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Comparison of stress induced in rats by four different anaesthetic regimens as recorded by urinary concentrations of corticosterone and testosterone

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Introduction

Stress in animals is not always easy to recognise or define. *Fraser et al.* (1975) defined stress as a state that occurs when an animal encounters adverse physical or emotional conditions which cause a disturbance of its normal physiological and mental equilibrium. Glucocorticoids are relased by the adrenal cortex in response to various stressors. Corticosterone is the major glucocorticosteroid secreted in laboratory rodents (mice and rats) and is frequently used as a marker to measure stress in animals. The main problem in assessing stress by means of glucocorticoids is that various factors can affect the levels of these hormones in both stressed and unstressed animals. Circulating testosterone levels have been suggested as a potential stress parameter in male primates (*Sapolsky* 1985) but this finding still remains to be confirmed and the potential usefulness in other species has not been examined.

Anaesthetic agents provide various levels of analgesia against pain and discomfort during a procedure. However, their use is associated with some risk of mortality and unpleasant effects during induction and recovery. These drugs frequently affect the cardiovascular, respiratory and thermoregulatory mechanisms including the central nervous system. *Whelan & Flecknell* (1991) defined surgical anaesthesia in laboratory animals as the state in which an animal is immobile, unaware of the procedure being performed and has an attenuated stress response.

The objective of this study was to compare four different anaesthetic regimens in order to assess which anaesthetic regimen is the least stressful in rats as measured by the secretion of urinary corticosterone and testosterone.

Materials and methods

A total of 12 male SPF Wistar rats (Charles River, UK) of 5–8 weeks of age were randomly divided into groups A, B and C, and allocated in pairs into metabolic cages (24.2 cm × 18 cm, area 500 cm^2) (Techniplast, Italy). Control animals (group A) were not handled except during cleaning and weighing procedures. Animals from groups B (2nd control group) and C (experimental group) were handled twice daily between 9.00 am and 4.00 pm. Group C contained four pairs of rats and each pair received one of the four different anaesthetic regimens on day 14 (Table 1). Food (RM1, Special Diet Service Ltd, Essex) and water were supplied *ad lib,* except for one hour each day when food was withheld. This simulated procedures prior to anaesthesia. The temperature range of the animal room was 20–23°C and relative humidity in the range of 45–62 %. The light variation consisted of a photoperiod of 12 h light : 12 h darkness. The rats were grouped 14 days prior to anaesthetic induction in order to recover from transport and adapt to their new environment. Food and water intake were measured throughout the experiment. Urine samples were collected every 24 hours throughout the experiment.

Table 1. Dose and duration of the different anaesthetic agents administered on day 14.

Group	Anaesthetic Agents	Concentration	Maintenance
C1	1 part Hypnorm, 2 parts H_2O & 1 part Midazolam mixture injected i.m.	Hypnorm = 2.7 mg/kg Midazolam = 2 mg/kg	Hypnorm 0.1 mg/kg i.m. after 30 mins
C2	Xylazine + Ketamine (1:30) injected i.p.	Xylazine = 3 mg/kg Ketamine = 90 mg/kg	Ketamine 25 mg/kg i.m. after 30 mins
C3	Methoxyflurane (volatile)	= < 4 %	0.4–1.0 %
C4	Halothane (volatile)	= 3–4 %	0.5–2 %

Experimental design

The animal were moved to the metabolic cages on day 0 of the experiment. On day 14 the animals in group C were anaesthetised for 45 minutes and returned to the metabolic cages following recovery. The animals were killed on day 22 by guillotine. Rats in group C were weighed and given atropine sulphate (0.04 mg/kg s.c.) thirty minutes prior to anaesthetic induction. Atropine sulphate reduces salivary and bronchial secretions. The first pair (C1) were injected i.m. with a mixture of Hypnorm and Midazolam. The second pair (C2) received firstly Xylazine and then Ketamine i.p. The third (C3) and fourth (C4) pairs were anaesthetised in an anaesthetic chamber with Methoxyfluarane and Halothane respectively. Table 1 summarises the concentrations used for induction and maintenance of anaesthesia for 45 minutes. Respiration was monitored every minute by direct clinical observation. Temperatures were recorded using a data logger (LOGIT, Griffin and George, Leicstershire UK). The rats were placed on cotton wool and on a heating pad during anaesthesia. Room temperature was maintained at 25°C until the rats were fully recovered.

Measurement of corticosterone and testosterone

The urine concentrations of corticosterone and testosterone were measured daily throughout the study period using commercial radioimmunoassay kits (BIOGENESIS LIMITED, Bournemouth and IMMUNODIAGNOSTIC SYSTEM LIMITED, Tyne

Table 2. Daily corticosterone (ng/ml) and testosterone (nmole/ml) in urine before and after anaesthesia.

	A	B	C1	C2	C3	C4
Corticosterone Before	2.41 ± 2.25	1.98 ± 1.9	1.75 ± 0.77	2.43 ± 1.1	1.98 ± 0.71	2.31 ± 0.78
After	1.5	2	4.5*	7*	3.5*	2.5
Testosterone Before	0.47 ± 0.13	0.65 ± 0.17	0.71 ± 0.18	0.72 ± 0.19	1.01 ± 0.99	0.66 ± 0.18
After	0.65	0.65	1.25*	0.9	0.4	0.65

* significantly elevated levels.

