VOL. 35, NO. 3
MARCH 1982

Research Communications in
Chemical Pathology and Pharmacology

EFFECTS OF ENKEPHALINS VERSUS OPIATES ON LOCOMOTOR ACTIVITY OF THE HORSE

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ABSTRACT

The enkephalins are small, pentapeptide neurotransmitter molecules which have reportedly been used in racing horses. In our experiments, DAla^{2}Metenkephalinamide and leucine enkephalin were administered to horses intravenously (IV) and intracisternally (IC). Leucine enkephalin had little effect on locomotor activity by either route at doses of 0.01 mg/Kg or less. Methionine enkephalinamide, an enzyme resistant enkephalin analog, had no significant effect when given IV (0.002 and 0.008 mg/Kg). Other experiments involving intracisternal dosing with this long acting form at higher levels (0.005-.01 mg/Kg), elicited an initial increase in locomotor activity, a rise in temperature, a marked increase in blood pressure, hyperventilation, the appearance of a rapid eye blinking reflex, lack of coordination and quivering. In contrast, dosing with fentanyl either IV (0.1 mg/Kg) or IC (0.0002 mg/Kg) produced a tenfold increase in locomotor activity without accompanying adverse clinical symptoms. The data suggest that very large doses of IV administered enkephalins or their analogs may be necessary to increase locomotor activity but such doses may also elicit a number of less desirable side effects.

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INTRODUCTION

Two pentapeptides, methionine- and leucine-enkephalin, have been postulated to be the endogenous ligands for the opiate receptor. When injected intracerebrally, they exert weak, morphine-like effects, but since they are rapidly destroyed by brain enzymatic activity, this effect is minimal and dissipates rapidly (Bergmann et al, 1977; Bhargava, 1979; Biggio et al, 1978; Tregear and Coghlan, 1980; Wei, Tseng, Loh and Li, 1977). To prolong their action, enkephalin analogs have been synthesized which are resistant to the peptidase activity. One of these, D-Ala^2-methionine-enkephalinamide, has been shown to stimulate dopamine turnover, an action that has been correlated with the analgesic effect, motor stimulation, tolerance and other opiate effects (Biggio et al, 1979; Katz, Carroll and Baldrighi, 1978).

The narcotic analgesics have been demonstrated to increase locomotor activity in a number of species including the horse and mouse (Combie et al, 1979; Katz et al, 1978; Tobin, 1981). Since the use of narcotics in racing horses is illegal, naturally occurring substances with opiate-like effects may be administered in an attempt to avoid detection of the "doping" agent. Reportedly, leucine enkephalin has been given to race horses in an attempt to improve their performance (McDonald, 1979).

Both of the long-acting enkephalin analogs have been shown to produce hyperactivity in mice (Katz et al, 1978) and CNS effects of these agents have been observed following systemic administration (Plotnikoff et al, 1976). In this study, we have administered both the naturally occurring and long-acting enkephalins to horses intravenously and intracisternally to determine the effectiveness of these

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agents. Locomotor activity of these horses was compared with that of horses given fentanyl and morphine, both of which increase locomotor activity and thus served as positive controls.

MATERIALS AND METHODS

A form of methionine enkephalin that is resistant to enzyme degradation (Tyr-D-Ala-Gly-Phe-Met-NH_2) (Pierce Chemical Co, Rockford, IL) and the natural form of leucine enkephalin acetate (Tyr-Gly-Gly-Phe-Leu) (Sigma Chemical Co, St. Louis, MO) were dissolved in distilled water or cerebrospinal fluid (CSF) immediately before use. Fentanyl citrate (Endo Laboratories, Inc, Garden City, NY) was obtained in crystalline form and dissolved in distilled water or CSF just prior to use. Morphine sulfate injectable (Eli Lilly & Co, Indianapolis, IN) was used as obtained from the manufacturer.

Locomotor activity of the horse was quantitated as previously described (Combie et al, 1979). Adult Standardbred and Thoroughbred mares and geldings were isolated in 4 x 4 m box stalls. Following a period of acclimitization to the surroundings, the animals were dosed and then observed until activity returned to baseline levels. The number of footsteps taken with the left foreleg was recorded for each 2 min period.

Treatment with the enkephalins was observed to produce a number of specific behavioral responses, including eating behavior, quivering, coordination changes and respiratory rate changes. Prominent among these changes was a very rapid (23/sec) blinking of the eyelids if a hand was held close to the eye. This rapid blinking response appeared to be characteristic of the actions of enkephalins in the horse, its elicitation by placing the hand in front of the eye of an animal is referred to as the eyeblink test and was graded subjectively.

