Gen. Pharmac. Vol. 16, No. 3, pp. 253–258, 1985
Printed in Great Britain.

0306-3623/85 $3.00 + 0.00

DOSE-RELATED EFFECTS OF FENTANYL ON AUTONOMIC AND BEHAVIORAL RESPONSES IN PERFORMANCE HORSES

STEVEN G. KAMERLING, DAVID J. DEQUICK, TIMOTHY J. WECKMAN and THOMAS TOBIN*
Department of Veterinary Science, 102 Animal Pathology Building, University of Kentucky, Lexington, KY 40546-0076, U.S.A.
(*Tel*: (606)257-4757)

(*Received* 29 *August* 1984)

Abstract—1. The dose-related effects of intravenously administered fentanyl (0.010, 0.005, 0.0025 mg/kg) and saline were studied in mature performance horses using a rigorous experimental protocol.

2. Fentanyl produced a dose-related prolongation of the skin twitch reflex latency but did not increase the hoof withdrawal reflex latency.

3. Dose related increases in stepping frequency, cardiac and respiratory rates were observed following fentanyl, while changes in rectal temperature and pupil area were not.

4. These data indicate that fentanyl, a prototypic mu-agonist, produces a syndrome characterized by analgesia, locomotor and sympathetic stimulation in the horse.

INTRODUCTION

The present study is part of a series of studies designed to characterize the effects of opiates in the performance horse. Unlike the human, the horse is behaviorally aroused, yet rendered analgesic following the administration of most opiates (Kalpravidh *et al.*, 1984a,b; Pippi *et al.*, 1979; Lumb *et al.*, 1983). Tobin *et al.* (1979) and Combie *et al.* (1979) demonstrated that locomotor behavior in the horse can be augmented in a dose-related manner by several opioid analgesics. Lumb *et al.* (1983) reported analgesia using the heat evoked foreleg flexion model following single doses of morphine and butorphanol in ponies, and after pentazocine and butorphanol in horses. Opiate induced tachycardia and tachypnea were also observed. Despite these reports, there have been no systematic attempts to concommittantly measure dose-related changes in nociception and autonomic function in performance horses following the administration of a prototypic short acting opioid analgesic. Due to excessive locomotor stimulation and spontaneous limb movements, we were unable to use the foreleg flexion model as an assay for narcotic analgesia. Consequently, a modification of the heat evoked skin twitch reflex was used (Houde and Wikler, 1951). Evidence is presented showing the superiority of this model over the foreleg flexion model as an analgesia assay for opioids which produce locomotor stimulation. The effects of increasing doses of fentanyl on nociceptive thresholds, locomotor behavior, cardiac and respiratory rates, pupil size, and rectal temperature were studied in Thoroughbred and Standardbred horses using a rigorous experimental protocol.

MATERIALS AND METHODS

Experiment 1

Mature Thoroughbred and Standardbred mares and geldings (400–600 kg) were used. All horses were acclimated to their stalls 24 hr prior to experimentation. In the first series of experiments, the horses were placed in a laboratory and confined to equine stockades. Nociceptive thresholds, cardiac and respiratory rates, pupil size, and rectal temperature were recorded concommittantly.

Nociceptive thresholds were measured using a modification of the methods of Hardy *et al.* (1940) and Pippi *et al.* (1979a). Radiant thermal stimuli were applied to 2 separate anatomical loci. At the first locus, stimuli were emitted from a manually activated heat lamp consisting of a 500 W projector bulb and condenser lens. A beam of light was focused upon the skin of the lateral aspect of the metacarpophalangeal joint at a fixed distance. In response to the noxious heat stimulus, the horse withdrew its forelimb. The time elapsed from illumination of the lamp to withdrawal of the limb was designated the hoof withdrawal reflex latency (HWRL). To deliver the thermal stimulus at the second locus it was necessary to fit each horse with a heat lamp, attached by flexible gooseneck connectors to a steel reinforced surcingle worn around the abdomen. The surcingle held the heat lamp in a stationary position caudal and dorsal to the withers (intrascapular area). The lamp contained a 500 W projector bulb and condensing lens that focused a beam of light to a point on the withers just lateral to the midline. Noxious radiant heat from the focused light beam evoked a reflex contraction of the cutaneous trunci muscle subserving the skin over the withers. The time from

Published as Kentucky Agricultural Experiment Station Article No. 84-4-248 with the approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Publication No. 106 from the Kentucky Equine Drug Research and Testing Programs, Department of Veterinary Science and the Graduate Center for Toxicology, University of Kentucky.

