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SMALL ADRENAL GLANDS IN CATS WITH FELINE INTERSTITIAL CYSTITIS

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ABSTRACT

Purpose: We documented the uncoupling of sympathetic nervous system activity from the hypothalamic-pituitary-adrenal axis in cats with feline interstitial cystitis (FIC). Altered hypothalamic-pituitary-adrenal activity was recently suggested in some humans with interstitial cystitis (IC) but to our knowledge no information exists on adrenal gland size and histopathology in this disease. To investigate further adrenal function in cats with FIC we determined cortisol responses to 125 μg synthetic adrenocorticotropic hormone (ACTH) as well as adrenal size and histology.

Materials and Methods: ACTH stimulation studies were performed in 11 healthy cats and 20 with FIC. Adrenal glands obtained at autopsy in 8 healthy cats and 13 with FIC were weighed, measured and examined histologically.

Results: Cats with FIC had significantly decreased responses to ACTH (2-way repeated measures ANOVA $p < 0.05$). Mean weight $\pm$ SD (58 $\pm$ 50 vs 241 $\pm$ 60 mg) and volume (264 $\pm$ 72 vs 410 $\pm$ 115 mm^3) of adrenal glands were significantly smaller in cats with FIC than in healthy cats ($p < 0.05$).

Conclusions: These results suggest that cats with FIC may have mild primary adrenal insufficiency. Decreased adrenal size has been observed in patients with chronic fatigue syndrome, which can be a co-morbid condition in some patients with IC. If these abnormalities are confirmed in humans with IC, hormone replacement therapy may be indicated in select patients.

KEY WORDS: adrenal glands; cats; cystitis, interstitial; sympathetic nervous system; corticotropin

Interstitial cystitis (IC) is a chronic pelvic pain syndrome of unknown cause and for which no generally accepted treatment is available.[1] The symptoms of IC include pain referable to the bladder, and/or increased urination frequency and urgency. IC may affect more than 700,000 American women[1] and a large number of men with prostatitis or prostatodynia.[2] A comparable disorder of domestic cats is a common veterinary problem. We have termed this syndrome feline IC (FIC).

We recently reported increased sympathetic nervous system activity in the absence of identifiable differences in hypothalamic-pituitary-adrenal (HPA) axis function in cats with FIC.[3] Nonstressed cats with FIC had significantly increased plasma norepinephrine compared with healthy cats but when challenged with corticotropin releasing factor, no differences in adrenocorticotropic hormone (ACTH) or cortisol were identified. Lutgendorf et al recently reported cortisol concentrations in the urine and saliva (measures of free cortisol) in humans with IC.[4] Although average urine or salivary cortisol did not differ between patients and controls, patients with IC and higher morning cortisol reported significantly less pain and urgency. In another study hypocortisolism was identified in patients with chronic pelvic pain and stress related bodily disorders.[5]

Although our initial studies did not identify large differences in HPA axis function between healthy cats and cats with FIC, the results suggested the possibility of a more subtle HPA abnormality. To investigate HPA axis function in cats with FIC further we performed a series of experiments to compare adrenal gland function, size and morphometrics in affected and healthy cats.

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METHODS

Animals. All cats with FIC were obtained as donations from clients due to a history of chronic recurrent stranguria, hematuria, pollakiuria and/or urination at inappropriate locations. They were evaluated at the veterinary teaching hospital at our institution. Evaluation consisted of physical examination (including body weight), complete blood count, serum biochemical analysis, urinalysis, urine bacteriological culture and cystoscopy. Cystoscopy was performed using a 9Fr rigid pediatric cystoscope (Karl Storz, Endoscopy America, Inc., Culver City, California) in female cats and a 3Fr flexible fiber optic cystoscope (Five Star Medical, Inc., San Jose, California) in male cats. The diagnosis of FIC was based on compatible history, and consideration of standard inclusion and exclusion criteria after obtaining the results of laboratory tests, including submucosal petechial hemorrhages (glomerulations) at cystoscopy.[3] Healthy, age matched cats determined to be free of disease and signs referable to the lower urinary tract according to the same diagnostic criteria as cats with FIC served as controls. All cats were housed in stainless steel cages in the college animal facilities and fed a standard commercial diet.

