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DIFFERENTIAL EFFECTS OF PHENYLBUTAZONE AND LOCAL ANESTHETICS ON NOCICEPTION IN THE EQUINE *

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The effects of procaine, mepivacaine and phenylbutazone on pain perception in the equine were studied using two behavioral assays of nociception; the thermal evoked hoof withdrawal reflex and skin twitch reflex. Pain perception threshold was measured as the latency from onset of thermal stimuli to reflex withdrawal of the forelimb or contraction of the cutaneous musculature. Procaine 2% and mepivacaine 2% prolonged the hoof withdrawal reflex latency when administered locally by producing a block of the palmar and metacarpal nerves. Significant analgesia lasted 90 min and 210 min for procaine and mepivacaine, respectively. Phenylbutazone (7.3 mg/kg) failed to alter pain thresholds measured over a 36 h post-treatment period. However, pain thresholds rose over time with successive trials. These data suggest that in the equine (1) phenylbutazone does not alter normal cutaneous pain perception, and (2) successive presentation of painful stimuli increases nociceptive thresholds.

Equine Pain Nociception Analgesic Procaine Mepivacaine Phenylbutazone

1. Introduction

Considerable debate surrounds the use of the non-steroidal anti-inflammatory drugs (NSAIDs) as pre-race medication in the racing equine. The basis for this debate stems from the contention that NSAIDs such as phenylbutazone (PBZ) alter pain perception (nociception) and consequently improve racing performance. Although somatic pain may impair performance, the effects of drugs on pain perception and performance have been difficult to assess in the equine. Attempts to objectively measure pain thresholds in this species have been limited to the studies of Pippi and coworkers (Pippi and Lumb, 1979; Pippi et al., 1979) using models of cutaneous, visceral and somatic pain. None of these studies have attempted to systematically characterize pain perception or correlate it with performance. In addition, results obtained from these studies were highly variable, and were carried out in ponies which are not performance equidae.

In the present study several fundamental questions were experimentally addressed. First, can techniques employed for measuring pain perception in standard laboratory animals be successfully adapted to performance horses? Secondly, can nociceptive reflexes be reproducibly obtained and

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objectively quantitated in these animals? Thirdly, can these techniques provide a valid assay of the efficacy of analgesic drugs? In the present study the effects of a prototypic NSAID, phenylbutazone, and two local anesthetics are described using two behavioral assays of nociception: the thermal evoked hoof withdrawal reflex and the skin twitch reflex.

2. Materials and methods

2.1. Measurement of pain perception

Mature Standardbred and Thoroughbred mares and geldings (400-800 kg) were used. All horses were acclimated to their stalls 24 h prior to experimentation and were confined to equine stockades at the time of the experiment. Radiant thermal stimuli were delivered by a heat lamp consisting of a projector bulb and condenser lens. A beam of light was focused upon the metacarpophalangeal joint or dorsal coronary band when the lamp was illuminated. In response to the noxious heat stimulus the horse withdrew its forelimb. The time from illumination of the lamp to withdrawal of the limb was designated the hoof withdrawal reflex latency (HWRL). This latency was measured by an electronic timer synchronized to the heat lamp and operated manually. Lamp intensity was rheostatically controlled and adjusted to produce a 5-7 s latency. Thermal stimuli were terminated when latencies of 1.6-1.8 times the pre-treatment (control) latency were achieved to prevent tissue damage.

During certain experimental sessions, each horse was fitted with a device that consisted of a dual heat lamp attached by flexible gooseneck connectors to a steel reinforced surcingle. A girth was placed around the abdomen which held the surcingle and heat lamp in a stationary position just caudal and dorsal to the withers. One of the lamps consisted of a projection bulb with a condensing lens that focused a beam of light to a point on the withers. This thermal stimulus evoked a reflex contraction of the cutaneous trunci muscle subserving the skin over the withers. The time from lamp illumination to skin twitch was designated the skin twitch reflex latency (STRL). This reflex was elicited just prior to or following the hoof withdrawal reflex in which identical rheostatic and timing devices were used. Skin temperature was measured preceding thermal stimulation of the withers by attaching a 33G microthermocouple (Bailey Instruments, Saddlebrook, NJ) to a small area of depilated skin with a thin film of spirit gum. Changes in skin temperature were monitored by a digital thermometer (Bailey Instruments) and recorded on a polygraph (Model 7D, Grass Instrument Co., Quincy, MA). The epidermal locus of radiant heat application was blackened with India Ink prior to stimulation. STRLs of around 7 s were obtained.

