EVALUATION OF THE EFFECTS OF STRESS ON THE SYMPATHETIC
NERVOUS SYSTEM AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN
CATS WITH FELINE INTERSTITIAL CYSTITIS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
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ABSTRACT

Interstitial cystitis (IC) is a chronic, debilitating pelvic pain syndrome, characterized by recurrent urinary frequency, urgency, and pain referable to the lower urinary tract. These symptoms often appear acutely, and follow a waxing and waning course. The etiology of this disease is unknown, and no acceptable long-term therapy exists. A spontaneously occurring disease analogous to IC occurs in domestic cats referred to as feline interstitial cystitis (FIC). This model of IC may be the most suitable model to investigate the disease because it is a naturally occurring disease, and clinical signs are exacerbated by stress, which occurs in the human counterpart.

To further investigate the stress response systems in the cat with FIC, we evaluated catecholamines and their metabolites as well as the hypothalamic-pituitary-adrenal axis in cats with FIC by evaluating urine cortisol/creatinine ratios and response of the adrenal gland to exogenous ACTH. When healthy cats were stressed, cortisol and catecholamines increased, but returned to baseline within hours to days. In our study, cats with FIC did not appear to have this capability. Their catecholamines remained elevated, while their adrenal cortex response was blunted. An apparent “dissociation” of the two major stress response systems was found. The overarching premise of our studies is that FIC is not just a bladder disorder but involves complex interactions of the
nervous, endocrine, and cardiovascular systems. How these systems communicate and manifest as FIC in some cats, but not in others remains to be determined. However, understanding that these interactions occur, is important for clinicians to help better evaluate and treat both humans and cats with IC.
Dedicated to my family for all their help and support throughout my training.
ACKNOWLEDGMENTS

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<td>alpha-2 adrenoceptors</td>
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<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
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<td>C:Cr</td>
<td>Cortisol:Creatinine ratio</td>
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<td>CCE</td>
<td>Catecholamines</td>
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<td>CFA</td>
<td>Complete Freund’s adjuvant</td>
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<td>CRF</td>
<td>corticotropin releasing factor</td>
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<td>CS</td>
<td>capsaiacin</td>
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<td>E</td>
<td>Epinephrine</td>
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<td>EAC</td>
<td>experimental autoimmune cystitis</td>
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<td>EM</td>
<td>Electron microscopic</td>
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<td>FIC</td>
<td>Feline Interstitial Cystitis</td>
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<td>Fluorescein</td>
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<td>GAG</td>
<td>Glycosaminoglycan</td>
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<td>HPA</td>
<td>Hypothalamic-Pituitary-Adrenal Axis</td>
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<td>IC</td>
<td>Interstitial Cystitis</td>
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<td>IR</td>
<td>Immunoreactivity</td>
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<td>LC</td>
<td>Locus Coeruleus</td>
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<td>LPS</td>
<td>compounds lipopolysaccharide</td>
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<td>NE</td>
<td>Norepinephrine</td>
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<td>noxious environmental stimuli</td>
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<td>NIDDK</td>
<td>National Institutes of Arthritis, Diabetes, Digestive and Kidney Diseases</td>
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<td>NK-1</td>
<td>neurokinin-1</td>
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<td>ovalbumin</td>
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<td>Poly IC</td>
<td>polyninosinic-polycytidylic acid</td>
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<td>Substance P</td>
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<td>TH</td>
<td>Tyrosine Hydroxylase</td>
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ABSTRACT

We systematically identified and evaluated various animal models that have been studied to help identify the underlying mechanisms of and possible treatment options for interstitial cystitis.

Models of interstitial cystitis published between 1983 and 2001 were obtained by searching MEDLINE and other Internet databases using cystitis and model as the primary key words. Models with characteristics of interstitial cystitis similar to those defined by National Institutes of Arthritis, Diabetes, Digestive and Kidney Diseases criteria were reviewed. Some articles describing animal models with similar pathological conditions in other organs were also included to enlarge the base of potentially relevant material.

We identified and evaluated some 16 animal models of interstitial cystitis, which we categorized as bladder inflammation induced by intravesical administration of an irritant or immune stimulant, systemic and environmentally induced inflammation, and a naturally occurring model of interstitial cystitis that occurs in cats. Some abnormalities identified in humans and cats with interstitial cystitis can be reproduced in healthy animals using luminal, systemic or environmental stimuli. At the level of the bladder the source of stimulation cannot be discriminated. Variability in the extent of bladder
distention complicated the interpretation of some studies. In addition, the noxious stimuli used can affect many epithelial surfaces as well as the urothelium, suggesting they are nonspecific responses to injury rather than specific to interstitial cystitis.

No model in bladder injury in healthy animals currently reproduces as many features of interstitial cystitis as the naturally occurring disease in cats. While induced models of relative injury may help to provide insight into the bladder response to injury, feline interstitial cystitis follows a similar chronic waxing and waning time course as does interstitial cystitis in humans, which may be more suitable for studying the effects of stressors on the severity of clinical signs as well as newly proposed therapies.

INTRODUCTION

Interstitial cystitis (IC) is a pelvic pain syndrome of unknown etiology. The clinical features of IC include chronic, recurrent urinary frequency, urgency, and pain referable to the lower urinary tract. These symptoms often appear acutely, and generally follow a waxing and waning course.\(^1,2\) Epidemiological studies reveal that more than half of IC patients report daily or constant pain, which is exacerbated by stressful circumstances.\(^3\) Inclusion and exclusion criteria for diagnosis of IC have been established by the National Institutes of Arthritis, Diabetes, Digestive and Kidney Diseases (NIDDK, Table 1-1)\(^4\) to assure that subjects in research studies of IC are reasonably comparable. These criteria are more stringent than those often used by practicing urologists.\(^5\) Clinical diagnosis usually includes the presence of compatible clinical features, and the absence of objective evidence of other diseases that could cause the symptoms. At least two variants of cystoscopic findings have been described in patients with IC.\(^6\)
In the more common non-ulcer form, only glomerulations (punctate submucosal petechial hemorrhages) are seen, whereas in the less common ulcer form fissures and scars that crack and bleed when the bladder is distended are present.

When IC is suspected in a patient, histopathology may be used to rule out other, better-defined disorders, but findings in the bladder of patients with IC are inconsistent. In non-ulcer IC, scattered glomerulations, small mucosal tears, and submucosal hemorrhages with mild or no inflammatory infiltrate can be seen, although abnormalities usually are limited to vasodilatation and submucosal edema. In the classic form, the “suburothelium shows chronic inflammation, fibrosis, dilatation of vessels with hemorrhage, neural proliferations and perineuritis.” These abnormalities occurred in only 3.9% of the 209 biopsy samples from the interstitial cystitis database study, and are not unique to IC. This study also reported that the histopathologic lesion severity did not correspond well with pain, urgency, or nighttime urinary frequency. Moreover, no reliable correlation has been found between the severity of cystoscopic findings and the clinical symptoms of IC, and the presence of glomerulations is not restricted to patients with IC. The understanding of IC is further complicated by the observation that symptoms may remain even after removal of the bladder, and that bladder lesions can occur in patients reporting significant improvement in clinical signs.
Affected patients also may have a variety of problems not related to the lower urinary tract. For example, epidemiological studies of patients with IC have reported that symptoms of dizziness, joint pain, abdominal discomfort, and chest pain are more prevalent in IC patients than in age matched controls. IC sufferers also report other chronic pain syndromes such as migraines, fibromyalgia, and irritable bowel syndrome. These observations suggest that the abnormalities responsible for IC extend beyond the urinary tract.

These complexities, in addition to the absence of identified causes and lack of generally effective treatments, make IC a particularly challenging disease to model. IC researchers have approached this problem in the bladder of healthy animals by using noxious intravesical, systemic, and environmental stimuli. A naturally-occurring lower urinary tract disease of cats with many features comparable to IC also has been investigated. This review describes animal models used to study cystitis and how they pertain to IC, and makes some suggestions for additional approaches to an improved understanding of the causes of IC using both induced models and cats with naturally-occurring disease.

MATERIALS AND METHODS

We used standard Internet medical databases (Medline and the Institute for Scientific Information’s Web of Science citation databases) to search for references pertaining to models of cystitis and bladder injury published during the period 1983-2001. Keywords used included variable combinations of bladder, chronic, cystitis, interstitial cystitis, model, neurogenic inflammation, pain and stress.
Models of cystitis were divided into induced models using intravesical, systemic and environmental stimuli to cause bladder inflammation, and the naturally-occurring model of IC in cats.

**Induced Models**

*Noxious intravesical stimuli*

The evaluation of patients with IC by urologists naturally led to an intensive focus on the lower urinary tract as the source of the problem. For this reason, many models of acute bladder injury in healthy animals have been investigated for their potential relevance to IC. For example, Ruggieri, et al.,\(^\text{18}\) used rabbits to investigate the possibility of some toxic constituent in the urine of IC patients. Instillation of urine from IC patients to 20 cm. of water pressure into rabbit bladders resulted in histopathological, but not functional, changes compatible with IC.\(^\text{19}\) However, more recent studies, instilling urine from IC patients (both in and out of remission) and from normal subjects into rabbit bladders using a similar protocol, but filling the bladder to only 10 -20% of capacity, did not result in differences in bladder capacity, urea permeability, or bladder histopathology between any of the groups. The authors concluded that disruption of the urothelial barrier by distention, rather than any toxic principle in the urine, was the most likely cause of the observed abnormalities.\(^\text{18}\) Another study in rabbits documented that filling the bladder to 90 –120% of capacity was sufficient to cause significant increases in dye penetration through the urothelium.\(^\text{20}\) As a consequence, we recommend that bladder pressures be reported so that more meaningful comparisons among studies may be made.
Despite evidence for absence of a toxic principle in urine of patients with IC, many studies have investigated the effects of intravesically-administered toxins on the bladder. For example, varying concentrations of acetone have been used to induce cystitis in rats, rabbits, and monkeys. Shimizu, et al. instilled 0.35 ml. of 10, 30, or 50% acetone into the bladders of rats for 90 seconds (pressures not reported) followed by washing with 0.6ml of saline. The 30% acetone treatment decreased time to micturition without altering threshold or micturition pressures. The micturition reflex was abolished completely with 50% acetone, resulting in incontinence. Inflammation was observed histologically in bladders treated with 30 and 50% acetone. The 50% group had more severe desquamation of the urothelium, but interestingly, the severity of inflammatory cell infiltrate was not different from the 30% group. Bladder strips from the 50% group also had a decreased contractile response to carbachol and electrical field stimulation, suggesting damage to the detrusor that has not been reported in patients with IC.

In 3.3 kg Green monkeys, 50 ml. of 50% acetone (~90% of bladder capacity) decreased bladder capacity and voiding volumes the first week after instillation. The monkeys were much less active in this week as well, suggesting some discomfort. Urea absorption increased from 22 to 67% after the acetone infusion, indicating increased bladder permeability. All parameters returned to normal by week four except for bladder compliance, which was still decreased.

Acid infusion also has been used to induce cystitis. Elgebaly et al. instilled 20ml of acidic phosphate-buffered saline (pH= 4.5; bladder pressure not recorded) into the bladders of female white New Zealand rabbits for 15 minutes, followed by a neutral
(pH=7.2) wash. Both ureters were ligated to avoid dilution of the acid by newly produced urine. Neutrophil chemotactic activity increased by 70% in treated animals. Electron microscopy revealed extensive neutrophil accumulation and edema in the acid-treated compared to control bladders. They also reported increased neutrophil chemotactic activity in urine from 11 IC patients compared to normal patients and patients with other lower urinary tract diseases. Results of biopsies from these patients were not reported, so the character and extent of cellular infiltrate could not be evaluated. In contrast to these results, Felson et al., could not detect any effect of urine from 10 IC patients on neutrophil chemotaxis.

Koltzenburg and McMahon studied the effects of bladder distention (6.5-19.5 cm H$_2$O), instillation of 2.5% mustard oil, and electrical stimulation of pelvic ganglia on plasma extravasation (Evans blue dye) in the urinary bladder of anesthetized rats. In this model, mustard oil and electrical stimulation, but not distention, increased plasma extravasation, suggesting that afferent neurons activated during normal distention and micturition differed from those that produced plasma extravasation. These findings suggested that a population of chemosensitive afferent fibers could play a role in visceral pain.

McMahon and Abel subsequently investigated the inflammatory responses of the bladder of female rats to instillation of 0.2ml of 25% turpentine, 2.5% mustard oil or 2% croton oil. Acute studies were conducted on anesthetized rats, and chronic (up to 4 days) studies were performed on decerebrate rats. All treatments resulted in plasma extravasation that resolved within 72 hours, but increased numbers of leukocytes still were present at that time. Cystometric evaluation identified bladder hyperreflexia for
variable periods that had resolved or begun to subside by 72 hours. Interestingly, responses to noxious somatic stimuli also were increased after irritant administration.

These studies documented the time course of development and resolution of acute inflammation in normal rodents. Although IC patients usually are diagnosed years after the onset of their symptoms, these experiments provided the basis for subsequent investigations of the mechanisms underlying acute bladder inflammation. For example, the nociceptive effects were found to result from activation of unmyelinated sensory neurons that were unresponsive to physiological changes in bladder pressure. Approximately 20% of unmyelinated sensory nerves in the rat bladder are sensitive to capsaicin (CS), the major pungent ingredient in hot peppers of the genus Capsicum. Capsaicin (and xylene) can be used to stimulate a population of unmyelinated "silent" afferent neurons in the pelvic nerve to help investigate the role of these fibers during acute inflammation of the bladder. One of the neurotransmitters contained in CS afferent neurons is Substance P (SP), a neuropeptide that binds preferentially to the neurokinin-1 (NK-1) receptor. Later studies also found that nerve growth factor was involved in the hyperreflexic response of the bladder, elicited dose-dependent plasma extravasation, and could sensitize thinly myelinated (aδ) and unmyelinated (c) sensory nerve fibers in the pelvic nerve. Increased amounts of nerve growth factor also have been found in the urinary bladder of women with IC.

In addition to noxious chemicals, inflammatory cystitis has been induced by immunological means. Bjorling and Saban used ovalbumin (OVA) sensitized guinea pigs to investigate immune-related responses of the bladder. This model resulted in a generalized increase in plasma extravasation and bladder histology that was somewhat
similar to the ulcer form of IC. It was used to explore the effects of intravesically administered SP, capsaicin, and restimulation with OVA on bladder inflammation. Histological changes after exposure to these compounds progressed with time (20 hours) from intense vasodilatation to marginalization followed by interstitial migration of leukocytes. They concluded that a variety of intravesical stimuli could induce the release of inflammatory mediators and neuropeptides, including SP, which could in turn initiate bladder inflammation and result in persistent, and even amplified cystitis after the inciting cause had been removed.

These and other investigators also have used immune sensitization to characterize additional features of the acute response of the bladder of normal animals to noxious luminal stimuli. For example, Luber-Narod, et al., compared instillation of xylene, or of the immune system-activating compounds lipopolysaccharide (LPS) or polyinosinic-polycytidylic acid (poly IC) to cystocentesis without fluid injection in rats. They found that LPS and poly IC rapidly (4 h) induced inflammation that was maintained for at least 7 days, and decreased the bladder content of immunoreactive SP by approximately 50%, suggesting enhanced release. Despite the presence of acute inflammation, no change in the urinary frequency was observed. They also found that an NK-1 receptor antagonist was unable to block the inflammation produced by poly IC, suggesting that SP was not the sole cause on the inflammation observed in this model.

Saban et al., recently have found that mice genetically deficient in the NK-1 receptor (NK-1 -/-) did not develop an inflammatory cell infiltrate or edema in response to intravesical infusion of 150ul. of dinitrophenyl 4-OVA after dinitrophenyl 4-human serum albumin sensitization, despite the presence of increased numbers of bladder mast
cells compared with wild-type mice and a comparable increase in the percentage of
degranulated mast cells. These results provide evidence for mandatory participation of
NK-1 receptors in the inflammation observed in this model of cystitis. Unfortunately, no
functional parameters were measured, so the effects, if any, on urinary or pain-related
behaviors are not known. Thus, the relevance of studies in NK-1 -/- mice to IC is as yet
unclear. An NK-1 independent component of edema formation in the skin of these mice
has been identified, and infusion of mustard oil into the colon of these animals resulted
in mucosal edema, pain-related behavior and referred hyperalgesia.