Drug injections were performed either intravenously or intracisternally. Intravenous injections were made into the jugular vein. Baseline locomotor activity after IV injections was determined by injecting saline IV and counting the footsteps taken during the next hr. To perform IC injections, the horse was restrained in a standing position. A 22-gauge, 3 inch spinal needle was inserted into the spinal column at the level of the cisterna magna. A small volume of CSF was withdrawn to verify the proper placement of the needle. The drug was then dissolved in this CSF and returned to the spinal canal. Baseline activity following the IC procedure was determined by withdrawing a small volume of CSF and then returning it to the cisterna magna and observing the locomotor activity for 16 min.

Rectal temperatures were measured using a digital read-out thermometer. Following IC injection of the long-acting methionine enkephalin (0.001 mg/Kg), blood samples were drawn into Vacutainer (Becton-Dickinson, Rutherford, NJ) tubes containing EDTA at intervals for 6 hr. The hematocrit and white blood cell differential counts were performed on these samples.

Cardiac effects of the long-acting methionine enkephalin and morphine were compared. Catheters were emplaced in the carotid artery and the right ventricle. The arterial and right ventricular pressures and electrocardiogram tracings were recorded on a Grass Model 7 polygraph (Grass Instruments, Inc, Quincy, MA) following IV injection of 0.002 mg/Kg of the enkephalin, 0.5 mg/Kg of morphine, and IC dosing with 0.005 mg/Kg of the enkephalin.

RESULTS

Intravenous injection of 0.01 mg fentanyl/Kg and IC dosing with 0.0002 mg fentanyl/Kg resulted in comparable locomotor activity as illustrated in Fig 1. Locomotor activity of the four horses dosed IV

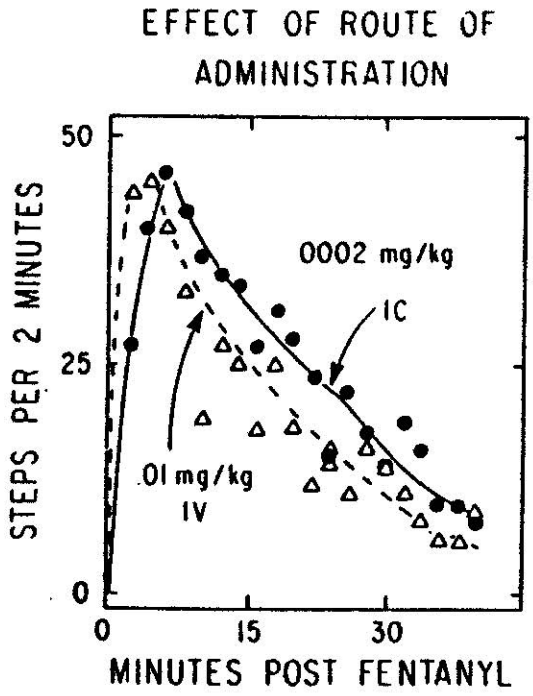

Fig 1 Locomotor activity of four horses dosed IV with 0.01 mg fentanyl/Kg is shown by the open triangles (Δ-Δ). On a separate occasion, the four horses were dosed IC with 0.0002 mg fentanyl/Kg; locomotor activity, in this case, is indicated by the closed circles (●-●).

peaked at 45 steps/2 min period, about 4 min after injection. This was an 11-fold increase over the average of 4 steps/2 min following saline injection. Aside from a propensity to eat hay, other behavioral effects were unremarkable. Activity returned to baseline levels by 40 min after dosing. Using 1/50 of the IV dose, four horses were dosed IC with 0.002

mg fentanyl/Kg. The peak of the locomotor activity of 46 steps/2 min occurred 6 min post-dosing. Again, all four horses showed an increased tendency to eat hay. Slight incoordination was noted in two of the four horses. Locomotor activity following withdrawal of CSF but without subsequent drug administration averaged about four steps/2 min.

Intravenous administration of leucine enkephalin (0.002 and 0.01 mg/Kg) and low doses of DAla^2-Met-enkephalinamide (0.002 mg/Kg) and fentanyl (0.001 mg/Kg) had little effect on locomotor activity (Fig 2). The leucine

