Evaluation of the effects of stress in cats with idiopathic cystitis

Jodi L. Westropp, DVM, PhD; Philip H. Kass, DVM, PhD; C. A. T. Buffington, DVM, PhD

Objective—To determine the effects of stress in cats with feline idiopathic cystitis (FIC) by evaluating bladder permeability, sympathetic nervous system function, and urine cortisol:creatinine (C:Cr) ratios during periods of stress and after environmental enrichment.

Design—Prospective study.

Animals—13 cats with FIC and 12 healthy cats.

Procedure—Cats subjected to an acute-onset moderate stressor for 8 days received IV injections of fluorescein. Serum fluorescein concentrations were determined and compared with those of controls to evaluate bladder permeability, and urine C:Cr ratios were compared to evaluate function of the hypothalamic-pituitary-adrenal (HPA) axis. Plasma catecholamine concentrations were analyzed in a subset of cats. After 8 days of moderate stress, cats were moved to an enriched environment, and tests were repeated after 21 days.

Results—Serum fluorescein concentrations were significantly higher in cats with FIC at all time points. In the cats in which plasma catecholamine concentrations were determined, concentrations of dihydroxyphenylalanine, norepinephrine, and dihydroxyphenylglycol were significantly higher in cats with FIC at all time points, whereas no differences in urine C:Cr ratio between groups were observed.

Conclusion and Clinical Relevance—Cats with FIC appeared to have altered bladder permeability, most notably during the period of initial stress. The increase in plasma dihydroxyphenylalanine concentration suggests that there may be stress-induced increase in the activity of tyrosine hydroxylase, which catalyzes the rate-limiting step in catecholamine synthesis. In contrast, no effects of stress on C:Cr ratios were observed, which suggests there was dissociation between the sympathetic nervous system and HPA-axis responses to stress. (Am J Vet Res 2006;67:731–736)

Disease of the lower portion of the urinary tract in cats is characterized by various combinations of stranguria, hematuria, periuria (inappropriate urination), and pollakiuria. Differential diagnoses for cats with these clinical signs include urolithiasis, urinary tract infection, and primary behavioral abnormalities. When results of diagnostic testing reveal no underlying cause for the signs, FIC is diagnosed. The etiology of FIC is unknown, and there are no known effective long-term treatments. Interstitial cystitis in humans is a spontaneously occurring disease that is analogous to FIC in cats.

Increased urothelial permeability is a feature of FIC and interstitial cystitis. The mucosal surface of the bladder should act effectively as a barrier to protect the urothelium from bladder contents. Defects in the mucosal lining could enhance reuptake of urine contents from the bladder and contribute to clinical signs of FIC and interstitial cystitis. In a study in humans, bladder wall permeability to urea instilled into the bladder lumen was observed, and results of an earlier study from the authors’ laboratory revealed increased permeability to sodium salicylate instilled into the bladder of cats with FIC. Orally administered fluorescein has been used as a marker for evaluating bladder permeability in humans with interstitial cystitis and has been reported to be a useful marker of altered permeability in a mouse model of cystitis when administered intravascularly. Fluorescein is a stable, low–molecular-weight molecule that diffuses into tissues readily. In clinically normal subjects, plasma fluorescein concentrations decrease after IV administration as fluorescein is excreted into urine. Fluorescein appears to be transported across membranes via a paracellular pathway, in which intercellular tight junctions in the urothelium are the rate-limiting barriers. Plasma concentrations of fluorescein after oral administration remain high for longer periods of time in women with interstitial cystitis, compared with healthy subjects; impairment of tight junctions has subsequently been reported in cats and humans with interstitial cystitis and may be the mechanism by which fluorescein returns to the general circulation down its concentration gradient after being excreted in urine.

