Vasopressin secretion in response to osmotic stimulation and effects of desmopressin on urinary concentrating capacity in dogs with pyometra

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Objective—To determine vasopressin (VP) secretory capacity during osmotic stimulation and the response to desmopressin treatment in dogs with pyometra and control dogs.

Animals—6 dogs with pyometra before and after ovariohysterectomy and 6 control dogs.

Procedure—Urine osmolality (Uosm) was measured during 12 hours. Values measured on the first day defined the basal Uosm pattern. On the second day, dogs were given desmopressin to induce a desmopressin-stimulated Uosm pattern. On day 3, the VP response to osmotic stimulation was examined.

Results—Median Uosm on day 1 was 340 mOsm/kg (range, 104 to 1,273 mOsm/kg) and 807 mOsm/kg (range, 362 to 1,688 mOsm/kg) in dogs with pyometra before and after surgery, respectively, and 1,511 mOsm/kg (range, 330 to 1,674 mOsm/kg) in control dogs. Median Uosm during desmopressin treatment was 431 mOsm/kg (range, 168 to 1,491 mOsm/kg) and 1,051 mOsm/kg (range, 489 to 1,051 mOsm/kg) in dogs with pyometra before and after surgery, respectively, and 1,563 mOsm/kg (range, 1,390 to 2,351) in control dogs. In dogs with pyometra, threshold for VP secretion was lower before surgery (median, 340 mOsm/kg; range, 331 to 386 mOsm/kg) than after surgery (median, 358 mOsm/kg; range, 343 to 439 mOsm/kg) or in control dogs (median, 347 mOsm/kg; range, 331 to 366 mOsm/kg). Highest maximum plasma VP values were found in dogs with pyometra.

Conclusions and Clinical Relevance—Dogs with pyometra had increased urine concentration in response to desmopressin but not to the degree of control dogs, whereas VP secretory ability was not reduced. (Am J Vet Res 2004;65:404–408)
effect of endotoxins on the kidneys or hypothalamus during pyometra in bitches has not been described.

Renal tubular abnormalities, including hyaline droplet degeneration, cellular swelling and vacuolization, basement membrane thickening, and accumulation of a lipofuscin-like pigment, have been reported in studies of dogs with pyometra. They may all play a role in the development of PU-PD in dogs with pyometra, but studies with control dogs of comparable age are lacking.

Specific staining of neurosecretory cells in the hypothalamus from bitches with pyometra and PU-PD in 1 study did reveal a reduction of staining material believed to represent VP granules, which contrasted with findings in another similar study. Thus, indirect evidence supports the hypothesis that the defect in concentrating ability is not related to a defect in VP production, but plasma VP concentrations and sensitivity of the osmoregulatory system have not been reported in bitches with pyometra. The objectives of the study reported here were to determine the VP secretory capacity in response to osmotic stimulation and the effects of desmopressin treatment on urinary concentrating capacity in dogs with pyometra and control dogs.

Materials and Methods

Animals—Six bitches with pyometra and 6 control bitches of comparable age were included in the study. After physical examination and laboratory tests were conducted, the diagnosis of pyometra was confirmed by use of ultrasonography. Additional inclusion criteria were polydipsia, a stable clinical condition that allowed for postponement of surgery, and willingness of owners to let their dogs participate in the study. Control dogs did not have a history of illness, and results of routine physical examination and laboratory tests did not reveal evidence of health problems. The signalment and clinical data of the dogs with pyometra in this study were similar in terms of clinicopathologic data and duration of disease to groups of dogs with pyometra described in other studies. Two dogs were difficult to classify with respect to PU-PD, which is consistent with another description of dogs with pyometra. Dogs with pyometra ranged from 7 to 10 years of age, whereas the control dogs were 7 to 9 years old. The WBC concentrations in the dogs with pyometra ranged from 4.3 to 10×10⁶ cells/L to 61.6×10⁶ cells/L (median, 18.0×10⁶ cells/L), whereas WBC concentrations in the control dogs ranged from 3.8 to 10×10⁶ cells/L to 13.0×10⁶ cells/L (median, 7.7×10⁶ cells/L). Serum creatinine concentration in dogs with pyometra ranged from 62 to 145 µmol/L (median, 72 µmol/L), whereas concentrations in control dogs ranged from 38 to 88 µmol/L (median, 77 µmol/L). For the 6 dogs with pyometra, duration of the condition was 1 to 14 weeks (median, 4 weeks), body temperature was 1 to 14 weeks (median, 4 weeks), body temperature was not recorded for 1 dog), and the protein-to-creatinine ratio ranged from 0 to 13 (median, 0). A clinical condition that was more severe than was apparent from the medical history and results of initial examination was discovered in 2 dogs with pyometra. One dog had extreme hypoalbuminemia that changed from 10 g/L on the day of admission to 6 g/L after surgery, although edema or ascites were not evident. That dog developed edema and azotemia during the first few months and uremia within 1.5 years after surgery. One dog was a clinical suspect for vasculitis at the time of testing but recovered uneventfully from surgery. That dog had PU-PD until it was lost from follow-up monitoring 1 year later.

