Clonidine in Horses: Identification, Detection, and Clinical Pharmacology*

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INTRODUCTION

In veterinary medicine, a number of the α₂-receptor agonist medications are marketed as sedatives, hypnotics, and analgesics, with their principal use being the chemical restraint of large and small animals. In human medicine, however, members of the α₂-receptor agonist family are used primarily as antihypertensive agents. In recent years, the usefulness of the α₂-adrenoceptor agonist drugs has been recognized in equine practice. For clinical purposes, these agents produce sedation and analgesia and, thus, are useful for premedication and markedly potentiate the effects of other sedative or analgesic agents.

The α₂-receptor agonists, of which clonidine is the best-known example, were first synthe-
In a recent study, it was suggested that various $\alpha_2$-receptor agonists were being dissolved in vitamin B$_{12}$ solutions containing alcohol and injected IV into horses before races. This is administered in small doses (about 0.02 mg/kg) shortly before post time to reduce the intensity of racing-related pulmonary hypertension and, by extension, the associated exercise-induced pulmonary hemorrhage (EIPH). The rationale for administering $\alpha_2$-receptor agonists is that most horses experience pulmonary hypertension during running, leading to EIPH, a considerable problem in the horseracing industry.

EIPH acutely interferes with the racing performance of horses by compromising the exchange of oxygen and carbon dioxide in the alveolar capillaries, and repeated bouts of EIPH result in chronic and cumulative damage to the lung. Known as an antihypertensive in human medicine, clonidine could reduce pulmonary arterial blood pressure in racing horses and, thus, potentially reduce the incidence or severity of EIPH. On the other hand, as an $\alpha_2$-agonist agent, clonidine may also have the ability to tranquilize or sedate horses and may also have some bronchodilator activity.

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Medications capable of improving the racing performance of horses are classified by the As-
Association of Racing Commissioners International (ARCI) based on their performance-enhancing potential. Clonidine is currently classified as an ARCI class 3 agent. As such, clonidine is considered to have the potential to influence the outcome of a race, and its administration to a horse shortly before post time would clearly contravene the rules of racing in most jurisdictions. As such, useful screening and confirmation methods for clonidine in equine serum, plasma, or urine are required.

Clonidine is a basic lipid-soluble drug with a high volume of distribution, and plasma concentration after administration is very low in humans and various animal species. Despite the numerous analytic methods described in the literature, the problem of quantitatively measuring clonidine in low concentrations in biologic matrices remains difficult. Furthermore, at this time, no validated and peer-reviewed analytic method exists for the quantitation of this drug in biologic fluids of racing horses to control inadvertent or intentional misuse.

The objective of this study was to develop and validate a peer-reviewed and peer-replicated sensitive and specific gas chromatography–mass spectrometry (GC/MS) quantitative method for clonidine in horses. In addition, we wanted to determine the duration of the pharmacologic effects of clonidine in performance horses to establish scientifically defensible withdrawal times and/or a threshold level for clonidine.

**MATERIALS AND METHODS**

**Horses**

Four mature Thoroughbred mares (7 to 8 years old) were used for this study. The animals described the detection, quantification, and pharmacokinetics of the related α₂-receptor agonist guanabenz in performance horses. It is essential for the welfare and integrity of the racing industry that accurate and specific analytic tests exist for the quantitation of this drug in biologic fluids of racing horses to control inadvertent or intentional misuse.
Figure 3. Electron impact mass spectrum of TBDMS derivative of clonidine (molecular weight, 343; A) and undervatized clonidine (B).
were maintained on grass hay and feed, which was 50:50 mixture of sweet feed and an alfalfa-based protein pellet (14% protein). Horses were fed twice a day. The animals were vaccinated annually and dewormed quarterly. A routine clinical examination was performed before each experiment to assure that the animals were healthy and sound. During experimentation, horses were provided water and hay ad libitum. Animals used in these experiments were managed according to the rules and regulations of the Tuskegee University Institutional Animal Care Use Committee, which also approved the experimental protocol.

