The pharmacokinetics, pharmacological responses and behavioral effects of acepromazine in the horse

S. BALLARD, T. SHULTS, A. A. KOWNACKI, J. W. BLAKE & T. TOBIN

Kentucky Equine Drug Research Program and the Graduate Center for
Toxicology, Department of Veterinary Science, College of Agriculture,
University of Kentucky, Lexington, Kentucky, U.S.A.

Ballard, S., Shults, T., Kownacki, A.A. & Tobin, T. The pharmacological responses and behavioral effects of acepromazine in the horse. *J. vet. Pharmacol. Therap.* 5, 21–31.

After intravenous (i.v.) injection, acepromazine was distributed widely in the horse (V_d = 6.6 litres/kg) and bound extensively ($\geq$99%) to plasma proteins. Plasma levels of the drug declined with an α phase half-life of 4.2 min, while the β phase or elimination half-life was 184.8 min. At a dosage level of 0.3 mg/kg acepromazine was detectable in the plasma for 8 h post dosing. The whole blood partitioning of acepromazine was 46% in the plasma phase and 54% in the erythrocyte phase.

Penile prolapse was clearly evident at doses from 0.01 mg/kg to 0.4 mg/kg i.v., and the duration and extent of protrusion were dose related. Hematocrit levels were significantly lowered by administration of 0.002 mg/kg i.v. (about 1 mg to a 500 kg horse) and increasing dosages resulted in greater than 20% lowering of the hematocrit from control levels. Pretreatment of horses with acepromazine also reduced the variable interval (VI 60) responding rate in all horses tested.

These data show that hematocrit changes are the most sensitive pharmacological responses to acepromazine, followed by changes in penile extension, respiratory rate, VI responding and locomotor responses. Acepromazine is difficult to detect in plasma at normal clinical doses. However, because of its large volume of distribution, its urinary elimination is likely prolonged, and further work on its elimination in equine urine is required.

Dr T. Tobin, Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, Kentucky 40546–0076, U.S.A.

INTRODUCTION

Acepromazine is a 'tranquilizer' which is widely used in equines. It is a neuroleptic drug which belongs to the broad clinical class of drugs known as phenothiazines. Typical of most phenothiazine 'tranquilizers', acepromazine produces behavioral changes which allow an animal to retain much of its alertness and co-ordination. This is important in equine medicine since many manipulations must be carried out on a standing animal. These

properties of acepromazine also lead to its use in competitive horses to manipulate an animal's performance. Since acepromazine is prohibited for use in competition horses by most racing commissions and show horse organizations and because of its frequent legitimate uses, it is important that its actions in the horse be well understood.

In a previous paper, we have detailed a method for the recovery and detection of acepromazine and chlorpromazine from biological fluids (Ballard & Tobin, 1981). Using this method, we now describe some characteristics of its disposition and pharmacology in the horse. Preliminary reports have been communicated (Tobin & Ballard, 1979; Tobin, 1981).

MATERIALS AND METHODS

Analysis and kinetics

Standard curves for acepromazine in plasma were obtained by spiking 4 ml of plasma and buffer samples with appropriate concentrations of acepromazine maleate in aqueous solution. Three millilitres of saturated tetraborate buffer solution and 2 ml of dichloromethane (DCM) were added, and the tubes were then capped and agitated on a Fisher Roto-Rack Model 343 for 1 h. Following this step, the tubes were centrifuged (3000 r.p.m. at $0°C$ for 1 h and the aqueous layer discarded. One millilitre of DCM was removed and evaporated to dryness. The dried residue was reconstituted with 50 μl of cyclohexane of which 5 μl aliquots were used for analysis.

Acepromazine levels in plasma were determined by drawing blood samples in 30 ml all-glass syringes containing approximately 2000 u. of Panheparin as an anticoagulant. The blood was centrifuged, and the plasma and red cell layers were separated. Seven millilitres of aliquots of this plasma were transferred to 15 ml screw-topped centrifuge tubes to which 3 ml of saturated tetraborate solution and 4 ml of dichloromethane were added. The tubes were capped and agitated at 7 r.p.m. for 1 h. Following agitation, the samples were centrifuged for 1 h at $0°C$ to break up the emulsions that formed. At this point, the aqueous layer was discarded

and 3 ml of the DCM was decanted into $15 × 75$ mm culture tubes and evaporated to dryness under a stream of nitrogen gas at room temperature. Samples were reconstituted with 10 μl of cyclohexane of which 5 μl were injected onto the gas chromatograph for analysis.

Plasma protein binding of acepromazine was determined by first placing 10 ml of plasma into a dialysis bag (Spectrapor® membrane number 4 tubing, molecular cutoff 12,000–14,000). The plasma was then spiked with the indicated concentration of drug, the bag sealed, and transferred to a 300 ml Erlenmeyer flask containing 200 ml of 50 mM phosphate buffer (pH 7.4). The flask and its contents were placed in a water bath at $37°C$ with constant agitation for 24 h. Buffer samples were analysed by combining 6 ml of buffer, 3 ml of saturated tetraborate, and 4 ml of dichloromethane in 15 ml screw-topped tubes and agitating them for 1 h. Plasma samples were analysed by combining 3 ml of plasma, 2 ml of saturated tetraborate buffer and 4 ml of dichloromethane in 15 ml screw-topped tubes and agitating them likewise for 1 h. Samples were then centrifuged at $0°C$ for 1 h, and 2 ml of the DCM were removed and evaporated to dryness. The residue was reconstituted with 10 μl of cyclohexane of which 5 μl were injected for analysis.