2.2. Experiment 1

In the first experiment 2% mepivacaine (Winthrop Labs., New York, NY), 2% procaine (A.H. Robins Co., Richmond, VA) or saline were administered to 6-8 animals at 10 day intervals between treatments. The anesthetics or pH adjusted saline were deposited subcutaneously as four 5.0 ml doses; 2 doses at the level of metacarpal bones 2 and 4 between the suspensory ligaments, and 2 doses at the distal ends of metacarpal bones 2 and 4. This procedure resulted in anesthetization of the palmar and metacarpal nerves. HWRLs were measured every 10 min for the first 30 min and at 20 min intervals thereafter. Three pre-treatment (control) latencies were obtained at 15 min intervals prior to saline or local anesthetic administration.

2.3. Experiment 2

In the second experiment 8 horses received an intravenous (i.v.) injection of an anti-inflammatory dose of PBZ (7.3 mg/kg) (Jensen-Salsbery Labs, Kansas City, MO) or an equal volume of saline according to a crossover design to which experimental observers were blind. HWRL, STRL, and skin, rectal and ambient temperatures were all obtained at the same intervals. Three latencies were obtained at 15 min intervals every 3 h for the first 15 h, and again at 24 and 36 h post-treatment. Three pre-treatment latencies were obtained just

prior to each injection. Post-treatment latencies were expressed as a percent of the mean pre-treatment (control) latency.

2.4. Statistical methods

Experiments were conducted according to a crossover design. Data were analyzed using a 2-way analysis of variance (ANOVA) for treatments and subjects. Significant linear time trends were determined by regression analysis. P values of 0.05 or less were considered significant.

3. Results

3.1. Local anesthetic effects

Fig. 1 shows that procaine produced a rapid onset nerve block within 10 min as evidenced by the significant increase in the HWRL. Peak post-procaine latency was approximately 1.8 times greater than the corresponding pre-treatment control latency. A 2-way ANOVA of area under the time action curve for the 90 min saline vs. procaine curves indicated a significant between treatments difference ($F = 19.90$, $P < 0.005$).

Fig. 2 illustrates that mepivacaine produced a similar rapid onset nerve block. Maximal responses were achieved earlier and sustained longer following mepivacaine than procaine. Latencies elicited 30-180 min after mepivacaine have identical means and variances since all subjects progressed to cut off latency (1.75 times pre-treatment control). A 2-way ANOVA of area under the saline and mepivacaine time action curves, for the 210 min post-injection period, indicated a significant between treatments difference ($F = 65.57$, $P < 0.005$). Mepivacaine analgesia was roughly 2-fold longer in duration than procaine analgesia.

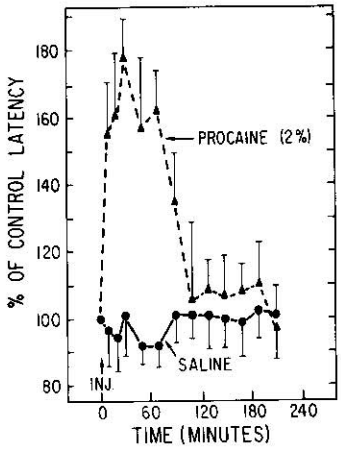

Fig. 1. Nerve blocking action of procaine (2%) on hoof withdrawal reflex latency. Values are mean post-treatment latencies (±S.E.M.) expressed as a percent of the mean pre-treatment (control) latency. Pre-saline latency = 8.3±0.6 s. Pre-procaine latency = 7.7±0.6 s. n = 6.

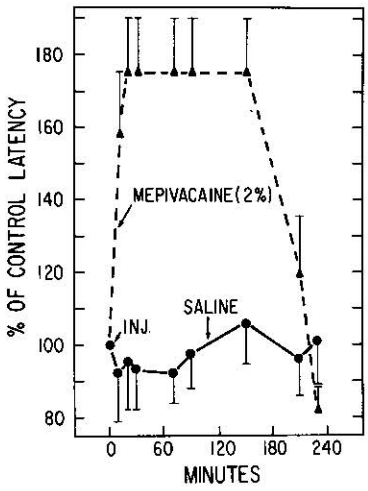

Fig. 2. Nerve blocking action of mepivacaine (2%) on hoof withdrawal reflex latency. Values are the mean post-treatment latencies (±S.E.M.) expressed as a percent of the mean pre-treatment (control) latency. Pre-saline latency = 8.4±0.6 s. Pre-mepivacaine latency = 6.3±0.4 s. n = 6.