For the pharmacokinetics study, clonidine was administered as a single IV dose at 0.025 mg/kg in 10% ethanol solution (prepared in isotonic saline solution), and blood samples were collected as described below. Clinical pharmacology of clonidine experiments followed a rigorous standard protocol to reduce variability from extraneous effects. Horses were placed in individual stalls in the early morning and allowed to acclimate to the stall for 7 hours before each experiment. For the determination of the sedative actions of clonidine, horses were dosed again with 0.025 mg/kg of clonidine as a single IV dose; the potency and duration of the sedative effects of clonidine in these horses were determined as described below. In addition, after a minimum 1-week washout interval following the completion of the sedation studies, the potency and duration of the analgesic effects of clonidine in these horses following a single IV dose at 0.025 mg/kg were determined as described below.

**Pharmacodynamic Parameters**

**Head Drop**

Sedation was assessed by measuring the degree of head drop following administration of clonidine. A pretreatment floor-to-chin height was determined at 30, 15, and 0 minutes before IV injection of clonidine to establish a baseline value in each horse. The degree of head drop was then measured at 5, 10, 15, 20, 25, and 30 minutes after injection and every 15 minutes thereafter until head drop measurements returned to baseline values. All related clinical signs associated with the sedative effects of clonidine in horses, such as ataxia and drooping of the eyelids and lower lip, were also reported. In a separate experiment to evaluate reversal of the effects of clonidine, the same protocol described above was followed, except that two horses received IV injections of 0.12 mg/kg yohimbine (an α₂-receptor antagonist) 20 minutes after injection of clonidine; the yohimbine powder was first dissolved in 2.5 ml of dimethyl sulfoxide (DMSO).

**Analgesia**

A heat projection lamp was used to determine thermal antinociception, which has been used as a measure of analgesia in another study. Briefly, focused radiant light or heat

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**TABLE 1. Interpretation of Fragments Observed in Clonidine Mass Spectrum Shown in Figure 3A**

<table>
<thead>
<tr>
<th>Mass (m/z)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>343 (345)</td>
<td>Molecular ion</td>
</tr>
<tr>
<td>286 (288)</td>
<td>Loss of t-butyl</td>
</tr>
<tr>
<td>252 (254)</td>
<td>Loss of C(CH₃)₂CH₂Cl by rearrangement</td>
</tr>
<tr>
<td>193</td>
<td>Loss of TBDMS and Cl</td>
</tr>
<tr>
<td>124</td>
<td>C₆H₃NCl fragment</td>
</tr>
<tr>
<td>93 (95)</td>
<td>C(CH₃)₂CH₂Cl+H by rearrangement</td>
</tr>
<tr>
<td>100</td>
<td>(CH₃)₂SiC(CH₃)₂</td>
</tr>
<tr>
<td>73</td>
<td>Si(CH₃)₃ by rearrangement</td>
</tr>
<tr>
<td>59</td>
<td>HSi(CH₃)₂</td>
</tr>
</tbody>
</table>
was used as a noxious stimulus and was directed at the pastern of the horse from a constant distance to elicit the classic flexion–withdrawal reflex. Hoof withdrawal reflex latency (HWRL) was defined as the time between initiation of lamp illumination and withdrawal of the hoof. The reflex times were adjusted by varying the intensity of the heat output with a rheostat so that the HWRL for control measurements was 3 to 4 seconds, with the actual HWRL recorded on an electronic timer built into the lamp. The duration of light exposure to the pasterns was limited to 10 seconds to prevent skin damage. A secondary unfocused light beam (sham light) was used to confound the horse, reducing the possibility that the flexion–withdrawal reflex was to visual rather than thermal perception of the focused light beam.

HWRL was measured at 30 and 15 minutes and immediately before injection of clonidine, and these times (−30, −15, and 0 minutes) were used to establish a mean baseline value for HWRL in each horse. The HWRL then was measured at 5 and 15 minutes after injection of clonidine and every 15 minutes thereafter until HWRL returned to control values. The HWRL was expressed as a percentage of the baseline values (100%), with 9 seconds being maximum analgesic effect (300%).

