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EVALUATION OF THRESHOLD DOSES OF DRUG ACTION IN THE HORSE USING HEMATOCRIT VALUES AS AN INDICATOR

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ABSTRACT

This study was designed to explore the use of hematocrit values as possible indicators of the threshold doses of adrenergic drugs in the performance horse. Acepromazine, detomidine, and fluphenazine were tested for their effects on hematocrit values, with the threshold dose for these effects investigated. Hematocrit values were shown to be quite sensitive to the administration of acepromazine with doses as low as 50 μg/horse producing detectable depressions in hematocrit values for up to 2 hours. Increasing the dose increased the magnitude of the effect, but did not appear to prolong it, while in contrast, reducing the dose to below 25 μg/horse totally eliminated the effect. The alpha-2 agonist detomidine produced a similar depression in hematocrit values, although doses of 10 μg/kg or approximately 5 mg/horse, were needed to produce a

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measurable effect. The anti-psychotic fluphenazine, which is believed to be an illegally administered drug in race horses, had no significant effect on hematocrit values when comparable doses were administered.

In addition, the results of monitoring the hematocrit values of six horses for 48 hours suggested that the variations seen may be partially related to circadian factors, with peak values occurring in the afternoon hours.

INTRODUCTION

The recent development of more sensitive and specific analytical tests for drugs in racing horses has given racing chemists the ability to detect small amounts of drugs for longer periods of time after their administration (Kwiatkowski, Sturma, Dai, *et al.* 1988; Tobin, 1989). While this sensitivity has greatly improved testing for illegal drugs in racing horses, it has given rise to problems with the detection of drug residues that were administered therapeutically and within the rules of racing. Inadvertent detection of legitimate therapeutic medications given days before post time creates regulatory problems for veterinarians, trainers and racing officials.

One approach to this problem is to identify threshold doses of drugs that are devoid of pharmacological effects, and thus, their corresponding blood or urine levels can be considered to be without forensic significance. With this approach, the most sensitive pharmacological response to a drug is identified and the threshold dose to produce this effect identified. This dose can then be correlated with blood concentrations that are unable to produce a pharmacological effect, and appropriate blood threshold levels for the drug in question can be established.

Earlier work in this laboratory with acepromazine showed that equine hematocrit values were particularly sensitive to the effects of this drug (Ballard, Shultz, Kowanacki, *et al.,* 1982). In studying the effects of acepromazine on several responses in the horse, Ballard and his co-workers found that hematocrit values were the most sensitive measurable parameter in response to acepromazine administration. In their study, the

hematocrit of a resting horse was found to normally lie in the range of 36 to 42%, with administration of about 1 mg per horse of acepromazine reducing these levels by approximately 10%. Other responses measured following acepromazine administration included respiratory rates, operant conditioned behavioral rates, penile protrusion in male horses, and the ability to block the locomotor response of fentanyl administration. All of these responses, however, required doses of acepromazine well above that needed to produce an effect on hematocrit values.

Due to the frequent use of acepromazine in equine medicine and surgery, and its use by horsemen in handling, training and shipping horses, we elected to repeat the hematocrit/acepromazine studies. In addition, we further evaluated the potential use of hematocrit values as an indicator of the threshold dose for effect of drugs in the performance horse.

MATERIALS AND METHODS

Horses

A total of ten Thoroughbred or Standardbred mares routinely maintained at pasture were used in all drug administration studies. The mares were placed in box stalls approximately 24 hours prior to testing and given grass hay and water as free choice. Additionally, the horses were given about 250 gm of clean dry oats at 8:00 AM each morning.