3.2. Phenylbutazone and successive trial effects

Figs. 3 and 4 show that a 7.3 mg/kg (or 3 g/1000 lbs) dose of PBZ had no analgesic activity when compared with saline according to the two nociceptive assays employed in this study. A 2-way ANOVA of areas under the time action curves for 36 h indicated no significant between treatments differences for the STRL ($F = 0.06$, NS) or HWRL ($F = 0.13$, NS). In addition, no significant differences between treatments were observed at any given time of day for either assay. However, a significant positive correlation (r) between STRL

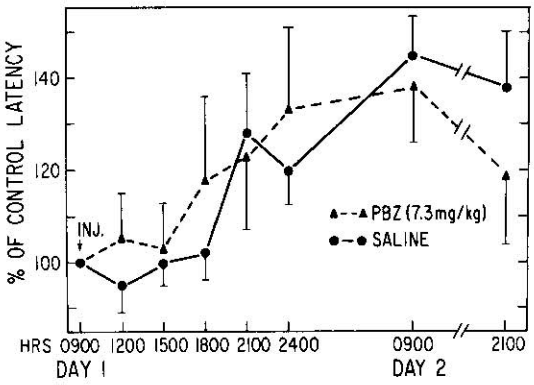

Fig. 3. Absence of analgesic effect of i.v. phenylbutazone (PBZ) on skin twitch reflex latency. Values represent mean post-treatment latency (±S.E.M.) expressed as a percent of the mean pre-treatment (control) latency. Pre-saline latency = 6.4±0.6 s. Pre-PBZ latency = 7.6±1.0 s. n = 8.

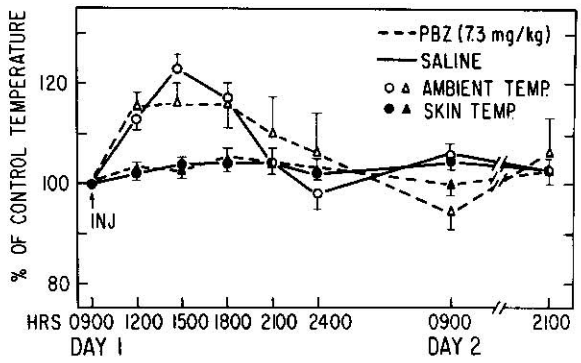

Fig. 5. Changes in ambient and basal skin temperatures in the phenylbutazone (PBZ) and saline treated group. Values represent mean temperatures (°C) ±S.E.M. of 8 horses expressed as a percent of the mean pre-treatment (control) temperatures. Mean pre-PBZ temperatures: ambient = 22.9±1.9°C, skin = 33.6±0.6°C. Pre-saline temperatures: ambient = 23.4±1.2°C, skin = 33.8±0.3°C.

and time of day was noted in the saline treated group over the 36 h post-treatment period ($r = 0.87$, $P < 0.01$), and in the PBZ treated group over the 24 h post-treatment period ($r = 0.95$, $P < 0.01$). Post-treatment STRL was significantly greater than control at 24 h ($F = 22.17$, $P < 0.005$) and 36 h ($F = 7.86$, $P < 0.05$) in the saline treated group; and at 24 h ($F = 11.34$, $P < 0.025$) in the PBZ treated group. A significant positive correlation between HWRL and time of day was observed over the 15 h post-treatment period in the saline treated group ($r = 0.86$, $P < 0.05$); and over the 24 h post-treatment period in the PBZ treated group ($r = 0.89$, $P < 0.01$). Post-treatment HWRLs were significantly greater than pre-treatment values at 15 h ($F = 9.91$, $P < 0.025$) in the saline treated group; and at 24 h ($F = 16.22$, $P < 0.025$) in the PBZ treated group.

