Effects of meloxicam and phenylbutazone on renal responses to furosemide, dobutamine, and exercise in horses

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Objective—To compare the effects of 2 NSAIDs (phenylbutazone and meloxicam) on renal function in horses.

Animals—9 Thoroughbred or Standardbred mares (mean ± SD age, 5.22 ± 1.09 years [range, 2 to 12 years]; mean body weight, 470 ± 25 kg [range, 442 to 510 kg]).

Procedures—A randomized blinded placebo-controlled crossover study was conducted to examine the effects of treatment with phenylbutazone, meloxicam, or a placebo (control solution) on renal responses to the administration of furosemide, dobutamine, and exercise (15 minutes at 60% of maximum heart rate). Renal function was assessed by use of bilateral ureteral catheterization for simultaneous determination of creatinine clearance, sodium excretion, and urine flow rate.

Results—Both phenylbutazone and meloxicam attenuated diuresis and natriuresis and reduced glomerular filtration rate, compared with results for the control solution, when horses were treated with furosemide. Mean arterial blood pressure, urine flow rate, and glomerular filtration rate were increased during or after (or both) dobutamine infusion. Both NSAIDs reduced urine flow rate and sodium excretion associated with dobutamine infusion and exercise but had no effect on glomerular filtration rate.

Conclusions and Clinical Relevance—Responses to meloxicam, a cyclooxygenase (COX)-2 preferential agent, appeared comparable to those detected after phenylbutazone treatment, which suggested that COX-2 was the mediator of prostanoid-induced changes to renal function in horses and indicated that COX-2–preferential agents would be likely to have adverse renal effects similar to those for nonselective COX inhibitors in volume-depleted horses. (Am J Vet Res 2014;75:668–679)

Cyclooxygenase (ie, PGH2 synthase) catalyzes the oxidation of arachidonic acid to the hydroperoxy endoperoxide PGG2 and its subsequent reduction to PGH2, which are the first 2 steps in prostanoid biosynthesis. Two isoforms of COX have been recognized since the early 1990s,1,2 and a third isoform (which is believed to be a splice derivative of COX-1) has been identified in the CNS.3 The constitutive expression of COX-1 in most cells was initially interpreted as evidence that this isoenzyme mediates homeostatic prostanoid functions, whereas the inducible production of COX-2 suggested this isoenzyme was responsible for proinflammatory responses. However, this dichotomous view of the function of COX isoforms was discredited with evidence that COX-2 is expressed in regions of nephrons responsible for prostanoid regulation of renal homeostasis, specifically the macula densa and adjacent cortical thick ascending limb of Henle.4 Other studies5–9 have indicated that although there are effects of species and diet on COX-2 expression, this isoenzyme is constitutively expressed in the renal tissues of all species that have been evaluated and is intimately involved in PG-dependent homeostatic regulation of renal hemodynamics, water excretion, and electrolyte balance.10–13

Arachidonic acid–derived prostanoids mediate renin release in the macula densa and contribute to tu

ABBREVIATIONS

<table>
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<tr>
<td>AVP</td>
<td>Arginine vasopressin</td>
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<td>COX</td>
<td>Cyclooxygenase</td>
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<tr>
<td>FE Na+</td>
<td>Fractional excretion of sodium</td>
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<td>GFR</td>
<td>Glomerular filtration rate</td>
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bular control of sodium, potassium, and water excretion. Prostacyclin (ie, PGL, PGE, and PGD, mediate a decrease in vascular resistance and redistribution of blood flow from the renal cortex to juxta medullary nephrons. Therefore, the function of COX-2 in the kidneys is of considerable clinical relevance because renal PGs are pro trophic of renal blood flow in the face of vasoconstrictive effects of renal sympathetic nerve activity, catecholamines, angiotensin II, and AVP. Prostaglandin E, also functions as an inhibitor regulator of AVP-induced reabsorption of water and sodium in the collecting tubule and thick ascending limb, respectively, whereby mediating diuretic and natriuretic effects. Consequently, inhibition of renal COX activity is associated with qualitative changes in urinary PG excretion and GFR, with adverse effects including retention of sodium, development of peripheral edema, and necrosis of the papillary crest. Renal adverse events after NSAID administration are more common for diseases characterized by a low circulating plasma volume.

To our knowledge, the distribution of COX-2 in the kidneys of horses has not been evaluated; similarly, the role of COX-2–derived prostanoids in renal homeostasis has not been determined in this species. Such information has important clinical implications because NSAIDs are used commonly in horses as anti-inflammatory or analgesic medications and often are used for conditions in which animals are likely to have a reduced effective circulating plasma volume. Renal adverse effects are not associated with prescribed dosing regimens for phenylbutazone or meloxicam in healthy euhydrated animals; however, renal effects of meloxicam have not been evaluated in compromised animals or under conditions whereby renal homeostatic mechanisms are challenged. In human patients with renal disease, meloxicam may have less nephrotoxic potential than nonselective COX inhibitors.

The effects of phenylbutazone on renal responses to exercise and furosemide in horses have been reported. Precise determination of urine output in horses can be achieved through ureteral catheterization, and this approach has been used to confirm that phenylbutazone treatment, which would provide evidence for a role of COX-2 in renal homeostasis in horses. We further hypothesized that dobutamine infusion would increase urine output and that this response would be unaffected by NSAID administration, which would suggest a direct sympathetomimetic effect on renal function independent of prostanoid production.

