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DOSE RELATED EFFECTS OF U-50,488H ON BEHAVIOR, NOCICEPTION AND AUTONOMIC RESPONSE IN THE HORSE

by

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SUMMARY

A major problem with the use of u agonist narcotic analgesics in the horse is that they produce marked central nervous stimulation. This stimulation appears as a marked locomotor response, and also as increased heart and respiratory rates. These effects are not desirable in most clinical situations, as they can interfere with ongoing anaesthetic, surgical, and recovery events. Therefore, a need exists for a narcotic analgesic which produces good analgesia with little or no locomotor or autonomic response, or other autonomic effects in the horse.

Current opiate receptor theory suggests that kappa agonists should fulfill this requirement. U-50,488H is an experimental narcotic analgesic that is a selective kappa agonist. In the present study, U-50,488H produced good analgesia in horses using both the skin twitch and hoof withdrawal reflex assays. Further, the analgesia was relatively long lasting (120 min) compared to other u-agonists tested in horses. The locomotor response to U-50,488H was less than observed with ethylketazocine and butorphanol, and has yielded the smallest locomotor response of any of the narcotic analgesics tested to date.

Other work showed that the autonomic responses to U-50,488H were less than those of other narcotic analgesics, and that the analgesic response to this drug was blocked by naloxone. Based on its ability to produce analgesia with little other stimulatory action, U-50,488H shows promise of becoming a useful narcotic analgesic in equine medicine.

INTRODUCTION

In a series of studies on the narcotic analgesics in the horse, we have shown that most of the u-agonist (morphine type) narcotic analgesics available for use in the horse produce marked locomotor stimulation (Combie, et al., 1981). This creates problems with the clinical use of these agents, since they may make horses difficult to control, and can interfere with recovery from surgery. Beyond this, the stimulating actions of these drugs on the autonomic nervous system leads to problems in control of cardiovascular function in horses under anesthesia. For these reasons a narcotic analgesic with fewer side effects would be very desirable in equine medicine.

Based on theoretical considerations, we have been investigating kappa agonists as possible candidates. In studies with ethylketazocine, a prototypic member of this group, we showed that while the analgesic response to this agent was good, the side effects of locomotor activity and sympathetic stimulation were much reduced (Kamerling, et al., 1986). Based on these observations, we examined the analgesic responses to U-50,488H a more selective kappa agonist, in the hopes that it would be a more clinically useful drug in the horse. The results presented here show good analgesic responses to U-50,488H in horses, minimal locomotor effect, and minimal cardiorespiratory action. Based on these considerations U-50,488H is an excellent analgesic candidate for clinical evaluation in the horse.

MATERIALS AND METHODS

Experimental Animals

Throughout all the experimental sessions mature thoroughbred and standardbred mares and geldings (400-600 kg) were used. These animals were kept at pasture until the week of their experimental session at which time

they were brought to the laboratory barn. They were housed in box stalls (180 sq ft) at least 24 hrs prior to experimental use to allow for aclimatization to confinement. While confined and awaiting experimental use each animal was provided hay and water ad libitum.

Locomotor Assay

The locomotor stimulatory potential or lack thereof of U-50,488H was determined by placing horses in enclosed box stalls equipped with one way viewing mirrors. This permitted observation of the animal's movement without detection. Locomotor responses were quantitated by observing and recording the number of footsteps taken with a taped right front forelimb, per 2 min observation period. A footstep was tallied each time the designated limb was lifted and returned to the ground accompanied by a positional change (Combie et al., 1979).

Initial studies to determine a manageable dose of U-50,488H in the horse revolved around a 200 ug/kg level. At this dose level pronounced ataxia lasting from 12-20 min and generalized muscle tremors occurred in some experimental subjects. While none of the horses subjected to this dose were rendered recumbent, the upper limit of dosage was reduced to 160 ug/kg to insure an adequate margin of safety for the animals and experimenters throughout the locomotor, nociceptive and physiologic assays. Doses of 160 ug/kg, 80 ug/kg, 40 ug/kg and 0.9% NaCl (saline) were administered to 8 horses over a four week period, individual treatments being determined by a Latin square crossover design. Locomotor responses were observed and recorded over a 16 min pre-injection period to establish an intra-experimental day baseline for each animal at each session. During the locomotor study footsteps were, after drug administration, tabulated every 2 min for 60 min.