Supported by a Grant entitled "Masking by Phenylbutazone in Equine Drug Testing: An Analysis", from the Kentucky Equine Drug Research Council of the Kentucky State Racing Commission.

*All correspondence should be addressed to Dr Tobin

lamp illumination to skin twitch was designated the skin twitch reflex latency (STRL). Both the HWRL and STRL were determined by a common electronic timer synchronized to the heat lamps that were operated manually. Lamp intensities were rheostatically controlled and adjusted to produce baseline (pretreatment) latencies of 6–9 sec. Stimuli were terminated when latencies of approximately 2 times the baseline latency were achieved to prevent tissue damage. Respiratory rate was determined from Polygraph (Grass Model 7, Quincy, MA) recordings of impedence changes between electrodes placed bilaterally below the diaphragm. Cardiac rate was obtained from polygraph recordings of an electrocardiogram. Pupil area was determined from photographs of the eye using a Polaroid CU-5 close-up camera, similar to the method of Marquart *et al.* (1967). Since the equine pupil is elliptical in shape, pupil area was calculated using the formula for the area of an ellipse (Area = vertical diameter + horizontal diameter $\div 2 \times \pi$). Rectal temperature was recorded from a deep rectal probe and digital thermometer (Sensortek; Saddlebrook, NJ).

Experiment 2

In the second series of experiments the horses were placed in standard box stalls (approximately 180 ft^2) which were closed on all sides. A window made of one-way mirrored glass located in each door permitted observers to record behavior without awareness by the animal. Locomotor behavior was quantitated 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).

Experimental design

Fentanyl citrate (0.010, 0.005 and 0.0025 mg/kg) and saline were administered intravenously to 8 subjects. Crossover experiments were conducted using replicate 4 × 4 Latin Square blocks. A different treatment was administered every 5 days to each horse such that all 4 treatments were delivered over 4 experimental sessions. Nociceptive thresholds, rectal temperature and pupil area were measured every 5 min for the first 30 min then at 45 and 60 min post-injection. Cardiac and respiratory rates were determined at 5 min intervals for 60 min post-injection. Three pre-treatment observations were obtained for each parameter at 15 min intervals prior to injection to establish a stable baseline.

Statistical analysis

Peak fentanyl effects occurred within 20–25 min of drug administration for most parameters. Therefore, post-treatment observations were summed for 20–25 min after injection for each horse and treatment giving a single, cumulative, post-treatment value per animal. These values were then analyzed using an analysis of variance (ANOVA) in which variance 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 regression analysis. Comparisons between saline and fentanyl were made using a Duncan's Multiple Range test. All calculations were performed on an IBM 3083 computer using a Statistical Analysis System program (Freund and Littel, 1981).

Drugs

Fentanyl citrate was dissolved in approximately 10 ml of sterile saline and administered as an i.v. bolus into the left jugular vein of each animal.The same volume of saline was administered as the control treatment. Fentanyl citrate was generously donated by McNeil Pharmaceutical, Spring House, PA.

RESULTS

Fentanyl produced a syndrome characterized by analgesia, locomotor stimulation and enhanced cardiac and respiratory rates. While the locomotor and cardiovascular enhancement occurred rapidly, the analgesic and respiratory responses occurred somewhat later and were more prolonged (Fig. 1). The skin twitch reflex latency was significantly increased in a linear dose-related manner ($P = 0.0001$, Table 1; Fig. 2). Significant differences among the treatments were also observed ($P = 0.0002$, Table 1). All doses of fentanyl produced significant increases ($P < 0.05$) in the STRL when compared to saline (Table 2). The highest dose of fentanyl produced around a 2-fold increase in mean reflex latency over the 20 min post-injection period (Table 2). Dose-related prolongation in the hoof withdrawal reflex was not observed ($P = 0.2768$, Table 1; Fig. 2).