Clinical signs associated with FIC and interstitial cystitis may be exacerbated by stressful circumstances. Chronic stress from internal or external sources may increase activity of TH, which catalyzes the rate-limiting step in catecholamine synthesis in the locus.
Materials and Methods

Thirteen cats (3 neutered males, 1 sexually intact female, and 9 spayed females) that were evaluated at The Ohio State University Veterinary Teaching Hospital were obtained as donations from clients because of the cats’ history of stranguria, hematuria, pollakiuria, inappropriate urination, or a combination of these signs. Initial evaluation consisted of complete physical examination, CBC, serum biochemical analyses, urinalysis, urine bacteriologic culture, and cystoscopy. Cystoscopy was performed by use of a 9-F rigid pediatric cystoscope in female cats; a 3-F flexible fiberoptic cystoscope was used in male cats. FIC was diagnosed on the basis of a compatible history and findings that were in accordance with described criteria, including observation of glomerulations (ie, pinpoint petechial mucosal hemorrhages) during cystoscopic imaging, after obtaining results of laboratory tests. Twelve clinically normal cats (3 sexually intact males, 1 neutered male, 7 sexually intact females, and 1 spayed female) of similar age were used as controls. Cats were housed in stainless steel cages in the animal colony and allowed to acclimate to the environment for at least 3 months. All experimental procedures were approved by The Animal Care and Use Committee of The Ohio State University.

A moderate stress protocol was designed, and each cat underwent the stress regimen on days 1, 3, and 8. Stressors included 12 hours of food deprivation, transport to the laboratory, and performance of test procedures, followed by changes in diet and housing. Diets were changed from the commercial dry food with which the cats were familiar to a new dry food, and cats were housed in a new environment (ie, metabolism cages in a different room of the vivarium). In random order, cats were brought into the laboratory in groups of 4 and placed in small holding cages while the initial tests were performed. Cats were weighed on the day of testing. The following variables were also measured: resting heart and respiratory rates and systolic blood pressure (measured by means of an ultrasonic Doppler blood flow detector with a number 3 cuff on the left hind limb). Cats were restrained in right lateral recumbency for the blood pressure measurements. A venous blood sample was obtained from the jugular vein. Serum and plasma were quickly frozen at −70°C for future analysis of catecholamines and metabolites. All plasma samples collected prior to administration of fluorescein were assayed for dihydroxyphenylalanine, dopamine, dihydroxyphenylacetic acid, NE, epinephrine, and dihydroxyphenylglycol by use of reverse high-performance liquid chromatography with electrochemical detection after partial purification by absorption on alumina according to a previously described protocol.

Fluorescein (230 µg/kg) was injected IV into the right cephalic vein and the time of administration recorded. Cats were returned to holding cages for 1 hour, after which blood was collected from the jugular vein for assessment of plasma fluorescein concentration. Fluorescein concentrations were determined by use of a fluorescent spectrophotometer operating at 494 nm. Samples were analyzed according to a described methodology.

After the venipuncture procedure, 20 µg of medetomidine/kg was administered IM into the epaxial muscles and the time was recorded. Ten minutes later, cardiovascular variables were assessed and recorded. Atipamezole (100 µg/kg) was administered IM to all cats after the degree of sedation, heart rate, and blood pressure were determined, to reverse any remaining effects of the medetomidine. Venipuncture and cardiovascular analyses were performed on days 1, 3, and 8.

After each group of 4 cats had undergone those procedures, cats were transferred to new metabolism cages and fed a new commercial diet as a continuation of moderately stressful circumstances. Each cat received 100 g of food/d, and intake was recorded daily. Urine was collected daily from the metabolism cages into bottles on dry ice. After thawing at 4°C, 2 aliquots were quickly refrozen at −70°C and another sample was allowed to warm to room temperature for complete urinalysis, including urine dipstick determinations. Urine pH was measured to the nearest 0.1 unit by use of a meter. Frozen samples were thawed to room temperature, and urine cortisol and creatinine concentrations were assayed by means of a standard chemiluminescent protocol in the teaching hospital laboratory.

