Urinary excretion of catecholamines, cortisol and their metabolites in Meishan and Large White sows: validation as a non-invasive and integrative assessment of adrenocortical and sympathoadrenal axis activity

Magali Hay, Pierre Mormède

Vet. Res. 29 (1998) 119–128

Original article

Urinary excretion of catecholamines, cortisol and their metabolites in Meishan and Large White sows: validation as a non-invasive and integrative assessment of adrenocortical and sympathoadrenal axis activity

Magali Hay, Pierre Mormède*

Laboratoire de neurogénètique du stress, Inserm-Inra, Institut François-Magendie, rue Camille-Saint-Saëns, 33077 Bordeaux cedex, France

(Received 31 October 1997; accepted 15 January 1998)

Abstract – Urinary free corticoids (cortisol and cortisone), catecholamines (norepinephrine or NE, epinephrine or E, dopamine or DA, and their O-methoxylated metabolites) as well as creatinine (Cr) were analysed in 42 spontaneously voided urine samples from Large White (LW, $n = 20$), Meishan (MS, $n = 6$), and LW × MS (F1, $n = 16$) lactating sows. The cortisol concentration in the urine of MS (28.1 pg/µg Cr) was five-fold greater than that of LW sows (6.2 pg/µg Cr, $P < 10^{-4}$). F1 were intermediate (12.0 pg/µg Cr). Mean cortisone concentration was also larger in MS (13.5 pg/µg Cr) compared to LW (7.1 pg/µg Cr, $P < 0.01$). Although the differences were less pronounced, the concentrations of the catecholamines were also greater in MS than in LW sows (norepinephrine: 25.4 versus 5.9 pg/µg Cr, epinephrine: 8.7 versus 2.8 pg/µg Cr and dopamine: 59.2 versus 17.8 pg/µg Cr, $P < 10^{-4}$). These results confirmed the hypercortisolism state of MS pigs previously shown by plasma cortisol assay and supported the hypothesis that the sympathetic nervous system is hyperactive in this breed. These urinary investigations may offer possible applications for the assessment of chronic stress. © Inra/Elsevier, Paris

pig / urine / cortisol / catecholamine / genetic

Résumé – Excrétion des catécholamines, du cortisol, et de leurs métabolites chez des truies Meishan et Large White : validation d'une approche non invasive et intégrée de l'activité de l'axe corticotrope et du système nerveux sympathique. Nous avons analysé les corticostéroïdes libres (cortisol et cortisone), les catécholamines (norépinéphrine, épinéphrine, dopamine et leurs dérivés O-méthoxylés) ainsi que la créatinine dans 42 échantillons d'urine de truies en lactation : 20 urines de truies Large White (LW), six urines de truies Meishan (MS) et 16 urines de

* Correspondence and reprints
Tel.: (33) 05 57 57 37 53; fax: (33) 05 57 57 37 52; e-mail: mormede@bordeaux.inra.fr

truies hybrides F1 (LW × MS). Les concentrations en cortisol étaient cinq fois plus importantes dans les urines des truies MS (28,1 pg/µg Cr) que dans les urines des truies LW (6,2 pg/µg Cr, $P < 10^{-4}$), les F1 se situant à un niveau intermédiaire (12,0 pg/µg Cr). Les concentrations en cortisone étaient également plus élevées pour les MS (13,5 pg/µg Cr) comparativement aux LW (7,1 pg/µg Cr, $P < 0,01$). Des différences dans l'excrétion des catécholamines ont été mises en évidence entre les truies MS et LW (norépinéphrine: 25,4 versus 5,9 pg/µg Cr, épinéphrine: 8,7 versus 2,8 pg/µg Cr et dopamine: 59,2 versus 17,8 pg/µg Cr, $P < 10^{-4}$). Ces résultats confirment l'hyperactivité de l'axe corticotrope des MS, qui avait déjà été établie par des mesures plasmatiques et sont en faveur d'une hyperactivité sympathique chez les animaux de cette race. Des applications de ces mesures urinaires sont envisagées pour l'évaluation des états de stress chronique. © Inra/Elsevier, Paris

porc / urine / cortisol / catécholamine / génétique

1. INTRODUCTION

Besides their well-known role in the regulation of metabolism [38], the hypothalamo-pituitary-adrenocortical (HPA) axis and the sympathetic nervous system (SNS) are markedly activated during stress states, leading to the secretion of glucocorticoids and catecholamines, respectively [33]. The measurement of the circulating levels of these hormones has provided a great deal of information on the effect of acute environmental, physical and psychological challenges in farm animals [1–3]. However, much less is known about the secretion of these hormones during long lasting stressful situations, e.g. chronic stress.

