Pharmacology, pharmacokinetics, and behavioral effects of caffeine in horses

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SUMMARY

Caffeine (4 mg/kg) was given by rapid IV injection to 4 horses. Plasma concentrations of the drug peaked at 10 μg/ml and decreased rapidly at first, and then more slowly, with an apparent β-phase half-life of 18.2 hours. Urinary concentrations of caffeine were remarkably consistent at about 3 times plasma values of the drug. Caffeine was detectable in both plasma and urine of the horses for up to 9 days after dosing.

After oral administration, caffeine was absorbed poorly with an apparent bioavailability of 39%. Although blood concentrations of caffeine peaked rapidly after oral administration, its apparent plasma half-life by this route was about 42 hours. These observations identify the possible existence of a slowly absorbed pool of caffeine in the gastrointestinal tract after oral administration.

When caffeine-treated horses were given fentanyl, the locomotor response to fentanyl was enhanced. This potentiation of the fentanyl response peaked at between 0 and 4 hours after dosing and was gone by 72 hours after caffeine dosing. The data indicate that the probability of behavioral stimulation due to caffeine by 72 hours after dosing may be small.

Caffeine is a member of the methylxanthine group of drugs and is one of the most widely used psychoactive agents in the world.[1] Caffeine and the closely related theophylline are both effective CNS stimulants. Experience in persons indicates that caffeine administration may act to reduce drowsiness and fatigue and produce a rapid, clear flow of thought. Caffeine stimulates the respiratory centers, produces complex cardiovascular effects, and relaxes bronchial smooth muscle. Theophylline is even more potent in this regard. The methylxanthines apparently increase the capacity for muscular work in persons, and they have well-known diuretic effects.[2]

The methylxanthines appear to produce their pharmacologic effects by antagonizing the CNS depressant actions of adenosine. Specific receptors have been demonstrated for adenosine in the CNS and the methylxanthines can be shown to bind to these receptors. Further, stable analogs of adenosine, which penetrate the brain, are powerful behavioral depressants, and their depressant actions are antagonized by the methylxanthines.[1]

Caffeine is, therefore, a substance which might be expected to affect the performance of horses and has been used as such.[3] For example, in the period 1947 to 1973, the Association of Official Racing Chemists determined that caffeine was the 2nd most-common substance reported by their laboratories.[4]

Recently, with improved detection methods for caffeine, use of the drug in race horses has decreased, although its use still occurs in other equine competitions, such as in pulling contests by draft horses and in show jumping. It is unusual, when considering caffeine's record as a doping agent, as well as a therapeutic agent, that no pharmacokinetic studies have been performed in the horse. The work presented here is intended to provide more complete information on the pharmacokinetic profiles of caffeine in the horse. Studies also were performed to determine the concentrations of caffeine which might act as a CNS stimulant, and which might, therefore, be expected to affect a horse's performance on the track.

Materials and Methods

A—Pharmacokinetics—Mature Thoroughbred and Standardbred mares and geldings were used throughout. All horses were kept at pasture and brought into box stalls preceding any experimental procedures.

Caffeine was administered by rapid IV injection to 4 horses in a solution of 1 part of sodium benzoate, 2 parts of caffeine, and 4 parts of water. Horses were dosed by injection into the left jugular vein. Subsequent blood samples were taken from the right jugular vein. For oral administration of caffeine, the caffeine dose was weighed, dissolved in 200 ml of distilled water, and given by stomach tube (followed by approx 2 cups of tap water). The stomach tube was removed, and blood and urine samples subsequently were obtained.

Sampling regimen—Blood samples were drawn into 10-ml Vacutainer tubes[a] (containing 20 mg of potassium oxalate + 25 mg of sodium fluoride) from the jugular vein. Urine was drawn from the mares via catheterization of the bladder. After administration of caffeine (time 0), blood samples were drawn at 5, 10, 20, and 40 minutes, at 1, 2, 4, 6, 12, and 24 hours, and then every 24 hours after administration until the drug was no longer detectable. In the case of oral administration, a blood sample also was taken at 30 minutes after dosing. After the blood sample was drawn, it was centrifuged and the plasma was harvested. The RBC were discarded. Urine was drawn at 1 hour after dosing, at 2, 4, 6, 12, and 24 hours, and every 24 hours until caffeine was no longer detectable

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[a] Becton, Dickinson & Co, Rutherford, NJ.

in the urine. The bladder was emptied after every sample was taken.

Preparation of samples—A given sample of plasma or urine (1 ml) was added to a centrifuge tube. (Each sample was analyzed in duplicate.) Saturated sodium tetraborate solution (1 ml) was added to each tube to adjust the pH to 9.2. Dichloromethane (2 ml) was added to each tube, and the tubes were mixed by rotation for 4 minutes. After centrifugation (1 hour at 1,600 × g), 1 ml of the dichloromethane was transferred to conically shaped centrifuge tubes. The tubes were placed in a water bath (65 C) until the dichloromethane had evaporated (approx 5 minutes) and then removed. When the tubes were cool, 50 μl of methanol[b] was added to each tube and the tube vortex-mixed (30 s). A portion of this solution (5 μl) was injected into the gas chromatograph.

Standards were prepared by adding 10 μl of a caffeine[c]:water solution of given concentrations to 1 ml of plasma or urine. The standards were made the day on which the horses were dosed, and analyzed on the day on which those horses' samples were analyzed. To check percentage recovery of caffeine, standards of given concentrations were prepared in methanol and compared with the standards prepared in the biological fluids.

Detection and quantitation of caffeine—Gas chromatography—A gas chromatograph equipped with a nitrogen detector[d] was used to quantitate caffeine in samples from horses given the drug by IV route. For the studies on samples from horses given caffeine orally, another model[e] gas chromatograph was used with identical equipment and matching settings.

