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THE PHARMACOKINETICS AND BEHAVIORAL EFFECTS OF FENTANYL AND OTHER NARCOTIC ANALGESICS IN THE HORSE

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SUMMARY

Narcotic analgesics have been used in horses for at least 100 years as a stimulant medication. Fentanyl, a potent narcotic analgesic, is listed by some racing authorities as a depressant, although narcotic analgesics have traditionally been considered to be stimulants in the horse. We therefore elected to study the effects of several narcotic analgesics on the locomotor activity of the horse.

All the narcotic analgesics tested induced a locomotor response which was quantitated by counting the number of steps that the horse took with its left foreleg. After saline injection horses averaged 4 steps per 2 mins. Narcotic analgesics increased locomotor activity up to 120 steps per 2 mins.

The step counting data was used to construct dose and time response curves for several narcotic analgesics. The potency as locomotor stimulants of all the narcotics tested paralleled their potency as analgesics in man. While the locomotor response to fentanyl was sharp and short-lived, the response to a large dose of morphine continued for up to 14 hrs.

Because the locomotor response to fentanyl was brief, the same locomotor response could be repeated at 90 min intevals, making it a useful tool to study the effects of other drugs. For example, by pretreating a horse with acepromazine and then challenging it with a series of fentanyl injections, the blockade of the locomotor response by acepromazine was readily quantitated.

INTRODUCTION

Narcotic analgesics have been used in horses for at least 100 years as a stimulant medication. However, since there is a lack of basic research on drugs in horses, racing authorities often have to rely on data obtained from man and laboratory animals when classifying drugs. Furthermore, track "folklore" about many drugs adds to the confusion. For example, fentanyl, a narcotic analgesic, is listed by some racing authorities as a depressant', despite the fact that track rumor insists a horse can be "moved up" with 1 mg or less of this potent drug. Therefore, at the Kentucky Equine Drug Research Laboratory, we are systematically evaulating effects of a wide range of drugs on the horse.

In early experiments one of us (T.T.) dosed Standardbred pacers with small amounts (1 mg) of fentanyl reportedly used on the track, but saw no consistent pharmacological effects. However, when we increased the dose to about 4 mg per horse to facilitate drug detection in blood samples, we observed marked signs of locomotor stimulation. In light of these facts, we established a step-counting procedure for quantitating the effects of narcotic analgesics on the behavior of horses.[3]

MATERIALS AND METHODS

Specially shielded box stalls were built with a 1 foot square window to allow observation of the horse. These 13½-foot square stalls had a straw covered earthen floor. The Thoroughbreds and Standardbreds were brought into these modified stalls 24 hours before an experiment for acclimatization to the surroundings. Water and grain buckets were removed before an experiment but hay was available in wall racks. To assist the observer in keeping track, the horse's left foreleg was wrapped in white tape. Only steps taken with that foot were counted. After intravenous injection of saline or drug, the observer recorded the number of steps on a hand held counter and logged the cumulative score for each 2-min period.

RESULTS

Figure 1 illustrates the effect of increasing doses of fentanyl on spontaneous motor activity in four horses. The straight line, representing about 4 steps/2-min period, is the characteristic baseline, obtained after saline injection. After 0.001 mg/kg fentanyl, equivalent to one-half mg per horse, there was no significant increase in locomotor activity. Shortly after intravenous (IV) injection of higher doses, 0.005, 0.010 and 0.020 mg/kg fentanyl, the horse began circling the stall. This activity peaked at 4-6 mins after the injection. The effect decayed rapidly for one-half hour and then more slowly to baseline at 1 hour. The peak response following dosing with 0.020 mg/kg fentanyl is 25 times the baseline level of activity. At the next higher dose, 0.040 mg/kg or approximately 18 mg per horse, three of the four horses in this experiment experienced such severe incoordination that they fell during the first few minutes. The curve appeared shifted to the right since no steps were counted while the horse was down. Fentanyl gives very reproducible results from horse to horse as illustrated by the fairly narrow ranges of the peaks.[3]