3.3. *Skin and rectal temperature*

Fig. 5 shows that phenylbutazone failed to alter basal skin temperature over time when compared with saline. A 2-way ANOVA of area under the

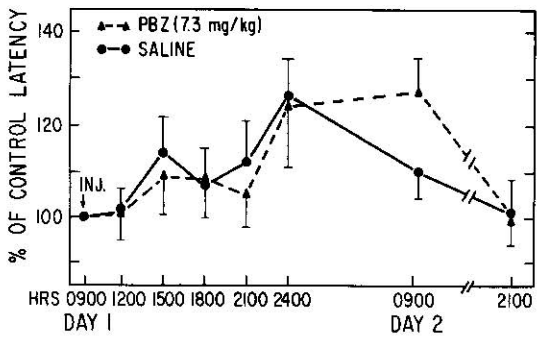

Fig. 4. Absence of analgesic effect of i.v. phenylbutazone (PBZ) on hoof withdrawal reflex latency. Values represent mean post-treatment latency (±S.E.M.) expressed as a percent of the mean pre-treatment (control) latency. Pre-saline latency = 6.9 ±0.6 s. Pre-PBZ latency = 7.1±0.5 s. n = 8.

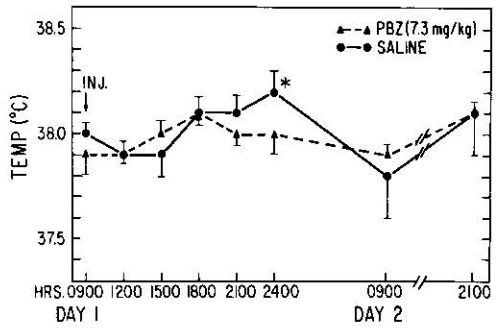

Fig. 6. Changes in rectal temperature as a function of time of day. Values represent mean rectal temperature (±S.E.M.) to the nearest 0.1°C in 8 horses. Values at 09:00 h (day 1) were obtained just prior to treatment. * This value is significantly different from the 24:00 h value in the PBZ treated group and from the 12:00 h value in the saline treated group.

time action curves for PBZ and saline showed no significant between treatments difference over the 36 h post-treatment period ($F = 1.07$, NS). In addition, skin temperature did not correlate with time of day for either the saline ($r = 0.38$, NS) or PBZ ($r = 0.02$, NS) treated groups. Significant between treatments differences in ambient temperature ($F = 8.21$, $P < 0.025$) at 09:00 h (day 2) were not paralleled by between treatments differences in skin temperature.

Rectal temperature showed a diurnal variation in the saline treated group (fig. 6). Day 1 temperature at 12:00 h (37.8°C) was significantly lower than the 24:00 h temperature (38.2°C) ($F = 42.48$, $P < 0.005$). A significant positive correlation was observed over the range of values between these two temperatures for the saline ($r = 0.94$, $P < 0.05$) but not for the PBZ ($r = 0.45$, NS) treated groups. In addition, the 24:00 h (day 1) saline treated group temperature was significantly greater than the corresponding PBZ treated group temperature ($F = 5.73$, $P < 0.05$) indicating that PBZ disrupted normal diurnal temperature variation.

4. Discussion

The hoof withdrawal and skin twitch reflexes are typical nocifensive reactions to locally aversive stimulation in the horse. They may be thought of as the counterpart of limb withdrawal in the rodent subjected to the hot plate (Woolfe and MacDonald, 1944) and to the thermal evoked skin twitch response in the dog (Andrews and Workman, 1941), respectively. These types of assays have been used to reproducibly measure changes in pain thresholds in numerous species under a variety of experimental conditions and drug treatments (Beecher, 1957). The radiant heat-evoked hoof withdrawal reflex has been used a test for the analgesic effects of narcotics in ponies by Pippi and coworkers (Pippi and Lumb, 1979; Pippi et al., 1979). However, these investigators reported large data variability among animals both within and between experimental days. The present study more systematically investigated normal, cutaneous thermal nociception in the horse by varying both the frequency of stimulation and the time of day during which stimuli were delivered. Since free ranging Thoroughbreds and Standardbreds were used, the data obtained better approximates responses in performance horses.

The large variability among subjects described by Pippi and coworkers (Pippi and Lumb, 1979; Pippi et al., 1979) was not encountered in the present study. HWRLs were similar among all subjects when comparable lamp intensities were used. Latencies seldom varied by more than 1 s among subjects regardless of sampling interval or treatment. We have observed that thermal stimuli may be delivered as often as every 5 min for 30-40 min without changing the latency.