This might be due to the practical and physiological limitations related to the measurement of hormones in plasma. Indeed, because of the pulsatility and the circadian variations in the secretion of these hormones, samples have to be collected very frequently (up to every 2 min) to obtain reliable basal values [40]. Moreover, it is well-known that the blood collection procedure may in itself elicit the activation of both corticosteroids and catecholamines secretion, thus interfering with the effect of the factors under study [11, 34]. This makes it difficult to detect differences in basal values, which are generally low.

Alternative sampling procedures include urine collection. This technique offers several advantages for the study of HPA axis and SNS activity. First, urine is the main elimination route for catecholamines, glucocorticoids and their metabolites [28, 30, 37]. Second, urine can be collected non-invasively, when it is spontaneously voided. Third, urinary glucocorticoids and catecholamines sum up over several hours, i.e. since the last miction or collection time. Thus, urine concentrations can be considered as more integrative than plasmatic ones, and it can be expected that small variations of HPA and SNS activity will be easier to detect from urinary excretion of glucocorticoids and catecholamines as compared to plasma measurements.

Urinary cortisol excretion has been shown to be highly correlated with the plasma free (i.e. active) concentrations in humans [5, 8, 12], cats [9], mice [27] and with total plasma cortisol in humans [7] and bighorn sheep [34]. One way to assess the reliability of urinary cortisol excretion as an evaluation of HPA axis function is to compare two breeds or strains known to differ in mean plasma cortisol concentrations [27]. Chinese Meishan pigs show plasma cortisol concentrations that are about two times as high as those of the European Large White [14] and Yorkshire [6] breeds. In the present study, we investigated whether such a difference could also be detected from the spontaneously voided urine of Large White (LW), Meis-

han (MS) and F1 (LW × MS) lactating sows. The excretion pattern of catecholamines for these three genotypes was also investigated. In addition, since plasma cortisol shows a marked circadian rhythm in pigs [14, 25], we investigated whether urinary cortisol concentrations differed according to the time of urine collection.

2. MATERIALS AND METHODS

2.1. Animals and housing

The experiment was conducted at the piggery of the Inra experimental research farm at Le Magneraud (Surgères, France). Forty lactating sows (20 Large White, 10 Meishan and 10 F1 – MS mother × LW father), aged between 2 and 4 years were included in the study. The sows, which had been housed in group pens during gestation, were moved into farrowing crates on day 107 of gestation. Food was provided twice daily (07:30 and 16:30 hours) and water was available continuously.

Spontaneously voided urine was collected on days 17 and 24 post partum, with three collection times per day: early in the morning (between 07:00 and 07:30 hours: h7), at midday (between 11:30 and 12:00 hours: h11) and in the end of the afternoon (between 16:30 and 17:00 hours: h16). The urine samples were then acidified using 6 M HCl (1 % of urine volume) and frozen at –80 °C in 5 mL aliquots. Using this procedure, 62 urine samples were collected: 40 from LW, 16 from F1 and six from MS.

2.2. Urinary analysis

Urinary cortisol and cortisone (the oxidized metabolite of cortisol) were assayed using a solid phase extraction procedure followed by high pressure liquid chromatography (HPLC) with UV absorbance detection (254 nm), as previously described [20]. Briefly, the filtered urine samples were laid on C18 cartridges mounted on a vacuum processing station. Following several washings, corticosteroids were eluted using absolute ethanol. After total evaporation of the ethanol, the dried residues containing corticosteroids were redissolved in the mobile phase before being injected into the HPLC system. Intra and inter assay coefficients of variation (%) were 7.4 and 10.6 for cortisol and 5.4 and 10.9 for cortisone.

Urinary free catecholamines (norepinephrine or NE, epinephrine or E, dopamine or DA) and metanephrines, the O-methylated metabolites of catecholamines (normetanephrine or NMN, metanephrine or MN, 3-O-methyldopamine or MD) were determined using liquid chromatography with electrochemical detection following an ion-exchange purification, as previously described [21]. Both catecholamines and metanephrines were obtained from the same urine sample, through the successive flow on cationic (catecholamines extraction) and then anionic (metanephrines extraction) ion-exchange columns. The eluates were monitered separately on an HPLC system with electrochemical detection, using an oxydizing potential of +0.65V for catecholamines and of +0.8V for metanephrines assessment. Intra and inter assay coefficients of variation (%) were 7.0 and 7.1 for NE, 6.5 and 11.6 for E, 3.8 and 5.8 for DA, 3.0 and 5.9 for NMN, 3.9 and 5.6 for MN, 3.4 and 3.6 for MD.