As shown in Fig. 2, following repeated dosing in the same horse, the spontaneous motor activity curve resulting from IV injection of fentanyl is very reproducible. For these experiments, each of three horses was dosed with 0.020 mg/kg fentanyl at 0, 90, 180 and 360 mins. In each case, activity peaked at four mins and only varied between 101 and 103 steps/2-min period. This reproducibility and the brevity of the behavioral effects of fentanyl make it a useful tool to

study the effects of other drugs.[2] For example, by pretreating these horses with acepromazine and then challenging them with a series of fentanyl injections, the usual locomotor response to fentanyl was partially blocked. Similarly, pre-treatment with naloxone essentially completely blocked the response to fentanyl. The naloxone blockade wore off slowly with only 50% of the normal locomotor response to fentanyl at 6 hours post-blockade. The naloxone block shows that the locomotor response to fentanyl requires occupation of opiate receptors and the acepromazine block suggests that the effect is also dependent on activation of dopaminergic systems.[2]

Testing morphine (Fig. 3), we found it had a longer duration of action at high doses and exhibited a cyclical behavior pattern[1]. Dosing with 0.1 mg/kg, peak activity was reached at 3 hrs and did not return to baseline until 14 hrs. To reduce observer fatigue when dosing with these longer acting drugs, we watched the horses for the first 2 hours following dosing and then only the last 16 mins of each succeeding hour until the activity returned to baseline.

These responses were apparently cyclical rather than the smooth dose-response curves we had seen with fentanyl, so we calculated our data in a different manner. It was found that by pooling all counts for 16-min periods and dividing by eight to give a mean response for a 2-min counting period, we came up with smooth response curves as illustrated by Fig. 4. After 0.6 mg/kg, the peak locomotor activity of about 30 steps/2-min period occurred 2 hrs after dosing. Increasing the dose to 1.2 mg/kg resulted in a mean response of about 50 steps/2-mins, while 2.4 mg/kg produced an average peak of 90 steps percounting period.

To compare the potencies of these narcotics, we took the peak of each of these motor activity curves and plotted them versus the dose of narcotic in mg/kg. The dose response curves generated in this way are represented in Fig. 5 and illustrate the peak locomotor responses for each dose of each drug tested and allow direct comparison of their potency as locomotor stimulants. This rank order of these drugs in inducing locomotor activity in the horse parallels their analgesic activity in man.[1]

We are currently investigating the exotic animal tranquilizer, etorphine, better known as M-99. The dose-response curve for this extremely potent drug is two full log units to the left of fentanyl. The peak response to this drug, 114 steps per 2-min period, is observed with as little as 0.0004 mg/kg etorphine.

Equally important as determining the effect of a drug on behavior, is detection and determination of the pharmacokinetics of the drug in the horse. We chose radioimmunoassay as the technique for detecting nanogram quantities of this potent drug. The methodology has been developed by Janssen Pharmaceutica. The assay is reportedly relatively specific for fentanyl and provides adequate sensitivity with a detection limit of 0.002 ng.[3]

The average fentanyl plasma level of four horses following injection of 0.020 mg/kg IV is seen in Fig. 6. The levels drop rapidly from 56 ng/ml at three minutes to less than half that at 6 mins. They then fell more slowly to 5 ng/ml at 1 hour. The levels stabilized at low values and decreased very slowly to 0.11 ng/ml at 12 hours with none detected at 24 hours. By feathering his curve, we determined that this pattern can be fitted by a 3-compartment system with half-lives of 6, 42, and 174 minutes. Fentanyl is a very lipid soluble drug, allowing rapid entry into the brain and then rapid redistribution to the rest of the body. The termination of action is determined by redistribution rather than metabolism.[5] Following dosing with only 0.001 mg/kg, we were able to detect fentanyl up to 4 hours in the plasma.