Noxious systemic stimuli

Many studies have focused on the effects of intravesically administered agents,
but absence of a demonstrated toxin in the urine of IC patients, the acute time course, and
the histopathological and functional alterations induced by toxins make these studies
difficult to relate to patients with naturally-occurring disease. Furthermore, similar
bladder abnormalities have been produced by other routes, suggesting that lesions found
in bladders represent a nonspecific reaction of this organ regardless of the insult applied.
For example, Bjorling et al., used two strains of mast-cell deficient mice and their
congenic, normal counterparts to investigate the role of systemic activation of mast cells
on bladder inflammation. They found that intravenous injection of SP or LPS induced
cystitis and increased plasma extravasation in normal, but not in mast cell-deficient mice,
indicating that mast cells participate in the inflammatory response of the bladder to
systemically administered SP and LPS in these mice. Whether these compounds acted
from the basal or luminal surface of the urothelium, or if they affected bladder function,
was not determined. A toxin from bracken fern causes a hemorrhagic cystitis even if
urine is not allowed to reach the bladder, demonstrating that toxins may result in bladder lesions from the basal side of the urothelium.\textsuperscript{45} Using an alternative approach, Jasmin et al.\textsuperscript{46} reported that injection of pseudorabies virus into the tail base of rats resulted in inflammation of the bladder (and of the colon and prostate gland as well in isolated cases) by activation of central nervous system circuits. Further studies identified the role of mast cells in this form of neurogenic cystitis.\textsuperscript{47}

The experimental autoimmune cystitis (EAC) model has reproduced one unusual histologic feature of non-ulcerative IC, the presence of edema and vascular congestion in the absence of an inflammatory infiltrate.\textsuperscript{48} Female Lewis rats (chosen because of their genetic predisposition to autoimmune disorders) injected in the tail base with bladder homogenate in Complete Freund’s adjuvant (CFA) was compared to animals injected with CFA alone. No difference in bladder capacity was identified, but urinary frequency at 7 weeks was significantly increased in the bladder homogenate vs. control group (21 vs. 8 urinations per 6 hours). Histological examination of the bladder revealed vascular congestion in the submucosa without an inflammatory infiltrate or increase in mast cell numbers only in the homogenate-injected group. No evidence of inflammation was observed in the CNS. In a subsequent report further characterizing the model,\textsuperscript{49} urinary frequency averaged over 9 weeks also was significantly increased in the bladder homogenate vs. control group (24 vs. 17 urinations per 6 hours). Although the authors also reported that urinary frequency progressed through two cycles of increase and decrease, this conclusion did not appear to be supported by the data presented. Additionally, no evidence of pain-related behavior was reported. The authors concluded that the significance of their model to IC was still an open question. Many of the features
of this model are non-specific, and it is not generally agreed that patients with IC are predisposed to autoimmune disease, as are these rats. It might be quite useful, however, to investigate potential immune-related mechanisms of increased urinary frequency, and the vascular congestion in the absence of an inflammatory infiltrate so commonly seen in patients with IC. For example, nociceptive fiber modification of L-selectin adhesion to neutrophils recently has been suggested as a mechanism of reducing inflammation that might be investigated using this model.\textsuperscript{50}

\textit{Noxious environmental stimuli}

Bladder abnormalities similar to those induced by noxious stimuli delivered by intravesical routes have also been demonstrated via both psychological and physical stressors applied to animals. Stressful external circumstances seem to aggravate symptoms of IC,\textsuperscript{51} and the effects of noxious environmental stimuli (NES) on the bladder have been studied using animal models. Spanos, et al.,\textsuperscript{52} subjected rats to 30 minutes of restraint stress, which resulted in activation of 75\% of bladder mast cells within 30 minutes. In contrast, the stressor activated 49\% of mast cells in animals treated with capsaicin as neonates, suggesting that the stress-induced bladder mast cell activation was mediated in part by CS neurons, as it is in many other tissues.\textsuperscript{53} Rabbit polyclonal anti-corticotropin releasing factor (CRF) serum failed to inhibit the inflammation. Approximately one- third of control animals also had activated mast cells in the bladder, suggesting that the stress of handling might have been adequate to induce these lesions. Restraint stress includes both physical and psychological stimuli, so environmental temperature and illumination have been manipulated to attempt to emphasize the influence of the psychological component. Ercan et al.,\textsuperscript{54} recently reported that 3 hours
of acute cold (4°C) stress in rats resulted in bladder mucosal edema, leukocyte infiltration, and mast cell degranulation. Electron microscopic (EM) abnormalities also were observed. They also found that stress-induced changes in rat bladder (and stomach and liver) could be prevented by CS given to neonates, or administered around the vagus or celiac nerves before exposure to cold immobilization stress. These studies feature nonspecific inflammatory changes in the bladder, but they also support the suggestion that activation of CS neurons may be involved in the bladder’s response to stressors (both internal and external). Unfortunately, urinary characteristics (e.g., voiding volumes, frequency) were not reported in these studies. Ultrastructural examination of the bladder of healthy rodents after prolonged exposure to 37°C temperature or constant illumination disclosed bladder abnormalities similar to those found in some patients with IC. The urothelium had abnormal cytoplasmic detail and loss of subapical vesicles. Disruption of tight junctions between superficial urothelial cells and desquamation of superficial cells also occurred, and this change exposes incompletely differentiated intermediate cells to the bladder lumen. Desquamation of superficial urothelial cells appears to be a non-specific bladder defense mechanism, also occurring after E. coli adherence, administration of LPS, ischemia, systemic administration of hydrocortisone or norepinephrine, or removal of calcium. Moreover, epithelial desquamation also occurs in the colon, intestine, lung, and skin in response to a variety of noxious stimuli. Desquamation may increase bladder permeability, permitting increased access of constituents of the urine to neurons and inflammatory cells in the submucosa, which may mediate part of the bladder’s response to threatening environmental stimuli (Figure 1-1). Increased bladder permeability occurs in some
patients (both human and feline) with IC,\textsuperscript{68-70} and altered epithelial (and endothelial) permeability also has been identified in the gingiva,\textsuperscript{71,72} skin,\textsuperscript{73} lungs,\textsuperscript{74} and gut\textsuperscript{64,65,75} of healthy subjects in response to a variety of physical and mental stressors.

These studies of NES suggest that complex mechanisms mediate CNS activation of local inflammation during stressful circumstances. The inflammatory response to acute NES may partially explain the observation of glomerulations in healthy women undergoing tubal ligation because they may have perceived this procedure as stressful.\textsuperscript{9} Increased numbers of mast cells have been observed in biopsy specimens from about 65\% of IC patients with ulcers and in 20\% of specimens from patients without ulcers,\textsuperscript{2} but the effects of stress on mast cells are not restricted to the bladder. Similar results have been found in brain, intestine, and skin.\textsuperscript{52}

Studies of healthy animals have thus provided an essential description of the complexity of responses of the normal bladder to a variety of insults. The relevance of these models to the etiology of IC, however, is less clear. The identified responses usually are not specific to the bladder, and when the stimulus is removed, healthy animals appear to return to normal.\textsuperscript{54}

**Naturally-occurring bladder disease in cats**

A spontaneously occurring disease analogous to IC also occurs in domestic cats. This disorder sometimes is referred to as Feline Interstitial Cystitis (FIC) to distinguish it from IC in humans.\textsuperscript{76-78} Cats with FIC meet all of the inclusion criteria, and all of the exclusion criteria for diagnosis of IC (Table 1) that can be applied to animals. Although criteria 1-3 cannot be tested in awake cats, we did not observe involuntary bladder contractions (at fill rates appropriate to cats) during urocystometric examination of 19
cats with FIC lightly anesthetized with ketamine and xylazine. Criterion 5 is difficult to apply to cats because they are non-diurnal animals, but client reports of increased frequency of urination are common. Criterion 18 restricts subjects to adults, and FIC most commonly is seen in adult cats. Thus, making allowances for species differences, cats with FIC meet the NIH criteria for IC.

FIC primarily resembles non-ulcer IC in humans, although ulceration and inflammatory infiltrates occasionally have been observed in cats. Abnormalities of local bladder factors, sensory (afferent) neurons, the central nervous system, and sympathetic (efferent) neurons occur in cats as they do in humans. For example, both humans and cats with IC seem to excrete smaller amounts of both total urinary glycosaminoglycan (GAG) and a specific GAG, (GP-51) than do normal individuals. Most, but not all, studies of human patients with IC have found increased bladder permeability. Bladder permeability to sodium salicylate was increased in an in vivo study of cats with FIC. Additionally, in vitro bladder epithelial urea permeability was significantly increased from normal in both undistended and distended bladders, water permeability was significantly increased in distended bladders, and transitional cell desquamation recently was identified in cats with FIC.

Increased neuronal SP immunoreactivity (IR) has been reported in the bladder of humans with IC in some but not all studies. In a preliminary study, we observed an increase in SPIR in the bladder of cats with FIC. Significant increases in the density of NK-1 receptors in the bladder of humans and cats with IC also have been reported. Similar increases in NK-1 receptor density have been reported in a variety of other chronic inflammatory processes.
A significant increase in tyrosine hydroxylase (TH) IR has been identified in the locus coeruleus (LC) of cats with FIC.\textsuperscript{95} Tyrosine hydroxylase is the rate-limiting enzyme of catecholamine synthesis. Bladder distention stimulates neuronal activity in the LC, and the LC (Barrington’s nucleus) is the origin of the descending excitatory pathway to the bladder.\textsuperscript{96} Moreover, chronic stress can increase TH activity in the LC,\textsuperscript{97} with accompanying increases in autonomic outflow.\textsuperscript{98,99} The LC contains the largest number of noradrenergic neurons, and is the most important source of NE, in the feline and human central nervous system. It is involved in such global brain functions as vigilance, arousal, and analgesia, and appears to mediate visceral responses to stress.\textsuperscript{100}

The increased THIR observed in the LC of cats with FIC may provide a clue to the observation that the clinical symptoms of IC follow a waxing and waning course in both cats and human beings, and are aggravated by environmental stressors.\textsuperscript{51,101,102} Corticotropin releasing factor (CRF) -containing projections from the amygdala that are activated as part of the stress response innervate the LC and hypothalamus.\textsuperscript{103,104} This activation increases CRF release in the LC, which has been proposed to stimulate the increased activity of the LC during stressful circumstances. This circuit provides a link between the environment and bladder function.\textsuperscript{100} If the nociceptive input resulting from an abnormal bladder somehow primes this circuit, it might become more sensitive to external activation. Preliminary studies in our laboratory have found increased bladder permeability in cats with FIC compared to controls when subjected to a mild stressor. In contrast to the apparent activation of the sympathoneural system found in cats with FIC, abnormalities of the hypothalamic-pituitary-adrenal axis have not been identified.\textsuperscript{105}
In addition to increased LC activity, cats with FIC also have increased plasma NE, enhanced stimulus-induced local NE release from the bladder and functional desensitization of central alpha-2 adrenoceptors (α-2 AR). In the LC, α-2 agonists inhibit NE release, whereas in the spinal cord they inhibit transmission of nociceptive input to the brain. The spinal receptors appear to be located on the central processes of sensory neurons. Although spinal α-2 AR activation can inhibit nociceptive input acutely, the receptors can become desensitized or downregulated after chronic stimulation.

Increased sympathoneural activity also may release inflammatory mediators associated with pain. For example, NE reportedly can induce local release of prostaglandins, which can in turn excite nociceptive C-fibers. Conversely, inhibition of sympathetic efferent activity appears to decrease inflammation in some circumstances. For example, sympathectomy reduced the severity of experimentally-induced urethral inflammation, arthritis, and colitis in rats. In both humans and cats with IC, amitriptyline, a tricyclic antidepressant with significant sympatholytic activity, has been shown to reduce the severity in some patients.

Although autonomic function has not yet been thoroughly evaluated in humans with IC, stressor-induced increases in sympathetic activity have been reported and increased density of bladder sympathetic fibers and THIR have been observed. Evidence for increased spinal sympathetic neuron activity also has been presented and increased urine NE excretion has been reported.
Like the induced models, FIC also has limitations as a model of IC in humans. One is the seemingly different gender distribution between affected males and females of the two species. In cats, both genders are affected roughly equally, whereas in humans, 90% of patients are women. One reason for the gender discrepancy may be related to differences in diagnoses rather than diseases. Men with IC symptoms are more likely to be diagnosed with non-bacterial prostatitis rather than with IC. Miller et al. recently reported that 8 of 20 men evaluated for non-bacterial prostatitis had cystoscopic findings compatible with IC, and “perhaps…should be given the diagnosis of IC”. If only half of the cases of non-bacterial prostatitis are the same disease as IC, the difference in gender distribution between humans and cats would disappear.

Another limitation to using cats with FIC to investigate etiologic mechanisms of IC is that affected animals are not easy to acquire without veterinarian and owner cooperation. This is particularly frustrating because some 4 million cats are destroyed annually in the United States for “elimination problems”, the majority of which are related to the urinary tract. Additionally, cats are more expensive to maintain in laboratory animal facilities than are rodents.

Despite these limitations, studies of cats with FIC have duplicated many results obtained in humans with IC. Moreover, a wealth of bladder and stress neuroscience research conducted in cats is available to compare anatomic and functional alterations in the CNS caused by IC in ways that would not be possible in humans or induced models.
Animal Welfare Considerations

Although models rarely show all the clinical, morphological, biochemical and functional features of the disease they resemble, they still can contribute significantly to a better understanding of the condition in humans.\textsuperscript{128} We also must remember that healthy animals used as experimental subjects serve as a means to our ends. Whether the end is alleviation of suffering in other animals and humans, or elucidation of basic biological mechanisms, the result is the same for the animal. Many cultures have decided that it is ethical to use one group of individuals as the means to another group’s ends. When such a decision is made, however, it carries with it the responsibility to treat the burdened group as humanely as possible. Mann, et al.,\textsuperscript{129} have pointed out that experimental design is an integral part of laboratory animal welfare. For example, if more animals are used than are necessary to adequately test a hypothesis, the extra animals used will have been wasted. In contrast, if too few are used and inadequate power is available to avoid a type II statistical error, all will have been wasted. A variety of experimental design strategies to reduce the number of animals used in research are available,\textsuperscript{129,130} including increasing effect size, reducing variability, appropriate use of controls, repeated measures from animals, using interim data analyses, applying 1-tailed rather than 2-tailed tests, using trend analysis and careful examination of the (blinded) data in addition to computer-based statistical analysis as appropriate.
Required Criteria
1. Glomerulations or Hunner’s ulcer on cystoscopic examination
   - examination for glomerulations should occur after distention of bladder under anesthesia to 80-100 cm water for 1-2 minutes
   - bladder may be distended up to two times before evaluation
   - glomerulations must be diffuse – present in at least three quadrants of the bladder
   - glomerulations must not be along the path of the cystoscope (since instrument contact can produce artifact)
2. Pain associated with the bladder or urinary urgency

Exclusion Criteria
(presence of any one of the following criteria excludes diagnosis of interstitial cystitis)
1. Bladder capacity of greater than 350 cc on awake cystometry using either a gas or a liquid filling medium.
2. Absence of an intense urge to void with the bladder filled to 1200 cc of gas or 150 cc of water during cystometry, using a fill rate of 30 to 100 cc/min.
3. The demonstration of phasic involuntary bladder contractions on cystometry using the fill rate described above.
4. Duration of symptoms less than 9 months.
5. Absence of nocturia.
6. Symptoms relieved by antimicrobials, urinary antiseptics, anticholinergics, or antispasmodics.
7. A frequency of urinations while awake, of less than eight times per day.
8. A diagnosis of bacterial cystitis or prostates within a 3-month period.
9. Bladder or ureteral calculi.
10. Active genital herpes.
11. Uterine, cervical, vaginal, or urethral cancer.
12. Urethral diverticulum.
13. Cyclophosphamide or any type of chemical cystitis.
14. Tuberculous cystitis.
15. Radiation cystitis.
16. Benign or malignant bladder tumors.
17. Vaginitis.
18. Age less than 18 years.