All urinary concentrations were expressed as a function of creatinine (Cr) excretion, to correct for the variable dilution related to water intake [13]. Creatinine levels were determined using a colorimetric quantitative reaction (Procedure 500, Sigma diagnostics, Saint-Quentin-Fallavier, France). This method is based on the destruction of the colour derived from the reaction between creatinine and alkaline picrate (the Jaffe reaction) when the mixture is acidified. Thus, the difference in colour intensity measured at 500 nm before and after acidification of the mixture is proportional to the creatinine concentration.

2.3. Statistics

The Crunch statistical package (Crunch Software Co., Oakland, CA) was used for all statistics. All data are expressed as mean ± standard error of the mean (s.e.m.). Cortisol, cortisone and all the ratios were analysed after log transformation to fit the Shapiro-Wilk test of normality. Data were submitted to an analysis of variance with genotype and collection time of the urine as 'between subjects' factors. Indeed, almost no sow could be sampled more than once on one day. We did not include the

day of collection as a 'within subject' factor in the analysis, since sows for which urine collection was successful on one day were not the same as on the other day. When significant, analysis of variance was followed by the Newman Keul multiple comparison test.

3. RESULTS

3.1. Urinary cortisol and cortisone *(figure 1)*

Neither time of collection nor the interaction between genotype and time of collection was significant (data not shown).

Genotype effect was highly significant for cortisol ($F_{2\text{-}50} = 32.1$, $P < 10^{-4}$) and cortisone ($F_{2\text{-}50} = 4.93$, $P = 0.01$). Mean cortisol concentration was almost five fold greater in the urine of MS compared to that of LW sows ($P < 10^{-4}$). F1 were at an intermediate level, differing from both MS ($P < 10^{-4}$) and LW ($P < 10^{-3}$). Although less pronounced, cortisone concentrations were also twice as high in MS compared to LW sows ($P < 0.007$). F1 crosses did not significantly differ from either of the parental breeds.

The ratio cortisol/cortisone, as depicted in *figure 2*, significantly differed between the genotypes ($F_{2\text{-}50} = 10.41$, $P < 10^{-3}$). It was higher for MS compared to LW and F1 ($P < 10^{-3}$ and $P < 0.006$, respectively). The mean ratio of F1 was intermediate but not significantly different from that of LW.

3.2. Urinary catecholamines and metanephrines *(figure 3)*

As for cortisol and cortisone excretion, no difference was found according to the collection time (data not shown).

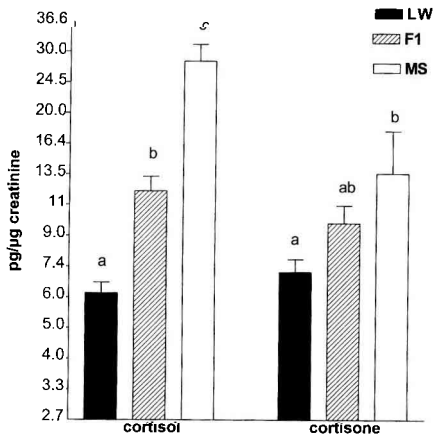

Figure 1. Mean urinary concentration of cortisol (left part of the figure) and cortisone (right part of the figure), expressed as a function of creatinine concentration, in Large White (LW), Meishan (MS) and F1 lactating sows. For each part of the figure, two genotypes with no common letter are significantly different (see text for detailed *P* values).

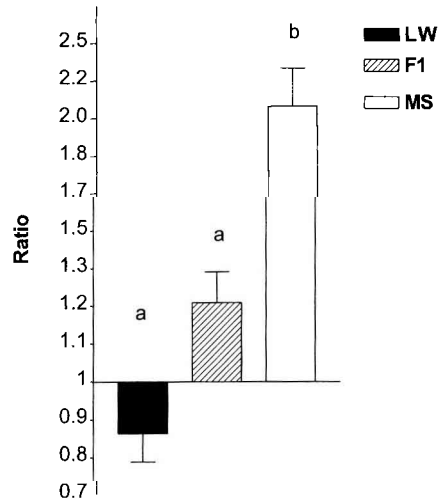

Figure 2. Mean cortisol/cortisone ratio in the urine of Large White (LW), Meishan (MS) and F1 lactating sows. Two genotypes with no common letter are significantly different (see text for detailed *P* values).

