Plasma concentration and local anesthetic activity of procaine hydrochloride following subcutaneous administration to horses

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Objective—To determine the durations of the local anesthetic effect and plasma procaine concentrations associated with 5- and 10-mg doses of procaine hydrochloride (with or without 100 µg of epinephrine) administered SC over the lateral palmar digital nerves of horses.

Animals—6 healthy adult horses.

Procedures—The hoof withdrawal reflex latency (HWRL) period was determined by use of a focused heat lamp before and after administration of procaine with and without epinephrine. Blood samples were collected immediately before determination of each HWRL period to assess plasma concentrations of procaine via liquid chromatography–mass spectrometry–mass spectrometry (LC-MS-MS).

Results—10 but not 5 mg of procaine alone and 5 and 10 mg of procaine administered with epinephrine significantly prolonged the HWRL period (mean durations of effect, 5, 120 and 180 minutes, respectively), compared with baseline values. Plasma procaine concentrations did not correlate well with local anesthetic activity; for example, although the HWRL was prolonged to the maximum permitted duration of 20 seconds at 60 to 180 minutes following administration of the 5-mg dose of procaine with epinephrine in certain horses, plasma procaine concentrations were less than the limit of quantitation of the LC-MS-MS assay.

Conclusions and Clinical Relevance—Small doses of procaine coadministered with epinephrine provided long-lasting local analgesia and resulted in plasma procaine concentrations that were not always detectable via LC-MS-MS. On the basis of these results, the use of regulatory limits or thresholds for procaine concentration in equine plasma samples obtained after racing should be seriously reconsidered. (*Am J Vet Res* 2007;68:495–500)

Pain is a perception that is perhaps shared by all species within the animal kingdom. Pain or nociception is a physiologic response that has been defined in animals as "an aversive sensory experience caused by actual or potential injury that elicits progressive motor and vegetative reactions, results in learned avoidance behavior, and may modify species specific behavior, including social behavior."[1] Nociceptive models of acute pain in animals are often classified by the type of stimulus used to evoke the sensation, and most fall into 1 of 4 categories: electrical, thermal, mechanical, or chemical. An ideal stimulus must be quantifiable, reproducible, and noninvasive.[2] Thermal models have been used to study acute pain in horses for many years.[3] Specifically, the efficacies of numerous analgesic agents commonly administered to horses have been studied by use of a focused radiant light-heat source directed onto the proximal interphalangeal region (pastern) of horses to elicit the classic flexion-withdrawal reflex.[4] In that heat latency model, the acute stimulation of primary afferent fibers (Aδ and C) evokes somatic and autonomic reflexes, which result in an organized escape behavior (hoof withdrawal) that is proportional to the intensity of the stimulus.[5]

Procaine hydrochloride is a relatively weak ester-type local anesthetic agent that has a potency of approximately one fourth that of lidocaine and a short duration of effect in most species. For example, by use of a thermal nociception model, doses of procaine as high as 320 mg reportedly induced local anesthesia in

Abbreviations	
LC-MS-MS	Liquid chromatography–mass spectrometry–mass spectrometry
HWRL	Hoof withdrawal reflex latency
d10-procaine	Deuterated procaine hydrochloride
m/z	Mass-to-charge ratio
LOQ	Limit of quantitation

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horses for approximately 60 minutes.[6,7] In those same studies, however, the duration of effect of procaine was significantly prolonged by coadministration of epinephrine (1:100,000 or 100 μg). Some horseracing jurisdictions in North America have adopted regulatory limits for procaine concentration in plasma samples obtained after racing, with no penalty assessed if the plasma procaine concentration in a sample is less than that limit.[8] However, plasma concentrations associated with small but locally effective doses of procaine have not been determined in horses. The purpose of the study reported here was to determine the durations of the local anesthetic effects and plasma procaine concentrations associated with 5- and 10-mg doses of procaine (with and without 100 μg of epinephrine) administered SC over the lateral palmar digital nerves of horses. Horses were administered small doses of the local anesthetic, and a heat latency model was used to evaluate the local anesthetic effects; additionally, an extremely sensitive LC-MS-MS method was developed and validated to quantify procaine concentration in plasma samples from those horses.

