J Comp Physiol B (2000) 170: 163–168

ORIGINAL PAPER

A. Teskey-Gerstl · E. Bamberg · T. Steineck · R. Palme

Excretion of corticosteroids in urine and faeces of hares (*Lepus europaeus*)

Accepted: 24 November 1999

Abstract Increased production of glucocorticoids by the adrenal cortex is found in mammals under stress. As cortisol itself is absent in the faeces, an enzyme immunoassay (11-oxoaetiocholanolone) measuring 11,17-dioxoandrostanes has already been established to measure faecal cortisol metabolites in ruminants for non-invasive monitoring of adrenocortical activity. The aim of this study was to establish route and delay of excretion of glucocorticoids in hares and to determine whether a cortisol-, corticosterone- or this new enzyme immunoassay is best suited to detect faecal glucocorticoid metabolites. In the first experiment radioactive-labelled glucocorticoids (^{14}C-cortisol and ^{3}H-corticosterone) were administered intravenously to two groups of three hares in metabolic cages. All voided urine and faecal samples were collected for 4 days. Metabolites of both steroids were found predominantly in the urine (91 ± 4%). Peak concentrations were observed in the first urinary sample following infusion (13 ± 6 h) and in the faeces with a delay of about 1 day (23 ± 7 h). Most of the radioactivity was not extractable with diethylether, indicating that the metabolites excreted in urine and faeces are mainly conjugated or polar unconjugated ones. This was confirmed by reverse-phase high-performance liquid chromatography separations of the metabolites, which also revealed marked differences concerning the metabolism of the two glucocorticoids injected. Compared with the cortisol and the corticosterone enzyme immunoassay, only the group-specific enzyme immunoassay for 11,17-dioxoandrostanes detected high quantities of immunoreactive metabolites. In a second experiment hares ($n = 20$) were stressed by rousing them three times (5 min, 10 min and another 5 min) with a 20-min break in-between. Faecal samples were collected 2 days before until 4 days after stress and analysed using the 11-oxoaetiocholanolone enzyme immunoassay. After stress significantly ($P < 0.001$) increased 11,17-dioxoandrostane concentrations were found. Based on these results, measuring 11,17-dioxoandrostanes in faeces enables non-invasive monitoring of disturbances in hares and thus provides a tool for field investigations elucidating the role of stress in hare populations.

Key words Hare · Non-invasive · Glucocorticoids · Faeces · Urine

Abbreviations *11,17-DOA* 11,17-dioxoandrostanes · *ACTH* adrenocorticotropic hormone · *EIA* enzyme immunoassay · *RP-HPLC* reverse-phase high-performance liquid chromatography

Introduction

During the last few years marked decreases in the hare population have been found in Europe (Klansek 1996). Although reasons are not fully elucidated, a correlation with increased stress mainly caused by poor environment (fragmented landscape) and by higher predation risk is discussed (Villafuerte et al. 1997). Negative effects of stress on reproduction (and indices of stress physiology) of the snowshoe hare were observed by Boonstra et al. (1998).

Stress increases the glucocorticoid production in the adrenal cortex mediated by the release of adrenocorticotropic hormone (ACTH) (Axelrod and Reisine 1984). Therefore, glucocorticoid concentrations in blood are widely used as a parameter of stress in

Communicated by G. Heldmaier

A. Teskey-Gerstl · E. Bamberg · R. Palme (✉)
Institute of Biochemistry and Ludwig Boltzmann
Institute of Veterinary Endocrinology,
University of Veterinary Medicine,
Veterinärplatz 1, A-1210 Vienna, Austria
e-mail: Rupert.Palme@vu-wien.ac.at
Tel.: +43-1-25077-4103; Fax: +43-1-25077-4190

