

Please

The Pharmacology of Narcotic Analgesics in the Horse III. Characteristics of the Locomotor Effects of Fentanyl and Apomorphine

T. Tobin, D.V.M., Ph.D.
J. Combie, B.S.
T. Shults, B.Sc.
J. Dougherty, Ph.D.

From the Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture, University of Kentucky; The Veteran's Affairs Medical Hospital, Lexington, KY (Dougherty); the Graduate Toxicology Program, University of Kentucky, Lexington, KY 40506.

Publication No. 38 from the Kentucky Equine Drug Research and Testing Programs, Department of Veterinary Science, University of Kentucky. Published as Kentucky Agricultural Experiment Station Article No. 78-4-143 with the permission of the Dean and Director, College of Agriculture.

The assistance of Edith Nuss and Barbara Schechter, who assisted with the counting procedure, is gratefully acknowledged. Supported by grants from the Kentucky Equine Research Fund, the Patricia Hewitt Foundation and a U.K.R.F. Graduate Fellowship.

A method for quantitating the locomotor response to stimulant drugs in the horse is presented. In this method a horse is isolated in a partially enclosed box stall and its left leg wrapped in white tape. It is then scored for the number of times it lifts this leg and completes a step. Saline-injected horses complete about four of these events per two-minute period. Horses injected intravenously (IV) with fentanyl or apomorphine show a marked increase in this behavior, peaking at about 100 steps/2 minutes for fentanyl and at about 150 steps/2 minutes for apomorphine. The event score is ambiguous, since the percentage of agreement between two different observers was 95.2%. The responses are dose-related, and for these drugs decline to control levels within 60 minutes. The results show that fentanyl and apomorphine are both potent locomotor stimulants in the horse with rapid onset of action after intravenous administration. The response to fentanyl after subcutaneous administration was erratic. When given a rapid IV injection, fentanyl is a reliable and rapidly acting motor stimulant.

Introduction

Although the narcotic analgesics have long been used in horses, there are few descriptions of their actions in horses. A recently synthesized, highly potent narcotic analgesic called fentanyl is reportedly widely used in racing horses in the United States,² but little data as to its effects on the horse exists. Standard textbooks of veterinary pharmacology have little information to offer on the action of narcotic analgesics per se in the horse,^{1,3} and one recent text even classifies and discusses pentazocine among the non-narcotic analgesics.⁵

In the human, fentanyl is a potent narcotic analgesic with a rapid onset and short duration of action. It has a profile of pharmacological activity similar to that of morphine except that it does not cause emesis and histamine release. It is considered to be about 150 times more potent than morphine.^{2,6} After IV injection in man, peak analgesia appears after about three to five minutes and lasts for 60 minutes.⁶ Fentanyl is a Schedule II controlled substance in the United States.

Leave

During the course of our work with fentanyl, it became clear that fentanyl is a potent behavioral stimulant in the horse. Fentanyl acts to induce a very clear-cut and specific locomotor response in the horse, and this activity lends itself to quantitation simply by counting the number of steps the animal takes in a two-minute period. In this paper we outline this method and show that it can be used to characterize the locomotor responses to fentanyl and apomorphine. Further work, to be presented later, shows that this method can be applied to other narcotic and central stimulant drugs.

Materials and Methods

Mature Thoroughbred or Standardbred horses of between four and 18 years of age and 400 to 550 kg body weight were used throughout. The animals were kept at pasture and brought into the specially shielded box stalls at least 24 hours prior to each experiment. The box stalls measured 13¼ feet square and had straw-covered earthen floors. The bulk of the work reported here was performed in these stalls, with plywood sheeting on the box grillwork to reduce interaction with horses in other stalls. A small glass window in the plywood shielding of about 12" × 12" was used for observation. In addition, a single brick box stall, well shielded from outside noise and equipped with a metal door and one-way glass window, was available to us. Data collected in this brick box stall was noticeably less variable than that collected in the modified barn box stalls. Prior to an experiment, all food and water buckets were removed from the stall.