Materials and Methods

Horses—Six university-owned Thoroughbreds (3 mares and 3 geldings) that were 3 to 8 years old and weighed 447 to 594 kg were used for this study. The study was approved by the University of Florida Institutional Animal Use and Care Committee. All horses were fed a commercially available grain mixture and hay twice daily and had free access to water at all times. Experiments were conducted in a climate-controlled space under artificial lighting.

Determination of the HWRL period—A heat latency model (previously described in detail by Kamerling et al[4]) was used in the study. Briefly, a focused radiant light–heat lamp (500-W incandescent projection lamp) was directed onto the pastern region of each horse as a noxious stimulus to elicit the classic hoof withdrawal reflex. To ensure that the heat absorption from the lamp was consistent for all horses, the hair on the dorsal and lateral sides of the forelimb pastern region selected for experimentation that day was clipped and the skin blackened with stamp pad ink. Both forelimbs were used during the study, but the treatment limb was chosen at random at the time of each experiment. In addition, the lateral side of the pastern region was marked with white ink in 6 distinct but adjacent sites to indicate where the heat lamp would be directed; this prevented undue tissue damage and ensured that the entire area of the pastern region was evaluated for nociception.

The HWRL period was defined as the interval between initiation of lamp illumination and withdrawal of the hoof. This interval was recorded (in seconds) by use of an electronic timer that was controlled by the lamp activation switch. A secondary unfocused light beam (sham light) illuminated randomly prior to and throughout testing was used to prevent response to visual cues rather than the thermal stimulus. The heat lamp intensity was adjusted for each horse to produce an HWRL period of 5 to 7 seconds' duration under untreated control conditions. Exposure of the skin to the heat stimulus was limited to 20 seconds to avoid induction of thermal injury.

The limb to be tested was randomly selected, and following skin preparation, the baseline HWRL period was determined just prior to the administration of each treatment by calculating the mean duration of 3 consecutively measured HWRL periods. The administration of treatment was recorded as time 0 minutes. During measurement of each HWRL period, the focused heat was directed onto 1 of the 6 sites marked with white ink. After administration of 1 of the 4 treatments, durations of subsequent HWRL periods were determined in the same manner.

The 4 treatments consisted of 5 and 10 mg of procaine (10 mg/mL) administered SC over the lateral palmar digital nerve at the level of the mid-lateral sesamoid bone in the presence and absence of epinephrine (1:100,000 or 100 μg). Each horse was administered each treatment in a random crossover fashion with at least 1 week between each administration. The volume administered via each injection was standardized at 1 mL; therefore, the 5-mg dose of procaine was diluted with 0.5 mL of sterile water. Following administration of 5 and 10 mg of procaine in the absence of epinephrine, HWRL periods were determined at 5, 10, 20, 30, 40, 50, 60, and 75 minutes after application and then every 15 minutes until the last recorded HWRL period duration was within ± 10% of the baseline period duration. Following administration of 5 and 10 mg of procaine combined with epinephrine, HWRL periods were recorded at 5, 10, and 30 minutes after application and then every 30 minutes until the HWRL period duration was within ± 10% of the baseline period duration.

Plasma samples—Blood samples were obtained before each drug administration and at time points corresponding to the HWRL period determinations. Blood samples were collected in duplicate via jugular venipuncture into partially evacuated plastic tubes containing a concentration of 2 mg of NaF/mL. Each one of the tubes was filled to capacity (6 mL) and was then centrifuged at 1,380 $\times$ g for 5 minutes immediately after collection. Three mL of each plasma sample was then immediately transferred into a new tube containing 2 mg of NaF/mL. The resulting final concentration of NaF in the plasma samples was approximately 6 mg/mL. The samples were then frozen at –20°C until analyzed via LC-MS-MS.

