Plasma concentrations of adrenocorticotropic hormone and α-melanocyte-stimulating hormone in ferrets (Mustela putorius furo) with hyperadrenocorticism

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Objective—To determine plasma concentrations of adrenocorticotropic hormone (ACTH) and α-melanocyte-stimulating hormone (α-MSH) in healthy ferrets and ferrets with hyperadrenocorticism.

Animals—16 healthy, neutered, privately owned ferrets, 28 healthy laboratory ferrets (21 sexually intact and 7 neutered), and 28 ferrets with hyperadrenocorticism.

Procedures—Healthy ferrets were used for determination of reference plasma concentrations of ACTH and α-MSH. Diagnosis of hyperadrenocorticism was made on the basis of history, clinical signs, urinary corticoid-to-creatinine ratios, ultrasonography of the adrenal glands, and macroscopic or microscopic evaluation of the adrenal glands. Blood samples were collected during isoflurane anesthesia. Plasma concentrations of ACTH and α-MSH were measured by radioimmunoassay.

Results—Plasma concentrations of ACTH in 23 healthy neutered ferrets during the breeding season ranged from 4 to 145 ng/L (median, 50 ng/L). Plasma concentrations of α-MSH in 44 healthy neutered or sexually intact ferrets during the breeding season ranged from <5 to 617 ng/L (median, 37 ng/L). Reference values (the central 95% of the values) for ACTH and α-MSH were 13 to 100 ng/L and 8 to 180 ng/L, respectively. Plasma concentrations of ACTH and α-MSH in ferrets with hyperadrenocorticism ranged from 1 to 265 ng/L (median, 45 ng/L) and 10 to 148 ng/L (median, 46 ng/L), respectively. These values were not significantly different from those of healthy ferrets. Plasma ACTH concentrations of sexually intact female ferrets in estrus were significantly higher than those of neutered females.

Conclusions and Clinical Relevance—Ferrets with hyperadrenocorticism did not have detectable abnormalities in plasma concentrations of ACTH or α-MSH. The findings suggest that hyperadrenocorticism in ferrets is an ACTH and α-MSH-independent condition. —(Am J Vet Res 2002;63:1395–1399)
with hyperadrenocorticism can be treated success-
fully with leuprolide acetate.11

Luteinizing hormone-dependent hyperadrenocor-
ticism and LH-dependent adrenal androgen-secreting
tumors have been described in humans.10 In LH-depen-
dent hyperadrenocorticism, hypercortisolism is associ-
ated with suppressed concentrations of ACTH. In LH-
dependent adrenal androgen-secreting tumors, both
cortisol and ACTH concentrations have been reported
to be within the reference range.11

Thus, there are several questions with regard to the
factors involved in the development of hypera-
drenocorticism in ferrets, such as the role of secretion
of ACTH and α-MSH. In ACTH-dependent hypera-
drenocorticism, one would expect increased plasma
concentrations of ACTH and possibly α-MSH. With
hypercortisolism, the LH-dependent form of hypera-
drenocorticism would lead to suppressed plasma
ACTH concentrations. To the authors’ knowledge,
there are no reports on plasma concentrations of
ACTH or α-MSH in ferrets with hyperadrenocorticism.

The purpose of the study reported here was to
determine plasma concentrations of ACTH and α-MSH
in 28 ferrets with hyperadrenocorticism. The values are
compared with those of 23 castrated and 21 sexually
intact healthy ferrets.

Materials and Methods

Control ferrets—A group of 23 neutered control ferrets
(13 female and 10 male) comprised 16 privately owned
ferrets that were house mates of the ferrets with hyperadreno-
corticism and 7 ferrets kept under laboratory conditions,
individually housed in outdoor suspended cages with a night
box. No additional lighting was provided. Water and ferret
pellets were provided ad libitum. The ages of the 23 ferrets
ranged from 1.5 to 8 years (median, 3 years). Blood samples
were collected between March 15 and September 29, 2000.
None of the ferrets had any clinical signs of hyperadrenocor-
ticism. All ferrets had urinary cortisol-to-creatinine ratio
< 1.7 X 10–6. Urinary corticoid concentrations were measured
by use of radio-immunoassay (RIA) for cortisol, as
described.12 The urinary corticoid concentration was related
to the urinary creatinine concentration (Jaffé kinetic method,
initial rate reaction) by calculation of its quotient (X 10–6).13

In addition, blood was collected on April 4, 2000 from 21
sexually intact ferrets, aged 2 to 3 years (14 female and 7
male), kept under the same laboratory conditions as described.