CONCLUSION

In conclusion: (1) We have developed a simple, rapid and reproducible step counting method for measuring the locomotor response to narcotic analgesics in the horse. (2) We have utilized this method and the locomotor response to fentanyl as a tool in the study of other drugs. (3) Using the step counting method, we have quantitated dose and time response data for several narcotic analgesics in the horse. (4) We have determined that the rank order, and relative potencies for locomotor effects of these narcotics in the horse, parallel their activity as analgesics in man. (5) We measured plasma and urine levels of fentanyl and found good correlation between plasma levels and the locomotor response. (6) Contrary to what some racing rule books say, we have shown the narcotic analgesics are potent locomotor stimulants in the horse.

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Figure 1. 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 of 0.001 mg/kg fentanyl is shown by the open triangles (Δ-Δ); 0.0005 mg/kg fentanyl by open circles (o-o); 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). At the highest dose tested all horses showed a loss of coordination resulting in a decrease in locomotion during the first 6 minutes. All points are the means of counts determined on four horses and the vertical bars represent SEMs.

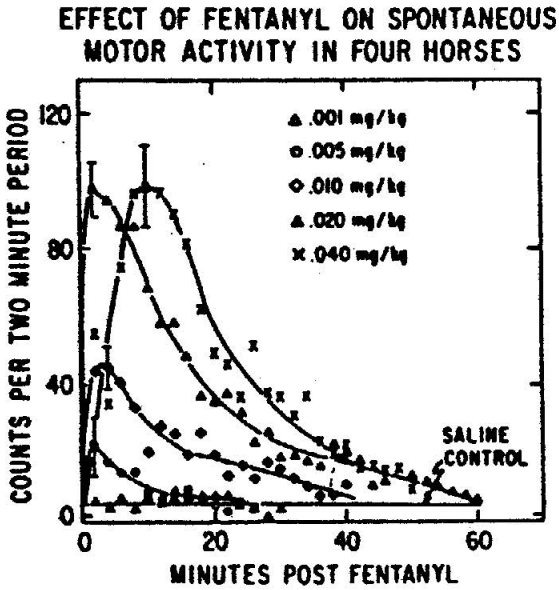


Figure 2. Blockade of the locomotor response to fentanyl by acepromazine and naloxone.

Horses were dosed IV with 0.1 mg/kg acepromazine or 0.015 mg/kg Narcan® at 15 and 5 minutes, respectively, before a series of 4 doses of 0.020 mg/kg fentanyl. The open circles (o-o) represent the locomotor response of horses dosed with fentanyl at 0, 1.5, 3 and 6 hours. The crosses (x-x) show the response to fentanyl following pre-treatment with acepromazine with naloxone. All points are the means of experiments on three horses.