Pharmacokinetic Parameters

Sample Collection

Horses were given a single IV dose of clonidine (0.025 mg/kg in 10% ethanol solution prepared in isotonic saline solution), and plasma concentrations were determined in a pharmacokinetics study. The skin over the left jugular vein was washed with povidone–iodine scrub (Poviderm, Burns Veterinary Supplies, Westbury, NY) and rinsed with ethanol. An IV catheter (Abbocath-T, 14-gauge × 5.5-inch, Abbott Animal Health, North Chicago, IL) was inserted into the left jugular vein and sutured in place. Clonidine was administered with great care as a single IV dose into the right jugular vein. Blood samples were collected from the left jugular vein for analyses at 0, 5, 10, 20, 30, and 45 minutes and 1, 2, 4, 6, 8, 10, 24, 48, 72, and 96 hours into heparinized Vacutainer plasma tubes (Becton Dickinson, Franklin Lakes, NJ) and then centrifuged at 4°C at 2,000 ×g for 15 minutes; the plasma was stored and refrigerated in 5-ml aliquots until assayed.
Analytic Detection of Clonidine

Standard solutions of clonidine (Sigma Chemical, St. Louis) and isoxsuprine (internal standard; Sigma Chemical) were prepared in N,N-dimethylformamide (DMF; Sigma Chemical) and stored in a refrigerator. These standards were allowed to come to room temperature (23°C) before use. With each analytic run, serial dilutions were made from the stock clonidine standard and added to blank plasma samples. Extraction standards were prepared by the addition of a known volume of a clonidine solution to blank plasma samples at a range of 1 to 250 ng/ml (0, 1, 2.5, 5, 10, 25, 50, 100, and 250 ng/ml). A known volume of an isoxsuprine standard (10 µl of 10 µg/ml in DMF) was added to each sample and standard and blank plasma samples as an internal standard.

The plasma standards and blanks (1 ml/sample) were placed in culture tubes. The plasma samples were alkalinized with 0.75 µl of ammonium hydroxide (concentrated) (Fisher Scientific, Suwannee, GA) solution.

Extraction of Clonidine

The analytic procedure was adapted from that described by Arrendale et al. Clean 16 × 150 mm Pyrex screw-cap culture tubes with polytetrafluoroethylene (PTFE) cap liners were silanized with trimethylchlorosilane (TMCS; Pierce Chemical, Rockford, IL) before use by rinsing them first with methanol, followed by a 5% solution of TMCS in hexane (Fisher Scientific), and twice more with methanol (Fisher Scientific). The silanized tubes were allowed to air-dry at room temperature.

The analytic method used was as follows: Plasma samples (1 ml) were pipetted into 15-ml screw-cap tubes. Then, 10 µl of 10 µg/ml internal standard was added to each tube, followed by 75 µl of ammonium hydroxide (concentrated) and dichloromethane/isopropanol.
ethylacetate (Fisher Scientific) (6:1:3). The tubes were shaken on a reciprocating shaker for 10 minutes and centrifuged at 300 \( \times g \) for 5 minutes. The aqueous layer was then aspirated to waste. The dichloromethane:isopropanol:ethylacetate extracts were decanted into silanized test tubes, DMF (15 µl) was added as a “keeper solvent,” and all extracts were evaporated under a stream of nitrogen at 40˚C in a waterbath.

For derivatization, each dried sample was dissolved in 50 µl of \( N \)-methyl-\( N \)-(tert-butyldimethyl-silyl)trifluoroacetamide + 1% tert-butyldimethylchlorosilane (MTBSTFA + 1% TBDMCS; Pierce Chemicals, Rockford, IL), vortex mixed briefly, incubated at 75˚C for 2 hours to produce the tert-butyldimethylsilyl (TBDMS) derivatives of both clonidine and internal standard, and then transferred to an autosampler vial equipped with a 200 µl spring-loaded insert. In addition, alternative derivatizations using \( N,O \)-bis-trimethylsilyltrifluoroacetamide plus 1% TMCS (BSTFA + 1% TMCS; Pierce Chemicals), Barb-Prep (Alltech Associates, Deerfield, IL), and \( N \)-methyl-\( N \)-trimethylsilyltrifluoroacetamide (MSTFA) + 1% TMCS (MSTFA + 1% TMCS; Pierce Chemicals) were evaluated for the detection and quantitation of clonidine.\(^3\) Each solution was transferred to a micro insert in an automatic liquid sampler vial (as described above), from which 1 µl was injected for chromatography.