During the experimental period the horse was scored for one event if he lifted his left leg and took a step. Movements of the left leg not resulting in relocation of the foot, such as scratching or pawing, were not counted. To assist the observer in tracking these events the left leg of the horse was wrapped in white tape. Each event was tallied on a hand counter^a and the cumulative score logged every two minutes.^b

The reliability and reproducibility of the observational measurement technique was verified by the use of two independent observers in one of the experiments concerning fentanyl (Figure 1). Percentage agreement between two observers was determined for each two-minute period by dividing the smaller of the event totals by the larger event total. These percentages were averaged across 35 two-minute periods and gave an overall percentage agreement of 95.17%. The appropriateness of this procedure was verified by calculating the correlation between the two observers' scores, which yielded a 0.998 correlation coefficient.¹

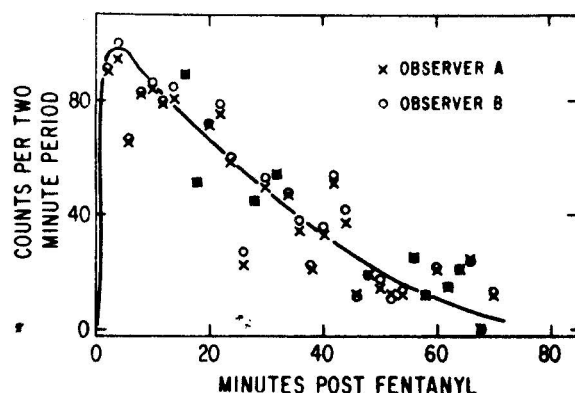


Figure 1. Locomotor response to fentanyl, IV, scored by two independent observers. A horse was injected with 0.02 mg/kg of fentanyl IV and scored by two independent observers as outlined in "Methods." The crosses (x) show counts as determined by Observer A, and the open circles (O) show counts by Observer B.

The fentanyl citrate was supplied by McNeil Laboratories, Fort Washington, PA. The apomorphine hydrochloride was from Eli Lilly and Company, Indianapolis, IN, in which 6 mg tablets were dissolved in sterile distilled water immediately before use.

Results

Early experiments on the administration of fentanyl to horses utilized doses of about 1.0 mg/1000 lbs, which are reportedly used in racing horses.^c With these doses no consistent pharmacological effects were observed or reported by trainers after double-blind administration experiments to Standardbred pacers in training. However, when the dose of fentanyl administered was raised about five-fold to facilitate detection of the drug in kinetic experiments in our laboratory, marked signs of locomotor stimulation were observed. This led to the development of the procedure previously outlined for quantitating this effect.

Figure 1 shows this method is highly reproducible from observer to observer. In this experiment, a horse was dosed with 0.02 mg/kg fentanyl IV and scored by two independent observers. Both scorers frequently assigned the same score for each two-minute period with the mean percent agreement being 92.5%. Correlation coefficient between observers was 0.998.

This scoring method allows accurate characterization of the response of individual horses. Figure 2 shows typical results which were obtained when a single horse was dosed with normal saline and increasing doses of fentanyl. On saline, the spontaneous locomotor activity of this horse was usually about 4 steps/2-minute period except for immediately after the IV injection, and this pat-

^a Veeder-Root, Inc., Hartford, CT.

^b Electronic Stop Watch, Cronus Precision Products, Santa Clara, CA.

^c Personal communications, to T. Tobin and T. Shults.

