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# The Effects of Kappa-Opioid Receptor Narcotics In the Horse: A New Class of Equine Analgesics

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ABSTRACT

Sensitive methods for measuring the analgesic, physiologic and behavioral effects of opioids in performance horses have recently been developed. Using these methods, a new class of narcotic analgesics have been identified which produce analgesia without the locomotor and behavioral stimulation observed with currently used narcotics. These agents appear to produce analgesia and sedation by agonistic actions at kappa-opioid receptors.

The existence of several opioid receptors has been postulated to explain the actions of various narcotic substances. Fentanyl, an opioid which acts at μ- or morphine receptors, produced dose-related analgesia, locomotor stimulation, tachycardia, tachypnea and behavioral arousal. However, ethylketazocine, which acts mainly at k- or ketocylazocine receptors, produced a similar degree of analgesia, and one-half to one-third the degree of locomotor stimulation observed with fentanyl. The experimental compound U50-488H, a selective kappa agonist, produced prolonged dose-related analgesia and sedation with little or no locomotor stimulation. No dose related changes in cardiac or respiratory rates were observed. These data indicate that narcotics which act at μ- or k-receptors are potent analgesics in the horse. However, the sedation, and minimal locomotor and autonomic stimulation produced by the k-receptor opioids is therapeutically preferred. Therefore, these agents represent a valuable class of compounds with distinct advantages over the μ-receptor narcotic analgesics currently used in the treatment of equine pain.

# INTRODUCTION

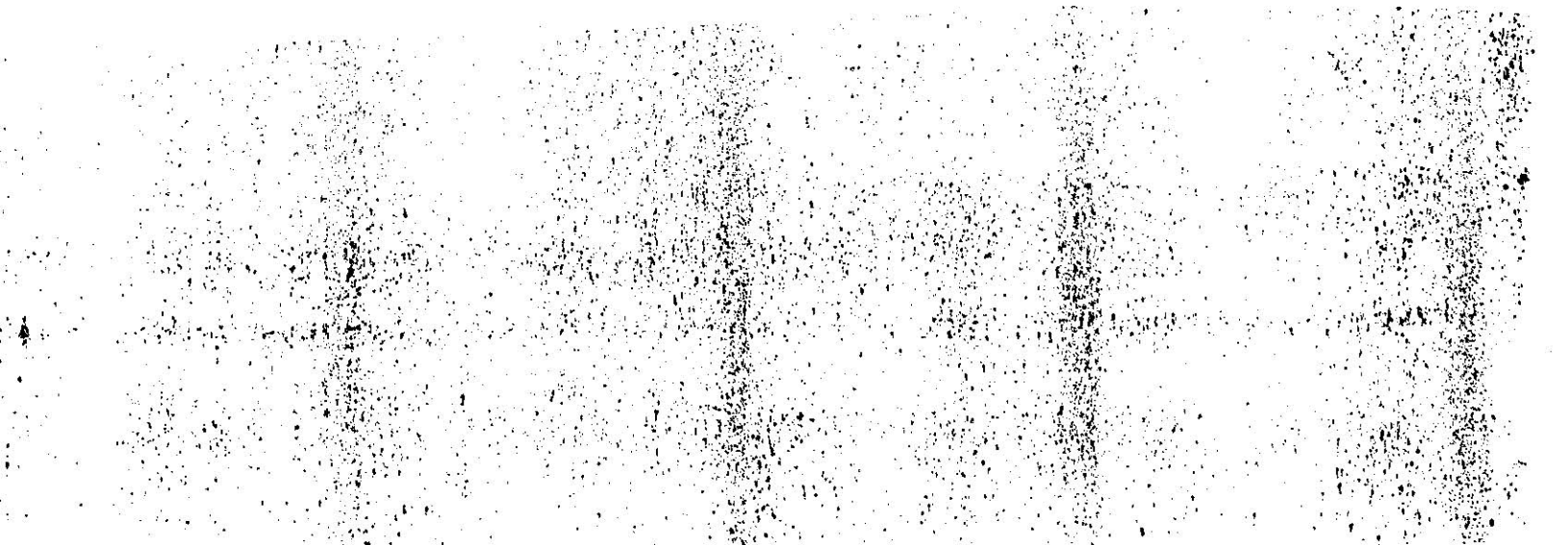
The existence of multiple opioid receptors is supported by biochemical, anatomical and pharmacological evidence. Different opioid receptor subtypes possess both different ligand binding characteristics and varying distribution in central and peripheral tissues. The inability of one opioid to suppress abstinence, or produce cross tolerance to another, has been demonstrated. There are differences in the quantity, location and type of opioid receptors found in a particular tissue or animal species. Different chemical classes of opioids produce distinct pharmacologic syndromes.[1,2]