For measuring the whole blood partitioning coefficient of acepromazine, 10 ml of whole blood (containing approximately 2000 u. of Panheparin as an anticoagulant) were spiked with 1–2 μg of the drug in aqueous solution. The samples were incubated for 2 h in a water bath at $37°C$ with mild agitation. At this point, the plasma and red cell fractions were separated by centrifugation and analysed separately. Three millilitres of the sample (plasma or red blood cells), 2 ml of saturated tetraborate solution, and 4 ml of cyclohexane were added to 15 ml tubes and rotoracked for 1 h. Tubes were then centrifuged at $0°C$ for 1 h, and 2 ml of the cyclohexane was removed and evaporated to dryness. To this dried residue, 10 μl of cyclohexane were added of which 5 μl were injected for analysis.

The analysis of plasma levels in the kinetics experiment was performed on a Perkin-Elmer 3920B gas-liquid chromatograph equipped

with a nitrogen/phosphorus detector. A six-foot glass column with a 3% OV-101 Gas Chrom Q 100/120 mesh packing was used, and operating temperatures were 250°C oven, 270°C injector port, 270°C manifold. For the partitioning and protein binding experiments, a Perkin-Elmer 900 model was used with the detector, column packing, and operating temperatures being the same. Carrier gas flow rates were 29 ml/min.

Spectrapor® membrane tubing was purchased from Spectrum Medical Industries, Inc., Los Angeles, Calif. Vacutainer® blood collection tubes were purchased from Becton-Dickinson, Rutherford, N.J. Fentanyl citrate was supplied by McNeil Laboratories, Fort Washington, Pa. Dichloromethane and cyclohexane were nanograde and obtained from Mallincrodt. Panheparin was purchased from Abbott Laboratories, North Chicago, Ill. Acepromazine was graciously donated by Ayerst Laboratories, New York, N.Y.

Pharmacological responses

The effect of acepromazine on penile protrusion was calculated by measuring penis length after intravenous acepromazine administration. Five dosage levels were used from 0.004 mg/kg to 0.4 mg/kg and measurements were taken at regular intervals until the penis had fully retracted. Values were expressed as a percentage of maximal protrusion seen in each horse and were means of determinations on four horses.

Measurement of hematocrit levels were performed on mature thoroughbred and standardbred horses. Before administration, each animal was brought into a stall and allowed to acclimate to its surroundings for 30 min. At this point, control hematocrits measured after which the animals were given acepromazine or a saline solution intravenously. Blood samples were drawn at regular intervals and immediately centrifuged to obtain packed cell (hematocrit) percentage values. Each dosage level was administered to four horses and the means and standard errors calculated.

Behavior

Operant behavior of the horse was measured by the use of modified 'Skinner' box apparatus

developed by Shults, Combie, Dougherty & Tobin, 1979 (Fig. 13). The animals were trained to bob their heads over a feed bucket which resulted in the interruption of a light beam and a consequent food reinforcement. Horses were trained on a 'fixed-interval' schedule in which they received a reinforcement of oats for a fixed number of responses (interruptions in the light beam). The animals readily learned this program and were later switched to a variable-interval (VI 60) schedule. When using the variable-interval program, reinforcements were independent of the number of responses but required a response based on a randomized predetermined time ratio. The horses readily adjusted and soon developed a stable baseline response on this schedule. The effect of acepromazine on this baseline response was measured when increasing doses of the drug and equal volumes of saline controls were administered to these horses 10 min before their introduction into the behavioral stalls.

RESULTS

Analysis and kinetics

Chromatograms of acepromazine from blood samples drawn in Vacutainer® tubes (Fig. 1) contained a prominent peak which
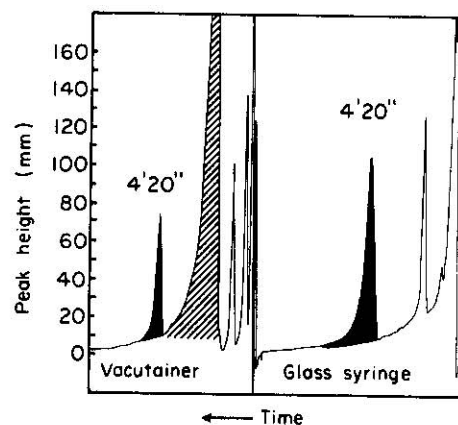