The chromatograph contained a 1.83-m glass column with 3% OV-101 mesh packing.[f] Oven temperature was kept constant at 200 C. The injection port and interface (manifold) temperatures were 275 C. The carrier gas flow (nitrogen) was 30 ml/min, the detector hydrogen flow was 3 ml/min, and the detector air flow was 30 ml/min. Attenuations varied from $\times 512 \times 10^{-10}$ to $\times 4 \times 10^{-10}$ at full scale. The nitrogen detector contained rubidium as the alkaline ion source and was set at 590 on the source potentiometer. The recorder was set at a chart speed of 10 mm/min, at a full-scale sensitivity of 0.5 mV. Peak height was the value used to quantitate the caffeine concentration.

Mass spectrometry—In several of the pharmacokinetic trials, caffeine standards and samples from horses dosed with caffeine were analyzed by GC-mass spectrometry to ensure that the substance quantitated was caffeine.[g]

Calculations—The following procedure was used for calculating the mean concentrations of caffeine in the plasma and urine of the horses given caffeine. For each time point, caffeine concentrations for the 4 horses in each study were averaged, and the mean value and SEM computed. In the event a horse had a nondetectable concentration of drug in the sample, the midpoint between zero and the lowest limit of detection was averaged in with the other measurable values. In this case, no SEM value was computed as one (or more) of the meaned values was not a true measure of the drug concentration in the biological fluid.[h]

B—Behavior—In the behavioral study, 4 horses (1 gelding and 3 mares) weighing between 430 kg and 480 kg were used. All horses were kept at pasture when not under experimental conditions. At 24 hours before an experiment, the horses were brought into specially designed, isolated box stalls (4 meters square). The stall was shielded to reduce interaction between the stalls and the outside environment. Observation of the horse in the stall was afforded by a 30-cm square glass window in the door. The horses were bedded with straw on clay floors and provided hay and water ad libitum. Immediately before a drug was to be injected, water and feed buckets were removed to prevent possible injury to the horse. Hay was constantly available in the hay rack. After every experimental period, water was offered to the horse.[5]

Spontaneous locomotor activity of the horse was measured by first wrapping the horse's left thoracic limb with a single piece of white tape to enhance its visibility. After injection of the drug to be tested, the observer immediately began to count, by use of a hand counter, the number of steps the horse took with the left thoracic limb. The cumulative number of footsteps was recorded every 2-minute period. A step was defined as a movement of the leg that resulted in a change of location of the foot. Therefore, scratching, pawing, and stamping were not counted. The observer also noted other signs the horse exhibited during this period, such as eating, sleeping, circling, and apparent agitation.[3]

In this behavioral study, the regimens of drug administration were performed 1 time in each of the 4 horses. The mean response of the 4 horses was then used in the evaluation of the results. To establish the base line of locomotor activity, each horse was injected with 1 ml of normal saline solution, and the locomotor response was recorded for 1 hour. To establish the control levels, the base-line activities of horses given fentanyl (0.01 mg/kg; in the form of fentanyl citrate[i]) were determined after IV administration. Locomotor activity was recorded until the number of footsteps/2-minute period was the same as that seen in the horse given saline solution—usually at an hour after fentanyl was injected. Previous studies[5] had shown that the locomotor response to fentanyl terminated by 1 hour and that subsequent injections of fentanyl did not alter the magnitude of the following fentanyl responses. Therefore, it was not necessary to show here that augmentation of the fentanyl response did not occur with serial administrations.

The base-line activities in each horse were determined after IV administration of caffeine (4 mg/kg; in a sodium benzoate solution [identical to the preparation used in the IV pharmacokinetic study]). Locomotor activity was recorded for 1 hour, beginning 15 minutes after caffeine injection. This hour was the only time period during which the locomotor activity was measured because by the end of 1-hour locomotor activity had decreased to that seen in controls (ie, given saline solution).

Caffeine (4 mg/kg) in a sodium benzoate solution was injected IV into the horse. After a period of 15 minutes, 0.01 mg/kg fentanyl was administered IV and this was recorded as time zero. The locomotor response was then recorded for 1 hour. Intravenous injection of fentanyl was repeated at 2, 4, 6, 10, 24, 48, and 72 hours after caffeine administration in each of 4 horses. The last administration (at 72 hours) was performed when caffeine was no longer seen to augment the motor response to fentanyl in any horse. The results were plotted as the mean number of footsteps/2-minute period in the 4 horses vs time after fentanyl administration.

C—Plasma protein binding—The percentage of plasma binding of caffeine was determined by the use of radiolabeled caffeine[j] as previously described.[6,g]

Results

Caffeine was readily detectable in equine plasma and urine, using dichloromethane extraction and gas chromatographic detection. The caffeine peaks were large and well defined (Fig 1), and peak height was linearly related to caffeine concentration over the range tested (Fig 2). The identity of the gas chromatographic peaks as caffeine was confirmed by mass spectrometry. The average recovery of caffeine from plasma was 96% and that from urine, 88%.

[b] Mallinkrodt Inc, Paris, Ky.
[c] Sigma Chemical Co, St Louis, Mo.
[d] Model 3920 gas chromatograph, Perkin-Elmer Corp, Norwalk, Conn.
[e] Model 900 gas chromatograph, Perkin-Elmer Corp, Norwalk, Conn.
[f] Gas Chrom Q, Applied Science Laboratories Inc, State College, Pa.
[g] Greene EW: *The Detection, pharmacokinetics and behavioral effects of caffeine in the horse.* MSc Thesis, Graduate Center for Toxicology, University of Kentucky: Lexington, 1980.
[h] Allen D, Department of Statistics, University of Kentucky, Lexington: Personal communication, 1980.
[i] McNeill Laboratories, Fort Washington, Pa.
[j] Amersham Corp, Arlington Heights, Ill.