Nociception Assay

Nociceptive (pain) thresholds were quantified using a modification of the methods of Hardy et al. (1940) and Pippi et al. (1979) as refined by Kamerling et al. (1985b). Two independent nocifensive stimuli were used, each involving a separate anatomical locus. A light beam was passed through a condensing lens and the intense radiant stimulus was focused on the skin of the lateral aspect of the right metacarpophalangeal joint at a fixed distance. The aversive movement (i.e. withdrawal of the affected foreleg) provided the end point of the stimulus. The time interval from initial illumination to shut down of the lamp upon limb flexion was designated as the hoof withdrawal reflex latency (HWRL) and recorded on a digital timer (Cole-Palmer Instrument Co., Chicago, IL). A second nociceptive parameter was also assayed using another intense beam of focused light emanating from an apparatus mounted on a saddle tree and affixed to the animal by a girth strap. This instrument was aligned at a fixed distance in such a manner as to deliver a nocifensive stimulus to the animal's withers. Elicitation of reflex contractive movements of the cutaneous musculature, upon sufficient heat loading, served as the end point for the stimulus. Here again, the time elapsed from initial illumination to reflex was designated the skin twitch reflex latency (STRL), which was recorded with the digital timer. Lamp intensities were adjusted to provide baseline reflex latencies of 7-9 s for the HWRL and 5-7 s for the STRL. Automatic cut-offs of 20 s and 15 s for the HWR and STR respectively, were set to avoid damage or urticaria at the stimulus loci.

Physiological Responses

Cardiac rate was recorded using a polygraph (Grass Instruments, Co., Quincy, MA) with bilateral electrodes attached ventrally at the animal's

heart girth. Respiration was recorded on the polygraph through impedence changes taken with an impedence converter (UFI, Main St., Morrow Bay, CA 93442). Pupil size was calculated by measuring vertical diameter of the elliptically shaped pupil from photographs of the eye, similar to methods of Marquart et al. (1967). Rectal temperature was recorded from a deep rectal probe and digital thermometer (Sensortek, Saddlebrook, NJ). Electromyogram recordings were taken on the polygraph adapted with a summating integrator (Grass Medical Instruments, Quincy, MA). Yawning frequency was tallied using a hand-held counter (Cole Palmer, Chicago IL).

All physiologic parameters (heart rate, respiration, pupil size, rectal temperature, EMG, and yawning frequency) were recorded simultaneously along with the measurement of nociceptive thresholds while horses were confined to stock restraints. Pre-treatment measurements were taken to establish an experimental day baseline for each animal at the outset of each experimental session. Post-treatment measurements were tabulated every 5 min for 60 min for the heart and respiratory rate. Pre-treatment measurements of the STR and HWR latency, pupil diameter, temperature, and yawning frequency were made every 15 min for 30 min. Post-treatment measurements of these parameters were then made every 5 min for 30 min, and at 45 and 60 min post-injection.

In a separate study of the duration of U-50,488H analgesia with or without naloxone (0.020 mg/kg IV) pre-treatment, pre- and post-treatment STRL's were recorded every 20 min for 40 min prior to drug administration and for 200 min after administration of: (1) naloxone and U-50,488H; (2) naloxone and saline; or (3) saline and U-50,488H.

U-50,488H was given intravenously at doses of 160, 80 and 40 ug/kg over a four week period according to a Latin square crossover design. Equal

doses were given in the locomotor assay as well as in the nociceptive assay. In the naloxone experiments, horses received the 160 ug/kg dose of U-50,488H.

Behavioral ques of sedation, head drop, ptosis of the lower lip, hind limb flacidity, and transient loss of postural tone and balance were subjectively evaluated and noted. Yawning frequency was quantified objectively as a separate index of sedation.