Materials and Methods

Animals—Nine Thoroughbred or Standardbred mares were included in the study. At the start of the study, mean ± SD age of the horses was 5.22 ± 1.09 years (range, 2 to 12 years), and mean body weight was 470 ± 25 kg (range, 442 to 510 kg). Horses were acclimatized to physical restraint in stocks and bilateral ureteral catheterization before commencement of experiments to ensure that they would tolerate ureteral catheterization and restraint for the duration of each experiment (3 to 4 hours). Horses were maintained at pasture during the interval between experiments, and their diet was supplemented with alfalfa hay. Horses were weighed each week immediately before ureteral catheterization. Horses were not fed or provided water during confinement in stocks and experiments. All procedures were approved by the Animal Care and Ethics Committee of Charles Sturt University.

Experimental design—A study population of 9 horses allowed use of a randomized block design based on a 3 × 3 Latin square. Power analysis suggested that 9 horses would enable us to discriminate differences in mean urine production as low as 5 mL/horse/min with a power of 95% (assuming an SD of 2.5 mL/horse/min, which was derived during preliminary studies with horses not included in the study reported here). The study was conducted as 5 sequential parts. Each of parts 1 through 4 was conducted over 3 consecutive weeks (12-week period), with a 1-week break between parts 3 and 4 to permit the horses to acclimatize to a treadmill. Part 5 was performed in experimental week 13. Experiments were commenced on the same day and at the same time (7 AM) each week, with the remaining 6 days allowed for drug washout and recovery.

Ureteral catheterization—On the day of an experiment, ureteral catheterization was performed as described elsewhere. Briefly, mares were restrained with their tails bandaged and elevated. The perineum of each mare was cleaned and the bladder emptied. Two gloved fingers of an investigator were passed through the urethra, and the ureteral orifices were identified by digital palpation. A 10F or 12F, 55-cm, silicone Foley catheter with stylette was advanced into one of the ureters; the stylette was then removed and the balloon inflated with 3 mL of air. The procedure was repeated for the contralateral ureter. After insertion of both catheters, 2 mL of lidocaine was infused locally into the vulva, and the catheters were sutured to the vulva. The catheters were evaluated to ensure they were patent (urine passing freely through both catheters), and each catheter then was connected to a separate closed collection system. After all urine samples were collected on the day of an experiment, the ureteral catheters were removed, and each mare received a single dose of penicillin G procaine (20 to 25 mg/kg, IM).

Experimental procedures—During each 3-week period for parts 1 through 4, horses were allocated to
receive phenylbutazone to receive (4.4 mg/kg, meloxicam to receive (0.6 mg/kg), or an equivalent volume of placebo (meloxicam vehicle only; control treatment) in accordance with a Latin square design. Treatments were administered orally 60 to 80 minutes before the administration of creatinine, Randomization of treatment order (by random number allocation and ballot) and administration of treatments were performed by an investigator (ALC) who was not responsible for collection of experimental data; other investigators were not aware of the treatment administered to each horse.

**PART 1 (EFFECT OF MELOXICAM AND PHENYLBUTAZONE ON RENAL FUNCTION)**

Urine flow rate, urine output, and creatinine clearance were determined after administration of an NSAID or the placebo and used to assess NSAID effects on renal function in healthy euhydrated horses. Catheters were placed in the ureters as described previously. A 16-gauge, 3.5-inch catheter was aseptically placed in a jugular vein of each mare to facilitate collection of multiple blood samples. Placebo or NSAID treatment was administered orally to each mare at –60 minutes. At –10 minutes, the urine collection bag of each mare was emptied, and urine then was collected for a 10-minute period (–10 to 0 minutes). At time 0, creatinine (65% of a total dose of 30 mg/kg) was administered SC in the cervical region, with the remainder of the creatinine (35% of a total dose of 30 mg/kg) administered SC at 35 minutes, as described elsewhere. The calculated dose of creatinine was dissolved in 200 mL of sterile compound sodium lactate solution (ie, Hartmann solution) by means of heating (approx 80°C) and agitation. The resultant solution was filtered and placed into 50-mL syringes. Creatinine solutions were prepared aseptically within 12 hours before administration.

Urine flow rate was determined at 35, 80, 100, and 120 minutes. Venous blood samples were collected at 80, 100, and 120 minutes. Paired serum and urine samples obtained at 80, 100, and 120 minutes were submitted for calculation of creatinine clearance. Sodium excretion and FE\textsubscript{Na} were determined in urine samples collected at 80, 100, and 120 minutes. The total volume of urine collected during the entire 130-minute collection period was recorded for each horse.