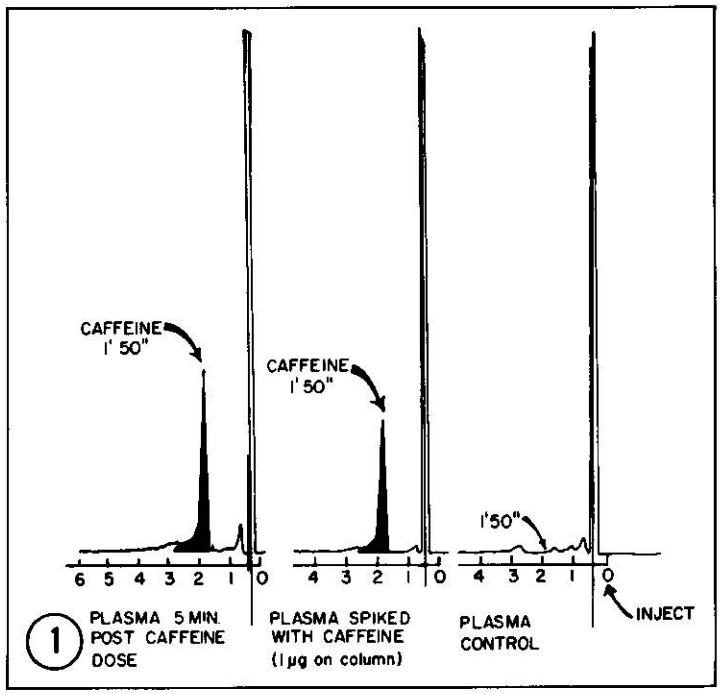

Fig 1—Detection of caffeine on a gas chromatograph equipped with a nitrogen detector. Blank plasma, plasma spiked with caffeine, and plasma from a horse dosed with caffeine were analyzed for caffeine as described in Materials and Methods. The solid peaks represent caffeine, which has a retention time of 1 minute 50 seconds under these conditions.

For IV injection of caffeine, it was found necessary to prepare the caffeine with sodium benzoate. When injected in this way, caffeine concentrations reached peak within 5 minutes after IV injection of the drug and then decreased slowly. The decreases of plasma and urinary concentrations were parallel, with urinary concentrations being consistently about 3-fold higher than plasma concentrations of the drug (Fig 3). Caffeine remained detectable for up to 9 days in the urine of horses given this dose of caffeine.

Figure 4 shows a kinetic analysis of the plasma concentrations in horses given caffeine IV. The data appear well fitted by a 2-compartment open model, with a β-phase half-life of about 18.2 hours, and an α-phase half-life of about 93 minutes. Other kinetic values for this model are described in Table 1. The β-phase half-life was consistent through about 3 orders of drug concentration, indicating that this measured β-phase was the terminal or metabolic phase.

Figure 5 shows plasma and urinary concentrations in horses orally given 4 mg of caffeine/kg. Plasma values peaked at about 2.7 µg/ml at 2 hours. From this point, plasma caffeine concentration fell until it was no longer detectable in the plasma of any horse by the 6th day after dosing. In 1 horse, plasma caffeine concentration was no longer detectable on the 3rd day; in another, by the 5th day; and in the other 2, by the 6th day.

Mean urinary concentrations in horses given caffeine orally were approximately 4.1 µg/ml at 1 hour and increased to about 7.2 µg/ml at 6 hours after dosing. From this point, the urinary values decreased until no horse showed measurable concentrations of drug (day 8). Caffeine disappeared from the detectable range in urine on days 4, 5, 6, and 8 in the different horses. In each horse, caffeine was detectable in the urine for an equal or greater time than it was detectable in plasma.

The relationship of the urinary and the plasma concentrations of caffeine after oral administration was not constant as it was after IV administration. Instead, urinary concentrations of caffeine were initially greater (1.5 to 4 times) than those in plasma in the first 24 hours. At 28 hours after the horses were given caffeine, urinary and plasma concentrations were nearly equal. After 48 hours, however, urinary concentrations of caffeine were lower than the concentrations in plasma and remained so for the subsequent samples drawn.

Figures 6 and 7 show a kinetic analysis of plasma caffeine values in horses given the drug orally. The declining portion of the plasma caffeine curve could be described by 2 decay constants, 1 with a half-time of 42 hours and 1 with a half-time of 6.6 hours. The lower plasma concentrations, and apparently longer plasma half-life of caffeine with IV route