Drugs

U-50,488H and naloxone* were dissolved in 10 ml of sterile saline at physiologic pH and injected into the left jugular vein. A volume of 10 ml of sterile saline was administered as a control treatment when called for in the experimental design. U-50,488H was generously supplied by Dr. Phillip VonVoightlander of the Upjohn Company, Kalamazoo, Michigan.

STATISTICAL ANALYSIS

The duration of the pharmacological effects of most significant parameters of concern with U-50,488H spanned a time course of 30 to 60 min. Statistical analysis was conducted based on the summation of data over the 60 min period post drug administration for the primary series of experiments. For the experiments involving the naloxone pre-treatment prior to U-50,488H administration, the data were summed over the 200 min time course post injection. All values were then analyzed using an ANOVA program in which variances among subjects, sessions and treatments as well as treatment by session variance was calculated. Linearity over treatments was determined by partitioning variance among treatments into linear and non-linear components and using a Duncan's Multiple Range test. All

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* Endo Laboratories Inc, Garden City, NJ.

calculations were then run on an IBM 3038 computer using a Statistical Analysis System program (Freund and Littell, 1981).

RESULTS

Fig. 1 shows the results of administration of increasing doses of U-50,488H on the hoof withdrawal reflex latency. After administration of 160 ug/kg, the largest dose tested, the hoof withdrawal latency increased by about 100%. At 20 min after administration, the effect had peaked and remained at the peak value for the remainder of the experiments. A similar response was observed with the skin twitch model (Fig. 2). However, analgesia developed more rapidly in that a near maximal effect was observed by 5 min after dosing. The STRL also remained increased for the full 60 min of the experiment.

Statistical analysis of the analgesic responses obtained during the experimental sessions involving the administration of U-50,488H to 8 subjects demonstrated many of the desired effects of a kappa-agonist analgesic. As measured by our two methods, analgesia was produced in a linear dose-related fashion for both the STRL model ($P=0.0004$) (Fig. 1) and the HWRL model ($P=0.0002$) (Fig. 2). At 160 ug/kg STRLs were elevated more than double that of the control values (14.5 ± 0.5). Similarly, at the highest dose (160 ug/kg) the HWRL values were doubled (11.2 ± 1.0) over the saline control levels. Significant ($P<0.05$) level of analgesia was also achieved using the 160 and 80 ug/kg levels of U-50,488H as revealed by the Duncan's Multiple Range groupings.

The locomotor response to U-50,488H was minimal, as shown in Fig. 3. At each dose tested up to 80 ug/kg, no locomotor response was detectable, while at the highest dose tested, the locomotor response was minimal. In addition, the locomotor response seen at 160 ug/kg was qualitatively

different from that observed with a typical u agonist. While the response with a typical u agonist was always a clearcut and well coordinated trot, described by clinicians as a "stall walk" the response to U-50,488H was noticably different. At high doses of U-50,488H the horse appeared to be drowsy and uncoordinated, and the small steps taken were postural adjustments to maintain balance, rather than steps associated with a true trotting response. Based on these observations, it appears that the locomotor response to this drug is minimal, and different in character from that seen with the classic u agonists. To emphasize the negligible nature of the locomotor response to this drug, the locomotor responses to approximately equi-analgesic doses of fentanyl and ethylketazocine are presented for comparative purposes (Fig. 3). While fentanyl produces a locomotor response peaking at 55 steps/2 min, and the response to ethylketazocine peaks at about 25 steps/2 min, the response to U-50,488H peaked at about 12 steps/2 min, and rapidly returned to control values. In fact, at lower doses (80 ug/kg) U-50,488H produces potent analgesia without any locomotor enhancement (Fig. 3).

Further analysis of data collected on the various physiological parameters revealed little in the way of undesired side effects (Fig. 4). Cardiac rate was not elevated in any subject over any of the three doses ($P=0.2651$). Respiration, similarly, was uneffected ($P=0.6159$) over the doses administered. Differences in pupil diameter were marginally non-significant ($P=0.0590$). Rectal temperature was not significantly effected over doses, ($P=0.4155$) but late-onset hyperthermia was observed at the high dose ($P<0.05$). EMG activity, although visually discernable in one of eight subjects at the 160 ug/kg dose, yielded no dose related significance ($P=0.3234$). The quantitated behavioral index of sedation (ie

yawning frequency) revealed a significant linear dose related (P=0.0004) response.