Drugs and Sampling

The drugs used were acepromazine (PromAce, Aveco Co. Inc., Fort Dodge, IA), fluphenazine (Prolixin, E.R. Squib and Sons, Inc., Princeton, NJ) and detomidine (Domosedan, Farmos Group Ltd., Turko, Finland). All pharmacological agents were administered by rapid IV bolus into the jugular vein from the right side of the horse. Blood samples were drawn by venipuncture from the left side and analyzed for hematocrit values in the laboratory located in the Veterinary Science research barn. Hematocrit readings were obtained by drawing each whole blood sample into three

heparinized capillary tubes (32mm × 0.6mm, Clay Adams Co., Parsippany, NJ). The samples were then centrifuged in an IEC micro hematocrit MB centrifuge for 3 minutes and the levels determined with an IEC circular micro-capillary tube reader (International Equipment Co., Needham Hts., MA). The three readings were then averaged to determine the reading for that time point.

Experimental Procedures

Drug (acepromazine, detomidine or fluphenazine) administration studies were conducted using within subjects designs. The treatments were counter balanced using a Latin Square cross-over with saline (2 ml/horse) used as the control treatment. Baseline levels were determined by sampling each horse at -30 and -5 minutes prior to treatment. Baseline circadian levels were obtained by placing six horses in box stalls for a 24 hour acclimation period, with hematocrit values were then determined every 4 hours for a period of 48 hours. The baseline data were analyzed for significance by ANOVA, while statistical significance between means in the drug administration studies were evaluated with the use of repeated t-tests with Bonferronie control of alpha.

RESULTS AND DISCUSSION

The variations seen in equine hematocrit values over a 48 hour period are illustrated in Figure 1. After the horses were allowed to acclimate in box stalls for 24 hours, hematocrit values for each horse were determined every 4 hours for a period of 48 hours.

The control values obtained are consistent with other reported values of equine hematocrit levels in the 36 to 42% range (Allen, Powell, Singleton, 1982; Ballard, Shultz, Kowanacki, *et al.*, 1982; Blackman, 1982; Mason and Kwok, 1977). In a seven year study conducted by Blackman and his colleagues factors contributing to the variances seen in equine hematocrit were analyzed (Blackman, 1982). They determined that individual genetic variation was responsible for 35% of the variation, while time of year was responsible for 12%, and unexplained factors contributed 29%. Additionally,

their analysis showed that location, age and sex had little or no effect on hematocrit values.

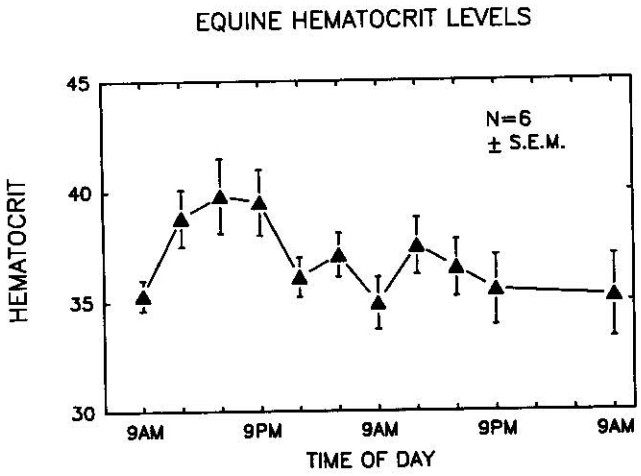

Figure 1. 48 hour profile of equine hematocrit values determined in six horses. Each point represents the mean hematocrit value readings for the group ± the standard error of that mean. Statistical analysis indicated that time of day was not a significant factor.

The data in Figure 1 suggests that there may be circadian factors as well. The hematocrit values were lowest in the morning hours and tended to climb in the afternoon, although statistical analysis by ANOVA does not indicate significance. The variations seen on the second day were much less marked which suggest that acclimation of the horses decreased the variations. It should be noted that these experiments were conducted in mid July in central Kentucky with ambient temperatures peaking at approximately 85 degrees Fahrenheit during the late afternoon and falling to about 60 degrees at night. While the stalls were equipped with ventilation fans and the horses provided with clean water free choice, slight dehydration in the afternoon may have occurred and could have thus contributed to the increased hematocrit levels.