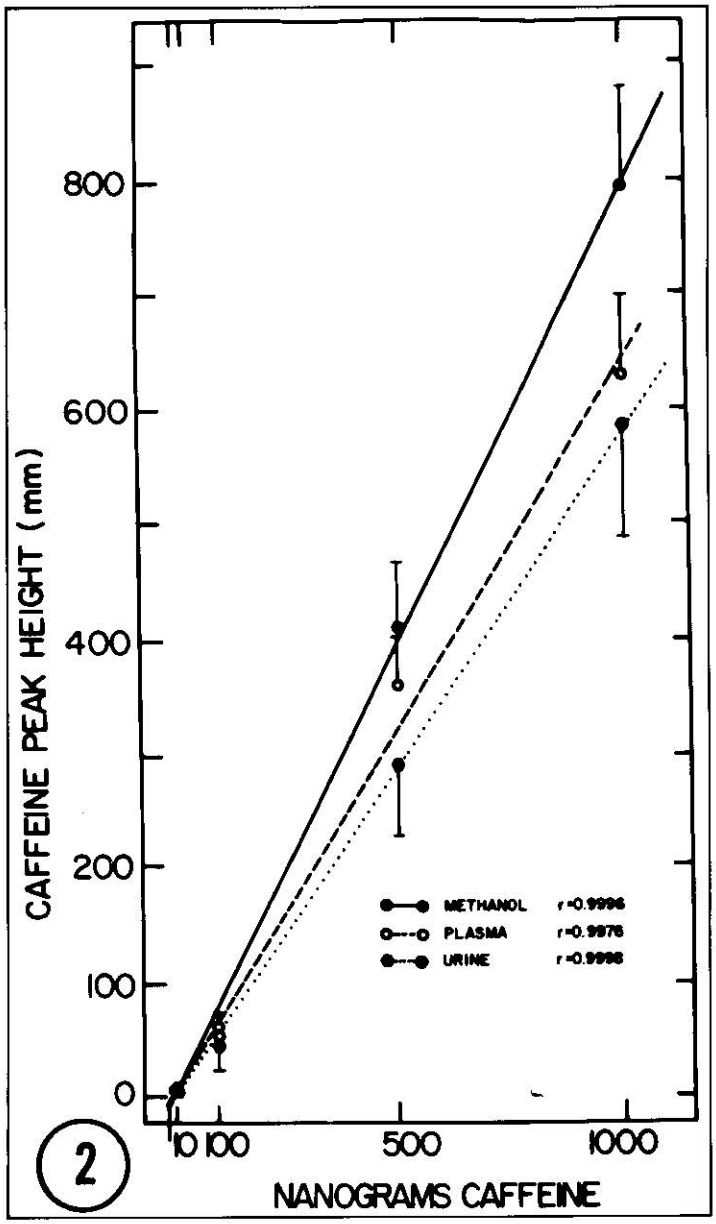

Fig 2—Caffeine standard curve, using a P-E 3920 gas chromatograph. The symbols represent the means of standard curves determined as described in Materials and Methods. The measured peak height of caffeine at a sensitivity setting of $\times 32 \times 10^{-10}$ on the chromatograph was plotted against the nanograms of caffeine injected on column. Correlation coefficients are given from the line representing the linear regression on the data points from the greatest to lowest amount of caffeine detected.

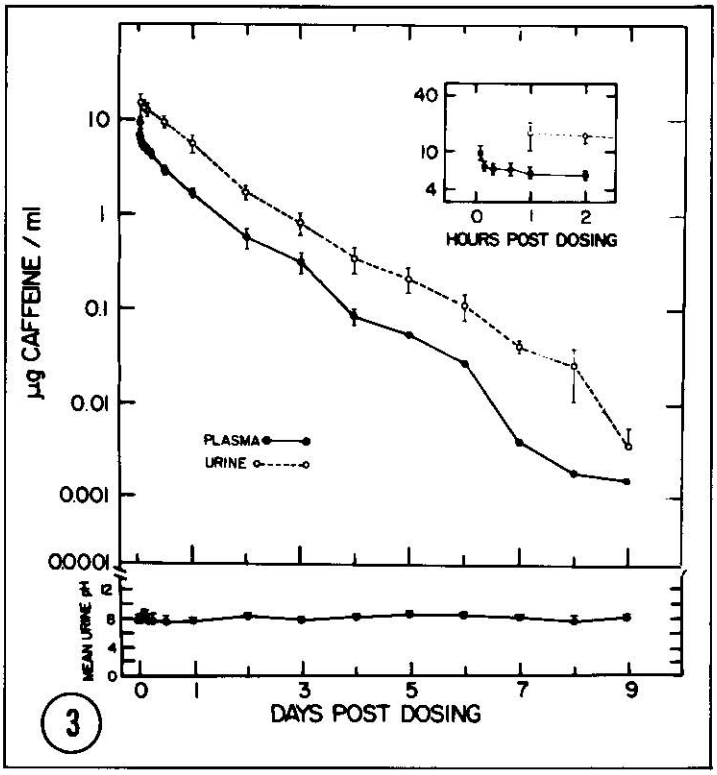

Fig 3—Mean plasma and urinary concentrations in 4 horses given caffeine (4 mg/kg) IV.
Inset—An expanded plot of the plasma and urinary values in the first 2 hours after dosing. Bottom panel: mean pH of the urine in 3 of the horses at the indicated times after caffeine dosing.

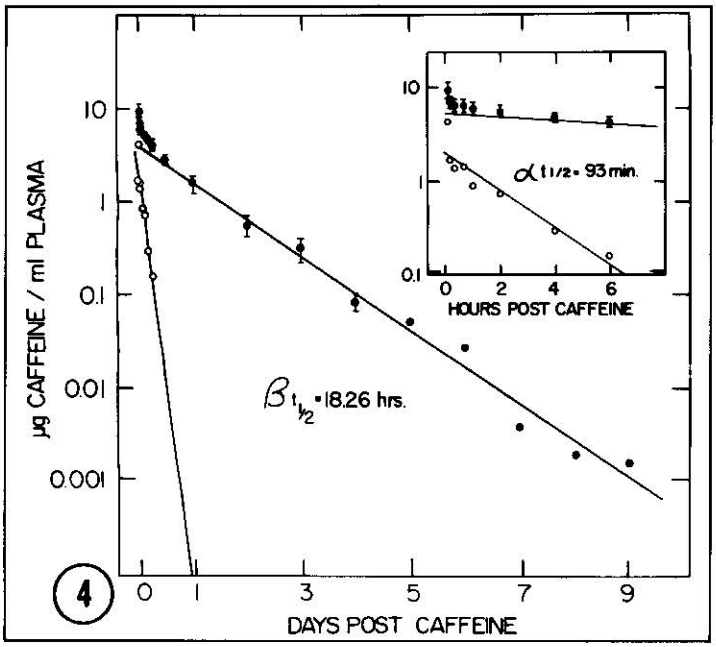

Fig 4—Kinetic analysis of plasma concentrations in 4 horses given caffeine (4 mg/kg) IV. Solid circles = mean plasma caffeine concentrations. The line fitted to the solid circles is the least squares fit of the logarithm of the plasma caffeine concentrations from 1 day to 9 days after dosing and represents the β- or elimination phase ($t_{1/2}$ = 18.26 hours). Open circles = least-squares fit to points representing the α- or distribution phase (half-life 93 min).
Inset—Early data points.

