EQUINE VETERINARY JOURNAL
Equine vet. J. (1989) **21** (1), 4-12

Review Article

Narcotic analgesics, their detection and pain measurement in the horse: A review

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Summary

Narcotic analgesics produce pharmacological effects by interacting with specific opiate receptors. At least five major types of opiate receptors have been recognised. These include μ (morphine) and kappa (ethylketazocine) receptor types. Narcotic analgesics which interact with μ receptors produce locomotor and autonomic stimulation at doses that produce little or no analgesia. Therefore, use of these drugs as analgesics in equine medicine has not been very satisfactory. Theoretical considerations suggested that the role of kappa agonists in equine analgesia be investigated. Using a pure kappa agonist, U-50, 488H, good analgesia was produced in the horse with little or no locomotor stimulation or autonomic effects. These data suggest that kappa agonists may be superior analgesics for clinical use in the horse. On the other hand, the locomotor stimulant effects of μ agonist analgesics enable their use as illegal medications. Specifically, these agents produce a good running response, signs of central nervous stimulation and analgesia, all potentially useful effects in a racehorse. Regulatory control of most narcotic analgesics can be obtained by high performance thin layer chromatographic screening. However, effective screening for the fentanyls and small doses of etorphine can only be achieved by use of immunoassay.

Introduction

NARCOTIC analgesics have long been used in the horse particularly to control the acute pain of spasmodic colic. More recently, they have been used illegally for their analgesic and stimulant actions in racehorses (Tobin 1981). For this reason the present authors have been investigating the pharmacology of narcotic analgesics in the horse which has led to a better understanding of their actions, the selection of more clinically useful agents and improved methods of their control in racehorses.

Narcotic analgesics have been associated with excitement or, as often stated, 'unpredictable' reactions in horses (Tobin 1981). Central nervous system stimulation and increased locomotor activity are highly predictable and dose related responses after the administration of most narcotic analgesics to horses (Combie, Dougherty, Nugent and Tobin 1979a). Different types of narcotic receptors and endogenous opiate ligands have been identified, resulting in a better understanding of the actions of opiate drugs in the horse (Martin, Eades, Fraser and Winkler 1964; Martin 1967, 1984).

This review presents recent advances in the understanding of narcotic analgesics in the horse and demonstrates how awareness of their basic actions can increase effectiveness of selection and use.

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Opiate receptors

Specific binding sites have been identified (Pert and Snyder 1973) which show all the characteristics required of pharmacological receptors for opiate analgesics. The binding is specific, saturable, of high affinity and the interaction is reversible. Binding sites are found at specific locations and have appropriate affinities for agonists and antagonists. Identification of these binding sites established the site of action of the narcotic analgesics (Snyder 1977).

Dissection and pathway studies have shown that these receptors are found at several levels of the pain pathways. In primates, the brain areas with high concentrations of the receptors are the intra-laminar nucleus of the thalamus, the hypothalamus, the periaqueductal grey matter and, particularly, the amygdala of the limbic system. Other areas include the guinea pig ileum and the mouse vas deferens, commonly used in *in vitro* systems for study of the actions of opiates (Kosterlitz, Waterfield and Berthoud 1973).

Hughes *et al* (1975) reported identification of two pentapeptides, leu- and met-encephalin, which were found to mimic morphine in its pharmacological actions. Further study showed that met-encephalin was part of a 91 amino acid chain of a previously discovered peptide, β-lipotropin (β-LPH) (Li and Chung 1976).

β-LPH is derived from a larger precursor molecule which contains the same amino acid sequence as adrenocorticotropic hormone (ACTH). The amino acid sequence 61-91 of β-LPH, β-endorphin, is also a very potent opioid compound. Other opioid compounds that have been identified include alpha (α) and delta (δ) endorphin and dynorphin. Dynorphin appears to possess very

dramatic behavioural and motor effects, but has limited analgesic properties (Nugent *et al* 1982).

The pharmacological effects of opioid peptides are similar to those of natural opiates. Responses to the peptides include analgesia, hypothermia, respiratory depression, tolerance, physical dependence and a number of behavioural changes. In addition, the opiates cause release of growth hormone, prolactin and ACTH, and decreased release of follicle stimulating hormone and luteinising hormone.

The principal difference between opiates, encephalins and endorphins is in their biological stability. Encephalins are unstable with actions lasting only for minutes. Longer chain endorphins are resistant to breakdown, and their effects last for up to 4 h. The duration of the action of the naturally occurring opiates is quite variable, but a single dose of morphine in the horse can act for 12 h or more (Combie *et al* 1979a).

Receptor subtypes

Even before the opiate receptors had been identified, evidence had been developed for the presence of more than one type of receptor (Martin and Eades 1964). For example naloxone, a potent narcotic antagonist, was relatively ineffective in antagonising the actions of cyclazocine, and the abstinence syndrome, which followed withdrawal from morphine, was different from that seen after nalorphine and cyclazocine. Martin (1984) suggested the existence of receptor dualism with the action of cyclazocine occurring at a different receptor from that of morphine. In a classic series of studies in dogs, Martin *et al* (1976) demonstrated pharmacological evidence for the existence of multiple opiate receptors. They suggested three different receptors, arbitrarily named mu (μ), kappa (κ), and delta (δ) receptors.

A prototypic μ receptor agonist such as morphine produces the analgesic triad of analgesia, sedation and respiratory depression in most animals. Other effects include decreased body temperature, decreased heart rate, pupillary constriction and anticonvulsent actions. All of these effects are blocked by naloxone, and cross-tolerance among μ agonists occurs.

