Pharmacokinetics and effects of aminorex in horses

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**Objective**—To investigate the pharmacokinetics and behavioral effects of aminorex administered IV and PO in horses.

**Animals**—7 Thoroughbreds.

**Procedures**—In a cross-over design, aminorex (0.03 mg/kg) was administered IV or PO. Plasma and urinary aminorex concentrations were determined via liquid chromatography–mass spectrometry.

**Results**—Decrease of aminorex from plasma following IV administration was described by a 3-compartment pharmacokinetic model. Median (range) values of α, β, and γ half-lives were 0.04 (0.01 to 0.28), 2.30 (1.23 to 3.09), and 18.82 (8.13 to 46.64) hours, respectively. Total body and renal clearance, the area under the plasma time curve, and initial volume of distribution were 37.26 (28.61 to 56.24) ml/min/kg, 1.25 (0.85 to 2.05) ml/min/kg, 13.39 (8.52 to 17.37) ng•h/ml, and 1.44 (0.10 to 3.64) L/kg, respectively. Oral administration was described by a 2-compartment model with first-order absorption, elimination from the central compartment, and distribution into peripheral compartments. The absorption half-life was 0.29 (0.12 to 1.07) hours, whereas the β and γ elimination phases were 1.93 (1.01 to 3.17) and 23.57 (15.16 to 47.45) hours, respectively. The area under the curve for PO administration was 10.38 (4.85 to 13.40) ng•h/mL and the fractional absorption was 81.8% (33.8% to 86.9%).

**Conclusions and Clinical Relevance**—Aminorex administered IV had a large volume of distribution, initial rapid decrease, and an extended terminal elimination. Following PO administration, there was rapid absorption, rapid initial decrease, and an extended terminal elimination. At a dose of 0.03 mg/kg, the only effects detected were transient and central in origin and were observed only following IV administration. (Am J Vet Res 2008;69:675–681)

Aminorex (2-amino-5-phenyl-2-oxazoline) is a substituted oxazoline derivative of amphetamine (Figure 1). It was originally synthesized in the early 1960s and marketed in Europe as an anorexic agent. This amphetamine analog increases norepinephrine concentrations in the CNS. Its use in Europe from 1967 to 1972 as an appetite suppressant led to an epidemic of weight control also caused increased incidence of pulmonary hypertension and valvular heart disease with subsequent fatal effects. The mechanism for the increase in pulmonary pressure remains elusive. Results of 2 studies suggest that anorexic agents can inhibit potassium current, cause membrane depolarization, and stimulate pulmonary vasoconstriction. Other investigators speculate that aminorex and similar drugs such as serotonin (5-hydroxytryptamine) are transporters of substrates that become translocated into pulmonary cells where, depending on the degree of drug retention and individual susceptibility, hypertension could develop as a response to high concentrations of these drugs or metabolites in circulation. Aminorex has pharmacologic actions similar to those of amphetamines in inducing CNS stimulation resulting in psychomotor and locomotor stimulation in mice and rats. Results of
those studies suggest that aminorex is a CNS stimulant that may have substantial potential for amphetamine-like abuse in humans.

Aminorex was detected, quantified, and confirmed in plasma and urine samples collected after racing from horses. Aminorex and related substances are prohibited in the horse-racing industry. After the initial report of aminorex use in racehorses, horses continued to yield positive results for the drug. To the authors' knowledge, the clandestine sources of aminorex remain unknown. The purpose of the study reported here was to investigate the pharmacokinetic and pharmacologic effects of IV or PO administration of aminorex in horses and relate these concentrations to the plasma and urinary concentration detected after race in an attempt to differentiate the concentrations inducing a pharmacologic effect from those that are pharmacologically and clinically irrelevant.

Materials and Methods

The study protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Seven Thoroughbreds, including 4 females and 3 males that were (mean ± SD) 7.4 ± 1.4 years old and weighed 504.4 ± 24.2 kg, were used in the study. Two days before the experiment, horses were brought from pasture into stalls and remained housed for the duration of the study. All experiments in this study started at 7 AM. Horses were fed grass hay and water ad libitum. Study horses were no longer actively racing but were otherwise in good health.