Experiments involving the pretreatment of four subjects with 0.02 mg/kg of naloxone 5 min prior to the injection of 160 ug/kg of U-50,488H showed an almost total blockade of the analgesic effect of U-50,488H. Subjects receiving U-50,488H (160 ug/kg) alone showed a 3-fold increase in STRL over either the naloxone-saline control or the naloxone-U-50,488H treatment sequence. Subjects injected with 0.02 mg/kg naloxone followed by 10 ml saline, showed reflex latencies which were indistinguishable from the naloxone/U-50,488H treatment sequence (Fig. 5). The peak analgesic effect for the STRL assay (15.0±0.0) was achieved at 40 min post injection and declined thereafter, returning to baseline values by approximately 180 min.

DISCUSSION

Results from the present study confirm and extend our earlier observations in the horse that k-receptor opioids produce analgesia with less attendant locomotor and sympathetic stimulation than u-opioids (Kamerling, et al., 1986). In fact, it appears that the greater the selectivity for the k-receptor, the more favorable the ratio of analgesia to locomotor effect. This is supported by comparing the ratios of the locomotor/analgesia ED_{50} values. The ratios for fentanyl, ethylketocyclazocine (EKC) and U-50,488H are 11.1, 19.2, and 82.5, respectively. The higher the ratio, the greater the proportion of analgesic to locomotor effect produced. These values indicate that U-50,488H produces roughly 4 times more analgesia than EKC and 7 times more analgesia than fentanyl per unit of locomotor activity. Furthermore, U-50,488H elevated pain threshold at doses which failed to produce any locomotor enhancement. The reduced propensity for locomotor stimulation is therapeutically advantageous in treating the pained equine patient.

An additional therapeutic advantage is the prolonged analgesia and sedation produced by U-50,488H. Elevation in pain threshold persisted for 2 hours. This would reduce the need for repeated dosing to maintain a given level of analgesia. We have observed that analgesia following the commonly used sedative/analgesic xylazine, lasts 30 mintues or less. Furthermore, U-50,488H analgesia outlasted that observed following fentanyl or EKC.

Both U-50,488H and EKC produced sedation according to the subjective criteria used in our studies. The most consistent observations included lowering of the head, yawning, hindlimb relaxation, and hypomotility. The apparent difference in sedative potency of these two agents may be that EKC possess some activity at the u-receptor. It is our current hypothesis that this receptor mediates the locomotor effects of the morphine-like narcotics in the horse. While both the u and k receptor appear to mediate analgesia, the k receptor probably mediates sedation rather than excitation.

The fact that naloxone blocked the analgesic response to U-50,488H suggests that this agent raised pain threshold by interacting with an opioid receptor.

A number of other pharmacological responses to U-50,488H were also less marked than those associated with fentanyl. The respiratory response, and the heart rate response were minimal after U-50,488H, and were not of a magnitude to significantly interfere with the use of this drug as a pre-anaesthetic medication. This is an important factor, since the cardiovascular responses to narcotic analgesics, particularly the classical u agonists are one of the principal factors which limit their usefulness during surgery.

Among the other physiological parameters measured, rectal temperature was only slightly increased at the highest dose administered. Other

parameters that were monitored included EMG and vertical pupil diameter. No significant effects on these parameters were observed.

The present study presents evidence for the existence of a new class of efficacious narcotic analgesics in the horse. Drugs such as U-50,488H which are selective agonists for the k-opioid receptor provide potent analgesia with fewer behavioral and autonomic side effects than currently used opiates.

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FIGURE 1

The time course of the effect of U-50,488H on hoof withdrawal reflex latency. Each point represents the mean response of eight horses.

