PHARMACOLOGY, PHARMACOKINETICS, AND BEHAVIORAL EFFECTS OF ACEPROMAZINE IN THE HORSE

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"Reprinted from proceedings of FOURTH INTERNATIONAL CONFERENCE on the Control of the use of Drugs in Racehorses", May 1981.
The Victoria Racing Club, Melbourne, Australia.

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ABSTRACT

Acepromazine is a phenothiazine tranquilizer that finds frequent use in equine medicine. When administered intravenously to mature thoroughbred and standardbred horses, acepromazine distributed according to the "two-compartment open model" with an A phase half-life of 4.17 minutes, a B phase half-life of 184.4 minutes, and a resulting volume of distribution of 6.6505 1/Kg. The percent of acepromazine bound to plasma proteins was determined as being greater than 99.0%. The drug distributed almost evenly between the plasma and erythrocyte phases in blood. Acepromazine exerted a profound effect upon hematocrit levels in the horse and was found to significantly depress packed cell volumes at doses as low as 0.002 mg/kg IV. Penile prolapse occurred at doses of 0.01 mg/kg of acepromazine. Higher dosages increased the duration and magnitude of the depression of respiratory rate. Therapeutic doses of acepromazine were shown to reduce the horse's capacity to respond in operant conditioning trials by as much as 50%. The fentanyl-induced locomotor response of the horse was also reduced. Hematocrit effects were by far the most sensitive response of the horse to acepromazine administration. Penile protrusion, operant behavior, and respiratory effects were approximately similar in sensitivity but required doses greater than 0.004 mg/kg.

Acepromazine, a phenothiazine derivative (Fig. 1), is a widely used tranquilizer in equine medicine. Like most phenothiazines, it blocks a range of central effects including locomotor activity, respiratory response, and control of body temperature[7]. Since horses retain much of their co-ordination and alertness while becoming easier to handle with acepromazine, the drug is frequently used in the transport of valuable or unruly animals. It may be used in competition in small doses to calm an otherwise excitable animal and allow it to perform in a more relaxed manner[8]. It is also used in veterinary medicine as a pre-anaesthetic during surgical procedures, sometimes in conjunction with other agents such as potent analgesics. Because acepromazine is widely used and is prohibited in most racing situations, sensitive and specific methods of detection are needed to test for its presence in performance horses[2]. Further, acepromazine metabolites may persist in urine for long periods[9]. We therefore elected to characterize the pharmacological effects of this drug in horses with particular reference to their duration of action.

In our initial recovery experiments, we were surprised to find that the recovery of acepromazine from equine plasma at pH 9.4 was relatively poor. When we repeated the experiments with human and ovine plasma much the same results were observed (Fig. 2). After a number of possible variables were investigated we were surprised to find that the recovery of acepromazine from plasma was atypical[3]. While recovery from buffer was essentially complete at basic pH, recovery from plasma was optimal at a pH of about 6.0. (Fig 3). This is despite the fact that acepromazine is a basic drug and might be expected to be readily available from plasma at basic pH values.

The availability of ^{3}H-chlorpromazine enabled us to determine directly the rate of movement of chlorpromazine from plasma to an apolar environment at different pH values. As shown in Fig. 4, the rate at which ^{3}H-chlorpromazine moved into DCM was most rapid at pH 6.0, and considerably slower at pH 9.2 and pH 11.0. The data show, however, that if the extraction period is prolonged that essentially complete extraction occurs at pH values of 9.2. Because of the technical convenience of buffering plasma samples to this value, all phenothiazine extractions from plasma in our laboratory are routinely performed at this pH, but with the extraction period prolonged to 1 hour.

Using this extraction method and the GC technique described by Ballard and Tobin (1981), we were able to follow the plasma levels of acepromazine for 8 hours after intravenous administration of 0.3 mg/kg of acepromomazine maleate. As shown in Fig. 5, acepromazine levels at first dropped rapidly after intravenous injection, with an apparent A phase T½ of about 4.2 minutes. Thereafter, the plasma half-life of this drug fell more slowly, with an apparent *B* phase T½ of about 185 minutes. Acepromazine was not detectable in equine plasma for more than 8 hours after dosing.

The most sensitive pharmacological response to acepromazine that we discovered was the effect on acepromazine on hematocrit in horses. As shown in Fig. 6, doses of acepromazine of as little as 0.002 mg/kg produced a significant decrease in the hematocrit of horses, which effect lasted for several hours. These are remarkably small doses of acepromazine to produce pharmacological effects in a horse.