At least two types of opioid receptors mediate analgesia. These have been classified as "μ" or morphine receptors, and "k" or ketocyclazocine receptors. Although both receptors are associated with opiate analgesia, they mediate different behavioral and autonomic responses. In the chronic spinal dog[3,4] morphine produced analgesia, miosis, bradycardia, bradypnea, hypothermia and behavioral indifference. However, only analgesia, miosis, and sedation occurred following ketocyclazocine administration. Furthermore, ketocyclazocine failed to suppress the abstinence syndrome in morphine-dependent animals. These observations lead to the postulation of multiple opioid receptors.[1,2] Since k-receptor opioids produced analgesia with fewer side effects in species such as the dog, a search for compounds with selective action at the k-site was begun.

The response to morphine in the horse differs markedly from that observed in man, rat and dog. Morphine produces marked sympathetic and

behavioral stimulation, rather than indifference or sedation. Tobin and co-workers[5,6] have demonstrated dose-related increases in locomotor activity following the administration of opioids which act predominantly at the μ-receptor. Despite their stimulant effects, μ-receptor opioids produce marked analgesia in horses and ponies.[7,8]

Most currently available narcotic analgesics are μ-receptor agonists (e.g. fentanyl, morphine, meperidine, methadone) or mixed agonist/antagonists (e.g. pentazocine, butorphanol). A propensity for producing sympathetic and locomotor effects has made their use hazardous and unpredictable. Clearly, a potent analgesic with fewer adverse effects is needed. Based on data from non-equine species, the k-opioids seemed likely candidates. To test this hypothesis, we administered increasing doses of fentanyl (a potent μ-agonist), ethylketazocine (a potent k-agonist with some μ-receptor activity), and U-50,488H (a selective k-receptor opioid) to the same group of performance horses. The time-course and dose-relationship of each agent on nociception, cardiac and respiratory rates, pupil size, body temperature and locomotor activity was determined. The following report describes the pharmacologic syndromes produced by μ- and k-receptor stimulation in the horse.



counting the number of footsteps taken per 2 min period.

Nociceptive thresholds and physiological responses were recorded concomittantly in horses placed in a laboratory and confined to equine stockades. Locomotor activity was assayed in the same subjects during a subsequent experimental session.

DRUGS

Fentanyl and ethylketazocine (EKC) were administered intravenously at doses of 0.010, 0.005, and 0.0025 mg/kg. U-50,488H was administered intravenously at doses of 0.040, 0.080 and 0.160 mg/kg. EKC was administered at doses of 0.003, 0.006, 0.012 mg/kg only during the locomotor session. Intravenous saline was administered as the control treatment.