Ferrets with hyperadrenocorticism—The 10 female
and 18 male ferrets with hyperadrenocorticism were evaluat-
ed between April 1998 and September 2000. All ferrets had
been neutered at 0.5 to 1 year of age. At the time of evalua-
tion, their ages ranged from 3 to 7 years (median, 5 years). In
22 ferrets, alopecia was the main clinical sign. Of the 6 other
ferrets, 5 were evaluated for swelling of the vulva, and in 1
ferret, a large adrenal mass was incidentally detected on the
left side when abdominal ultrasonography was performed for
other reasons. After 2 months this ferret died with metastases
in the adrenal tumor. Histologic examination (9) provided additional support for the diag-

In 21 ferrets there was unilateral enlargement of the
adrenal gland, on the left side in 15 ferrets and on the right
side in 6 ferrets. In all these ferrets, the contralateral adrenal
gland was of normal size (ie, no atrophy). Seven ferrets had
bilateral adrenal enlargement. In 24 of 26 ferrets, use of
ultrasonography identified the alterations in the adrenal
glands correctly.

Histologic examination of the adrenal glands of 26 fer-
rets (10 females and 16 males) revealed hyperplasia (n = 9),
adrenoma (15), and metastasized adenocarcinoma (2). In 1
ferret, the adrenal tissue was lost for histologic examination,
and in 1 ferret the adrenal tumor was left in place because of
its growth into the caudal vena cava.

Blood collection—In affected ferrets and the control
ferrets, blood was collected between 2:30 and 4:00 PM
from the anterior vena cava by use of isoflurane anesthe-
sia. Blood was immediately placed in chilled EDTA-coated

![Figure 1—Dilution curves (concentration vs counts per minute (cpm)) of 2 ferret plasma samples (△, ▲) and a human adreno-
corticotrophic hormone (ACTH) standard (■) analyzed by use of radioimmunoassay.](image1.png)

![Figure 2—Dilution curves (concentration vs cpm) of a ferret plas-
ta sample (▲) and a human melanocyte-stimulating hormone
(α-MSH) standard (■) analyzed by use of radioimmunoassay.](image2.png)
tubes and centrifuged at 4°C. Plasma was stored at −20°C pending analysis. Samples were analyzed within 6 months after collection.

**Hormone determination**—Plasma ACTH concentrations were measured by use of a commercially available immunoradiometric assay as per manufacturer’s instructions. The interassay coefficient of variation was 7.8%, and the sensitivity was 1 ng/L. There was no cross-reaction with α-MSH.

Plasma concentrations of α-MSH were measured by use of RIA with antiserum to synthetic human α-MSH, as described. This antiserum had full cross-reactivity with desacyl-α-MSH but < 0.1% cross-reactivity with ACTH1-39 and 4% cross-reactivity with ACTH1-24. Synthetic human α-MSH was used as a standard. The limit of detection was 5 ng/L, and the intra- and interassay coefficients of variation were 10 and 23%, respectively.

Both assays were validated for use in ferrets. Dilution curves were made from a ferret plasma sample and compared with standards for ACTHb and α-MSHc. For both hormones, the curves followed the standard pattern (Fig 1 and 2).

**Statistical analyses**—The percentile method was used to establish the reference ranges for plasma ACTH and α-MSH concentrations. The percentiles P 2.5 and P 97.5 were determined with a probability of 95%. The Wilcoxon rank sum test was used for comparison of plasma ACTH and α-MSH concentrations of neutered and sexually intact ferrets. Results were graphically represented in box-and-whisker plots. In these figures, the box represents the interquartile range from the 25th to 75th percentile, the horizontal bar through the box indicates the median, and the whiskers represent the main body of data, which in most instances is equal to the range. For all comparisons, a value of P < 0.05 was considered significant.

**Results**

**Healthy ferrets**—Plasma ACTH concentrations in 23 neutered healthy ferrets ranged from 4 to 143 ng/L (median, 50 ng/L; range, 13 to 100 ng/L). In the 21 sexually intact ferrets kept under laboratory conditions, plasma ACTH concentrations ranged from 38 to 144 ng/L (median, 88 ng/L; range, 53 to 109 ng/L). The plasma ACTH concentrations in the sexually intact ferrets were significantly (P < 0.01) higher than in the neutered ferrets.

Plasma ACTH concentrations in sexually intact female ferrets (n = 14; median, 94.5 ng/L; range, 62 to 144 ng/L) were also significantly (P < 0.01) higher than in neutered female ferrets (13; median, 44 ng/L; range, 8 to 145 ng/L; Fig 3). The difference in plasma ACTH concentrations between sexually intact male ferrets (n = 7; median, 68 ng/L; range, 38 to 133 ng/L) and neutered male ferrets (10; median, 66 ng/L; range, 4 to 100 ng/L) was not significant.