Drug administration and sample collection—Aminorex (0.03 mg/kg) was administered IV or PO to horses in a crossover, randomized design. A minimum of 2 weeks elapsed before the alternative drug administration was repeated in the same horse. Blood samples were collected by use of a 14-F catheter placed in a jugular vein. For IV administration, a catheter was also placed in the contralateral vein. Prior to placement of catheters, the areas over the jugular veins were clipped, washed with surgical soap, and rinsed with a viricide and 70% isopropyl alcohol. The aminorex solution administered IV was prepared by dissolving aminorex in 1 mL of dimethyl sulfoxide, and that solution was diluted with 15 mL of sterile water and administered via the contralateral jugular vein. Administration of saline (0.9% NaCl) solution (130 mL) was started, aminorex was injected within 30 seconds, and saline solution administration continued for approximately 10 seconds to ensure that all of the aminorex had been administered. Blood samples were collected at 0, 2, 5, 10, 30, and 45 minutes and 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, and 36 hours after drug administration.

The orally administered solution was prepared by suspending aminorex in 10 mL of water to which a palatable sweetener was added, resulting in a total volume of 15 mL. Blood samples were collected at 0, 15, 30, and 45 minutes and 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, and 36 hours after administration. Potassium oxalate with sodium fluoride was used as anticoagulant. Blood samples were centrifuged (2,500 X g for 15 minutes) to obtain plasma.

In the 4 female horses, a sterile indwelling 24-F self-retaining catheter placed in the bladder and attached to a drainage bag for continuous collection of urine, after washing the vulva with surgical soap and rinsing with a viricide. Total volume of urine excreted was measured at 1, 2, 4, 6, 8, 10, and 12 hours after drug administration, and total quantity of aminorex excreted was calculated for up to 12 hours. Plasma and urine samples were collected at the same time. Urine samples were also collected at 16, 20, 24, and 36 hours. Aliquots of 2 mL of plasma and urine were immediately frozen at −20°C and later stored at −70°C until analyzed. Each aliquot of urine or plasma was used once to eliminate any effect of freeze-thaw cycles on the concentration of aminorex in the sample. Changes in heart rate were monitored by use of a telemetric ECG signal and behavioral changes were recorded with a video camera.

Quantification of aminorex—Aminorex standard solutions were prepared with 30:50:1 acetonitrile:water:formic acid at 1, 2.5, 5.0, 10, 25, 50, and 100 ng/mL. All reagents used were of HPLC grade. A calibration curve was prepared daily by adding 0.1 mL of the standard solutions to 0.9 mL of drug-free horse plasma containing 1% NaF/mL. Positive control samples were also prepared in duplicate at 0.5 and 5 ng/mL. Calibrators and control samples were analyzed in duplicate. Drug-free negative control plasma (with and without internal standard) was analyzed with each batch. Standard solutions were stable throughout the course of the study.

Sample preparation and liquid-liquid extraction—One milliliter of grade ammonium hydroxide solution in a 1:1 (vol/vol) ammonium hydroxide:water solution was added to 1 mL of calibrators, control samples, and study samples. Internal standard of 0.1 mL of 100 ng of clenbuterol d6/mL was also added as the internal standard (IS) to all calibrators, control samples (except 1 of 2 negative control samples), and study samples before vortexing for 15 seconds was performed. Five milliliters of methyl tert-butyl ether was added to all tubes. The tubes were capped and roto-racked for 10 minutes; the samples were then centrifuged at 1,750 X g for 10 minutes. The organic (top) layer was transferred.
to clean borosilicate tubes and evaporated to dryness at 65°C under air. The residues were reconstituted in 100 μL of 0.1% formic acid in water and transferred to 2-mL autosampler vials fitted with 0.2-mL-volume inserts.

HPLC-electrospray ionization tandem mass spectrometry—Analysis of the sample extracts was performed by use of a linear ion trap interfaced to a liquid chromatograph and auto-sampler. Analysis was performed in positive ion electrospray mode. The HPLC mobile phase consisted of 2.35M aqueous formic acid (pH 5) and 0.1% formic acid-acetonitrile. The pump was operated in a linear gradient mode from 15% formic acid to 100% formic acid-acetonitrile at 0.2 mL/min and at ambient temperature for 4 minutes with 1 minute hold at 100% formic acid-acetonitrile. The system was re-equilibrated to initial conditions for 1 minute. Total cycle time per sample was 7.5 minutes. Chromatographic separation of analytes was achieved by use of a carbon 18 column (3-μm particle size; 3.0 X 50 mm).