FIG. 1. Contaminant in plasma drawn in vacutainer tubes. The left panel shows a chromatogram of acepromazine extracted from plasma which was drawn in vacutainer tubes. The solid colored peak represents acepromazine while the shaded peak is a contaminant believed to represent tri(2-butoxyethyl)-phosphate. The right panel shows the chromatogram of an extracted sample of plasma drawn in a glass syringe with Panheparin present as an anticoagulant.

interfered with the measurement of the drug peak. Since this interference was not present when blood was drawn in all-glass syringes (Fig. 2), all analytical sampes were samples drawn in glass syringes. Retention times of acepromazine were variable with changes in the carrier gas flow rate, and in the experiment of Fig. 1, a 29 ml/min flow rate resulted in a retention time of 4 min and 20 sec. The peaks were symmetrical with a mild degree of 'tailing' which became more pronounced as the retention times were increased. Other experiments showed that recoveries were 98.4% complete. Standard curves obtained from spiked plasma and buffer samples (Fig. 3) were linear with correlation values of 0.9999 and 0.9982, respectively.

When mature thoroughbred and standard-bred horses were administered 0.3 mg/kg of acepromazine i.v. the unchanged drug was detected in plasma for 8 h (Fig. 4). When these data were analysed by an SAS computer program using the Marquandt method, a very close fit to a biphasic (two compartment) model equation (Fig. 5) was found with a correlation coefficient of 0.9966. The β phase, elimination, half-life was determined to be 4.2 min. Volume of distribution was found to be 6.6 l/kg and clearance was 24.8 l/min. Other kinetic parameters for the distribution

FIG. 3. Acepromazine standard curves from plasma and 50 mM phosphate buffer solutions. (○) Recovery from plasma solutions while recoveries from buffer solutions are shown as (●). Regression coefficients were 0.9999 for plasma extractions and 0.9982 for buffer extractions. Analysis was made through the use of a gas-liquid chromatograph equipped with a nitrogen detector.

of acepromazine in the horse are given in Table I.

When drug concentrations were determined in the plasma and buffer phases after dialysis, the percentage protein binding was calculated using the following formula: Percentage binding = [(drug concentration in plasma − drug concentration in buffer)/(drug concentration in plasma)].

As shown in Fig. 6 acepromazine is almost completely bound to plasma proteins at normal physiological levels. At 0.1 μg/ml, 99.6% of the drug is bound. At 0.5 μg/ml, 99.0% of acepromazine remains bound to the plasma proteins. As the concentrations of acepromazine are increased from this point, the percentage of drug binding decreases dramatically to 66.7% at a dose level of 10 μg/ml. The corrected volume of distribution, based on the free drug concentration in the plasma, therefore, in 1650 litres/kg or about 831,000 litres/horse.

The partitioning of acepromazine in whole blood is given in Fig. 7. Acepromazine distributed almost equally between plasma and erythrocytes. After incubation, plasma was found to contain 46.47% of the drug while the red cell fraction contained 53.45%.
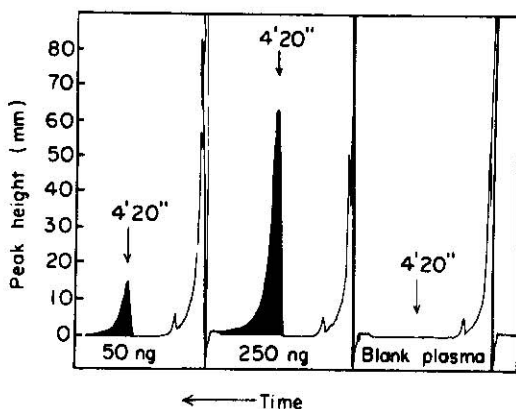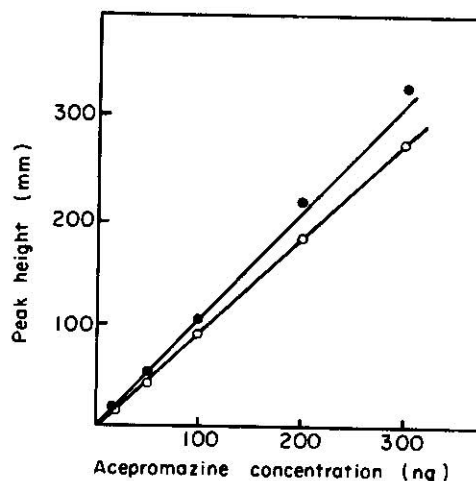