**PART 2 (EFFECT OF MELOXICAM AND PHENYLBUTAZONE ON RENAL FUNCTION AFTER THE ADMINISTRATION OF FUROSEMIDE)**

Similar to the procedures in part 1, catheters were placed in the ureters, a catheter was placed in a jugular vein, treatment was orally administered to each mare at –60 minutes, and urine was collected for 10 minutes (–10 to 0 minutes). At time 0, creatinine (65% of a total dose of 30 mg/kg) was administered SC in the cervical region; creatinine was prepared as described in part 1. Urine flow was determined at 0 and 10 minutes, and a dobutamine infusion (5 µg/kg/min) was administered from 10 to 40 minutes. The remainder of the creatinine (35% of a total dose of 30 mg/kg) was administered SC at 35 minutes (5 minutes before the end of the dobutamine infusion). Urine flow rate was determined at 20 (10 minutes after the start of the dobutamine infusion), 40 (immediately after completion of the dobutamine infusion), 80, 100, and 120 minutes. Total urine output was determined for the entire 130-minute collection period in each horse. Paired serum and urine samples obtained at 10 minutes (prior to start of dobutamine infusion), 20 minutes (10 minutes after the start of the dobutamine infusion), and 100 minutes (60 minutes after completion of the dobutamine infusion) were used for calculation of creatinine clearance and FE\textsubscript{Na}. Renal sodium excretion and FE\textsubscript{Na} were determined from urine samples collected at 10, 20, and 100 minutes.

The MAP was determined noninvasively at 0, 10, 20, 40, 50, 70, 100, and 120 minutes by means of indirex oscillometric sphygmomanometry with a cuff placed over the coccygeal artery. MAP was based on a minimum of 3 separate, repeatable measurements. The HR was recorded at these same times.

**PART 3 (EFFECT OF MELOXICAM AND PHENYLBUTAZONE ON RENAL RESPONSES TO DOBUTAMINE INFUSION)**

Similar to the procedures in parts 1 and 2, catheters were placed in the ureters. A 16-gauge, 3.5-inch catheter was aseptically placed in both jugular veins of each mare (one catheter was used for collection of blood samples, and the other was used for administration of dobutamine). Treatment was orally administered to each mare at –60 minutes, and urine was collected for 10 minutes (–10 to 0 minutes). At time 0, creatinine (65% of a total dose of 30 mg/kg) was administered SC in the cervical region; creatinine was prepared as described in part 1. Urine flow was determined at 0 and 10 minutes, and a dobutamine infusion (5 µg/kg/min) was administered from 10 to 40 minutes. The remainder of the creatinine (35% of a total dose of 30 mg/kg) was administered SC at 35 minutes (5 minutes before the end of the dobutamine infusion). Urine flow rate was determined at 20 (10 minutes after the start of the dobutamine infusion), 40 (immediately after completion of the dobutamine infusion), 80, 100, and 120 minutes. Total urine output was determined for the entire 130-minute collection period in each horse. Paired serum and urine samples obtained at 10 minutes (prior to start of dobutamine infusion), 20 minutes (10 minutes after the start of the dobutamine infusion), and 100 minutes (60 minutes after completion of the dobutamine infusion) were used for calculation of creatinine clearance and FE\textsubscript{Na}. Renal sodium excretion and FE\textsubscript{Na} were determined from urine samples collected at 10, 20, and 100 minutes.

**PART 4 (EFFECT OF MELOXICAM AND PHENYLBUTAZONE ON RENAL RESPONSES TO EXERCISE OF MODERATE INTENSITY)**

Horses were acclimatized to exercise on a high-speed treadmill inclined at 6° by 3 runs (speed, 4 to 5 m/s) during the 10-day period before commencing this part of the study. The HRmax for each horse was determined at this time by an incremental maximal exercise test. Similar to the procedures in the preceding parts, catheters were placed in the ureters, a catheter was placed in a jugular vein, and treatment was orally administered to each mare at –60 minutes. At 40 minutes, each mare received 30 mL of sterile compound sodium lactate solution (ie, Hartmann solution) by means of heat-
Urine flow rate was determined at 0 minutes (immediately before commencing exercise), 20 (5 minutes of recovery after the 15-minute exercise period; during the recovery period, horses were moved from the treadmill to collection stocks), 30, and 50 minutes. Total urine volume was determined for the entire 60-minute collection period. Creatinine clearance and \( FE_{\text{Na}} \) were calculated from paired urine and serum samples obtained at 0, 20, and 50 minutes. Renal sodium excretion and \( FE_{\text{Na}} \) were calculated from urine samples collected at 0, 20, and 50 minutes.

**Part 5 (Urine flow rate and creatinine clearance at rest)**

Part 5 was conducted to reevaluate urine flow rates and to identify differences attributable to the 2 creatinine clearance protocols used in parts 1 through 4. Similar to the procedures in the preceding parts, catheters were placed in the ureters, and a catheter was placed in a jugular vein. At time 0, 4 mares received a single dose of creatinine (20 mg/kg, IV). Urine flow rate was determined for these 4 mares at 0, 20, 40, 60, and 80 minutes, and creatinine clearance was calculated from paired urine and serum samples obtained at 20, 40, 60, and 80 minutes. At time 0, the remaining 5 mares received an SC administration of creatinine (65% of a total dose of 30 mg/kg); those 5 mares received the remainder of the creatinine (35% of a total dose of 30 mg/kg) via SC administration at 35 minutes. Urine flow rate was determined at 0, 10, 30, 50, 70, and 90 minutes. Creatinine clearance and \( FE_{\text{Na}} \) were calculated from paired urine and serum samples obtained at those same times.

**Analysis of samples**—Urine flow rate was calculated from the volume collected from both ureters during each designated collection period. Total urine output was the volume of urine collected during an entire collection period in each part of the study.