Table 1.1: Criteria for diagnosis of IC established by the National Institutes of Arthritis, Diabetes, Digestive and Kidney Diseases.
Figure 1.1: A conceptual model of the response of the bladder to noxious stimuli. The bladder lumen is on the far right. Damage or malfunction of the glycosaminoglycan (GAG) layer or the urothelium may permit constituents of the urine to activate sensory neurons (C-Fibers) in the submucosa, which transmit action potentials to the spinal cord (SC) that are perceived as painful by the brain. Sensory fibers also can generate a local axon reflex without propagating an action potential. The axon reflex results in release of peptide neurotransmitters such as Substance P (SP) by the nerve endings. Interaction of SP with receptors on vessel walls results in vascular leakage, which can be augmented by SP-induced release of histamine (His) by mast cells (MC). These actions may give rise to the submucosal petechial hemorrhages observed at cystoscopy. Receptors for SP also occur on smooth muscle, which when activated stimulate muscle contraction. In addition to luminal insults, increased vascular permeability and desquamation of the superficial cells of the urothelium also can result from systemic stimuli and in response to environmental stimuli, and activate C-fibers and mast cells.
REFERENCES


CHAPTER 2

EFFECTS OF STRESS ON CATS WITH INTERSTITIAL CYSTITIS

ABSTRACT

Interstitial cystitis (IC) in humans can have a waxing and waning course and clinical signs are usually exacerbated by stress. Feline interstitial cystitis (FIC), a naturally occurring model of IC, has a similar clinical presentation. Altered bladder permeability appears to be a feature in both species as well. To investigate the effects of stress in cats with FIC we subjected affected cats and healthy controls to an acute, mild stressor for eight days. During that time period, we compared serum fluorescien concentrations to evaluate bladder permeability, responses to medetomidine, in order to evaluate the effects on the α2-AR, and urine cortisol excretions (cortisol:creatinine - C:Cr) to evaluate the hypothalamic-pituitary-adrenal axis. In a subset of cats, catecholamine concentrations [CCE], were also analyzed. Thirteen FIC and 12 normal cats went through this protocol and samples were obtained on day 1, 3, and day 8 to assess habituation to the stressor. On day 9, all cats were moved to an “enriched” environment and testing was once again repeated on day 35 to investigate this form of therapy. The effects of experimental group (FIC, healthy controls), repeated factors time (1, 3, 8, and 35 days) and treatment (pre versus post), and their interactions were simultaneously analyzed using repeated measures analysis of variance.
Fluorescein concentrations were significantly higher in FIC cats at all times, but were highest after the acute stress, suggesting increased bladder permeability which was worse with during the initial stress. Cats with FIC showed a significant decrease in response to the α2-AR agonist, medetomidine, in regards to pupil dilation and per cent change in heart rate. No differences were noted in level of sedation, or blood pressure. In the group of cats where plasma [CCE] were available, plasma DOPA, NE, and DHPG concentrations were significantly increased in FIC cats at all times. In contrast, no differences between groups were identified in C:Cr at any time.

Elevated serum [FL] suggests increased permeability in cats with FIC, which appears to be exacerbated by stress. The differences noted between groups after the α2-AR agonist was administered could be due to abnormalities in the post synaptic α2a-AR or other downstream mechanisms. The marked increment in [DOPA] suggests the possibility of a stress-induced increase in activity of tyrosine hydroxylase (TH), the rate-limiting step in CCE synthesis. In contrast, no effects on C:Cr were identified, suggesting an uncoupling of these two parameters of the stress response. Similar findings have been reported in human patients with chronic pelvic pain, panic disorder, and post-traumatic stress disorder.
INTRODUCTION

Interstitial cystitis (IC) is a chronic, debilitating pelvic pain syndrome characterized by variable combinations of increased urinary frequency, urgency, and lower urinary tract pain. The etiology of this disease is unknown, and no acceptable long-term therapy exists. A spontaneously occurring disease analogous to IC occurs in domestic cats, referred to as feline interstitial cystitis (FIC) to distinguish it from IC in humans.\textsuperscript{1} Cats with FIC meet all the inclusion and exclusion criteria for diagnosis of IC that can be applied to animals.\textsuperscript{2} Although most human cases of IC are diagnosed in women, both sexes are represented in FIC, and newer studies suggest the disease is prevalent in men as well.\textsuperscript{3}

One feature of IC is increased urothelial permeability. Parson’s et al., reported increased permeability to urea instilled into the bladder of human patients\textsuperscript{4} and our lab has reported increased permeability to sodium salicylate instilled into the bladder of cats with FIC.\textsuperscript{5} Oral fluorescein (FL) has also been used as a marker to evaluate permeability in humans with IC, and has been reported to be a good marker of altered permeability in a mouse model of cystitis when administered intravesically.\textsuperscript{6}

In addition to bladder abnormalities, clinical signs in both FIC and IC are exacerbated by stressful circumstances. Chronic stress (both internal and external) can increase TH activity, the rate-limiting step in catecholamine synthesis, in the locus coeruleus (LC)\textsuperscript{7} with accompanying increases in autonomic outflow. Increased TH immunoreactivity in the LC\textsuperscript{8} and bladders\textsuperscript{9} of cats with FIC has been reported. Furthermore, increased plasma norepinephrine (NE) concentrations\textsuperscript{10} were found in these cats. NE and the LC play an integral part in modulating the body’s response to stressful
circumstances, and the sympathetic nervous system mediates this response primarily through a rich supply of alpha-2 adrenoceptors (α2-AR) located in the LC. Neurons to the LC are relatively inactive during periods of reduced stress, however during periods of anxiety, these same neurons increase firing and greater amounts of NE are released throughout the LC. Most α2-AR are prejunctional inhibitory autoreceptors, however other studies have shown that some α2-AR are post synaptic, such as those which mediate their effect on blood vessels and the iris. Previous studies have reported abnormalities in the α2-AR of cats with FIC and women with IC.

To determine the effects of stress on cats with FIC we conducted a series of experiments to analyze bladder membrane permeability using an intravenous injection of sodium FL. We also evaluated the functional sensitivity of the α2-AR by assessing sedation, heart rate, blood pressure, and pupil diameter after administration of the selective α2-AR agonist, medetomidine. Finally, we analyzed the two primary limbs of the stress response system in cats by analyzing plasma catecholamine concentrations and urine cortisol/creatinine (C:Cr) ratios during various stress and environmental enrichment time periods to investigate the effects of stress on membrane permeability and the α2-AR.

**MATERIALS AND METHODS**

Thirteen cats (3 neutered males (MN), 1 intact female (FI) and 9 spayed females (FS)) obtained as donations from clients due to a history of stranguria, hematuria, pollakiuria, inappropriate urinations, or a combination of these signs were evaluated at The Ohio State University Teaching Hospital. Evaluation consisted of a complete physical examination (including body weight), CBC, serum biochemical analysis, urinalysis, urine bacteriologic culture, and cystoscopy. Cystoscopy was performed, using
a 9-F rigid pediatric cystoscope (Karl Storz, Endoscopy America Inc, Culver City, Calif.) in female cats; a 3-F flexible fiber optic cystoscope (Five Star Medical Inc, Charlottesville, VA) was used in the male cats. A diagnosis of FIC was made on the basis of compatible history, and standard inclusionary and exclusionary criteria (including the presence of glomerulations at cystoscopy) after obtaining the results of the above laboratory tests. Twelve normal cats (3 MI, 1 MN, 7 FI and 1 FS) of similar age were used as controls. All cats were initially housed in stainless steel cages in the animal colony and allowed to acclimate to their environment for at least 3 months. The Animal Care and Use Committee of The Ohio State University approved all the experimental procedures.

A moderate stress protocol was designed so each cat had the following procedures performed on days 1, 3, and 8. The stressors included 12 hours of food deprivation, transport to the laboratory, administration of test procedures, followed by changes in both diet and housing; diets were changed from their familiar commercial dry food to a novel dry food, and cats were housed in a novel environment (metabolism cages in a different room of the vivarium). Cats were brought into the laboratory in groups of four and placed in small holding cages while the initial tests were performed. All cats were weighed the day of testing. A lux meter (Lutron Electronic Enterprise Co, Ltd, Taiwan) was placed next to the right eye and each cat was allowed to acclimate to the light for 1-2 minutes. A mid-lateral pupil measurement was obtained to the nearest millimeter. All lux readings were approximately 430 for each cat. Cats also had the following parameters measured: resting heart and respiratory rate, indirect systolic blood pressure measured using the Ultrasonic Doppler Flow Detector (Model 811-BL, Aloha, Oregon) with a
number 3 cuff on the left rear leg. All cats were placed in right lateral recumbency for this measurement. Following this information, a venous blood sample was obtained from the jugular vein. The serum and plasma were quickly frozen at -70°C for future analysis of catecholamines and metabolites. All pre-FL plasma samples were assayed for dihydroxyphenylalanine (DOPA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE), epinephrine (E), and dihydroxyphenylglocol (DHPG) using reverse high performance liquid chromatography with electrochemical detection after partial purification by absorption on alumina as previously described.\textsuperscript{10}

Fluorescein (250ug/kg) was then injected into the right cephalic vein and the time of administration recorded. Cats were placed back in their holding cages for one hour. After 60 minutes, a blood sample was obtained from the jugular vein and plasma FL was assessed using a fluorescent spectrophotometer at 494nm. (Turner Quantech Fluorometer, FM109515, Barnstead/Thermolyne, Dubuque, Iowa). Fluorescein samples were analyzed as previously described.\textsuperscript{18}
After the venipuncture, 20ug/kg of medetomidine was administered intramuscularly into the epaxial muscles and the time recorded. Exactly 10 minutes later, sedation level was evaluated using a standardized scale for each cat based upon previous studies in the literature.\textsuperscript{19} Pupil diameter was also assessed at that time. The time period and dose of medetomidine was chosen based on previous studies in the literature,\textsuperscript{19,20} personal communication with a boarded veterinary anesthiologist, and our experience administering this drug to more than 50 healthy cats at various doses (10-40ug/kg). At 20mcg/kg, medetomidine appeared to achieve moderate sedation in healthy cats without causing pronounced cardiovascular abnormalities. Atipamezole (300 ug/kg) was administered to all cats after assessing sedation, HR and BP to remove any delayed effects of the medetomidine. This venipuncture and cardiovascular analyses were performed on day one, day three, and for a third time on day eight.
After each group of four was completed, the cats were placed into clean metabolism cages to continue a moderate stressful situation for them. A new standard feline commercial diet (Iams® maintenance chicken based dry formula) was fed to all cats. Each cat received 100 grams per day and the intake was recorded daily. Urine was collected daily on dry ice, allowed to thaw at 4 degree C. Two aliquots were quickly frozen at -70° C and another sample was allowed to come to room temperature. A complete urinalysis including urine dipstick (chemstrip 9, Boehringer, Mannheim, Indianapolis, IN) and pH was measured by meter (PHM95 pH/ION METER, radiometer analytical A/5, Copenhagen, Denmark) to the nearest 0.1 meter. The previous frozen samples were then thawed to room temperature and urine cortisol and creatinine concentrations were assayed by a standard chemiluminescent protocol in our hospital laboratory.

At the end of the moderate stress period (day 9), cats were moved to an "enriched environment." (Figure 2.1) The cages were larger and each cage contained a covered bed, two types of approved toys, and a larger litter pan. The cats were not only fed the commercial dry food as before, but were also given a choice of canned food. The food was weighed daily and intake of each type recorded. In addition to the cage changes, all cats had interaction with humans (in addition to the animal caretakers) at least 15 minutes per day. The interaction for each cat was appropriate for that particular cat's disposition. Music was also played in the room for them. After three weeks, food was pulled the night before testing and all the previous testing described above were repeated once more.
STATISTICS

The effects of experimental group (FIC, healthy controls), repeated factors time (1, 3, 8, and 35 days) and treatment (pre versus post), and their interactions were simultaneously analyzed using repeated measures analysis of variance. When dependent measures were ordinal scores the Mann-Whitney test was used to compare experimental groups conditional on time. P-values less than 0.05 were considered statistically significant and p-values between 0.1 and 0.05 were considered marginally statistically significant.

RESULTS

No differences in food intake were found between the two groups at any time during the study (data not shown). Abnormalities pertaining to the lower urinary tract were subsequently found in one healthy cat (an intact male) shortly after the study began. This cat’s data were not included in the statistical analysis and he was removed from the study. Some blood samples could not be obtained from various cats during the study period for several reasons (e.g., poor jugular veins, uncooperative cat). The number of samples analyzed for each day and test are provided for each parameter we assessed.

Urinalysis. During the eight days the cats were in metabolism cages, microscopic hematuria was present in 30% of samples from cats with FIC, compared with 7% of samples from healthy cats (p=0.003, Figure 2.2). No difference in cortisol/creatinine ratios was identified between the two groups (Figure 2.3).

Plasma fluorescein concentrations. The plasma FL concentrations were evaluated on all days in all 13 FIC and 11 healthy cats except for day 35, when only 10 healthy cats were analyzed. FIC cats had significantly higher mean plasma FL
concentrations on all days. There was a significant difference between groups (p=0.001) and across days (p<0.0001, Figure 2.4). Both groups had higher mean plasma FL concentrations on day 1 (297ng/ml ± 103ng/ml vs. 142ng/ml ± 99ng/ml; mean ± sd) and continued to decrease over the study period to their lowest reading on day 35 (191ng/ml ± 90ng/ml vs. 83ng/ml ± 43ng/ml). In addition, FIC cats had significantly higher plasma FL concentrations on day 1 compared to subsequent days (p=0.01).

Medetomidine Testing of α2-AR: All 13 FIC and 11 healthy cats had their in vivo α2-AR function tested on all days except for days 8 and 35, when medetomidine analyses could not be obtained in one healthy cat on each day due to injection errors.

Heart rate: The decline in heart rate following medetomidine administration was significantly greater in FIC cats compared to healthy cats, although this difference was attenuated by day 35 (p=0.05, figure 2.5A and 2.5B; Table 2.1).

Respiratory Rate: The FIC cats had significantly lower respiratory rates compared to healthy cats (p=0.03), throughout the study. Both groups had a significant decrease in respiratory rate after the medetomidine was administered (p<0.0001, Figure 2.6A and 2.6B). No significant difference was noted across days (p=0.43).

Blood pressure: No significant difference in blood pressure was identified between groups (p=0.42). There was a significant difference between pre and post values (p<0.0001), and all cats mean blood pressures were significantly lower ten minutes after medetomidine was given (Figure 2.7A and 2.7B).

Sedation Score: No significant difference in sedation score was identified between the two groups (p=0.15; Figure 2.8).
Pupil Size: The per cent change in pupil diameter was significantly greater on days 3, 8, and 35 compared to day 1 (p=0.003), and the increase in pupil diameter following medetomidine administration was greater in healthy cats compared to FIC cats (p=0.004; Figure 2.9A and 2.9B).

Catecholamine concentrations Due to a sample processing error, only 6 FIC cats and 5 healthy cats had plasma catecholamines and metabolites analyzed. In this group, plasma DOPA, NE, DHPG concentrations were significantly increased in FIC cats at all times (p=0.04, p=0.03, p=0.04 respectively; Figures 2.10, 2.11, 2.12). Plasma DA and DOPAC were oftentimes elevated in FIC cats compared to healthy cats (p=0.09, p=0.08 respectively; Figures 2.13, 2.14). No significant difference in plasma EPI concentrations between the two groups was identified (p=0.15; Figure 2.15).