STATISTICAL ANALYSIS

Treatments were administered at weekly intervals according to a Latin square crossover design. Prior to treatment, baseline responses were recorded for 30 min. There were no significant differences among baseline responses indicating a stable pre-treatment period. Post-treatment measurements were made every 5 min for the first 30 min, then at 45 and 60 min. Since most responses peaked within 20-30 min of drug administration, post-treatment responses were averaged over that time for each horse, treatment, and parameter. All values were then analyzed using an analysis of variance (ANOVA) in which variance among subjects, session, treatments, and interaction variances were calculated. Linearity over treatments was determined using a linear contrast test. Differences between a given dose-effect and saline were determined using

a test of least squares difference or Duncan's Multiple Range Test. All calculations were performed on an IBM 3083 computer using the (SAS) General Linear Models procedure.[11]

## RESULTS

### EFFECTS OF FENTANYL

Fentanyl produced a pharmacologic syndrome which differed markedly from that observed after EKC or U-50,488H. The results in Table 1

TABLE 1

Effects of fentanyl and saline on 5 physiologic parameters

| | Parameter | | | | |
|---|---|---|---|---|---|
| TREATMENT | S | H | F | C | R |
| Fentanyl | | | | | |
| 0.010 mg/kg | 12.0 ± 0.8* | 10.5 ± 1.0 | 38 ± 12* | 48 ± 3* | 50 ± 4 |
| 0.005 mg/kg | 10.1 ± 1.5* | 8.9 ± 0.7 | 27 ± 11 | 42 ± 2 | 43 ± 5 |
| 0.0025 mg/kg | 8.5 ± 0.9* | 9.9 ± 0.7 | 17 ± 11 | 39 ± 2 | 44 ± 5 |
| Saline | 5.9 ± 0.5 | 9.6 ± 1.1 | 12 ± 7 | 40 ± 2 | 40 ± 5 |
| Treatment effect[a] (P-value) | 0.0002** | 0.2266 | 0.0270** | 0.0004** | 0.2298 |
| Linear contrast[b] (P-value) | 0.0001** | 0.2768 | 0.0005** | 0.0016** | 0.0667 |

Treatment values represent the mean response (± SEM) of 8 horses obtained over a 20-25 min sampling period for each parameter.

Parameters: S = skin twitch reflex latency (s);
H = hoof withdrawal reflex latency; F = footsteps/2 min;
C = cardiac rates (beats/min);
R = respiratory rate (breaths/min);

*Indicates drug values are significantly different from saline ($P<0.05$)
**Indicates statistical significance ($P<0.05$)
[a]If $P < 0.05$, there is a significant difference among treatments
[b]If $P < 0.05$, there is a significant linearity over treatments

indicate that fentanyl produced significant dose-related increases in the skin twitch reflex latency (STRL), footstepping frequency and cardiac rate. Differences among treatments were also significant. A linear increase in respiratory rate approached significance ($P = 0.067$). Pain threshold and locomotor activity showed the greatest augmentation. All three doses of fentanyl produced analgesia when compared with saline. A 2-fold increase in threshold was produced by the highest dose. Due to the large variance among subjects ($P = 0.0002$) only the 0.10 mg/kg produced significant locomotor enhancement when compared to saline. However, a 3-fold increase in footstepping frequency was observed at that dose. Pupil diameter and rectal temperature were inconsistantly increased (not shown). Equine behavior under fentanyl was characterized by restlessness, spontaneous locomotor activity, vocalization, and hyper-responsiveness to visual, auditory, and tactile stimuli. Changes in most physiological responses generally occurred within minutes of drug administration. Analgesia, tachycardia, and locomotor enhancement peaked within 10 minutes and recovered within 25-30 minutes. Inconsistent but slight increases in respiratory rate and rectal temperature occurred around 20 minutes post-dose.