The electrospray ionization source was operated with a positive spray voltage of 5.5 kV and sheath gas setting of 18 (high purity nitrogen) arbitrary units. Capillary temperature was 350°C. Ion trap settings for MS-MS analyses were as follows: protonated molecular ion for aminorex [M+H]+ was m/z 163, with collision energy (% of 20): protonated molecular ion for hydroxyaminorex [M+H]+ was m/z 179.1, with collision energy (% of 30), wideband; and protonated molecular ion for IS [M+H]+ was m/z 286.2, with collision energy (% of 23), wideband. Product ions monitored for quantitative analysis were aminorex (m/z 120), hydroxyaminorex (m/z 136), and IS (m/z 204, 268).

Analytes were quantified by use of liquid chromatography acquisition software. A linear unweighted calibration curve was used for aminorex. No method or instrument carryover was detected within the reporting range of results (approx 5 pg/mL). Intra-assay precision and accuracy were evaluated by analyzing the control samples before and after each run for all batches. Precision was expressed as CV%, and accuracy was expressed as percentage of the target concentration. Recovery was determined by comparison of neat working stock solutions with fortified negative plasma responses following preparation by the extraction method as described. Recovery was indicated by the ratio of the obtained calibration line slopes. Instrument recovery was determined by analysis of a given quality control sample > 10 times. Method recovery was approximately 70%. The limits of quantification and confirmation were 0.01 ng/mL. The standard operating procedures for the quantification of analytes by this laboratory meet requirements for accreditation by the American Association for Laboratory Accreditation and ISO 17025 International Guidelines.

Pharmacokinetic analysis—The plasma concentration versus time data curve following IV or PO administration of aminorex was analyzed by use of non-linear least squares regression analysis. Data from the IV study were analyzed in each horse by use of a 2-compartment model and a 3-compartment mammary model. The number of compartments required to best describe the data were based on the appearance of the curve, the reduction in the sums of squares, and the minimization of fractional SD of each compartmental parameter. The software allows translation between the compartmental models and equivalent exponential forms. The intercompartmental microconstants for an IV 3-compartment mammary model with elimination from the central compartment (k12) and distribution to compartments 2 (k21, k23) and 3 (k31, k32) were converted to macroconstants exponents α, β, and γ as described.

The plasma concentration versus time data from the PO study were analyzed by the use of 2 models. A 2-compartment model with first-order absorption (k01) and elimination from the central compartment (k12) with intercompartmental rate constants reflecting distribution into peripheral compartments (k21, k23) and a 1-compartment model with absorption (k01) and elimination (k12) were fit to the data. The compartmental microconstants were converted to macroconstants exponents: absorption (k01) β and γ elimination or k12 elimination (k12), respectively. A number of weighting schemes [W(K)] were used for IV and PO data during the fitting process. The fractional SD was in the form of W(K) = 1/C*QO(K)**2, where QO(K) was the kth observed datum and C was the fractional SD. The SD weighting scheme was of the form W(K) = 1/C*QO(K)**2. The fractional SD weighting process favors the terminal phase of the decay curve, where the SD favors the larger and intermediate data points. The fitting process (iterations) ceased when the improvement in the sums of squares of the last iteration was < 1%. The A, B, and C coefficients (ng/mL) for the IV administration were calculated from the dose, Vc, and the relevant compartmental rate constants. Half-lives were calculated as the ln2 divided by the exponential constants. Plasma concentration at 0 time (C0) was the sum of the coefficients A, B, and C. The total AUC0-24 from 0 to 24 was calculated by use of the trapezoidal rule, and the fractional amount absorbed was calculated as follows:

$$\text{AUC}_{0\rightarrow t} = \frac{\text{AUC}_{0\rightarrow \infty}}{\text{AUC}_{0\rightarrow t}}$$