At the end of the moderate stress period (evening of day 8), cats were moved to an enriched environment. Cages were larger, and each cage contained a covered bed, 2 types of toys, and a larger litter pan. Cats were fed the same commercial dry food as before but were also offered canned food. The food was weighed daily, and intake of each type was recorded. Cats also

Figure 1—Relationship between hematuria and number of days of exposure to a moderate stress protocol in 13 cats with FIC (crosshatched bars) and 11 healthy cats (black bars).
had interaction with humans (in addition to the animal caretakers) for ≥15 min/d. The nature of the interaction with each cat was appropriate for each cat’s disposition. Music was also played in the room. After 3 weeks, food was withheld on the night before testing and all previous procedures were repeated.

Statistical analysis—The effects of experimental group (ie, cats with FIC vs healthy controls), time (1, 3, 8, and 35 days), treatment (pre- vs post-treatment), and interactions among those variables were simultaneously analyzed by use of 3-way repeated-measures ANOVA. When dependent measures were ordinal scores, the Mann-Whitney test was used to compare experimental groups conditional on time. Values of $P < 0.05$ were considered significant.

Results

No differences in food intake were observed between cats in the 2 groups at any time. Abnormalities pertaining to the lower portion of the urinary tract were detected in 1 healthy cat (a sexually intact male) subsequent to commencement of the study; data from that cat were not included in statistical analyses, and the cat was released from the study. Some blood samples were not obtained from various cats during the study for several reasons (eg, impaired jugular veins or uncooperative cat).

Urinalyses and urine C:Cr ratios—During the 8 days the cats spent in metabolism cages, microscopic hematuria was detected in 30% of samples from cats with FIC, compared with 7% of samples from healthy cats ($P = 0.003$; Figure 1). No differences between the groups in urine C:Cr ratios were observed (Figure 2).

Plasma fluorescein concentrations—Plasma fluorescein concentrations were evaluated on all test days in all 13 cats with FIC and in the 11 healthy cats except on day 35, when only 10 healthy cats were analyzed. Cats with FIC had significantly higher mean plasma fluorescein concentrations on all days; differences were significant between groups ($P = 0.001$) and across days ($P < 0.001$; Figure 3). Mean ± SD plasma fluorescein concentrations...
centrations were highest in cats with FIC and healthy cats on day 1 (297 ± 103 ng/mL vs 142 ± 99 ng/mL, respectively) and decreased over the study period to their lowest values on day 35 (191 ± 90 ng/mL vs 83 ± 43 ng/mL in cats with FIC and healthy cats, respectively). In addition, cats with FIC had significantly (P = 0.01) higher plasma fluorescein concentrations on day 1, compared with concentrations on subsequent days.

Catecholamine concentrations—Because of sample processing error, plasma catecholamines and metabolites were analyzed in only 6 cats with FIC and 5 healthy cats. In those cats, plasma concentrations of dihydroxyphenylalanine (P = 0.04), NE (P = 0.03), and dihydroxyphenylglycol (P = 0.04) were significantly higher at all time points in cats with FIC than in healthy cats; Figures 4–6). Plasma concentrations of dopamine and dihydroxyphenylacetic acid were often higher in cats with FIC, compared with healthy cats, although the differences were not significant (P = 0.09 and P = 0.08, respectively; Figures 7 and 8). No significant difference between the groups in plasma epinephrine concentration was detected (P = 0.15; Figure 9). Data pertaining to cardiovascular variables, pupillary diameter measurements, and medetomidine analyses have been published elsewhere.16

Discussion
Plasma fluorescein concentrations were significantly higher at all time points in cats with FIC, compared with healthy cats, and were highest after the acute period of stress. Variables improved after the cats’ stay in an enriched environment. In cats in which plasma catecholamine concentrations were determined, concentrations of dihydroxyphenylalanine, NE, and dihydroxyphenylglycol were significantly higher in cats with FIC at all times and decreased after initiation of environmental enrichment. In contrast, there were no differences between groups in the urine C:Cr ratio at any time, suggesting uncoupling of the SNS and HPA system.