of injection, indicated that caffeine may be slowly and incompletely absorbed after oral administration of the drug. Calculating the areas under the curve by the trapezoidal rule,[7] the bioavailability of caffeine given to horses orally was only 39% of that available in horses given the same drug by IV administration.

The rate constant of absorption (k_{ab}) and the absorptive half-life ($t_{1/2ab}$) of caffeine after oral administration were determined, using the method of residuals.[8] The least squares fit of the terminal portion of the curve in Figure 6 (solid circles, dotted line; 48 hours to 120 hours) gave rise to a line defining the elimination of caffeine. The extrapolated values of this line were subtracted from the corresponding true, initial, values of caffeine concentration (5 minutes to 24 hours) and the resulting residual caffeine concentrations were plotted in Figure 6 (open circles). A least-squares fit of the terminal linear portion (2 to 24 hours) of these residual data points resulted in a line defining the α-portion, or distributive portion, of the curve. This is illustrated by the dotted line among the open circles in Figure 6 and this has been replotted in Figure 7, shown by the solid line among the solid circles. By subtracting the ascending residual plasma caffeine concentrations (5 minutes to 2 hours) from

TABLE 1—Pharmacokinetic parameters* of caffeine after IV administration

$t_{1/2}\ \alpha$ = 1.55 hr	V_1 = 133.33 L
$t_{1/2}\ \beta$ = 18.26 hr	= 0.267 L/kg
k_a = 0.093 hr^{-1}	
k_{12} = 0.184 hr^{-1}	V_2 = 191.47 L
	= 0.383 L/kg
k_{21} = 0.210 hr^{-1}	V_d = 324.8 L
	= 0.650 L/kg
	Clearance = 0.205 L/min

* Based on a 2-compartment open model with elimination occurring from the central compartment.

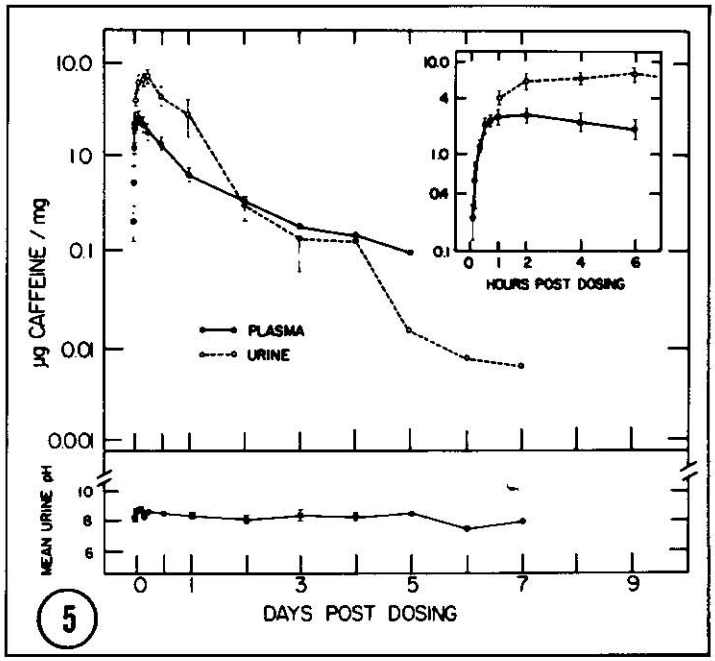

Fig 5—Four horses dosed with 4 mg/kg caffeine orally. Mean plasma and urinary concentrations in 4 horses given caffeine 4 mg/kg orally.
Inset: An expanded plot of the plasma and urinary values taken in the first 6 hours after dosing. Bottom panel: Mean pH of the urine in the 4 horses at the indicated times after caffeine dosing.

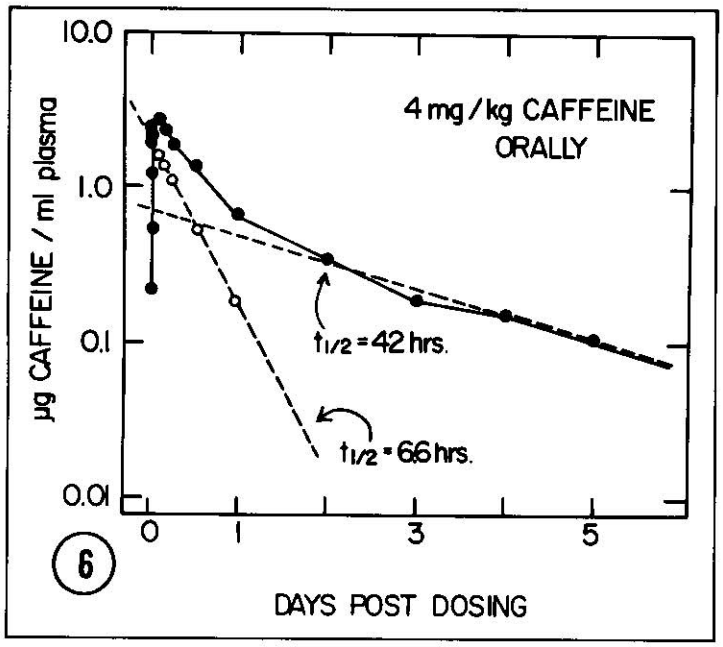

Fig 6—Plasma half-life of caffeine given orally to 4 horses. Solid circles = mean plasma concentrations of caffeine. A least-squares fit of the values in the terminal linear phase resulted in a line (– – –) from which a half-life of 42 hours for caffeine was calculated (r = 0.9841). Values on this extrapolated line were subtracted from the plasma caffeine concentrations seen during the initial rapid decline of caffeine concentrations in plasma and the data points, represented by the open circles, resulted. A linear regression on these values resulted in a line from which a half-life of 6.6 hour for caffeine was calculated (r = 0.9940).