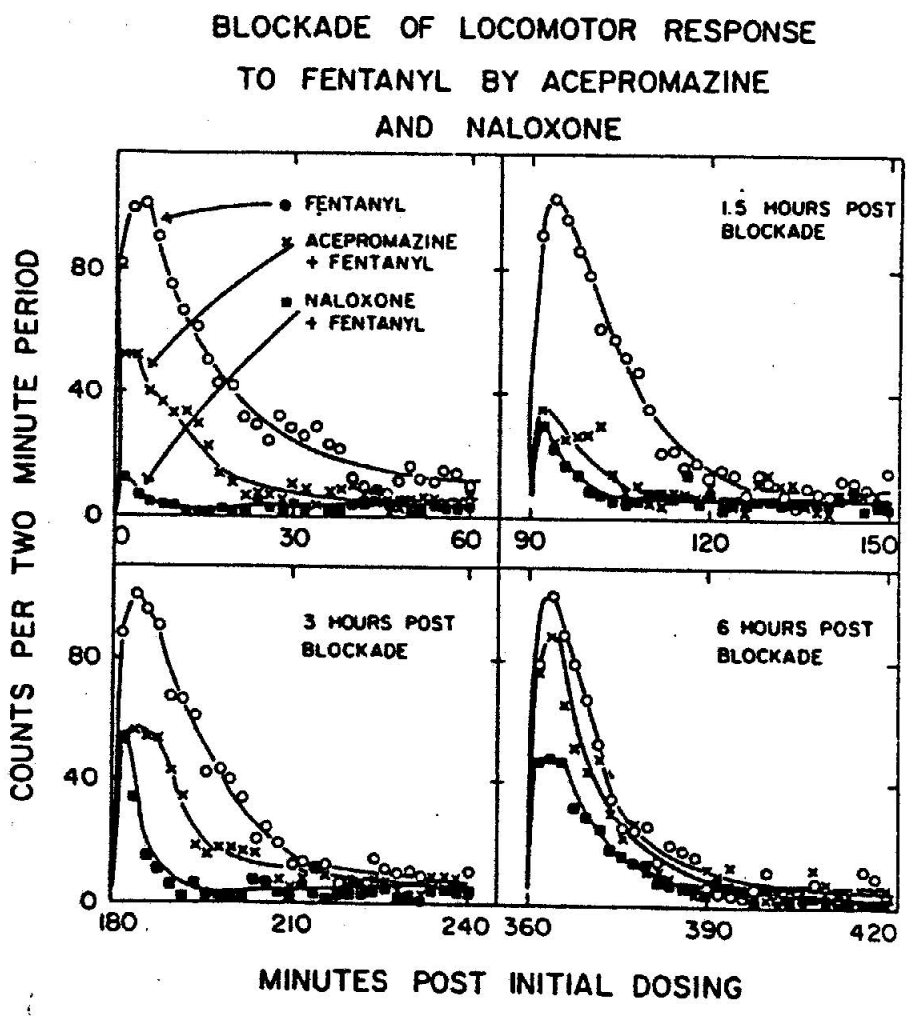


Figure 3. Effect of morphine on spontaneous motor activity in a single horse.

A horse was injected with saline and the indicated doses of morphine IV. The number of times the horse lifted its left front foot in each 2-minute period was recorded as an indication of motor activity. Activity following saline is shown by closed circles (●-●); 0.1 mg/kg morphine by open triangles (Δ-Δ); 0.3 mg/kg morphine by open diamonds (◇-◇); 1.2 mg/kg morphine by closed triangles (▲-▲); and 2.4 mg/kg morphine by teardrops (◊-◊).

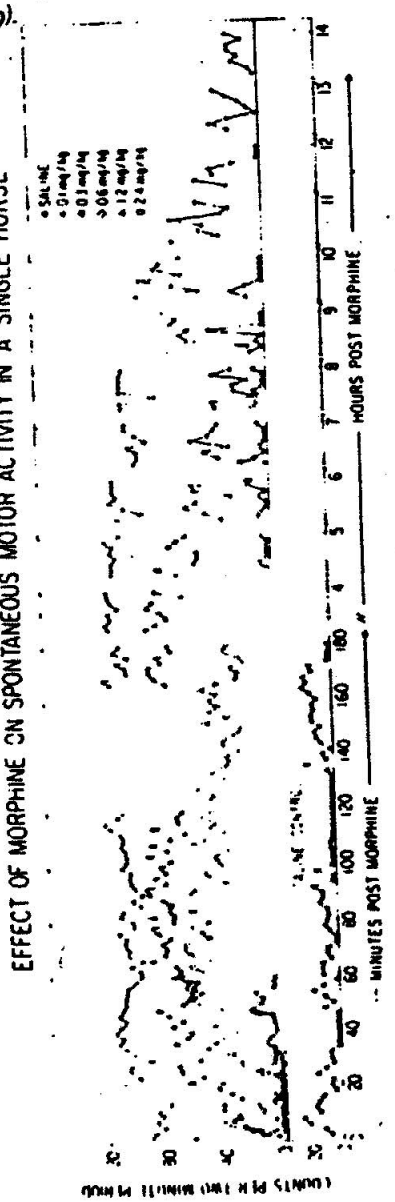


Figure 4. Effect of morphine on spontaneous motor activity in the horse.