Detection of procaine in plasma via LC-MS-MS—Procaine concentrations in equine plasma samples were determined via LC-MS-MS. Deuterated procaine hydrochloride[a] for use as an internal standard and procaine hydrochloride[b] were purchased. Standard solutions of procaine and d10-procaine were prepared by dissolving the hydrochloride salt in high-performance liquid chromatography–grade methanol to a concentration of 0.1 mg/mL. Working solutions were prepared by diluting the standard solutions in high-performance liquid chromatography–grade methanol to concentrations of 10, 0.1, and 0.01 μg/mL. Working solutions of drug and internal standards were prepared fresh daily and stored in a refrigerator (3°C) when not in use. Calibration and

positive and negative control samples were prepared in drug-free equine plasma. Calibration samples were prepared from the working solutions of procaine at concentrations of 0.1, 0.2, 0.5, 1.0, 1.5, 3.0, 6.0, 10.0, 20.0, and 40.0 ng/mL. Positive control samples were prepared from the working solutions of procaine in duplicate at concentrations of 0.5, 3.0, and 10.0 ng/mL in drug-free equine plasma. To prevent degradation of procaine, 250 µL of the NaF stock solution (30 µg/µL) was added to the calibration and positive and negative control sample tubes. Frozen plasma samples were allowed to thaw at room temperature (approx 22°C), and the internal standard (d10-procaine) was added to 1-mL aliquots of each of the samples at a volume sufficient to provide a final concentration of 50 ng of d10-procaine/mL. Two milliliters of a 1M bicarbonate-carbonate buffer solution (pH, 11) was then added to each tube, and the samples were vortex mixed. Five milliliters of dichloromethane was then added, and the tubes were mixed by inversion for 10 minutes. The samples were centrifuged, and the organic supernatant was removed and evaporated in a water bath (40°C) under a continuous stream of nitrogen. The resulting residue was reconstituted in 50 µL of deionized water and analyzed via LC-MS-MS.

Both the procaine and the d10-procaine internal standard had a mean retention time of 4.32 minutes. The retention times of the sample peaks were required to be within ± 0.2 minutes of the corresponding retention times for the standard and calibrator peaks to be considered acceptable for analysis. The molecular ions for procaine and d10-procaine were isolated and fragmented, and the resultant full MS-MS spectra were collected. Product ions at m/z 100.0, 119.9, and 163.9 were used for the qualitative identification of procaine. Product ions at m/z 110.0, 119.9, and 163.9 were used for the qualitative identification of d10-procaine. The m/z 100.0 and 110.0 product ions from procaine and d10-procaine, respectively, were used for quantitation. Data analysis was carried out by use of computer software.[c]

In addition to the requirement for retention times of the sample peaks to be within ± 0.2 minutes of the corresponding retention times for the standard and calibrator peaks, the retention times for each peak were not acceptable for analysis if they differed from the mean of all the peaks by more than ± 5% over the course of the run. Positive control samples, comprising duplicates at 2 concentrations, were analyzed before and after each set of test samples. To be acceptable for analysis, the ratios of the retention times of the ions used for quantitation could not differ by more than ± 20% between 2 positive control samples. Those positive control samples were dispersed among the test samples. In addition, the ratios of the retention times of the ions used for quantitation of the calibration samples could not differ by more than ± 20% from the positive control samples. Samples that produced area ratios that differed by more than ± 20% were excluded from data analysis. Positive control samples that produced area ratios that differed by more than ± 20% from the calibration samples provoked a repeat analysis of the sample. Regression analysis of the calibration curve was required to produce a correlation coefficient (r^2) of 0.98 for the curve to be acceptable for data analysis. The LOQ was defined as the lowest concentration that had a precision varying less than ± 20%. The LOQ for this study was determined to be 0.2 ng/mL.

Statistical analysis—Data are presented as mean ± SD. Effects of each of the 2 doses of procaine (5 and 10 mg) with and without coadministration of epinephrine were compared with the times for maximum local anesthetic effect that followed each administration for all 6 horses (n = 24) by use of a 2-way ANOVA. A least squares means test followed as a post hoc correction. Significance was set at a value of $P < 0.05$.

Results

In the present study, the mean HWRL period (an indication of a local anesthetic response) after administration of 5 mg of procaine without epinephrine SC over the lateral palmar digital nerves in horses did not differ significantly from the mean HWRL period measured before treatment because of the large degree of variation in the HWRL responses of the horses (**Figure 1**). For example, the HWRL period was prolonged to the maximum permitted duration of 20 seconds in only 4 of the 6 horses following administration of 5 mg of procaine. In contrast, 10-mg doses of procaine without epinephrine produced a maximum mean prolongation of the HWRL period to 19 ± 2 seconds 5 minutes after administration in 6 of 6 horses (**Figure 2**). Although the effects after administration of the 10-mg dose of procaine did last longer in individual horses, for the group overall, this effect dissipated by 30 minutes when the mean HWRL period was no longer significantly different from the mean baseline HWRL period.

