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# Dose-related effects of ethylketazocine on nociception, behaviour and autonomic responses in the horse

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Sensitive methods for measuring the analgesic, physiological and behavioural effects of opioids in the horse have recently been developed. Fentanyl, a prototypic μ-opiate receptor agonist, has been previously shown to produce a syndrome characterized by marked analgesia and locomotor stimulation as well as tachycardia, tachypnoea and behavioural arousal. To determine whether other opiate receptors mediate some of the actions of the narcotic analgesics in the horse, an agent with activity at κ- and to a lesser extent μ-receptors was studied using a vigorous experimental protocol. Like fentanyl, ethylketazocine (EKC) (0·0025–0·012 mg $kg^{-1}$ i.v.) produced marked dose-related analgesia to noxious thermal stimuli. Modest dose-related increases in locomotor activity, pupil diameter and rectal temperature were also observed. However, in contrast to fentanyl, EKC failed to produce any change in cardiac or respiratory rates and produced behavioural sedation rather than arousal. These data suggest that μ- and possibly κ-receptors can mediate the actions of narcotics in the horse.

The existence of multiple opiate receptors in a variety of species is strongly supported (see review by Martin 1984). Recent observations in this laboratory showed that fentanyl, a short acting μ-opioid agonist, produced analgesia, locomotor stimulation, tachycardia, and tachypnea in the horse (Kamerling et al 1985). These responses occurred in a dose-related fashion, over a range of intravenous doses from 0·0025–0·010 mg $kg^{-1}$. The analgesia and locomotor enhancement were attributed to actions on the central nervous system, while the tachycardia, tachypnea and behavioural arousal were thought to reflect sympathetic stimulation. Most opiates used clinically or illicitly in the horse have been μ-agonists (e.g. morphine, fentanyl, meperidine, oxymorphone and methadone) or mixed agonists/antagonists (e.g. butorphanol, and pentazocine). Most studies of μ-agonists in this species indicate that drugs of this class produce varying degrees of analgesia using the forelimb flexion model (Pippi et al 1979) or balloon induced colic model (Pippi et al 1979; Lowe 1969). Cardioacceleration, respiratory stimulation (Muir et al 1978, 1980; Lumb et al 1983), behavioural arousal, and enhanced locomotion (Tobin 1981) have also been reported following the administration of μ-agonists. The mixed agonists–antagonists produce qualitatively similar effects (Muir et al 1978; Tobin 1981; Lumb et al 1983).

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Studies in species other than the horse suggest that κ-opioid agonists produce a qualitatively different physiological syndrome from that obtained with the μ-opioid agonists (Martin et al 1976; Hayes & Tyers 1983; Gilbert & Martin 1976). In some species μ-agonists produce greater analgesia following noxious thermal stimuli than κ-agonists, whereas the latter are more selective against noxious visceral or mechanical stimuli (Tyers 1980, 1982; Upton et al 1982, 1983; Gilbert & Martin 1976). Furthermore, κ-agonists generally produce less locomotor stimulation and greater sedation than μ-agonists in species excited by the latter (Tepper & Woods 1978; Woods et al 1978). Many of the cardiopulmonary effects typically produced by μ-agonists are not observed with the κ-agonists (Gilbert & Martin 1976; Martin 1984; Hayes & Tyers 1983).

Although μ-agonists are potent analgesics in the horse, their use is limited by the attendant locomotor and sympathetic stimulation at analgesic doses. Since κ-agonists clearly produce analgesia in several pain models with apparently less autonomic and behavioural stimulation, we examined the effects of ethylketazocine (EKC), using two recently developed equine nociception assays. The effects of EKC on respiratory and cardiac rates, pupil diameter, locomotor behaviour, rectal temperature, and gross behaviour were also studied. The following report describes the syndrome observed in the horse following EKC administration.

## METHODS

### *Animals*

Mature Thoroughbred and Standardbred mares and geldings (400–600 kg) were used. The animals were kept at pasture and allowed free access to food and water. The horses were placed in standard box stalls (approximately 180 sq ft) 24 h before each experiment for acclimatization to the laboratory.

### *Locomotor assay*

For this measurement horses were placed in box stalls which were enclosed on all sides. A window made of one-way mirrored glass located in each door permitted observers to record behaviour without detection by the animal. Locomotor behaviour was quantified by counting the number of footsteps taken per 2 min period. A footstep was scored each time the right foreleg was lifted off the ground and returned along with a positional change (Combie et al 1979).