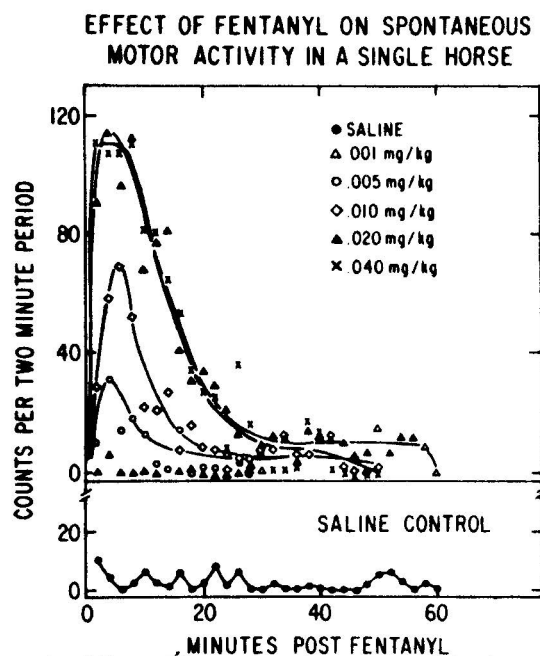


Figure 2. Effect of fentanyl on locomotor activity in a single horse. A horse was injected with saline or increasing doses of fentanyl IV. The number of times the horse lifted its left front foot and placed it in a different position in a two-minute period was recorded as an indication of locomotor activity. Activity following IV saline injection is shown by solid circles (●-●); 0.001 mg/kg fentanyl by open triangles (Δ); 0.005 mg/kg by open circles (○-○); 0.010 mg/kg fentanyl by open diamonds (◇-◇); 0.020 mg/kg fentanyl by solid triangles (▲-▲); and 0.040 mg/kg fentanyl by crosses (x-x).

tern was consistent over the one-hour control period. The lowest dose of fentanyl, 0.001 mg/kg or approximately 0.5 mg to a 1000 lb horse, produced no observable behavioral effects. A five-fold increase in dose produced a small (30 steps/2-minute period) increase in locomotor activity which peaked at four minutes and then declined toward the control level. Similarly, increasing doses produced further increases in both the rate and duration of the response, the effect peaking at about 110 steps/2-minute period and then decaying away. In this particular horse, no difference in response between the two highest doses tested was seen.

Figure 3 shows the mean response of four horses to the same doses of fentanyl. As previously, the pooled points are well fitted by a single line and show very smooth and reproducible dose and time response effects. Again, no effect was seen at the lowest dose tested, and at all doses up to 0.02 mg/kg the locomotor response had peaked between two and four minutes postdosing. At the highest dose tested (0.04 mg/kg) all the horses in this group became incoordinated and either staggered or fell during the first six minutes. The time of peak response was thus delayed by the incoordination produced at higher doses, and the rate of locomotion was no higher than that observed at 0.02 mg/kg.

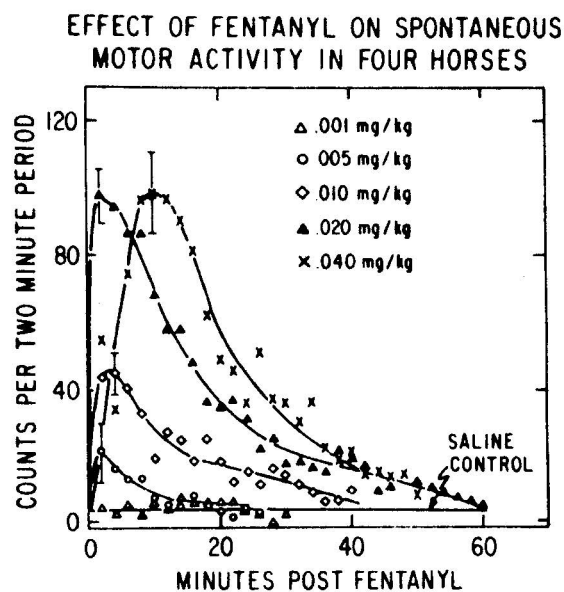


Figure 3. Effect of fentanyl on locomotor activity in four horses. Horses were injected with saline or increasing doses of fentanyl IV. The average counts per two-minute period following saline injection are shown by the straight line near the bottom of the graph. The response to 0.001 mg/kg fentanyl is shown by the open triangles (Δ-Δ); 0.005 mg/kg fentanyl by open circles (○-○); 0.010 mg/kg fentanyl by open diamonds (◇-◇); 0.020 mg/kg, solid triangles (▲-▲); and 0.040 mg/kg fentanyl by crosses (x-x). At the highest dose tested all horses showed a loss of coordination resulting in a decrease in locomotion during the first six minutes. All points are the means of counts determined on four horses and the vertical bars represent SEM's.