Initially, changes in plasma procaine concentrations paralleled the local anesthetic effects. For example, following administration of the 5-mg dose of procaine without epinephrine, the maximum mean peak plasma concentration of procaine (2.6 ± 2.3 ng/mL) and the mean maximum duration of the HWRL period occurred simultaneously 10 minutes after injection (Figure 1). Following the administration of 10 mg of procaine without epinephrine, the onset of local anesthetic

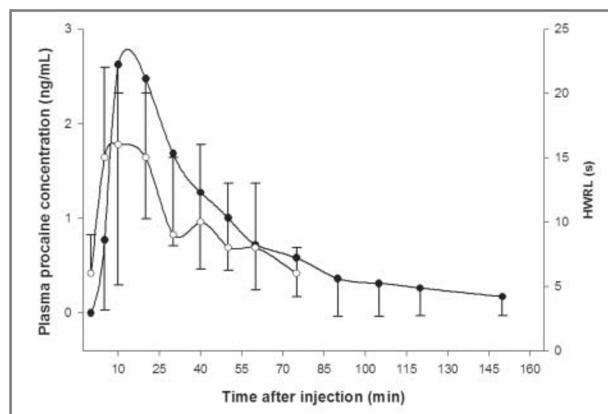

Figure 1—Plasma procaine concentrations (black circles) and duration of HWRL periods (white circles) at intervals before (baseline) and after administration (at time 0) of a 5-mg dose of procaine SC over the lateral palmar digital nerves of 6 horses. Values represent mean ± SD.

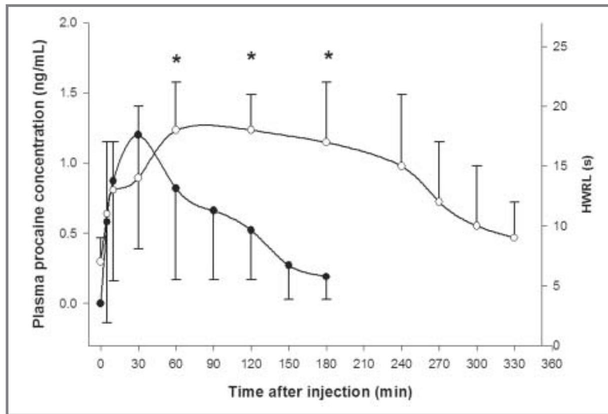

Figure 2—Plasma procaine concentrations (black circles) and duration of HWRL periods (white circles) at intervals before (baseline) and after administration (at time 0) of a 10-mg dose of procaine SC over the lateral palmar digital nerves of 6 horses. Values represent mean ± SD. *Duration of HWRL period at this time point was significantly ($P < 0.05$) different from baseline period.

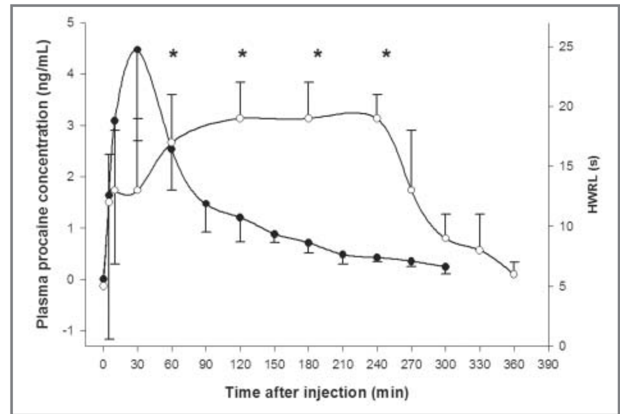

Figure 4—Plasma procaine concentrations (black circles) and duration of HWRL periods (white circles) at intervals before (baseline) and after administration (at time 0) of a 10-mg dose of procaine with epinephrine (10 µL/mL) SC over the lateral palmar digital nerves of 6 horses. Values represent mean ± SD. *See* Figure 2 for key.