Dogs with pyometra were rehydrated during a period of 2 to 4 hours prior to ovariohysterectomy. During surgery, cystocentesis of the bladder was performed, and a renal biopsy specimen and uterine swab specimen were obtained for bacteriologic culture. The renal biopsy specimen was stained by use of H&E and periodic acid–Schiff stains and examined by use of light microscopy. Only 2 of the dogs had bacteriuria, but bacteria were cultured from the uterus of each of the 6 dogs with pyometra (4 with Escherichia coli, 1 with Streptococcus canis, and 1 with Pasteurella multocida). Results of histologic examination of renal tissues were considered normal in 1 dog with pyometra, whereas, as the other 5 had evidence of pathologic changes (2 with low-grade membranous glomerulonephritis, 1 with severe interstitial pyelonephritis, 1 with subacute glomerulitis and tubulointerstitial nephritis, and 1 with interstitial nephritis).

Testing procedures—Clinicopathologic data were obtained on the day of arrival at the clinic. Urine osmolality (Uosm) was measured every 2 hours for a 12-hour period on day 1 of the study. Dogs had ad libitum access to water during the measurement period. Values measured on day 1 were used to define the basal pattern.

On day 2, serial measurements of Uosm were performed at the same time points; however, dogs were treated with desmopressin (1 drop in the conjunctival sac at the beginning of the measurement period and again 6 hours later). These values were used to define the desmopressin-stimulated Uosm pattern.

On day 3 of the testing procedure, the VP response to osmotic stimulation was investigated by IV infusion of hypertonic saline (20% NaCl) solution throughout a 2-hour period at a rate of 0.03 mL/kg/min. One dog with pyometra was inadvertently given hypertonic saline solution as a bolus injection, causing maximal VP secretion in the middle of the test procedure.

Blood samples for measurement of plasma VP concentration and Posm were collected at 20-minute intervals from a jugular vein of each dog into chilled EDTA-coated tubes and immediately placed on ice until centrifuged. Plasma was obtained by centrifugation at 4°C and stored at −20°C until assayed. The 3-day testing procedure was performed twice in the dogs with pyometra (before and 1 month after ovariohysterectomy). The testing procedure was performed only once in the control dogs.

Measurement of Posm and Uosm—Plasma osmolality and Uosm were measured in duplicate by use of the freezing-point depression technique. Vasopressin was extracted by adding 5.2 mL of chilled (4°C) 96% ethanol to 0.8 mL of plasma; samples were then incubated during end-over-end rotation for 30 minutes at 4°C. After incubation, samples were centrifuged at 5,000×g for 30 minutes at 4°C. Supernatant was collected and dried overnight by use of a rapid vacuum concentrator. Extracts were dissolved in 0.8 mL of assay buffer. Mean ± SD recovery of VP was 75 ± 1%. Vasopressin concentrations were measured by use of a radioimmunoassay validated for samples obtained from dogs. Serial dilutions of an extract of canine plasma from a dog with a high VP concentration were plotted, which resulted in a curve parallel to the standard curve. Limit of detection of the radioimmunoassay was 1 pmol/L. The intra-assay coefficient of variation was 12% at 8 pmol/L, and the interassay coefficient of variation were 20% at 1.5 and 4 pmol/L and 10% at 8.5 pmol/L.

Data analysis—The effect of desmopressin treatment on urinary concentrating capacity was evaluated by calculating the mean of Uosm values for each of the dogs for days 1 and 2. Median values of individual means within each group of dogs were compared for days 1 and 2. The relationship between Posm and plasma VP concentration was determined by use of regression analysis. The slope of the regression line was used to describe the sensitivity of the osmoregulatory system, and the intercept with a value of 5 pg/mL provided a measure of the threshold value.

Because of the limited number of dogs and wide variability in results among bitches, we did not perform statistical testing between groups with regard to changes in Uosm values, plasma VP concentrations, osmotic threshold, or sensitivity of the VP response.
Results

Median Uosm for the dogs with pyometra on day 1 was 340 mOsm/kg (range, 104 to 1,273 mOsm/kg), compared with a median value of 807 mOsm/kg (range, 362 to 1,688 mOsm/kg) for those same dogs 1 month after ovariohysterectomy and 1,511 mOsm/kg (range, 830 to 1,674 mOsm/kg) for the control dogs. Median Uosm during desmopressin treatment on day 2 was 431 mOsm/kg (range, 168 to 1,491 mOsm/kg) for the dogs with pyometra, compared with 1,051 mOsm/kg (range, 489 to 1,680 mOsm/kg) for those same dogs 1 month after ovariohysterectomy and 1,563 mOsm/kg (range, 1,390 to 2,351 mOsm/kg) for the control dogs (Table 1).