Locomotor behavior or stepping frequency was enhanced in a linear dose-related manner ($P = 0.0005$, Table 1, Fig. 2). A significant difference among treatments was also observed ($P = 0.0270$, Table 1). Fentanyl (0.010 mg/kg) produced a roughly 3-fold increase in mean stepping frequency over the 24 min post injection period. The stepping frequency observed at this dose was significantly different ($P < 0.05$) from saline (Table 2). As can be seen in Fig. 1, locomotor stimulation consistently peaked within 4 min of fentanyl administration resulting in qualitatively similar time response curves. This similarity was not readily observed in any of the other parameters examined.

Cardiac rate was elevated in a linear dose-related manner ($P = 0.0016$, Table 1; Fig. 2). Significant differences among treatments was also observed ($P = 0.0004$, Table 1). Fentanyl produced a 20% increase in heart rate at the highest dose, which was significantly different from saline ($P < 0.05$, Table 2). The tachycardia occurred rapidly with peak effects observed within 4 min of injection (Fig. 1). This response was particularly marked at the high dose.

Tachypnea was not as consistently observed as the previously described responses. While no significant difference among treatments was noted ($P = 0.2298$, Table 1) a linear trend over treatments was suggested ($P = 0.0667$, Table 1; Fig. 2). A mean increase of 10 breaths/min was observed following the high dose of fentanyl when compared to saline, however, this was not significant (Table 2). These data suggest that higher doses of fentanyl are required to elicit a more consistent tachypneic response.

No consistent changes in pupil area or rectal temperature were observed (Tables 1 and 2, Figs 1 and 2).

DISCUSSION

Fentanyl produced a syndrome characterized by analgesia, sympathetic stimulation and behavioral arousal. Data obtained from the skin twitch reflex (STR) showed that fentanyl produced potent, albeit short duration, analgesia of such magnitude that each dose could be statistically distinguished from saline. Further, the analgesic response was linear over doses. These data suggest that fentanyl is an efficacious