Horses were injected IV with saline and increasing doses of morphine. 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 0.6 mg/kg morphine is shown by the open diamonds (◇-◇); 1.2 mg/kg morphine by the closed triangles (▲-▲); and 2.4 mg/kg morphine by the crosses (x-x). Averaged counts per 2-minute period were determined for the 10-minute interval immediately preceding the time indicated. All points are the means of such averaged counts on four horses.

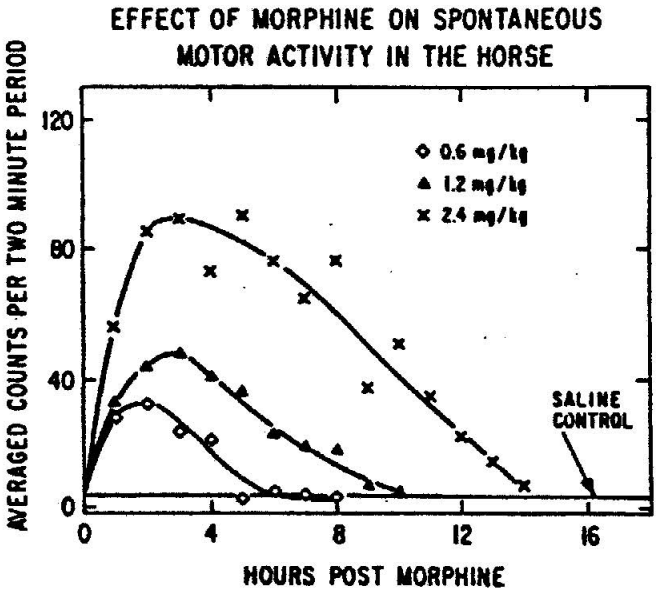


Figure 5. Dose response curves for locomotor activity following narcotic analgesics in the horse.

Horses were intravenously dosed with saline and increasing amounts of the indicated drugs. The average number of steps taken during the peak 2-minute period were plotted for etorphine, fentanyl and apomorphine. For all other drugs, average steps per 2-minute period were determined for the 16-minute interval of peak activity. Three to ten horses were used in the experiments on etorphine, fentanyl, apomorphine, methadone, morphine and pentazocine. One horse was used to determine each dose response curve for hydromorphone, anileridine and meperidine. The average counts per 2-minute period for the saline control are shown by the straight line at the bottom of the graph.

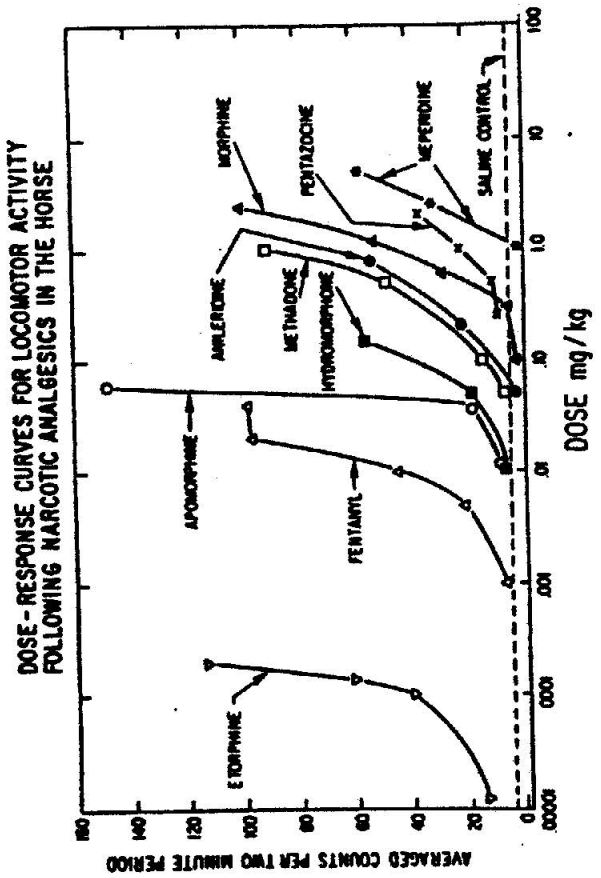
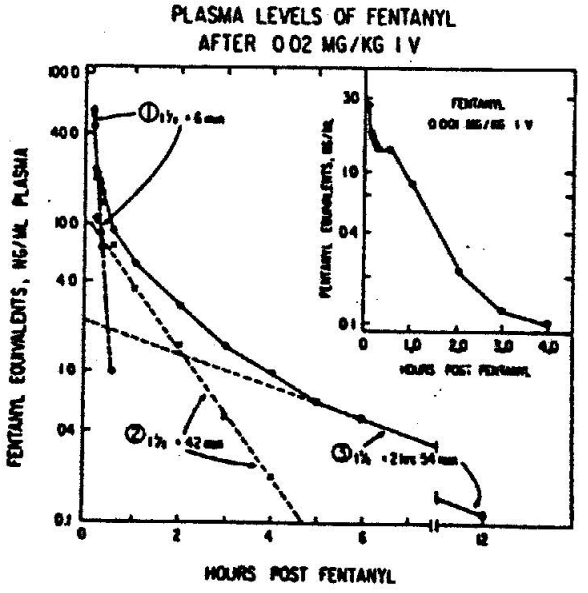