The next most sensitive pharmacological response to acepromazine was penile protrusion[5]. While doses of 0.004 mg/kg had no effect on penile protrusion, this response appeared at doses of about 0.01 mg/kg and was apparently maximal at doses of 0.4 mg/kg. Even at these large doses, however, this fairly sensitive pharmacological response did not last longer than about 10 hours. (Fig. 7).

The respiratory rate of horses is well known to be sensitive to acepromazine and Fig. 8 shows the response observed to increasing doeses of acepromazine. As with the penile response, about 0.04 mg/kg was required for a good effect, and 0.4 mg/kg produced the maximal response observed[6].

The availability of a variable interval responding apparatus in our laboratory enabled us to test the effects of acepromazine on CNS function in our horses. In those trials, acepromazine was adminstered to these horses intravenously 10 minutes prior to each trial. As shown in Fig. 9, horses were relatively resistant to the central effects of acepromazine, about 0.4 mg/kg or the maximal dose tested being required for a significant inhibition in the responding rate of these animals.

Figure 10 shows a family of dose response curves for these responses. Half-maximal inhibition of hematocrit occurred at doses of about 1 mg/horse, while 5 mg/horse was required for penile protrusion and between 5 and 50 mg/horse for effects on variable interval responding and respiration.

In summary, these experiments have shown that acepromazine extracts very slowly from equine plasma, and that under the usual extraction conditions for basic drugs up to 1 hour extraction period is required. After its intravenous administration to horses, it has a plasma half-life of about 3 hours and was no longer detectable by our methodology at 8 hours post-dosing. Among pharmacological responses to acepromazine its depressant effect on the hematocrit was the most sensitive, requiring only about 1mg/horse for 50% inhibition. The next most sensitive response was penile protrusion, requiring about 5mg/horse, follwed by respiratory depression and inhibition of variable interval responding, both of which required about 5 to 50 mg/horse for 50% inhibition.

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FOOTNOTE

Published as Kentucky Agricultural Experiment Station Article No. 81-4-143 with approval of the Dean and Director, College of Agricultute and Kentucky Agricultural Experiment Station.

Publication 74 from the Kentucky Equine Drug Research Program and the Graduate Toxicology Programme, University of Kentucky, Lexington, KY 40546.

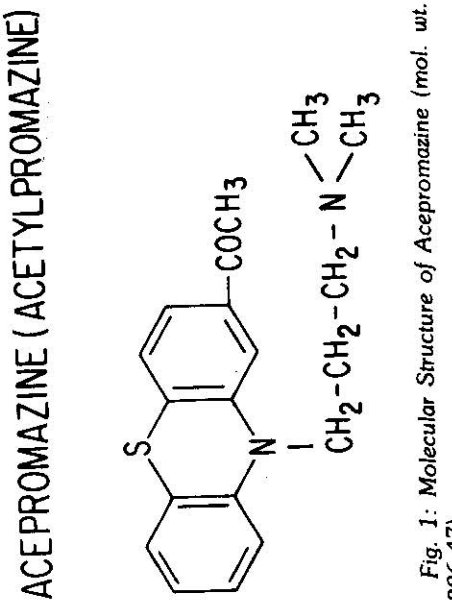

Fig. 1: Molecular Structure of Acepromazine (mol. wt. 326.47).

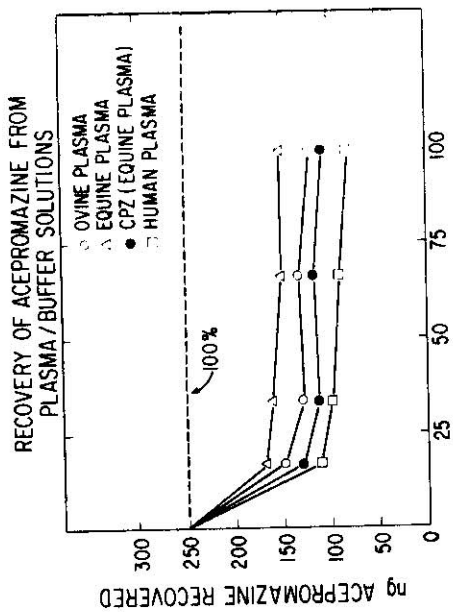

Fig. 2: Partial Recovery of Acepromazine from Plasma.

250 ug of acepromazine were added to 3ml of plasma and sufficient 50 mM phosphate buffer (pH 7.4) added to yield the indicated dilutions of plasma. The samples were then adjusted to pH 9.2 and extracted into dichloromethane. The symbols show recovery of acepromazine from different dilutions of ovine (O), equine (Δ), and human (□) plasmas and also the recovery of chloropromazine from equine plasma (•).