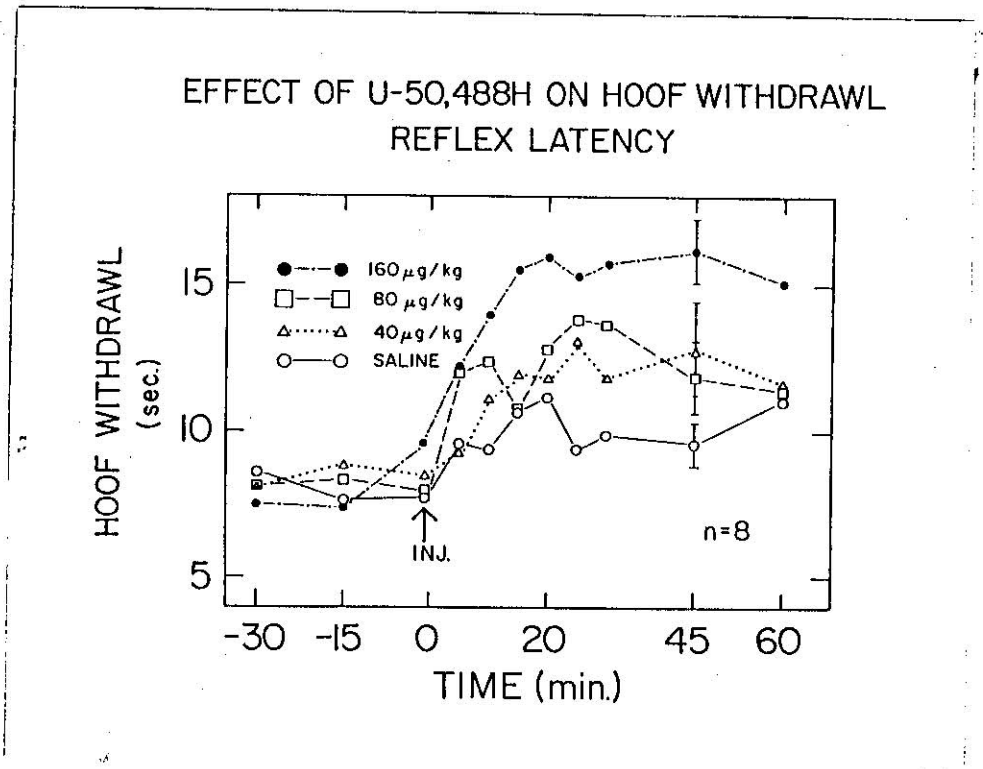

FIGURE 2

The time course of the effect of U-50,488H on skin twitch reflex latency. Each point represents the mean response of eight horses.