FIG. 2. Detection of acepromazine with a gas-liquid chromatograph equipped with a nitrogen/phosphorus detector. Chromatograms of plasma extractions as described in Methods show plasma containing 50 ng and 250 ng of drug and drug-free plasma. Retention times were 4 min and 20 sec for acepromazine.
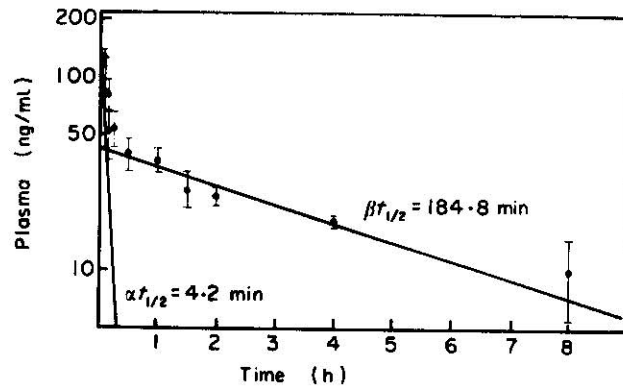
FIG. 4. Plasma levels after rapid intravenous injection of 0.3 mg/kg acepromazine maleate. The data points (●) show plasma concentrations of acepromazine after rapid i.v. administration of 0.3 mg/kg of the drug. The β phase half-life was determined by computer analysis and found to be 184.8 min. The α phase half-life was also determined and found to be 4.2 min. All data points are means ± standard errors of the means of determinations on five horses. Reproduced with permission from Tobin (1981).
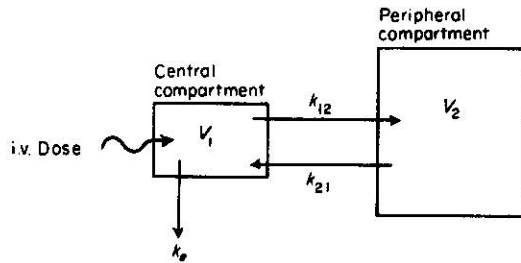

FIG. 5. Possible two-compartment open model for the distribution and elimination of acepromazine in the horse.

TABLE I. Plasma kinetic values calculated from i.v. administration of 0.3 mg/kg acepromazine in the horse

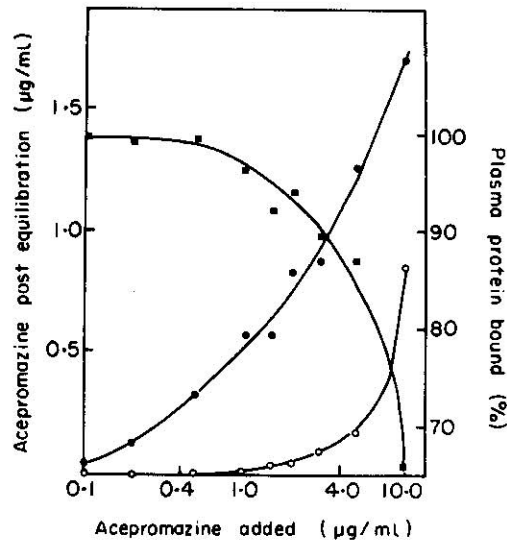| Parameter	Value
A	133.5 ng/ml
B	42.4 ng/ml
α	0.166 min^{-1}
β	0.004 min^{-1}
$\alpha t_{1/2}$	4.2 min
$\beta t_{1/2}$	184.8 min
k_{21}	0.043 min^{-1}
k_{21}	0.112 min^{-1}
k_e	0.145 min^{-1}
V_1	1.71 litres/kg
C_p	175.0 ng/ml
V_d (area)	6.6 litres/kg
V_d (corrected)	1650.0 litres/kg or 831,000 litres
Cl	24.8 litres/min

FIG. 6. Percentage plasma protein binding of acepromazine. Ten millilitres of plasma containing the indicated concentration of acepromazine was placed in a section of dialysis tubing, sealed, and immersed in 200 ml of 50 mM phosphate buffer solution (pH 7.4). The entire contents were agitated for 24 h at 37°C and then analysed for acepromazine content as described in Methods. (○) Indicate the concentration of free drug, while (●) represent the concentration of bound drug. (■) Indicate the percentage of drug bound at the various concentrations of acepromazine.
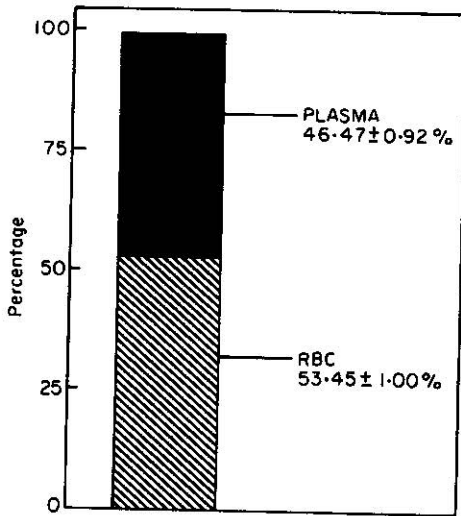
FIG. 7. Whole blood partitioning coefficient of acepromazine. Ten millilitre samples of whole blood were spiked with 1–2 μg of acepromazine. Samples were incubated at 37°C with agitation for 2 h. Three millilitre samples of plasma and red cells were removed and analysed for acepromazine content. Values are means of three experiments ± standard errors of the means.

Pharmacological responses

The most readily apparent pharmacological response of male horses to acepromazine is penile protrusion or penile prolapse. As shown in Fig. 8, penile protrusion was greatest at the dose of 0.4 mg/kg of acepromazine when the peak response was reached at 30 min and did not begin to subside until 4 h post dose. Penile length did not return to control levels until 10 h post dosing. The smallest dose that resulted in prolapse was 0.01 mg/kg.