The GFR was determined by serum creatinine clearance, adapted from a method described elsewhere. Creatinine clearance was determined by evaluation of paired serum and urine samples by use of the following equation:

\[
CL_{\text{cr}} = \frac{\text{[urine volume} \times \text{Cr}_{\text{urine}}]}{\text{body weight}} \times \frac{1}{\text{time}}
\]

where \( CL_{\text{cr}} \) is the clearance of creatinine, \( \text{Cr}_{\text{urine}} \) is the urine creatinine concentration, and \( \text{Cr}_{\text{serum}} \) is the serum concentration of creatinine.

Sodium excretion was calculated on the basis of urine volume and sodium concentration in urine samples obtained at specified time points. The \( FE_{\text{Na}} \) was calculated by use of the following equation:

\[
FE_{\text{Na}} = 100 \times \left( \frac{\text{Na}^+_{\text{urine}} \times \text{Cr}_{\text{serum}}}{\text{Na}^+_{\text{serum}} \times \text{Cr}_{\text{urine}}} \right)
\]

where \( \text{Na}^+_{\text{urine}} \) is the urine sodium concentration and \( \text{Na}^+_{\text{serum}} \) is the serum sodium concentration. Sodium concentrations were determined with an ion-selective electrode on samples that had been stored at \(-20^\circ\text{C}\) prior to analysis.

**Statistical analysis**—Data were initially evaluated by use of descriptive analyses. Data were analyzed by use of a linear mixed model with restricted maximum likelihood. The model was as follows: response + mean + treatment + time + treatment-by-time interaction + horse + day.

Horse and day were fitted as random terms, and treatment, time, and the treatment-by-time interaction were fixed terms. Total urine volume was analyzed similarly without inclusion of time and the treatment-by-time interaction. Model assumptions were that factor-level variances were equal (as determined by use of the Hartley test) and that residuals were normally distributed (as determined by use of the Shapiro-Wilk test of normality), had constant variance, and were independent. When treatment or factor-level variances were unequal, additional terms were fitted in the model to account for extra variability. When necessary for a variable (ie, urine flow rate, total urine output, MAP, and Na+ clearance), data were logarithmically transformed to meet other model assumptions. Predicted values were assigned a rank on the basis of the Tukey family of pairwise differences with a family confidence level of 5%. Unless indicated otherwise, results were reported as predicted values, and SE was determined by use of the linear mixed model. Values were considered significant at \( P < 0.05 \).

**Results**

**Part 1 (effect of meloxicam and phenylbutazone on renal function)**—Eight horses completed this part of the study. One horse was fractious in the stocks and was able to complete only the first 2 weeks of this experiment; thus, data for that horse were not included in the analysis for this part of the study. Time of collection had a significant (\( P < 0.001 \)) effect on urine flow rate and creatinine clearance (Figure 1). Mean ± SEM urine flow rate was less, but not significantly (\( P = 0.052 \)) different, following both meloxicam (11.6 ± 1.1 µL/kg/min) and phenylbutazone (12.6 ± 1.1 µL/kg/min) treatment, compared with urine flow rate for the control treatment (13.9 ± 1.1 µL/kg/min). There was not a significant effect of the treatment-by-time interaction for urine flow rate (\( P = 0.952 \)) or creatinine clearance (\( P = 0.996 \)). Urine flow rate decreased as time progressed, with mean values for all treatments being significantly lower at 80, 100, and 120 minutes than the values at 0 minutes. Conversely, creatinine clearance increased significantly (\( P < 0.001 \)) for the 3 collection times. Treatment had no significant (\( P = 0.231 \)) effect on creatinine clearance. Total urine output was less, but not significantly (\( P = 0.250 \)) different, following administration.
of both phenylbutazone and meloxicam, compared with total urine output after the control treatment. Values for FENa+, determined for 3 placebo-treated horses ranged between 0.046% and 1.046%.

Part 2 (effect of meloxicam and phenylbutazone on renal function after the administration of furosemide)—There was not a significant (P = 0.283) effect of the treatment-by-time interaction. However, furosemide administration had a significant (P < 0.001) effect on urine flow rate, which caused a significant increase in urine flow rate at 20, 35, 80, and 100 minutes, compared with the urine flow rate at time 0 (Figure 2). For all sample collection times, mean ± SEM urine flow rate for the control treatment (78.3 ± 1.1 µL/kg/min) was significantly (P < 0.001) greater than the urine flow rate after phenylbutazone (61.6 ± 1.1 µL/kg/min) or meloxicam (55.5 ± 1.1 µL/kg/min) administration. Peak urine flow rate was detected 35 minutes after treatment with phenylbutazone or meloxicam, compared with 20 minutes after administration of the control treatment. Treatment also had a significant (P = 0.002) effect on total urine output during the 120-minute collection period after treatment. Compared with the mean urine output after administration of the control treatment (10.33 ± 0.56 L), mean urine production was reduced after administration of phenylbutazone (9.10 ± 0.67 L) and meloxicam (7.89 ± 0.42 L); however, the reduction...
was only significantly different after meloxicam administration. Treatment, but not time of sample collection, had a significant ($P < 0.001$) effect on creatinine clearance after furosemide administration. Administration of both phenylbutazone ($1.3 \pm 0.10$ mg/kg/min) and meloxicam ($1.46 \pm 0.10$ mg/kg/min) caused a decrease in creatinine clearance, compared with that for the control treatment ($1.80 \pm 0.10$ mg/kg/min). Furosemide treatment caused a significant ($P < 0.001$) increase in mean $\text{FE}_\text{Na}^+$ ($6.60 \pm 1.36\%$; range, $1.71\%$ to $16.84\%$) at 80 minutes in all horses, but NSAID treatment did not have a significant ($P = 0.141$) effect on $\text{FE}_\text{Na}^+$. Similarly, sodium excretion was significantly ($P < 0.001$) increased at 80 minutes in all horses ($281.68 \pm 1.33$ mmol; range, 64.6 to 800.0 mmol), and there was a significant ($P = 0.041$) effect of treatment across all sampling times. Urine sodium concentration after phenylbutazone (mean, $39.7 \pm 1.3$ mmol; range, 2.2 to 714.0 mmol) and meloxicam (mean, $37.2 \pm 1.3$ mmol; range, 1.1 to 434.9 mmol) treatment was significantly less than that after the control treatment (mean, $60.8 \pm 1.3$ mmol; range, 5.9 to 800.0 mmol).