### Gas Chromatography–Mass Spectrometry Analysis

Samples were analyzed using Perkin Elmer AutoSystem XL Gas Chromatography and TurboMass Mass Spectrometer (Perkin Elmer, Norwalk, CT) set in positive ion mode. The gas chromatography conditions used for identification of the corresponding clonidine peak were as follows: column, HP-5ms [(5% phenyl)-95% methylpolysiloxane)] 30 m, 0.50-µm film thickness, 0.25-mm internal diameter (Agilent Technologies, Wilmington, DE) (helium flow rate: 1.5 ml/min); injector, 250˚C; transfer line, 250˚C; the oven was temperature programmed from 70˚C (2 minutes at initial temperature) with a 20˚C/min rate of increase to 280˚C (holding temperature, held for 10 minutes). The mass spectrometer was set to acquire from mass:charge ratio (\( m/z \))

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**TABLE 2. Accuracies and Within-Run and Between-Run Precisions of GC/MS Assay Used to Quantify Clonidine in Horse Plasma Samples**

<table>
<thead>
<tr>
<th>Theoretic Concentration (ng/ml)</th>
<th>Measured Concentration (ng/ml; mean ± SD; n = 6)</th>
<th>Accuracy (%; mean ± SD)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within-run</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.40 ± 0.146</td>
<td>96.13 ± 5.87</td>
<td>6.11</td>
</tr>
<tr>
<td>25</td>
<td>25.13 ± 2.25</td>
<td>103.2 ± 5.66</td>
<td>5.48</td>
</tr>
<tr>
<td>200</td>
<td>203.48 ± 6.04</td>
<td>101.8 ± 2.99</td>
<td>2.93</td>
</tr>
<tr>
<td>Mean</td>
<td>100.38</td>
<td></td>
<td>4.84</td>
</tr>
<tr>
<td><strong>Between-run</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.53 ± 0.167</td>
<td>101.3 ± 6.80</td>
<td>6.71</td>
</tr>
<tr>
<td>25</td>
<td>23.83 ± 1.947</td>
<td>95.33 ± 7.92</td>
<td>8.3</td>
</tr>
<tr>
<td>200</td>
<td>204.5 ± 6.09</td>
<td>102.3 ± 3.28</td>
<td>3.2</td>
</tr>
<tr>
<td>Mean</td>
<td>99.64</td>
<td></td>
<td>6.07</td>
</tr>
</tbody>
</table>
In quantitative experiments, selected ion monitoring (SIM) was performed for ions \( m/z \) 93, 252, 254, and 286 for clonidine and \( m/z \) 178 for internal standard. The \( m/z \) 178 of TBDMS–isoxsuprine derivative is the major fragmentation product and was monitored as the internal standard ion. For TBDMS–clonidine derivative, quantitation was based on the most abundant ion, \( m/z \) 252. Mass Spec Calculator Pro (Version 4.03, 1998, Quadtech Associates, Fairfield, CA) was used to assist in the interpretation of full-scan spectra where necessary.

For chromatographic and mass spectrometric identification of clonidine and isoxsuprine, Chromatographic and Mass Spectrometric Criteria for Identification Guidelines (available from the Association of Official Racing Chemists [AORC], Lexington, KY) were used.\(^4\)

A standard curve was constructed by plotting standard clonidine concentration versus the ratio of clonidine:internal standard peak areas. Standard curves were generated with Sigma Plot for Windows (Aspire Software International, Leesburg, VA). The areas of peaks corresponding to clonidine and internal standard were recorded, and the internal standard values were used to normalize the clonidine areas. Integrated peak values were entered into QuattroPro for Windows (Borland Software Corporation, Scotts Valley, CA) for statistical analysis of standards and for interpretation of unknown amounts of clonidine. An estimate of clonidine concentration in unknown samples was obtained by comparing unknown:internal standard area ratio obtained from the unknown sample and interpolated on the standard curve.

### Validation of the Assay

The GC/MS method for the quantitation of clonidine was validated by applying criteria in the most recent Standard Operating Procedure available to us from Dr. Rick Sams.\(^8\) The quantitative method of clonidine was validated by examining the measurement of consistency of results (within-run and between-run), correlation (coefficient of determination of the standard curve), and extraction efficiency of the assay. The within-run precision was calculated from similar responses from six repeats of three control samples (2.5, 25, and 200 ng/ml) in one run. The between-run precision was determined by comparing the calculated response

### TABLE 3. Extraction Efficiencies and Measured and Expanded Uncertainties of GC/MS Assay Used to Quantify Clonidine in Horse Plasma Samples\(^a\)