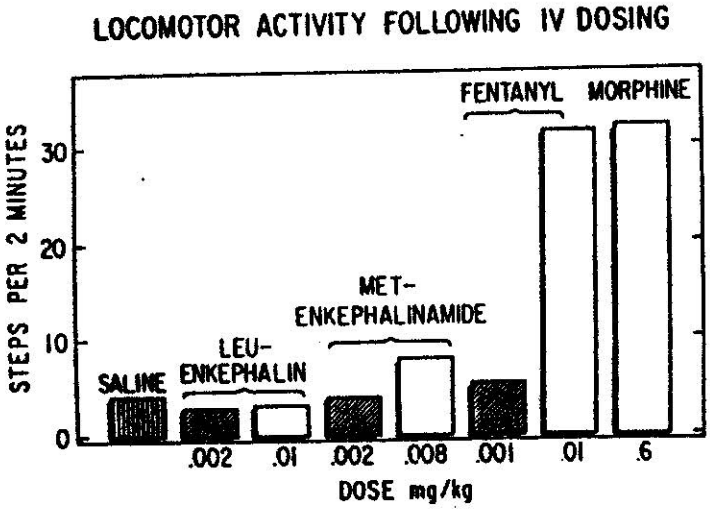

Fig 2 The average number of steps taken per 2 min counting period during the first 16 min post-IV dosing with the indicated amounts of leu-enkephalin, the long acting met-enkephalinamide, fentanyl and morphine are shown. Four horses given saline served as controls.

enkephalin resulted in almost steady eating activity. Consumption of hay was less marked although present following dosing with the long-acting met-enkephalin and the low dose of fentanyl. Larger doses of these

compounds (D-Ala^2-Met-enkephalinamide, 0.008 mg/Kg; fentanyl 0.01 mg/Kg; and morphine (0.6 mg/Kg) resulted in a slight increase in locomotor activity for the enkephalin and a marked increase for the two narcotics.

There was no significant increase in locomotor activity following IC administration of either level of leu-enkephalin (Fig 3). D-Ala^2-Met-enkephalinamide at a dose of 0.002 mg/Kg induced no significant increase

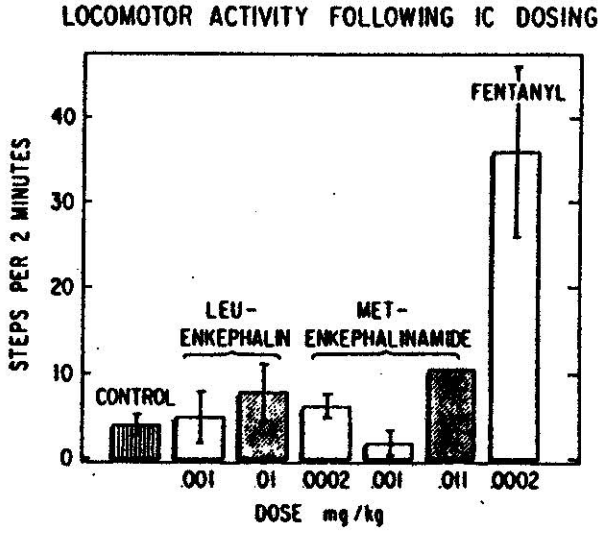

Fig 3 At least three horses were dosed IC with the indicated amounts of leu-enkephalin, met-enkephalinamide or fentanyl (except one horse was used for the 0.011 mg met-enkephalinamide/Kg dose). Controls were four horses that had spinal fluid withdrawn but no drug given. The average number of footsteps taken by the horses per 2 min during the first 16 min after dosing is shown. The vertical bars represent ± SEM.

in locomotor activity. However, one of the three horses in this experiment was slightly incoordinated and exhibited a positive eye blink test 16 min after dosing. The 0.001 mg/Kg dose of this enkephalin actually appeared to result in a decrease in locomotor activity, although this was not

statistically significant (1-way ANOVA, $F = 2.098$, $\alpha = .05$). The reason for this decrease appeared to be a marked propensity for all three horses to eat hay. In fact, two of the horses never left the hay rack during the step-counting period. All three horses exhibited signs of neuromuscular involvement. By 16 min post-dosing, the whole body of one horse was shaking. The muzzles of the other two horses were twitching rapidly by 30 min. Rapid eye blinking, characteristic of the enkephalins, was positive at 16 and 30 min post-dosing, equivocal at 1 and 3 hr, and negative at 6 hr. The horse exhibiting the most distressed clinical picture had a pulse rate of 42 per min at 27 min after dosing and the pulse rate was too weak to take at 48 min. The animal spent prolonged periods of time standing in one place with his head lowered. When forced to walk, he appeared stiff and turned with difficulty, although there was some improvement after a few min of exercise. After 1 hr, the experiment was terminated by the IV injection of 10 mg naloxone. Within 55 sec all clinical signs had returned to normal. Hematocrit and white blood cell differentials did not change over the 6-hr period following IC dosing with this long-acting enkephalin.

One horse was given 0.011 mg of the D-Ala^2-Met-enkephalinamide per Kg IC. Although there was a significant increase in locomotor activity for the first 8 min, this activity ceased as a lack of coordination and other clinical signs increased. By 6 min, the first signs of incoordination were noted and the horse's nose was quivering. After 10 min, there was essentially no more spontaneous locomotor activity for the duration of the experiment. The horse maintained a wide stance behind and a narrow one in front while periodically looking back at his side. The

horse was still sweating, hyperventilating and walking wide in the hind end at this time. By 24 hr post-dosing, all clinical signs had returned to normal.