Figure 6. Plasma levels of fentanyl after 0.020 mg/kg IV.

Horses were dosed intravenously with 0.020 mg/kg fentanyl and blood samples drawn at intervals for 24 hours. Plasma levels were determined by RIA methodology and are represented by the solid circles (●-●). Curve peeling revealed a 3 compartment system with half-lives of 6, 42 and 174 minutes. The inset illustrates plasma levels following dosing of horses with 0.001 mg/kg fentanyl.



DISCUSSION

MAYLIN: Once again I would think it should be fentanyl equivalents you should be measuring rather than fentanyl.

COMBIE: I should have said that; it says fentanyl equivalents on the slide.

MAYLIN: We did some preliminary work before we completely understood the metabolism of fentanyl and I think the second compartment you are describing is probably metabolism. If you do a basic extraction and then count this extract in the radioimmunoassay, it's markedly different than if you just use the whole plasma.

COMBIE: Thank you.

SMITH: I'd like to congratulate you on your very elegant piece of work. Is there any correlation between the behavioral half-lives of the compounds you've investigated and their plasma half-lives? Do you have any notion on how big the first-pass effect in general is for the horse? You are making broad comparisons between man and the horse and yet we must remember that so much of the human data is obtained by oral administration whereas for the horse most data is obtained from intravenous or other parenteral route studies. How big is the first pass effect in the horse for drugs that are given orally?

COMBIE: To answer your first question, the behavioral effect has an α-phase t½ of 10 mins. and the β-phase half-life is 30 mins. following dosing of the horse with 0.020 mg/kg fentanyl. We superimposed the plasma and activity levels and found good correlation for the first two phases. The prolonged plasma elimination phase starts once fentanyl levels have fallen below about 5 ng/ml which apparently is insufficient to induce locomotor activity.

TOBIN: I would just point out that if you take rank order of the potencies at maximum effect, they exactly parallel the rank order of analgesic activity in man. But if you take the areas under the curves, the ranks of the orders are very different which certainly suggests that the pharmacological half-life is different and presumably also the metabolic half-life differs between the horse and man. The most clear-cut example is morphine which has the greatest area under the curve in the horse, and is therefore the most potent analgesic in terms of the time period over which analgesia is maintained. By the way, Joan, would you like to briefly state what you found with butorphanol or Stadol® because there is a considerable interest in this drug at the moment.

COMBIE: With butorphanol we have dosed one horse with up to 0.4 mg/kg. We found very little increase in locomotor activity. Dosing with 0.1, 0.2 and 0.4 mg/kg resulted in no significant difference in footstep counts (paired data t test, $p<.01$). The peak of activity was only 25 steps/2 minute period.