In most dogs, mean Uosm increased during desmopressin stimulation. However, substantial individual variation in response to desmopressin was evident in all groups. In 1 dog with pyometra before ovariohysterectomy, there was a minimal increase in Uosm during desmopressin treatment. After ovariohysterectomy, Uosm decreased during desmopressin treatment in 2 dogs, whereas in a third dog, Uosm was virtually identical before and during desmopressin treatment. In the group of control dogs, Uosm decreased during desmopressin treatment in 1 dog, whereas Uosm was virtually identical before and during desmopressin treatment in another dog (Table 1).

Table 1—Effects of desmopressin treatment on urinary concentrating capacity and vasopressin (VP) secretory capacity (osmotic threshold and sensitivity of VP secretion) in response to osmotic stimulation in 6 dogs with pyometra before and 1 month after ovariohysterectomy and in 6 control dogs of comparable age

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<tr>
<td>Median Uosm</td>
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</table>

For all dogs, median 12-hour mean Uosm was 901 mOsm/kg (range, 104 to 2,219 mOsm/kg), median osmotic threshold was 348 mOsm/kg (range, 331 to 439 mOsm/kg), median sensitivity was 0.18 (pg/mL)/(mOsm/kg) (range, 0.04 to 0.48 [pg/mL]/[mOsm/kg]), and median maximum VP concentration was 7.7 mg/mL (range, 2.7 to 20.1 mg/mL).

Table 1: Effects of desmopressin treatment on urinary concentrating capacity and vasopressin (VP) secretory capacity (osmotic threshold and sensitivity of VP secretion) in response to osmotic stimulation in 6 dogs with pyometra before and 1 month after ovariohysterectomy and in 6 control dogs of comparable age

| Mean urine osmality (Uosm) without (day 1) and with (day 2) desmopressin in treatment (mOsm/kg) | Osmotic threshold (mOsm/kg) | Sensitivity (pg/mL)/(mOsm/kg) | Maximum VP concentration in plasma (pg/mL) |
| Dog | Pyometra | Before surgery | After surgery | Control dogs | Pyometra | Before surgery | After surgery | Control dogs | Pyometra | Before surgery | After surgery | Control dogs |
|-----|----------|----------------|---------------|--------------|----------------|---------------|---------------|--------------|--------------|----------------|---------------|--------------|--------------|
| Day 1 | Day 2 | Day 1 | Day 2 | Day 1 | Day 2 | Day 1 | Day 2 | Day 1 | Day 2 | Day 1 | Day 2 | Day 1 | Day 2 |
| 1 | 783 | 1,491 | 1,430 | 1,546 | 1,393 | 1,396 | 336 | NA | 334 | 0.37 | NA | 0.27 | 11.6 | NA | 12.8 |
| 2 | 266 | 329 | 393 | 1,680 | 1,628 | 2,219 | 335 | 369 | 360 | 0.22 | 0.21 | 0.06 | 10.9 | 6.8 | 6.0 |
| 3 | 1,273 | 533 | 1,688 | 1,546 | 910 | 1,621 | 343 | 343 | 350 | 0.28 | 0.23 | 0.18 | 14.0 | 12.9 | 8.0 |
| 4 | 413 | 583 | 362 | 519 | 830 | 1,431 | 366 | 439 | 353 | 0.11* | 0.04* | 0.17 | 4.4 | 2.7 | 7.7 |
| 5 | 104 | 169 | 722 | 557 | 1,667 | 2,251 | 321 | 348 | 325 | 0.48 | 0.19 | 0.45 | 20.1 | 7.3 | 19.3 |
| 6 | 120 | 168 | 390 | 489 | 1,662 | 1,506 | 365 | 358 | 343 | 0.05* | 0.12* | 0.16 | 6.4 | 4.6 | 6.5 |
| Median | 340 | 431 | 807 | 1,051 | 1,511 | 1,563 | 340 | 358 | 347 | 0.25 | 0.18 | 0.18 | 11.3 | 6.8 | 7.9 |

*Values for the regression line did not differ significantly (P < 0.05) from 0.

For all dogs, median 12-hour mean Uosm was 901 mOsm/kg (range, 104 to 2,219 mOsm/kg), median osmotic threshold was 348 mOsm/kg (range, 331 to 439 mOsm/kg), median sensitivity was 0.18 (pg/mL)/(mOsm/kg) (range, 0.04 to 0.48 [pg/mL]/[mOsm/kg]), and median maximum VP concentration was 7.7 mg/mL (range, 2.7 to 20.1 mg/mL).

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NA = Not available.