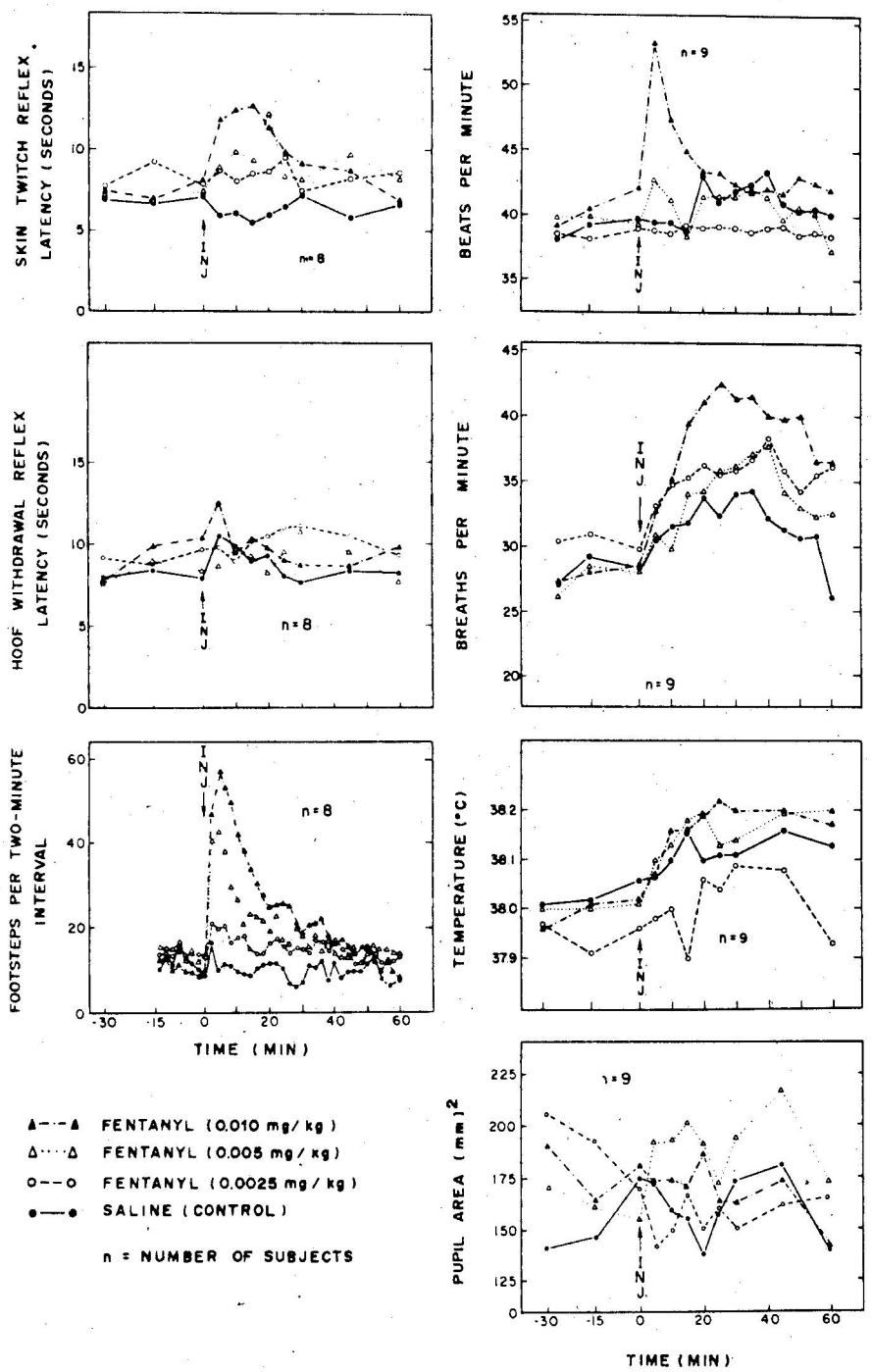

Fig. 1. Time course of the effects of fentanyl on skin twitch and hoof withdrawal reflex latencies, cardiac and respiratory rates, locomotor activity, rectal temperature and pupil area. Each point represents the mean response of 8–9 horses.

analgesic in the horse and that the STR model is a valid and sensitive assay for antinociception. Changes in the nociceptive threshold following drug adminstration could not be detected using the hoof withdrawl reflex (HWR) model. Locomotor stimulation, characterized both by spontaneous flexion of the forelimbs and postural changes, confounded the measurement. During periods of peak locomotor stimulation and STR prolongation, hoof withdrawal could not be distinguished from spontaneous flexion particularly at the high dose of fentanyl. This observation makes data obtained from other laboratories (Lumb *et al.*, 1983) using the HWR as a measure of opiate analgesia, difficult to reconcile.

The time course and magnitude of the fentanyl-induced locomotor stimulation observed in the present study agrees with previous findings from this laboratory. Tobin *et al.* (1970) and Combie *et al.*

Table 1. Analysis of variance of post-treatment responses

Source of variance	d.f.	Parameter S	H	F	C	R	T	P
Subjects	7	0.0326	0.0014	0.0002	0.0008	0.0040	0.0008	0.0721
Sessions	3	0.0120	0.0500	0.3180	0.0347	0.0477	0.1721	0.3460
Treatments	3	0.0002	0.2266	0.0270	0.0004	0.2298	0.4424	0.6580
Linearity	1	0.0001	0.2768	0.0005	0.0016	0.0667	0.3039	0.4816
Non-linearity	2	0.3698	0.1696	0.0867	0.3008	0.7704	0.3896	0.4680
Treatment * session	6	0.1584	0.5278	0.9924	0.0072	0.2820	0.5987	0.8942

Values in table are probability estimates (P).
Parameters: S—skin twitch reflex latency (sec); H—hoof withdrawal reflex latency (sec); F—footsteps/2 min period; C—cardiac rate (beats/min); R—respiratory rate (breaths/min); T—rectal temperature (°C); P—pupil area (mm^2).
d.f. = degrees of freedom.

