In vivo evaluation of $\alpha_2$-adrenoceptors in cats with idiopathic cystitis

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**Objective**—To evaluate the in vivo response of $\alpha_2$-adrenoceptors to medetomidine administration in cats with feline idiopathic cystitis (FIC) during periods of stress and after environmental enrichment.

**Animals**—13 cats with FIC and 12 healthy cats.

**Procedures**—Cats were subjected to an acute-onset moderate stressor for 8 days. After stress, 20 µg of medetomidine/kg was administered IM on days 1, 3, and 8. Heart rate, blood pressure, pupil diameter, respiratory rate, and level of sedation were evaluated before and after administration of the drug. After day 8, cats were moved to an enriched environment, and tests were repeated on day 35.

**Results**—Heart rate decreased and pupil diameter increased significantly after medetomidine administration in healthy cats, compared with cats with FIC. Cats with FIC had significantly lower respiratory rates. No significant differences in blood pressure or sedation level were found.

**Conclusions and Clinical Relevance**—Increased plasma catecholamine concentrations during the enrichment phase, which have been reported elsewhere, may have contributed to the differences in $\alpha_2$-adrenoceptor responses detected in cats with FIC. (Am J Vet Res 2007;68:203–207)

Diseases affecting the lower portion of the urinary tract of cats are characterized by variable combinations of stranguria, hematuria, urinations in inappropriate locations (peruria), and pollakiuria. There are several differential diagnoses for these clinical signs, including urolithiasis, urinary tract infections, and primary behavioral abnormalities. When no underlying cause for these clinical signs can be found, a diagnosis of FIC is often made. The etiology of this disease is unknown, and it appears to affect male and female cats equally.

The clinical signs of FIC wax and wane and are often reported by owners to be exacerbated by stressful circumstances. Chronic stress from internal and external sources can increase tyrosine hydroxylase activity, the rate-limiting step in catecholamine synthesis, in the LC of cats with FIC. 

**Abbreviations**

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<th>Abbreviation</th>
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<tr>
<td>FIC</td>
<td>Feline idiopathic cystitis</td>
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<td>LC</td>
<td>Locus coeruleus</td>
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<tr>
<td>$\alpha_2$-AR</td>
<td>$\alpha_2$-Adrenoceptor</td>
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<td>MEMO</td>
<td>Multimodal environmental modification</td>
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The LC plays an integral part in modulating the body’s response to stressful circumstances, mediated largely through norepinephrine-containing neurons of the sympathetic nervous system. Release of norepinephrine from these neurons is controlled in part by a rich supply of presynaptic autoinhibitory $\alpha_2$-ARs. Postsynaptic $\alpha_2$-ARs also are present on some norepinephrine-containing neurons, such as those modulating cardiovascular function and iris diameter. Neurons in the LC are relatively inactive during periods of quiescence. During periods of arousal, however, the firing rate of these neurons increases and greater amounts of norepinephrine are released. The $\alpha_2$-ARs are important in modulating those responses, and in the spinal cord, they can help reduce nociceptive input to the brain. The antinociceptive effects of the $\alpha_2$-ARs are mediated via inhibition of adenylcyclase activity, activation of K+ currents, inhibition of Ca2+ channels, and increased mitogen-activated protein kinase phosphorylation through both pre- and postsynaptic neuronal membrane receptors.

Because high plasma catecholamine concentrations have been detected in cats with FIC, we hypothesized that $\alpha_2$-ARs could become downregulated because of increased exposure to their agonist. Results of preliminary studies suggest abnormalities in the $\alpha_2$-ARs of cats with FIC and women with interstitial cystitis, an analogous disease in humans.
The purpose of the study reported here was to evaluate the in vivo response of α₂-AR function by assessing sedation, heart rate, blood pressure, and pupil diameter in healthy cats and cats with FIC after administration of the selective α₂-AR agonist medetomidine. Because clinical signs of FIC can increase during stress, cats were evaluated at various times during and after a moderate stress protocol, as previously described.

**Materials and Methods**

Thirteen cats (3 neutered males, 1 sexually intact female, and 9 spayed females) obtained as donations from clients because of a history of severe, recurrent stranguria; hematuria; pollakiuria; and periuria or a combination of these signs were evaluated at The Ohio State University Veterinary Teaching Hospital. Twelve clinically normal cats (3 sexually intact males, 1 neutered male, 7 sexually intact females, and 1 spayed female) of similar age were used as controls. 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. Cats received a diagnosis of FIC or were deemed healthy on the basis of evaluations that have been reported. The Animal Care and Use Committee of The Ohio State University approved all experimental procedures. Other results from these cats have been reported.