DISCUSSION

The most important findings in this study include the increased fluorescein concentrations which were significantly higher in FIC cats at all times, but were highest after the acute stress. In addition to increased permeability, cats with FIC showed a significant decrease in response to the α2-AR agonist, medetomidine, in regards to pupil dilation and per cent change in heart rate. These findings improved after the environmental enrichment time period. In the group of cats where plasma [CCE] were available, plasma DOPA, NE, and DHPG concentrations were significantly increased in FIC cats at all times and also began to decline after environmental enrichment. In contrast, no differences between groups were identified in C:Cr at any time suggesting an uncoupling of these two stress response systems.
Increased bladder permeability has been reported in cats with FIC\textsuperscript{5} as well in humans and other models of cystitis\textsuperscript{6,21} by assessing plasma concentrations of a drug after intravesical administration. Fluorescein, a fluorescent dye of molecular weight 325, has been used to assess membrane permeability. Buffington et al.,\textsuperscript{18} evaluated orally ingested FL in women with IC and found plasma FL concentrations were significantly higher in IC patients than in control subjects. Urine FL excretion was significantly lower in IC patients compared to control subjects, suggesting that increased FL may be a useful marker of altered membrane permeability.

Due to slight individual differences in gastrointestinal transit time that can occur, variability in drug absorptions for oral medications, and the need for anesthesia for intravesical drug administration, we choose to administer fluorescein intravenously in cats to assess membrane permeability. No adverse reactions were encountered in any cat during the course of the study, and to our knowledge, only one report of an adverse reaction to this drug in cats exists.\textsuperscript{22} Increased bladder permeability might allow FL to be reabsorbed, delaying its excretion. Due to the study design, we were unable to collect urine to assess urine FL concentrations, however all plasma concentrations were higher in FIC cats similar to what is reported in women with IC.\textsuperscript{18}

The cause of the altered membrane permeability is not fully understood. The glycosaminoglycan (GAG) layer, which lies on top of the apical membrane of the bladder, can act as a permeability barrier, but the contribution of this layer remains under debate. Decreased GAG excretion,\textsuperscript{2,23} as well as increased total GAG to sulfated GAGs ratios\textsuperscript{23} has been reported in the urine of cats and humans respectively with IC, suggesting a mechanism for increased permeability. Leaky tight junctions could also
increase permeability in cats with FIC. Lavelle et al.\textsuperscript{24}, evaluated urinary bladders from FIC cats, and found marked increases in permeability under hydrodistention. Scanning electronmicroscopy revealed patches devoid of umbrella cells and disruption of tight junctions that worsened after hydrodistention.

Studies have also shown that psychological stress can alter bladder membrane permeability. Veranic et al.,\textsuperscript{25} reported altered urothelial desquamation in rats exposed to constant illumination. Psychological stress can lead to an increase in cytokine production and inflammation. Both groups of cats had higher plasma FL concentrations during the stress portion of the study, suggesting that stress might play a role in membrane permeability.

In addition to increased membrane permeability, FIC cats also had abnormalities in their SNS. In the subset of cats where [CCE] were performed, plasma DOPA and NE were significantly higher compared to controls. Other CCE and metabolites were elevated in the FIC cats as well. The marked increase in DOPA concentrations suggests a stress-induced increase in activity of TH, the rate-limiting step in catecholamine synthesis, of which DOPA is the first reaction product. Reche et al.\textsuperscript{8} have reported increased TH immunoreactivity in the LC of cats with FIC. An elevation in TH immunoreactivity in the bladders of FIC cats also has been reported.\textsuperscript{9}

We hypothesized that elevated NE would adversely affect the \(\alpha_2\)-ARs in FIC cats due to chronic agonist stimulation. \(\alpha_2\)-AR have a wide variety of functions in the body, particularly in the cardiovascular system and in pain modulation. Alpha adrenoceptors were originally subdivided based on synapse location (ie, presynaptic vs. post synaptic), however later studies found that \(\alpha_2\)-ARs could be found at pre- and post-synaptic...
locations. In general, α2-ARs are responsible for platelet aggregation (via the α2a-AR), neurotransmitter release, vasoconstriction and even antinociception. Four pharmacological subtypes of the α2-ARs (A, B, C and D) have been identified in the literature based on ligand affinity studies. The α2d subtype has been reported in rat and bovine tissues and is considered analogous to the α2a in humans. The subtypes are well conserved across mammalian species. All members of the α2-AR family inhibit adenylate cyclase through coupling to an inhibitory G-protein, although some are capable to couple to stimulatory G-proteins. Most α2-ARs conduct their activity by inhibiting voltage-gated Ca\(^{2+}\) channels and opening K\(^{+}\) channels. NE and EPI bind to these receptors at the presynaptic terminal and exert a negative feedback control over CCE release. In the bladder the α2-ARs are found primarily in the urethral submucosa and bladder mucosa (not the muscle) suggesting a role in the regulation of blood flow, urethral lubrication, but not contraction. However, the α2-ARs function as autoreceptors and can modulate NE outflow, and alter contractility through this mechanism.

The specific functions of each α2-AR subtype functions have been documented via specific knockout mice models such as the α2a-D79N and the α2a-KO. Most of the “classic” effects (e.g. hypotension, sedation, analgesia, and hypothermia), of the α2-AR effects can be attributed to the α2a-AR subtype. The α2a subtype is a key receptor involved in cardiovascular function, and modulating the release of NE, DA, and serotonin. It is also the most widely distributed subtype of the α2-AR in the frontal cortex and hypothalamus. The hypotensive effects are due to stimulation of the α2a-AR in the ventrolateral medulla, decreasing sympathetic outflow, which will then cause a
reduction in blood pressure as well as heart rate. Based on mRNA coding studies, the α2a-AR is found in the locus coeruleus and other noradrenergic nerve terminals, which suggests it plays a crucial role in NE release. These studies also suggest that the sedative effects of α2-AR agonists are due to stimulation of the α2a-AR in the LC and reducing NE. Furthermore, the α2a-AR appears to be the predominant subtype involved in antinociception. Less is known about the roles of the α2b and α2c receptor subtypes, but it is thought the α2b plays an important role in salt induced hypertension and possibly developmental processes. The α2c-AR does not play a crucial role in cardiovascular regulation, but possibly contribute by regulating the dopaminergic system and the startle response.

We used medetomidine to evaluate the functional sensitivity of the α2-AR in our cats. Medetomidine is a bridge-methylated derivative of detomidine used often in veterinary medicine for animal sedation and restraint and does appear to have more selectivity for the α2a-AR. Medetomidine is a potent and selective pre- and post-synaptic α2-AR agonist. We choose 20µg/kg IM based on previous administration of this drug to healthy, middle aged cats, which resulted in the majority of cats demonstrating moderate sedation at this dose (data not shown). Although medetomidine has a much stronger α2/α1 selectivity ratio, than other α2-AR agonists, we avoided higher doses because it could potentially stimulate α1-AR, which would counteract the sedation.

The differences noted between the two groups when evaluating HR and pupil dilation after medetomidine administration could have occurred secondary to chronic agonist stimulation with NE at the α2-AR sites. Heart rate is controlled, in part, by central post-synaptic α2- ARs in the nucleus of the solitary tract. Pupil dilation occurs
via stimulation of centrally located, post-synaptic α2-AR located in the Edinger-Westfal nucleus. Stimulation of these receptors results in pupil dilation due to CNS inhibition of the parasympathetic tone to the iris.38,39 After medetomidine administration to both groups of cats, both of these parameters were significantly different in FIC cats compared to healthy ones. These differences were less after environmental enrichment, when NE had dramatically decreased.

No differences were noted between the two groups in regards to sedation and blood pressure after medetomidine. Sedative effects of α2-AR agonists are mediated from stimulation of the presynaptic α2-AR located primarily on the LC in the pons and lower brainstem,40 whereas HR and pupil dilation are primarily post-synaptic. We also noted a variable response in sedation in both groups. This could be due to variable drug absorption when medetomidine is administered IM. Similar problems have been reported in dogs when evaluating medetomidine given IM.41 Moreover, if abnormalities exist in the α2-ARs, this could affect peripheral perfusion and absorption of the drug itself. In future studies, the drug should be dosed and administered intravenously to avoid this potential problem. Although both groups had a significant decrease in blood pressure after medetomidine, no differences were noted between the groups. However, although not statistically significant, the FIC cats decreased their blood pressure by only 9% and 8% on days 1 and 3 respectively, whereas the healthy controls decreased their blood pressure by 15% and 12% on each of these days. Perhaps if we evaluated a larger number of cats, the differences might be more apparent.
Interestingly, cats with FIC had significantly lower RR compared to healthy cats although both groups decreased significantly after medetomidine was administered. We expected FIC cats to have higher respiratory rates as a reflection of higher perceived stress. RR may not be a good indicator of stress and some cats may reflect their anxiety by inactivity and minimal movements. This could be a defense mechanism so as not to be seen by predators.

The high noradrenergic tone in FIC cats could lead to a desensitization of the $\alpha_2$-AR, whereby there is a rapid reduction in the receptor’s response. *In vitro* studies in FIC cats evaluating electrical field stimulation studies on bladder strips,\textsuperscript{16} revealed that atipamezole, an $\alpha_2$-AR antagonist, did not alter the relaxing effect of NE, providing further evidence for abnormalities in these receptors. The reason for this is unclear at this time, but could be caused by receptor desensitization due to persistent elevation in NE.

In other species, chronic stress has also been reported to cause desensitization of the $\alpha_2$-AR. For example, *in vitro* studies evaluating tree shrews under chronic stress had decreased binding site numbers in various brain sections to the $\alpha_2$ antagonist, $3[H]$rauwolscine, than their counterparts, suggesting downregulation of the $\alpha_2$-AR due to high noradrenergic activity.\textsuperscript{29} *In vivo* work by Gomez et al. provided evidence that chronic stress leads to desensitivity of the $\alpha_2$-AR (post synaptic) in rats when evaluating the clonidine induced jaw-opening reflex.\textsuperscript{42} Others have shown functional desensitization central post-synaptic $\alpha_2$-AR in rats after administration of cumulative doses of clonidine or repeated tail pinching as a form of chronic variable stress.\textsuperscript{43} Studies in mice have shown rapid functional down regulation of pre- and post-synaptic $\alpha_2$-ARs after treatment with the antidepressant, sibutramine. The initial down regulation was
only moderately increased by subsequent drug administration. In these studies, the post-synaptic α2-AR appears to functionally desensitize first. Desensitization is usually rapid and reversible; the receptors are internalized but not lost.

However, one must realize the α2-ARs are complex receptors, coupled to G proteins (usually Gi) and many second messenger systems to carry out the effects of the agonists. Abnormalities in other mechanisms have been documented in other chronic stressful diseases, which commonly occur in humans with IC. For example, hypofunctional G proteins have been reported in humans with migraine headache and fibromyalgia. Abnormalities in the α2-AR, the G protein, or second messengers could allow for increased nociceptive input to the brain and various other abnormalities that could perpetuate clinical signs of FIC and IC, especially during periods of stress. In humans with IC, adrenergic receptor gene polymorphisms have also been reported.

Although the [CCE] were elevated in FIC cats, no differences were noted in the urine cortisol/creatine ratios. We hypothesized that the cortisol/creatine would be elevated in FIC cats as well because this test is a sensitive indicator of the adrenal gland’s response to stress and a reflection of the hypothalamic-pituitary-adrenal axis. However, we found that FIC and healthy cats had similar ratios. Glucocorticoids have been reported to decrease plasma levels of CCEs, inhibit CCE synthesis and can attenuate plasma CCE response to some stressors. This implies that glucocorticoids can inhibit sympathoneuroal outflow and our studies suggest that the lack of cortisol may also be perpetuating or causing the elevated SNS we observed. The elevated SNS could also help explain that symptoms of FIC follow a waxing and waning course in both cats and human beings, and are aggravated by environmental stressors. Because of its
significant sympatholytic activity, amitriptyline and other tricyclic antidepressants have been used to reduce the severity of the chronic disease in both humans and cats\textsuperscript{50,51}

This study has several limitations concerning the plasma FL concentrations and CCE analyses. In regards to the permeability testing, although plasma concentrations were higher in FIC cats at all times, we did not assess urine FL concentrations simultaneously, which would have provided information on fluorescein excretion in the cat. Plasma CCE concentrations were obtained by external jugular venipuncture, which could provide an acute stress for the cat and “artificially” increase the concentrations at that moment. However, the means and standard deviations we found for healthy cats were comparable to other studies in cats where plasma NE\textsuperscript{10} concentrations were evaluated in cats after jugular catheters were placed and cats were allowed to acclimate to the collection process. Due to the study design, placing jugual catheters was not possible, but this did hinder our ability to collect blood samples from every cat throughout the course of the study at each designated sampling time.

Unfortunately, in this study we were unable to match both groups for gender. Although the male and female numbers in general were similar, the control population contained more intact animals. The cats with FIC were obtained as donations and most had been neutered or spay, whereas many of the control cats were purchased and had not been altered at the time of this study. No data in the literature was found to evaluate the differences in $\alpha_2$-ARs in male, female, intact or spayed cats, but studies in pre and post-menopausal women as well as healthy men may provide some insight. Studies in healthy pre-menopausal women have demonstrated a greater $\alpha$-AR sensitivity than men because a lower infusion rate of phenylephrine (primarily an $\alpha_1$-AR agonist) was required to
increase systolic blood pressure. In other studies, responses to phenylephrine and clonidine (an α2-AR agonist), showed significant dose-related vasoconstriction in men, but not women. Other studies demonstrated no gender differences in α2-AR testing between genders. Furthermore, studies have evaluated the α2a-AR binding sites in post-menopausal women compared to healthy women of reproductive age. Although differences were found in imidazoline receptors, no differences were found in platelet α2a-AR densities and no differences were noted with estrogen replacement therapy.

Gender (intact vs. spay) could affect CCE concentrations. When evaluating the sympathoadrenal response in post menopausal women, Menozzi et al., reported that no differences in diastolic blood pressure, heart rate, and epinephrine concentrations were found with transdermal estradiol supplementation, however NE concentrations were significantly lower during estradiol therapy. When analyzing catecholamines in our cats, we had more female spayed cats in the FIC group, therefore, it is possible the effects of estrogen may have artificially lowered the NE concentrations in the control group. However, previous work by our laboratory support that elevated NE as well as increased TH-immunoreactivity is found in cats with FIC. Similar findings are reported in women with interstitial cystitis. No differences in cortisol excretion between men and women is reported.

In conclusion, the stress protocol we designed was used to see what, if any, affects a moderate stress would have in cats with FIC. Clinical signs (e.g. hematuria) were noted in many cats during the stress period as well as significantly higher plasma fluorescein concentrations. DOPA and NE elevations in cats with FIC were also noted and could contribute to the differences in α2-AR responses we found in cats with FIC.
Continual agonist stimulation could cause desensitization of the receptors. Furthermore, a lack of cortisol could contribute to the elevated SNS. The three week environmental enrichment protocol was helpful to evaluate if this therapy could improve the previously documented abnormalities. By day 35, flourescein and catecholamine concentrations were lower and only mild differences in per cent pupil dilation were noted by that time and further studies should be implemented in client owned FIC cats to evaluate this treatment option.
Figure 2.1: Environmental enrichment setting for all cats which began after day 8.

![Environmental enrichment setting for all cats](image)

Figure 2.2: Urine microscopic hematuria from FIC and Healthy Cats.

![Hematuria chart](chart)
Figure 2.3: Urine Cortisol/creatinine ratios from FIC and healthy cats during the seven days these cats were in the metabolism cages during their moderate stress protocol. No differences were found between the two groups.
Figure 2.4: Plasma Fluorescein in 13 FIC and 11 healthy cats. There was a significant difference between groups (p=0.0011) and across days (p<0.0001). In addition, FIC cats had significantly higher plasma fluorescein concentrations on day 1 compared to subsequent days. The squares and triangles represent the mean fluorescein concentrations and bars represent the standard deviation. The moderate stress protocol ended on day nine, when the enrichment part of the study began.
Figures 2.5A and 2.5B: Heart rate taken before (A) and after (B) 20mcg/kg of medetomidine was administered into the epaxial muscle of cats. The decline in heart rate following medetomidine administration was significantly greater in FIC cats compared to healthy cats, although this difference was attenuated by day 35 (p=0.048). The moderate stress protocol ended on day nine, when the enrichment part of the study began.
Table 2.1: Percent change in heart rate (HR) after 20mcg/kg of medetomidine was administered intramuscularly to cats. Healthy cats had a significantly (*) greater decrease in HR after the alpha-2 agonist, medetomidine, was administered on all days, except day 35. The moderate stress protocol ended on day nine, when the environmental enrichment part of the study began.