<table>
<thead>
<tr>
<th>Theoretic Concentration (ng/ml)</th>
<th>Extraction Efficiency (%; mean ± SEM; ( n = 6 ))</th>
<th>Measured Uncertainty (%)</th>
<th>Expanded Uncertainty (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>96 ± 9.6</td>
<td>3.92</td>
<td>9.79</td>
</tr>
<tr>
<td>25</td>
<td>80 ± 6.0</td>
<td>2.45</td>
<td>6.12</td>
</tr>
<tr>
<td>100</td>
<td>75.5 ± 6.1</td>
<td>2.49</td>
<td>6.22</td>
</tr>
<tr>
<td>Mean</td>
<td>83.83</td>
<td>2.95</td>
<td>7.38</td>
</tr>
</tbody>
</table>

\( ^a\)Uncertainty was determined using the method of A2LA. (Uboh C: Personal communication, Equine Toxicology and Research Laboratory, University of Pennsylvania, School of Veterinary Medicine, Kennet Square, PA, 2002)

Mean ± expanded uncertainty = 95% confidence range.
of the low (2.5 ng/ml), middle (25 ng/ml), and high (200 ng/ml) control samples over three consecutive daily runs (total of six runs). The assay accuracy for within-run and between-run was established by determining the ratio of calculated response to expected response for low (2.5 ng/ml), middle (25 ng/ml), and high (200 ng/ml) control samples over six runs.

Standard curve correlability was measured by the mean coefficient of determination ($r^2$) for six consecutive daily runs. The extraction efficiency was determined by comparing the response (in area) of low (2.5 ng/ml), middle (25 ng/ml), and high (200 ng/ml) control samples over six runs.

In addition to this determination of the LOD, an alternate calculation was performed utilizing the analyte’s peak height compared to the baseline noise in the $m/z$ 252 fragmentation chromatogram. By this method, the LOD was defined as the lowest concentration of analyte producing a peak greater than or equal to three times the baseline noise of the ion chromatogram. The lower limit of quantitation (LOQ) was defined as the concentration calculated from the mean of the zero responses plus five times the standard deviation.

**RESULTS**

We developed a sensitive quantitative GC/MS method for clonidine employing a liquid extraction procedure and derivatization with MTBSTFA + 1% TBDMCS to detect and quantify clonidine in plasma samples from horses. Figure 1 shows the chemical structure and expected isotopic profile of underivatized clonidine. The selected ion chromatogram of TBDMS derivative of clonidine extracted from plasma spiked with 250 ng/ml of clonidine standard is shown in Figure 2. Figure 3 and Table 1 present the full-scan mass spectrum of derivatized clonidine peak as shown in Figure 2, as well as the underivatized clonidine standard (AORC library entry) and the interpretation of the derivatized clonidine spectrum. The chemical structure of the TBDMS derivative of clonidine along with its predicted isotopic profile is shown in Figure 4. For quantitation, the intense ion at $m/z$ 178 was chosen for SIM-MS of TBDMS isoxsuprine derivative (internal...
standard, data not shown) and the intense ion at $m/z$ 252 was chosen for SIM-MS of TBDMS clonidine derivative. The resultant standard curve was linear from 1 ng/ml to 250 ng/ml, with the $r^2$ value of the assay ($n = 6$) being $0.997 \pm 0.005$ (SD). Figure 5 presents a typical standard curve for clonidine, with the LOD for this method being 0.3 ng/ml and the LOQ being 0.6 ng/ml. In addition, an alternate calculation for LOD was performed utilizing the analyte’s peak height compared with the baseline noise in the $m/z$ 252 fragmentation chromatogram. By this method, the LOD was defined as the lowest concentration of analyte producing a peak greater than or equal to three times the baseline noise of the ion chromatogram and was 0.0154 ng on column (1 ng/65 µl, data not shown).

For within-run, the coefficient of variation (CV) for 2.5 ng/ml ($n = 6$) was 6.11%, for 25 ng/ml ($n = 6$) was 5.48%, and for 200 ng/ml ($n = 6$) was 2.93%, with the mean CV of 4.84% (Table 2) less than the 15% CV value acceptable for the assay validation. In addition, the within-run accuracies for this method ($n = 6$ for each value) were 96.13% ± 5.87 (SD), 103.2% ± 5.66 (SD), and 101.8% ± 2.99 (SD) for 2.5, 25, and 200 ng/ml standard solutions, respectively (Table 2); the accuracy requirements for validation of the assay are between 85% and 115%.