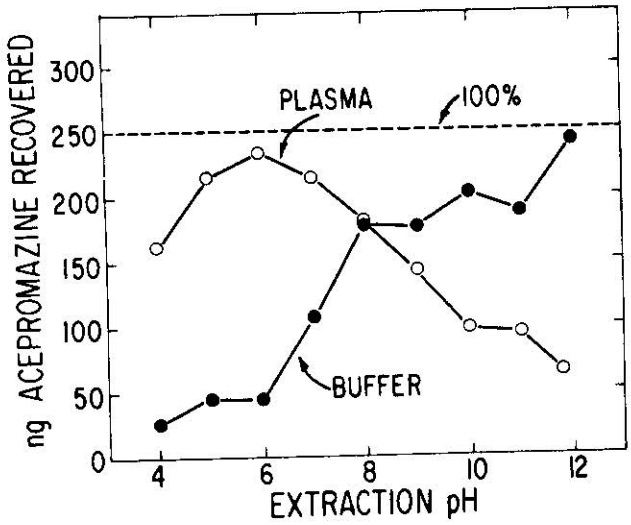

Fig. 3: Recovery of Acepromazine from Spiked Plasma and Buffer Samples at Different pH Values.

250 ug of acepromazine were spiked into 3 ml of equine plasma or water, buffered to the indicated pH, and then extracted into dichloromethane. The open circles (0) show recovery of acepromazine from plasma, while the solid circles (•) show recovery from buffer.

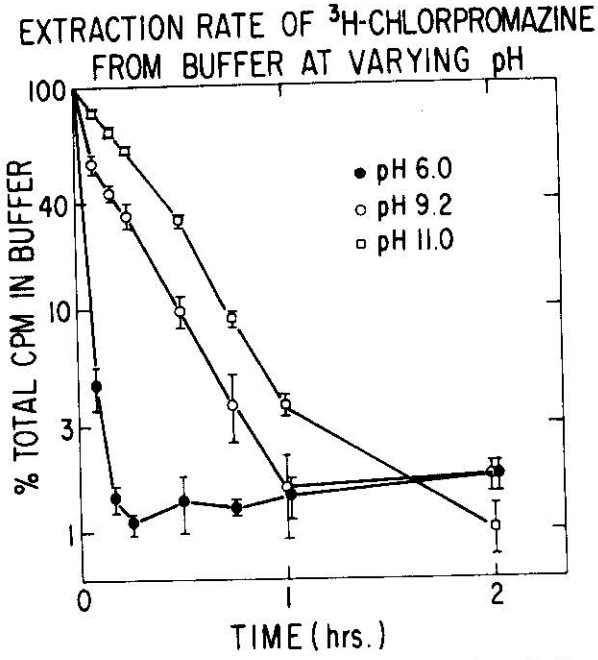

Fig. 4: Extraction Rate of Chlorpromazine from Buffer at Varying pH.

50 ug of ³H-chlorpromazine were added to 5 ml of phosphate buffer at the indicated pH values. The symbols show the time course of disappearance of ³H-chlorpromazine from the buffer solutions at pH 6.0 (solid circles), 9.2 (open circles), and pH 11.0 (open squares).

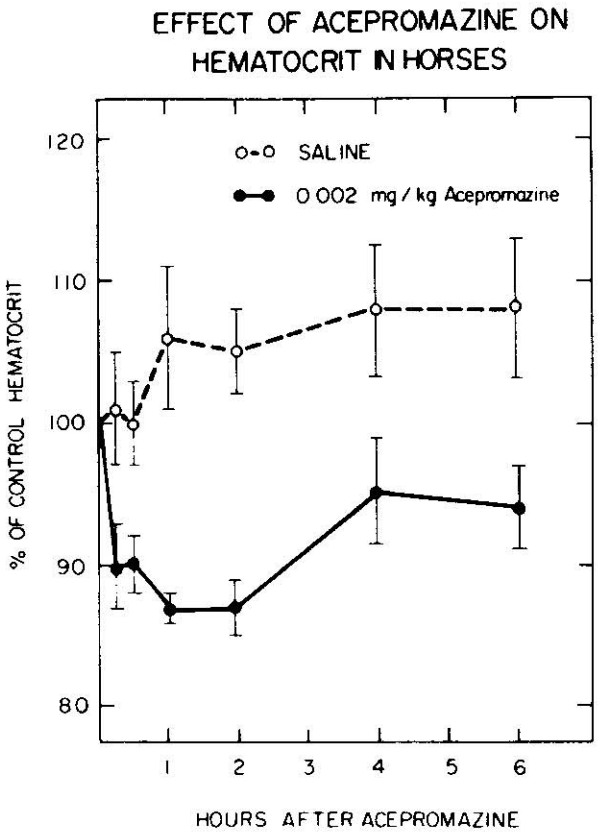

Fig. 6: Effect of Acepromazine on Hematocrit in Horses.