EFFECTS OF EKC

Data in non-equine species suggest that EKC is primarily active at the k-receptor and to a lesser extent at the μ-receptor. This hypothesis was supported by our results. Table 2 shows that EKC produced

TABLE 2

Effects of ethylketazocine (EKC) and saline on 5 physiologic parameters

| | Parameter | | | | | |
|---|---|---|---|---|---|---|
| TREATMENT | S | H | T | P | TREATMENT | F |
| EKC (mg/kg) | | | | | EKC(mg/kg) | |
| 0.010 | 10.5 ± 1.2* | 13.5 ± 0.5* | 38.0 ± 0.1* | 9.4 ± 1.0† | 0.012 | 18 ± 7 |
| 0.005 | 8.0 ± 0.7* | 13.2 ± 0.8* | 37.9 ± 0.1 | 8.4 ± 0.7 | 0.006 | 12 ± 3 |
| 0.0025 | 6.0 ± 0.7 | 11.8 ± 0.8 | 37.85± 0.1 | 7.8 ± 0.5 | 0.003 | 12 ± 4 |
| Saline | 5.2 ± 0.8 | 10.5 ± 1.5 | 37.7 ± 0.1 | 8.4 ± 1.0 | Saline | 5 ± 1 |
| Treatment effect[a] (P-value) | 0.0008** | 0.0083** | 0.2270 | 0.0492** | | 0.3373 |
| Linear contrast[b] (P-value) | 0.0001** | 0.0012** | 0.0231** | 0.0231** | | 0.0476** |

Treatment values represent the mean response (± SEM) of 8 horses obtained over a 20 min sampling period for each parameter excluding T, which was sampled 25-30 min post-treatment.

Parameters: S = skin twitch reflex latency (s)
H = hoof withdrawal reflex latency
T = rectal temperature (°C)
P = vertical pupil diameter (mm)
F = footsteps/2 min

*Indicates drug values are significantly different from saline ($P<0.05$)
**Indicates statistical significance ($P<0.05$)
[a]If $P < 0.05$, there is a significant difference among treatments
[b]If $P < 0.05$, there is a significant linearity over treatments
†Indicates significant difference ($P<0.05$) from the low dose

dose-related increases in both the STR and HWR latencies. Significant differences among treatments were also observed. Both the high and medium doses produced significant increases in latency compared to saline. Comparison of the analgesic $ED_{50}$ (Table 4) shows that EKC was slightly less potent on a µg/kg basis than fentanyl. A test for

parallelism showed that the slopes of the dose-effect curves of these two opioids were not significantly different. This suggests that fentanyl and EKC may have acted at a common receptor to produce analgesia. The analgesic effects of fentanyl could not be compared using the HWR assay, because of the propensity of fentanyl to produce spontaneous foreleg flexion.

Despite its analgesic effects, EKC produced only a slight increase in locomotor activity and no spontaneous foreleg flexion. Although the locomotor response was linear over treatments, EKC effects could not be distinguished from saline at any dose. Furthermore, there was no significant difference among treatments (Table 2). Table 5 shows that the slopes of the locomotor dose-effect curves of EKC and fentanyl were significantly different or non-parallel. This suggests that fentanyl and EKC exerted their locomotor effects at different receptors.

EKC failed to alter cardiac or respiratory rates (Table 2). However, slight linear increases in rectal temperature and pupil diameter were observed. At the low dose, EKC tended to constrict pupils, while at the high dose, it produced dilation. These values were significantly different from each other, but not from saline. A significant difference among treatments was observed for pupil diameter. Rectal temperature was significantly elevated only at the high dose.

Although a few horses showed initial signs of excitement at the high dose of EKC, most animals showed progressive signs of sedation. A brief period of ataxia and repetitive yawning was observed just after drug administration. This progressed to head drop, muscle relaxation, ptosis, and inactivity which lasted around 20-25 min.