T. Steineck
Research Institute of Game Biology and Ecology,
University of Veterinary Medicine,
Savoyenstraße 1, A-1160 Vienna, Austria

animals (e.g. Morton et al. 1995). However, blood collection itself causes stress, especially in wildlife species such as hares, as it is accompanied by trapping and handling (Boonstra and Singleton 1993). To evaluate disturbances in those animals, it is necessary to establish non-invasive methods for the determination of glucocorticoids or their metabolites. Collection of faecal samples is easy and feedback-free as there is no need to handle the animal, which is especially suited for practical use under field conditions. Glucocorticoids are metabolised in the liver (Brownie 1992) and the metabolites are excreted via the urine and the faeces. In addition, intestinal bacteria in the gut can affect the structure of the metabolites (Macdonald et al. 1983). Infusion studies (^{14}C-cortisol) in sheep (Palme et al. 1996) and characterisation of the faecal metabolites resulted in the establishment of a group-specific enzyme immunoassay (EIA) for 11,17-dioxoandrostanes (11,17-DOA; 11-oxoaetiocholanolone-EIA) by Palme and Möstl (1997), the biological relevance of which has already been proven in ruminants (Palme et al. 1999, 2000).

To our knowledge, literature on glucocorticoid metabolism and excretion in hares is not available. Because of pronounced inter-species differences concerning both the metabolism and excretion of glucocorticoids (Taylor 1971; Palme et al. 1996) it is not possible to draw analogous conclusions from other studies. The aim of this study was to establish route and delay of excretion of glucocorticoid metabolites after intravenous (i.v.) administration of radioactive-labelled glucocorticoids (^{14}C-cortisol and ^{3}H-corticosterone) to two groups of three hares. Furthermore, metabolites should be tentatively characterised and their immunoreactivity tested in various EIAs (11-oxoaetiocholanolones-, cortisol- and corticosterone-EIA, respectively) to determine that which is best suited. In addition, the biological relevance of such a non-invasive method was reviewed by determining whether elevated concentrations of the glucocorticoid metabolites in the faeces of hares can be monitored following stress.

Materials and methods

First experiment (radioinfusion)

Animals and housing

For the first experiment a total of six (three male, three female) healthy brown hares (*Lepus europeus*) from the Research Institute of Game Biology and Ecology, University of Veterinary Medicine, Vienna, aged 15–30 months (all were mature and females not pregnant) were used. Ten days before the study started, the hares were transferred to a stable with metabolic cages (59 cm length, 50 cm width, 40 cm height; made of refined steel) at the same facility to become accustomed to the new environment. The animals received their usual feed (hay, pellets of concentrated feed). Fodder was refilled once a day and available all day. They were allowed to drink water ad libitum. Although the hares were kept in cages, they were not used to being handled by human beings.

Administration of radioactive-labelled glucocorticoids

^{14}C-Cortisol (NEC-163) and ^{3}H-corticosterone (NET-399) were obtained from New England Nuclear (Dreieich, Germany). Before the injection, their purity (>98%) was checked by thin-layer chromatography (Palme et al. 1996). A total of 370 kBq (=10 μCi) of one of two radioactive-labelled glucocorticoids was administered in 2 ml of sterile NaCl solution containing 10% ethanol at around 11:00 a.m. ^{14}C-Cortisol was administered to two does and one buck and ^{3}H-corticosterone was administered to one doe and two bucks. The labelled glucocorticoids were injected into the saphenous vein, located laterally on the hind leg, as this vein is big enough to administer 2 ml of the injection solution and a better immobilisation of the hare is possible. The whole manipulation (catching, fixation, administration, putting the hare back into the cage) lasted 5–10 min.

Collection of samples and determination of radioactivity

In the infusion experiment all animals were checked once an hour for 4 days and all voided faeces and urine were collected separately. Whereas the urine ran through the wire grating into a metal tube, the faeces were caught by it. All samples were stored at −20 °C.

To determine amounts of radioactivity in urine and faeces the same method was used as described by Palme et al. (1996). Briefly, 0.5-g aliquots per 5 g of wet faeces of each sample were extracted with 3 ml of methanol and 2 ml of distilled water for 30 min on a vortex. After centrifugation (2500*g*, 15 min) a 0.5-ml aliquot of the supernatant (in duplicate) was mixed with 10 ml of scintillation fluid (Quicksafe A, No. 1008000, Zinsser Analytic, Maidenhead, UK) and measured in a liquid scintillation counter (Packard Tricarb 4640, Warrenville, Ill., USA). To improve the recovery of apolar steroid metabolites a further extraction step was included, using a higher percentage of methanol. Therefore, 2.5 ml of the supernatant were discarded and another 5 ml of methanol (100%) added to the remainder and extracted as before. Duplicates of urine samples (0.1 ml) were mixed with scintillation fluid (6 ml) and measured directly.