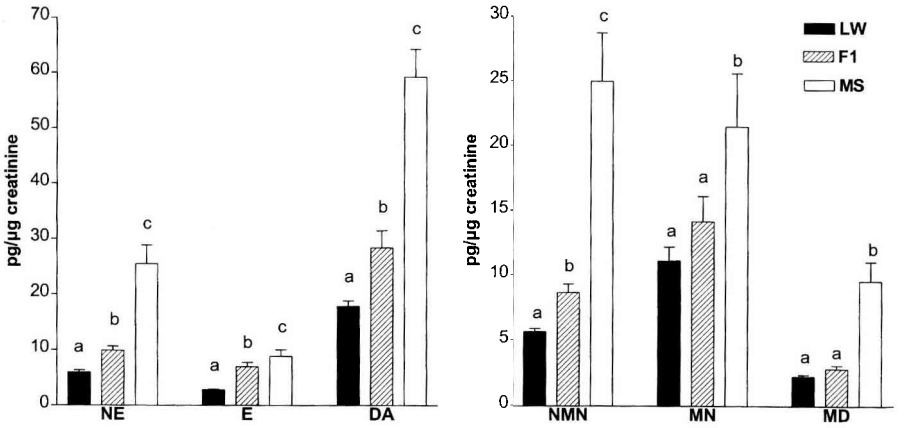

Figure 3. Mean urinary concentration of catecholamines (left panel of the figure) and metanephrines (right panel of the figure), expressed as a function of creatinine concentration, in Large White (LW), Meishan (MS) and F1 lactating sows. NE: norepinephrine, E: epinephrine, DA: dopamine, NMN: normetanephrine, MN: metanephrine, MD: 3-O methyldopamine. For each group of bar charts, two genotypes with no common letter are significantly different (see text for detailed *P* values).

Genotype effect was highly significant for NE ($F_{2\text{-}50} = 67.0$, $P < 10^{-4}$), E ($F_{2\text{-}50} = 43.0$, $P < 10^{-4}$), DA ($F_{2\text{-}50} = 56.5$, $P < 10^{-4}$), NMN ($F_{2\text{-}50} = 76.2$, $P < 10^{-4}$), MN ($F_{2\text{-}50} = 5.5$, $P = 0.007$) and MD ($F_{2\text{-}50} = 88.2$, $P < 10^{-4}$). Urinary catecholamine concentrations in MS were about three times greater than that of LW sows for NE ($P < 10^{-4}$), E ($P < 10^{-4}$) and DA ($P < 10^{-4}$). F1 crosses showed intermediate values (maximal *P* value < 0.02).

Differences between MS and LW sows were also highly significant for all metanephrines concentrations (maximal *P* value < 0.0025). As opposed to catecholamines, F1 crosses did not differ from LW sows for MN and MD concentrations, but remained under the level of MS for both compounds (MN: $P < 0.02$, MD: $P < 10^{-4}$). F1 differed however from both LW ($P < 0.02$) and MS ($P < 10^{-4}$) for NMN.

The ratio O-methoxylated/parental amine *(figure 4)* was very different according to the amine considered. It was high for E (above 2), intermediate for NE (about 1) and very low for DA (below 0.2). Differences between genotypes were found only for MN/E and MD/DA ratio ($F_{2\text{-}50} = 3.6$, $P < 0.035$ and $F_{2\text{-}50} = 3.2$, $P < 0.05$, respectively), but the differences were rather small.

4. DISCUSSION

The main result of this study was the larger urinary cortisol concentration found in MS compared to LW sows. This confirmed the difference in adrenocortical axis activity previously found using plasma measurements [6, 14, 35]. These studies reported a mean cortisol concentration of about twice as much in MS compared to the reference breeds (LW, Yorkshire, Landrace). In urine, we report a five-fold difference in cortisol concentration between LW and MS sows. At least three hypotheses can be put forward to explain this amplification of the differ-