Preliminary studies with EKC indicated that intravenous doses in excess of 0·012 mg $kg^{-1}$ produced severe ataxia and recumbency in some subjects. However, none of the horses staggered or fell at the 0·012 mg $kg^{-1}$ dose. Therefore, it was chosen as the maximum, behaviourally tolerable dose. Doses of 0·012, 0·006, 0·003 mg $kg^{-1}$ and 0·9% NaCl (saline) were administered over four weekly sessions to each horse, according to a Latin square crossover design. Locomotor activity was quantified for 15 min before each injection to establish a steady pretreatment baseline. Since no significant difference between the four pretreatment curves ($P = 0{\cdot}5104$) was obtained, they were averaged and plotted as one line on the time action curve. Footstepping frequency was recorded every 2 min for 60 min after injection (Fig. 1).

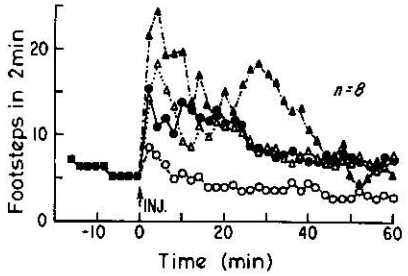


FIG. 1. Time course of the effects of EKC on locomotor activity. Each post-injection point represents the mean response of 8 horses. Each pre-injection point represents the mean response of 8 horses over 4 pretreatments. Key: (▲), EKC 0·012 mg $kg^{-1}$; (△), EKC 0·006 mg $kg^{-1}$; (●), EKC 0·003 mg $kg^{-1}$; (○), saline control; (■), $\bar{x}$ pretreatment values.

### *Nociception assay*

Nociceptive thresholds were measured using a modification of the methods of Hardy et al (1940) and Pippi et al (1979). A beam of intense light was focused upon the skin of the lateral aspect of the metacarpophalangeal joint at a fixed distance. In response to this noxious heat stimulus, the horse withdrew its foreleg. The time interval from lamp illumination to removal of the limb from the stimulus was designated the hoof withdrawal reflex (HWR) latency. A second beam of intense light was focused upon the withers which evoked a reflex contraction of the cutaneous trunci muscle when the stimulus became noxious. The time interval from lamp illumination to skin twitch was termed the skin twitch reflex (STR) latency. Lamp intensities were adjusted to produce a baseline HWR latency of 9–10 s, and an STR latency of 5–6 s. Cut-off latencies of 20 s for the HWR and 15 s for the STR were chosen to prevent tissue damage.

### *Physiological responses*

Cardiac rate was obtained from polygraph (Grass Model 7, Quincy, Mass.) recordings of an electrocardiogram. Respiratory rate was determined from polygraph recordings of impedence changes between bilaterally placed abdominal electrodes. Pupil size was quantified by measuring the vertical diameter of the elliptically shaped pupil from photographs of the eye, similar to the methods of Marquart et al (1967). Rectal temperature was recorded from a deep rectal probe and digital thermometer (Sensortek; Saddlebrook, NJ).

Nociceptive thresholds, cardiac and respiratory rates, pupil size, and rectal temperature were recorded concomitantly from horses placed in a laboratory and confined to equine stockades. Pretreatment measurements were made to obtain a steady baseline. There were no significant differences between pretreatment responses within any parameter. Therefore, all pretreatment data were plotted as mean responses on the time action curves of each parameter (Fig. 2). Pre- and post-treatment measurements were made every 5 min for 60 min for cardiac and respiratory rates. Pretreatment measurements of STR and HWR latency, pupil diameter and temperature were made every 15 min for 30 min. Post-treatment measurements were then obtained at 5 min intervals for the first 30 min, and at 45 and 60 min post-injection.

Ethylketazocine was administered at doses of 0·010, 0·005 and 0·0025 $mg^{-1}$ over four weekly sessions according to a Latin square crossover

design. Doses used in the physiological studies were slightly lower than those used in the locomotor assay because 0·010 mg kg$^{-1}$ was the maximum allowable dose for management of the horses in the laboratory.

Sedation was evaluated subjectively. Yawning, head-drop, unilateral hindlimb flaccidity, bouts of muscle relaxation and momentary loss of postural tone were considered symptomatic of sedation and drowsiness.