Characterisation of radioactive metabolites

Firstly, amounts of ether-soluble/insoluble steroids were determined as described by Palme et al. (1996). Briefly, a 0.5-ml aliquot of urinary samples ($n = 5$ from each hare) was extracted twice with 5 ml diethylether. After extraction of faeces ($n = 18$ from each hare) the supernatants of the two extraction steps were combined, concentrated (to approximately 1.5 ml) and extracted with three times 5 ml diethylether. Radioactivity was measured in the combined ether extracts and the remaining aqueous phases.

Secondly, faecal samples containing peak radioactivity were subjected to a clean-up procedure with Sep-Pak C_{18} columns (Palme et al. 1997). Briefly, 1 g of faeces was suspended in 20 ml of methanol/water (4 + 1) and the supernatant diluted with 0.2 mol l^{-1} sodium acetate buffer (pH 4.8; 1 + 3) and passed through a Sep-Pak C_{18} cartridge (1 g; Waters, Milford, Mass., USA). The cartridge was washed with 10 ml of aqua bidest. and dried with a stream of N_2. Elution was performed using 10 ml of dichloromethane, ethyl acetate/methanol (4 + 1) and methanol successively. The dichloromethane fraction (containing unconjugated metabolites) and the ethylacetate/methanol fraction (containing mainly conjugated or polar unconjugated steroids) were separately injected onto a Novapak C_{18} column (3.9 mm × 150 mm) with a Mini-Guard-column (C18). The flow rate was 1 ml min^{-1} and a mixture of methanol/water was utilised as mobile phase for the reverse-phase high-performance liquid chromatography (RP-HPLC). For the separation of unconjugated metabolites, the initial concentration of methanol was 50%. A linear gradient up to 75% within 40 min (and afterwards up to 100% in another 5 min) was applied. In the case of conjugated metabolites methanol concentration was 20% for the first 5 min

(isocratic) and increased linearly up to 100% during the next 30 min. In both cases fractions (three per minute) were collected and an aliquot counted to determine the presence of radioactive steroids. In addition the immunoreactivity of metabolites was tested in a cortisol-, corticosterone- and 11,17-DOA-EIA (Palme and Möstl 1997). Aliquots of conjugated corticoid metabolites (present in HPLC fractions) were dissolved in 0.5 ml of acetate buffer (pH 4.8) and treated with *Helix pomatia* intestinal juice (0.5 ml of a 1:500 diluted mixture of β-glucuronidase/arylsulfatase; Merck 4114) to determine the amounts of hydrolysable steroids by ether extraction after hydrolysis (40 °C; 12 h).

Second experiment (biological relevance)

To test the biological relevance of a determination of corticoid metabolites in the faeces, another experiment was performed. The hares were kept in the open air in their original, individual cages (155 cm length, 77 cm width, 90 cm height), which were made of wood with a roofing felt on the top and wire gratings on the ground. The cages were situated in two rows with 21 hares in each row. A total of 20 hares (5 from each row and sex) were chosen. All of them were mature and healthy and between 15 months and 32 months old. They were fed as described above. The females were not pregnant. To separate the urine from the faeces additional wire nettings were installed below. In order to determine basal levels of faecal 11,17-DOA, 2 days before the hares were stressed, their faeces were collected once a day in the morning as defecations took place only during evening, night and morning. To stress the hares, they were roused three times (5 min stress:20 min break:10 min stress:20 min break:5 min stress). The faeces of the stressed hares were collected once a day in the morning for another 4 days. After mixing, aliquots of individual samples were stored at −20 °C until analysis with the EIA following extraction with 80% methanol (5 ml) as previously described (Palme and Möstl 1997). Statistical analysis (Friedman and Tukey Test) was done using the SigmaStat software package (SPSS ASC, Germany). As the concentrations of the steroids were not normally distributed on all days, they are shown as boxplot diagrams (Fig. 4).