Figure 4 shows the effect of apomorphine administered intravenously on spontaneous locomotor activity in three horses. A dose of 6 mg produced no significant alteration from control, while 18 mg produced a small (four-fold) increase in locomotion. Increasing the dose to 30 mg produced a very sharp increase in the response to apomorphine, with the peak rate of about 150 steps/2-minute period. This response shows that these horses in their stalls are capable of a peak locomotor response considerably greater than the peak observed with fentanyl, suggesting that the ceiling to this response observed with fentanyl is an inherent property of the drug and not a limitation of the experimental method.

Figure 5 shows the response obtained when horses were dosed subcutaneously with fentanyl. Two horses showed behavioral responses which were essentially baseline responses, only one of which is plotted for the sake of clarity. Three horses showed clear behavioral responses. Of these responses, two were erratic, while one was a relatively smooth response. All of these responses peaked between 20 and 30 minutes after dosing, as compared with four minutes after IV administration. It appears that after subcutaneous administration, the behavioral response to fentanyl is delayed, erratic and much less intense than after its IV administration.

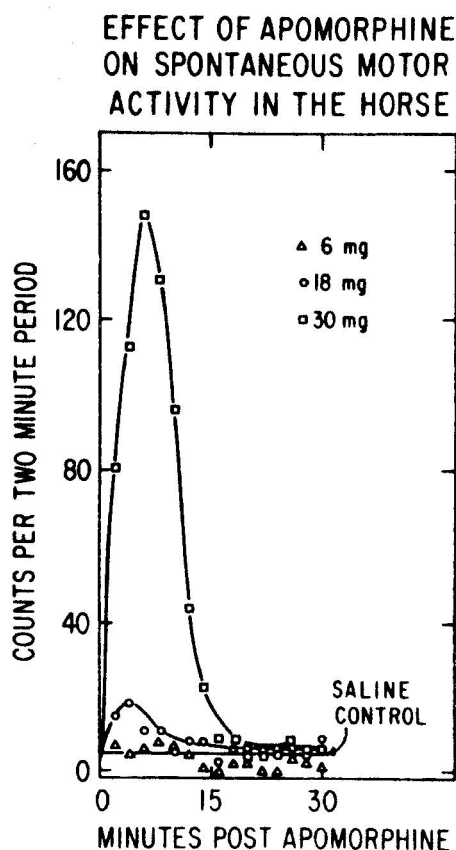


Figure 4. Effect of apomorphine on locomotor activity in the horse. Three horses were injected with saline or increasing doses of apomorphine. The average counts per two-minute period following saline injection are shown by the straight line at the bottom of the graph. The response to 6 mg apomorphine is shown by the open triangles (Δ); 18 mg apomorphine by open circles (\circ); 30 mg apomorphine by open squares (\square). All points are the means of counts determined on three horses.