A moderate stress protocol was administered for 8 days and additional procedures, including administration of medetomidine, were performed on each cat on days 1, 3, and 8, as reported. Prior to medetomidine administration, a lux meter was placed next to the right eye and each cat was allowed to acclimate to the light for 1 to 2 minutes. A midlateral pupil measurement was obtained to the nearest millimeter. All values were approximately 430 lux for each cat. Resting heart and respiratory rates and indirect systolic blood pressure measured with an ultrasonic Doppler flow detector with a No. 3 cuff on the left pelvic limb were also evaluated (cats were placed in right lateral recumbency for this measurement). As part of the stress protocol, after these measurements, a venous blood sample was obtained from the jugular vein for analyses of catecholamine and sodium fluorescein concentrations, as reported.

After venipuncture, 20 µg of medetomidine/kg was administered IM into the epaxial muscles of each cat and the time was recorded. Exactly 10 minutes later, heart rate, blood pressure, pupil measurement, respiratory rate, and level of sedation were recorded. Level of sedation was assessed from 0 to 20 by evaluation of a number of variables including the posture of the cat, resistance to being placed in lateral recumbency, and degree of jaw relaxation. A higher value was interpreted to indicate a higher total sedation score. Atipamezole (300 µg/kg) was then administered to counteract any delayed effects of the medetomidine.

Cats were tested in groups of 4, after which they were placed in clean metabolism cages to continue the stress protocol. Each cat received 100 g/d of a standard feline commercial diet and intake was recorded daily. At the end of the moderate stress period (day 9), the cats were moved to an enriched environment. Cages were larger, and each cage contained a covered bed, 2 types of approved toys, and a larger litter pan. The cats were fed the commercial dry food as before and also were offered a commercial canned cat food. All food was weighed daily, and the intake of each type was recorded. All cats had interaction with humans (in addition to the animal caretakers) ≥15 min/d. Music also was played in the room during the day. On day 35, food was withheld the night before testing and all the previous testing procedures were repeated.

**Statistical analysis**

The effects of experimental group (ie, cats with FIC vs healthy controls), time (1, 3, 8, and 35 days), and treatment (before vs after treatment) and their interactions were simultaneously analyzed by use of 3-way repeated-measures ANOVA. For dependent measures with ordinal scores, the Mann-Whitney test was used to compare groups at each time point. Values of P < 0.05 were considered significant.

**Results**

No differences in food intake were detected between the 2 groups at any time during the study. Abnormalities pertaining to the lower portion of the urinary tract were found in 1 healthy cat (a sexually intact male) shortly after the study began. The cat was removed from the study, and data obtained from the cat were not included in the statistical analysis. In all 13 cats with FIC and 11 healthy cats, all variables were evaluated on all testing days except for days 8 and 35; variables could not be determined in 1 healthy cat on each of these days because of injection errors.

Heart rates before administration of medetomidine were not different between groups (Figure 1). After administration of medetomidine, the decrease in heart rate was significantly greater in healthy cats, compared with cats with FIC, except on day 35 (Table 1). Cats with FIC had a significantly (P = 0.03) lower respiratory rate, compared with that of healthy cats, throughout the study. Both groups had a significant (P < 0.001) decrease in respiratory rate after medetomidine was administered (Figure 2). No significant difference was detected across days (P = 0.43).

No significant difference in resting blood pressure was identified between groups (P = 0.42). Mean blood pressure was significantly lower in healthy cats than in cats with FIC throughout the study (Figure 3).
pressure was significantly \( P < 0.001 \) lower 10 minutes after medetomidine was administered (Figure 3) in both groups. No differences were detected in percent age change in blood pressure between the 2 groups, although the change was slightly greater in healthy cats.

No significant difference in sedation score was identified between the 2 groups \( (P = 0.15; \text{Figure 4}) \). The increase in pupil diameter was significantly \( (P = 0.004) \) greater in healthy cats, compared with cats with FIC, after medetomidine administration (Table 2). Furthermore, the percentage change in pupil diameter was significantly \( (P = 0.003) \) greater on days 3, 8, and 35 in healthy cats, compared with that recorded on day 1.