C1 = Hypnorm/Midazolam, C2 = Xylazine/Ketamine, C3 = Methoxyflurane, C4 = Halothane.

& Wear UK respectively). These assays are based on the competitive double antibody technique.

Statistical evaluation

Differences in food and water intake between groups were evaluated by one way analysis of variance. P-values < 0.05 were considered statistically significant. When comparing pre-treatment corticosterone and testosterone levels with post-treatment, values different from the mean $\pm$ 2 s. d. were considered significantly different.

Results

There was no significant variations in food or water intake in the control and experimental groups throughout the study.

There was no significant difference in pre-treatment corticosterone levels in urine between handled and non handled animals (Table 2).

A significant increase in corticosterone levels after anaesthesia in groups C1, C2 and C3 was recorded. There was no significant difference in the corticosterone levels of the control groups and group C4 (Table 2).

Testosterone concentrations increased significantly following anaesthesia in group C1 only (Table 2).

Discussion

Glucocorticoid levels in body fluids are often used as indicators of acute stress whereas they are not useful as measures of chronic stress and this has been reviewed recently by *Manser* (1992). Minor stress e.g. blood sampling results in increased plasma levels of glucocorticoids and in order to avoid this and eliminate the diurnal variation we chose to measure corticosterone in urine collected in metabolic cages. Handling can cause significant increases in glucocorticoids in animals not habituated to it. The rats from groups B and C were handled twice daily for fourteen days, prior to anaesthesia in order to reduce stress associated with handling.

Three of the four anaesthetics used (Table 2) resulted in increased urine levels of corticosterone. Hypnorm/Midazolam and Xylazine/Ketamine are injectable anaesthetics and resulted in greater increases in corticosterone synthesis than induction with Methoxyfluarane which is a volatile anaesthetics. There was no significant increase in corticosterone levels when Halothane (volatile) was used as an anaesthetic. This was probably due to the short fast induction of Halothane. Volatile anaesthetics probably provide the safest means (in experienced hands only) of anaesthetising small mammals. The depth of anaesthesia can be adjusted relatively quickly and easily and normally the animals recover rapidly (*Flecknell* 1991). When anaesthetising small animals, injection of the drug intravenously is often difficult and another route must be chosen. The effect of the anaesthetic is slower and the depth more difficult to ascertain when using intraperitoneal or intramuscular routes. Administration of an antagonist can often be necessary. Some antagonists reduce the analgesia provided by the anaesthetic and the animal may experience some discomfort.

The present results demonstrate that corticosterone, which is the active glucocorticoid in rodents, may be a useful indicator of short term stress associated with anaesthetic regimens in rats.

Transiently elevated levels of testosterone have been recorded in baboons following acute stress (*Sapolsky* 1985). In the present study elevated levels of testosterone in urine were only observed after Hypnorm/Midazolam anaesthesia. Although testosterone does not seem to be as sensitive a parameter as corticosterone to measure acute stress in male rats, it may prove to be a useful supplement.

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Summary
Glucocorticoid levels in body fluids are frequently used as indicators of stress. Corticosterone is the major glucocorticosteroid secreted in laboratory rodents. Urinary concentrations of corticosterone were measured as indicators of stress induced in rats by four different anaesthetic regimens. Testosterone levels were also measured, as a potential stress parameter.
Three of the four anaesthetic regimens used resulted in increased urine levels of corticosterone. Elevated levels of testosterone were only observed in one of the treatments.
In conclusion, corticosterone may be a useful indicator of stress associated with anaesthetic regimens in rats. However testosterone does not seem to be as sensitive a parameter as corticosterone but it may prove to be a useful supplement.

Sammendrag
Glucocorticoid-niveauer i blod og/eller urin er hyppigt anvendt som stress-indikatorer. Corticosteron er det mest betydende glucocorticosterid hos gnavere. Corticosteron-koncentrationen blev målt som indicator for stress induceret i rotter i forbindelse med fire forskellige anaestesimetoder. Testosteron niveauer blev ligeledes målt som mulig stress parameter.
Tre af de fire anaestesimetoder resulterede i øgede niveauer af corticosteron i urin. Øgede niveauer af testosteron blev kun observeret i forbindelse med een anaestesimetode.
Det konkluderes, at corticosteron kan være en brugbar indikator for stress i forbindelse med anaestesi af rotter. Imidlertid ser testosteron ikke ud til at være så følsom en parameter som corticosteron i denne sammenhæng, men testosteron kan måske bruges som et supplement til andre stress-markører.

Yhteenveto / K. Pelkonen
Kortikosteroidien määrää ruumiin nesteissä pidetään usein stressin osoittajina. Kortikosteroni on laboratoriojyrsijöiden ensisijaisesti erittämä glukokortikosteroidi. Työssä seurattiin rotan virtsan kortikosteronipitoisuutta neljan erilaisen nukutustavan aiheuttaman stressin osoittajina. Samanaikaisesti mitattiin myös testosteronin määrää, mahdollisena stressimuuttujana.
Kolmessa neljästä tutkitusta nukutustavasta virtsan kortikosteronin määrä nousi. Testosteronimäärä nousi vain yhdessä nukutustavassa.
Pääteltiin että kortikosteroni sopii rotilla nukutusstressin osoittajaksi. Testosteroni ei näytä olevan yhti herkkä, mutta saattaa osoittautua käyttökelpoiseksi havainnon varmistajaksi.

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