Four horses were dosed intravenously with 0.002 mg/kg of acepromazine in one trial and an equal volume of saline in another trial. The open circles (O) show the hematocrits of saline-treated animals, while the solid circle (•) show the effects of acepromazine. Hematocrits are expressed as a percent of the control (initial) hematocrit values. Zero time hematocrits in these horses averaged about 32.5%. All values are means — the standard errors of the means.

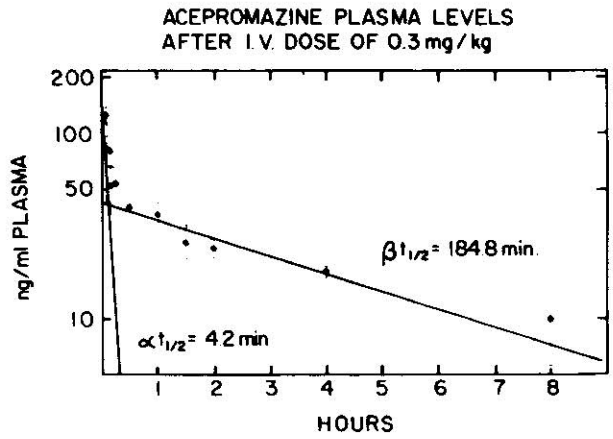

Fig. 5: Plasma Levels After Rapid Intravenous Injection of 0.3 mg/kg Acepromazine Maleate.

The data points (•) show plasma concentrations of acepromazine after rapid I.V. administration of 0.3 mg/kg. The B phase half-life was determined by computer analysis and found to be 184.8 min. The A phase half-life was also determined and found to be 4.17 min. All data points are means — standard errors of the means of determinations on 5 horses.

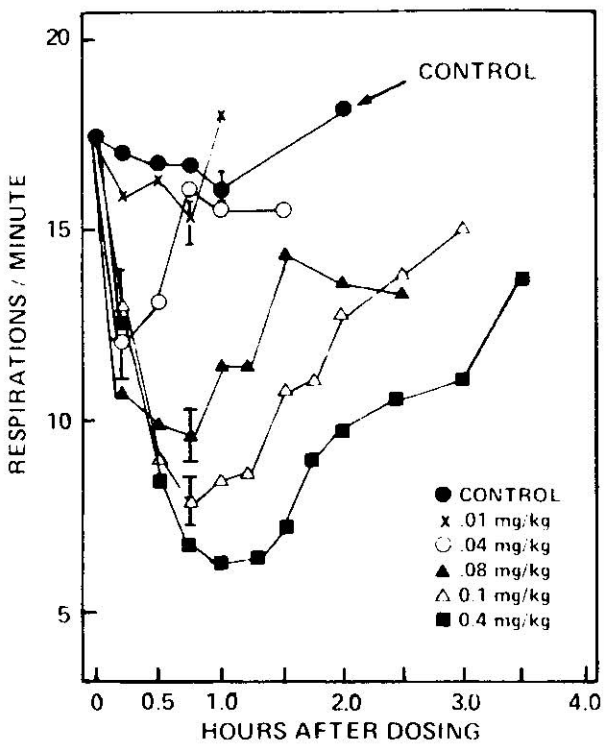

Fig. 8: Effects of Acepromazine I.V. on Respirtory Rate in the Horse.

The closed circles (•) show the respiratory rate in control horses, while other symbols show the respiratory rates observed in these horses after the indicated doses of acepromazine I.V. All data points are the means of determinations on 4 horses and the vertical bars represent standard errors of the means.

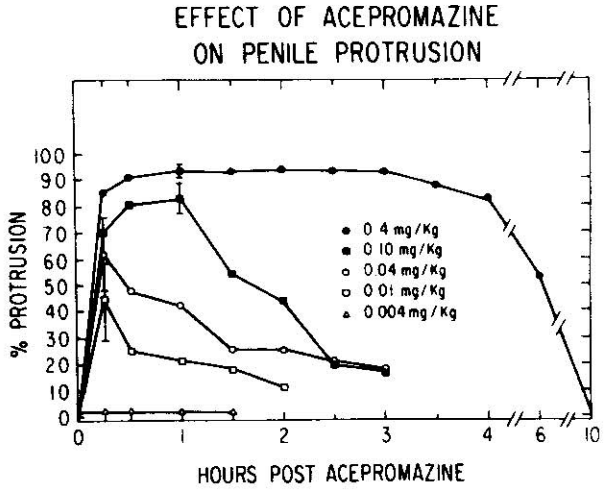

Fig. 7: Effect of Acepromazine on Penile Protrusion in Geldings.