Kappa receptor agonists such as ketocyclazocine produce behavioural sedation, analgesia and miosis, but have little effect on heart rate, body temperature or the skin twitch reflex (Kamerling, Weckman, Donahoe and Tobin 1985c) A κ agonist will not suppress abstinence or precipitate withdrawal in the morphine-dependent dog, and will not substitute for morphine in the dependent monkey.

The sigma (σ) receptor is a third distinct type of narcotic receptor for which evidence was gained in the chronic spinal dog model. The prototypic agonist is n-allyl-normorphine (Martin *et al* 1976).

Other receptors are the δ receptor in rat vas deferens, which has a uniquely high affinity for encephalins, and a unique receptor in the central nervous system for the endorphins, the epsilon (ε) receptor (Schulz, Faase, Wuster and Herz 1979). These different receptor types and their properties are summarised in Table 1.

A number of narcotics exist which have actions at more than one receptor. These include the partial μ agonists buprenorphine, profadol and propiram; and the agonist-antagonists pentazocine, cyclazocine, butorphanol, nalbuphine and nalorphine. In small doses these agents are partial agonists at the μ receptor but become competitive in larger doses. Many of these agents are currently marketed for clinical use as narcotic analgesics in the horse (Martin *et al* 1976; Houde 1979).

Models for studying narcotic analgesics in the horse

An ideal analgesic should produce effective analgesia without any behavioural stimulation or cardiovascular effects. Attainment of these goals has been difficult because of the absence of suitable models for assessing the pharmacological effects of different narcotic analgesics in the horse. Further, where effective means of measuring the actions of these drugs were available, few dose response studies were carried out, making comparisons between drugs difficult.

While physiological parameters could be readily measured, the analgesic response was difficult to evaluate. Three models of pain perception in the horse have been reported. The balloon colic model of Lowe (1969, 1978) utilises a caecal fistula into which a balloon is inserted and inflated. As the pressure in the caecum increases, the horse shows corresponding signs of discomfort, which are scored by the observer. The principal disadvantage of this model is that the method is subjective, with no clear end point and further, the method relies on pressure and as such imitates flatulent colic, whereas most colics have a component of inflammatory pain. Narcotic analgesics have been shown to suppress colonic motor responses (Roger, Bardon and

TABLE 1: Proposed classes of opiate receptors; their putative agonists and antagonists, and functions

	Mu (μ)	Kappa (κ)	Sigma (σ)	Delta (δ)	Epsilon (ε)
Agonist					
	Morphine and related opiates	Ketocyclazocine	SKF 10,047	Leu-encephalin Met-encephalin	Endorphins
	Fentanyl Sufentanil Etorphine Levorphanol Meperidine	Ethylketazocine Bremazocine Mr 2034 U-50, 488H Dynorphin	Phencyclidine Benzomorphans	Several short opioid peptides	α-endorphin δ-endorphin δ-endorphin
Effects					
	Analgesia	Analgesia-spinal	Autonomic stimulation	Analgesia	Analgesia
	Locomotor activity		Dysphoria	Behavioural effects	
Antagonists					
	Naloxone Naltrexone	Mr 2266 WIN 44, 447 Naloxone			

(Adapted from DeQuick 1984)

Ruckebusch 1985), which may account for the apparent analgesia seen in Lowe's model.

Superficial pain is readily measured by a radiant light method (Beecher 1957), using the lateral surface of the forelimb as the test area (Pippi and Lumb 1979; Pippi, Lumb, Fialho and Scott 1979), such that when the horses withdraw their forelimbs, they are considered to have signalled their perception of pain. The problem with this model is that some drugs also produce locomotor responses which are difficult to distinguish from pain responses.

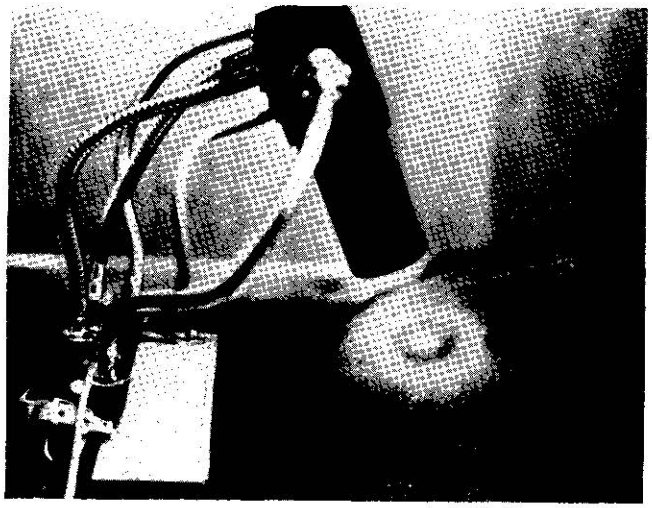
In a refinement of the radiant heat method Kamerling, Weckman, DeQuick and Tobin (1985b) designed a 'withers' superficial pain perception model (Fig 1) whereby light is directed onto the withers area and a skin twitch, caused by contraction of the subcutaneous trunci muscle, signals the end point. Because there is no interaction with locomotor responses, the present authors consider this to be the superior radiant heat pain perception model.

Other pain perception models reported are the dental enamel model of Scott and the periosteal model of Pippi (DeQuick 1984). No data on the dental enamel model have been reported, and Pippi has pointed out that the reliability of the periosteal model is not clear (Pippi and Lumb 1979). However, these models deserve further investigation, because pain perception studies in other species have shown that the effectiveness of drugs is often dependent on the model against which they are assayed (Chapman *et al* 1985).