Volume of the central compartment was calculated as follows:

$$V_c = \frac{D_i}{C_p}$$

where D_i is the dose administered IV and C_p is the plasma concentration at zero time. Ratios of the interdepartmental rate constants k21/k31 and k23/k32 times Vc were used to calculate Vc and Vc, and Vc, following IV administration was calculated as the sum of all compartments:

$$V_{c, \text{dss}} = \left[ \frac{k_{21}k_{32} + k_{21}k_{31} + k_{23}k_{31}}{k_{21} \times k_{31}} \right] V_c$$
Total body clearance was calculated as follows:

\[ \text{Cl}_b = \frac{\text{Div}}{\text{AUC}_i} \]

Urinary excretion rate \((R_u)\) was calculated as urinary concentration \((\text{ng/mL})\) times urine flow \((U_f; \text{mL/min})\), and \(\text{Cl}_r\) was calculated as follows:

\[ \text{Cl}_r = \frac{R_u}{C_p} \]

Statistical analysis—Pharmacokinetic parameter estimates of aminorex were expressed as median and range, and nonparametric Wilcoxon/Kruskal-Wallis rank sum tests were used for statistical comparisons of parameters. ANOVA was used for parametric analysis. The plasma concentrations of aminorex are expressed as mean ± SD. A value of \(P < 0.05\) was considered significant.

Results

IV administration—Decrease of the plasma concentration-time curve of aminorex administered IV was best described by a 3-compartment model (Figure 2). Plasma concentrations were \(11.99 ± 4.58\) ng/mL, \(0.05 ± 0.03\) ng/mL, and \(0.04 ± 0.02\) ng/mL at 2 minutes, 24 hours, and 36 hours, respectively. The initial decline of the plasma concentration was rapid followed by a long terminal half-life. Pharmacokinetic parameter estimates and inter-compartmental rate constants were determined (Tables 1 and 2). The \(t_{1/2g}, t_{1/2ph},\) and \(t_{1/2ph}\) half-lives were 0.04, 2.30, and 18.82 hours, respectively. Aminorex was quantifiable in all horses at 24 hours but in only 5 of the 7 horses at 36 hours. The median (range) of the fractional SD for all compartmental parameter estimates used in defining the IV model was 0.074 (0.023 to 1.84). There was a significant \((P < 0.001)\) difference between \(V_L\) and \(V_C\) for IV administration. There were also significant \((P < 0.01)\) differences in the \(\text{AUC}_i\) for IV versus PO administrations \((13.9 \pm 10.3\) ng•h/mL, respectively).

PO administration—Increases and decreases in the plasma concentrations following PO administration were best described by an absorption and 2-compartment model with elimination from the central compartment and distribution into peripheral compartments (Figure 2). Pharmacokinetic parameter estimates and the intercompartmental rate constants were determined (Tables 2 and 3). The maximum concentration and the time to maximum concentration following PO administration were \(2.85 ± 1.07\) ng/mL and \(0.85 ± 0.13\) hours, respectively. Aminorex was quantifiable in all horses at 24 hours and in 5 of 7 horses at 36 hours. Plasma concentrations at 24 and 36 hours were \(0.07 ± 0.03\) ng/mL and \(0.05 ± 0.04\) ng/mL, respectively. The median (range) of the fractional SD for all compartmental parameter estimates used in defining the PO model was \(0.060\) (0.013 to 4.05). Median fractional amount absorbed was 81.8% with a range of 33.8% to 86.9%.

Urinary concentrations—The urinary concentrations following IV or PO administrations peaked at 2 and 6 hours, with \(132.94 ± 52.36\) ng/mL and \(87.38 ± 76.93\) ng/mL, respectively (Figure 3). Aminorex was