<table>
<thead>
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<th>Per cent change in HR after medetomidine administration</th>
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<td>Day 1</td>
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<td>Day 35</td>
<td>20%</td>
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**Figure 2.6A and 2.6B:** Respiratory rate taken before (A) and after (B) 20mcg/kg of medetomidine was administered into the epaxial muscle of cats. The FIC cats had significantly lower respiratory rates compared to healthy cats (p=0.033), throughout the study. Both groups had a significant decrease in respiratory rate after the medetomidine was administered (p<0.0001). No significant difference was noted between days (p=0.43). The moderate stress protocol ended on day nine, when the enrichment part of the study began.
Blood pressure before medetomidine

Blood pressure after medetomidine

**Figures 2.7 A and 2.7B**: Blood pressure measurements assessed both before (A) and after (B) 20mcg/kg of medetomidine was administered. There was no significant difference in blood pressure between groups (p=0.42). All cats mean blood pressures were significantly lower ten minutes after medetomidine was given (p<0.0001). The moderate stress protocol ended on day nine, when the environmental enrichment part of the study began.
Figure 2.8: Sedation score in FIC and healthy cats taken 10 minute after 20mcg/kg of medetomidine was administered intramuscularly. No significant difference was found between the two groups (p=0.15). The moderate stress protocol ended on day nine, when the environmental enrichment part of the study began.
Figure 2.9A and 2.9B: Pupil size before (A) and after (B) 20mcg/kg of medetomidine was administered intramuscularly to FIC and healthy cats. The percent change in pupil diameter was significantly greater on days 3, 8, and 35 compared to day 1 (p=0.0031), and the increase in pupil diameter following medetomidine administration was greater in healthy cats compared to FIC cats (p=0.0039; Figure 2.8A and 2.8B). The moderate stress protocol ended on day nine, when the enrichment part of the study began.
Figures 2.10-2.15: Catecholamine and metabolite concentrations in 6 FIC and 5 healthy cats. Plasma dihydroxyphenylalanine (DOPA), norepinephrine (NE), dihydroxyphenylglocol (DHPG) concentrations were significantly increased in FIC cats at all times (p=0.04, p=0.03, p=0.04 respectively). Although not statistically significant, plasma dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) were oftentimes elevated in FIC cats compared to healthy ones (p=0.09, p= 0.08 respectively). There was no significant difference in plasma EPI concentrations between the two groups (p=0.17). The moderate stress protocol ended on day nine, when the enrichment part of the study began.
REFERENCES


35. Ghelardini C., Galeotti N., Bartolini A. Antinociception induced by amitriptyline and imipramine is mediated by alpha2A-adrenoceptors. *Jpn J Pharmacol* 2000;**82**:130-137


CHAPTER 3

SMALL ADRENAL GLANDS IN CATS WITH FIC

ABSTRACT

We documented an uncoupling of sympathetic nervous system activity from the hypothalamic-pituitary-adrenal (HPA) axis in cats with feline interstitial cystitis (FIC). Altered HPA activity recently was 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 further investigate adrenal function in cats with feline IC (FIC), we determined cortisol responses to 125 µg of synthetic adrenocorticotrophic hormone (ACTH), as well as adrenal size and histology.

ACTH stimulation studies were performed on 11 healthy and 20 FIC cats. Adrenal glands obtained at necropsy of 8 healthy and 13 FIC cats were weighed, measured, and examined histologically.

FIC cats had significantly reduced responses to ACTH (P<0.05; 2-way repeated measures ANOVA). Weight (60±49 vs. 247±46 mg; mean±s.d.) and volume (263±52 vs. 453±104 mm³) of adrenal glands were significantly smaller in cats with FIC (P<0.05) than in healthy cats.
These results suggest that cats with FIC may have mild primary adrenal insufficiency. Reduction in adrenal size was observed in patients with chronic fatigue syndrome, which can be a comorbid condition in some IC patients. If these abnormalities are confirmed in human IC patients, hormone replacement therapy may be indicated in selected cases.

**INTRODUCTION**

Interstitial cystitis (IC) is a chronic pelvic pain syndrome of unknown cause and for which no generally accepted treatment is available. The symptoms of IC include pain referable to the urinary bladder, or increased frequency and urgency of urination, or both. IC may affect more than 700,000 American women, and a large number of men with prostatitis or prostatodynia. A comparable disorder of domestic cats is a common veterinary problem; we have termed this syndrome feline interstitial cystitis (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. Non-stressed FIC cats had significantly increased plasma norepinephrine concentrations compared to healthy cats, but when challenged with corticotropin releasing factor, no differences in ACTH or cortisol concentrations were identified. Lutgendorf, et al., recently reported cortisol concentrations in the urine and saliva (measures of free cortisol) of humans with IC. Although average urine or salivary cortisol concentrations did not differ between patients and controls, IC patients with higher morning cortisol concentrations reported significantly less pain and urgency. In other studies, hypocortisolism has been identified in patients with chronic pelvic pain and stress-related bodily disorders.
Although our initial studies did not identify large differences in HPA axis function between healthy cats and cats with FIC, the results did suggest the possibility of a more subtle HPA abnormality. To investigate HPA axis function in cats with FIC further, we conducted a series of experiments to compare adrenal gland function, size and morphometrics in affected and healthy cats.

MATERIALS AND METHODS

Animals:

All cats with FIC were obtained as donations from clients due to a history of chronic recurrent stranguria, hematuria, pollakiuria, or urinations in inappropriate locations, or a combination of the above symptoms, and were evaluated at The Ohio State University Veterinary Teaching Hospital. Evaluation consisted of a complete physical examination (including body weight), CBC, serum biochemical analysis, urinalysis, urine bacteriologic culture, and cystoscopy. Cystoscopy was performed, using a 9-F rigid pediatric cystoscope (Karl Storz, Endoscopy America Inc, Culver City, Calif.) in female cats; a 3-F flexible fiber optic cystoscope (Five Star Medical Inc, San Jose, CA) was used in the male cats. The diagnosis of FIC was based on compatible history, and consideration of standard inclusion and exclusion criteria after obtaining the results of the above laboratory tests, including the presence of submucosal petechial hemorrhages (glomerulations) at cystoscopy. 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 were used 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 responsive to ACTH was tested in 11 healthy cats and 20 cats with FIC. Cats were evaluated at various times during the day between 1000 and 1600 hours (cats do not have diurnal cortisol rhythms). ACTH stimulation tests were performed within 2-4 days of the cat’s arrival at the university, which is considered a moderately stressful time for our patients. A pre-test blood sample was collected from an external jugular vein and placed immediately in a non coagulant-containing glass tube. Serum cortisol concentrations were analyzed using a chemiluminescent method in the hospital laboratory. All cats were then given the standard feline dose (125 µg) of synthetic ACTH, (Cortrosyn®, Organon Inc., West Orange NJ), by intramuscular injection. Blood was collected at 30 and 60 minutes after ACTH administration and analyzed for cortisol concentration in the exact same manner as the pre-test sample.

**Adrenal Gland Collection:**

Adrenal glands were evaluated in 5 FIC and 3 healthy cats where ACTH stimulation tests were performed. An additional 8 cats with FIC and 6 healthy cats where ACTH stimulation tests were not available were also evaluated. Not every cat that had an ACTH stimulation test could be evaluated by necropsy; only those cats that were being euthanized for other studies were analyzed. All cats were euthanized by one of two methods: 10 of the cats were deeply anesthetized with sodium pentobarbital (25 mg/kg IV, Abbott Laboratories, North Chicago, IL) and perfused transcardially through the ascending aorta with saline followed by a fixative containing 4% paraformaldehyde in phosphate buffer. The adrenal glands were removed and placed in the fixative for 8 hours and then transferred to 0.1M phosphate buffer containing 30% sucrose (pH 7.3) for
48 hours. 11 of the cats were euthanized using 120mg/kg Beuthanasia-D Special solution (Shering-Plough Animal Health Corp., Kenilworth, NJ). Immediately after euthanasia, the adrenal glands were carefully removed and placed in neutral buffered formalin (1:10, Accra Lab, Swedesboro, NJ). 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 milligram. Cats were weighed to the nearest gram 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 Adobe Photoshop Elements (Adobe Systems, Inc., San Jose, CA) to measure the areas of the three zones of the cortex and the medulla as a percentage of total adrenal gland section area. Comparisons between groups for adrenal gland measurements were made using the Student’s t–test. A two-way repeated measures ANOVA and Tukey Kramer post hoc test for adjusted pairwise comparisons was used to compare cortisol concentrations between groups after ACTH stimulation. The relationship between baseline, 30 minute (T30), and 60 minute (T60) serum cortisol concentrations and adrenal gland size (expressed as a % of body weight) was tested by examining the correlations between the cortisol concentrations and the size of the glands. (Prism, GraphPad Software Inc., San Diego, CA).
RESULTS

All cats used in these studies were domestic short haired cats and no differences in breed were found. Significant differences in gender distribution, age of the cats in the ACTH stimulation studies, and weight in the cats that underwent full necropsies were found (Table 3-1). Cortisol responses of healthy cats to ACTH stimulation were within hospital and published normal ranges. Cats with FIC had significantly reduced serum cortisol responses to ACTH (Figure 3-1. mean±s.d. group effect P<0.005, time effect P<0.0001; 2-way repeated measures ANOVA). The weight (158±50 vs. 241±60 mg) and volume (264±72 vs. 410±115 mm³) of adrenal glands were significantly smaller in cats with FIC (P<0.01 for all comparisons by 2-tailed t-test) than in healthy cats. Cats with FIC had significantly smaller glands when adrenal weight was normalized to the cat’s body weight (3.2%±1.6% vs. 6.5%±1.7% body weight, p<0.0001, Figure 3.2). Absolute gross adrenal weights from cats with FIC were also significantly smaller compared to healthy cats (.159±.014 vs. .244±.021 gm, p=0.003, Figure 3.3). No correlation was found between serum cortisol concentrations and adrenal gland size (baseline r²=0.22, T30 r²=0.08, T60 r²=0.11).

Although no obvious histological abnormalities were identified, the relative areas of the zonae fasciculata and reticularis were significantly smaller (P=.0004) in sections of glands from cats with FIC than from healthy cats (Table 3-2). No differences between groups were found in the relative area of the medulla.
DISCUSSION

The most significant finding in this study was the dramatic reduction in the size of the adrenal glands of cats with FIC. The individual cat ACTH stimulation tests results were variable, however, and did not correlate well with the cat’s adrenal gland size. We previously reported that we could not identify differences in ACTH and cortisol concentrations between 4 healthy cats and 4 cats with FIC after injection of corticotrophin releasing factor under minimally stressful circumstances. A larger number of cats evaluated under maximal stressful conditions in this study could have allowed the differences to become evident. Although group differences in cortisol responses to ACTH were statistically significantly smaller in cats with FIC than in healthy cats, the individual differences would not have been considered clinically abnormal in all cases. We have however observed that plasma ACTH concentrations increased in cats with FIC when stressed, suggesting that the hypothalamic and pituitary components of the HPA axis responded appropriately and the abnormalities we observed are most likely localized to the adrenal gland itself. These findings suggest that adrenal gland function may be relatively normal under unstressed conditions, but not respond adequately during stressful circumstances.
Baseline ACTH and cortisol concentrations in both healthy cats and humans appear to vary widely among studies. The ACTH and cortisol responses to provocative stimulation also are quite variable, depending on the type of test, the dose of the agent used, and even the type of ACTH used. The variability in results renders the predictive value of the available tests inadequate for diagnosis of marginal adrenal insufficiency. In these circumstances, direct visualization of the adrenal gland resulted in identification of significant differences.

The relationship of ACTH release and adrenal cortical responsiveness has been debated in other diseases wherein small adrenal gland size with or without hypocortisolism sometimes occurs. In humans, a comparable reduction in adrenal gland size has been reported in patients with chronic fatigue syndrome. These authors suggested that small size might have been the result of primary or secondary adrenal insufficiency. They concluded that the defect was more likely to be secondary, due to reduced ACTH release.

Histological evaluation of the cat adrenal glands revealed that the reduction 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 both groups. Preliminary morphometric evaluation found significant reductions in the size of the zonae fasiculata and reticularis (p<0.0004). Although no significant differences were noted in the zona glomerulosa, preliminary data from our lab 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, reduced stimulation by, or sensitivity to, ACTH, and immune-mediated (e.g., antibodies to the 21α hydroxylase enzyme, infectious, or inflammation induced degeneration. Pre-existing hypoplasia could not be ruled out in the present study, but might be investigated in relatives of affected individuals. The difference in adrenal gland size did not appear to be due to on-going immune-mediated, infectious, or inflammation induced degeneration based on histopathologic examination of the glands, although the possibility of a previous occurrence causing the damage could not be excluded. Because IC previously has been suggested to have features of auto-immune disease, we investigated the presence of circulating anti-adrenal antibodies in six human patients (because this test is currently not available for cats) and found all to be negative (data not shown). Thus, whether the small adrenal glands are a cause, consequence or unrelated to FIC remains to be determined.

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

Similar to humans, anxiety is commonly associated with lower urinary tract problems in cats. We found that 60% of patient cats with no clinical signs other than urinating outside the litter box had lesions consistent with FIC at cystoscopy in one study.
Also, cats restricted to indoor living are some 5 times more likely to develop urinary problems than cats allowed outdoors.\textsuperscript{16} Thus, a similar combination of physiological and behavioral abnormalities may affect both cats and humans with IC.

The present study has important limitations. First, the dose of ACTH, although standard, was supraphysiologic, and may not have been appropriate for identification of the relatively moderate extent of adrenal insufficiency present in these cats. Similar observations have been made in humans.\textsuperscript{17} Other limitations include the variability in age and neuter status of the healthy cats compared with the 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 have been reported to increase in size with age and neutering.\textsuperscript{18} Females were reported to have slightly larger adrenal glands in that study as well. Therefore, these differences are less likely to explain the results we observed. Based on the distribution of the adrenal gland size data (Figure 3-2), it is possible that two populations may have been represented within each group, although no significant differences in age or weight were found to account for them. Studies to specifically address all of these limitations are in progress.

Reduced HPA axis function may play a role in the pathophysiology of some of the signs, including the alterations in sensory\textsuperscript{19} and sympathetic function\textsuperscript{20} and possibly some of the “autoimmune” aspects of IC. Based on the lack of predictive value of currently available tests of HPA axis function, we suggest that studies of the HPA axis of patients with IC include evaluation of adrenal size and function.
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<td>0.13</td>
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<tr>
<td>Weight</td>
<td>3.8 ± 1.1</td>
<td>5.2 ± 1.6</td>
<td>0.05</td>
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<tr>
<td>ACTH Stim</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gender</td>
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<td>9 MN, 1 FI, 10 FS</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2.8 ± 2.0</td>
<td>6.9 ± 4.3</td>
<td>0.005</td>
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<tr>
<td>Weight</td>
<td>5.1 ± 1.4</td>
<td>5.3 ± 1.4</td>
<td>0.82</td>
</tr>
</tbody>
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**Table 3.1:** Age, weight and gender distributions in healthy cats and cats with FIC. Although some (3 healthy and 5 FIC) cats had both ACTH stimulation tests and full necropsies, it was not possible to obtain necropsy results on all cats where stimulation tests were performed.