ACTH stimulation test. The serum cortisol response to ACTH was tested in 11 healthy cats and 20 with FIC. Cats were evaluated at various times during the day between 10:00 a.m. and 4 p.m. (cats do not have diurnal cortisol rhythms[6]). ACTH stimulation tests were performed within 2 to 4 days of cat arrival at the university, which is considered a moderately stressful time. A pretest blood sample was collected from an external jugular vein and placed immedi-

ately in a noncoagulant containing glass tube. Serum cortisol concentrations were analyzed using a chemiluminescent method at the hospital laboratory. All cats were then given 125 μg Cortrosyn (Organon, Inc., West Orange, New Jersey) synthetic ACTH by intramuscular injection. Blood was collected 30 and 60 minutes after ACTH administration and analyzed for cortisol concentration in the same manner as the pretest sample.

Adrenal gland collection. Adrenal glands were evaluated in 5 cats with FIC and 2 healthy cats in which ACTH stimulation tests were performed. An additional 8 cats with FIC and 6 healthy cats for which ACTH stimulation tests were not available were also evaluated. Not every cat that underwent an ACTH stimulation test could be evaluated at autopsy. Only cats that were euthanized for other studies were analyzed. All cats were euthanized by 1 of 2 methods. Ten cats were deeply anesthetized intravenously with 25 mg/kg sodium pentobarbital (Abbott Laboratories, North Chicago, Illinois) and perfused transcardially through the ascending aorta with saline, followed by fixative containing 4% paraformaldehyde in phosphate buffer. The adrenal glands were removed, placed in the fixative for 8 hours and then transferred to 0.1 M phosphate buffer containing 30% sucrose (pH 7.3) for 48 hours. Another 11 cats were sacrificed using 120 mg/kg Beuthanasia-D Special Solution (Shering-Plough Animal Health Corp., Kenilworth, New Jersey). Immediately after sacrifice the adrenal glands were carefully removed and placed in neutral buffered formalin (Accra Laboratories, Swedesboro, New Jersey) (1:10). After the tissues were fixed all glands were meticulously cleaned to remove all fat and surrounding adherent tissue, blotted dry on absorbent paper and weighed to the nearest mg. Cats were weighed to the nearest gm to permit calculation of adrenal weight as a percent of body weight. Because some adrenal glands were used for other studies, only 4 healthy cats and 7 cats with FIC had adrenal glands sectioned transversely, paraffin embedded, cut and stained with hematoxylin and eosin for histological evaluation. All sections were examined by a board certified veterinary pathologist for any abnormalities or peculiarities. They were then digitized and evaluated using Photoshop Elements (Adobe Systems, Inc., San Jose, California) to measure the area of the 3 zones of the cortex and medulla as a percent of total adrenal gland section area.

Comparisons between groups for adrenal gland measurements were made using the Student's t test. Two-way repeated measures ANOVA and the Tukey Kramer post hoc test for adjusted pairwise comparisons were used to compare cortisol between the groups after ACTH stimulation. The relationship between baseline, 30-minute and 60-minute serum cortisol, and adrenal gland size expressed as a percent of body weight was tested by examining the correlations between cortisol concentration and gland size using Prism (GraphPad Software, Inc., San Diego, California).

RESULTS

All cats used in these studies were domestic shorthaired cats and no differences in breed were found. Significant differences were found in gender distribution, age of the cats in the ACTH stimulation studies and weight in the cats that underwent full autopsy (table 1). Cortisol responses of healthy cats to ACTH stimulation were within hospital and published normal ranges. Cats with FIC had significantly decreased mean serum cortisol responses to ACTH (group effect 2-way repeated measures ANOVA $p < 0.005$ and time effect $p < 0.0001$, fig. 1). Weight (158 ± 50 vs 241 ± 60 mg) and volume (264 ± 72 vs 410 ± 115 mm^3) of the adrenal glands were significantly smaller in cats with FIC than in healthy cats (all comparisons 2-tailed t test $p < 0.01$). Cats with FIC had significantly smaller glands when adrenal weight was normalized to body weight (3.2% ± 1.6% vs 6.5% ± 1.7%, $p < 0.0001$, fig. 2). No correlation was found between serum cortisol and adrenal gland size at baseline, or 30 or 60 minutes ($r^2 = 0.22$, 0.08 and 0.11, respectively). Although no obvious histological abnormalities were identified, the relative areas of the zonae fasciculata and reticularis were significantly smaller in sections of glands from cats with FIC than in those from healthy cats ($p = 0.0004$, table 2). No differences between groups were found in the relative area of the medulla.