Table 1—Pharmacokinetic parameter estimates of aminorex (median and range) following a single IV administration of 0.03 mg/kg to 7 horses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (ng/mL)</th>
<th>Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13.45</td>
<td>6.68–23.25</td>
</tr>
<tr>
<td>α (h⁻¹)</td>
<td>17.86</td>
<td>2.47–49.91</td>
</tr>
<tr>
<td>t₁/2α (h)</td>
<td>0.04</td>
<td>0.01–0.28</td>
</tr>
<tr>
<td>β (h⁻¹)</td>
<td>0.30</td>
<td>0.22–0.56</td>
</tr>
<tr>
<td>t₁/2β (h)</td>
<td>2.30</td>
<td>1.23–3.09</td>
</tr>
<tr>
<td>τ (h⁻¹)</td>
<td>3.03</td>
<td>0.41–5.74</td>
</tr>
<tr>
<td>γ (h⁻¹)</td>
<td>0.054</td>
<td>0.13–4.87</td>
</tr>
<tr>
<td>Vₘ (L/kg)</td>
<td>0.037</td>
<td>0.015–0.085</td>
</tr>
<tr>
<td>Vₜ (L/kg)</td>
<td>18.82</td>
<td>8.13–46.64</td>
</tr>
<tr>
<td>Cl (L/min/kg)</td>
<td>37.26</td>
<td>28.61–56.24</td>
</tr>
<tr>
<td>Cl (L/min/kg)</td>
<td>1.25</td>
<td>0.85–2.05</td>
</tr>
<tr>
<td>AUCᵢ (ng•h/mL)</td>
<td>13.39</td>
<td>8.22–17.37</td>
</tr>
<tr>
<td>Vₘ (L/kg)</td>
<td>1.44</td>
<td>0.10–3.64</td>
</tr>
<tr>
<td>Vₜ (L/kg)</td>
<td>1.07</td>
<td>0.95–7.12</td>
</tr>
<tr>
<td>Vₘ (L/kg)</td>
<td>12.63</td>
<td>3.85–54.63</td>
</tr>
<tr>
<td>Vol (L/kg)</td>
<td>22.89</td>
<td>8.39–44.11</td>
</tr>
</tbody>
</table>

Table 2—Compartmental rate constant estimates of aminorex (median and range) and mean ± SD of fractional SD of parameters following a single IV or PO administration of 0.03 mg/kg to 7 horses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median estimates</th>
<th>Range</th>
<th>Fractional SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV administration*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k₁</td>
<td>1.26</td>
<td>0.62–7.51</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td>k₂</td>
<td>12.69</td>
<td>1.22–27.14</td>
<td>0.09 ± 1.06</td>
</tr>
<tr>
<td>k₃</td>
<td>1.96</td>
<td>0.62–5.69</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>k₄</td>
<td>0.43</td>
<td>0.09–10.68</td>
<td>0.15 ± 0.15</td>
</tr>
<tr>
<td>k₅</td>
<td>0.05</td>
<td>0.01–0.11</td>
<td>0.23 ± 0.31</td>
</tr>
<tr>
<td>Oral administration*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k₁</td>
<td>1.27</td>
<td>0.65–5.75</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>k₂</td>
<td>0.15</td>
<td>0.05–0.33</td>
<td>0.08 ± 0.05</td>
</tr>
<tr>
<td>k₃</td>
<td>0.06</td>
<td>0.04–0.45</td>
<td>0.10 ± 0.11</td>
</tr>
<tr>
<td>k₄</td>
<td>0.03</td>
<td>0.01–0.11</td>
<td>0.10 ± 0.07</td>
</tr>
</tbody>
</table>

*Three-compartment mammary model. TAbsorption and 2-compartment model with elimination from the central compartment. k = Intercompartmental fractional rate constants.
Table 3—Pharmacokinetic parameter estimates of aminorex (median and range) following a single PO administration of 0.03 mg/kg to 7 horses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{\text{a}} ) (h)</td>
<td>2.37</td>
<td>0.65–5.75</td>
</tr>
<tr>
<td>( t_{\text{α}} ) (h)</td>
<td>0.29</td>
<td>0.12–1.07</td>
</tr>
<tr>
<td>( t_{\text{\text{β}}} ) (h)</td>
<td>0.36</td>
<td>0.22–0.69</td>
</tr>
<tr>
<td>( t_{\text{\text{γ}}} ) (h)</td>
<td>1.93</td>
<td>1.01–3.17</td>
</tr>
<tr>
<td>( \text{Cl}_{\text{\text{α}}} ) (mL•min/kg)</td>
<td>0.74</td>
<td>0.59–2.41</td>
</tr>
<tr>
<td>( \text{AUC}^0 ) (ng•h/mL)</td>
<td>10.38</td>
<td>4.85–13.40</td>
</tr>
</tbody>
</table>

\( t_{\text{a}} \) = absorption half-life. \( t_{\text{\text{α}}} \) = elimination half-life. \( t_{\text{\text{γ}}} \) = elimination half-life.