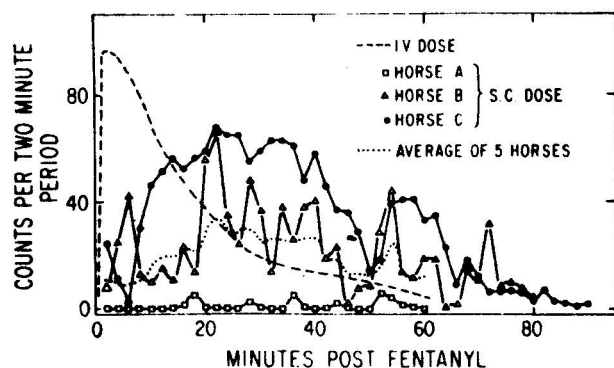


Figure 5. Locomotor response to subcutaneous dosing with fentanyl. Horses were dosed subcutaneously with 0.020 mg/kg fentanyl. Three representative responses are shown by the open squares (\square), open triangles (Δ) and solid circles (\bullet). The dotted line (.....) is the mean response of five horses. The dashed line (---) is the mean response to the same dose administered IV, replotted from Figure 3.

Discussion

The results presented here show that the simple step-counting procedure described in "Methods" allowed accurate and reproducible quantitation of the stimulant effects of a narcotic drug in the horse. The equipment required for this method was minimal, consisting of a shielded stall, a stopwatch, event counter, and some white tape. The event counted is unambiguous and the consistency of counting between observers is excellent (Figure 1). In order to insure that behavioral measurement procedures involving human observers are reliable and replicable, the determination of agreement between independent observers is essential. In addition, a detailed description of the rating and scoring procedure is also necessary.⁴ Using this method, the motor responses to both fentanyl and apomorphine in the horse were characterized. Both of these drugs produced a sharp increase in motor activity which peaked within minutes and then declined. The intensity and duration of the response was dose-related, and peak responses of up to 40 times baseline levels were observed. For fentanyl, this response was limited by the action of this drug at high doses to cause incoordination. The response to high doses of apomorphine, which did not cause incoordination, was greater than that of fentanyl, showing that the fentanyl response was limited by factors associated with the action of the drug on the horse and not by any limitation of the measuring method. The data, in general, show little scatter (Figure 2), and pooled data (Figure 3) form a very useful representation of the pharmacological action of these drugs. With minor changes in handling and presentation of data, this method is being used to characterize the locomotor responses to a representative cross section of narcotic drugs and central stimulants in the horse.

This method makes quite clear the fact that fentanyl is a potent locomotor stimulant drug in the horse. Our information from supposedly knowledgeable track sources on this subject was contradictory, and the "California Rules of Racing" has listed fentanyl as a depressant drug.⁷ In contrast with these reports, fentanyl in our hands was a very potent and, if given IV, reliable and effective stimulant of certain behavior. The spontaneous activity of our horses at the lowest dose tested (0.001 mg/kg) was not significantly different from that observed after saline injection, so any depressant effect of fentanyl must occur at doses lower than this. Increasing the dose to 0.005 mg/kg showed clear-cut behavioral stimulation which was seen at all doses tested up to 0.04 mg/kg. At 0.04 mg/kg these horses were initially incoordinated and fell, but the incoordination was clearly associated with signs of central nervous system (CNS) stimulation.

The time course of the pharmacological action of fentanyl after its rapid IV injection, combined with its high lipid solubility, suggests that the onset and offset of the actions of fentanyl after its IV administration are primarily due to a "bolus" effect. Thus, when injected rapidly IV the delay in onset of drug action is small (< 1 minute). The peak of drug effect occurs rapidly, within two to four minutes, and the drug effect is over within 60 minutes. This time course of action suggests that rapid entry of fentanyl into the brain from a high concentration "bolus" of drug in the blood is responsible for the initial sharp peak of drug effect, and that the pharmacological action of fentanyl is terminated primarily by redistribution of the drug from the CNS and not necessarily by metabolism. These suggestions are supported by observations of Figure 5, which show that the pharmacological response to fentanyl after its subcutaneous administration is erratic and slower in onset, with peak drug effect taking between 20 and 30 minutes to develop. It appears likely, therefore, that the smooth, sharp, and highly reproducible characteristics of the pharmacological response to IV fentanyl are primarily determined by swift administration of the IV dose and the characteristics of cerebral blood flow.