Increased bladder permeability has been reported in cats with FIC as well as in other animal models of cystitis17 by assessing plasma concentrations of a drug after intravesical administration. Fluorescein is a fluorescent dye that has been used to assess membrane permeability. In an earlier study,15 investigators observed that oral administration of fluorescein resulted in significantly higher plasma fluorescein concentrations in women with interstitial cystitis than in control subjects. Urinary fluorescein excretion was significantly lower in patients, compared with control subjects, suggesting that high plasma fluorescein concentrations may be a useful marker of altered bladder permeability. In those patients, creatinine clearance was normal, suggesting that decreased renal blood flow was not the reason underlying the decreased fluorescein excretion.

Because of individual differences in gastrointestinal transit time, variability in drug absorption rates for orally administered medications, and the need for anesthesia in administering drugs intravesically, we administered fluorescein IV to assess bladder permeability in our cats. No adverse reactions were encountered in any cat during the course of the study, and to our knowledge, only 1 report18 of an adverse reaction to fluorescein in cats has been reported. Increased bladder permeability may allow fluorescein to be reabsorbed, delaying its excretion. As a result of technical problems with the urine fluorescein assay, we were not able to assess urine fluorescein concentrations; however, all plasma concentrations were higher in cats with FIC, a finding similar to that reported in women with interstitial cystitis.15

The etiology of altered bladder permeability is not fully understood. However, activation of the SNS may increase epithelial permeability, permitting substances...
encountered in the environment greater contact with sensory afferent neurons; increased activation of sensory afferent neurons could subsequently lead to development of local inflammation.28,29 Altered bladder permeability has been reported in cats with FIC1 and may be mediated via the SNS. Sympathoneural-epithelial interactions appear to play an important role in permeability. For example, 1 group of investigators20 revealed that application of NE to strips of urinary bladder tissue induced release of nitric oxide from the urinary bladder epithelium. Application of capsaicin also induced nitric oxide release from the bladder epithelium as well as from nervous tissue in the urinary bladder. In light of other reports21,22 in which it was proposed that nitric oxide may increase urethral permeability, those results suggest that some of the alterations in permeability associated with SNS activation may be mediated by NE via this mechanism. In addition, psychologic stress may lead to increased cytokine production and development of inflammation. In the present study, both groups of cats had higher plasma fluorescein and catecholamine concentrations during the stress portion of the study, suggesting that stress plays a role in membrane permeability. We cannot rule out the possibility that the high fluorescein concentrations detected were not a result of decreased renal blood flow because renal blood flow was not evaluated. However, the ability to concentrate urine and findings of serum renal variables in reference range were part of the inclusion criteria for cats in our study.

In the subset of cats for which plasma catecholamines were measured, plasma concentrations of dihydroxyphenylalanine and NE were significantly higher, compared with concentrations in controls. High serum concentrations of other catecholamines and metabolites were detected in cats with FIC, as well. The marked increase in dihydroxyphenylalanine concentrations suggested that there was a stress-induced increase in serum TH activity because dihydroxyphenylalanine is the first reaction product in the catecholamine synthetic pathway. Increased TH immunoreactivity in the locus coeruleus of cats with FIC23 and high TH immunoreactivity in the bladders of humans with interstitial cystitis24 have been reported.