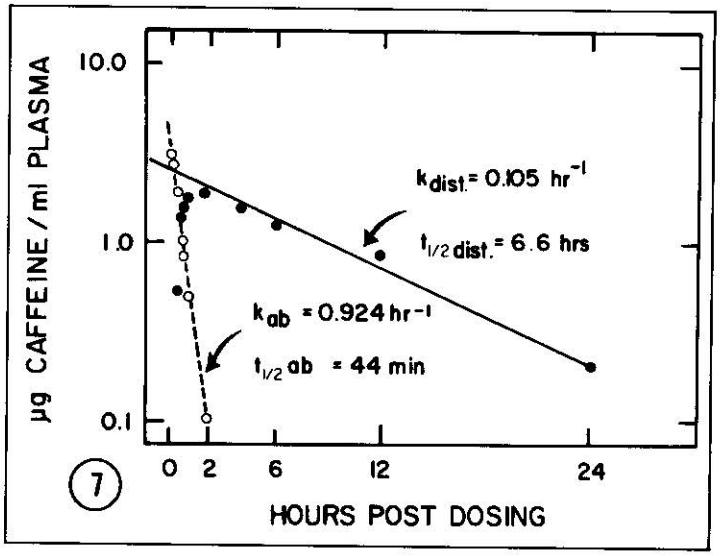

Fig 7—Absorption of caffeine (4 mg/kg) given by stomach tube to 4 horses. This figure plots the mean residual plasma concentrations (solid circles) shown in Figure 6 as (open circles). Subtraction of the ascending plasma concentrations (5 min to 2 hr) from the least-squares fit of the residual caffeine concentrations of the distributive phase (solid circles) resulted in the values represented by the open circles. A least-squares fit of the values (open circles) gave an absorption rate constant (k_{ab}) of 0.924 hour^{-1} from which an absorptive half-life ($t_{1/2ab}$) of 44 minutes was determined.

the corresponding values on the extrapolated line of the distribution phase, a 2nd set of residual caffeine concentrations was obtained. These are shown in Figure 7 by the open circles. A least-squares fit of these latter points (the dotted line in Figure 7) resulted in the line describing the absorptive phase of caffeine. From the slope of this line (slope = $k_{ab}/2.303$) the rate constant of absorption (k_{ab}) was obtained and was equal to 0.9424 hour^{-1}. Using the equation $t_{1/2ab} = 0.693/k_{ab}$, the absorptive half-life of caffeine was found to be 0.735 hours or 44 minutes. Table 2 reviews the pharmacokinetic parameters of caffeine after oral administration.

TABLE 2—Pharmacokinetic parameters of caffeine after oral administration

Administered dose = 4 mg/kg of body weight
Fraction of absorbed dose (F) = 39.03%
Absorbed dose = 1.56 mg/kg
Percentage of caffeine excreted unchanged in the urine
After oral administration = 1.2%
After IV administration = 3.0%
Absorption
Rate constant = 0.9424 hr^{-1}
Half-life = 44 min
Distribution
Rate constant = 0.1045 hr^{-1}
Half-life = 6.6 hr
Elimination
Rate constant = 0.0165 hr^{-1}
Half-life = 42 hr

TABLE 3—Percentage of plasma protein binding of caffeine

Concentration of unlabeled caffeine added to plasma	Percentage of plasma protein binding (mean ± SEM)
10 µg/ml	0.22% ± 0.22
5 µg/ml	1.18% ± 0.66
1 µg/ml	2.00% ± 0.96
0.5 µg/ml	1.87% ± 0.94
0.1 µg/ml	0.40% ± 0.23

Over a range of from 0.1 to 10 µg/ml of caffeine in plasma, the maximal plasma protein binding of caffeine was about 2% (av 1.13%; Table 3).

The effects of caffeine on locomotor responses are shown in Figure 8. Injection of caffeine alone produced a locomotor response which was significantly greater than that in horses given saline solutions. Administration of fentanyl to caffeine-treated horses produced the most marked increase in locomotor activity in the period between 30 to 60 minutes after fentanyl administration, at 4 hours after caffeine was given. As shown in Figure 9, this effect was observable and was statistically significant ($P < 0.01$) at 0, 2, 4, 6, and 10 hours after caffeine, but was not observable at 72 hours.

At the other times of measurement of the locomotor response (24 and 48 hours after caffeine), the response was increased, but this increase was not statistically significant when compared with the response to fentanyl alone. By 72 hours after horses were given caffeine, there was no measurable elevation of the locomotor response compared with the response to fentanyl alone.

Figure 10 shows the locomotor response to saline solution, fentanyl, caffeine, and caffeine plus fentanyl. In this experiment, the horses were treated with fentanyl 4 hours after they were given caffeine. The response to caffeine and fentanyl together in the last 30 minutes after fentanyl injection was greater than the sum of the response to these agents alone, showing that caffeine potentiated the locomotor response to fentanyl.