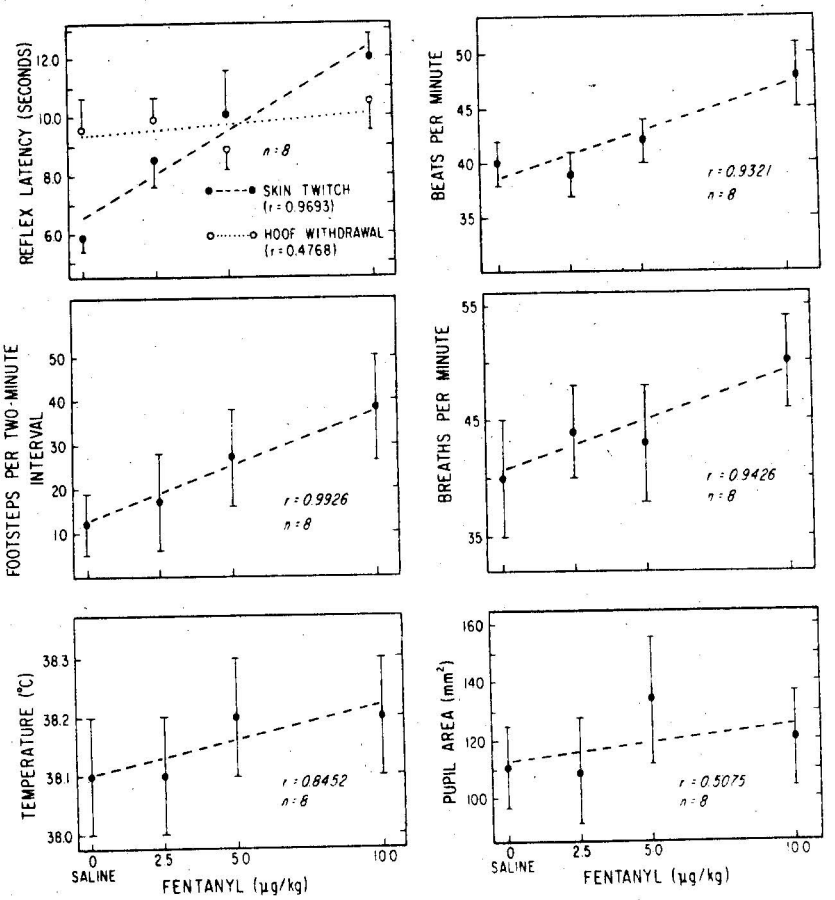

Fig. 2. Dose–response curves for fentanyl (0.010, 0.005, 0.0025 mg/kg) and saline on skin twitch and hoof withdrawal reflex latencies, cardiac and respiratory rates, locomotor activity, rectal temperature and pupil area. Each point represents the mean response (± SEM) of eight horses. r = regression coefficient.

Table 2. Effects of fentanyl and saline on 7 physiologic parameters

Treatment	Parameter S	H	F	C	R	T	P
Fentanyl 0.010 mg/kg	12.0 ± 0.8*	10.5 ± 1.0	38 ± 12*	48 ± 3*	50 ± 4	38.2 ± 0.1	121 ± 16
Fentanyl 0.005 mg/kg	10.1 ± 1.5*	8.9 ± 0.7	27 ± 11	42 ± 2	43 ± 5	38.2 ± 0.1	134 ± 22
Fentanyl 0.0025 mg/kg	8.5 ± 0.9*	9.9 ± 0.7	17 ± 11	39 ± 2	44 ± 5	38.1 ± 0.1	109 ± 18
Saline	5.9 ± 0.5	9.6 ± 1.1	12 ± 7	40 ± 2	40 ± 5	38.1 ± 0.1	111 ± 14
Sampling period (min)	$T_{5\text{-}20}$	$T_{5\text{-}20}$	$T_{2\text{-}24}$	$T_{5\text{-}20}$	$T_{5\text{-}25}$	$T_{5\text{-}25}$	$T_{5\text{-}65}$

Values represent the mean response (± SEM) of 8 subjects averaged over the sampling periods (min) indicated for each parameter.
*Indicates responses are significantly different ($P < 0.05$) from saline according to an ANOVA and Duncan's Multiple Range Test.
Parameters: S—skin twitch reflex latency (sec); H—hoof withdrawal reflex latency (sec); F—footsteps/2 min period; C—cardiac rate (beats/min); R—respiratory rate (breaths/min); T—rectal temperature (°C); P—pupil area (mm^2).