The effects of acepromazine on the hematocrit in horses is shown in Fig. 9, where the percentage change in the control (initial) hematocrit values was plotted for horses dosed intravenously with 0.002 mg/kg acepromazine or a saline solution. Paired *t* tests were performed on these data comparing saline *v.* control, control *v.* acepromazine-treated, and saline *v.* acepromazine-treated. The results of this analysis, given in Table II, show that the control hematocrits were significantly ($P < 0.05$) different from the hematocrits of treated animals, until 4 h post dose when the levels returned to control. When comparing saline-treated animals to acepromazine-treated, the hematocrits were significantly different until 6 h post dose, with the greatest difference occurring at the 2 h mark. When saline was compared with controls there was no significant difference throughout the 6 h time interval.

As the dosage of acepromazine was increased, the effect on the hematocrit increased. When 0.01 mg/kg of acepromazine was administered, the hematocrit was reduced by 25% within 30 min post dose. When the dosage was increased to five times this amount, the hematocrit was not depressed any further, but the duration of the effect lasted much

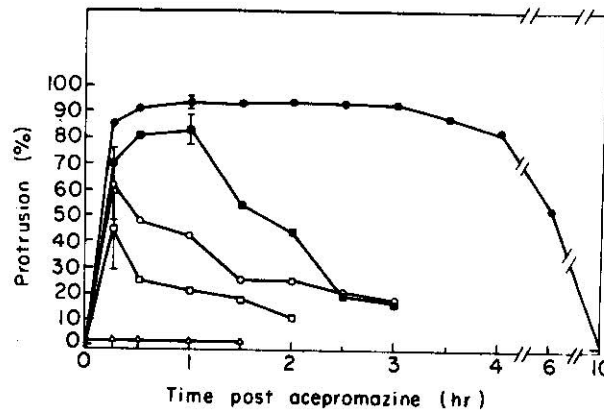
Time post acepromazine (hr)

FIG. 8. Effect of acepromazine on penile protrusion in geldings. Acepromazine at 0.4 mg/kg was administered intravenously to four geldings and the maximal length of penile protrusion measured. The symbols show the penile protrusion measured after each subsequent dose of acepromazine, expressed as a percentage of the maximal protrusion seen in each horse. Tobin (1981). (●) 0.4 mg/kg; (■) 0.10 mg/kg; (○) 0.04 mg/kg; (□) 0.01 mg/kg; (△) 0.004 mg/kg.
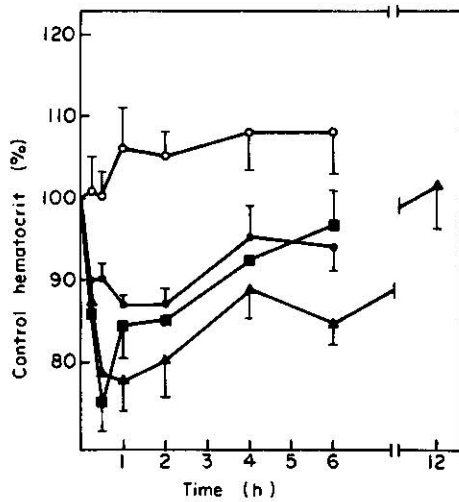
FIG. 9. Effect of acepromazine on hematocrit in horses. This figure shows the effects of increasing dosages of acepromazine on hematocrits. Saline-treated horses are shown as (O), while (●) show the effects of 0.002 mg/kg of acepromazine. Dosages of 0.01 mg/kg and 0.05 mg/kg of acepromazine are represented by (■) and (▲), respectively. All values shown are means ± the standard errors of the means of determinations on four horses.
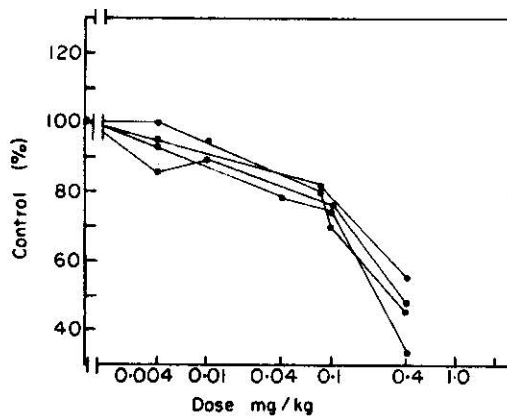

FIG. 10. Effect of acepromazine on variable interval responding. Acepromazine was administered intravenously to four horses, 10 min before each trial. (●) Represent the response of each horse expressed as a percentage of saline control performance for the same horse.

longer and levels did not return to control until 12 h post dosing.

Behavior

When acepromazine was administered to horses before performing operant conditioned (VI 60) behavioral tests, a decreased rate of responding resulted (Fig. 10). At doses as low as 0.004 mg/kg, one horse was found to have a responding rate of 15% below controls. The other three horses were not greatly affected with one horse showing no deviation from control. The rate of responding decreased as the dosage of acepromazine increased with a 20%—30% decrease from control responding rate when a dose of 0.1 mg/kg was administered. As the dose was increased to 0.4 mg/kg, the rate of responding was further depressed to a range of 55%—37% of control responding rate.