While hematocrit values are apparently a sensitive indicator of adrenergic drug actions, it is a difficult parameter to work with as the pharmacological effect cannot be described as robust. Horses are quite sensitive to changes in their surroundings with their degree of hydration likely to be a factor in baseline hematocrit levels (Hinton, 1978). Nevertheless, carefully controlled studies can be conducted to determine threshold doses of adrenergic drugs and statistically significant results obtained in drug administration studies.

The effects of IV administration of acepromazine on equine hematocrit levels at doses of 0.01, 0.05, 0.1, 1.0 and 10 μg/kg body weight are presented in Figure 2. The 0.1, 1.0 and 10 μg/kg doses produced statistically significant ($p<.05$) decreases at the 0.5 and 1 hour time points post administration, with the levels returning to baseline values at 2 and 3 hours. Figure 2 additionally shows that the 0.01 and 0.05 μg/kg, as well as the saline control dose, did not produce any significant effect on the hematocrit levels. The threshold dose for pharmacological effect of acepromazine on hematocrit values in the horse is plotted in a dose-response format in Figure 3, and shows the threshold dose to be approximately 0.1 μg/kg or about 50 μg/horse.

These data are in good agreement with previously discussed studies on acepromazine and equine hematocrit, with the effects of acepromazine on hematocrit values being the most sensitive measurable parameter of acepromazine administration. For example, operant conditioning is an extremely sensitive indicator of subtle behavioral effects of drug administration (Wood, Stanley and Tobin, 1989). However, doses of acepromazine of 100 μg/kg needed to be reached before significant changes in rate of behavioral could be seen (Tobin, 1989). More recently, with the development of very sensitive immunoassays for acepromazine allowing the detection of an apparent subclinical dose of 10 μg/kg for brief periods of time, the extreme sensitivity of the hematocrit of the horse to acepromazine administration is further illustrated (Kwiatkowski, Sturma, Dai, *et al.*, 1988).

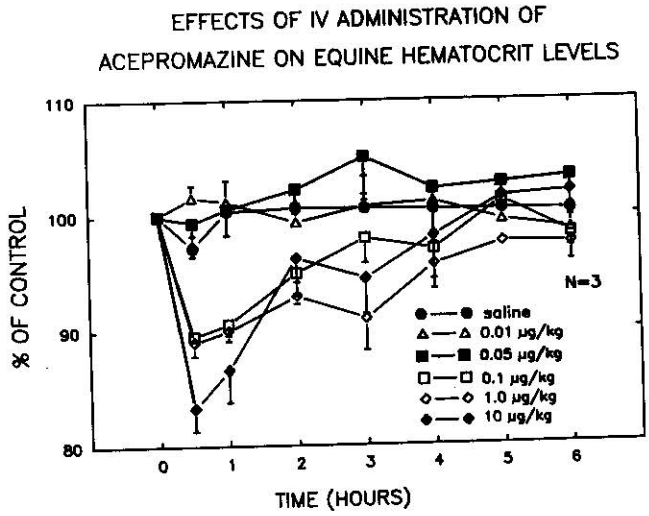

Figure 2. Effects of IV administration of acepromazine on equine hematocrit levels. Treatments consisted of saline (2 ml) or acepromazine at the following dose levels; 0.01, 0.05, 0.1, 1.0 and 10 µg/kg. The points represent the means ± the standard error. The 0.5 and 1 hour time points for the 0.1, 1.0, and 10 µg/kg doses are significantly ($p<.05$) different from the corresponding saline time points.