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n=4)</th>
<th>FIC (n=7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Mean Medulla ± SD</td>
<td>12 ± 5</td>
<td>18 ± 6</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Mean Glomerulosa ± SD</td>
<td>11 ± 3</td>
<td>13 ± 1</td>
<td>0.07</td>
</tr>
<tr>
<td>% Mean Fasciculata &amp; Reticularis ± SD</td>
<td>79 ± 3</td>
<td>70 ± 5</td>
<td><strong>0.004</strong></td>
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</tbody>
</table>

**Table 3.2:** Comparison of adrenal gland morphology between healthy cats and cats with FIC. The areas of the zones of the cortex and the medulla were calculated as a percentage of total adrenal section area. Cats with FIC had significantly smaller zones consisting of the fasciculata and reticularis, whereas there was a trend for the zona glomerulosa to be larger. No significant differences were noted in the relative area of the medulla.
**Figure 3.1:** Serum cortisol responses (mean ± S.D) to 125 µg synthetic ACTH administered intramuscularly to FIC and healthy. The cortisol response of FIC cats was significantly less than that observed in healthy cats (P<0.05, two way repeated measures ANOVA). Results of Tukey-Kramer post hoc tests comparing groups at each time point are presented in the figure.

**Figure 3.2:** Adrenal gland weight as a percent of cat body weight. (the horizontal line demarks the mean of each group). Weights of adrenal glands from cats with FIC were significantly smaller compared to healthy cats. (p<0.0001)
Figure 3.3: Absolute gross adrenal gland weight. (the horizontal line demarks the mean of each group). Weights of adrenal glands from cats with FIC were significantly smaller compared to healthy cats. (p=0.003)
REFERENCES


   *J Urol*, **168**: 1054, 2002

   with interstitial cystitis. *J Urol*, **165**: 2051, 2001

4. Lutgendorf, S. K., Kreder, K. J., Rothrock, N. E., Hoffman, A., Kirschbaum, C.,
   Sternberg, E. M. et al.: Diurnal cortisol variations and symptoms in patients with

   pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology, **25**: 1,
   2000

6. Kemppainen, R. J., Peterson, M. E.: Domestic cats show episodic variation in plasma
   concentrations of adrenocorticotropic, alpha-melanocyte-stimulating hormone (alpha-
   MSH), cortisol and thyroxine with circadian variation in plasma alpha-MSH


   insufficiency. *J Clin Endocrinol Metab*, **79**: 923, 1994

   glands in chronic fatigue syndrome: a preliminary computer tomography study.
   Psychoneuroendocrinology, **24**: 759, 1999

    Trends Endocrinol Metab, **13**: 373, 2002

    Autoantibodies to steroidogenic enzymes in autoimmune polyglandular syndrome,
    Addison's disease, and premature ovarian failure. *J Clin Endocrinol Metab*, **81**: 1871,
    1996

    Journal of Urology*, **167**: 694, 2002
CHAPTER 4

COMPUTED TOMOGRAPHY, NOT ULTRASOUND, IDENTIFIES SMALL ADRENAL GLANDS IN CATS WITH INTERSTITIAL CYSTITIS

ABSTRACT
To evaluate the feasibility of identifying small adrenal glands in cats with feline interstitial cystitis (FIC) using computed tomography (CT) and ultrasonography.

Adrenal glands of 10 cats with FIC and 11 healthy cats were evaluated using CT. 8 FIC cats and 9 healthy cats also had ultrasound evaluations.

Cats were anesthetized with isoflurane and CT scans pre and post-contrast medium-enhancement, were performed. Adrenal gland volumes were calculated and normalized to body weight for each cat. Ultrasound measurements were obtained and adrenal gland volumes calculated using the following formula: \((L \times W \times H) \times \frac{\pi}{6}\). Both of these measurements were compared to a group of 15 FIC and 11 healthy cats where necropsies were available and actual adrenal volume measurements could be obtained.

Cats with FIC had significantly smaller adrenal gland volumes compared to healthy cats when evaluated by CT or necropsy. Adrenal gland volumes calculated by ultrasound were not significantly different from normal cats.

Results of this study suggest that CT scans can be used to accurately evaluate adrenal gland volumes in cats. Caution should be used when utilizing ultrasound using the standard formula in the literature. The reason for the small adrenal glands remains
unknown at this time, but future studies to evaluate the hypothalamic-pituitary-adrenal axis should be done in cats with FIC. CT scans of their adrenal glands could be used as a diagnostic tool if sensitivity and specificity are determined.

**INTRODUCTION**

Interstitial cystitis (IC) is a chronic pelvic pain syndrome in women for which there is no known cause or generally accepted treatment.¹ The symptoms of IC include pain referable to the urinary bladder, or increased frequency and urgency of urination, or both. IC may affect more than 700,000 American women,² and a large number of men with prostatitis or prostatodynia.³ A comparable disorder of domestic cats is a common veterinary problem; we have termed this syndrome feline interstitial cystitis (FIC).⁴

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.⁵ Non-stressed FIC cats had significantly increased plasma norepinephrine concentrations compared to healthy cats, but when challenged with corticotropin releasing factor, no differences in ACTH or cortisol concentrations were identified between groups. Lutgendorf, et al., recently reported cortisol concentrations in the urine and saliva (measures of free cortisol) of humans with IC.⁶ Although average urine or salivary cortisol concentrations did not differ between patients and controls, IC patients with higher morning cortisol concentrations reported significantly less pain and urgency.
We have determined a sub-maximal cortisol response in FIC cats under stressful conditions after 125µg of synthetic ACTH is administered, suggesting a decreased adrenal cortical response in FIC cats under these conditions. In this study we also reported that adrenal glands weighed significantly less in cats with FIC compared to healthy cats. To determine if ante mortem identification of small adrenal glands in cats with FIC was feasible, we conducted a series of experiments to compare adrenal gland size by computed tomography (CT) and ultrasound.

MATERIALS AND METHODS

Animals:

All cats with FIC were obtained as donations from clients due to a history of chronic recurrent stranguria, hematuria, pollakiuria, urinations in inappropriate locations, or a combination of the above symptoms, and were evaluated at The Ohio State University Veterinary Teaching Hospital. Evaluation consisted of a complete physical examination (including body weight), CBC, serum biochemical analysis, urinalysis, urine bacteriologic culture, and cystoscopy. Cystoscopy was performed, using a 9-F rigid pediatric cystoscope (Karl Storz, Endoscopy America Inc, Culver City, CA.) in female cats; a 3-F flexible fiber optic cystoscope (Five Star Medical Inc, San Jose, CA.) was used in the male cats. The diagnosis of FIC was based on compatible history, and consideration of standard inclusion and exclusion criteria after obtaining the results of the above laboratory tests, including the presence of submucosal petechial hemorrhages (glomerulations) at cystoscopy. Healthy, age-matched cats free of disease and signs referable to the lower urinary tract according to the same diagnostic criteria as cats with FIC were used as controls. All cats were housed in stainless steel cages in the college
animal facilities and fed a standard commercial diet. The Animal Care and Use Committee of The Ohio State University approved all the experimental procedures described.

**Computed tomography**

10 FIC and 11 healthy cats were anesthetized with isoflurane and positioned in sternal recumbency on the bed of a fourth generation CT scanner (Picker PQS, Philips Medical Systems, N.A., Bothell, WA.). The acquisition parameters were as follows: kVp - 130; mA - 125; algorithm – standard. All image sequences were acquired while manually insufflating the lungs to prevent respiratory motion. A dorsal pilot image of the abdomen was made for image acquisition planning. For the first phase, helical slices (5 mm collimation) of the abdomen were made from the cranial aspect of the liver through the caudal aspect of the left kidney. The initial sequences were reviewed to locate the adrenal glands and plan for the second phase of data acquisition. For the second phase, helical slices (2 or 3 mm collimation) of the adrenal glands (and surrounding structures) were obtained. The second phase started in the caudal part of the liver, cranial to the right kidney and ended with the mid aspect of the left kidney. For the third phase, phase 2 was repeated after contrast medium administration. Iohexol 240 mgI/ml (Omnipaque®, Nycomed Inc., Princeton, NJ) was injected intravenously via a cephalic vein catheter using a dosage of 0.45 ml/kg (1.0 ml/lb).

**CT Image Analysis**

Using the post - contrast medium - enhanced images (Figures 4-1 and 4-2), the volume of each adrenal gland was calculated similar to methods for volume estimation of the spleen. On each slice that contained adrenal gland, the margin of the adrenal gland
was traced and the CT computer generated the area (mm$^2$) contained within this region of interest (ROI). The area from each ROI was multiplied by the slice thickness, and the results of all slices were added, to determine the volume of each adrenal gland (mm$^3$).

**Ultrasonography**

Eight FIC cats and nine healthy cats were anesthetized with isoflurane and positioned in dorsal recumbency. The adrenal glands were imaged using an 8.5 MHz curved array transducer (Acuson Sequoia, Siemens Medical Systems, Ultrasound Division, Mountain View, CA.) using described techniques.$^{10}$ On longitudinal images, the length (L) and height (H) of each adrenal gland was determined using the ultrasound machine electronic calipers. On transverse images, the height (H) and width (W) of each adrenal gland was determined. The volume of each adrenal gland was calculated using the following formula for a prolate ellipse: $\text{volume} = (L \times W \times H) \times \pi/6^{11}$. The largest height value was used for calculations.

**Adrenal Gland Volume Measurements:**

We previously reported adrenal gland weight as a percentage body weight in 13 cats with FIC and 8 healthy cats.$^{7}$ Two other FIC and three healthy cats were euthanized and adrenal glands were obtained in the same manner we previously reported. To evaluate the accuracy of the CT and ultrasound measurements, volumes were obtained on all adrenal glands by performing measurements made at the widest portion of the glands for length, width and thickness.

**Statistical analysis**

Comparisons between groups were made using the Student’s two-tailed t tests. (Prism, GraphPad Software Inc., San Diego, CA.)

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RESULTS

In all groups, FIC cats were older and heavier than healthy cats, so adrenal gland volumes were standardized to body weight. Gender distribution also was different in the group of cats who had ultrasounds and those where full necropsies were performed (Table 4-1). Using CT, the adrenal gland volume (per kg body weight) was significantly lower in FIC cats (47.9 ±14.0 vs. 85.6.0±13.3; mean vol/BW (kg) ±s.d., p<0.001; Figures 4-3). A similar pattern was found when the data were not normalized to body weight (216.8 ± 21.5 vs. 278.0 ± 24.3, p=0.07, Figure 4-3). Cats with FIC had significantly smaller adrenal gland volumes/body weight (kg) (46.6 ± 4.3 vs. 101.0 ± 13.1 body weight, p<0.0001; Figure 4-4). No significant difference in adrenal gland volume/body weight was present between FIC and healthy cats using ultrasound derived volumes (15.1 ± 1.8 vs. 19.5 ± 3.9; mean vol/BW (kg) ± s.d., p=.3364).

DISCUSSION

We recently reported a decrease adrenocortical response to ACTH in FIC cats, but the individual differences in the ACTH stimulation tests did not appear clinically abnormal in several cases and adrenal gland size was more reliable in distinguishing healthy cats from ones with FIC.⁷ We have now documented that CT is a reliable, non-invasive means to measure adrenal gland volumes in cats. This finding is important for two reasons. First, adrenal gland CT may be used as a non-invasive screening tool for detection of FIC and possibly other disorders in cats. Second, it might now be possible to serially evaluate cats with FIC relatively non-invasively. This would permit investigation of relationships between adrenal gland volume and urinary bladder lesions and clinical signs in cats with FIC. If adrenal gland size does not increase with complete resolution
of clinical signs, it would suggest the small adrenal glands were present early in the disease course (possibly prior to the onset of clinical signs). We have only had the opportunity to assess adrenal gland volume by CT in two cats that have been free of clinical signs for several years. The sizes of their adrenal glands were still in the range of cats with FIC we reported in this study.

Significant differences in adrenal gland volume / body weight between healthy cats and FIC cats were found using CT data but not using ultrasound data. One reason for the difference is the methods used to calculate the adrenal gland volume. Using CT, we were able to trace the contour of the adrenal glands on multiple thin slices and add the area of these slices to determine adrenal gland volume. The mean percentage error of volume calculations using the sum – of – areas CT technique was 3.9% for eight dog kidneys.\textsuperscript{12} Irregular contours of an organ are more easily accounted for using the sum – of – areas CT technique. Using ultrasound, the dimensions of the adrenal glands were estimated and the volume of each gland was calculated using the formula for a prolated ellipse. A sum – of – areas technique is currently not possible using ultrasonography. Because CT scanning is almost completely automated, it is not subject to error related to manual operation as in ultrasonography.\textsuperscript{12} In people, CT is the modality of choice for imaging adrenal glands,\textsuperscript{13} although three-dimensional ultrasound may improve image analysis.\textsuperscript{10,14}
When using gross specimen measurements as a gold standard for adrenal gland size, CT appeared to accurately measure these organs. This is contrary to reports in people, where CT scans underestimated the size of adrenal tumors.\textsuperscript{15} We may have been more accurate because cat adrenal glands are elliptical whereas human adrenal glands are “boomerang” shaped making them more difficult to measure.

Abnormalities in the HPA axis have been reported in humans with chronic fatigue syndrome,\textsuperscript{16} a common comorbid condition in humans with interstitial cystitis. Using CT, Scott et al., documented significant reductions in adrenal width in patients with chronic fatigue syndrome compared with healthy patients.\textsuperscript{16} We did not evaluate whether or not adrenal gland width was significantly different between healthy and FIC cats because volume determination was a more global parameter, and because of the difference in the shape of the glands between species.

We previously reported that histological evaluation of the cat adrenal glands revealed the reduction in size was limited to the adrenal cortex. Variable degrees of nodular hyperplasia were observed in some glands from cats in both groups. Morphometric evaluation found significant reductions in the size of the zonae fasciculata and reticularis (p=0.03).\textsuperscript{7}

The etiology of the small adrenal glands was not identified. Potential explanations for the small glands include genetic or developmentally related hypoplasia,\textsuperscript{17} reduced stimulation by, or sensitivity to ACTH, and immune-mediated (e.g., antibodies to the 21\alpha hydroxylase enzyme,\textsuperscript{18} infectious, hemorrhage, necrosis or inflammation induced degeneration. Pre-existing hypoplasia could not be ruled out in the present study, but might be investigated in relatives of affected individuals. The difference in adrenal gland
size did not appear to be due to on-going disease induced degeneration based on histopathologic examination of the glands, although the possibility of a previous insult causing the damage could not be excluded. Thus, whether the small adrenal glands are a cause, consequence or unrelated to FIC remains to be determined.

The present study has important limitations. Some variability in age, neuter status, and weight of the healthy cats compared with the cats with FIC was present. Nemeroff et al. reported no correlation between adrenal gland size and age in humans\textsuperscript{19} and adrenal glands increased in size with age and neutering in sheep\textsuperscript{20} Douglass et al. reported when canine adrenal glands were evaluated ultrasonographically, that there was a significant positive correlation with age and adrenal the length of the left adrenal gland.\textsuperscript{21} Even though the FIC cats were older, their adrenal glands were actually smaller; therefore these differences are less likely to explain our results.

Other limitations include 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 for FIC. Studies are currently underway to address these issues.

CONCLUSIONS

Cats with FIC had smaller adrenal glands than healthy cats when evaluated by CT. The CT measurements were very similar to another group of cats where gross evaluation and measurement of the glands was possible. The reason for the small adrenal glands is unknown at this time, but the areas in the cortex that produce cortisol, androgens and neurosteroids were smaller. CT could be used as a screening tool for cats suspected of having FIC. Once the sensitivity and specificity are evaluated, CT scans of
adrenal glands could be a useful diagnostic tool. In addition to analyzing adrenal gland size, future studies should evaluate of the HPA axis in cats with FIC including a more comprehensive analysis of adrenal hormone concentrations.
Table 4.1: Gender, age, and weights of cats in each group that were evaluated. A larger number of spay animals were evaluated in the FIC group throughout. FIC cats were also heavier and older in the CT group. Cats who had ultrasounds were older in the FIC group and those cats who were necropsies were heavier in the FIC group. MI=male intact. MN=male neutered. FI=female intact. FS=female spay.