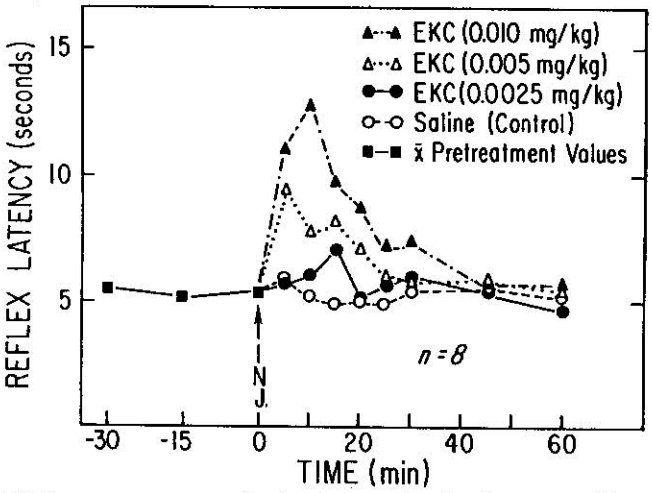
The only behavioural model reported for the narcotic analgesics is that of Tobin, Combie, Shults and Dougherty (1979b). In this model, horses are dosed with the drugs in a box stall, allowed to walk free and the number of footsteps that they make with their left foreleg scored. Most narcotic analgesics, and particularly the μ agonist type, produce a good locomotor response that is easily quantitated by this method. Using this method, Combie *et al* (1979a) produced the first dose and time response curves (Fig 2) for behavioural responses to narcotic analgesics in the horse. These studies showed that μ agonists first produce an eating response, then a locomotor response and finally incoordination and collapse. Plotting these data as dose response curves yielded a series of parallel dose response curves, which enabled accurate comparison of the potencies of different narcotic analgesics. This led Tobin (1981) to suggest that locomotor stimulation was an integral part of the action of narcotic analgesics, and that the locomotor effect was closely related to the analgesic effect. Tobin (1981) disputed the historic concept that narcotic analgesics are erratic and unreliable in the horse. Rather, they appeared to produce a very reliable central nervous stimulation, which was the reason they had fallen into disuse in the horse.

The fact that narcotic analgesics are stimulants in the horse has led to a continued search for a narcotic analgesic which would yield good analgesia, but little central excitation. In general, the approach has been to test new narcotic analgesics against clinically used doses of other drugs. However, because there was little information on which to base the doses of either drug, these studies often compared ineffective or poorly comparable doses of drugs. One major advantage of the behavioural and dose response work outlined in Fig 2 is that it provides the first logical basis for the selection of doses of these drugs.

Fig 1. Withers superficial pain model



(a) Withers model apparatus affixed by girth strap to experimental subject



(b) Dose-response curves showing increases in reflex latency to withers stimulus after treatment with ethylketazocine. Time of injection of control or drug (arrow)

Specific opiates

Morphine

Morphine, the prototypic μ agonist, is the narcotic analgesic against which all others are compared. Its pharmacology in the horse has been described by a number of workers (Amadon and Craige 1937; Muir, Skarda and Sheehan 1979; Combie *et al* 1979a; Tobin 1981; Combie, Nugent and Tobin 1983; Kalpravidh,

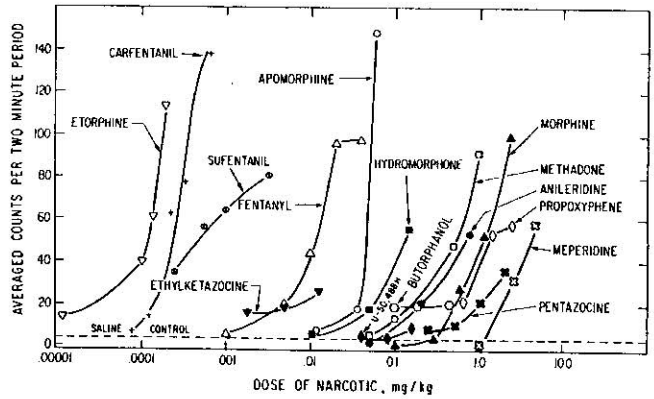


Fig 2. Locomotor effects of selected narcotics in the horse. The symbols show the peak locomotor responses as steps/2 mins to the rapid intravenous injection of the indicated doses of each agent. Apomorphine, not a narcotic analgesic, is included for comparative purposes

Lumb, Wright and Heath 1984).

The actions of morphine in the 'pain-free horse' at a dose level of 0.12 mg/kg bodyweight (bwt) or about 5 mg/horse, have been studied (Muir *et al* 1979). This dose produced no discernible behavioural effects in the present authors locomotor stimulation model (Fig 2) and, apart from producing transient stimulation of the heart, respiration and blood pressure had virtually no effect in Muir's *et al* (1979) horses. This author also suggested that the horses experienced euphoria and dysphoria following administration of morphine. In retrospect, the dose used by Muir *et al* (1979) appears to have been too small to produce significant cardiovascular or behavioural responses or, probably, any analgesic response.

The actions of morphine in the horse at about 0.66 mg/kg bwt or about 300 mg/horse have been determined (Kalpravidh *et al* 1984). Although high by clinical standards, this dose is only about 50 per cent of the ED50 for locomotor stimulation by morphine in the horse. At this dose, morphine stimulated locomotor activity for 4 to 5 h, but appeared to suppress superficial and intestinal pain for only 30 to 60 mins. These data suggest that the locomotor stimulant effects of morphine are more marked than its analgesic effects.

The cardiovascular effects of the doses of morphine used by Kalpravidh *et al* (1984) were also marked. Morphine increased systolic pressure at this dose for 4 h, with no sign of returning to control. Similarly, heart rate was significantly increased for 4 h, but no effects on central venous pressures were seen. Morphine also increased the respiratory rate for 4 h. The data suggest that the analgesic response, at least as measured by Kalpravidh *et al* (1984), was poorly responsive to morphine in comparison with the locomotor and cardiovascular responses. Although it is not clear how much of this effect was due to the difficulty of measuring analgesic responses in the horse, the data suggest, at least, that the behavioural and cardiovascular effects of morphine are stronger than its analgesic effects.