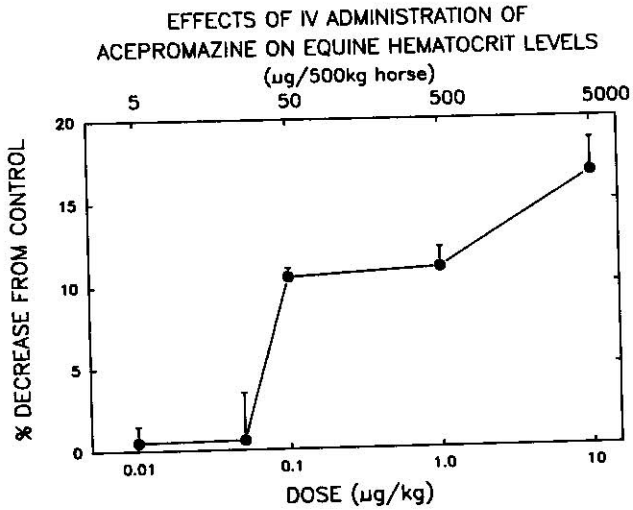

Figure 3. The acepromazine/hematocrit data plotted as a dose-response curve illustrating that the threshold dose for effect is 0.1 µg/kg or approximately 50 µg/horse.

In addition to acepromazine, we tested the effects of the adrenergic alpha-2 agonist detomidine. Detomidine is a newly developed sedative agent that causes sedation by pre-synaptically inhibiting the release of epinephrine and norepinephrine (Clark and Taylor, 1986; Ruskoaho, 1986). When we tested the effects of detomidine on equine hematocrit levels we encountered a sensitive and dose-dependent effect comparable to that of acepromazine, as presented in Figure 4. While detomidine is considered to be a more potent sedative than acepromazine, it was less effective in its effects on hematocrit levels. A dose of 10 μg/kg was needed to produce significant decreases in hematocrit. Detomidine is thought to exert its peripheral pharmacological effects by reducing the tone of the sympathetic outflow from the spinal cord (Ruskoaho, 1986). The results of our hematocrit studies would appear to be consistent with the proposal that the predominantly CNS active detomidine has limited direct peripheral effects.

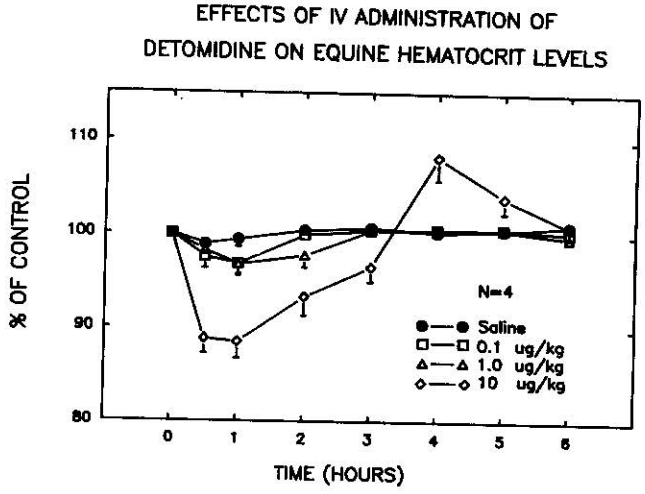

Figure 4. Effects of IV administration of detomidine on equine hematocrit. The treatments consisted of saline (2 ml) or detomidine at the following dose levels; 0.1, 1.0, or 10 μg/kg. The points represent the means of the group ± the standard error. The 0.5 and 1 hour time points for the 10 μg/kg dose are significantly ($p<.05$) lower than the corresponding saline time points.

Fluphenazine is an anti-psychotic drug used in human psychotherapy with a recommended dose in human medicine in the order of 1 or 2 μg/kg (Gilman, Goodman, Rall, *et al.*, 1985). It is suspected of being illegally administered to race horses for its potentially subtle sedative properties and was thus included in this study. The results of our study on the effects of fluphenazine administration on the hematocrit values in horses are presented in Figure 5. The data show that IV administration of fluphenazine had no significant effect on equine hematocrit values at any of the dose levels tested. These data are consistent with the belief that fluphenazine has primarily dopaminergic and seratonergic blocking properties. Most physiologists agree that hematocrit values are predominantly controlled by adrenergic receptors located on the spleen, and it follows that one would not expect a dopaminergic agent to exert a significant influence on hematocrit values.