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>FIC</th>
<th>P Value</th>
</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>8 FI, 3 FS</td>
<td>3 MN, 7 FS</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>3.7 ± 2.8</td>
<td>6.9 ± 4.5</td>
<td>0.066</td>
</tr>
<tr>
<td>Weight (kg)</td>
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<td></td>
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<td>8 FS</td>
<td></td>
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<tr>
<td>Age (yr)</td>
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<td>6 MN, 1 FI, 8 FS</td>
<td></td>
</tr>
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<td>Weight (kg)</td>
<td>3.8 ± 1.0</td>
<td>5.2 ± 1.3</td>
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</tbody>
</table>

Figure 4.1: Dorsal plane reconstruction, CT image of the mid cranial abdomen of an FIC cat made after the intravenous injection of iohexol. The left adrenal gland (white arrowhead) is the ovoid soft tissue density left and lateral to the aorta. The right kidney (RK) is adjacent to the left kidney (LK).
Figure 4.2: Transverse image of the mid cranial abdomen of an FIC cat made after the intravenous injection of iohexol. The right adrenal gland (white arrowhead) is located in the mid dorsal aspect of the abdomen, right lateral to the caudal vena cava.

Adrenal gland volumes assessed by CT

Figure 4.3: Absolute adrenal volume assessed by CT scan in FIC and healthy cats. Adrenal volume was lower in FIC cats (p=0.07).
Figure 4.4: Adrenal gland volumes as a percent body weight when analyzed by necropsy, CT scans, and ultrasound. The horizontal line demarks the mean of each group. Upon gross examination, the adrenal volume (per kg body weight) was significantly lower in FIC cats (p=0.0034). Similarly, volumes calculated by CT scans were significantly smaller in FIC cats (p<0.0001). No differences were noted in the gland size when evaluated by ultrasound. Volumes obtained by CT were comparable to those obtained at necropsy, but ultrasound data were not.
REFERENCES


CHAPTER 5

EFFECTS OF STRESS ON URINE NERVE GROWTH FACTOR IN CATS WITH INTERSTITIAL CYSTITIS

ABSTRACT

Interstitial cystitis (IC) is a chronic pelvic pain disorder in humans and a comparable disease has been described in the domestic cat (FIC). Nerve growth factor (NGF) has been investigated in IC because it is associated with nociceptive processing, mast cells, and chronic inflammatory processes, which have all be reported in patients with IC. NGF concentrations can also be altered by stress. To investigate the effects of stress on NGF in cats with FIC, food was removed 12 hours prior to evaluating 8 FIC cats and 8 healthy cats. All cats were then placed in metabolism cages and urine was collected daily for one week. A complete urinalysis including specific gravity was performed on all urine samples and 24-hour urine volume was recorded. NGF was analyzed by ELISA (NGF Emax® Immunoassay, Promega Corp.) on urine supernatants on days 1, 2 and 7. On day one, hematuria was detected in the FIC cat. Two FIC cats developed hematuria that persisted, but lessened throughout the one-week period. One healthy cat (a genetic sister to an FIC cat) also had persistent hematuria. On day one, the FIC cat had higher urinary pH when evaluated by urine dipstrip. FIC cats had significantly lower urine pH values on day 4 (6.8 vs 7.4; p=0.01). One FIC cat were considered an outlier and these data
were removed. On day 2, cats with FIC had significantly lower 24-hour urine NGF concentrations compared to healthy cats (4043 ± 2001 vs. 7788 ± 3271; mean± std dev; p=0.03). No significant differences were found on days 1 or 7.

Urinary excretion of NGF was lower in FIC cats compared to healthy cats only during the initial part of the study, suggesting that stress decreases NGF excretion in these cats. A small study reported higher NGF concentrations in the urine of IC patients. Others reported no differences in NGF concentrations in the seminal fluid of men with chronic pelvic pain, and increased NGF in the bladder of cats with FIC. Because it appears that stress can alter NGF concentrations in the urine, protocols for analyzing NGF should be standardized.

INTRODUCTION

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 urinary bladder, or increased frequency and urgency of urination, or both. IC may affect more than 700,000 American women,² and a large number of men with prostatitis or prostatodynia.³,⁴ A comparable disorder of domestic cats is a common veterinary problem; we have termed this syndrome feline interstitial cystitis (FIC).⁵ Cats exhibit similar clinical signs such as hematuria, urinary frequency, and exacerbations under stressful conditions. Furthermore, based on their behaviors, it also appears that pain is a prominent feature of FIC as well.

Nerve growth factor (NGF) has been investigated in IC because it is associated with nociceptive processing, mast cells, and chronic inflammatory processes, which have all be reported in patients with IC. NGF is essential for the development of all primary
sensory nociceptors, but its function is not restricted to prenatal development. NGF can bind to its receptors, trkA (high affinity) or p75 (low affinity), and continues to be expressed in the adult nervous system. It is associated primarily with sympathetic neurons.\textsuperscript{6,7} The neurotrophins, in particular NGF, might play a significant role in both the clinical and pathological features of IC.

The expression of NGF, as well as other neurotrophins could increase in diseases such as IC, and alter the nerves that innervate the bladder. This can contribute to a positive feedback loop for the inflammatory cytokines in this organ. A small number of patients evaluated by Okragly et al.\textsuperscript{8} reported that NGF, NT-3, and glial derived neurotrophin factor, were increased in a few women with IC and bladder cancer. Lowe et al.,\textsuperscript{9} reported increased NGF concentrations in bladder biopsies from patients with chronic pelvic pain or sensory urgency, and immunohistochemistry revealed NGF concentrations in the urothelium where sensory fibers terminate. Others (personal communication with Dr Lori Birder) have reported elevated NGF in the bladder mucosa of cats with FIC, but not in the smooth muscle.
To evaluate the effects of stress on NGF concentrations in humans, Hadjiconstantinou et al.\textsuperscript{10} evaluated plasma NGF concentrations in people who provided continued care for a cognitively impaired spouse. When the caregivers perceived high stress or depression, elevations in NGF were also noted compared to well-matched controls. Previous studies have also found elevations in NGF in acute stress models.\textsuperscript{11} These studies could be pertinent to patients with IC because stress has been shown to increase clinical signs (hematuria and stranguria) in both cats and women with this disease.\textsuperscript{12} It is unknown if altered NGF concentrations occur during stressful conditions in FIC cats.

The purpose of this study was to determine if NGF concentrations could be evaluated non-invasively in the urine of cats and also to analyze the effect of stress on urine NGF concentrations in cats with FIC.

**MATERIALS AND METHODS**

8 FIC cats and 8 healthy cats were placed in metabolism cages. Cats were diagnosed with FIC based on previous published criteria.\textsuperscript{5} Due to space limitations, 4 cats from each group were placed into the metabolism cages; the second group of cats was analyzed three weeks later. Food was removed 12 hours prior to moving the cats to their new environment. All cats were then fed and watered by their usual caretakers and cages cleaned so the only stressor that was applied occurred at the beginning of the week. Previous studies (see chapter 2) from our lab have shown this stressor can induce clinical signs in cats with FIC and cause alterations in various stress response parameters. Urine was collected from each cat (if available) between 9am and 11am beginning on day 2. The total volume of urine collected was recorded and complete urinalysis including
specific gravity was performed. Aliquots of urine were centrifuged at 2000g for 20 minutes; the supernatant was removed and frozen at –80ºC until analysis.

NGF analysis:

Urine supernatant samples were thawed and centrifuged at 2000g for 20 minutes at 4ºC and urine NGF was assayed according to the manufacturers instructions (Promega NGF Emax ImmunoAsasy System, Madison, WI). Briefly, urine dilutions were made up to 1:50 to find the dilution that most consistently fit the standard curve; a urine dilution of 1:2 was then chosen.

Standard 96 well ELISA plates were coated with the anti-NGF polyclonal antibody and incubated overnight at 4ºC. All wells were then washed with TBST buffer (20mM Tris-HCl pH=7.6; 150 mM NaCl) and blocked with a 1:5 dilution of block and sample buffer. The plates were incubated for one hour at room temperature. After blocking the plates once more, the standard curve was prepared and added to the appropriate wells. The urine samples were then added and the plate was incubated for six hours at room temperature with shaking at 500rpm. The anti-NGF monoclonal antibody was added and plates were incubated overnight at 4ºC. The next day, all wells were washed five times and the anti-rat IgG HRP conjugate was added. After 2.5 hours of incubation at room temperature with shaking (500rpm), the color development was added. The reaction was stopped with 1N HCL and absorbance was recorded at 450 nM. Urine NGF concentrations were multiplied by the total urine volume to calculate 24-hour urine NGF.
STATISTICS

Comparisons between groups were made using the Student’s two-tailed t tests. (Prism, GraphPad Software Inc., San Diego, CA). A p value of < 0.05 was considered significant.

RESULTS

No significant differences were noted in age and weight between the two groups of cats however a larger number of spayed female cats were present in the FIC group (table 1). On day one, urine was collected from one cat in each group, but urine was available from most cats on each subsequent day (Figure 5-1). On day one, hematuria was detected in the FIC cat. Two FIC cats developed hematuria that persisted, but lessened throughout the one- week period. One healthy cat (a littermate to an FIC cat) also had persistent hematuria (Figure 5-2). On day one, the FIC cat had higher urinary pH when evaluated by urine dipstrip. FIC cats had significantly lower urine pH values on day 4 (6.8 vs 7.4; p=0.01; Figure 5-3). No significant differences in urine specific gravity were detected between the two groups. (Figure 5-4).

24- hour urine NGF concentrations were analyzed in cats on days 1, 2 and 7 to see the effects of stress on this neurotrophin. One healthy cat was considered an outlier and these data were removed. On day 2, cats with FIC had significantly lower 24-hour urine NGF concentrations compared to healthy cats (4043 ± 2001 vs. 7788 ± 3271; mean± std dev; p=0.03). No significant differences were found on days 1 or 7 (Figure 5-5).
DISCUSSION

Based on previous studies, it was not surprising that most cats did not urinate within the first 24 hours. Similar studies from our lab have demonstrated that cats do become stressed in new environments and previous data have also demonstrated that a moderate stressor will affect both groups, but usually the FIC cats are affected more severely (see chapter 2).

Cats with FIC do exhibit more clinical signs under stressful conditions and two cats with FIC developed persistent hematuria that lessened slightly over the seven-day study. One healthy cat developed hematuria and interestingly, she was a genetic relative of an FIC cat.

The most interesting finding from this study was that cats with FIC had significantly lower urine NGF concentrations after the initial moderate stressful event (removal of food and change of environment). It is possible that this neurotrophin is bound to its receptor under stressful conditions only in FIC cats, which is why less is detected in the urine. Data from Birder el al. (personal communication) supports this hypothesis, whereby FIC cats had elevated NGF concentrations in the bladder mucosa compared to healthy cats. These tissues were obtained under similar (albeit slightly more stressful) conditions as the urine from our study.
Various studies analyzing NGF in bladder tissues, plasma, seminal fluid and urine have yielded variable results, which could be due to differing protocols. To the author’s knowledge, only one study is published evaluating NGF concentrations in the urine. Although elevated NGF concentrations were found in the urine of some patients with IC, it was not specific for the disease and only four patients were analyzed. No bladder tissue from those patients was evaluated under similar circumstances for comparison.

Miller et al. evaluated NGF concentrations in seminal fluid from men with chronic prostatitis/chronic pelvic pain syndrome, a disease that has similar characteristics of IC in women. They found no significant differences in NGF concentrations in affected men compared to healthy controls, but NGF concentrations did correlate directly with pain severity. In one study evaluating the effects of chronic stress on plasma NGF, significant increases in plasma NGF were detected in the affected group. We were not able to evaluate plasma NGF in our cats because the high content of immunoglobulins in plasma would cross react with the primary antibody with the system we used.
The type of stressor encountered and the age of an individual can alter the effects of NGF concentrations. On day seven of our study, no differences were found between the two groups, suggesting that measuring NGF concentrations in the urine should be done under moderate to severe stressful conditions to differentiate the groups. Under these conditions, abnormalities in the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system have been documented in cats with FIC as well. Under minimal stress, many of these abnormalities in FIC cats were not apparent. Therefore, similar to catecholamine analyses, it is prudent to recommend that NGF concentrations should be analyzed under similar conditions in all groups of patients.

Studies in humans with chronic pelvic pain have revealed NGF concentrations in the urothelium where sensory fibers terminate and NGF is associated primarily with sympathetic nociceptive neurons. Increased numbers of sympathetic nerve fibers detected by tyrosine hydroxylase immunoreactivity are present in bladders from humans with IC, suggesting a greater amount of sympathetic nerve fibers are present in the bladders of IC patients. If NGF is bound in close proximity to these fibers, this can promote thermal hypersensitivity and mechanical hyperalgesia even in the absence of a strong cellular inflammatory component, which is typically seen in bladder biopsies from IC patients.

The present study has important limitations. First, few cats were available to assess, and perhaps a larger sample size would demonstrate a more pronounced difference between the two groups. Second, as mentioned earlier, immunoglobulins from plasma can cross react with the monoclonal antibody and cause false elevations in NGF concentrations. To avoid this possibility, all urine samples were centrifuged twice in
order to remove any contaminants and decrease this risk. Moreover, more cats with FIC had hematuria and their NGF concentrations were lower on day one suggesting this was not a problem with our study. Third, more intact females were present in the control group, and estrogen has been reported to influence the onset and course of neurogenic inflammation of the bladder. Although an estrogen antagonist has been reported to cause no alterations in NGF concentrations, it has been reported to decrease NGF mRNA. Because more intact females were in the control group, this variable should not have affected our outcome.

Studies evaluating NGF have been documented in several acute and chronic inflammatory conditions. It is possible that NGF may be protective and necessary to help in the healing process, however it may act as an "alert molecule" which is able to prime the immune system of potential noxious stimuli. The effect of NGF depends on the surrounding environment and could be difficult to predict, just as the effect of many of the cytokines on certain cells are difficult to predict. The neuroimmune and endocrine systems are tightly connected and may vary from patient to patient in certain diseases. It is not known if NGF is primarily responsible for the pain that occurs, especially during a stressful event. Future studies should include evaluation of urine and bladder mucosal biopsies simultaneously from patients with IC as well statistics to evaluate if and how NGF correlates with the clinical signs of IC and FIC.
<table>
<thead>
<tr>
<th>NGF</th>
<th>Sex</th>
<th>Age</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>3 MN, 3 FI, 1 FS</td>
<td>3.14 ± 1.07</td>
<td>4.40 ± 2.12</td>
</tr>
<tr>
<td>FIC</td>
<td>2 MN, 1 FI, 5 FS</td>
<td>5.50 ± 3.82</td>
<td>4.94 ± 1.29</td>
</tr>
</tbody>
</table>

**Table 5.1:** Gender, age, and weights of cats in both groups. No significant differences were noted in age and weight, however more spayed female cats were present in the FIC group.

![24 Hr Urine Volumes](image)

**Figure 5.1:** 24 hour urine volumes collected from each FIC and healthy (H) cats. The horizontal line demarks the mean for each group. No significant differences were noted in urine volumes during the 7 day study.
**Microscopic Hematuria**

![Graph showing microscopic hematuria](image)

**Figure 5.2:** Hematuria recorded from FIC and healthy (H) cats. There were two FIC cats who developed hematuria that waxed and waned throughout the 7 days. One healthy cat also had hematuria; this cat is a genetic sister to an FIC cat.

**24 Hour Urine pH**

![Graph showing 24 hour urine pH](image)

**Figure 5.3:** Urine pH analyzed by dipstrip from FIC and healthy (H) cats. The horizontal line demarks the mean of each group. The FIC cat’s pH was higher on day one. On day four, FIC cats had significantly lower urine pH compared to healthy cats (p=0.01).
Figure 5.4: Urine specific gravity detected from FIC and healthy (H) cats. The horizontal line demarks the mean of each group. No significant differences were detected throughout the study.