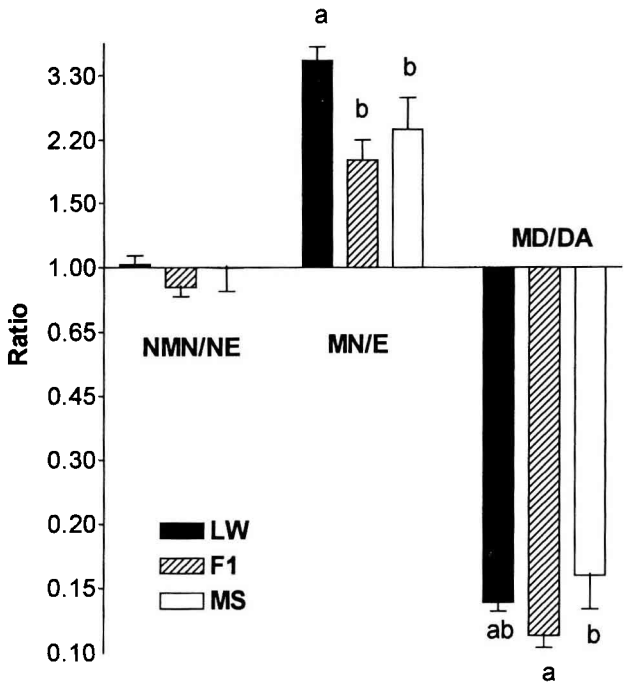

Figure 4. Mean metanephrines/catecholamines ratio in the urine of Large White (LW), Meishan (MS) and F1 lactating sows. NE: norepinephrine, E: epinephrine, DA: dopamine, NMN: normetanephrine, MN: metanephrine, MD: 3-O methyldopamine. For each group of bar charts, two genotypes with no common letter are significantly different (see text for detailed *P* values).

ence between the two breeds. First, the plasma studies mentioned above involved 6-week-old piglets [14, 35] or 8-month-old pregnant gilts [6], compared to the at least 2-year-old lactating sows we used. Second, creatinine excretion is known to depend mainly on the muscle mass [23]. Since the composition of MS pig carcass reveals a lower muscle content when compared to weight matched LW (30.5 % versus 47.3 %) [39], we could expect that creatinine output of MS would be about 64 % that of LW. Thus, although we cannot exclude additional genetic differences in the metabolism of creatine, this overestimation of cortisol excretion in MS sows alone does not explain by itself the 2.5-fold amplification of the difference found in plasma levels between the two breeds. Finally, differences in the metabolic rates of cortisol may account for the different levels between breeds.

11β-Hydroxysteroid dehydrogenase isoform 2 (11β-HSD2) is a microsomal enzyme which catalyses the dehydrogenation of the active cortisol into the inactive cortisone [24]. This enables aldosterone to bind to its specific mineralocorticoid receptors, otherwise occupied with equal affinity by cortisol [42]. Urinary cortisone excretion has received little attention so far. However, it was shown that most of it was formed from the cir-

culating cortisol in the kidneys [22, 44]. Combined with that of cortisol, its assessment might provide additional information about adrenocortical activity [26] and also 11β-HSD2 activity [36]. The larger cortisol/cortisone ratio found in MS sows can be explained either by a reduced activity of 11β-HSD2 in this breed or by a saturation of this enzyme due to the high plasma cortisol concentration. However, human case studies of hypercortisolism states resulting from ectopic adrenocorticotrophic hormone (ACTH) and the Cushing syndrome revealed that 11β–HSD2 has considerable capacity for the conversion of cortisol to cortisone [36]. Thus, the difference might come from genetic differences in the enzyme activity, which remains to be confirmed.

We were not able to detect any difference in cortisol and cortisone between the three collection times, despite the evidence of a circadian rhythm of cortisol secretion in the pig, peaking at the beginning of the day (between 06:00 and 10:00 hours) [14, 25]. Since cortisol is cleared from the peripheral circulation in approximately 2 h [9], we expected higher values for h7 and h11 as compared to h16 samples. Several reasons can explain the lack of circadian variations for urinary cortisol in our study. First, we do not know the interval between the miction collected and the preceding one. Second, since almost no sow could be sampled three times in a given day, we were unable to take into account individual variations, which can markedly affect the profile of the circadian variations, as has been shown in humans [7]. Third, the lactating status of the sows might have interfered with the circadian rhythmicity of the cortisol secretion, although plasma corticosterone exhibited normal circadian variations in lactating rats [43]. We plan to study more accurately the circadian profile of cortisol excretion with animals fitted with vesical catheters.

Cortisol concentrations in the urine of F1 sows was intermediate to that found in both parental breeds. The results of Désautés et al. [15] suggest a dominance in the transmission of the hypercortisolism trait of MS. Indeed, they found that F1 (MS mother) and reverse F1 (LW mother) had similar plasma cortisol levels compared to MS. However, although non-significant, cortisol concentrations of F1 were lower than that of MS and reverse F1 pigs. Similarly, Bergeron et al. [6] found that reverse F1 (Yorkshire mother) had plasma cortisol concentrations close to that of MS gilts, whereas F1 (MS mother) were intermediate between the MS and Yorkshire parental breeds. This compares favourably with our results, since our F1 were born from a MS mother.