Figure 1—Vasopressin (VP) secretion in response to osmotic stimulation with hypertonic saline (20% NaCl) solution in 6 dogs with pyometra before (A) and 1 month after (B) ovariohysterectomy and in 6 control dogs of comparable age (C). Each symbol represents results for 1 dog. Posm = Plasma osmolality.
also have been attributable to hypovolemia associated with the VP response caused by osmotic stimulation may not reach 400 mOsm/kg after ovariohysterectomy in 2 dogs with pyometra. In some dogs with pyometra, VP release seemed to be increased, but it returned to expected values after ovariohysterectomy. The threshold value for VP secretion was decreased and sensitivity of the VP response was increased in some dogs with pyometra, compared with values for the control dogs. Analysis of the data suggests that VP secretory ability is adequate in dogs with pyometra. Changes in threshold and sensitivity of the osmoregulatory system have been reported for several endocrine disorders, including the syndrome of inappropriate VP release. The results observed in our study could potentially result from interference with the endocrine secretory feedback system, a direct mechanical or biochemical interference with VP receptors, or interference with the formation of aquaporin water channels in renal tubular cells. An increase in the VP response caused by osmotic stimulation may also have been attributable to hypovolemia associated with polyuria.

We did not detect a substantial increase in plasma VP concentration during osmotic stimulation before or after ovariohysterectomy in 2 dogs with pyometra. In both dogs, only a small increase in Uosm was detected during desmopressin treatment, excluding deficiency of VP as the sole cause of PU-PD. Values for Uosm did not reach 400 mOsm/kg after ovariohysterectomy in these 2 dogs, whereas values of 700 to 1,700 mOsm/kg were reached after ovariohysterectomy in the other dogs with pyometra. Examination of renal biopsy specimens obtained during ovariohysterectomy revealed tubulointerstitial nephritis and pyelonephritis in these 2 dogs, whereas membranous glomerulonephritis or no abnormalities were found in the other dogs with pyometra. Thus, polyuria after ovariohysterectomy in these 2 dogs may have been attributable to permanent renal damage. Both dogs were in poorer clinical condition than was apparent from the medical history and initial clinical examination. The severity of their condition may explain why these dogs did not have good urinary concentrating capacity during control testing after ovariohysterectomy.

Renal lesions in dogs with pyometra are believed to be temporary because PU-PD in most affected dogs improves within a few weeks after surgery. However, polyuria may remain after surgery, as was seen in the 2 dogs of our study. Thus, the question remains as to whether polyuria is a direct result of the renal lesions. Tubular abnormalities in the kidneys of dogs with pyometra may interfere with the cellular action of activated VP receptors. However, the glomerular and tubular lesions described in dogs with pyometra have typically been based on the results of studies in which investigators did not include an age-matched control group. In 1 study with age-matched control dogs, changes were similar in the kidneys of healthy dogs and dogs with pyometra, suggesting that renal lesions in dogs with pyometra are not directly associated with polyuria.

The low Uosm values during serial measurements on day 1 clearly documented polyuria in the dogs with pyometra. After ovariohysterectomy, Uosm improved in 4 of 6 dogs. In control dogs and dogs with pyometra, desmopressin treatment increased urine concentration. Therefore, it can be concluded that the healthy dogs did not concentrate urine to a maximal extent. Although endogenous VP production was sufficient, administration of desmopressin increased urine concentration. Administration of desmopressin did not return Uosm to normal values in dogs with pyometra, suggesting partial renal resistance to VP. These data are compatible with the downregulation of VP receptors, interference with VP receptor function, or interference with the formation of aquaporin water channels in renal tubular cells. Administration of desmopressin 1 month after ovariohysterectomy did not cause Uosm values to improve and be similar to values of the control dogs, suggesting that renal resistance to VP may not have been fully reversed at the time of testing.

Although we did not measure circulating endotoxin concentrations in this study, results of bacterial culture make it unlikely that all of the bitches with PU-PD were endotoxemic. Administration of E coli toxin to clinically normal dogs induces decreased renal concentrating ability and polyuria, which persists as long as exposure to the toxin is maintained. Two studies have revealed varying prevalence of endotoxemia in bitches with pyometra by use of differing methods for detection. In 1 of those studies, the plasma endotoxin concentration was significantly higher in dogs with pyometra that had more severe clinical effects. Although E coli predominates, other bacteria (including gram-positive bacteria) are involved in pyometra, as observed here and in other studies. In some dogs with pyometra, blood endotoxin concentrations are low, and in dogs in which E Coli are not isolated, polyuria is also evident.

Urine concentration was increased in dogs with pyometra in response to desmopressin but did not reach the Uosm values of the control dogs. Pyometra was not associated with decreased ability to secrete VP. These results are consistent with interference with the
function of VP receptors or interference with the formation of aquaporin water channels in renal tubular cells of dogs with pyometra.

References