**Discussion**

Significant differences in pupil and heart rate responses to medetomidine were detected between cats with FIC and healthy cats. Medetomidine, a bridge-methylated derivative of detomidine used often in veterinary medicine for sedation and restraint, is a potent and selective pre- and postsynaptic \( \alpha_2 \)-AR agonist and appears to have more selectivity for the \( \alpha_2a \)-ARs than for other ARs. The dose \( (20 \mu g/kg, \text{IM}) \) was chosen on the basis of results of previous administration of this drug to healthy, middle-aged cats, which resulted in moderate sedation in most cats, as well as from doses reported in the literature.\(^{13,14}\) Although medetomidine has a much stronger \( \alpha_2 \)-to-\( \alpha_1 \) AR selectivity ratio than other \( \alpha_2 \)-AR agonists do, higher doses were not used to attempt to reduce the likelihood of stimulating \( \alpha_1 \)-ARs, which would counteract the sedation.\(^{12}\)

The \( \alpha_2 \)-ARs have a wide variety of functions, particularly in the cardiovascular and nociceptive systems. In addition to modulating vasoconstriction and iris diameter, \( \alpha_2 \)-ARs are involved in platelet aggregation (via the \( \alpha_2 \)-ARs), neurotransmitter release, and antinociception.\(^{15}\) In the urinary bladder, \( \alpha_2 \)-ARs are found primarily in the urethral submucosa and bladder mucosa (not the muscle), suggesting a role in the regulation of blood flow and urethral lubrication, but not on smooth muscle.\(^{16}\) However, \( \alpha_2 \)-ARs function as autoreceptors to decrease norepinephrine outflow and could alter contractility through this mechanism. Alternatively, the mucosa could influence contractility via its sensor and transducer functions.\(^{17}\)

Decreased response to medetomidine administration was found in cats with FIC, compared with healthy cats, for heart rate and pupil dilation, but the reason for these differences was unclear. We hypothesized that high norepinephrine concentrations would adversely affect the \( \alpha_2 \)-ARs in cats with FIC because of chronic agonist stimulation, although the exact mechanism for the differences could not be elucidated from this study. Heart rate is controlled, in part, by central postsynaptic \( \alpha_2 \)-ARs in the nucleus of the solitary tract.\(^{18}\) Pupil dilation occurs via stimulation of centrally located, post-

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<tr>
<td>1</td>
<td>23 ± 15</td>
<td>34 ± 10</td>
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<tr>
<td>3</td>
<td>15 ± 14</td>
<td>37 ± 12</td>
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<tr>
<td>8</td>
<td>24 ± 9</td>
<td>32 ± 13</td>
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<td>35</td>
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synaptic \(\alpha_2\)-ARs located in the Edinger-Westfal nucleus. Stimulation of these receptors results in pupil dilation caused by CNS inhibition of the parasympathetic tone to the iris.\(^{19,20}\) After medetomidine administration to both groups of cats, both of these variables were significantly different between groups. Throughout the stress protocol, catecholamine concentrations increased in cats with FIC to a far greater extent than in healthy cats. This difference was diminished after environmental enrichment, a time during which norepinephrine (and other catecholamine) concentrations substantially decreased.\(^3\)

Results of an in vitro study\(^4\) of electrical field stimulation of bladder strips from cats with FIC indicated that atipamezole, an \(\alpha_2\)-AR antagonist, does not alter the relaxing effect of norepinephrine, providing further evidence for abnormalities in these receptors. In other species, chronic stress is also reported to cause desensitization of the \(\alpha_2\)-ARs. For example, results of an in vitro study\(^21\) evaluating various brain sections from tree shrews subjected to chronic stress indicate that the number of binding sites for the \(\alpha_2\) antagonist 3[H]rauwolscine is decreased, suggesting downregulation of the \(\alpha_2\)-ARs because of high noradrenergic activity. An in vivo study by Gomez et al\(^{22}\) also provides evidence that chronic stress may lead to desensitization of the postsynaptic \(\alpha_2\)-ARs in rats via evaluation of the clonidine-induced jaw-opening reflex. Other authors\(^3\) have detected functional desensitization of central postsynaptic \(\alpha_2\)-ARs in rats after repeated tail pinching as a form of chronic variable stress. In that study, the postsynaptic \(\alpha_2\)-ARs appeared to become functionally desensitized first. Desensitization is usually rapid and reversible, suggesting that receptors are internalized but not lost.