To our knowledge, no direct comparison of plasma catecholamine concentrations between adult LW and MS pigs has been published up to now. Le Dividich et al. [29] found 60 % lower epinephrine plasma concentrations in newborn MS piglets compared to LW, but the norepinephrine concentrations did not differ between the two breeds. Plasma concentrations of norepinephrine and epinephrine in 6–8-week-old MS piglets were found to be 258 and 225 pg/mL, respectively [35]. These values are larger than those found by Barrand et al. [2] in 6–10-week-old LW piglets (80–180 pg/mL for norepinephrine and 10–30 pg/mL for epinephrine), and than those found by Fernandez et al. [18] in 100-kg LW pigs (75–105 pg/mL for norepinephrine and 80–115 pg/mL for epinephrine). It is, however, unwise to draw conclusions from these results because it is not likely that the values mentioned in these studies reflect resting levels, especially when they were obtained by venipuncture [11, 35]. Despite the possible overestimation of catecholamine excretion in MS due to creatinine, as discussed above, and the possible existence of genetic differences in

catecholamines metabolism between the two breeds, our results were in favour of a hyperactivity of the SNS in MS compared to LW sows. It is unlikely that this comes from the the fact that we assayed free (i.e. unconjugated) catecholamines, since we previously showed that the conjugated fraction of catecholamines and metanephrines was small in the urine of lactating sows (0 to 35 % according to the amine) and that total (free plus conjugated) catecholamine estimation was highly correlated with the free fraction [21]. The interpretation of the higher DA excretion from MS sows was made difficult by the fact that the origin and functional relevance of urinary DA remain unclear. Urinary DA probably results from the glomerular filtration and especially from the active secretion by the renal tubular epithelium [19].

Metanephrines assessment provides additional information about sympathoadrenal activity to that provided by catecholamines [17]. This is especially true for MN, which is a major excretion product of E [28]. Thus, we can suppose that the sum of both compounds might provide a more accurate estimation of the secretion of E by the adrenal medulla. As opposed to MN, the O-methylation by catechol-O-methyltransferase (COMT) is not the main pathway for the metabolism of NE, since urinary NMN accounts for as little as 4.5 % of the total NE metabolites in humans [28]. Nevertheless, because COMT is localized mainly in non-neuronal tissues, NMN is a useful marker of NE release (as opposed to other metabolites which can also be formed from the unreleased, i.e. inactive NE) [17]. The usefulness of the information provided by the assessment of these compounds in genetic and stress studies needs further evaluations. Interestingly, we found that the ratio MN/E was much larger than the NMN/NE and MD/DA ratio independent of the genotype. This confirmed the preference of E over NE for the COMT route of metabolism, a result already found by Eisenhofer and Finberg [16] from spillover studies in rat. MD is mainly formed within gastrointestinal tissues, spleen and stomach, where the deamination pathway is predominant (Eisenhofer, pers. comm.). This probably explains the low MD/DA ratio that we found.

Our results showed that it is possible to assess structural differences in the activity of both the HPA and SNS axes from the measurement of glucocorticoids and catecholamines in the spontaneously voided urine of sows. This approach might also offer new perspectives for the assessment of chronic stress states in pigs, as it has already been done in cats [10], dogs [4], pigs [41] and monkeys [31, 32]. However, the study of environmental influences on the excretion levels of stress hormones will have to take into account the individual variations under basal condition. This approach might be relevant in the context of the increasing concern about farm animal welfare and the need for objective criteria for welfare assessment.

ACKNOWLEDGEMENTS

This study was supported by a special grant from Inra (AIP 'Bien-Etre et Sciences du Comportement'). The authors wish to thank C. Désautés, J.C. Caritez and all the team of the 'Domaine Experimental du Magneraud' for their active collaboration. M. Hay is the recipient of a fellowship from the National Veterinary School of Toulouse.

REFERENCES

[1] Arnone M., Dantzer R., Does frustration induce aggression in pigs? Appl. Anim. Ethol. 6 (1980) 351–362.

[2] Barrand M.A., Dauncey M.J., Ingram D.L., Changes in plasma noradrenaline and adrenaline associated with central and peripheral thermal stimuli in the pig, J. Physiol. 316 (1981) 139–152.

[3] Becker B.A., Nienaber J.A., Christenson R.K., Manak R.C., DeShazer J.A., Hahn G.L., Peripheral concentrations of cortisol as an indicator of stress in the pig, Am. J. Vet. Res. 46 (1985) 1034–1038.