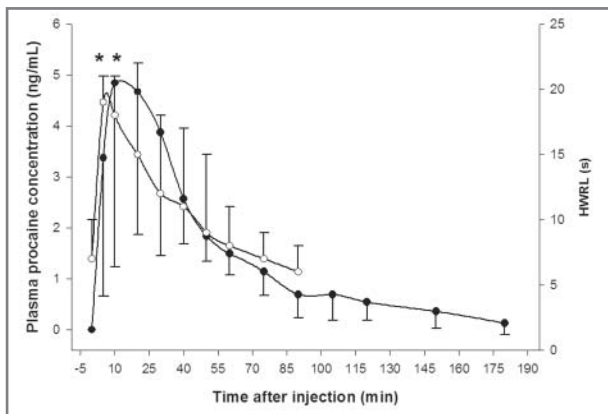

Figure 3—Plasma procaine concentrations (black circles) and duration of HWRL periods (white circles) at intervals before (baseline) and after administration (at time 0) of a 5-mg dose of procaine with epinephrine (10 µL/mL) SC over the lateral palmar digital nerves of 6 horses. Values represent mean ± SD. *See* Figure 2 for key.

activity (as determined by significant prolongation of the HWRL period, compared with the baseline HWRL period) and the mean peak plasma concentration of procaine (4.8 ± 3.6 ng/mL) occurred simultaneously 5 minutes after injection (Figure 2). After determination of the peak value, mean plasma procaine concentration decreased rapidly and was correlated closely with the decrease in the duration of the HWRL period. However, the mean plasma procaine concentration remained higher than the LOQ of the assay (0.2 ng/mL) for approximately 120 and 150 minutes after administration of 5 and 10 mg of procaine, respectively. This was far longer than the mean duration of effect for the 10-mg dose of procaine, which was only 5 minutes. Although the 5-mg dose of procaine did not reliably induce local anesthesia in all of the study horses, it did nevertheless result in plasma procaine concentrations that were readily detectable by the analytic method used in the study.

Compared with findings following injection of procaine alone, administration of 5- and 10-mg doses of procaine with epinephrine SC over the lateral palmar digital nerves of horses resulted in local anesthetic effects that were slower in onset but much longer in duration. In addition, changes in plasma procaine concentrations did not correlate with the local anesthetic effects. For example, when a 5-mg dose of procaine was administered with epinephrine, the peak plasma procaine concentration (1.2 ± 0.8 ng/mL) was detected 30 minutes after administration, but the HWRL period was not significantly different from the baseline period until 60 minutes after injection (**Figure 3**). In a similar manner, the peak plasma procaine concentration following administration of 10 mg of procaine with epinephrine was also detected 30 minutes after administration (4.5 ± 1.8 ng/mL), but the duration of the HWRL period was not significantly different from the baseline period until 60 minutes after injection (**Figure 4**). The mean durations of effect following the administration of 5- and 10-mg doses of procaine with epinephrine were approximately 120 and 180 minutes, respectively; these values were significantly longer than the durations of effect in the absence of epinephrine.

When either the 5- or 10-mg dose of procaine was coadministered with epinephrine, the HWRL period remained significantly greater than the baseline period for extended intervals, despite rapid decreases in plasma procaine concentrations (Figures 3 and 4). In 3 of the 6 horses, the local anesthetic effect persisted from 60 to 180 minutes after administration of the 5-mg dose of procaine with epinephrine, despite plasma procaine concentrations that were less than the LOQ of the analytic method used in the study. In contrast, administration of a 10-mg dose of procaine with epinephrine resulted in mean plasma procaine concentrations that were greater than the LOQ of the analytic method for 300 minutes after injection, whereas the mean duration of the HWRL period was significantly greater than the baseline period from 60 to 240 minutes after injection. In addition, plasma procaine concentrations were greater than the assay LOQ at all time points at which a local anesthetic effect was evident as a significant prolongation in HWRL period, compared with the baseline HWRL period, in all of the horses after treatment with 10 mg of procaine with epinephrine.

Discussion

The results of the present study in horses indicated that procaine is a potent local anesthetic agent and that the duration of the drug's anesthetic effects can be quite prolonged in the presence of epinephrine. Although 5 mg of procaine alone did not result in a significant prolongation of the mean HWRL period (compared with the baseline period), that same dose of procaine in the presence of epinephrine significantly increased the duration of the HWRL period for 120 minutes beginning 60 minutes after injection. In a similar manner, the addition of epinephrine increased the mean duration of effect of 10 mg of procaine from 5 to 180 minutes after injection, compared with the baseline value.