Figure 5.5: 24 hour urine NGF from urine obtained on days 1,2 and 7 from FIC and healthy (H) cats. The horizontal line demarks the mean of each group. Cats with FIC had significantly lower NGF concentrations on day 2 compared to healthy cats (p=0.03).
REFERENCES


Chapter 6

Conclusion and Treatment Options for Cats with FIC

Interstitial cystitis (IC) in humans has been characterized as a chronic lower urinary tract syndrome of unknown cause and with no generally accepted treatment. The symptoms of IC include variable combinations of dysuria, hematuria, and chronic pelvic pain. The diagnosis of IC still remains a diagnosis of exclusion and no one test is available yet to help identify IC patients. One of the most valuable resources for understanding a cryptogenic disorder like IC is the availability of a naturally occurring disease model in another species that permits investigations not possible in the target species. The most relevant naturally occurring model of IC in humans is feline interstitial cystitis (FIC) in cats. Until an identified etiology, specific pathogenesis, or pathognomonic symptom complex is identified in patients with IC, the relevance of induced animal models can only be evaluated in the context of a bladder injury and the body's response to whatever "threat" was imposed on it. Most of these models evaluate acute responses of the bladder to injury, a circumstance where the correlation between pain and inflammation is reasonably good.\(^1\) The relevance of these models to chronic pain syndromes may not be applicable to patients with IC.
Two presentations of IC are recognized based on cystoscopic evaluation of the bladder (although the necessity of cystoscopy for the diagnosis of IC is a matter of some debate among urologists). As in cats, only submucosal petechial hemorrhages are observed in most IC patients (Type I), whereas ulcers occur within the dome and lateral walls of the bladder in a minority (15-20%) of patients (Type II). Cats fit all of the inclusion and exclusion criteria that can be applied to animals for Type I IC in humans. Converging evidence suggests that these two types of IC may be distinct entities. This is particularly important for studies of therapy, since the two forms appear to respond differently to a variety of treatments. For example, both the sodium pentosan polysulfate and analgesic doses of tricyclic drugs reportedly are more effective in patients with Type I than with Type II disease, whereas patients with Type II IC appear to respond more favorably to treatment with transcutaneous electrical nerve stimulation, and appear to obtain significant symptomatic relief after removal of the bladder; the pain in the patients with Type I IC is not usually diminished by this procedure. This difference suggests that the cause of the pain in patients with Type II disease may be nociceptive, whereas the pain of patients with Type I IC and cats with FIC may be neuropathic. Nociceptive pain arises from persistent stimulation of sensory afferent fibers, and is relieved by removal of the stimulus. Examples of nociceptive pain include the pain of a toothache that is relieved by extraction of the affected tooth, and that associated with severe osteoarthritis of the hip joint, which is relieved by hip replacement. In contrast, neuropathic pain arises from an abnormality attributable to the nervous system, and although generally attributed to a body structure, can remain after removal of that structure.
In addition to neuropathic pain, humans with IC, and cats with FIC also appear to suffer symptoms referable to other organ systems. For example, lower urinary tract signs have been reported in cats with separation anxiety syndrome, hypertrophic cardiomyopathy, and obesity. It is possible that a non-bladder etiology for FIC might be related to alterations in the two primary stress response systems in the body, the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis. The abnormalities we documented could have detrimental effects on various organ systems, and result in a variety of clinical signs.

Recent studies have begun to map the pathways that transduce stress responses into cellular dysfunction. Events external to the central nervous system, from both within and without the body, are conveyed to the brain by hormones and sensory neurons. Afferent signals are conveyed to the thalamus, where they are evaluated and forwarded to the cortex for further processing prior to activation of the appropriate motor program. Potentially threatening events also can activate the emotional motor system directly via the amygdala, bypassing the cortical (inhibitory) pathway.
Based on our research in cats with severe, recurrent FIC, we have documented an enhanced activation of the stress response system, primarily the SNS. Normal regulation of the stress response systems is complex, but in summary, once stimulated by higher brain structures responding to the perception of a threat, corticotropin releasing factor (CRF) is released from the hypothalamus. CRF acts as a hormone to stimulate the anterior pituitary to release ACTH. CRF also acts as a neurotransmitter to stimulate neurons in the brainstem, including the locus coeruleus, to activate the sympathetic nervous system. Under mildly stressful conditions, I found that cats with FIC had a significantly higher plasma concentrations of DOPA, NE and other catecholamine metabolites compared to healthy cats (see chapter 2). These results support previous work documenting elevated tyrosine hydroxylase, the rate limiting step in catecholamine synthesis, protein content in the brainstem of cats with FIC.\textsuperscript{17}

Activation of the stress response system also can increase epithelial permeability, permitting environmental agents greater access to sensory afferent neurons, which could result in increased sensory afferent firing and local inflammation.\textsuperscript{18} Sympathetic neural-epithelial interactions appear to play an important role in permeability. For example, Birder et al.,\textsuperscript{19} have shown that the application of NE to urinary bladder (UB) strips induces release of nitric oxide from UB epithelium. In light of reports that nitric oxide may increase urothelial permeability,\textsuperscript{20,21} these results suggest that some of the sympathetically mediated alteration in permeability may be mediated by NE via this mechanism. The increased permeability related to increase SNS activation does not require direct interaction with epithelial cells, nor is it restricted to the urinary bladder. Neuronal release of NE is but one of a variety of mechanisms whereby stress-related
activation of the efferent SNS can activate mast cells, which can in turn increase epithelial permeability.\textsuperscript{22} Afferent sensory neurons too may increase epithelial permeability by releasing neurotransmitters at the peripheral process of the nerve via sympathetic-sensory coupling, dorsal root reflexes, and axon reflexes.\textsuperscript{23}

However, the presence of inflammation and altered permeability is not well correlated with pain \textit{per se}. In the bladder, we have reported the presence of submucosal petechial hemorrhages in cats with no signs referable to the lower urinary tract\textsuperscript{24} and others have identified urothelial disruption and increased presence of iNOS (and presumably increased permeability) in painless bladder conditions.\textsuperscript{25} Moreover, emotional and environmental factors such as stress or depression can modulate the experience of pain through descending pathways from the midbrain.\textsuperscript{26} Thus, even the increased activity of afferent nerves noted in cats with FIC\textsuperscript{27} could result in differences in perceived bladder sensations depending on the effects of the emotional state of the animal on descending inhibitory and facilitory balance.

In addition to permeability abnormalities, elevated plasma concentrations of catecholamines and their metabolites during stressful circumstances could lead to a functional desensitization of the $\alpha_2$-AR. In the LC, $\alpha_2$-agonists inhibit NE release, whereas in the spinal cord they inhibit transmission of nociceptive input to the brain.\textsuperscript{28,29} Although spinal $\alpha_2$-AR activation can inhibit nociceptive input acutely, the receptors can become desensitized or downregulated after chronic stimulation (i.e., by continuously elevated NE).\textsuperscript{30,31} Post-synaptic central $\alpha_2$-ARs can mediate antinociceptive responses; the $\alpha_2a$-AR subtype has been documented to play a major role in the control of nociceptive responses.\textsuperscript{32} Pain is a prominent feature in humans with IC and, based on
client history and clinical signs, of cats with FIC as well. Abnormalities of α2a-AR function could be detrimental to patients. In humans with IC, adrenergic receptor gene polymorphisms have been reported that may contribute to the predisposition to IC. Adrenoceptor abnormalities also may be secondary to the increased SNS activity of cats with IC, since I found that catecholamine concentrations declined and the functional heart rate response to stimulation of post synaptic α2-ARs by medetomidine normalized after environmental enrichment.

These data could provide an explanation for the association of symptom flares with stressful circumstances in cats with FIC as well as women with IC. Such a mechanism also could explain the magnitude of the placebo response in IC and FIC drug trials, which is approximately 50%, and provide an alternative mechanism of action for a popular drug used in treatment of IC - sodium pentosan polysulfate. Knowledge of this “placebo” response can be very useful for the clinician because maximizing it through environmental enrichment strategies as described in chapter 2, could result in a more successful outcome for the patient.

In contrast to the elevations in the SNS we documented, we found the cortisol response to ACTH stimulation was reduced during stressful periods in cats with FIC. We also found that adrenal gland size (analyzed both by gross sections and computed tomography) was significantly smaller in cats with FIC that in healthy cats. Microscopic examination of the adrenal glands by a board-certified veterinary pathologist excluded the presence of obvious fibrosis, hemorrhage, inflammation, infection, or necrosis as causes of the reduced size; the primary abnormality identified was a reduced size of the fasciculata and reticularis zones (the zones responsible for cortisol and other steroid
hormone secretions). These results, when combined with our earlier observations of increased concentrations of CRF and ACTH (data not shown) in response to stress in the absence of a comparable increase in plasma cortisol concentrations, strongly support the presence of decreased adrenocortical reserve in cats with FIC.

Decreased adrenal steroid synthesis might also have adverse affects on the SNS. Cortisol normally restrains SNS outflow from the LC, and also inhibits its own release by feedback inhibition at the level of the anterior pituitary. Furthermore, cortisol plays a role in epithelial permeability by enhancing tight junction integrity to reduce permeability. This and other adrenocortical steroid-related protective mechanisms may be less efficient in hypocortisolemic states. Hyopcortisolemia could lead to further elevations in catecholamines and more apparent clinical signs as the disease progresses. These two stress responses appear to be uncoupled from one another, whereby enhanced sympathetic tone, but decreased adrenocortical function occurs simultaneously. This “uncoupling” abnormality has been described in several other chronic stressful conditions in humans.

The most parsimonious explanation currently available to explain the combination of increased CRF, ACTH, and SNS activity in the presence of reduced adrenocortical response and small adrenal fasciculata and reticularis zones without other apparent abnormalities is a genetic disorder or a developmental accident (or some combination of the two). A number of recent reviews have explored the consequences of subjecting pregnant females to threatening stressors for the developing fetus. If the stressor is sufficiently harsh, the hormonal products of the ensuing stress response may cross the placenta and affect the course of fetal development. A reduction in adrenal size may
result from glucocorticoid-mediated suppression of release of ACTH by the fetal anterior pituitary. Recently Leavitt et al., reported that glucocorticoid injection during late gestation in baboons inhibited fetal pituitary ACTH release and adrenal cortical ACTH receptor expression. They subsequently determined that this effect blocked development of the fetal transitional (cortisol-producing) zone. Pre- and post-natal stressors also can result in persistently increased central CRF activity. Regardless of the cause, decreased biological activity of glucocorticoids may have a variety of adverse effects on bodily function, possibly related to their role in restraining activation of the immune system and other components of the stress response, including the SNS and CRF.

The apparent lack of long term benefit of glucocorticoid therapy in patients with FIC suggests that inadequate production of other steroids might also play a role in the pathophysiology of this disease. The adrenal cortex is responsible for many different hormones and we have only investigated the most common one, cortisol. The adrenal gland secretes many other hormones responsible for many different functions. Evidence suggests that part of the stress response may include maintaining production of cortisol (primarily through the Δ-4 pathway which results in cortisol production) at the expense of the 17,20 lyase which results in dehydroepiandrosterone (DHEA) production. Products of the 17-α hydroxylase enzyme such as DHEAS, the longer lived metabolite of DHEA, may become deficient when the stressor is severe or adrenocortical reserve is inadequate. Preliminary studies in humans with IC have suggested alterations in adrenocortical hormone ratios, namely a ~3-fold increase in the cortisol/DHEAS ratio in female patients during flare. Adrenocortical
function also has been evaluated in humans with other chronic, waxing and waning pain conditions (e.g., chronic fatigue syndrome - CFS) by measuring the cortisol/DHEAS ratio which was 2-3 fold higher in CFS patients than in normal controls. Kizildere, et al., have suggested that serum concentrations of DHEAS may be low in patients with inflammatory and non-inflammatory diseases due to an activated SNS. They concluded that sympathetic hyperactivity may be a common denominator for low levels of DHEAS in both inflammatory and non-inflammatory diseases. Currently, we have not investigated this in cats with FIC. In the meantime, we do not advocate the use of glucocorticoids as a sole treatment for FIC, based on clinical experience that cats do not seem to improve with the current anti-inflammatory doses commonly administered.

_Treatment Strategies for FIC_

Currently, the HPA axis abnormalities found in cats with FIC are not fully understood. Our current therapy is aimed at reducing the output of the SNS in hopes of decreasing sympathetic tone, neurogenic inflammation, and altered permeability. Any treatment strategy to decrease SNS outflow may be important in reducing these abnormalities. Having the proper tools to help owners understand FIC is important in maintaining client satisfaction when beginning any treatment regimen.

The development of chronic FIC requires the presence of a susceptible cat in a provocative environment. Our challenge is to identify what is provoking this susceptible cat in hopes of preventing future bouts. Early intervention strategies to help stop the progression of the pain cycle seem prudent, because enhanced stress response system activity seems to be central to maintaining the chronic
neuroinflammatory process. As mentioned above, I have documented an increase in catecholamines and their metabolites in cats with FIC, which may be altering the responses of the α2-AR as well. When healthy cats are stressed, cortisol and catecholamines increase, but return to baseline within hours to days. In our study, cats with FIC did not appear to have this capability. Their catecholamines remained elevated, while their adrenal cortex response was blunted. Because it is known that symptoms increase in both cats and humans with stressful events, it is possible that any treatment strategy that normalizes the SNS may be important in reducing the clinical signs of FIC.

The sensitivity of cats to their surroundings has long been recognized.\textsuperscript{52,53} Recent ethological studies in zoos,\textsuperscript{54} research laboratories,\textsuperscript{55} and boarding facilities\textsuperscript{56} have documented that cats subjected to impoverished or unpredictable environments have decreased activity levels and increased hiding behaviors. The indoor environment of some housed cats also may be monotonous and predictable, which could be stressful.\textsuperscript{57} While reducing the risk of infectious disease and accidental injury, indoor housing has been associated with increased risk (odds ratio) for development of lower urinary tract signs, calcium oxalate urolithiasis, odontoclastic resorptive lesions, obesity, and hyperthyroidism.\textsuperscript{58} Our clinical experience with cats with severe FIC suggests that environmental enrichment to attempt to reduce the cat’s perception of threat often is sufficient to eliminate recurrence of lower urinary tract signs. In our lab’s recent studies, we have documented that environmental enrichment strategies that consisted of owner education about their cats’ disease, proper litterbox management strategies, dietary alterations, modification of the indoor environment to
reduce anxiety, and working with owners in multicat households to reduce conflict resulted in highly statistically and clinically significant remission of lower urinary tract signs and abnormal behavioral signs. Changes to the cat’s environment were implemented slowly and alterations were tailored for each individual cat (in a similar manner as our study cats) according to limitations of each owner and household.

Another strategy to possibly decrease the SNS we have used includes phermonotherapy. Pheromones are fatty acids that seem to transmit highly specific information between animals of the same species. Although the exact mechanisms of action are unknown, pheromones reportedly induce changes in both the limbic system and the hypothalamus that alter the emotional state of the animal. Feliway® (Ceva Sante Animale, Libourne, France), a synthetic analogue of this naturally occurring feline facial pheromone, was developed in an effort to decrease anxiety-related behaviors of cats. Although not specifically tested in cats with FIC, treatment with this pheromone has been reported to reduce the amount of anxiety experienced by cats in unfamiliar circumstances, a response that may be helpful for FIC patients and their owners. Others have reported decreased spraying in multi-cat households, and a significant decrease in scratching behavior. Although Feliway® is not a panacea for unwanted cat behaviors or FIC, based on reports in the literature and the improvement we noted in cats after environmental enrichment, we have used it successfully in combination with environmental enrichment in some cats with FIC.

Conclusion
FIC no longer appears to be a syndrome that is solely isolated to the bladder. Based on our studies in cats and evidence from studies of humans with IC, identification of involvement of other organ systems\textsuperscript{63-65} suggests a role for a central neuroendocrine abnormality in at least some patients. FIC and the other unexplained clinical conditions with which it can be comorbid are so complex that it seems unlikely that all, or even most, cases will be explained by a single underlying etiology. Even if a neuroendocrine imbalance only explains a subset of cases, however, it could improve care for these patients, and hopefully lead to alternative hypotheses for those cats who suffer from FIC resulting from other etiologies.
REFERENCES


LIST OF REFERENCES


204. Piletz, J. E., Halbreich, U. Imidazoline and alpha(2a)-adrenoceptor binding sites in postmenopausal women before and after estrogen replacement therapy. Biol Psychiatry 2000;48:932-939


228. Schott, G. D. Delayed onset and resolution of pain: some observations and implications. *Brain* 2001;**124**:1067-1076