Noxious radiant heat delivered at various cutaneous loci selectively excites afferents, spinal neurons, and ascending pathways which subserve pain (VyKlicky, 1979). This type of stimulation has the advantage of reproducibly eliciting a relatively 'pure' pain sensation without involving other sensory modalities. These properties were particularly useful in making objective comparisons of onset, intensity and duration of analgesic action of mepivacaine and procaine. Mepivacaine analgesia clearly outlasted that of procaine. However, both agents produced longer lasting analgesia than that described in man using the ulnar-block technique and pinch test (Albert and Lofstrom, 1965). The efficacy of a nerve block in the equine is typically determined by palpation or pin prick of the limb region distal to the site of local anesthetic deposition. This manipulation invariably activates other sensory modalities (i.e. touch and pressure) which can elicit limb flexion in the absence or presence of pain and yield inaccurate information on antinociception. However, the thermal evoked hoof withdrawal reflex was a sufficiently sensitive assay to detect quantitative differences in the analgesic capacities of both of the agents tested. In addition, it is clear from the stability of the saline curves over time (figs. 2 and 3) that repeated thermal stimuli may be administered as frequently as every 20 min for $3\frac{1}{2}$ h without producing hyper- or hypoalgesia.

Considerable debate has occurred over the years concerning the detection of the anti-nociceptive effects of NSAIDs. The majority of studies in a variety of species have generally shown that PBZ

is a weak or ineffective analgesic at anti-inflammatory doses in assays which measure responses to noxious thermal stimuli (Carroll, 1959; Crepax and Silvestrini, 1963; D'Amour and Smith, 1941). However, PBZ is clearly efficacious as an analgesic in the presence of experimentally induced inflammatory pain (Crepax and Silvestrini, 1963; Winder et al., 1962). It had not yet been established whether this agent alters pain perception in normal non-inflamed tissue in the performance equine. Data from the present study suggest that the horse behaves as other species do in its lack of analgesic response to therapeutically anti-inflammatory doses of PBZ. Peak anti-inflammatory response to a single dose of PBZ generally occurs 10-12 h post-dose in the equine, a time during which prostaglandin (PG) synthesis inhibition should occur in the tissue (Tobin, 1979). Since there was no significant difference between saline and PBZ treatments, particularly 9 h after PBZ administration, it is unlikely that PG is involved in normal thermal pain perception in the equine.

Skin temperature was recorded prior to each stimulus to assess local hyperthermia, as an index of cutaneous inflammation. Since skin temperature did not increase with repeated stimulation over time or differ between treatments, local inflammation can be excluded as a confounding variable in the STRL determinations. While PBZ did not alter normal pain perception, its ability to suppress inflammatory cutaneous pain has yet to be determined using the STR model. Despite rather wide changes in ambient temperature and diurnal variation in rectal temperature, skin temperature fluctuations were minor. This suggests that (a) the thermocouple placed at the skin/air interface closely approximately actual skin surface temperature, (b) skin temperature does not follow a diurnal rhythm, and (c) skin temperature does not passively follow moderate changes in ambient temperature.

Both STRL and HWRL rose linearly over the 15 to 24 h sampling period in the drug and saline treatment groups. This suggests that some repetitive aspect of the experimental procedure produced an increasing elevation in pain threshold. The routine presentation of painful stimuli may have been sufficiently stressful to stimulate the release of endogenous opioid peptides (e.g. endorphin) which in turn raised nociceptive thresholds (Millan and Emrich, 1981). Stress or pain induced analgesia, and its partial or complete reversal by naloxone, has been reported in mice and rats (Amir and Amit, 1978; Grau et al., 1981; Chesher and Chan, 1977). In addition, diurnal differences in opioid peptide levels have been correlated with nociceptive thresholds in mice. Highest and lowest sensitivity to pain were reported in the early morning and late afternoon, respectively (Wesche and Frederickson, 1979). Similar diurnal differences in the equine may exist. Such differences may have been confounded by the schedule of stimulus presentation used in the present study. The role of endogenous opioid peptides in the performance equine remains to be elucidated.

Phenylbutazone appeared to disrupt the diurnal variation in rectal temperature. This was evidenced by the lack of temperature elevation at the 15 h post-dose trial in the PBZ treated group. This was unexpected since it is generally believed that PBZ is antipyretic only in febrile animals (Crepax and Silvestrini, 1963). There are no reports of the effects of PBZ in diurnal temperature variation. In addition, a role for PG in normal diurnal temperature regulation in the equine has not been described. Data from the present study suggest that PG synthesis may be involved in establishing daily peaks in temperature fluctuation. Further study will be necessary to better identify this relationship.

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