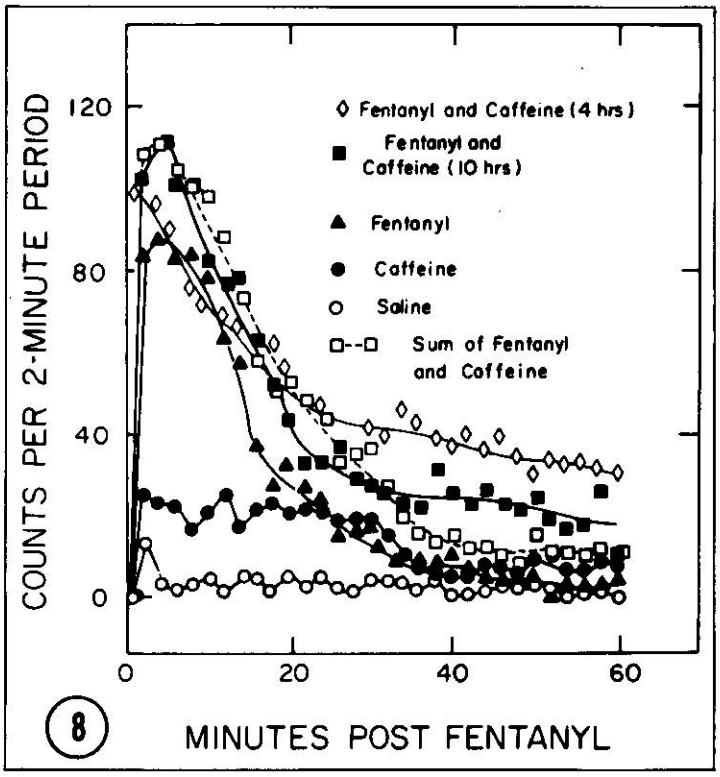

Fig 8—Locomotor response (No. of footsteps/2-min period) in horses given fentanyl and caffeine. Caffeine (4 mg/kg) in a sodium benzoate solution) was injected IV and after 15 minutes, 0.01 mg of fentanyl/kg was injected; the latter was time zero. The locomotor response was recorded for 60 minutes. The symbols show the mean response of the 4 horses tested. Fentanyl was administered again at various time intervals including 4 hours (◇) after caffeine administration and at 10 hours (■) after caffeine administration. For comparison, the locomotor responses after the following independent drug administrations were recorded; saline solution (○), caffeine (●), fentanyl (▲), and the summed responses of caffeine and fentanyl (□).

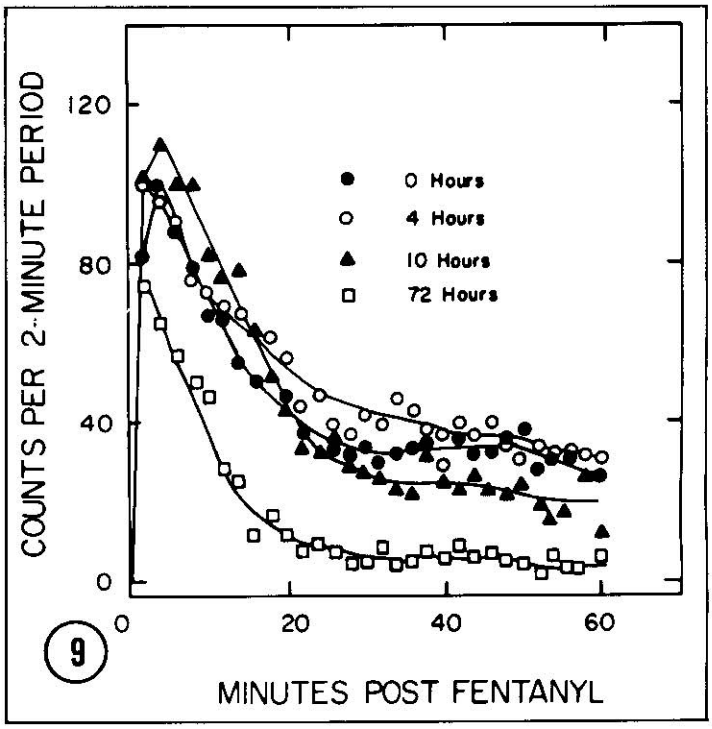

Fig 9—Locomotor response (No. of footsteps/2-min period) in horses given fentanyl and caffeine. Caffeine (4 mg/kg; in a sodium benzoate solution) was injected IV and after 15 minutes, was followed by administration of fentanyl at various time periods. This figure gives the number of footsteps/2-minute period seen in the hour after fentanyl administration when fentanyl was injected at 0 hour (●), 4 hours (○), 10 hours (▲), and 72 hours (□) after caffeine administration. The symbols show the mean response in the 4 horses.

Discussion

Gas chromatography with nitrogen-phosphorus detection proved a satisfactory method of detecting caffeine in the plasma and urine of horses. The gas chromatographic peaks for caffeine were clear and sharp, and there were no interfering peaks. The method was sensitive down to 0.1 ng of caffeine injected into the gas chromatograph. Caffeine could be detected in the plasma and urine of horses for up to 9 days after administration, long after detectable behavioral effects of the drug had disappeared.[8]

After horses were given caffeine IV, plasma concentrations of the drug decreased rapidly for about 4 hours and then more slowly, with an apparent plasma half-life of about 18 hours. This 2nd β- or elimination phase was log-linear as the plasma values decreased from about 5 to 0.005 μg of caffeine/ml. The prolonged linearity of this phase indicates that this β-phase represents a true terminal or metabolic phase for caffeine in the horse. The pharmacokinetics of caffeine in the plasma of the horse were well described by a 2-compartment open model with the kinetic values described in Table 1.