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 dosage of acepromazine and expressed as a percentage of the maximal protrusion seen in each horse.

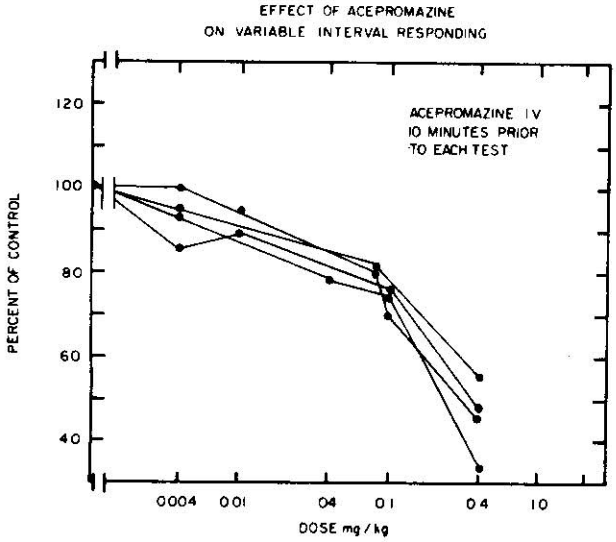

Fig. 9: Effect of Acepromazine on Variable Interval Responding.

Acepromazine was administered intravenously to 4 horses 10 min. prior to each trial. Closed circles (●) represent the response of each horse expressed as percent of saline control performance for the same horse.

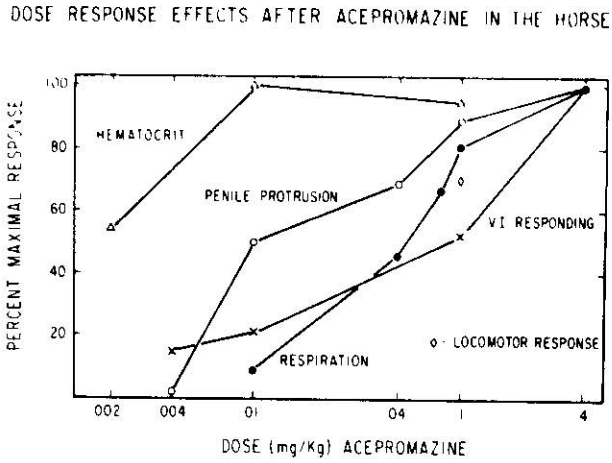

Fig. 10: Dose Response Effects After Acepromazine in the Horse.

The above graph shows the percent maximal observed response of 5 different pharmacological and behavioral parameters as related in intravenous dosage of acepromazine. Hematocrit effects are shown by open triangles (Δ), penile protrusion as open circles (0), respiration as closed circles (●), variable interval responding (X), and locomotor response as open diamonds (◊). Only one dosage level was used for locomotor response.

DISCUSSION

MASON: This is not so much a question to Dr. Tobin but a comment. Your report underlines the relationship between the veterinarian and analyst. On a number of occasions we have noted clinical symptoms of penile protrusion in horses. We have referred this matter to the laboratory on the basis that these were the clinical signs shown, suspecting the use of a tranquilliser. I think you have clarified a number of doubts I had about our laboratory, with apologies to our analyst.

REILLY: You were talking about 150 mg doses. Did you do any administrations of lower levels, say 5 mg. per horse, and were you able to detect the drug at those levels?

TOBIN: We did administrations as low as 1 mg for the pharmacological studies. There were no chemical studies at those levels. We consider ourselves fortunate to be able to detect it in plasma at the higher doses. We are doing some work on metabolites in urine, but that is a much slower proposition.

MELDRUM: Your graph showed penile protrusion at 5 mg per horse. We use this drug quite often for standing castrations and fail to get much response under about 20 mg. For the penis to be fully protruded you must use about 30 mg. What degree of penile protrustion do you get with a 5 mg dose?

TOBIN: It is about 50% at 0.01 mg per kg, a 5 mg per horse dose.

GERBER: Respiratory rates are of doubtful significance if they are above 16 or so at the start of observations. In our studies normally it is 8 to 12. The age of horses has some effect.

TOBIN: From my experience in our horses in Kentucky that are mature mares the rates are around 16. We have dosed horses with cocaine and got very clean dose reponse curves, and, in the case where exponentially the rate of decay of the respiratory rate changes as the dose increases, it always decayed back to about 16 breaths per minute.