Results

Overall amounts of radioactivity recovered in urine and faeces after injection were 90 ± 5%. Most of the recovered radioactivity of both glucocorticoids was found in the urine (91 ± 4%; see also Table 1 for individual values). Peak urinary radioactivity was monitored in the first sample after infusion, showing a delay of about half a day (13 ± 6 h). Levels subsequently returned to baseline. In faeces both maximal radioactivity (23 ± 7 h) and background levels were reached later. During the protracted decline some smaller peaks of radioactivity were observed (Fig. 1).

Table 1 Excretion of radioactivity (%) and delay of peak radioactivity (h) in urine and faeces of individual hares (*f* female, *m* male)

Hare no. (sex)	Corticosteroid	% Excretion		Peak (h)	
		Urine	Faeces	Urine	Faeces
1 (f)	^{14}C-cortisol	85.3	14.7	7.9	19.0
2 (m)	^{14}C-cortisol	94.8	5.2	18.5	29.5
3 (f)	^{3}H-corticosterone	90.2	9.8	6.4	13.4
4 (m)	^{3}H-corticosterone	91.3	8.7	10.4	18.2
5 (f)	^{14}C-cortisol	97.5	2.5	18.3	29.3
6 (m)	^{3}H-corticosterone	89.8	10.2	18.2	29.2
	Mean	91.47	8.53	13.3	22.8
	SD	4.2	–	5.7	7.4

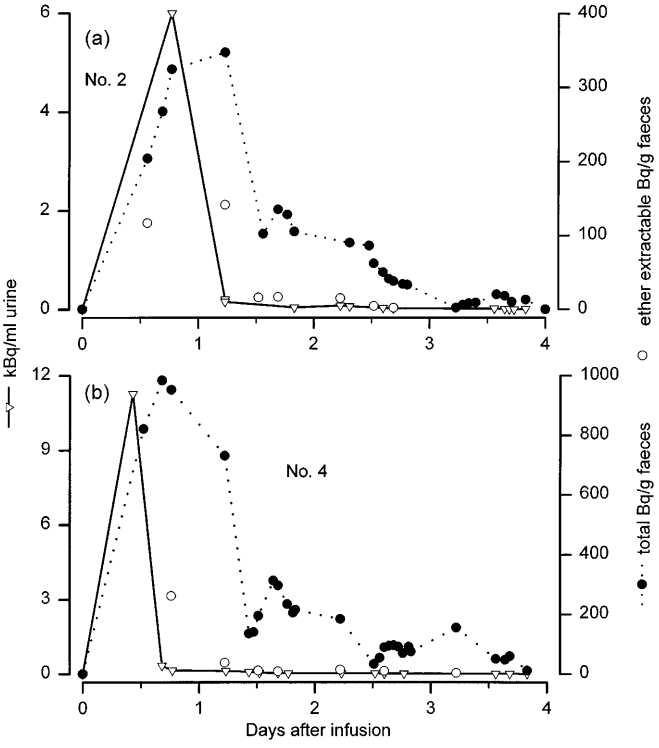

Fig. 1 Time-course of excretion of radioactivity in urine and faeces in one hare each, following infusion of ^{14}C-cortisol (**a**) and ^{3}H-corticosterone (**b**)

Only small portions of the radioactive metabolites both in urine and faeces were ether extractable (3.4–14.5%; median 7.5%, and 1.0–57.1%; median 9.6%, respectively). Highest amounts of ether extractable metabolites were found in samples around peak radioactivity and percentage tended to decline with time (Fig. 1). RP-HPLC separations revealed the presence of a large number (>20) of radioactive metabolites. There were marked differences in metabolite formation between the two glucocorticoids infused (Figs. 2, 3). Metabolites of the dichloromethane fraction of both chromatograms (Figs. 2a, 3a) mainly showed more polar metabolites (fractions 4–15) but there were additional ^{14}C-metabolites present in fractions 30–45. The biggest of them showed highest immunoreactivity in the 11-oxoaetiocholanolone-EIA and co-eluted with 11-oxoaetiocholanolone, as proven by a separately injected standard. In the case of the ethylacetate/methanol fraction metabolites of ^{14}C-cortisol were very polar (fractions 6–20) but ^{3}H-corticosterone metabolites (Figs. 2b, 3b) were less so (eluting mainly between fractions 22 and 40). Only in the latter (fractions 30–37) could substantial portions (80–90%) be hydrolysed with the β-glucuronidase/arylsulfatase, whereas this percentage was low (3–20%) in the case of the other conjugated metabolites. Very small portions of non-metabolised cortisol or corticosterone were found. Highest amounts of