Figure 3—Urine concentration (mean ± SD) of aminorex following IV (hatched bars) and PO (black bars) administration in 4 horses.

Aminorex has been studied in dogs,23–25 pigs,26,27 and monkeys.28 The main aim of those studies was to develop a model to study anorexic-induced pulmonary hypertension. Treatment dose for aminorex administered PO or IV in these species ranged from 0.25 to 15 mg/kg. At these doses, transient increases in systemic and pulmonary pressures were detected but no cumulatively sustained hemodynamic effects were observed in dogs, and tolerance to the drug was reported in dogs.27 Changes in behavior described in the dogs, pigs, calves, and monkeys included nervousness, restlessness, hyperactivity, appetite suppression, and weight loss. Prolonged oral administration to dogs and rats did not induce pathologic evidence of hypertensive vascular disease.28 Most of the administrations of aminorex quantified in urine in all horses following IV administration at 24 and 36 hours at 5.95 ± 3.77 ng/mL and 2.40 ± 1.58 ng/mL, respectively, and following PO administration at 6.12 ± 4.73 ng/mL and 2.14 ± 2.82 ng/mL, respectively. The percentage of the total dose of aminorex excreted in urine within 12 hours was 1.7 ± 0.3% and 1.5 ± 1.1% for IV and PO, respectively. The differences in \( \text{Cl}_{\text{\text{α}}} \) following IV (1.25 mL•min/kg) and PO (0.74 mL•min/kg) administrations (Tables 1 and 2) were not significant.

Behavior—Within 1 minute following IV administration of aminorex, all horses had pinning of the ears, fixation of the head position, apparent lack of awareness of the environment, and an increase in blinking, all of which were noted for approximately 4 to 5 minutes. There were no changes in heart rate following IV administration. Changes in behavior and heart rate were not observed following PO administration.

Discussion

Aminorex has been identified as a so-called designer drug of abuse that is synthesized in clandestine laboratories and classified as a Drug Enforcement Agency Schedule 1 drug. Aminorex and methylenaminorex are sometimes illegally sold as methamphetamine. Both compounds have central stimulant activity similar to amphetamine, and pulmonary hypertension has been associated with recreational use of 4-methyl-aminorex in humans.31,33 Aminorex and methylenaminorex are prohibited in the horse-racing industry.

Aminorex was detected, quantified, and confirmed in plasma and urine samples collected from horses after racing in Pennsylvania beginning in December 2005 and detected at all 4 race tracks in Pennsylvania up to November 2006. It was first detected and reported in a racing jurisdiction adjacent to Pennsylvania prior to December 2005 and in other jurisdictions after 2006. To date, the source of aminorex as well as how and when the horses were administered or contaminated with the drug have not been determined.

For the present study, aminorex was not commercially available and was specifically synthesized.4 Means (range) of the plasma and urinary concentrations quantified and confirmed in samples collected after racing were 0.20 (0.02 to 1.54) and 2.43 (0.44 to 121.7) ng/mL, respectively. One of the aims of this study was to compare the concentration of aminorex that induced pharmacologic and clinically observable effects in horses with the aforementioned concentrations in an attempt to distinguish pharmacologically relevant from irrelevant concentrations.

Quantification of aminorex in plasma or urine has not previously been published, and to the authors' knowledge, this is the first study to determine the pharmacokinetics of aminorex in any species, including humans. Following IV administration, a 3-compartment model was the best fit for all 7 horses. Total body clearance of 37.26 mL•min/kg was rapid. The large \( V_{\text{ss}} \) of 22.89 L/kg caused the rapid decline in the plasma concentration following IV administration despite the long \( \gamma \) terminal half-life. In the first 12 hours, only approximately 1.5% of aminorex administered was accounted for in the urine. This reflects a \( \text{Cl}_{\text{\text{α}}} \) of only 1.25 and 0.74 mL•min/kg for the IV and PO administrations, respectively. Much of the renal excretion was in the form of the hydroxyaminorex metabolite, the main metabolite of aminorex identified in horses. It was not quantified because its authentic standard was not available.

