Hormonal stress response of laboratory mice to conventional and minimally invasive bleeding techniques

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Abstract

Conventional bleeding of small laboratory animals is often associated with stress and injuries that can cause haematomas, inflammation and ultimately the death of animals under investigation. Here, we used faecal glucocorticoid metabolites as an indicator of stress imposed on laboratory mice (Mus musculus domesticus) when bled in three different ways: puncture of the tail vein following mechanical immobilisation; puncture of the retro-orbital plexus following chemical immobilisation; and a minimally invasive technique using blood-sucking bugs (Reduviidae, Heteroptera) without any immobilisation. We hypothesised that blood-sucking bugs provoke a lower hormonal stress response than conventional bleeding techniques because laboratory animals are not handled and because the mechanical stimulus of an insect sting is supposedly weak. Each of the 16 mice was bled using one of the three methods at a time in a random order with seven days of recovery between subsequent bleeding events. To monitor the stress hormones, we determined corticosterone metabolites in faecal samples of mice collected one day before, 8 h after and one day after the bleeding event. Concentrations of faecal glucocorticoid metabolites increased in all three treatment groups compared with baseline values. However, average concentration of stress hormone metabolites after bleeding was higher by a factor of about 1.5 when conventional bleeding techniques were applied than when bugs were used. We conclude that blood-sucking bugs may offer a gentle alternative for obtaining blood samples from small animals such as mice.

Keywords: animal welfare, blood samples, corticosterone, Dipetalogaster maximus, faecal samples, Mus musculus

Introduction

Animal welfare requirements necessitate that pain imposed on laboratory vertebrates should be minimised, particularly during bleeding (Monash University Animal Welfare Committee 2008; GV Solas 2009). This is most often accomplished by adjusting the specific technique of bleeding to the species in question, individual animal characteristics (eg age, sex), the minimum volume of blood needed for analysis and the requirements of the experimental design. Yet, during vein puncturing with conventional needles, it is inevitable that skin tissue and blood vessels are damaged. This may lead to haematomas or even more severe complications (Hoff 2000). Delicate veins or arteries of small animals, such as mice and birds, may complicate bleeding (Voigt et al 2006). As a consequence, small animals may have to be punctured repeatedly, which may even increase the risk of injuries. Also, animals have to be either mechanically or chemically immobilised for bleeding, which often causes stress (Gärtner et al 1980; Vahl et al 2005). Stressful handling may activate the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis and this may lead to increased levels of circulating stress hormones. Generally, the acute release of catecholamines and glucocorticoids should help organisms to respond quickly to new situations for immediate survival. In case of chronic stress, however, the release can lead to significant negative effects such as suppression of the immune system, reproduction and vitality of animals (Riley 1981; Holst 1998; Kloet et al 1999; Touma & Palme 2005).

In laboratories, glucocorticoids of domestic and wild animals have been validated as indicators for an animal’s body condition, health and welfare, making them an important tool for ecological and laboratory studies. The use of this tool, however, requires accurate measurement of baseline levels for unstressed animals (Arnold et al 2008). Unfortunately, the determination of baseline corticosterone