*Statistical analysis*

Peak EKC effects occurred within 20 min of drug administration for most parameters. Therefore, post-treatment observations were summed for 20 min after injection for each horse and treatment, giving a single, cumulative post-treatment value per animal. Since peak temperature effects occurred later, these values were summed at 25–30 min post-injection. All values were then analysed using an analysis of variance (ANOVA) in which variances among subjects, sessions and treatments as well as treatment by session variance were 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 calculations were performed on an IBM 3083 computer using a Statistical Analysis System program (Freund & Littell 1981).

*Drugs*

Ethylketazocine methanesulphonate was dissolved in approximately 10 ml of sterile saline and acidified with lactic acid. After dissolution, it was adjusted to physiological pH with bicarbonate and administered as a bolus into the left jugular vein. An equal volume of saline was administered as the control treatment. EKC was generously supplied by Sterling-Winthrop Research Institute, Rensselaer, NY.

## RESULTS

Ethylketazocine produced a marked but brief period of analgesia (Fig. 2). The STR was prolonged in a linear dose-related manner ($P = 0·0001$, Fig. 2) and differences among treatments were significant ($P = 0·0008$) over the 20 min post-dose period. EKC (0·010 mg kg$^{-1}$) produced a maximal 2-fold increase in STR latency (10·5 ± 1·2 s) compared with control (5·2 ± 0·8 s). Both this dose and 0·005 mg kg$^{-1}$ (8·0 ± 0·7 s) produced increases in latency which were significantly different ($P < 0·05$) from saline. A linear dose-related increase in the HWR latency was also observed ($P = 0·0012$). However, the magnitude of the increases at the middle and high doses were much less than those obtained in the STR assay. Only a 1·3-fold increase in HWR latency above control was observed at the high dose. Despite smaller dose-related changes in the HWR, significant differences among treatments ($P = 0·0083$) were found. Latencies obtained at the middle (13·2 ± 0·8 s) and high dose (13·5 ± 0·5 s) were significantly greater ($P < 0·05$) than saline (10·5 ± 1·5).

The effects of EKC on pupil diameter were complex. Wide fluctuations in pupil size were observed over the entire time course of drug action, particularly at the lower and middle doses (Fig. 2). Despite these fluctuations, significant differences ($P = 0·0492$) among treatments were obtained. The overall mydriatic effect of EKC was small since none of the doses could be distinguished from saline. However, pupil diameter was significantly larger ($P < 0·05$) at the high dose (9·4 ± 1·0 mm) when compared with that obtained at the lower dose (7·8 ± 0·5 mm). These data suggest a trend toward mydriasis and miosis at the high and low doses, respectively.

EKC produced a significant dose-related increase in rectal temperature ($P = 0·0231$). However, this response was observed at 25–30 min after injection. Despite the late onset, only the high dose (38·0 ± 0·1 °C) produced significant hyperthermia with respect to the saline control (37·7 ± 0·1 °C) ($P < 0·05$). This was the only parameter which showed a distinctly delayed response to EKC.

The effects of EKC on locomotion were modest. A linear dose-related increase in stepping frequency was observed ($P = 0·0476$). However, these changes were of such small magnitude that differences among treatments ($P = 0·3373$) and differences between the various doses and saline could not be statistically distinguished. The EKC-induced locomotor response was qualitatively different from that observed after fentanyl. Locomotion following EKC appeared to be a more compensatory response to ataxia, and an effort to stabilize and maintain postural tone. Locomotor stimulation induced by fentanyl was characterized by rapid onset, repetitive circling.

Although a few horses showed signs of behavioural arousal following administration of the high dose of EKC (0·012 mg kg$^{-1}$), most animals showed progressive signs of sedation. A brief period of ataxia and repetitive yawning were observed within 5 min of drug administration. This progressed to head drop, muscle relaxation (particularly in the hindlimbs), ptosis and motionlessness. Two horses exhibited muscle tremor at the high dose.