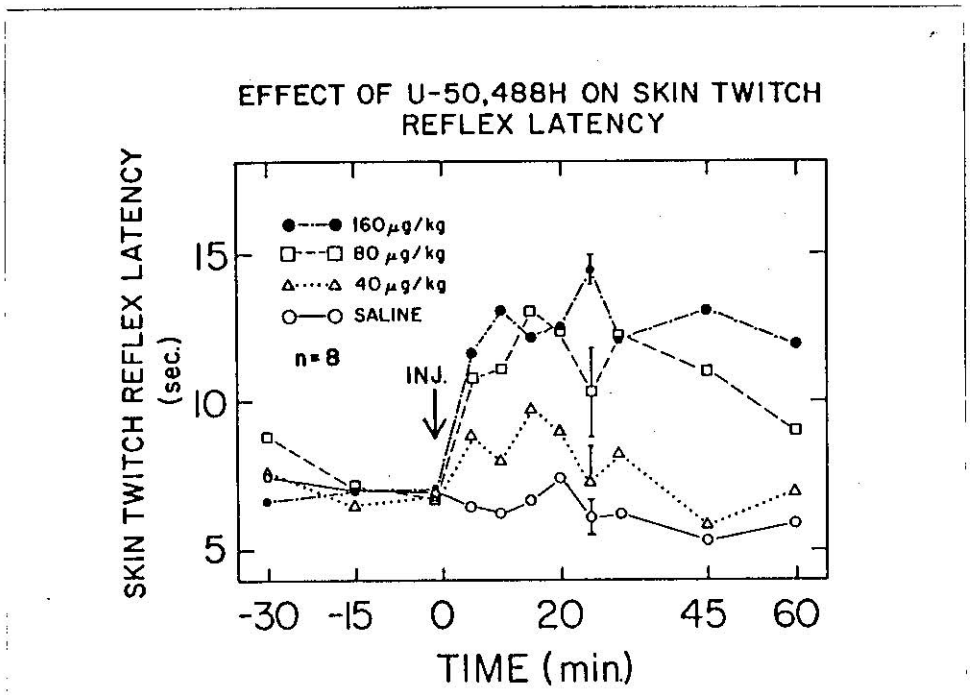

FIGURE 3

Time course of the effects of U-50,488H at 160, 80, and 40 ug/kg, fentanyl (0.010 mg/kg) and ethylketazocine (0.012 mg/kg) on spontaneous locomotor activity. Each point represents the mean response of eight horses.

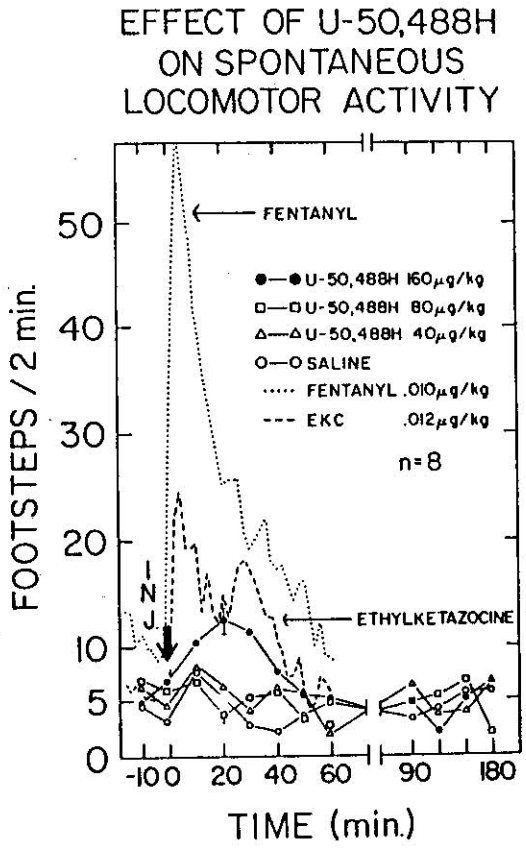

FIGURE 4

Effects of U-50,488H on pupil diameter, EMG activity, yawning frequency, rectal temperature and cardiac and respiratory rates. Each point represents the mean response of eight horses over four treatment sessions. Key: (O) 160 ug/kg; ( ) 80 ug/kg; ( ) 40 ug/kg; (O) saline control.

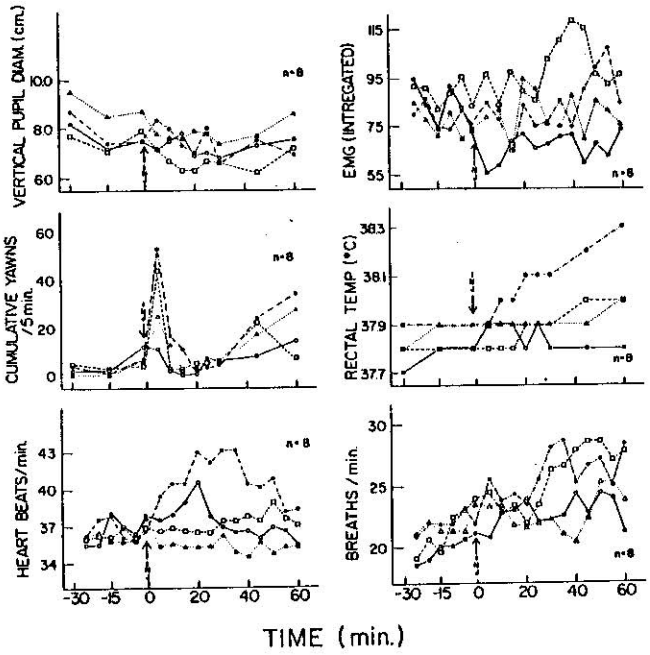

FIGURE 5

Time course of the effect of U-50,488H with and without naloxone pre-treatment. Each point represents the mean response of four horses and the vertical bars indicate standard error of the mean.