**Part 3 (effect of meloxicam and phenylbutazone on renal responses to dobutamine infusion)**—Dobutamine infusion was associated with a significant ($P < 0.001$) increase in MAP in all horses, regardless of treatment, with no concomitant increase in HR (Figure 3). Although time did not have a significant effect on HR, there was a significant ($P = 0.002$) effect of NSAID treatment on HR. Treatment with both phenylbutazone ($36.83 \pm 1.13$ beats/min) and meloxicam ($37.31 \pm 1.13$ beats/min) was associated with a significantly lower HR, compared with results after the control treatment ($38.99 \pm 1.13$ beats/min). There were significant effects of NSAID treatment ($P = 0.009$) and time of sample collection ($P = 0.001$) on urine flow rate, but the treatment-by-time interaction did not have a significant effect on urine flow rate. Across all treatments, urine flow rate peaked 10 minutes after the start of Figure 3—Heart rate (A), MAP (B), urine flow rate (C), urine output (D), creatinine clearance (E), and sodium excretion (F) for 9 horses after SC administration of phenylbutazone, meloxicam, or a placebo (control treatment) at time 0 and IV infusion of dobutamine (5 µg/kg/min for 30 minutes from 10 to 40 minutes) in part 3. Values reported in panels A, B, C, E, and F represent mean ± SEM. In panel A, treatment had a significant ($P = 0.002$) effect on HR. In panel B, time had a significant ($P < 0.001$) effect on MAP. In panel C, time and treatment each had a significant ($P = 0.001$ and 0.009, respectively) effect on urine flow rate. In panel D, treatment did not have a significant effect on urine volume. In panel E, time had a significant ($P < 0.001$) effect on creatinine clearance, but treatment did not have a significant effect on creatinine clearance. In panel E, time and treatment each had a significant ($P = 0.038$ and 0.002, respectively) effect on sodium excretion. **Values with different letters differ significantly ($P < 0.05$). See Figure 1 for remainder of key.
the dobutamine infusion and then decreased such that mean urine flow rates for all treatments at 40, 80, and 120 minutes were significantly less than the peak urine flow rate. Throughout the urine collection period, there was a significant reduction in urine flow rate after treatment with phenylbutazone (13.5 ± 1.2 µL/kg/min), compared with the urine flow rate for the control treatment (16.4 ± 1.2 µL/kg/min). Urine flow rate was also reduced after meloxicam treatment (14.7 ± 1.2 µL/kg/min), but this reduction was not significantly different from results after phenylbutazone treatment or the control treatment. Total urine output was not significantly (P = 0.199) affected by treatment. Dobutamine infusion was associated with a significant increase in creatinine clearance 10 minutes after the start of the dobutamine infusion (ie, time = 20 minutes) and 1 hour after completion of the dobutamine infusion (ie, time = 100 minutes). This response was not significantly (P = 0.352) affected by the administration of phenylbutazone or meloxicam, and there was not a significant effect for the time-by-treatment interaction. Although NSAID treatment did not have a significant (P = 0.074) effect on FE\textsubscript{Na}, there was a significant (P = 0.002) effect of time on FE\textsubscript{Na} for all treatments. The FE\textsubscript{Na} 1 hour after dobutamine infusion (mean ± SEM, 0.16 ± 1.49%; range, 0.03% to 0.73%) was significantly less than the value at other times. Significant effects of time (P = 0.038) and NSAID treatment (P = 0.020) on sodium excretion were detected. The mean value obtained after meloxicam treatment (13.99 ± 1.62 mmol), but not after phenylbutazone treatment (19.11 ± 1.57 mmol), was significantly less than the mean value obtained after the control treatment (36.63 ± 1.55 mmol). The treatment-by-time interaction was not significant.