TABLE 3

Effects of U-50,488H and saline on 5 physiologic parameters

| | Parameter | | | | |
|---|---|---|---|---|---|
| TREATMENT | S | H | F | T | Y |
| U-50,488H (mg/kg) | | | | | |
| 0.060 | 12.9 ± 0.4* | 15.0 ± 0.4* | 8.4 ± 0.6* | 38.2 ± 0.1* | 2.3 ± 0.5* |
| 0.080 | 11.2 ± 0.5* | 12.2 ± 0.4* | 4.7 ± 0.4 | 38.0 ± 0.1 | 1.5 ± 0.4 |
| 0.160 | 8.0 ± 0.4 | 11.7 ± 0.5 | 5.3 ± 0.5 | 37.9 ± 0.1 | 1.5 ± 0.3 |
| Saline | 6.3 ± 0.3 | 10.1 ± 0.3 | 4.3 ± 0.4 | 37.8 ± 0.0 | 0.8 ± 0.2 |
| Treatment effect[a] (P-value) | 0.0001** | 0.0001* | 0.1305 | 0.0116** | 0.1058 |
| Linear contrast[b] (P-value) | 0.0001** | 0.0001* | 0.0363** | 0.0014** | 0.0002** |

Treatment values represent the mean response (± SEM) of 8 horses obtained over a 60 min sampling period for each parameter excluding temperature, which was sampled 30-60 min post-treatment.

Parameters: S = skin twitch reflex latency (s)
H = hoof withdrawal reflex latency
F = footsteps/2 min
T = temperature (°C)
Y = yawning frequency

*Indicates drug values are significantly different from saline ($P<0.05$)
**Indicates statistical significance ($P<0.05$)
[a]If $P < 0.05$, there is a significant difference among treatments
[b]If $P < 0.05$, there is a significant linearity over treatments

EFFECTS OF U-50,488H

The effects of U-50,488H were qualitatively similar to those obtained after EKC. Linear dose-related increases in the STRL and HWRL were observed over a 60 minute period (Table 3). Differences among

treatments were also significant. Elevations in threshold were seen at the high and medium dose for the STR assay, and at all three doses for HWR assay. U-50,488H produced longer lasting analgesia than either fentanyl or EKC. At the higher doses, this effect persisted for 60-100 min. Although the slopes of the analgesia dose-response curves did not differ among the opioids, the U-50,488H slope was roughly twice that of fentanyl and EKC (Table 4). Furthermore, data in Table 4 indicates that U-50,488H produced a greater percent of maximal analgesia than EKC or fentanyl, despite the fact that it was less potent (μg/kg) than the latter two opioids.

TABLE 4

Analgesic effects of μ- and κ-opioids

| DRUG | DOSE (μg/kg) | % MAXIMAL ANALGESIA | SLOPE | PARALLELISM |
|---|---|---|---|---|
| Fentanyl | 2.5 | 56 ± 5 | 0.4032 | vs. EKC (NS) |
| | 5.0 | 62 ± 9 | | vs. U-50,488H (NS) |
| | 10.0 | 69 ± 4 | | |
| EKC | 2.5 | 38 ± 3 | 0.5271 | vs. Fentanyl (NS) |
| | 5.0 | 46 ± 4 | | vs. U-50,488H (NS) |
| | 10.0 | 56 ± 5 | | |
| U-50,488H | 40.0 | 53 ± 5 | 1.1095 | vs. Fentanyl (NS) |
| | 80.0 | 75 ± 8 | | vs. U-50,488H (NS) |
| | 160.0 | 84 ± 5 | | |

Percent maximal analgesia was calculated from the formula; Σ STRL (obs)/ Σ STRL (max) x 100. Eight STRLs (observed) were summed over 30 minutes. The maximum (max) possible latency was 15 sec/observation.

NS indicates that the slopes of the dose-response lines are not significantly different.

Table 3 shows that U-50,488H produced a modest degree of locomotor stimulation which was linear over treatments. However, differences among

treatments could not be statistically distinguished. Only the highest dose produced significant stimulation when compared to saline, but this increase is of questionable biological significance. Table 5 indicates that U-50,488H produced the smallest percent of maximal locomotor stimulation. Table 6 shows that the $ED_{50}$ for locomotor stimulation was at least an order of magnitude greater than EKC and 2 orders of magnitude greater than fentanyl. The slope of the U-50,488H locomotor-effect curve differed significantly from fentanyl but not from EKC. This suggests that U-50,488H and EKC acted at a common opioid receptor which differed from that mediating the action of fentanyl.