TABLE 1. *Age, weight and gender distribution in healthy cats and cats with FIC*

	Healthy	FIC	p Value
Autopsy:			
No. gender	Male intact 4, female intact 3, female spayed 1	Male neutered 7, female intact 1, female spayed 5	
Mean age ± SD	4.5 ± 1.7	6.6 ± 3.1	0.13
Mean wt ± SD (mg)	3.8 ± 1.1	5.2 ± 1.6	0.05
ACTH stimulation:			
No. gender	Male intact 1, male neutered 5, female intact 2, female spayed 3	Male neutered 9, female intact 1, female spayed 10	
Mean age ± SD	2.8 ± 2.0	6.9 ± 4.3	0.005
Mean wt ± SD (mg)	5.1 ± 1.4	5.3 ± 1.4	0.82

Three healthy cats and 5 with FIC underwent ACTH stimulation tests and autopsy but it was not possible to obtain autopsy results in all cats in which stimulation tests were performed.

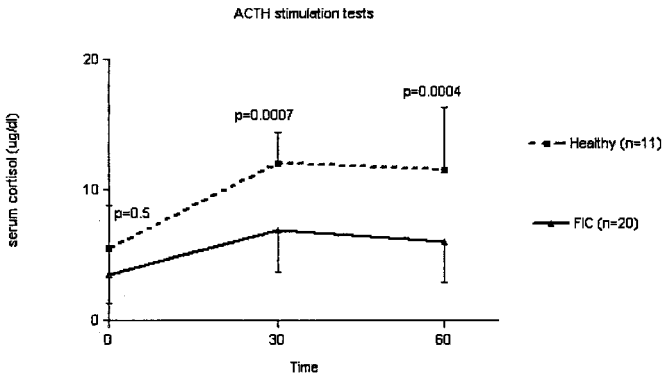

FIG. 1. Mean serum cortisol response ± SD to 125 μg synthetic ACTH administered intramuscularly was significantly less in cats with FIC than in healthy cats (2-way repeated measures ANOVA $p < 0.05$). Tukey-Kramer post hoc tests were used to compare groups at each time point.

DISCUSSION

The most significant finding in this study was the dramatic decrease in the size of the adrenal glands of cats with FIC.

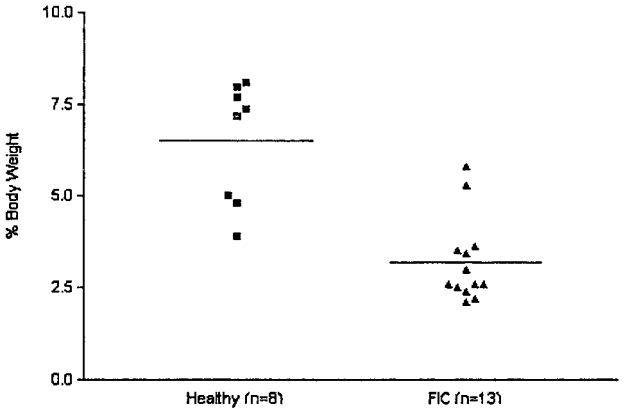

FIG. 2. Adrenal gland weight as percent of cat body weight. Weight of adrenal glands in cats with FIC was significantly less than that in healthy cats ($p < 0.0001$). Horizontal lines indicate mean.

TABLE 2. *Adrenal gland morphology in healthy cats and cats with FIC*

	Healthy	FIC	p Value
No. cats	4	7	
% Mean medulla ± SD	12 ± 5	18 ± 6	0.1
% Mean cortex ± SD			
Glomerulosa	11 ± 3	13 ± 1	0.07
Fasciculata + reticularis	79 ± 3	70 ± 5	0.004

Cortex and medulla area was calculated as a percent of total adrenal section area and cats with FIC had significantly smaller fasciculata and reticularis zones, whereas there was a trend for the zona glomerulosa to be larger and no significant differences were noted in relative medulla area.

However, individual cat ACTH stimulation tests results were variable and did not correlate well with cat adrenal gland size. We have previously reported that we could not identify differences in ACTH and cortisol between 4 healthy cats and 4 with FIC after injection of corticotrophin releasing factor under minimally stressful circumstances.[3] A larger number of cats evaluated under more stressful conditions in this study could have allowed the differences to become evident. Although group differences in cortisol responses to ACTH were statistically significantly less in cats with FIC than in healthy cats, individual differences would not have been considered clinically abnormal in all. However, we have observed that plasma ACTH increases in cats with FIC when stressed, suggesting that the hypothalamic and pituitary components of the HPA axis responded appropriately and the abnormalities that we observed were most likely localized to the adrenal gland.[7] These findings suggest that adrenal gland function may be relatively normal under unstressed conditions but they might not respond adequately during stressful circumstances.