DISCUSSION

Analysis and kinetics

A gas chromatograph equipped with a

nitrogen/phosphorus detector was utilized in these experiments because of its sensitivity and selectivity for nitrogen-containing compounds and for its wide range of linear response. Chromatograms obtained through the described methods gave very sharp peaks with no interference (Fig. 2). Detector responses throughout the working range of this assay were linear from both phosphate buffer and plasma solutions with correlation coefficients of 0.9982 and 0.9999, respectively (Fig. 3). Under the chromatographic conditions used, the retention time for acepromazine was 4 min and 20 secs and, in most cases, acepromazine was the only peak present after 2 min (Fig. 2). A moderate amount of 'tailing' was noted with acepromazine, but this could be reduced by increasing the carrier gas flow rates which would consequently reduce the retention times.

A large contaminating peak was noted in blood and plasma samples that had been drawn in Vacutainer tubes (Fig. 1). This is presumably the same contamination noted by Jatlow & Bailey (1975) and Bailey & Jatlow (1976) who believed it to represent tri(2-butoxyethyl)-phosphate. For our purposes, blood samples were drawn in 30 ml glass syringes with Panheparin used as an anticoagulant. This method eliminated the contaminating peak and allowed more sensitive drug detection.

Plasma concentrations of acepromazine

after rapid intravenous administration of 0.3 mg/kg are given in Fig. 4. Levels of the drug were present in detectable quantities for only 8 h post dose. Many drugs when given by rapid i.v. administration behave according to the 'two-compartment open model' (Greenblatt & Koch-Weser, 1975). According to this model, a drug distributes in two phases: the α phase, where the drug distributes from the central compartment (consisting of the serum or blood volume together with the extracellular fluid of some highly perfused tissues) to the peripheral compartment (the less perfused body tissues into which the drug moves more slowly), and the β phase, which is the elimination of the drug from the central compartment, usually by metabolism or excretion (Fig. 5). A biexponential expression of this model (Baggot, 1977) combining these two phases would be as follows:

$$C_p = Ae^{-t} + Be^{-t}$$

When the data in Fig. 5 were compared with this model equation by computer analysis (SAS, Marquardt method) a very close correlation was found ($r = 0.9966$). The α phase half-life was found to be 4.2 min while the β phase half-life was determined as being 184.8 min. Other kinetic parameters are given in Table I.

The volume of distribution of acepromazine was found to be 6.6 litres/kg, an unexpectedly large number indicating that the drug may be accumulating in peripheral compartments in the body. Similar data have been reported by Guyton, Missaghi & Marshall (1976) who showed that in calves, the liver and kidneys retained phenothiazine at concentrations greater than those found in blood. The accumulation of the drug by these tissues could easily account for this high volume of distribution estimate. At normal theoretical plasma levels of acepromazine, 0.1 to about 0.5 μg/ml, the percentage of acepromazine bound was 99.6%, 98.9% and 99.0%, respectively. These data are similar to those of Curry (1970) who reported that chlorpromazine, a phenothiazine of similar structure to acepromazine, was more than 90% bound to human plasma proteins at a range in concentration from 0.008 to 15.1 μg/ml. The corrected volume of distribution (V_d) of the drug, assuming 99.6% protein binding, was found to be 1650 litres/kg. When the concentration

of acepromazine was increased beyond this level, the percentage of plasma binding decreased to 66.7% at 10.0 μg/ml. This decrease in binding with increased acepromazine concentration would suggest a component of the plasma which has a high affinity and a relatively low capacity for binding acepromazine. The nature of this binding is not clear at this time.

Clinically effective doses of acepromazine in horses are usually in the range of 0.04 mg/kg to 0.1 mg/kg. At these therapeutic doses, however, the blood levels of this drug are low and detection is very difficult. When Noonan, Blake, Murdick & Ray (1972) administered 50 mg intravenously to horses, the plasma levels at 1 h were no higher than 1.7 ng/ml. At these levels, kinetic data would be difficult to obtain with the methods used in these experiments. In order to achieve measurable blood levels of acepromazine, we found it necessary to administer the relatively high dose of 0.3 mg/kg. Marked clinical signs of the actions of acepromazine were observed in all these horses. The animals sweated profusely and some exhibited ataxia. In male horse, penile protrusion occurred and seemed to correlate well with the animals' apparent state of sedation (see Fig. 8).

Pharmacological responses

A sensitive and very obvious response of the horse to acepromazine is extension of the penis. As shown in Fig. 8, penile prolapse occurred at a dose of 0.01 mg/kg and higher. This effect was dose-related, with higher doses resulting in greater penile protrusion. The duration of this effect was also influenced by increasing doses of acepromazine. At 0.4 mg/kg, the penis was prolapsed for 4 h and did not retract completely until 10 h post dose. A dose of 0.004 mg/kg was evidently below the threshold dose for this effect as it produced no measurable penile prolapse.