DISCUSSION

From the data gathered in this study, it appears that, under certain conditions, the enkephalins may affect the locomotor activity of the horse (Fig 2 & 3). The three most important variables appear to be the form of the enkephalin used, the route of administration, and the size of the dose.

The enzyme-resistant form of the enkephalins is generally accepted as being far more potent (30-100X) than the naturally occurring forms (Aloisi, DeCarolis and Longo, 1980; Biggio et al, 1978; Rigter et al, 1980; Vaught and Takemori, 1979). Clearcut pharmacological effects are therefore more likely after dosing with the enzyme-resistant form of met-enkephalin than after leucine enkephalin.

In the present study, several clinical effects were induced by the administration of the long-acting methionine enkephalin analog that were not observed with the narcotics. Movement of an object toward the eye of a horse dosed with fentanyl or morphine caused the horse to blink a few times. Under the influence of the enkephalin, an extremely rapid blinking or twitching, lasting for the duration of movement near the eye, was observed. The trembling and marked lack of coordination elicited by the IC administered enkephalin have not previously been observed following IV and IC dosing with narcotic analgesics with the exception of high levels of the partial agonist, pentazocine (2.0 mg/Kg IV) (Combie et al, 1979).

Other researchers have found qualitatively different effects when the pentapeptides were administered by different routes. For example, analgesic activity of the enkephalins following systemic dosing is seldom demonstrable (Pert et al, 1976), but the anti-amnesic effect is mediated through the opiate receptors (Rigter, 1978). In rats, it has been found that several enkephalins with no opiate activity after peripheral injection did reduce passiveness (Kastin et al, 1978). Intraperitoneal administration of enkephalin to rats learning a maze demonstrated behavioral effects which Kastin (1976) dissociated from their opiate-like effects. Peripheral stores of enkephalins have been found (DiGiulio et al, 1978) and it is possible that the behavioral activity of systemically administered enkephalins is initiated at a peripheral site (Rigter et al, 1980). This could account for qualitatively different effects seen between central and peripheral administration of the pentapeptides.

It is likely that a much larger dose is required for effect after systemic administration. The data from dosing with fentanyl (Fig 1) indicates that 50 times as much drug must be administered IV to obtain equivalent effects from IC dosing. Since fentanyl is highly lipid soluble and penetrates readily into the CNS, it is likely that a much greater ratio might be necessary for the enzyme-resistant analog of met-enkephalin to penetrate the CNS and elicit clear cut central responses comparable to those obtained from the IC dosing with .011 mg/Kg.

Of most interest to racing authorities are the possible effects of these compounds on locomotor activity. It has been shown that narcotic analgesics increase the locomotor activity of the horse (Combie et al,

1979; Tobin, 1981). Several reports suggest that enkephalins and their analogs may also facilitate an increase in locomotor activity (Katz et al, 1978; Plotnikoff et al, 1976; Wei et al, 1977), especially in those species in which morphine produces excitement - mice, cats and horses (Broekkamp, Phillips and Cools, 1979; Carroll and Sharp, 1972; Combie et al, 1979; Katz et al, 1978). Although the enkephalins did not elicit dramatic increases in locomotor activity in the horses in this study, two points should be remembered. Reports indicate that many of the opiates are more potent than the enkephalins. For example, leu-enkephalin has one-tenth the potency of morphine (Bhargava, 1978) and D-Ala^2-Met-enkephalinamide has one-half the potency of morphine (Pert et al, 1976). Therefore, far larger doses than were used in this study could produce more dramatic results. The high cost of the enkephalins may play a major role in the extent of their use on the race track. The enzyme-resistant form of met-enkephalin costs $25./mg. That translates to $100. for the 0.008 mg/Kg that produced a very minimal response following IV dosing (the only practical route for racing horses). However, this minimal response under our laboratory conditions likely would be magnified under actual racing conditions. Other drugs reportedly used on the track in low doses have been found to be ineffective in the laboratory, likely due to the fact that environmental demands may greatly increase a subject's sensitivity to a drug (Doull, Klaassen, Amdur, 1980).

In summary, therefore, clinical effects elicited by long-acting met-enkephalin administered IC are unlike those elicited by fentanyl. Although no significant effects were observed following IV dosing with the

enkephalins, the fentanyl data suggest that a minimum of 50 times as much (and likely far more) pentapeptide must be given IV to obtain the same effects of an IC dose.

ACKNOWLEDGEMENT

Publication #90 from the Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture and the Graduate Toxicology Program, University of Kentucky, Lexington, KY 40546-0076. Published as Kentucky Agricultural Experiment Station article No. 82-4- with the permission of the Dean and Director, College of Agriculture.

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