[4] Beerda B., Schilder M.B.H., Janssen N.S.C.R.M., Mol J.A., The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs, Horm. Behav. 30 (1996) 272–279.

[5] Beisel W.R., Cos J.J., Horton R., Chao P.Y., Forsham P.H., Physiology of urinary cortisol excretion, J. Clin. Endocrinol. 24 (1964) 887–893.

[6] Bergeron R., Gonyou H.W., Eurell T.E., Behavioral and physiological responses of Meishan, Yorshire and crossbred gilts to conventional and turn-around gestation stalls, Can. J. Anim. Sci. 76 (1996) 289–297.

[7] Brandenberger G., Follenius M., Variations diurnes de la cortisolémie, de la glycémie et du cortisol libre urinaire chez l'homme au repos, J. Physiol. 66 (1973) 271–282.

[8] Burke C.W., Beardwell C.G., Cushing's syndrome. An evaluation of the clinical usefulness of urinary free cortisol and other urinary steroid measurements in diagnosis, Q. J. Med. XLII (1973) 175–204.

[9] Carlstead K., Brown J.L., Monfort S.L., Killens R., Wildt D., Urinary monitoring of adrenal responses to psychological stressors in domestic and nondomestic felids, Zool. Biol. 11 (1992) 165–176.

[10] Carlstead K., Brown J.L., Strawn W., Behavioral and physiological correlates of stress in laboratory cats, Appl. Anim. Behav. Sci. 38 (1993) 143–158.

[11] Castagné V., Coder R., Gaillard R., Mormède P., Stress-induced changes of circulating neuropeptides Y in the rat: comparison with catecholamines, Regul. Pept. 19 (1987) 55–63.

[12] Cope C.L., Black E.G., The reliability of some adrenal function tests, Br. Med. J. 2 (1959) 1117–1122.

[13] Crockett C.M., Bowers C.L., Sackett G.P., Bowden D.M., Urinary cortisol responses of longtailed macaques to five cage sizes, tethering, sedation and room change, Am. J. Primatol. 30 (1993) 55–74.

[14] Désautés C., Réactivité comportementale et neuroendocrinienne au stress chez le porc : étude génétique, thèse d'Université, Rennes, France.

[15] Désautés C., Bidanel J.-P., Mormède P., Genetic study of behavioral and pituitary-adrenocortical reactivity in response to an environmental challenge in pigs, Physiol. Behav. 62 (1997) 337–345.

[16] Eisenhofer G., Finberg J.P.M., Different metabolism of norepinephrine and epinephrine by catechol-O-methyltransferase and monoamine oxidase in rats, J. Pharmacol. Exp. Ther. 268 (1994) 1242–1251.

[17] Eisenhofer G., Friberg P., Pacak K., Goldstein D.S., Murphy D.L., Tsigos C., Quyyumi A.A., Brunner H.G., Lenders J.W.M., Plasma metadrenalines: do they provide useful information about sympatho-adrenal function and catecholamine metabolism? Clin. Sci. 88 (1995) 533–542.

[18] Fernandez X., Meunier-Salaün M.-C., Mormède P., Agonistic behavior, plasma stress hormones, and metabolites in response to dyadic encounters in domestic pigs: interrelationships and effect of dominance status, Physiol. Behav. 56 (1994) 841–847.

[19] Goldstein D.S., Clinical assessment of catecholaminergic function, in: Goldstein D.S. (Ed.), Stress, Catecholamines, and Cardiovascular Disease, Oxford University Press, New York, 1995, pp. 234–286.

[20] Hay M., Mormède P., Improved determination of urinary cortisol and cortisone or corticosterone and 11-dehydrocorticosterone by high-performance liquid chromatography with ultraviolet absorbance detection, J. Chromatogr. B. 702 (1997) 33–39.

[21] Hay M., Mormède P., Determination of catecholamines and methoxy-catecholamines excretion patterns in pig and rat urine by ion-exchange liquid chromatography with electrochemical detection, J. Chromatogr. B. 703 (1997) 15–23.

[22] Hellman L., Nakada F., Zumoff B., Fukushima D.K., Bradlow H.L., Gallagher T.F., Renal capture and oxidation of cortisol in man, J. Clin. Endocrinol. 33 (1971) 52–62.

[23] Heymsfield S.B., Arteaga C., McManus C., Smith J., Moffitt S., Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method, Am. J. Clin. Nutr. 37 (1983) 478–494.

