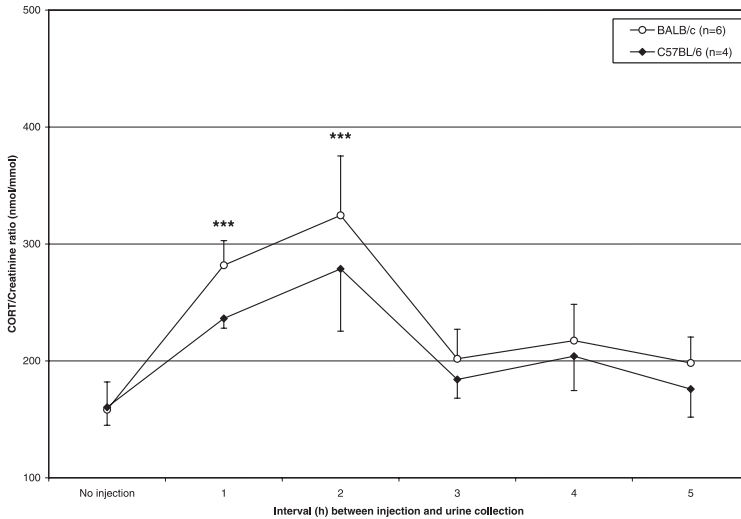


FIGURE 1 uCORT/Creatinine ratios of two strains of mice at baseline and 1 to 5 hr after intraperitoneal injection. Asterisks indicate a significant difference from baseline. Data are presented as means \pm SEM.

However, despite the high baseline values, the increase of uCORT found in the first 2 hr after the mice had been subjected to an IP injection indicates that the effects of an acute, mild stressor can be detected in urine. Likewise, peak concentrations of CORT metabolites have been found in urine approximately 2 hr after CORT injection (Touma, Sachser, Mostl, & Palme, 2003). The variation at the 2-hr interval was increased compared with the 1-hr interval (see Figure 1). At this point, the method seems less accurate. Some of the mice might have urinated between 1 hr and 2 hr after injection, voiding the peak concentrations of uCORT that had accumulated in the bladder.

The volume of saline injected into the peritoneal cavity might have influenced voiding. If the intention is to measure its effect with the lowest possible variation, it seems advisable—on the basis of these results—to collect urine about 1 hr after the animal has been subjected to an acute stressor. Alternatively, if one wants to avoid experimental bias due to the effects of acute stressors, urine should not be collected within the first 3 hr. The effects of the acute stressor are likely to disappear after this period.

In conclusion, our results show that uCORT can be used to measure the effects of acute stress. Therefore, the use of this parameter can be seen as a useful refinement of routine practice for animals in the laboratory. The increased variation of uCORT values at the 2-hr time point can, however, be considered as a drawback for the use of this parameter.

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