(1979, 1981) observed a dose-dependent increase in stepping frequency following morphine and fentanyl, two prototypic mu agonists. However, opioid-induced locomotor enhancement in the horse has been attributed to stimulation of both opiate and dopamine receptors. This is supported by the observations that the locomotor effects of fentanyl and morphine can be antagonized by naloxone, haloperidol, and acepromazine (Tobin *et al.*, 1979; Combie *et al.*, 1981). Furthermore, locomotor stimulation can be produced by apomorphine alone, a putative dopamine agonist (Tobin *et al.*, 1979). Increased motor activity following opioid administration is thought to occur via the central release of dopamine from neurons in the nigrostriatal pathway. Several morphine-like analgesics increase motor activity at low doses in the mouse, a species like the horse which is particularly excited by morphine (Holdstein and Sheehan, 1969). This hyperactivity is enhanced by drugs which potentiate synaptically released catacholamines in the brain (Hollinger, 1969). These data suggest that fentanyl probably acts through a monoaminergic synapse to elicit the observed locomotor enhancement.

Like the horse, the response to morphine in the cat is characterized by analgesia, excitement and behavioral signs of sympathetic stimulation (Tovat and Akcash, 1956; Kayaalp and Kaymakcalan, 1966; McKensie and Beechey, 1962; Stewart and Rogoff, 1922). Morphine increases heart rate and blood pressure in unanesthetized and sedated cats (Wallenstein, 1979; Kayaalp and Kaymakcalan, 1966). However, this effect is not consistent over all doses. The tachycardia and hypertension observed at the higher doses is however prevented by adrenalectomy (Wallenstein, 1979). Furthermore, morphine releases catacholamines from the adrenal medulla in this species (Outschourn, 1952). These data suggest that morphine induced cardiovascular changes in the cat are primarily due to adrenal stimulation and subsequent peripheral release of catacholamines. Although plasma catacholamine levels were not measured in our experiments, we hypothesize a similar mechanism to explain the dose-related tachycardia observed after fentanyl administration in the horse. However, a central mechanism cannot be excluded. Morphine-induced hypertensive fluctuations, observed in unanesthetized cats, are absent in spinal transected cats and after anesthetic is administered (Kayaalp and Kaymakcalan, 1966). Therefore, morphine can exert an excitatory influence on central vasomotor centers in the cat and possibly in the horse as well.

Respiratory stimulation was highly variable among horses. A trend toward linearity ($P = 0.066$, Table 1) over treatments is suggested from our data. However, no statistical differences among the doses of fentanyl and saline could be distinguished. In most species, morphine produces respiratory depression by inhibiting respiratory neurons and decreasing the sensitivity of the respiratory center to carbon dioxide (see review by Martin, 1984). However, morphine produces respiratory stimulation in the cat (Stewart and Rogoff, 1922; Florez *et al.*, 1968). Earlier studies linked the tachypnea to hyperthermia (Stewart and Rogoff, 1922), possibly through an action on the caudal hypothalamus (McCrum and Ingram, 1951). However, our observations following fentanyl in the horse agree with those of Florez *et al.* (1968) following morphine in the cat, in that respiratory frequency was augmented without modification in rectal temperature. It is possible that respiratory stimulation resulted from fentanyl-induced catacholamine release both centrally and peripherally. In the rat, decreases in brain NE levels were associated with respiratory depression, while increases in NE levels antagonized this depression (Meldrum and Isom, 1981).

Depending on the dose and locus of administration, mydriasis and miosis can be produced in the cat (Wallenstein, 1979). Mydriasis appears to involve participation by the adrenals and could thus be a sequela of catacholamine release. However, morphine produces miosis in adrenalectomized cats, an action which is predominantly central in origin. The lack of significant mydriasis in the horse is difficult to reconcile in light of other signs of sympathetic stimulation. However if peripheral and central mechanisms are antagonistic, then recruitment by fentanyl of both the peripheral and central nervous systems could result in a cancellation of effects.

Acknowledgements—The authors wish to thank John Turbek for his statistical assistance and Diane Haughey for preparation of this manuscript.

REFERENCES

Combie J., Dougherty J., Nugent E. and Tobin T. (1979) The pharmacology of narcotic analgesics in the horse. IV. Dose and time response relationships and behavioral responses to morphine, meperidine, pentazocine, anileridine, methadone, and hydromorphone. *J. Equine Med. Surg.* **3,** 377–385.

Combie J., Shults T., Nugent E., Dougherty J. and Tobin T. (1981) Pharmacology of narcotic analgesics in the horse: Selective blockade of narcotic-induced locomotor activity. *Am. J. Vet. Res.* **42,** 716–721.