The pharmacokinetics of morphine in the horse have been determined by Combie, Blake, Ramey and Tobin (1981) who gave 0.1 mg/kg bwt (45 mg/horse), which was considered to be a dose without significant pharmacological effect, and found plasma levels of morphine dropping from about 600 ng/ml shortly after injection through a three phase compartment system to become undetectable in plasma after about 48 h. The initial urinary concentrations of free morphine and its major glucuronide metabolite were about 21 µg/ml, which became undetectable six days after dosing. This long plasma half-life of morphine is consistent with its relatively prolonged pharmacological effects in the horse, as shown by the duration of the behavioural and cardiovascular changes induced by this drug.

Based on the prolonged clearance of morphine in the horse, Combie *et al* (1981) recommended that morphine should not be administered to racehorses within one week of racing.

Fentanyl

Fentanyl is a potent short acting narcotic analgesic of the µ agonist type which is marketed as a short acting analgesic in human medicine. It is not approved for use in animals but, because of its potency, it has been used illegally in racehorses in the United States. This led to the behavioural studies on which the data of Fig 2 are based, and fentanyl has a central place in the understanding of the pharmacology of narcotic analgesics in the horse (Tobin 1978; Tobin *et al* 1979a,b).

When administered by rapid intravenous (iv) injection fentanyl produces a sharp increase in locomotor activity. The effect is short lived, peaking within 5 mins of administration and returning to background within 60 mins (Tobin *et al* 1979b). The response is remarkably stable, reproducible and repeatable (Combie, Dougherty, Shults and Tobin 1978).

The relationship between locomotor, analgesic, and autonomic responses to fentanyl have been studied (Kamerling, DeQuick, Weckman and Tobin 1985a). At doses of 2.5, 5 and 10 µg/kg bwt, fentanyl produces a dose related increase in the skin twitch latency, but no effects on hoof withdrawal latency. At these doses it also causes dose related increases in stepping frequency and cardiac and respiratory rates. Kamerling *et al* (1985a) interpreted this data to mean that fentanyl produces analgesia and sympathetic and locomotor stimulation in the horse at approximately equivalent doses.

The analgesic response to fentanyl was brief, lasting about the same period (30 mins) as the locomotor response. Effects on heart rate were brief, but the effects on respiratory rate and rectal temperature lasted longer. The data suggest that the effects of fentanyl, like those of morphine, are predominantly autonomic and behavioural, with relatively modest and transient effects on pain perception.

The actions of fentanyl in the horse at a dose of 0.22 mg/kg bwt (about 90 mg/horse) have been reported (Pippi and Lumb 1979). At this dose, they reported it to be the most effective drug in the treatment of superficial pain. However, correlation of these results with the present authors' is difficult because the reported dose is about five times that which caused collapse in the present authors' horses. Also, the fetlock model did not show an analgesic response to fentanyl in the present authors' study (Kamerling *et al* 1985a).

The pharmacokinetics of fentanyl in the horse are not well understood. The only test for blood levels of this drug is radioimmunoassay (RIA), the ability of which to distinguish between various forms of the drug is not clear. For this reason, kinetic studies on 'fentanyl' are usually described in terms of fentanyl equivalents and are, at best, estimates of the true kinetics of fentanyl in the horse.

Using this system, it has been shown that plasma levels of 'fentanyl' fell with an initial half-life of about 3 mins, then rapidly entered a second phase with a half-life of about 42 mins, followed by a third phase with a half-life of about 180 mins (Combie, Shults and Tobin 1979b). The rapid initial decrease corresponds with its relatively brief pharmacological action, suggesting that its pharmacological actions are terminated by redistribution.

Fentanyl or its metabolites can be detected in equine urine by commercial RIA for about four days. It is metabolised in the horse to the β-keto acid and despropionyl fentanyl, with about 90 per cent being β-keto acid (Combie *et al* 1979b). Despropionyl fentanyl is the form of fentanyl used in forensic detection of this drug, with the despropionyl form being reconstituted to fentanyl in the assay. This test, which was introduced in 1979, has served to control the use of fentanyl in racehorses in North America (Tobin and Combie 1984).

More recently, a number of different fentanyl analogues have been developed some of which are being introduced in human medical practice (Janssen 1985). These include sufentanil and alfentanil. Other fentanyls which have been developed include carfentanil and lofentanil, while α-methylfentanyl and 3-methylfentanyl have appeared in the illicit synthesis or so-called 'designer' drug market (Baum 1985). All of these agents are presumably classic µ agonists, and probably have the same spectrum of pharmacology as fentanyl in the horse, and also the same abuse potential.

In summary, fentanyl is a potent and rapidly acting narcotic analgesic in the horse. It produces marked locomotor and autonomic effects, and moderate analgesia. It is detected easily by RIA, and at least four days should be allowed for fentanyl to clear in the urine of a horse before urine testing. More recently, analogues of fentanyl have become available, and the ability to

screen for these agents is not as readily available as that for fentanyl.

Etorphine

Structurally similar to morphine, etorphine is a synthetic opiate synthesised from the pharmacologically inactive alkaloid thebaine. It is administered as the hydrochloride, usually in combination with a phenothiazine tranquiliser, for the immobilisation of large wild animals. For this reason it is referred to in the United States as 'elephant juice'. It has also been used in Great Britain to prepare domestic animals for surgery. The recommended dose for the horse is 25 µg/kg bwt (Booth and McDonald 1982).