Baseline ACTH and cortisol in healthy cats and humans appear to vary widely among studies. ACTH and cortisol responses to provocative stimulation are also quite variable depending on the type of test, the dose of the agent used and even the type of ACTH used.[8] The variability in results renders the predictive value of the available tests inadequate for diagnosing marginal adrenal insufficiency. In these circumstances direct visualization of the adrenal gland resulted in the identification of significant differences.

The relationship of ACTH release and adrenal cortical responsiveness has been debated in other diseases in which small adrenal gland size with or without hypocortisolism sometimes occurs. In humans a comparable decrease in adrenal gland size has been reported in patients with chronic fatigue syndrome.[9] In that series it was suggested that small size might have been the result of primary or secondary adrenal insufficiency. The conclusion was that the defect was more likely to be secondary and due to decreased ACTH release.

Histological evaluation of the cat adrenal glands revealed that the decrease in size was limited to the adrenal cortex. No histopathological abnormalities were identified in the adrenal glands, although variable degrees of nodular hyperplasia were observed in some glands from cats in each group. Preliminary morphometric evaluation revealed significant reductions in the size of the zonae fasiculata and reticularis ($p < 0.0004$). No significant differences were noted in the zona glomerulosa, and preliminary data at our laboratory suggested a normal aldosterone response to corticotropin releasing factor administration.

The etiology of the small adrenal glands was not identified. Potential explanations include genetic or developmentally related hypoplasia,[10] decreased stimulation by or sensitivity to ACTH and immune mediated (eg antibodies to the 21α hydroxylase enzyme[11]), infectious or inflammation induced degeneration. Preexisting hypoplasia could not be ruled out in the current study but it might be investigated in relatives of affected individuals. The difference in adrenal gland size did not appear to be due to ongoing immune mediated, infectious or inflammation induced degeneration based on histopathological examination of the glands, although the possibility of a previous occurrence causing the damage could not be excluded. Because IC has previously been suggested to have features of autoimmune disease, we investigated the presence of circulating anti-adrenal antibodies in 6 humans (because this test is currently not available for cats) and found that all results were negative (data not shown). Thus, whether the small adrenal glands are a cause, consequence or unrelated to FIC remains to be determined.

Although bladder abnormalities in cats and humans with IC are similar,[12] symptoms of IC extend beyond the bladder, affecting many body systems in the 2 species. In 4 studies of patients with IC investigators found significantly higher frequencies of allergic/immune, cardiopulmonary, dermatological, endocrine, gastrointestinal, genitourinary, musculoskeletal and neurological abnormalities in those with IC than in the reference population used.[13] Increased rates of irritable bowel syndrome, fibromyalgia and chronic fatigue syndrome have also been reported.[14]

As in humans, anxiety is commonly associated with lower urinary tract problems in cats.[15] We found that 60% of cats with no clinical signs other than urinating outside of the litter box had lesions consistent with FIC at cystoscopy in 1 study.[16] Also, cats restricted to indoor living are about 5 times more likely to have urinary problems as cats allowed outdoors.[17] Thus, a similar combination of physiological and behavioral abnormalities may affect cats and humans with IC.

The current study has important limitations. Although the dose of ACTH was standard, it was supraphysiological and may not have been appropriate to identify the relatively moderate extent of adrenal insufficiency present in these cats. Similar observations have been made in humans.[18] Other limitations include variability in age and neuter status of healthy cats compared with cats with FIC, the fact that we only examined cats with severe disease and the absence of comparison groups of cats with other disorders to determine the sensitivity and specificity of the abnormality. With regard to the differences in age and neuter status in other studies adrenal glands were reported to increase in size with age and neutering.[19] Females were also reported to have slightly larger adrenal glands in that study. Therefore, these differences are less likely to explain the results that we observed. Based on the distribution of adrenal gland size data (fig. 2) it is possible that 2 populations may have been represented within each group, although no significant differences in age or weight were found to account for them. Studies to address specifically all of these limitations are in progress.

Decreased HPA axis function may have a role in the pathophysiology of some signs, including alterations in sensory[20] and sympathetic function,[21] and possibly some autoimmune aspects of IC. In conclusion, based on the lack of predictive value of currently available tests of HPA axis function we suggest that studies of the HPA axis in patients with IC should include evaluation of adrenal size and function.

Dr. Edward Feldman provided Cortrosyn and Dr. Charles Capen performed the adrenal gland histopathological evaluation.

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