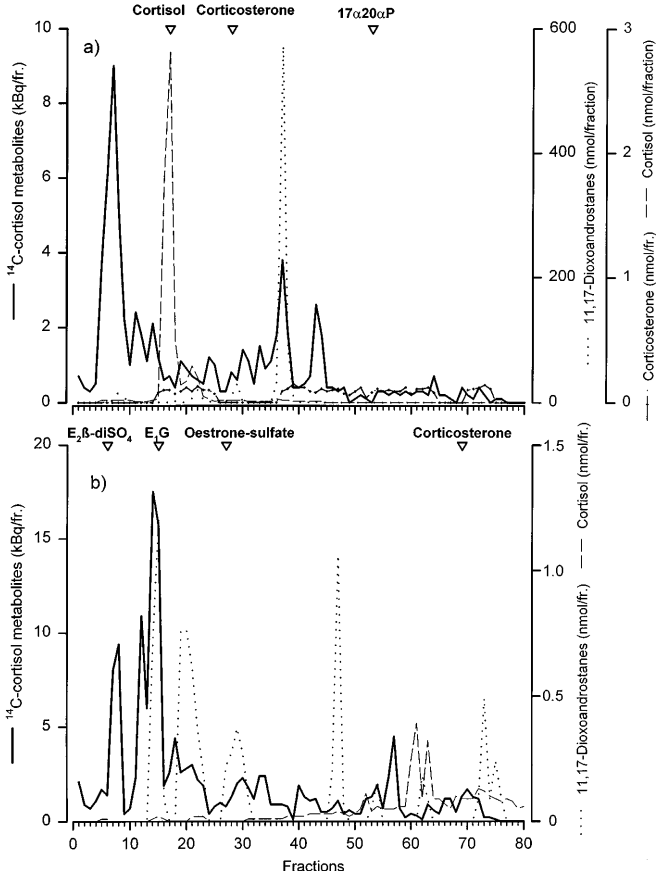

Fig. 2 Reverse-phase high-performance liquid chromatography (RP-HPLC) separation of unconjugated (**a**) and conjugated (**b**) ^{14}C-cortisol metabolites- and their immunoreactivity tested in a cortisol; corticosterone- and 11-oxoaetiocholanolone-enzyme immunoassay (EIA). Fractions marked with *open triangles* represent the approximate elution time of respective standards (*17*α,20α*P* 17α,20α-dihydroxyprogesterone, E_1 oestrone, E_2β oestradiol-17β, E_1G oestrone-glucuronide)