[24] Idrus R.B.H., Mohamad N.B., Morat P.B., Saim A., Kadir K.B.A., Differential effect of adrenocorticosteroid dehydrogenase bioactivity at the anterior pituitary and hypothalamus in the rat, Steroids 61 (1996) 448–452.

[25] Janssens C.J.J.G., Helmond F.A., Wiegant V.M., The effect of chronic stress on plasma cortisol concentrations in cyclic female pigs depends on the time of day, Domest. Anim. Endocrinol. 12 (1995) 167–177.

[26] Jenner D.A., Richards J., Determination of cortisol and cortisone in urine using high-performance liquid chromatography with UV detection, J. Pharmaceut. Biomed. Anal. 3 (1985) 251–257.

[27] Kley H.K., Herberg L., Krüskemper H.L. Evaluation of adrenal function in mice by measurement of urinary excretion of free corticoids, J. Steroid Biochem. 7 (1976) 381–385.

[28] Kopin I.J., Catecholamine metabolism: basic aspects and clinical significance, Pharmacol. Rev. 37 (1985) 333–364.

[29] Le Dividich J., Mormède P., Catheline M., Caritez J.C., Body composition and cold resistance of the neonatal pig from european (Large White) and chinese (Meishan) breeds, Biol. Neonate. 59 (1991) 268–277.

[30] Lindner H.R., Enterohepatic circulation and pattern of urinary excretion of cortisol metabolites in the ewe, J. Endocrinol. 52 (1972) 19–20.

[31] Mason J.W., Harwood C.T., Rosenthal N.R., Influence of some environmental factors on plasma and urinary 17-hydroxycorticosteroid levels in the Rhesus monkey, Am. J. Physiol. 190 (1957) 429–433.

[32] Mason J.W., Mougey E.H., Kenion C.C., Urinary epinephrine and norepinephrine responses to chair restraint in the monkey, Physiol. Behav. 10 (1973) 801–804.

[33] Mendl M., Deag J.M., How useful are the concepts of alternative strategy and coping strategy in applied studies of social behaviour? Appl. Anim. Behav. Sci. 44 (1995) 119–137.

[34] Miller M.W., Hobbs N.T., Sousa M.C., Detecting stress responses on Rocky Mountain bighorn sheep (Ovis canadensis canadensis): reliability of cortisol concentrations in urine and feces, Can. J. Zool. 69 (1991) 15–24.

[35] Mormède P., Dantzer R., Bluthe R.M., Caritez J.C., Differences in adaptative abilities of three breeds of Chinese pigs. Behavioural and neuroendocrine studies, Genet. Sel. Evol. 16 (1984) 85–102.

[36] Palermo M., Shackleton C.H.L., Mantero F., Stewart P.M., Urinary free cortisone and the assessment of 11β-hydroxysteroid dehydrogenase activity in man, Clin. Endocrinol. 45 (1996) 605–611.

[37] Palme R., Fischer P., Schildorfer H., Ismail M.N., Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock, Anim. Reprod. Sci. 43 (1996) 43–63.

[38] Picon L.O., Régulation hormonale du métabolisme énergétique, in: Dupouy J.P. (Ed.), Hormones et Grandes Fonctions, Ellipses, Paris, 1993, pp.158–200.

[39] Poilvet D., Bonneau M., Caritez J.C., Legault C., Carcass tissu composition in Meishan pigs (MS), Large White (LW) and F1 (MSxLW) pigs, in: Molenat M., Legault C. (Eds.), Symposium sur le porc chinois, Inra editions, Jouy-En-Josas, 1990, p. 238.

[40] Rushen J., Problems associated with the interpretation of physiological data in the assessment of animal welfare, Appl. Anim. Behav. Sci. 28 (1991) 381–386.

[41] Stefanovic M.P., Bayley H.S., Slinger S.J., Effect of stress on swine: heat and cold exposure and starvation on vanilmandelic acid output in the urine, J. Anim. Sci. 30 (1970) 378–381.

[42] Stewart P.M., Edwards C.R.W., Specificity of the mineralocorticoid receptor. Crucial role of 11β-hydroxysteroid dehydrogenase, Trends Endocrinol. Metab. 1 (1990) 225–230.

[43] Walker C.D., Lightman S.L., Steele M.K., Dallman M.F., Suckling is a persistant stimulus to the adrenocortical system of the rat, Endocrinology 130 (1992) 115–125.

[44] Whitworth J.A., Stewart P.M., Burt D., Atherden S.M., Edwards C.R.W., The kidney is the major source of cortisone in man, Clin. Endocrinol. 31 (1989) 355–361.