After IV administration of caffeine to the horse, the decrease of caffeine concentrations in the urine paralleled the decline of caffeine concentrations in the plasma. This indicated that caffeine was being excreted in the urine at a rate similar to its elimination from the plasma. This conclu-

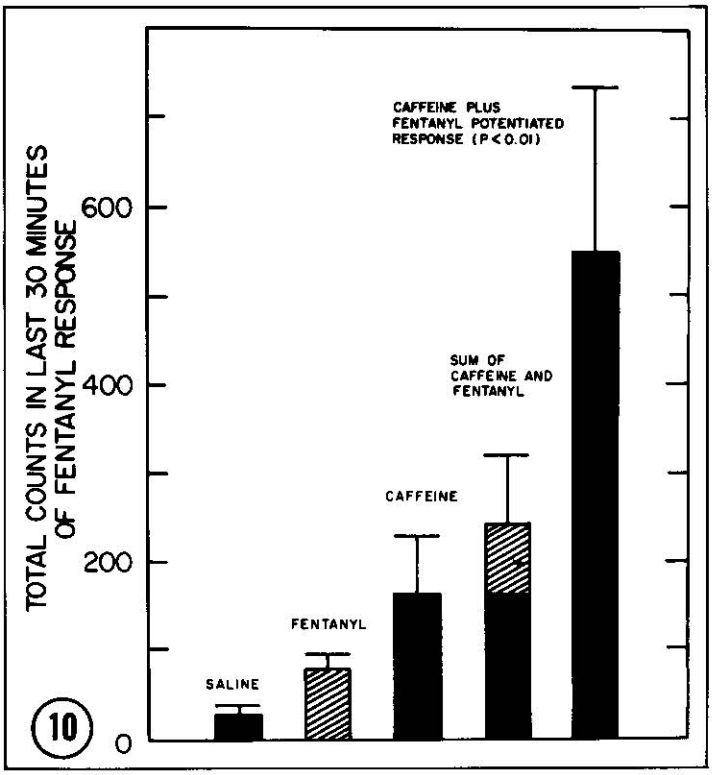

Fig 10—Potentiation of fentanyl response (No. of footsteps/last 30 min) in horses pretreated (4 hours) with caffeine (4 mg/kg). The bars show the mean number of footsteps in 4 horses recorded in the last 30 minutes.

sion is supported by similarities in the elimination and urinary half-lives of caffeine, 18.25 and 19.51 hours, respectively. Examination of the relationship of the concentrations of caffeine in the plasma to those in the urine showed that urinary concentrations of caffeine remained at a level almost uniformly 3 times those in the plasma at the time of each sampling. Other research workers reported a similar 3-fold elevation of urinary caffeine concentrations over those in the plasma of the horse.[9,k]

After oral administration of caffeine in horses, the pharmacokinetics of caffeine are consistent with partial and delayed absorption of the drug from the gastrointestinal tract. Comparison of areas under the curves for IV injection and oral administration show that the bioavailability of caffeine after oral administration in horses was only about 39%. This partial absorption was confirmed by comparing the amount of caffeine excreted in the urine after caffeine was administered IV and orally. It seems likely, therefore, that caffeine is poorly absorbed from the gastrointestinal tract, although the possibility of first-pass effects in the liver and/or substantial biliary excretion of the drug cannot be ruled out at this time.

Kinetic analysis of the plasma concentrations of caffeine seen in the horse after oral administration led to a determination of the time over which caffeine was absorbed from the gastrointestinal tract. The initial rate of absorption of caffeine was fast, with an apparent half-life of about 44 minutes. This rapid initial rate of absorption gives rise to the rapid initial peak blood values for caffeine, which thereafter decline rapidly. The final plasma half-life for caffeine, however, is a slower 42-hour apparent plasma half-life, considerably longer than its plasma half-life after IV injection. This prolonged plasma half-life of caffeine is consistent with delayed absorption of caffeine from the gastrointestinal tract. Since caffeine can be rapidly absorbed, this prolonged half-life may represent a poorly available form of caffeine, perhaps in the form of a poorly soluble precipitate in the gastrointestinal tract.

With some important exceptions, the pharmacologic effects of any drug cease within a period of hours to days after a single dose, although traces of the drug may remain detectable in blood or urine for days or weeks. Caffeine appears to be typical of drugs in this regard, in that it can be relatively easily detected in blood or urine. These detections, however, bear no clearcut relationship to the pharmacologic effects. For this reason, we elected to determine the duration of the pharmacologic actions of caffeine so that we might compare this duration with the period for which caffeine can be detected in blood or urine samples. The goal of this approach would be to determine blood concentrations of caffeine which are not associated with CNS stimulant effects.

As shown in Figure 8, caffeine alone produced an increase in locomotor activity, but this effect was small and only occurred shortly after IV injection. Since it appeared likely that the behavioral response to caffeine would last longer than the 1 or 2 hours indicated by this data, we elected to study the effects of caffeine on fentanyl-stimulated locomotor activity. We were encouraged in this approach by previous work which showed that pretreatment with methamphetamine potentiated the locomotor response to fentanyl, particularly later in the fentanyl response.[3]

As shown in Figures 9 and 10, caffeine also potentiated the locomotor response to fentanyl, and this potentiation was most marked in the last 30 minutes of the fentanyl response. For example, the locomotor response to fentanyl was improved to a statistically significant degree at 0, 2, 4, 6, and 10 hours after caffeine (Fig 10), when compared with either the response when fentanyl was administered alone (Fig 8) or the 72-hour response. These data indicate that the behavioral response to IV caffeine has essentially ceased within 72 hours of administration. These data then are consistent with the hypothesis that the blood levels of caffeine found 72 hours after administration are not likely to be associated with any significant pharmacologic effect.

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