Combie *et al* (1978) demonstrated that in low doses (100 µg/horse) etorphine produced the classic locomotor response of the narcotic µ agonists. They observed a peak locomotor response with a dose as small as 0.4 µg/kg bwt. Etorphine is characterised by extremely high potency (Anon 1977), rapid onset and short duration of action (Fig 2). Rosenbaum, Holford and Sadee (1984) and Combie *et al* (1979a) have shown that the analgesic effects are rapidly reversed by naloxone, diprenorphine, and nalorphine. Pharmacological studies have shown responses in the horse to include tachycardia, depression of respiratory rate, spastic rigidity of the limbs, and muscle tremors (Booth and McDonald 1982). These studies all suggest that etorphine is predominately a µ agonist.

Until recently, there was no reliable screening test for etorphine in the horse. Within the last three years an RIA analysis for this drug has become available which can detect etorphine doses of 10 µg/horse. Recently, another RIA has been developed which has improved on the sensitivity of this test, and can detect etorphine equivalents in post race urine for five days after a dose of 100 µg/horse (Tai *et al* 1987). Because the clinical dose of etorphine is about 100 times this dose, it appears that the clearance time for etorphine in the horse after use in this way is likely to be substantially more than five days (Tai *et al* 1987). It should be noted, however, that these tests have been empirically seen to detect doses of etorphine as low as 10 µg per horse, and thus comprise a highly effective screening procedure, though the chemical nature of the analyte has not yet been conclusively established.

Pentazocine

Pentazocine is a mixed narcotic agonist-antagonist which is marketed as an analgesic in veterinary medicine. Its pharmacology in the horse has been studied fairly extensively.

Pentazocine is a benzomorphan derivative that is reported to exert potent analgesic actions and has between one half and one sixth of the analgesic actions of morphine in man, with a low incidence of side effects. Its pharmacokinetics in the horse have been reported by Tobin and Miller (1979) and Davis and Sturm (1970) and its actions on pain by Lowe (1969, 1978).

Studies on pentazocine showed that the locomotor response to this drug did not appear until about 0.5 mg/kg bwt had been administered. The ED50 for the locomotor response was about 1.0 mg/kg bwt; the response peaked at about 2 mg/kg bwt with a response of about 40 steps/2 mins. This response is less than half that obtained with the prôtotypic µ agonists, morphine or fentanyl. The data show that pentazocine is somewhat less potent than morphine, and has much less efficacy in producing a locomotor response (Tobin and Miller 1979).

The locomotor response to pentazocine was also clearly different from that of the prototypic µ agonists in its tendency to cause muscle tremors and ataxia. The cardiopulmonary effects of pentazocine in the horse were reported by Muir *et al* (1978). The dose of pentazocine used was 0.9 mg/kg bwt or about the ED50 for this drug on locomotor response. This had no effect on respiratory rate, and a significant effect on heart rate and cardiac output for only 15 mins. However, both systolic and diastolic blood pressure were significantly increased for 30 mins after this dose.

In studies on the actions of pentazocine, using the balloon colic model, Lowe (1978) dosed his horses with about 1 mg/kg bwt, about the same dose as was used by Muir *et al* (1978). Lowe (1978) considered that this dose of pentazocine produced no observable analgesia in either trial. Pippi and Lumb (1979) also studied the actions of pentazocine on pain in the horse, at a dose of 2.2 mg/kg bwt, and did not consider it to be particularly effective.

Lowe (1969) showed that pentazocine at about 6 to 7 mg/kg bwt produced good analgesia for up to 2 h in his colic model. This dose, however, was more than six times the manufacturer's recommended dose, and Lowe reported side effects of muscle tremors, hypertonicity and unsteadiness.

It has been shown that the locomotor response to pentazocine in horses is very short lived at 1.0 mg/kg bwt, declining to less than control values within 20 mins (Tobin and Miller 1979). Only at 2.0 mg/kg bwt was a marked increase in locomotor activity seen, lasting for 2 h, which concurred with the observations made on the colic model (Lowe 1978).

The pharmacokinetics and urinary clearance of pentazocine in the horse have been studied (Davis and Sturm 1970; Tobin and Miller 1979). Davis and Sturm (1979) treated ponies with 3 mg/kg bwt intramuscularly (im) and followed blood levels of the drug for 7 h. They reported peak blood levels of about 0.5 µg/ml at 30 mins after dosing, after which plasma levels declined exponentially to become undetectable after 7 h.

Tobin and Miller (1979) reported that after a dose of 1 mg/kg bwt iv, pentazocine distributed relatively slowly in the horse, with an α-phase half-life of about 27 mins, and a β-phase half-life of about 138 mins. In agreement with other published data (Davis and Sturm 1970) blood levels of pentazocine peaked 30 mins after im injection of the drug, declining in a complex multi-exponential fashion. About 30 per cent of the administered dose was eliminated in the urine as a glucuronide metabolite. Following complete hydrolysis of the glucuronide metabolite in urine, pentazocine was detectable in equine urine for up to six days. Based on these observations, Tobin and Miller (1979) recommended that pentazocine should not be given to horses within at least six days of racing .

Butorphanol

Butorphanol is a synthetic narcotic of the nalorphine-cyclazocine series of narcotic agonist-antagonist compounds which is comparable or superior in potency to morphine. It is marketed for use in the horse as Stadol (Bristol Laboratories, New York). Its locomotor response, analgesic actions and cardiopulmonary effects have been studied in the horse (Heel, Brogden, Speight and Avery 1978; Tobin 1981).

Work by Tobin (1981) has shown that the maximal locomotor response to butorphanol in the horse is about 20 steps/2 mins, about one fifth of the maximal response to morphine (Fig 2). The data suggest that the stimulant effects of Stadol in the horse are small, and apparently poorly related to dose.