Long term or permanent paralysis of the retractor penis muscle, although very rare, has been known to occur following acepromazine administration (Lucke & Sansom, 1979). This effect seems to be dose-related and appears to occur more frequently in stallions than geldings (Gibbs, 1978; Jones, 1979). For this reason it has been recommended that the use

of acepromazine in valuable breeding stallions be kept to the minimum possible dosages and injected intramuscularly instead of i.v. to prevent possible unwarranted side-effects (Jones, 1979).

Analysis of the data in Fig. 9 shows that acepromazine produces significant changes in the hematocrit levels in the horse at the extremely low dose of 0.002 mg/kg. This is the most sensitive pharmacological response of the horse to this drug that we have found. This dosage amounts to about 1 mg of acepromazine i.v. to a 500 kg horse. As shown in Table II, when a paired *t* test was performed on these data, there was no significant difference between the hematocrits of saline-treated animals and their control (initial) hematocrits. When controls were compared with acepromazine-treated animals, the difference in hematocrits were significant at all time points except the 6 h samples.

This effect of acepromazine on the hematocrit was first noted in our laboratory as we performed analysis of blood samples drawn in our kinetic experiments. Lang, Eglen & Henry (1979) noted a similar action of acepromazine in the dog. They found that 0.07 mg/kg of acepromazine by intramuscular (i.m.) administration reduced the number of circulating erythrocytes by 23% at 1 h post dose. These values are very similar to our own data in the horse for a dosage of 0.05 mg/Kg i.v. Lang also noted a 20% fall in leucocyte counts and a similar drop in the number of lymphocytes and platelets. The spleen of the dog, like that of the horse, is highly innervated by the adrenergic nervous system and possesses a large number of muscularly controlled capsules. Acepromazine, being an adrenergic blocker may cause relaxation of these capsules and result in the sequestering circulatory blood cells (Lang *et al.*, 1979).

Behavior

The effect of acepromazine on variable interval responding is the most sensitive behavioral test that we have found. When horses were given an intravenous dose of 0.004 mg/kg of acepromazine, three out of four horses responded with a decreased responding rate, with one horse showing a 15% decrease. Increasing dosages, as one would expect, produced a more profound depression. At the dosage of 0.4 mg/kg, the highest dose administered, responding rates were less than 50% of control values.

The dose sensitivity of these behavioral effects appeared quite close to two other central effects of acepromazine in the horse studied in our laboratory. Work on the inhibition of respiratory response by acepromazine

TABLE II. Paired *t* tests results of comparisons of the hematocrits in acepromazine-treated horses

Time post dose (h)		*t*	d.f.	$\bar{\Delta}$	SΔ	*P*
Control ≠ treated	0.25	3.3988	3	0.0975	0.05737	0.97875
	0.30	4.7354	3	0.1050	0.04430	0.99107
	1.00	9.6959	3	0.1275	0.02630	0.99880
	2.00	5.9880	3	0.1325	0.04425	0.99530
	4.00	1.5016	3	0.0550	0.07326	0.88489
	6.00	2.1567	3	0.0650	0.06027	0.94000
Saline ≠ treated	0.25	4.8100	3	0.1118	0.04650	0.99140
	0.30		3	0.1050	0.07590	0.96510
	1.00		3	0.1875	0.07270	0.99290
	2.00		3	0.1770	0.02500	0.99960
	4.00		3	0.1357	0.08278	0.97680
	6.00		3	0.1400	0.12880	0.9409
Saline ≠ control	0.25	−0.3274	3	−0.0143	0.08706	0.61750
	0.30		3	0.0000	0.05944	0.50000
	1.00		3	−0.0600	0.09626	0.84949
	2.00		3	−0.0450	0.05196	0.90915
	4.00		3	−0.0807	0.09005	0.91460
	6.00		3	−0.0750	0.11030	0.86650

has shown that this response is half-maximally inhibited at about 0.4 mg/kg, closely paralleling the sensitivity of VI responding in these horses. Similarly, inhibition of fentanyl stimulated locomotor response (Fig. 11) appears to be similarly sensitive to acepromazine. It, therefore, appears that the horse is relatively resistant to the central actions of acepromazine, as compared with the cardiovascular actions of this agent.

In summary, acepromazine distributes very widely in the horse, and has a very large volume of distribution. This large volume of distribution means that the free or effective concentration of acepromazine present at drug receptor sites must be very small indeed. The most sensitive response found to acepromazine was in actions on hematocrit, a 50% of maximal reduction in hematocrit being achieved with doses of the order of 1 mg/horse. The next most sensitive response was penile protrusion, which response was half-maximal at about 5 mg/horse. Respiratory depression and VI responding and, from very limited data, locomotor response were all shown to have about the same response to acepromazine, requiring about 20 mg/horse for a half-maximal response.

The small amounts of this drug injected into horses and its very large volume of distribution make acepromazine a difficult drug to detect in plasma. On the other hand, its large volume of distribution in the horse means that elimination of this drug by the horse may be relatively slow. Good quantitative studies on the 'clearance' of this drug in horse urine are required, since acepromazine is an agent with many legitimate uses in equine medicine and likely a fairly prolonged half-life in equine urine.