Part 4 (effect of meloxicam and phenylbutazone on renal responses to exercise of moderate intensity)—Treatment (meloxicam, phenylbutazone, or placebo) was administered (mean ± SEM) 72 ± 12.83 minutes before the onset of exercise. Both phenylbutazone and meloxicam had significant (P < 0.001) effects on renal response to exercise (Figure 4). Mean urine flow rate was significantly reduced after administration of meloxicam (7.3 ± 1.1 µL/kg/min) or phenylbutazone (6.6 ± 1.2 µL/kg/min), compared with results for the control treatment (10.5 ± 1.2 µL/kg/min). Despite an apparent increase in urine flow rate during or immediately after exercise, time did not have a significant (P = 0.103) effect because of the large variation in responses to exercise within horses, and the treatment-by-time interaction also was not significant. There was not a significant effect of treatment on total urine output (P = 0.160) or creatinine clearance (P = 0.156). There was not a significant effect of time (P = 0.152) on creatinine clearance. Treatment, but not time of sample collection, had a significant (P = 0.001) effect on FE\textsubscript{Na}. Values obtained after treatment with meloxicam (mean ± SEM, 0.06 ± 1.27%; range, 0.02% to 0.24%; logarithmically transformed predicted mean ± SEM, −2.88 ± 0.24), but not after treatment with phenylbutazone (mean, 0.08 ± 1.27%; range, 0.02% to 0.21%; logarithmically transformed predicted mean ± SEM, −2.56 ± 0.24), were significantly less than values obtained after administration of the control treatment (mean, 0.10 ± 1.27%; range, 0.03% to 0.33%; logarithmically transformed predicted mean ± SEM, −2.29 ± 0.24). Similarly, time did not have a significant (P = 0.152) effect on sodium excretion, but treatment had a significant (P = 0.003) effect on sodium excretion. Mean sodium excretion after the administration of both meloxicam (4.82 ± 1.24 mmol) and phenylbutazone (5.19 ± 1.24 mmol) was less than that after the control treatment (8.30 ± 1.24 mmol). Red discoloration of urine, which was suggestive of hematuria or pigmenturia, was evident in

Figure 4—Urine flow rate (A), urine output (B), creatinine clearance (C), and sodium excretion (D) for 9 horses after SC administration of phenylbutazone, meloxicam, or a placebo (control treatment) at time 0 and exercise of moderate intensity from 0 to 15 minutes in part 4. In panel A, treatment had a significant (P < 0.001) effect on urine flow rate, but time did not have a significant effect on urine flow rate. In panel B, treatment did not have a significant effect on urine volume. In panel C, neither treatment nor time had a significant effect on creatinine clearance. In panel D, treatment had a significant (P = 0.005) effect on sodium excretion, but time did not have a significant effect on sodium excretion. See Figure 1 for remainder of key.
appeared comparable to those for phenylbutazone, it is fusion and exercise. Because responses to meloxicam excretion, but not GFR, associated with dobutamine in- mide. Both NSAIDs reduced urine flow rate and sodium attenuation diuretic and natriuretic responses to furose-

results indicated that both NSAIDs reduced GFR and the case following a single intervention. Analysis of the robust perspective on renal responses than would be rate challenges to renal homeostasis providing a more information on renal function, with the inclusion of 3 sepa- mation on renal function, with the inclusion of 3 sepa- rateous characterization of both glomerular and

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Discussion

In the study reported here, we evaluated effects of a nonselective (phenylbutazone) and COX-2–prefer-ential (meloxicam) inhibitor of PG synthesis on renal function in horses by comparing changes in GFR, so-
dium excretion, and urine flow in response to furose-
mide, dobutamine, and moderate exercise after NSAID administration. Each part of the study was designed as a separate intervention to provide complementary information on renal function, with the inclusion of 3 separate challenges to renal homeostasis providing a more robust perspective on renal responses than would be the case following a single intervention. Analysis of the results indicated that both NSAIDs reduced GFR and attenuated diuretic and natriuretic responses to furose-
mide. Both NSAIDs reduced urine flow rate and sodium excretion, but not GFR, associated with dobutamine infusion and exercise. Because responses to meloxicam appeared comparable to those for phenylbutazone, it is likely that COX-2 mediated prostanoinduced changes caused by pharmacological and physiologic challenges in the present study.

Oral administration of NSAIDs was selected for the present study because phenylbutazone and meloxicam are both commonly administered via this route and because our research group has evaluated pharmacokinetics of both drugs following oral administration to adult horses. Physiologic or pharmacological challenges and subsequent samples were timed such that horses would have plasma concentrations between 200 and 1,000 ng/mL (meloxicam14,21) or 5 and 20 µg/mL (phenylbutazone14,22), which are likely to represent therapeutic plasma concentrations. However, because plasma drug concentration, tissue (renal) penetration, and biological effects of NSAIDs are variable and not in phase, hysteresis in the concentration-effect relationship is a feature of these agents, and it is difficult to predict the relationship between plasma concentration and biological effects. The COX-2 selectivity of meloxicam is decreased at plasma concentrations of approximately 1,200 ng/mL or greater; thus, it was considered important to ensure that plasma meloxicam concentrations did not exceed this value. It is possible that IV administration or repeated oral administration may have achieved higher or more predictable tissue concentrations and a greater biological effect than was observed. Administration of both agents has been associated with biological effects in previous studies in horses16,17 and other species,24,25 which suggests that tissue concentrations of each agent were sufficient to influence renal PG synthesis and would be of clinical relevance.

The present study increased the information available about renal responses to the sympathomimetic drug dobutamine in conscious animals and effects associated with NSAID administration in response to dobutamine administration. However, we did not detect natriuretic effects after furosemide administration and renal effects on exercise, which suggested that experimental protocols (timing of sample collection or exercise intensity) were not optimized or that IV administration or more protracted dosing schedules may be appropriate in future studies.