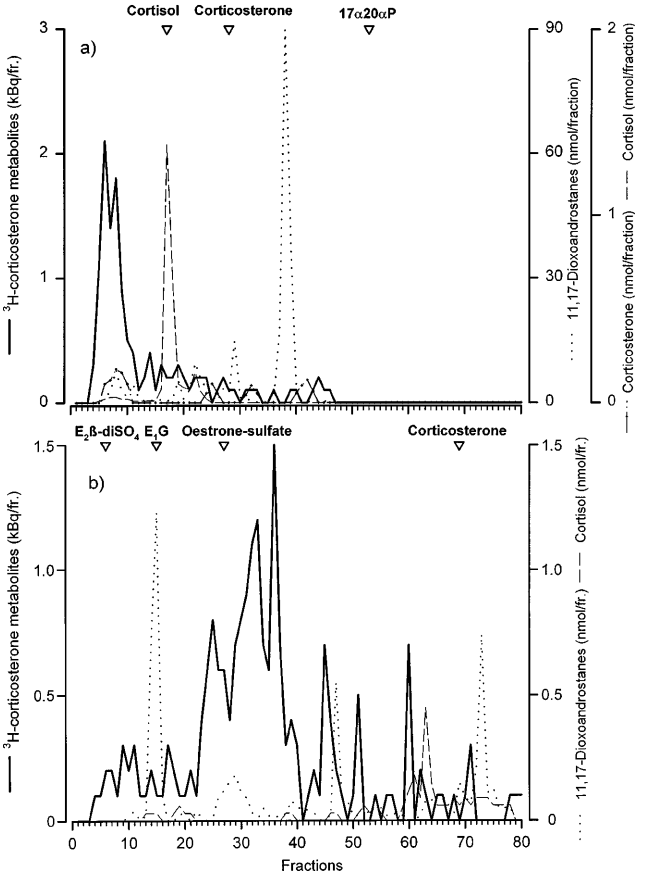

Fig. 3 RP-HPLC separation of unconjugated (**a**) and "conjugated" (**b**) ^{3}H-corticosterone metabolites, and their immunoreactivity tested in a cortisol; corticosterone- and 11-oxoaetiocholanolone-EIA. Fractions marked with *open triangles* represent the approximate elution time of respective standards (*17*α,20α*P* 17α,20α-dihydroxyprogesterone, E_1 oestrone, E_2β oestradiol-17β, E_1G oestrone-glucuronide)

immunoreactive metabolites in the fractions were detected with a group-specific EIA for 11,17-DOA (as compared with cortisol- and corticosterone-EIAs).

In the second experiment, levels of faecal 11,17-DOA concentrations of hares during the first 2 days ranged from 43 nmol kg^{-1} to 274 nmol kg^{-1} faeces (median 100). Following stress, levels in individual hares increased by up to fivefold on one of the following 2 days. Concentrations were significantly higher ($P < 0.001$) on these days than starting levels (first 2 days). Levels decreased and reached starting concentrations by day 4 (Fig. 4). There was a tendency (higher mean values on all days) towards higher levels of faecal cortisol metabolites (though not statistically significant; Student's *t*-test) in females than in males.

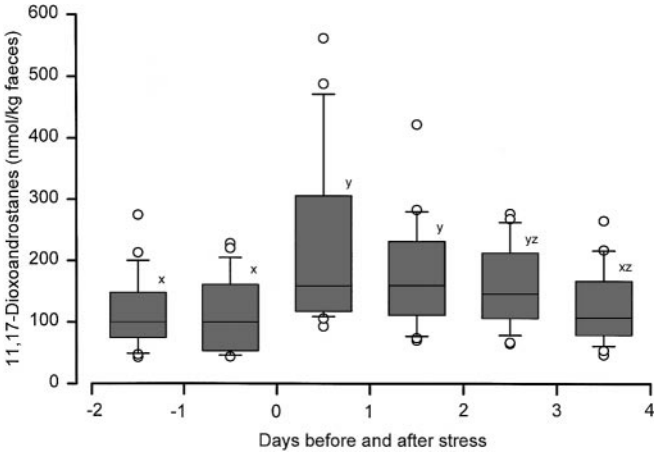

Fig. 4 Concentration of 11,17-DOA (nmol kg^{-1} faeces) in faecal samples of hares ($n = 20$) before and after stress (rousing). Times (days) differ significantly ($P < 0.05$) in the following cases: superscript x versus y or yz, and y versus xz, respectively

Discussion

The aim of this study was to gain basic information concerning the excretion of glucocorticoids in hares. This should advance efforts to establishing non-invasive

glucocorticoid monitoring procedures in this species. Special attention was given to the faecal samples, because these can be collected in the field.

Previous infusion studies of ^{14}C-cortisol in different animals (Bahr et al. 2000; Graham and Brown 1996; Palme et al. 1996; Palme and Möstl 1997; Möstl et al. 1999) have demonstrated pronounced inter-species differences, concerning route of excretion, delay time of faecal peak radioactivity and metabolites formed.