REFERENCES

Baggot, J.D. (1977) *Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology*. W. B. Saunders Company, Philadelphia.
Bailey, D.N. & Jatlow, P.I. (1976) Gas-chromatographic analysis of therapeutic concentrations of imipramine and desipramine in plasma, with use of a nitrogen detector. *Clinical Chemistry*, 22/10, 1697–1701.
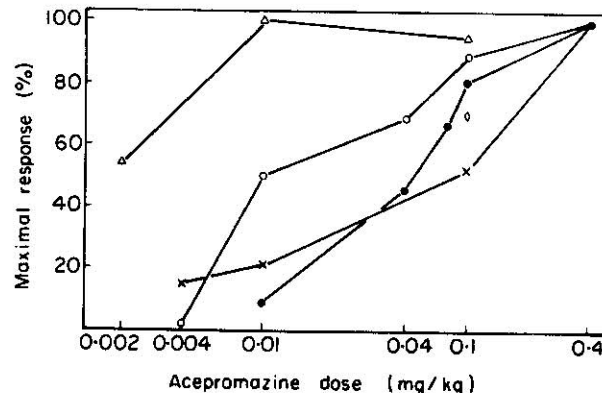

FIG. 11. Dose-response effects after acepromazine in the horse. The above graph shows the percentage maximal observed response of five different pharmacological and behavioral parameters as related to intravenous dosage of acepromazine. Hematocrit effects (△); penile protrusion (○); respiration (●); variable interval responding (×); locomotor response (◇). Only one dosage level was used for locomotor response. The respiratory and locomotor data are replotted from Tobin & Ballard, 1979.

Ballard, S. & Tobin, T. (1981) Atypical conditions for quantitative recovery of acepromazine and chlorpromazine from plasma. *Journal of Toxicology and Environmental Health*, 7, 745–751.

Casper, R., Garver, D.L., Dekirmenjian, H., Chang, S. & Davis, J.M. (1980) Phenothiazine levels in plasma and red blood cells. *Archives General Psychiatry*, 37, 301–305.

Curry, S.H. (1970) Plasma protein binding of chlorpromazine. *Journal of Pharmacy and Pharmacology*, 22, 193–197.

Garver, D.L., Davis, J.M., Dekirmenjian, H., Jones, F.D., Casper, R. & Haraszti, J. (1976) Pharmacokinetics of red blood cell phenothiazine and clinical effects. *Archives General Psychiatry*, 33, 862–866.

Gibbs, M. (1978) Acetylpromazine meleate (letter). *Veterinary Record*, 102(13), 291.

Greenblatt, D.J. & Koch-Weser, J. (1975) Medical intelligence: drug therapy, clinical pharmacokinetics. *New England Journal of Medicine*, 293, 702–705.

Guyton, C.L., Missaghi, E. & Marshall, P.M. (1976) Metabolic disposition of radioactive phenothiazine in calves: a balance study. *American Journal of Veterinary Record*, 37(11), 1287–1289.

Jatlow, P.I. & Bailey, D.N. (1975) Gas-chromatographic analysis for cocaine in human plasma, with use of nitrogen detector. *Clinical Chemistry*, 21/13, 1918–1921.

Jones, R.S. (1979) Acepromazine in male horses (letter). *Veterinary Record*, 105(17), 405.

Lang, S.M., Eglen, R.M. & Henry, A.C. (1979) Acepromazine administration: its effects on caine haematology. *Veterinary Record*, 105, 397–398.

Lucke, J.N. & Sansom, J. (1979) Penile erection in the horse after acepromazine. *Veterinary Record*, 105(1), 21–22.

Muir, W.W. & Hamlin, R.L. (1975) Effects of acepromazine on ventilatory variables in the horse. *American Journal of Veterinary Record*, 36, 1439.

Noonan, J.S., Blake, J.W., Murdick, P.W. & Ray, R.S. (1972) Bromination of phenothiazine tranquilizers: a method for sensitive and specific detection. *Life Sciences*, 11(2), 363–373.

Shults, T., Combie, J., Dougherty, J. & Tobin, T. (1979) Variable interval scheduling in the horse: a sensitive measure of behavior. In: Proceedings of the Third International Symposium on Equine Medication Control, Eds. T. Tobin, J. W. Blake and W. E. Woods. Published by the International Equine Medication Control Group and the Department of Veterinary Medicine, University of Kentucky.

Tobin, T. (1981) *Drugs and the performance horse*. Charles C. Thomas, Publisher, Springfield, Illinois.

Tobin, T. & Ballard, S. (1979) Pharmacology review: The phenothiazine tranquilizers. *Journal of Equine Medicine and Surgery*, 3, 460–466.

Tobin, T., Combie, J., Shults, T. & Dougherty, J. (1979) The pharmacology of narcotic analgesics in the horse. III. Characteristics of fentanyl and apomorphine. *Journal of Equine Medicine and Surgery*, 3, 284–288.