Ureteral catheterization was tolerated well by horses in the present study, and use of this technique yielded urine flow rate and total urine output findings consistent with those in previous studies. Similarly, the use of creatinine clearance to assess GFR provided control values consistent with findings from earlier studies. Tubule function was evaluated by determination of FENa, and sodium excretion during timed urine collections. This approach permitted simultaneous characterization of both glomerular and tubular function in response to NSAID treatment and the various interventions.

Part 1 of the study revealed that there was a significant reduction in urine flow rate, consistent with renal
conservation of water. This reduction in urine flow rate was associated with increased tubular reabsorption of water because creatinine clearance (GFR) increased significantly, which was a response that would increase filtrate volume. The results from part 5 of the study indicated that neither the protracted sample collection period nor the various creatinine clearance protocols had a significant effect on variables of renal function determined during the study because results were consistent with values reported in the literature and with results from part 1 of the present study.

Urine flow rate and total urine output in part 1 were lower after administration of phenylbutazone or meloxicam; however, observed differences were not significant, and NSAID treatment had no effect on creatinine clearance. Prostaglandins do not mediate renal blood flow or tubular function in healthy euhydrated animals; however, administration of NSAIDs has been associated with dose-related increases in sodium and water retention.10 An increase in GFR, as suggested by the increase in creatinine clearance, may have elicited increased renal release via tubuloglomerular feedback. Whereas the findings for the present study did not support a subsequent homeostatic reduction in glomerular filtration, tubular reabsorption may have increased by activation of the renin-angiotensin-aldosterone axis. Because tubuloglomerular feedback is mediated by adenosine, PGE2, and nitrous oxide, NSAIDs may subsequently influence this response.

Furosemide is freely filtered in the glomeruli and secreted in the proximal tubules, where it increases urine flow by competitively binding to the chloride binding site of the Na+/K+/2Cl− cotransporter.31 Consequently, inhibition of reabsorption of sodium and chloride ions in the thick ascending limb of the loop of Henle results in the delivery of large volumes of isotonic fluid to the distal tubules. In addition to effects on tubular reabsorption, furosemide induces changes in renal hemodynamics25 and glomerular filtration32 by stimulating PGE2 production in the thick ascending limb of the loop of Henle34 and via direct stimulation of renin secretion34 associated with increased PGI1 synthesis35 (or independent of PGI1 synthesis36). The response detected in the present study was consistent with that in other reports in horses in which there was an increase in urine flow after the administration of furosemide,17 with peak flows 15 to 30 minutes after IV administration of furosemide. Consistent with reports in horses and other species whereby the nonspecific COX inhibitors phenylbutazone17 and indomethacin24,25 attenuated the diuretic effects of furosemide, findings in the present study indicated that the COX-2–preferential agent meloxicam also had a significant effect on urine production. Whereas phenylbutazone reduced total urine volume and urine flow rate to approximately 80% that of the values for the control treatment, the response after meloxicam administration was approximately 76% that of the values for the control treatment. This magnitude of response was consistent with findings considered to be of clinical relevance in other species in which the administration of NSAIDs typically reduces the response to loop diuretics by 15% to 20%.13 These observations are in contrast to findings in human patients with chronic heart failure in which meloxicam did not influence the response (cumulative urine output or creatinine clearance) to chronic administration of furosemide.30

The administration of both meloxicam and phenylbutazone in part 2 was associated with a significant reduction in creatinine clearance after furosemide treatment, which suggested that PG-dependent mechanisms can modulate glomerular filtration in horses and that COX-derived prostanoids such as PGE2 are important mediators of this effect. Because the magnitude of response to both meloxicam and phenylbutazone appeared similar, it is likely that the changes were mediated primarily by COX-2. Furosemide administration was associated with a significant increase in sodium excretion, which has been reported in other equine studies.30,37 Whereas NSAID administration has been reliably found to attenuate the natriuretic response to furosemide in other species,30 responses in horses have been less well characterized. In 1 study,16 in horses, phenylbutazone attenuated the natriuretic response to furosemide, and this effect was most evident during the first 60 minutes after furosemide administration. Although determination of sodium excretion (FESNa, sodium excretion at 80 minutes) in the present study included analysis of urine collected at ≥35 minutes after furosemide administration, it is possible that analysis of sodium excretion during the first 30 minutes after furosemide administration might have provided a better opportunity to assess NSAID-induced changes in renal handling of sodium. Alternatively, drug doses or administration may not have been optimized for a peak effect on tubule function, or renal effects on sodium handling may be more resilient to pharmacological modification than the effects for water handling. Investigators in an earlier study17 similarly failed to detect an effect of phenylbutazone administration on sodium excretion after furosemide treatment. It is worthy to mention that responses in the present study after administration of meloxicam were lower than those after administration of phenylbutazone or the control treatment, which suggested that further investigation of the effects of meloxicam on tubule function is warranted.