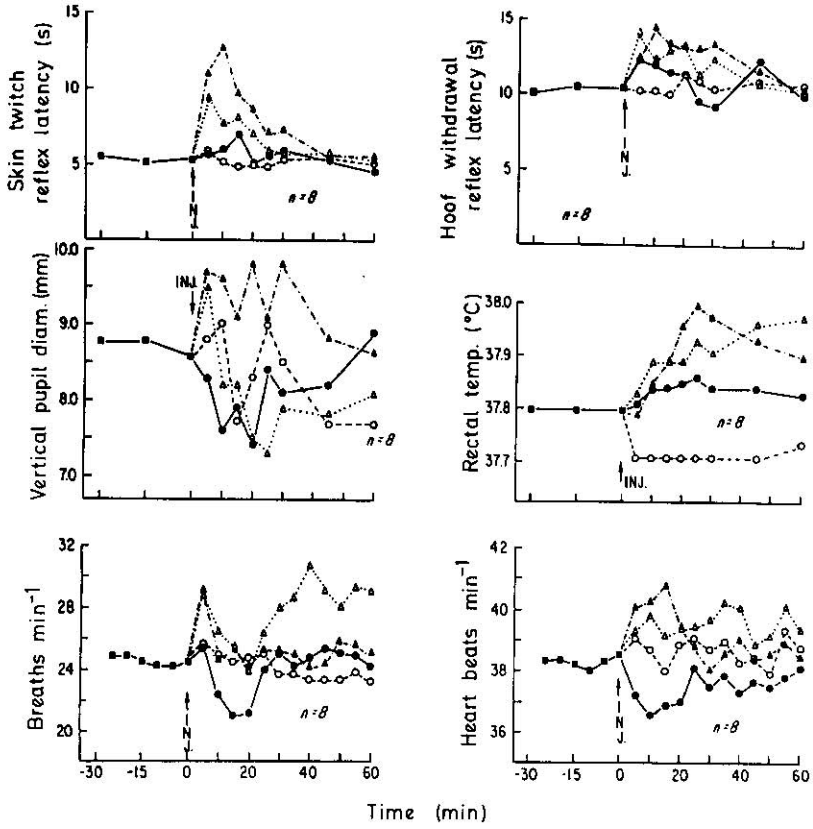


FIG. 2. Time course of the effects of EKC on skin twitch and hoof withdrawal reflex latencies pupil diameter, rectal temperature and cardiac and respiratory rates. Each post-injection point represents the mean response of 8 horses. Each pre-injection point represents the mean response of 8 horses over 4 pretreatments. Key: (▲), EKC 0·010 mg kg$^{-1}$; (△), EKC 0·005 mg kg$^{-1}$; (●), EKC 0·0025 mg kg$^{-1}$; (○), saline control; (■), $\bar{x}$ pretreatment values.

## DISCUSSION

Ethylketazocine produced a syndrome characterized by marked analgesia, sedation, and a modest amount of locomotor stimulation, mydriasis, and hyperthermia. The influence of EKC appeared greater on nociception than any other parameter studied. The fact that nociceptive thresholds rose linearly over treatments, differences among treatments were statistically significant, and STR and HWR latencies were elevated above control at the middle and high doses, argues that EKC is a potent analgesic in the horse.

We have recently observed that fentanyl (0·0025, 0·005, 0·010 mg kg$^{-1}$ i.v.) produced a dose-related increase in STR latency of approximately the same magnitude and duration as that obtained with EKC. Recent studies in other species, however, suggest that μ- and κ-agonists differ in their potencies against various types of nociceptive stimuli. Studies in the mouse, rat and dog showed that κ-agonists, such as EKC, MR 2033, and ketacyclazocine were more potent against noxious chemical and pressure stimuli, and less potent or inactive against noxious thermal stimuli (Gilbert & Martin 1976; Tyers 1980, 1982; Upton et al 1982, 1983). On the other hand, μ-agonists were effective in all three types of analgesic tests. This was clearly not the case in the horse. Both the HWR and STR latency were elevated in a dose-related manner following EKC administration, and both assays employed heat as the noxious stimulus. One explanation for this discrepancy is that EKC-induced analgesia might have occurred via μ-receptor stimulation. There is good evidence that EKC and other κ-agonists produce some of their effects by interacting with μ-receptors and are therefore weak μ-agonists (Martin et al 1976; Hayes & Tyers 1983; Pickworth & Sharpe 1979; Gilbert & Martin 1976). The specific

role of the κ-receptor in mediating thermal analgesia in the horse cannot be established until more selective κ-agonists are tested on the equine model. Furthermore, since no attempts were made to antagonize the analgesia with naloxone, an action of EKC at non-opioid receptors cannot be excluded.