TABLE 5

Effects of μ- and κ-opioids on locomotor activity

| DRUG | DOSE (μg/kg) | % MAXIMAL LOCOMOTOR STIMULATION | SLOPE | PARALLELISM |
|---|---|---|---|---|
| Fentanyl | 2.5 | 18 ± 11 | 0.8313 | vs. EKC* |
| | 5.0 | 28 ± 11 | | vs. U-50,488H* |
| | 10.0 | 41 ± 12 | | |
| EKC | 3.0 | 12 ± 4 | 0.3434 | vs. Fentanyl* |
| | 6.0 | 12 ± 4 | | vs. U-50,488H (NS) |
| | 12.0 | 18 ± 7 | | |
| U-50,488H | 40.0 | 7 ± 3 | 0.4287 | vs. Fentanyl (P<0.05) |
| | 80.0 | 5 ± 2 | | vs. EKC (NS) |
| | 160.0 | 12 ± 3 | | |

Percent maximal locomotor stimulation was calculated from the formula; $\bar{X}$ footsteps per 2 min (obs)/$\bar{X}$ footsteps per 2 min (max) x 100. Footsteps/2 min were averaged over 20 minutes. The maximal number of attainable footsteps/2 min = 100, which was determined emperically in the locomotor laboratory.

*Indicates that the slopes of the dose-response lines are significantly different (P<0.05).

TABLE 6

$ED_{50}$ for analgesic and locomotor responses to μ- and κ-opioids

| DRUG | ANALGESIA (a) $ED_{50}$ (μg/kg) | LOCOMOTOR (1) $ED_{50}$ (μg/kg) | THERAPEUTIC RATIO $ED^{1}_{50}/ED^{a}_{50}$ |
|---|---|---|---|
| Fentanyl | 1.4 | 15.5 | 11.1 |
| EKC | 6.5 | 125.1 | 19.2 |
| U-50,488H | 33.7 | 2778.8 | 82.5 |

$ED^{a}_{50}$ = dose (μg/kg) which produced 50% maximal analgesia.

$ED^{1}_{50}$ = dose (μg/kg) which produced 50% maximal locomotor stimulation.

$ED^{1}_{50}/ED^{a}_{50}$ = ratio of analgesia to locomotor effect. The higher the ratio, the greater the proportion of analgesia to locomotor stimulation produced.

U-50,488H produced a behavioral syndrome similar to that observed after EKC. However, signs of sedation were more pronounced and prolonged. Repetitive yawning episodes were particularly common. Quantitation of this behavior showed a linear dose-related increase. However, yawning frequency was distinguished from saline only at the highest dose (Table 3).

Dose-related increases in rectal temperature and significant differences among treatments were also observed with U-50,488H. However,

this opioid had no appreciable effect on pupil diameter, cardiac rate or respiratory rate.

## DISCUSSION

Results from this study suggest that the equine possess both μ- and k-opioid receptors, which mediate distinct pharmacologic syndromes. Mu-receptors stimulation (via fentanyl) is associated with analgesia, increased locomotor activity, sympathetic stimulation and behavioral arousal. Kappa-receptor stimulation (via U-50,488H and EKC) mediates analgesia and sedation.

### ANALGESIA

Studies in non-equine species have demonstrated qualitative and quantitative differences in the analgesia produced by μ- and k- opioids. These differences may reflect dissimilar receptor affinities or varying receptor locations within the central nervous system[12-15]. Studies in the dog, rat and mouse suggest that k-opioids produce greater analgesia to noxious chemical and pressure stimuli, and lesser and more variable analgesia to noxious thermal stimuli[2,12,16-19]. On the other hand, μ-opioids are apparently active against all types of nociceptive stimuli. Although non-thermal pain stimuli were not tested in our study, both U-50,488H and EKC produced potent analgesia in the heat evoked STR and HWR assays. In fact, U-50,488H, the most selective k-agonist used[20], produced stronger and more lasting analgesia than either EKC or fentanyl.