Although plasma catecholamine concentrations were high in cats with FIC, no differences between affected and healthy cats were detected in the urine C:Cr ratios. We had hypothesized that the urine C:Cr ratio would be high in cats with FIC because the test is a sensitive indicator of adrenal response to stress and results reflect HPA-axis activity. However, the ratios were similar in healthy and affected cats. In an earlier study,7 we found that cortisol release in response to ACTH stimulation was reduced during stressful periods in cats with FIC. In that study, adrenal gland size was significantly smaller in cats with FIC than in healthy cats. Microscopic examination of the adrenal glands did not reveal fibrosis, hemorrhage, inflammation, infection, or necrosis as causes of the reduced size; the primary histologic abnormality observed was that the zona fasciculata and zona reticularis (the zones responsible for production of cortisol and other steroid hormones) were markedly smaller than in healthy cats. These results, combined with observations of increased plasma concentrations of corticotrophin-releasing factor24,25 and ACTH26 in response to stress in the absence of a comparable increase in plasma cortisol concentrations, suggest that there is decreased adrenocortical reserve in cats with FIC.

Glucocorticoid administration reportedly decreases plasma catecholamine concentrations, inhibits synthesis of catecholamines, and may attenuate the increased plasma catecholamine concentrations induced by certain stressors.27 These findings suggest that glucocorticoids may inhibit sympathoneural outflow activity; results suggest that lack of cortisol resulted in or perpetuated the observed heightened SNS activity. An increase in SNS outflow activity could also explain why clinical signs of FIC follow a waxing and waning course in cats and humans and are aggravated by environmental stressors.28,29 Amitriptyline and other tricyclic antidepressant drugs with sympatholytic activity have been used to ameliorate the severity of the chronic form of the disease in both species.25,26

The present study had several limitations concerning plasma fluorescein concentrations and catecholamine analyses. In regard to the bladder permeability testing, although plasma concentrations of fluorescein were higher in cats with FIC at all times, we did not assess urine fluorescein concentrations simultaneously; a procedure that would have yielded information about fluorescein excretion in cats. Plasma catecholamine concentrations were determined on blood samples obtained via external jugular venipuncture, which could have been an acute stressor for the cat and resulted in artificially increased plasma concentrations at that moment. However, mean and SD values derived from the cats in this study were comparable to values from other studies in which plasma NE concentrations were evaluated after jugular catheters were placed and cats had been allowed to acclimate to the noninvasive collection process.12 Because of study design, placement of jugular catheters was not possible in our cats, but this hindered our ability to collect blood samples from every cat at every designated sampling time over the course of the study.

It was not possible to match groups according to sex. Although the numbers of male and female cats were similar, the control population contained more sexually intact cats. Cats with FIC were obtained as donations, and most had been neutered or spayed, whereas many of the control cats were purchased and had not been sexually altered at the time of enrollment into the study. It is known that sex may influence plasma catecholamine concentration. In a study27 evaluating the sympathoadrenal response to estradiol administration in postmenopausal women, there was no difference in plasma epinephrine concentrations after transdermal estradiol supplementation; however, plasma NE concentrations were significantly lower during estradiol treatment. In the group of cats in which plasma catecholamines were analyzed, there were more spayed female cats in the FIC group and it is possible that the effects of estrogen artificially decreased NE concentrations in the control group. However, results of previous work in our laboratory support that high concentrations of NE28 and high TH
immunoreactivity in the locus coeruleus may be observed in cats with FIC. Similar findings have been reported in women with interstitial cystitis. Urinary cortisol excretion in men is similar to that in women.

The experimental protocol in the present study was designed to determine what, if any, effects a period of moderate stress would have in cats with FIC. Clinical signs of exacerbated disease (e.g., hematuria) and significantly higher plasma fluorescein concentrations were observed in many cats during the stress period. Increased plasma concentrations of dihydroxyphenylalanine and NE in cats with FIC were also observed, despite any differences in the urine C:Cr ratios between the 2 groups. Housing cats for 3 weeks in an enriched environment appeared to decrease plasma fluorescein and catecholamine concentrations, suggesting that further studies should be implemented in cats with FIC to evaluate this as a treatment option.

References
16. Westropp JL. Evaluation of the sympathetic nervous system and hypothalamic pituitary adrenal axis in cats with interstitial cystitis. Columbus, Ohio: Department of Veterinary Clinical Sciences, The Ohio State University, 2005:166.