In hares only a small portion of glucocorticoids was excreted via the faeces. However, this does not limit the measurement itself, as faecal metabolite concentrations depend also upon steroid production rate and amounts of produced faeces and as sensitive EIAs enable the measurement of low concentrations of metabolites.

In addition, differences in the excretion route of various steroids in a given species were found in domestic livestock (Palme et al. 1996). However, from the described injection of two glucocorticoids in hares this could not be statistically proven for the same class of steroid hormones, as only six animals in total were used and individual differences and sex (Palme et al. 1996) also probably exert an influence.

Glucocorticoids are heavily metabolised (Brownie 1992). In contrast to domestic livestock (Palme et al. 1996) the majority of not only urinary but also faecal metabolites was not extractable with diethylether, indicating the predominance of conjugated or polar unconjugated metabolites, which in the faeces was confirmed by RP-HPLC separations. This fact may be due to a different metabolism or a different intestinal bacterial flora in hares, as metabolites undergo an additional metabolism by bacteria (Macdonald et al. 1983). In our study we did not concentrate on the conjugated metabolites, as our EIAs detect mainly unconjugated steroids in hares (Figs. 2, 3). To measure conjugated steroids an additional hydrolysis step is necessary, which reduces the practicability of the measurement. Unfortunately most of the conjugated metabolites in hare faeces were not even hydrolysable.

As reported in domestic livestock (Palme and Möstl 1997; Möstl et al. 1999) almost no cortisol (or corticosterone) was found in the faeces of hares. Likewise, and underlined by HPLC immunograms, specific cortisol- and corticosterone-EIAs are not suited for measuring faecal glucocorticoid metabolites in hares. In this species the EIA for 11,17-DOA yielded the highest amounts of immunoreactivity measured. Similar results were obtained in other species such as sheep (Palme and Möstl 1997), horses, pigs (Möstl et al. 1999) and primates (Bahr et al. 2000). In addition, in sheep and horses (Palme and Möstl 1997; Möstl et al. 1999; Palme et al. 1999) measured faecal metabolites (11,17-DOA) reflected adrenocortical activity quite well.

Interestingly there were marked differences between infused cortisol and corticosterone concerning both conjugated and unconjugated faecal metabolites. According to HPLC immunograms only metabolites of ^{14}C-cortisol but not of ^{3}H-corticosterone were measured with the newly developed EIA. As immunoreactivity did not co-elute with radioactive peaks of ^{3}H-corticosterone metabolites, these metabolites are derived from endogenous cortisol. This is underlined by the fact that following ^{14}C-cortisol infusion such metabolites could be detected after HPLC separation (e.g. metabolites measured mainly by the 11-oxoaetiocholanolone-EIA eluting between corticosterone and 17α,20α-dihydroxyprogesterone). This observation corresponds very well with the fact that enzymes (desmolase) responsible for the side-chain cleavage, and thus for the formation of 11,17-DOA, require a 17α-hydroxy group (Macdonald et al. 1983), which is present in the case of cortisol but not with corticosterone. However, this fact does not matter, as cortisol – as stated by Boonstra and Tinnikov (1998) – is the main glucocorticoid in hares and ^{3}H-corticosterone was only injected for comparison as it predominates in other lagomorphs such as rabbits.

The presence of small peaks of radioactivity after maximum concentrations could be due to mixing in the intestine (especially caecum) and/or an enterohepatic recirculation, as was demonstrated in domestic livestock (Palme et al. 1996), and/or coprophagy, which might not have been prevented totally. The decrease in the amount of unconjugated metabolites during that time underlines the latter two possibilities. Due to the protracted excretion, short-term changes might not be well reflected in faecal corticoid metabolite concentrations. However, as mainly the unconjugated metabolite fraction, which decreases more quickly, is measured by the 11-oxoaetiocholanolone-EIA, that should not matter. However, even the disadvantage of dampening short-term changes may turn into an advantage if overall long-term stress-related levels of corticoids could be evaluated. Therefore, determination of faecal corticoid metabolites may be very well suited to determine both short-term (unconjugated metabolites) and overall levels (polar metabolites) of disturbances in a population.