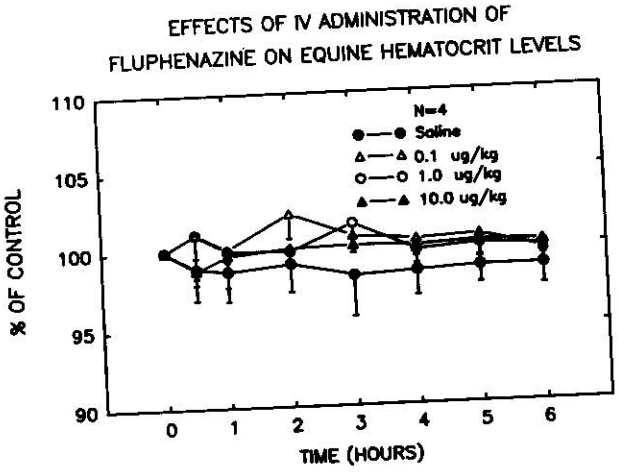

Figure 5. Effects of IV administration of fluphenazine on equine hematocrit levels. The treatments consisted of saline (2 ml) or fluphenazine at the following dose levels; 0.01, 0.1, 1.0, or 10 μg/kg. The points represent the means of the group ± the standard error. The change from control levels for all doses are not significantly ($p>.05$) different from the saline time points.

In summary, our analysis of equine hematocrit values over a 48 hour time period revealed considerable fluctuations in baselines values, with circadian or environmental factors possibly playing a role in these variations. Despite these variations, however, we believe that our data indicate that equine hematocrit values are quite useful and sensitive in evaluating the administration of adrenergic drugs.

REFERENCES

Allen, B., Powell, D., and Singleton, W. (1982) Value and limitations of haematology in viral infections in horses. Vet. Rec. (110) 348.

Ballard, S., Shultz, T., Kowanacki, A., Blake, J.W. and Tobin, T. (1982) The pharmacokinetics, pharmacological responses and behavioral effects of acepromazine in the horse. J. Vet. Pharmacol. Therap. (5) 21-31.

Blackman, D.J. (1982) The biochemistry and haematology of inherent performance. In Equine Exercise Physiology. Snow, Persson and Rose (eds.) Granta Editions, Cambridge. 344-353.

Clark, K.W. and Taylor, P.M. (1986) Detomidine: a new sedative for horses. Equine Vet. J. 18: 366-370.

Gilman, A.J., Goodman, L.S., Rall, T. and Murad, F. (1985) The Pharmacological Basis of Therapeutics. MacMillan Publisher Co.

Hinton, M. (1978) On the watering of horses. A review. Equine Vet. J. (10) 27-31.

Kwiatkowski, S., Sturma, L., Dai, M-R., Tai, H-H., Watt, D., Tai, C-L., Woods, W.E., Weckman, T., Yang, J-M., Wood, T., Chang, S-L., Blake, J.W., Tobin, T., Prange, C., Brockus, D., Stobert, D., Wie, S., Chung, R., McDonald, J., Bass, V., Merchant, S., Artemenko, M. and DeLeon, B. (1988) Immunoassay detection of drugs in racing horses. VII. Detection of acepromazine in equine urine and blood by ELISA and PCFIA. Res. Comm. Chem. Path. Pharmacol. (61) 391-412.

Mason, D. and Kwok, H. (1977) Some haematological and biochemical parameters in race horses in Hong Kong. Equine Vet. J. (9) 96-99.

Ruskoaho, S.W. (1986) Subtypes and functions of alpha-adrenoceptors. Acta. Vet. Scand. (82) 17-28.

Shults, T., Combie, J., Dougherty, J., and Tobin, T. (1982) Variable interval conditioning in the horse: A sensitive measure of behavior. Am. J. Vet. Res. (43) 1143-1146.

Tobin, T. (1989) The effects of drugs on equine performance and the use of ELISA tests in equine medication control. J. Equine Vet. Science. (3) 160-168.

Wood, T., Stanley, S. and Tobin, T. (1989) Operant conditioning and its applications in equine pharmacology. J. Equine Vet. Science. (3) 124-130.