EKC-induced increases in locomotor activity, pupil diameter and rectal temperature were modest yet dose-related. The maximum dose of 0·012 mg $kg^{-1}$ used in the locomotor assay was selected because higher doses occasionally caused recumbency and compromised the precision of the assay. The high dose should have elicited a maximal response thereby optimizing chances for observing any locomotor effect over the range of doses tested. Despite the appearance of a dose-related increase in stepping frequency, this augmentation was small and even the effects of the high dose could not be distinguished from saline at any point along the time action curve. The major behaviours observed following drug administration were yawning, head drop, hind limb relaxation and degrees of ataxia. These behaviours were not observed following saline administration. We have previously observed marked dose-related increases in locomotor activity following injection of a variety of μ-agonists over a wide range of doses (Tobin et al 1979; Combie et al 1979, 1981). Behavioural arousal and vocalization were typically observed along with locomotor enhancement. It is likely that the modest degree of EKC-induced locomotor stimulation reflected weak μ- rather than κ-receptor stimulation. This hypothesis is supported by studies which showed that μ- and δ- but not κ-receptors mediated opiate-induced increases in murine dopamine turnover in the striatum (Wood et al 1980). Central release of dopamine from neurons in the nigrostriatal pathway is thought to mediate opiate-induced locomotor stimulation (Hollinger 1969).

On the other hand, ataxia and the signs of sedation occurring after the weak locomotor effect were probably κ-receptor mediated. This observation is consistent with studies in the mouse which, like the horse, is excited by morphine (Goldstein & Sheehan 1969). In the mouse, morphine increased while EKC decreased locomotor activity particularly at higher doses (Tepper & Woods 1978; Woods et al 1978). Ataxia and an immobile posture were also reported (Tepper & Woods 1978).

The effect of opiates on pupil size in the horse have not been systematically studied. Recent attempts by this laboratory to measure pupil area following fentanyl administration failed to reveal any consistent changes. The effects of EKC on this parameter are complex. The data suggest that at low doses EKC may be a weak miotic and at high doses a mydriatic. This duality of action may be a consequence of stimulation of both μ- and κ-receptors. However this has not been observed in other species. Both the μ- and κ-agonists produce miosis in the dog. In fact EKC is a more potent miotic than morphine (Martin 1984; Gilbert & Martin 1976). In the rat, EKC and morphine both produced mydriasis (Adler 1982), while in the mouse EKC was without effect (Hayes & Tyers 1983). If the qualitatively similar effects of μ- and κ-agonists obtained in other species can be extended to the horse, then μ-receptors may mediate EKC-induced mydriasis.

κ-Receptor agonists had little or no effect on body temperature in the mouse (Hayes & Tyers 1983) or dog (Martin et al 1976; Gilbert & Martin 1976; Pickworth & Sharpe 1979). However, multiple opioid receptor mechanisms have been postulated for temperature regulation (Martin 1984; Geller et al 1983). Recent studies in the rat have shown that fentanyl and morphine produced naloxone reversible hyperthermia at low doses and hypothermia at high doses. On the other hand, EKC and other κ-agonists produced only hypothermia, which was somewhat resistant to naloxone blockade (Geller et al 1983). In the present study, a dose-related increase in rectal temperature was observed 25–30 min after drug injection and this increase was significant only at the high dose. Although we did not observe a significant increase in rectal temperature following fentanyl administration, it is not clear whether μ- or κ-receptors mediated the EKC-induced hyperthermia in the present study.

The lack of significant change in respiratory or cardiac rates following EKC is consistent with the lack of potency of EKC on these parameters in the dog (Gilbert & Martin 1976; Hayes & Tyers 1983). However, it contrasts with the fentanyl-induced tachycardia and tachypnea we have observed previously. These data argue that μ-receptor stimulation by EKC was not sufficient to produce the cardiac and respiratory stimulation observed after equi-analgesic doses of fentanyl. The role of κ-receptor stimulation in equine cardiorespiratory function is not known.

In conclusion, EKC produced marked dose-related analgesia and weak locomotor stimulation, mydriasis, and hyperthermia, and sedation. Both the heat-evoked STR and HWR were significantly prolonged indicating that EKC is a potent analgesic against noxious thermal stimuli in the horse. The lack of selectivity of EKC for κ-receptors prevents

attribution of the above effects to a particular opiate receptor. However, the lack of effect on the cardiorespiratory system, the mixed effect on pupil diameter, and signs of sedation suggest a degree of κ-receptor involvement in some of the actions of EKC in the horse.

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