Florez, J., McCarthy L. E. and Borison H. L. (1968) A comparative study in the cat of the effects of morphine injected intravenously and into the cerebrospinal fluid. *J. Pharmac. exp. Ther.* **163,** 448–455.

Freund R. J. and Littell R. C. (1981) *SAS for Linear Models: A Guide to the ANOVA and GLM Procedures*, SAS Institute, Inc., Cary, NC.

Goldstein A. and Sheehan P. (1969) I. Tolerance to the "running fit" caused by levorphanol in the mouse. *J. Pharmac. exp. Ther.* **169,** 175–184.

Hardy J. D., Wolff H. G. and Goodell H. (1940) Studies on pain. A new method for measuring pain threshold. Observations on spatial summations of pain. *J. Clin. Invest.* **19,** 649–657.

Hollinger M. (1969) Effect of reserpine, alpha-methylparatyrosine and pargyline on levorphanol-induced running activity in mice. *Archs int. Pharmacodyn. Ther.* **179,** 419–424.

Houde R. W. and Wikler A. (1951) Delineation of the skin twitch response in dogs and the effects thereon of morphine, thiopental, and mephenesin. *J. Pharmac. exp. Ther.* **103,** 243–248.

Kalpravidh M., Lumb W. V., Wright M. and Heath R. B. (1984a) Effects of butorphanol, flunixin, levorphanol, morphine, and xylazine in ponies. *Am. J. Vet. Res* **45,** 217–223.

Kalpravidh M., Lumb W. V., Wright M. and Heath R. B. (1984b) Analgesic effects of butorphanol in horses: Dose response studies. *Am. J. Vet. Res.* **45,** 211–216.

Kayaalp S. O. and Kaymakcalan S. (1966) A comparative study of the effects of morphine in unanesthetized and anesthetized cats. *Br. J. Pharmac.* **26,** 196–204.

Lumb W. V., Pippi N. L. and Kalpravidh M. (1983) Evaluation of analgesic drugs in horses. *Animal Pain Perception and Alleviation*, pp. 179–205. American Physiological Society, Bethesda.

Marquart W. G., Martin W. R. and Jasinski D. R. (1967) The use of the Polaroid CV camera in pupillography. *Int. J. Addict.* **2,** 301–304.

Martin W. R. (1984) Pharmacology of opioids. *Pharmac. Rev.* **35,** 283–323.

McCrum W. R. and Ingram W. R. (1951) The effects of morphine on cats with hypothalamic lesions. *J. Neuropath. exp. Neurol.* **10,** 192–203.

McKensie J. S. and Beechey N. R. (1962) The effects of morphine and pethidine on somatic evoked responses in the midbrain of the cat and their relevance to analgesia. *Electroenceph. Clin. Neurophysiol.* **14,** 501–519.

Meldrum M. J. and Isom G. E. (1981) Role of monoaminergic systems in morphine-induced respiratory depression. *Neuropharmacology* **20,** 169–175.

Outschourn A. S. (1952) The hormones of the adrenal medulla and their release. *Br. J. Pharmac.* **7,** 605–615.

Pippi N. L., Lumb W. V., Fialho S. A. G. and Scott R. J. (1979) A model for evaluating pain in ponies. *J. Equine. Med. Surg.* **3,** 430–435.

Stewart G. N. and Rogoff J. M. (1922) The influence of morphine on normal cats and on cats deprived of the greater part of the adrenals with special reference to body temperature, pulse and respiratory frequency and blood sugar content. *J. Pharmac. exp. Ther.* **19,** 97–130.

Tobin T., Combie J., Shults T. and Dougherty J. (1979) The pharmacology of narcotic analgesics in the horse. III. Characteristics of the locomotor effects of fentanyl and apomorphine. *J. Equine Med. Surg.* **3,** 284–288.

Tovat S. and Akcash A. (1956) The relation between excitation caused by analgesic drugs in the cat. *Archs int. Pharmacodyn. Ther.* **108,** 92–101.

Wallenstein M. C. (1979) Biphasic effects of morphine on cardiovascular system of the cat. *Eur. J. Pharmac.* **59,** 253–260.