The hemodynamic effects of dobutamine have been sufficiently characterized in anesthetized horses, ponies, and foals17–41; however, the effects of this agent on systemic arterial blood pressure and renal function in conscious horses have been poorly characterized. In the present study, the administration of dobutamine increased MAP with no effect on HR. Dobutamine is an inotropic agent that increases cardiac output via direct stimulation of β2-adrenergic receptors, with lesser affinity for β1- and α-adrenergic receptors.37 An effect of NSAID treatment on HR was detected because both phenylbutazone and meloxicam were associated with a significant reduction in HR relative to values for the control treatment. Although the difference was small and likely of no physiologic importance, experiments in other species have indicated a complex interaction between adrenergic mechanisms and COX activation on cardiac function.42

To the authors’ knowledge, the study reported here represented the first evaluation of renal responses to dobutamine infusion in conscious adult horses. A transient and modest increase in urine flow rate was
observed during the first 10 minutes of dobutamine infusion, and creatinine clearance was increased after dobutamine treatment. These findings suggested that dobutamine infusion may support renal function in horses, although sodium excretion was decreased and caution must be exercised in extrapolating these findings from healthy subjects to hypovolemic or hypotensive animals or to animals with intrinsic renal dysfunction. Renal function after dobutamine infusion, in combination with norepinephrine administration, has been studied in normotensive foals; no significant difference in urine output, creatinine clearance, or fractional excretion of electrolytes was detected in that study. Investigators have reported divergent results for other species. In dogs, pigs, and humans, dobutamine infusion did not increase GFR or renal blood flow in adult patients or healthy volunteers. Conversely, studies in newborn pigs and human neonates revealed an increase in renal arterial blood flow in response to dobutamine infusion. Studies in compromised patients revealed an increase in renal blood flow in humans with congestive heart failure or low-output cardiac insufficiency and increased urine output in dogs with experimentally induced endotoxic shock.

In the study reported here, pretreatment with both phenylbutazone and meloxicam ameliorated increases in urine flow rate associated with dobutamine infusion. Although a significant treatment-by-time interaction was not detected, the response to phenylbutazone and meloxicam appeared to differ at 120 minutes. Physiologic or pharmacological mechanisms that might explain this observation were not examined in the present study. The increase in creatinine clearance induced by dobutamine infusion, which likely was attributable to an increase in renal (glomerular) blood flow, was not affected by NSAID treatment, and changes in renal handling of sodium were not associated with dobutamine infusion with or without NSAID treatment. Strenuous exercise in horses is associated with diversion of renal blood flow to support perfusion of exercising muscle, with a consequent decrease in urine flow and GFR, which is followed by a transient increase in urine flow. Conversely, exercise of moderate intensity increases urine output and associated with increased systemic blood pressure and endocrine mediators of renal function, including atrial natriuretic peptide, renin, aldosterone, and AVP. Phenylbutazone can abolish this response and also can attenuate the transient increase in urine flow after strenuous exercise. To our knowledge, possible effects of COX–2–preferential NSAIDs have not been evaluated in horses, and results of the present study indicated that the response to meloxicam treatment was indistinguishable from that after phenylbutazone treatment. Similar to results reported in another study, there was considerable within- and between-treatment variation in the urinary excretory responses to exercise in the present study. This is attributable to variation in work effort associated with standardized exercise protocols; therefore, it is likely that the intensity of exercise used in the present study (50% to 67% of HRmax) further confounded this variability. Exercise at 60% of maximal aerobic capacity represents the approximate transition from moderate intensity to high intensity, as defined by endocrine responses such as ACTH and glucocorticoid secretion. Hence, although exercise intensity was greater than moderate for most of the horses in the present study, as indicated by gross hematuria or pigmentation, the exercise protocol was not clearly moderate intensity or high intensity. After the control treatment, some horses responded with an increase in urine flow after exercise (typical of exercise of moderate intensity), whereas others responded with little or no change in urine flow. Our ability to discriminate effects during exercise from those evident after exercise was further reduced by the physical limitations that prevented collection of urine produced during exercise on the treadmill (urine flow rate was calculated at 20 minutes, which included urine output for 5 minutes of recovery following cessation of exercise). Regardless of these limitations, it is clear that the administration of phenylbutazone and meloxicam abolished changes in urine output that occurred in response to exercise.

Overall, analysis of the findings for the present study suggested that COX–2–preferential NSAIDs were likely to affect renal function in horses in a manner comparable to that for nonselective COX inhibitors, and risks for renal adverse effects are therefore likely to be similar. For this reason, drugs such as meloxicam should be used with caution in horses with a reduced effective plasma volume. Although characterization of COX expression was outside the scope of the present study, the findings supported a role of COX-2 in regulating glomerular and tubular function in horses. This could be further evaluated in studies of gene expression and by the use of COX-1–selective agents. Evaluation of renal and urine PG concentrations would provide direct evidence of the mechanism by which effects are mediated. The technique of ureteral catheterization with simultaneous determination of creatinine clearance and sodium excretion offers the opportunity to evaluate glomerular and tubule function in vivo in this species. Improved characterization of renal responses to NSAIDs could be achieved by administration of higher doses, by use of IV administration, and by administration of repeated doses. Determination of renal drug concentration would allow characterization of pharmacodynamic responses to treatment, which has been found to correlate with the severity of pathological changes.

c. MILA International Inc, Erlanger, Ky.
d. Oraljet P-butazone Paste, Virbac (Australia Pty Ltd), Milperra, NSW, Australia.
e. Meloxicam oral suspension, Troy Ilium, Glenedenbing, NSW, Australia.
f. C4253, Sigma-Aldrich Pty Ltd, Castle Hill, NSW, Australia.
g. MillisGP, 0.22-µm PES filter, Millipore Australia Ptd Ltd, North Ryde, NSW, Australia.
h. Avant 2120 pulse oximeter with noninvasive blood pressure monitor, Nonin Medical Inc, Plymouth, Minn.
i. Beltalong, Horse Technology, Euroa, VIC, Australia.