These interpretations are well supported by studies on the pharmacokinetics of fentanyl in man and rabbits.^{3,9} In these studies the pharmacokinetics of fentanyl were best described by a three-compartment model. In this system, the first compartment was assumed to be blood, in which fentanyl concentrations fell to very low levels within about five minutes. The second compartment was thought to be well perfused lipophilic tissues (such as brain), where fentanyl is taken up in high concentrations between about five and 20 minutes. This time course corresponds approximately with the behavioral effects observed in Figures 1 through 3, suggesting that a similar mechanism operates in the horse. Finally, the third phase

lasts from about 20 minutes on. This phase consists of redistribution to poorly perfused tissues and transformation and elimination of the drug, and is not associated with any clear-cut behavioral effects.

The fact that the pharmacological actions of fentanyl after its IV administration are determined by redistribution makes fentanyl a highly suitable drug for behavioral experiments in the horse. It means that repeated doses of fentanyl can be given over a short period, with minimal danger of error due to drug cumulation. Experiments utilizing this principle are in progress. Because the locomotor response is short, the behavioral question posed by challenge with fentanyl is rapidly answered, which makes fentanyl a useful tool to probe behavior and drug effects in the horse.

The relationship between the behavioral responses reported in this paper and the analgesic effects due to fentanyl are not clear, but is likely to be close. Because this behavioral response to fentanyl is blocked by naloxone (experiments in progress), its expression requires occupancy of narcotic receptors in the CNS.¹⁰ Because fentanyl is a highly lipid soluble drug,^{8,9} it is likely to have access to all narcotic receptors in the CNS. It is therefore not clear from these experiments whether or not the behavioral effects are due to effects on opiate receptors, which specifically produce motor responses, rather than analgesia. Nevertheless, it is encouraging to note that in the human, peak analgesia after fentanyl occurs within three to five minutes and lasts for about 30 to 60 minutes.⁸ The correlation between this time course and that observed in Figures 1 through 3 is striking. It appears possible, therefore, that the dose response and time course of the analgesic response due to fentanyl in the horse may be very similar to that of the motor response measured in this report.

References

1. Brander, G.C. and Pugh, D.M.: *Veterinary Applied Pharmacology and Therapeutics*. 3rd ed., Lea & Febiger, Philadelphia, (1977).
2. Goodman, S. and Gilman, A.: *The Pharmacological Basis of Therapeutics*. 5th ed., MacMillan Publishing Co., New York, (1975).
3. Hess, R., Stiebler, G., and Herz, A.: Pharmacokinetics of Fentanyl in Man and the Rabbit. *Eur J Clin Pharm*, **4**, (1972): 137-141.
4. Johnson, S.M. and Bolstad, O.D.: Methodological Issues in Naturalistic Observation: Some Problems and Solutions for Field Research. In *Behavior Change: Methodology, Concepts and Practice*. L.A. Hamerlynck, L.C. Handy, and E.J. Mash, Eds. Research Press, Champaign, IL (1973): 7-67.
5. Jones, L.M., Booth, N.H., and McDonald, L.E.: *Veterinary Pharmacology and Therapeutics*. 4th ed., The Iowa State University Press, (1977).
6. Longrigg, R.: *The History of Horse Racing*. Stein & Day, New York (1972).
7. *Medication Procedures and Related Instructions*. State of California Horse Racing Board, (March 1977): 1-4.
8. *Remington's Pharmaceutical Sciences*. 15th ed. J.E. Hoover, Ed., Mack Publishing Co., Easton, PA (1975).
9. Schleimer, R., Benjamini, E., Eisele, J., and Henderson, G.: Pharmacokinetics of Fentanyl as Determined by Radioimmunoassay. *Clin Pharm Ther*, **23**, (1978): 188-194.
10. Tobin, T.: Pharmacology Review: Narcotic Analgesics and the Opiate Receptor in the Horse. *J Eq Med Surg*, **9**, (1978): 397-399.