In order to test this technique for field studies (environmental or man-made stress) a pilot study was performed on hares, which were subjected to rousing stress. Levels of faecal cortisol metabolites were significantly elevated after stressing the animals. The use of homogenised morning samples may have smoothed peak concentrations and thus have diminished the response as reflected by faecal 11,17-DOA concentrations.

From our study we conclude that measuring faecal 11,17-DOA should be a valuable tool for non-invasive monitoring of disturbances in hares. This could help to gain more information about stress in this species under field conditions, and thus may be a great step toward elucidating reasons for the decrease in the hare population in Europe.

Acknowledgements The authors thank Mrs. A. Kuchar-Schulz and Mrs. M. Stark for excellent assistance in the laboratory. The financial support of the "Hochschuljubiläumsstiftung der Stadt Wien" (Proj. No.: H-00086/97) is gratefully acknowledged. The animal experiments were conducted with the permission of the respective national authority (GZ 68.205/11-Pr/4/97).

References

Axelrod J, Reisine TD (1984) Stress hormones: their interaction and regulation. Sci 224: 452–459

Bahr N, Palme R, Möhle U, Hodges K, Heistermann M (2000) Comparative aspects of the metabolism and excretion of cortisol in three individual nonhuman primates. Gen Comp Endocrinol (in press)

Boonstra R, Singleton GR (1993) Population declines in the snowshoe hare and the role of stress. Gen Comp Endocrinol 91: 126–143

Boonstra R, Tinnikov A (1998) Increased corticosteroid binding capacity of plasma albumin but not of corticosteroid-binding globulin caused by ACTH-induced changes in free fatty acid concentrations in snowshoe hares and rabbits. J Endocrinol 156: 205–212

Boonstra R, Hik D, Singleton GR, Tinnikov A (1998) The impact of predator-induced stress on the snowshoe hare cycle. Ecol Monogr 68: 371–394

Brownie AC (1992) The metabolism of adrenal cortical steroids. In: James WHT (ed) The adrenal gland. Raven Press, New York, pp 209–224

Graham LH, Brown JL (1996) Cortisol metabolism in the domestic cat and implications for noninvasive monitoring of adrenocortical function in endangered felids. Zoo Biol 15: 71–82

Klansek E (1996) Application of zoological research results in order to increase biodiversity in the agricultural landscape. Ekologia (Bratislava) 15: 419–422

Macdonald IA, Bokkenheuser VD, Winter J, McLernon AM, Mosbach EH (1983) Degradation of steroids in the human gut. J Lipid Res 24: 675–700

Möstl E, Meßmann S, Bagu E, Robia C, Palme R (1999) Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. J Vet Med A 46: 621–632

Morton DJ, Anderson E, Foggin CM, Kock MD, Tiran EP (1995) Plasma cortisol as an indicator of stress due to capture and translocation in wildlife species. Vet Rec 136: 60–63

Palme R, Möstl E (1997) Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int J Mammal Biol 62 [Suppl II]: 192–197

Palme R, Fischer P, Schildorfer H, Ismail MN (1996) Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim Reprod Sci 43: 43–63

Palme R, Möstl E, Brem G, Schellander K, Bamberg E (1997) Faecal metabolites of infused ^{14}C-progesterone in domestic livestock. Reprod Dom Anim 32: 199–206

Palme R, Robia C, Baumgartner W, Möstl E (2000) Transport stress in cattle as reflected by an increase in faecal cortisol metabolites. Vet Rec 146: 108–109

Palme R, Robia C, Meßmann S, Hofer J, Möstl E (1999) Measurement of faecal cortisol metabolites in ruminants: a noninvasive parameter of adrenocortical function. Wien Tierärztl Mschr 86: 237–241

Taylor W (1971) The excretion of steroid hormone metabolites in bile and faeces. Vitam Horm NY 29: 201–285

Villafuerte R, Litvaitis JA, Smith DF (1997) Physiological responses by lagomorphs to resource limitations imposed by habitat fragmentation: implications for condition-sensitive predation. Can J Zool 75: 148–151