Unlike other species, k-opioids appear to be at least as effective as μ-opioids against thermal cutaneous pain in the horse. The reason for

this discrepancy is not readily apparent. Studies in the mouse, dog and rat suggest that μ-receptor analgesia is supraspinally mediated, while k-receptor analgesia occurs primarily in the spinal cord[4,5,13,16,21-23]. It may be that the spinal cord plays a greater role in pain modulation in the equine than do supraspinal sites. Unfortunately, the type and distribution of opioid receptors in the equine is not known. Another explanation is that μ- and k-opioids act as a common "analgesia-receptor". The fact that we could not demonstrate a difference between any of the slopes of analgesia dose-response curves supports this notion. However, experiments using additional doses of agonists and antagonists would be necessary to adequately test this hypothesis.

LOCOMOTION

The μ- and k-opioids clearly showed a difference in potency in producing locomotor stimulation. Significant differences in slopes between the fentanyl dose-response curve and the U-50,488H and EKC curves suggests that μ- rather than k-receptors, mediate narcotic-induced locomotor stimulation in the hores. This hypothesis is supported by behavioral and neurochemical studies in other species. In the mouse, a species excited by morphine, k-opioids produce a decrease in locomotor activity and no Straub tail[23-27]. Furthermore, μ- but not k-opioids increased dopamine turnover in the murine striatum[24]. Kappa-opioids have no apparent agonist activity on striatal dopamine neurons, and may even antagonize morphine-induced locomotor stimulation[28]. Mu-agonists such as morphine and fentanyl are thought to augment locomotor activity by releasing dopamine from neurons in the

nigrostriatal pathway[29]. Opioid-induced locomotion can be disrupted by dopamine receptor antagonists[6,30].

THERAPEUTIC RATIO

The relatively high analgesic potency and low locomotor potency of the k-opioids is therapeutically desirable. This is best illustrated by the "therapeutic ratio", calculated for each opioid in Table 6. Note that the ratio of analgesic to locomotor effects is highest for U-50,488H, followed by EKC, then fentanyl. According to several assays selectivity for the k-receptor follows the same order[14,15,20]. These data indicate that U-50,488H is more likely to produce greater analgesia with lesser locomotor stimulation than either EKC or fentanyl. The additional therapeutic advantages of the k-opioids are that (1) they produce sedation at analgesic doses rather than behavioral arousal, and (2) they do not alter cardiovascular or respiratory activity.

AUTONOMIC RESPONSES

The tachycardia, tachypnea and arousal produced by μ-opioids in the horse is well documented[8,31,32] and corroborates our findings with fentanyl. These sympathetic effects probably reflect narcotic-induced release of catecholamines from the adrenal medulla, as well as central excitation of vasomotor ccenters. Morphine-induced analgesia, excitement, and sympathetic stimulation in the cat have been attributed to the above mechanisms[33,34]. Based on the lack of sympathetic signs, it seems unlikely that EKC or U-50,488H released catecholamines except possibly at the highest doses.

The k-opioids have little effect on body temperature in the mouse[35], or dog[3,36]. However, EKC and other k-agonists produced

hypothermia in the rat, while fentanyl produced hyperthermia at low doses and hypothermia at high doses[37]. Our data suggests that k-receptors may be involved in temperature regulation in the horse, since both U-50,488H and EKC produced dose-dependent hyperthermia.

Pharmacologic evidence presented in this study supports the hypothesis that at least two types of opioid receptors exist in the horse. While stimulation of both μ- and k-receptors with preferential agonists produces analgesia, each receptor appears to mediate a distinct autonomic and behavioral syndrome. The k-opioids represent a potentially valuable class of narcotic analgesics for the treatment of pain in horses.

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