The results of the present study also indicated that in both the absence and presence of epinephrine, plasma procaine concentrations do not correlate well with the local anesthetic activity of the drug in horses. In the absence of epinephrine, plasma procaine concentrations following administration of the 5- and 10-mg doses of procaine remained greater than the LOQ of the assay (0.2 ng/mL) long after the HWRL period duration had returned to baseline value. In contrast, when procaine and epinephrine were administered together, local anesthetic activity persisted in some horses even when plasma procaine concentrations were less than the LOQ of the analytic method. It has been hypothesized that epinephrine prolongs the duration of effect of local anesthetics by causing local vasoconstriction, which decreases the elimination rate of the drug from the tissues.[9] However, epinephrine is also known to enhance the potency of lidocaine, and both pharmacodynamic and pharmacokinetic mechanisms have been proposed for this effect.[10] The results of the study reported here confirm that the effect of epinephrine on the duration of local anesthetic activity can be profound.

In the present study, the effect of 5 but not 10 mg of procaine in the absence of epinephrine was similar to that determined in a previous study[7] in horses. For example, the results of that investigation and our study indicated that administration of a 5-mg dose of procaine SC over the lateral palmar digital nerve of horses did not significantly increase the mean duration of the HWRL period from baseline.[7] However, this finding may be misleading because the HWRL period did increase to the maximum permitted duration of 20 seconds in 4 of the horses in the present study; the mean HWRL period was not significantly different from the baseline value because of the high degree of variation in the responses among the horses. This variation was not surprising given the small dose (5 mg) and volume (1.0 mL) that were administered via SC injection that was guided only by digital palpation of the nerves. There may also be some variation in the sensitivity of individual horses to the local anesthetic itself. In our study, the administration of a 10-mg dose of procaine resulted in a mean duration of effect of only 5 minutes. In contrast, in the previous study,[7] the mean duration of effect of 10 mg of procaine was 15 minutes. The reason for the comparatively shorter duration of effect detected in the present study is unclear because the experimental protocols in the 2 studies were identical to the extent that could be achieved feasibly. However, given the relatively small doses of procaine administered and the high degree of interhorse variability in our study, the difference is not surprising.

Our data have important ramifications for some racing jurisdictions in North America. Several jurisdictions in Canada and the United States have adopted regulatory limits for procaine concentrations in plasma samples obtained from horses after racing, with no penalty levied if the procaine concentration in a plasma sample is less than that limit. In Canada, the limit is 25 ng of procaine/mL of plasma in samples obtained after racing, but this plasma concentration is not related to any administered dose of procaine or specific withdrawal period.[8] In fact, prior to completion of the present study, plasma concentrations associated with small but locally effective doses of procaine had not been determined because of the lack of a suitably sensitive analytic method.[7] The use of an LC-MS-MS method with an LOQ of 0.2 ng/mL in the study reported here resulted in detection of procaine for as long as 300 minutes following administration of 10 mg of procaine with epinephrine. Of critical regulatory importance, however, was the finding that a 5-mg dose of procaine with epinephrine prolonged the HWRL period to the maximum permitted duration of 20 seconds, although the plasma procaine concentrations were less than the LOQ of the assay. Because the detection of exogenously administered epinephrine would be extremely difficult, it would not be possible to determine whether procaine had been administered with or without epinephrine. In addition, plasma concentrations of procaine in our study did not exceed 10 ng/mL, which is much less than the concentration permitted by some racing jurisdictions. Altogether, these data have indicated that procaine at any concentration in plasma could be associated with a clinically important local anesthetic effect in horses. Therefore, given these findings, the continued use of regulatory limits set for procaine concentrations in plasma samples obtained from horses after racing has to be questioned.

a. Deuterated procaine hydrochloride, Neogen Corp, Lansing, Mich.
b. Procaine hydrochloride, Sigma Chemical Co, St Louis, Mo.
c. Finnigan Xcalibur qualitative analysis software, Thermo Fisher Scientific, Waltham, Mass.

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