For between-run, the CV for 2.5 ng/ml ($n = 6$) was 6.71%, for 25 ng/ml ($n = 6$) was 8.30%, and for 200 ng/ml was 3.20%, with the mean CV of 6.07% (Table 2). In addition, the between-run accuracies for this method ($n = 6$ for each value) were 101.3% ± 6.80 (SD), 95.33% ± 7.92 (SD), and 102.3% ± 3.28 (SD) for 2.5, 25, and 200 ng/ml standard solutions, respectively (Table 2). The extraction efficiency was determined in three different concentrations ($n = 6$ for each value): low (2.5 ng/ml) = 96% ± 9.6 (SD), middle (25 ng/ml) = 80% ± 6.0 (SD), and high (100 ng/ml) = 75.5% ± 6.1 (SD; Table 3). The measurement uncertainty and expanded uncertainty were determined in three different concentrations ($n = 6$ for each value): low (2.5 ng/ml) = 3.92% and 9.79%, middle (25 ng/ml) = 2.45% and 6.12%, and high (100 ng/ml) = 2.49% and 6.22%, respectively (Table 3).

In addition, alternative derivatizations using BSTFA + 1% TMCS, Barb-Prep, and MSTFA + 1% TMCS were evaluated for the detection and quantitation of clonidine using the GC/MS and extraction method indicated in Materials and Methods. Clonidine was not detected following its derivatization with Barb-Prep for 2 hours. Derivatization of clonidine with BSTFA + 1% TMCS for 2 hours generat-
ed both mono- and bis-trimethylsilyl (TMS) derivatives of clonidine (data not shown), and, therefore, this derivatization agent was not further evaluated for the detection and quantitation of clonidine. Derivatization of clonidine with MSTFA + 1% TMCS for 2 hours generated bis-TMS derivative of clonidine (data not shown). The highly specific ion $m/z$ 338 of the bis-TMS derivative of clonidine (data not shown) was a much smaller percentage of the total mass spectral intensity than was that of the $m/z$ 252 ion of the TBDMS derivative of clonidine. Correspondingly, areas acquired during SIM acquisition of the bis-TMS derivative were only about one-tenth those of the TBDMS derivative, and, therefore, the TBDMS derivative was chosen over the TMS derivative for quantitative purposes.

In clinical pharmacology studies, horses did not have any locomotor activity for 30 to 45 minutes after IV administration of clonidine at 0.025 mg/kg. Clonidine produced rapid, profound sedation as evidenced by relaxation of the lower lip, drooping of eyelids, and extreme head drop (Figure 6). As suggested by the rapidity of the head drop, the horses were clinically sedated within minutes of clonidine administration. The maximum sedative effect of clonidine was observed within 15 to 20 minutes after IV injection. Head height remained approximately 50% of the pretreatment value for about 45 minutes after clonidine injection. Although horses remained standing and were able to walk 45 minutes after IV clonidine administration, the head drop persisted for 2.5 to 3 hours after injection (Figure 6). Figure 7 illustrates the rapid reversal of the sedative action of clonidine following yohimbine injection in two horses given 0.025 mg/kg of clonidine. In this experiment, 2 to 3 minutes after IV injection of yohimbine (0.12 mg/kg), head height was approximately 50% of pretreatment value; by 5 to 10 minutes after injection, head height was within normal limits and the horses were clinically alert.

Figure 8 illustrates the rapid onset of analgesia (within 5 minutes) following IV injection of 0.025 mg/kg clonidine. IV administration of 0.025 mg/kg of clonidine failed to provide maximal analgesia (300% of control value) in all horses, although the limited analgesic effect persisted for about 60 minutes. Clonidine induced its maximum analgesic activity within 30 minutes after administration in all horses included in this study, and analgesia returned to control values by 75 to 90 minutes after administration.

The mean plasma concentration (±SD) of clonidine following 0.025 mg/kg single IV injection of clonidine is shown in Figure 9; as shown, the plasma concentration of clonidine...
was relatively closely distributed among the four horses included in this study. The plasma concentration of clonidine rapidly declined following IV administration; at 30 minutes after administration, the mean plasma concentration of clonidine was 1.4 ± 0.45 ng/ml. The plasma concentration of clonidine 40 minutes after administration was above the lowest standard curve concentration (1 ng/ml) only in one of the horses; the plasma concentration of clonidine in this horse was around 1.1 ng/ml. Clonidine was still detectable up to 2 hours after a single IV injection (0.025 mg/kg), although the plasma concentrations were lower than 1 ng/ml after 40 minutes postadministration.