In the present study, no differences were detected between the 2 groups in regards to sedation and blood pressure after medetomidine administration. Sedative effects of \(\alpha_2\)-AR agonist responses are mediated from stimulation of the presynaptic \(\alpha_2\)-AR located primarily on the LC in the pons and lower portion of the brainstem,\(^{24}\) whereas heart rate,\(^8\) blood pressure,\(^{12}\) and pupil dilation\(^{26}\) are primarily postsynaptic. A variable response in sedation in both groups was also evident. This could be attributable to variable drug absorption when medetomidine is administered IM, as has been reported in dogs.\(^8\) Sex or individual animal differences in \(\alpha_2\)-AR susceptibility to norepinephrine-mediated desensitization may also have been present. Moreover, if abnormalities exist in the \(\alpha_2\)-ARs, this could affect peripheral perfusion and absorption of the drug. In future studies, the drug dose should be calculated and the drug administered IV to avoid this potential problem.

Although both groups had a significant decrease in blood pressure after administration of medetomidine, no differences were detected between the groups. Blood pressure of cats with FIC decreased by only 9% and 8% on days 1 and 3, respectively, whereas it decreased by 15% and 12% on these days in the healthy cats, although this difference was not significant. Interestingly, cats with FIC had a significantly lower respiratory rate than did healthy cats, although respiratory rates decreased significantly in both groups after medetomidine was administered. We expected cats with FIC to have higher respiratory rates because of their greater stress responsiveness.\(^{29}\) Respiratory rate alone thus may not be a good indicator of stress. Some cats may respond to stress with inactivity and minimal movements as part of a defense mechanism to avoid detection by predators. A lower respiratory rate also has been reported in humans\(^27\) after chronic stress.

The \(\alpha_2\)-ARs are complex receptors; they are coupled to G proteins (usually inhibitory [Gi proteins]) and use second messenger systems to carry out the response to agonist binding. Abnormalities of some of these downstream effectors systems have been detected in other chronic stressful diseases that commonly occur in humans with interstitial cystitis. For example, hypofunctional G proteins have been reported in humans with migraine headaches and fibromyalgia.\(^{20,29}\) Abnormalities in \(\alpha_2\)-ARs, G protein, or second messengers could permit increased nociceptive input to the brain and various other abnormalities that could perpetuate clinical signs of FIC and interstitial cystitis, especially during periods of stress. Adrenergic receptor gene polymorphisms also have been reported in humans with interstitial cystitis\(^{10}\) and in cats with FIC.\(^{30}\)

Unfortunately, in this study, we were unable to match the groups regarding sex. Although the male and female numbers in general were similar (FIC, 3 males and 10 females; healthy, 3 males and 8 females), the control population contained more sexually intact cats. The cats with FIC were obtained as donations, and most had been neutered, whereas many of the control cats were purchased and had not been neutered. No data in the literature were found to evaluate the differences in \(\alpha_2\)-ARs in male versus female or sexually intact versus neutered cats. Results of a study\(^{31}\) in healthy premenopausal women indicate greater \(\alpha_2\)-AR sensitivity than in men because a lower infusion rate of phenylephrine (primarily an \(\alpha_2\)-AR agonist, not an \(\alpha_1\)-AR agonist) was required to increase systolic blood pressure. Responses to phenylephrine and clonidine (an \(\alpha_2\)-AR agonist) included substantial dose-related vasoconstriction in healthy men but not in healthy women.\(^{32}\) However, 1 study\(^33\) compared \(\alpha_2\)-AR binding sites in postmenopausal women with those of healthy women of reproductive age. Although differences were found in imidazoline receptors, no differences were found in platelet \(\alpha_2\)-AR densities and no differences were detected during estrogen replacement therapy.

In the present study, the increases in dihydroxyphenylalanine, norepinephrine, and dihydroxyphenylglycol concentrations\(^3\) could have contributed to the decreased \(\alpha_2\)-AR responses detected in cats with FIC. Continual agonist stimulation could cause desensitization of the receptors. By day 35, catecholamine concentrations were lower and only mild differences in percentage pupil dilation were detected between the 2 groups of cats. On the basis of these data, treatment strategies aimed at decreasing sympathetic tone in hopes of improving \(\alpha_2\)-AR function have been developed and tested in client-owned cats with FIC.\(^{34}\) In this study, owners of 46 indoor-housed cats with FIC were offered recommendations for MEMO based on a detailed environmental history. Cats had follow-up for 10 months via client contact to determine the effect of
MEMO on lower urinary tract signs and other clinical signs. Significant reductions in lower urinary tract signs, fearfulness, nervousness, and signs referable to the respiratory tract were identified. These results suggest that MEMO may be a useful adjunctive approach to therapy for indoor-housed cats with FIC caused by decrements in noradrenergic outflow.


References