The cardiopulmonary effects of Stadol in the horse have been studied by Robertson, Muir and Sams (1981) who used the same doses as Tobin (1981). In accordance with the behavioural data, no significant changes were seen in heart rate, EEG interval, cardiac output, diastolic and mean pulmonary arterial pressures, respiratory rate, blood gases, and pH. However, arterial blood pressure was elevated in one experiment.

The behavioural effects of butorphanol in the horse have been reported by Robertson *et al* (1981). At high doses, signs of 'euphoria' were noted in all horses, with increased locomotor activity and ataxia lasting for up to 2 h. They also reported that their horses were less responsive to painful stimuli, although how this was determined was not described.

The actions of butorphanol on pain in horses at a dose of 0.22 mg/kg bwt have been studied (Kalpravidh *et al* 1984). At this dose level butorphanol produced virtually no effect on superficial pain, but had apparantely good effects on visceral pain, reducing its intensity for up to 4 h. Robertson *et al* (1981) reported that this dose of butorphanol significantly increased heart rate, but had virtually no other effects on the cardiovascular system.

In summary, 0.22 mg/kg bwt butorphanol appears to be effective in the treatment of visceral pain for up to 4 h but has little effect on cardiovascular responses, and apparently little central stimulant or locomotor activating effects. As a mixed agonist-antagonist, its analgesic actions appear to have been enhanced relative to its autonomic and central stimulant actions.

Ethylketazocine

Ethylketazocine is the prototype of a group of drugs that interact with κ receptors (Martin *et al* 1976). In general, κ agonists are more effective against noxious visceral or mechanical stimuli, and tend to produce less locomotor stimulation and more sedation than μ agonists (Kosterlitz *et al* 1973). Because members of this group of analgesics had never been tested in the horse, the present authors determined their effects on nociception, autonomic responses, and locomotion.

Ethylketazocine produced good dose related analgesia in the thermal stimulus model, modest changes in locomotor activity, and no increase in cardiac or respiratory rate (Kamerling, DeQuick, Weckman and Tobin 1986a). The data suggest that, in the horse, κ receptor stimulation differs from μ receptor stimulation, and that κ agonists may be useful therapeutic agents (Kamerling *et al* 1985c).

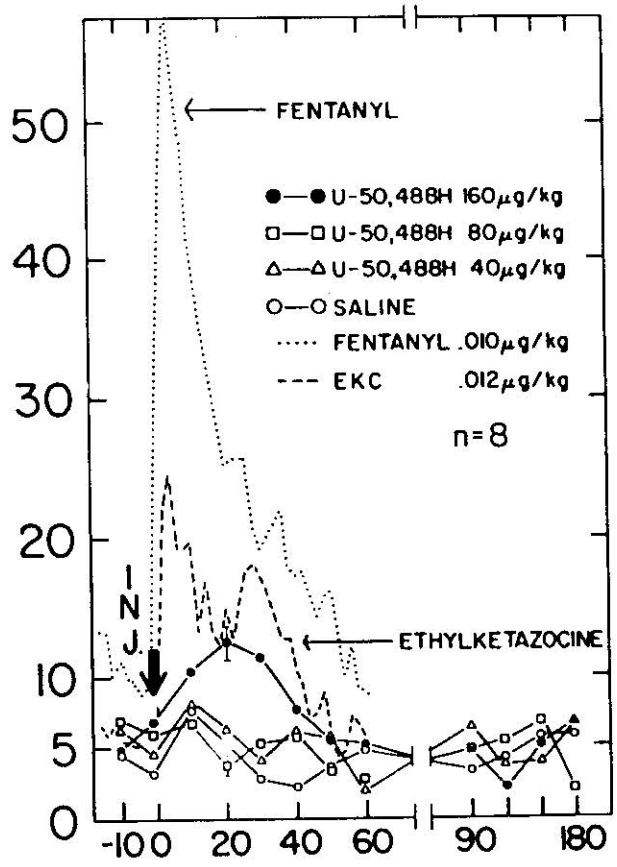
U-50,488H

U-50,488H is a structurally novel and selective κ opioid agonist (Vonvoigtlander, Lahti and Ludens 1983). It displays analgesic effects in a number of different analgesic assays, which are blocked by naloxone. It shows no cross tolerance with morphine, and does not cause morphine type physical dependence. These observations suggest that the analgesic actions of U-50,488H are mediated by the so-called κ receptors which are different to those at which morphine produces its effects. U-50,488H also produces sedation and diuresis (Vonvoigtlander *et al* 1983).

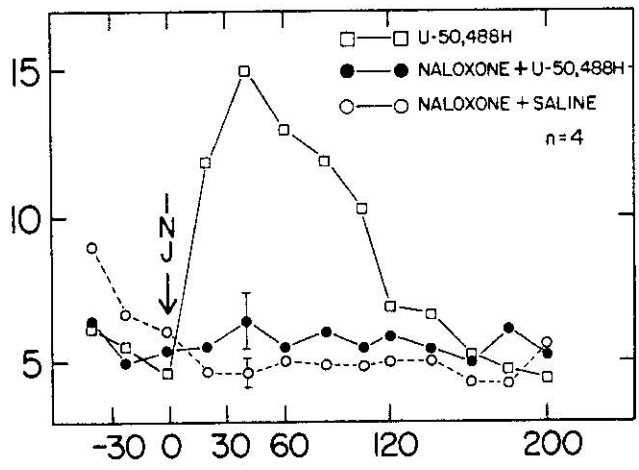
As shown in Fig 3a, U-50,488H produced virtually no locomotor stimulation in the horse, in sharp contrast with the marked locomotor response to fentanyl, and the more modest but still substantial response to ethylketazocine (Kamerling *et al* 1985a, 1986). As well as showing a reduced locomotor response, U-50,488H produced good analgesic response that lasted about 2 h after iv administration (Fig 3b). This analgesic response was accompanied by an apparent sedative response, in contrast with the marked excitement produced by most μ agonists. Few signs of autonomic stimulation were seen, supporting suggestions that this drug could be a useful sedative and analgesic in the horse (Kamerling *et al* 1985c, Kamerling, Weckman, Donahoe and Tobin 1988).