Plasma α-MSH concentrations of sexually intact (n = 21) and neutered (23) ferrets did not differ significantly, nor was there a significant difference in the sexually intact ferrets between males and females; therefore, the data were combined to establish reference values. Plasma α-MSH concentrations in the 44 ferrets ranged from < 5 to 617 ng/L (median, 37 ng/L; range, 7 to 180 ng/L).

**Ferrets with hyperadrenocorticism**—Plasma ACTH concentrations in the 28 neutered ferrets with hyperadrenocorticism (median, 45 ng/L; range, 1 to 144 ng/L) were significantly (P < 0.01) higher than in the healthy ferrets. The plasma ACTH concentrations in the sexually intact ferrets were significantly (P < 0.01) higher than in the healthy ferrets.
265 ng/L) were not significantly different from those in healthy neutered ferrets (Fig 4). In 6 hyperadrenocorticotid ferrets, the plasma ACTH concentration exceeded the upper limit (10 ng/L) of the reference range of healthy ferrets.

The plasma α-MSH concentrations in ferrets with hyperadrenocorticism (n = 27; median, 46 ng/L; range, 10 to 148 ng/L) were not significantly different from those in healthy ferrets (Fig 3). The plasma α-MSH concentration did not exceed the upper limit (180 ng/L) of the reference range of healthy ferrets in any of the hyperadrenocorticotid ferrets.

Discussion

In this study, plasma ACTH concentrations of healthy neutered ferrets ranged from 4 to 145 ng/L, which is similar to the ranges reported for dogs, cats, and horses.1-3,15-20 Plasma ACTH concentrations in sexually intact ferrets were significantly higher than in neutered ferrets. This may be attributable to the fact that the blood samples were collected during the breeding season. In female frogs, secretory activity of ACTH cells increases during the breeding season.27 The question remains whether this is due to the arousal associated with the breeding season or to a direct effect of the activated pituitary-ovarian axis on the corticotrophic cells. The latter is the most likely explanation because the plasma ACTH concentrations of sexually intact male ferrets were not significantly different from those of neutered male ferrets.

In our study, basal plasma α-MSH concentrations in ferrets were similar to those in cats, which are somewhat higher than in dogs.22,23 The values in ferrets may have been artificially increased by placement of a mask and induction of isoflurane anesthesia, because isoflurane has an unpleasant odor. It is known that, particularly in induction of isoflurane anesthesia, because isoflurane has been artificially increased by placement of a mask and induction of isoflurane anesthesia, because isoflurane has an unpleasant odor. It is known that, particularly in this study, plasma ACTH concentrations of sexually intact ferrets were not significantly different from those of neutered male ferrets.

In addition, ACTH and α-MSH are released in a pulsatile manner in dogs.24 Consequently, an occasional high value might be found with single measurements, although, particularly with α-MSH, the pulse frequency is low.24 To our knowledge, there are no reports on the release patterns of ACTH and α-MSH in ferrets, but pulsatile release is likely because it has been found in all species studied. These considerations should be taken into account when individual values are interpreted. In our study, group values were evaluated instead of individual samples. We believe that the effects of possible pulsatile hormone release were outweighed by the numbers of animals per group.

Because we found that plasma concentrations of ACTH and α-MSH of ferrets with hyperadrenocorticism are essentially identical to those of healthy neutered ferrets, we believe that the adrenocortical changes and clinical signs cannot be ascribed to hypersecretion of ACTH. In addition, it is likely that there was no primary hypercortisolism either, because this finding should be associated with either decreased or increased plasma ACTH concentrations. Consequently, the increased corticoid-to-creatinine ratios might not be a reflection of hypercortisolism but rather the result of other urinary steroid hormones or steroid hormone metabolites cross-reacting in the cortisol assay. The antibody used in the assay for measurement of urinary cortisol is known to cross-react with other endogenous corticosteroids such as 21-deoxycorticoster (62%), cortisone (11%), cortisone (2%), 11-deoxycorticoster (1.3%), deoxycorticosterone (1.3%), and 17α-hydroxyprogesterone (0.1%).24

Thus, hyperadrenocorticism in ferrets should probably be regarded as a normocortisolemic and corticotrophin-independent hypersecretion of androgens. Primarily, many different ectopic and abnormal hormone receptors have been found in the adrenal cortices of humans with hyperadrenocorticism.6 In ferrets with adrenocortical hyperfunction, involvement of the LH receptor is likely. Recent preliminary immunohistochemical studies by Wagner et al13 and our group14 have revealed that adrenal tumors possess cells with LH receptors.

Results of our study suggest that hyperadrenocorticism in ferrets may be an ACTH- and α-MSH-independent condition accompanied by unchanged plasma cortisol concentrations.

References

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