The relevant factors in the rapid decline in the plasma concentration of aminorex were the large volume of distribution and the rapid transfer into tissue compartments and not \( \text{Cl}_{\text{\text{α}}} \). The distribution into deeper compartments caused the long and similar terminal half-life for the IV and PO administrations.

Aminorex has been studied in dogs,23–25 pigs,26,27 and monkeys.28 The main aim of those studies was to develop a model to study anorexic-induced pulmonary hypertension. Treatment dose for aminorex administered PO or IV in these species ranged from 0.25 to 15 mg/kg. At these doses, transient increases in systemic and pulmonary pressures were detected but no cumulatively sustained hemodynamic effects were observed in dogs, and tolerance to the drug was reported in dogs.27 Changes in behavior described in the dogs, pigs, calves, and monkeys included nervousness, restlessness, hyperactivity, appetite suppression, and weight loss. Prolonged oral administration to dogs and rats did not induce pathologic evidence of hypertensive vascular disease.28 Most of the administrations of aminorex...
in those experimental studies were oral, but 0.25 mg/kg administered IV was considered an effective dose in calves that weighed approximately 75 kg. This induced a transient increase in systemic blood pressure and variable changes in heart rate but no increases in pulmonary pressure.

The dosage of aminorex administered to the horses in the present study was 0.03 mg/kg, which was a dose of approximately 15 mg. The behavioral changes noted following IV administration were transient and minimal. Consistently observable effects in all horses were pinning of the ears, reduction of head movement, and increased blinking. This is atypical behavior for horses, and control observations in the same horses included constant movement of the ears and turning and bobbing of the head in response to background sounds. The changes in facial appearance can be described as a so-called lost facial expression, which was interpreted to suggest a loss of responsiveness to the horses' surroundings. Neurologically, this appearance has been correlated with preictal aura, which is a premonitory sign of a seizure. Cessations of the transient central behavioral effects were more difficult to establish than their onset because the horses returned to normal behavioral patterns within 4 to 8 minutes, as indicated by movement of the head and ears as a general response to noises and the immediate environment and the return of alert facial and eye expressions. The initial rapid decrease in plasma concentration, during the 2-, 5-, and 10-minute periods following IV administration when central behavioral effects were noted were 11.9, 6.8, and 5.2 ng/mL, all of which were higher than those quantified in samples obtained after racing. This result suggested that either the dosage of aminorex to which racehorses in Pennsylvania were exposed was much lower than 0.03 mg/kg or that the horses were administered aminorex much in advance of the time of racing.

In horses, pharmacologic effects were not observed following PO administration. The maximum plasma concentration attained following PO administration of aminorex was approximately 3 ng/mL, and the highest concentration attained in 1 horse was 4.4 ng/mL. Orally administered doses of 25 and 50 mg were also without observable pharmacologic effects. The lowest dose administered PO to a horse was 12.5 mg, and aminorex was quantified in plasma and urine at 24 hours at 0.048 and 4.66 ng/mL, respectively.

The pharmacokinetics of aminorex indicated a rapid distribution phase and initial rapid decrease in the plasma concentration following IV administration and rapid absorption with a large fraction of the drug absorbed following PO administration. The terminal elimination phases after IV and PO administration were prolonged, indicating distribution into deeper peripheral tissues and a subsequent slower release. At a dose of 0.03 mg/kg, the only effects noted were transient CNS effects that were only detected following IV administration of aminorex.

d. Chlorhexidine diacetate, Fort Dodge Health, Fort Dodge, Iowa.


g. Hewlett-Packard Telemetry, Atlanta, Ga.

h. Cerrilliant, Round Rock, Tex.

i. Fisher Scientific, Fair Lawn, NJ.

j. LNXQLC and auto-sampler, ThermoFisher Scientific, San Jose, Calif.

k. Mac-Mod Analytical, Chad Fords, Pa.

l. Excalibur software, version 2.0, Quan Browser, ThermoFisher Scientific, San Jose, Calif.


n. JMP, version 6.0, SAS Institute Inc, Cary, NC.

References