An unexpected response to U-50,488H was a yawning reaction soon after iv injection, which reappeared later; its significance is unclear. Although U-50,488H has been shown to have diuretic effects in mice, no signs of a diuretic response were seen in any of these experiments.

Fig 3. Locomotor and analgesic effects of selected narcotics



(a) Spontaneous locomotor activity after fentanyl, ethylketazocine and U-50,488H. The dotted line (...) shows the locomotor response of four horses after the medicated dose of fentanyl was administered intravenously. The dashed line (---) shows the response after the indicated dose of ethylketazocine, while the symbols show the response observed after the indicated doses of saline or U-50,488H



(b) analgesic effect of U-50,488H and its antagonism by naloxone. The open squares (□-□) show the analgesic response to U-50,488H after iv administration of 160 μg/kg, in the presence of naloxone, 20 μg/kg (●-●) administered iv 5 mins before U-50,488H. Time of injection of control or drug (arrow)

In summary, U-50,488H produced good analgesia in the horse, with little locomotor, autonomic or central nervous stimulation. It does not produce morphine type physical dependence or substitute for morphine. It should, therefore, have little abuse potential in man. These qualities are in sharp contrast with the characteristics of typical μ agonist analgesics.

The endogenous opiates

The potential for abuse of endogenous opiates and encephalins has been a problem for racing regulators. The encephalins are seen as compounds which could mimic the actions of narcotic analgesics in horses, but would be difficult to regulate. Therefore, they could be used to produce central stimulant and analgesic effects, but avoid regulatory control because the agents are normal constituents of horse urine.

Because of these concerns the present authors investigated the actions of D-ala^2-metencephalinamide and leucine encephalin in horses (Nugent *et al* 1982). These agents were administered both iv and intracisternally (ic) and their pharmacological actions compared with those of classic narcotic analgesics. The results suggested that these agents are not likely to be useful stimulants in racehorses.

Leucine encephalin had little effect on locomotor activity after either iv or ic administration at the doses tested. Methionine encephalin had no significant effect when given iv but when given ic it produced an increase in locomotor activity, a rise in temperature, hyperventilation and a marked increase in blood pressure. Other signs included a rapid eyeblinking, lack of coordination and quivering. Overall, the response would not appear to be useful in racehorses, and the ic route of administration would be difficult to use in a clinical situation. Based on these observations, it is unlikely that these agents could be used successfully to improve the performance of racehorses, and appear more likely to reduce the iv performance (Nugent *et al* 1982).

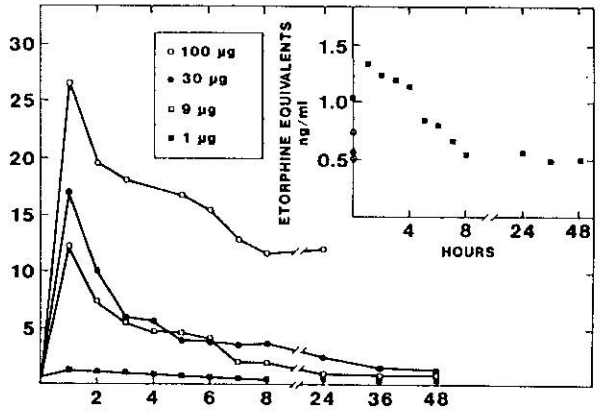
Screening for opiates

High performance thin layer chromatography

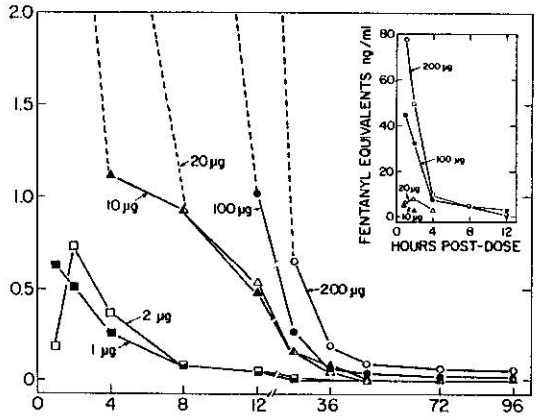
Thin layer chromatography (TLC) has historically been used as a drug detection technique. More recently the necessity for a very sensitive, rapid screening test for morphine congeners prompted the development of high performance thin layer chromatographic (HPTLC) procedures (Poole, Khatib and Dean 1986). High performance thin layer chromatography has several advantages compared to 'standard' thin layer adsorption techniques. It generally requires less sample, yields better sensitivities and resolution, and development distances are shorter than for standard TLC plates. The narcotic screening tests employ HPTLC in an adsorptive distribution mechanism. The silica gel on such plates is very fine grained with narrow particle size diameters. Because phenolic and/or alcoholic substituents are common to morphine-like drugs, biotransformation is frequently to a conjugated species with glucuronic acid.