DISCUSSION AND CONCLUSION

Clonidine has been marketed for human use for a number of years as a centrally acting α₂-receptor agonist to decrease blood pressure.8 Other centrally mediated effects of clonidine include sedation10,11 and analgesia,12,13 and, as such, clonidine has the potential to produce sedative or tranquilizing effects as well as analgesia in horses.

In a recent study, it was suggested that various α₂-receptor agonists were being dissolved in vitamin B₁₂ solutions containing alcohol and injected IV into horses before races.17 The rationale for administering α₂-receptor agonists is that most horses experience pulmonary hypertension during running, leading to EIPH, a considerable problem in the horseracing industry. Known as an antihypertensive in human medicine, clonidine could reduce pulmonary arterial blood pressure in racing horses and, thus, potentially reduce the incidence or severity of EIPH. On the other hand, as an α₂-agonist agent, clonidine may also have the ability to tranquilize or sedate horses and may also have some bronchodilator activity. Clonidine is currently classified as an ARCI class 3 agent. As such, clonidine is considered to have the potential to influence the outcome of a race, and its administration to a horse shortly before post time would clearly contravene the rules of racing in most jurisdictions.

In this study, it was shown that IV injection of clonidine at 0.025 mg/kg produced rapid, profound sedation as evidenced by relaxation of the lower lip, drooping of eyelids, and extreme head drop. As suggested by the rapidity of the head drop, the horses were clinically sedated within minutes of clonidine administration. The maximum sedative effect of clonidine was observed within 15 to 20 minutes after IV injection and persisted for 2.5 to 3 hours after injection. Administration of yohimbine quickly reversed the sedative action of clonidine, which is consistent with the suggestion that the major
pharmacologic responses to clonidine are mediated through $\alpha_2$-receptors.

Clonidine was also able to produce analgesic effects following IV administration but failed to provide maximal analgesia (300% of control value) in all horses; however, the limited analgesic effect persisted for about 60 minutes. Clonidine induced its maximum analgesic activity within 30 minutes after administration in all horses included in this study, and analgesia returned to control values by 75 to 90 minutes after administration.

In conclusion, clinical pharmacology studies suggest that an IV dose of clonidine at 0.025 mg/kg induces sedation as quickly as that produced by other $\alpha_2$ agonists and that sedation and analgesia were generally intense and considerably long lasting. These experiments suggest considerable clinical potential for clonidine as a sedative and a relatively long-lasting analgesic in equine medicine.

The pharmacokinetic parameters of clonidine have been explained by using two- and three-compartmental open body models with a first-order elimination process in humans and various animal species. Clonidine is a basic lipid-soluble drug with a high volume of distribution, and the plasma concentration is very low following its administration both in humans and various animal species. Therefore, the evaluation of the pharmacokinetic behavior of this drug has been hampered by the lack of sensitive and specific analytic methods capable of measuring concentrations in the picogram to nanogram range. In this study, we developed a relatively sensitive analytic method for the detection and quantification of clonidine, with the LOD being 0.3 ng/ml and LOQ being 0.6 ng/ml. Even though this analytic method is relatively sensitive, we were not able to quantify clonidine in plasma samples of horses 40 minutes after a single dose at 0.025 mg/kg IV. Clonidine was still detectable up to 2 hours after a single IV injection at 0.025 mg/kg, although the plasma concentrations at all time points after 40 minutes postadministration were lower than our lowest standard concentration, which was 1 ng/ml.

This study suggests that clonidine is a highly lipid-soluble drug that is rapidly distributed from vascular spaces into tissue spaces, making the analytic detection of this drug very difficult in plasma samples of horses. For safety reasons, higher doses of clonidine were not investigated in this study to determine the pharmacokinetic parameters of clonidine in performance horses. Further studies are required to determine the exact pharmacokinetic parameters of clonidine in horses. Because we were not able to quantify clonidine in plasma samples of horses 40 minutes after IV administration, it was difficult to establish the relationship between dose and plasma concentration and between plasma concentration and pharmacodynamics. In addition, since the primary goals of this study were to determine the pharmacokinetic parameters and clinical pharmacology of clonidine, we did not attempt to quantify clonidine in urine samples. It is possible that detection of clonidine in urine samples might be a more useful screening method than using plasma, and urine samples might be useful to...
control inadvertent or intentional misuse of clonidine in racing horses.

REFERENCES