Preparation of biological fluids for HPTLC analyses initially requires enzyme hydrolysis with β-glucuronidase. The most effective hydrolytic enzymes are those from the marine mollusc *Patella vulgata*. This enzyme is effective at 65°C, allowing a hydrolysis time of 3 to 4 h to release 100 per cent of the conjugated drug (Combie, Blake, Nugent and Tobin 1982). Partitioning of the release drug into organic solvents separates the more lipophilic narcotics from other constituents in the biological fluid. Two extracts of the hydrolysate, one with a solvent blend of moderate polarity (petroleum ether:methylene chloride [2:1 v/v]), and the second with a solvent of high polarity (methylene chloride:isopropanol [6:1]), effectively separate the more lipophilic drugs from the less lipophilic. Back extraction of the initial organic solvent blend further aids in 'cleaning-up' the extract before chromatography (Blake, Tobin and Chang 1986).

Fig 4. Immunoassay for narcotic analgesics in equine urine



(a) Concentrations of etorphine equivalents in horses after dosing with 100 μg/horse (○-○), 30 μg/horse (●-●), 9 μg/horse (□-□), and 1 μg/horse (■-■ insert). All values represent experimental points with one horse only. Reproduced with permission from Woods et al *(1986b)*



(b) Concentrations of fentanyl equivalents in fentanyl treated horses. The symbols show the levels of fentanyl equivalents in horses after iv dosing with 200 μg/horse (○), 100 μg/horse (●), 20 μg/horse (△), 10 μg/horse (▲), 2 μg/horse (□), and 1 μg/horse (■). Fentanyl equivalents were determined by ^{125}I-fentanyl RIA. All values represent single experimental points. Reproduced with permission from Woods et al *(1986a)*

Substituent groups available on the narcotic molecules determine the proper colorimetric overspray(s) to employ for drug detection on the HPTLC plate. The two groups common to most of these drugs are a phenolic NH_2 and nitrogen at various sites. Generally, sensitive spray techniques for either or both of these groups can be chosen. Two valuable sprays are modified Folin-Denis and Ludy Tenger's, in combination with NH_4OH and $NaNO_2$, respectively. Detection of 2 to 10 nanograms (ppb) of a narcotic analgesic on the HPTLC plate is possible with the above oversprays (Blake, Tobin and Roberts 1978).

Immunoassay

The most sensitive screening method for high potency narcotic analgesics is RIA. Commercial RIAs will detect etorphine, fentanyl, sufentanil and alfentanil in horse urine after doses of as little as 1 μg/horse (Fig 4a), which is too low to be pharmacologically effective. Because of this, RIA is the screening method of choice where sensitive detection methods are required.

Commercially available RIA detects etorphine equivalents in urine at a dose as low as 1 μg/horse of etorphine (Fig 4a). It requires about 100 μg of etorphine to produce a good pharmacological effect in a horse and therefore this test is sensitive enough for most screening purposes. It is somewhat superior in sensitivity to the HPTLC test for etorphine described earlier, but suffers from the disadvantage that is has to be run specifically for etorphine (Simon, Hiller and Edelman 1973; Woods *et al* 1986b), and it is relatively expensive, costing about $5/test, compared with less than $0.50 for each HPTLC test (Tobin, Woods and Blake 1985; Tobin 1986).

Similarly, RIA for fentanyl is extremely sensitive (Michiels, Hendriks and Heykants 1977), and relatively expensive. A commercially available RIA kit for fentanyl will detect about 1 ng/ml of fentanyl equivalents in equine urine. More recently, however, the sensitivity of this test has been improved by the use of an [^{125}I] analogue of fentanyl (Tobin *et al* 1986) (Fig 4b) and concentrations of fentanyl or its metabolites as low as 100 pg/ml can now be detected in horse urine. This concentration is much less than those likely to be found in the urine of horses after pharmacologically effective doses of this drug. Based on the sensitivity of this test, it is practical to pool urine samples from a number of horses, screen the pooled urines, and then rescreen individual samples if a suspicious test appears.

A large number of fentanyls are now available both from the pharmaceutical industry and from the illegal market for use in horses (Baum 1985; Janssen 1985). Legitimate medications that are more potent than fentanyl include sufentanil, carfentanil and alfentanil. An antibody which would react to all of these fentanyl analogues would be very useful because all of these agents have the potential to be abused in racehorses.

A problem with the use of RIA for routine screening of horse urine is the propensity of horse urines to give high background levels. For example, preliminary experiments with a commercial etorphine assay showed background levels approaching 1 ng/ml occurring in control horse urines. Such high backgrounds are not readily distinguishable from low level positives, and may be mistaken for positives by inexperienced observers (Woods *et al* 1986b). Because of this, it is extremely important to determine the background or noise levels likely to be found in horse urine with any immunoassay. For example, other work on the modified fentanyl test, showed that the background levels found in horse urine were significantly higher than those found in human urines. For this reason, anyone using RIA systems in routine post race analysis of horse urine needs to be aware of the likelihood of higher than usual backgrounds in horse urine, and evaluate carefully the background characteristics of the test system in the horse (Woods *et al* 1986a).

Authors' note. – Since this paper was submitted for publication, the use of non-isotopic immunoassays for high potency drugs in racing horses has increased dramatically. Recently, Tobin and his colleagues (Tobin *et al* 1988) have reported on the development of non-isotopic immunoassays for about forty high potency drugs reportedly abused in racing horses. The introduction of these assays into routine pre- and post race testing has been enormously successful, with multiple positives being called for buprenorphine, oxymorphone and other related opiates, mazindol, cocaine, sufentanil and acepromazine.

Acknowledgements

Published as Kentucky Agricultural Experiment Station Article 86-4-174 with the approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station. Publication number 127 from the Kentucky Equine Drug Testing and Research Programs, Department of Veterinary Science and the Graduate Center for Toxicology, University of Kentucky. Supported by a grant from the Kentucky Equine Drug Research Council and the Kentucky State Racing and Harness Racing Commissions.

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Received for publication 19.8.86
Accepted 6.5.88