Proc 5th Intn'l Conf, Cont use of drugs in race horses, Toronto Canada, June 1983.

PHENYLBUTAZONE: LACK OF EFFECT ON NORMAL CUTANEOUS PAIN PERCEPTION IN THE HORSE

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ABSTRACT

The effects of xylazine, mepivicaine, phenylbutazone and saline on cutaneous pain perception were investigated. A pain stimulus was delivered by focusing a light beam on the fetlock area. The light was adjusted so that the animal voluntarily withdrew the foot after about a 5-second exposure. The time from light exposure to withdrawal of the foot was the reflex latency. Exposures did not extend beyond 15 seconds. Latency in the absence of treatement was determined for each experiment. After injection of xylazine (Rompun®), 1 mg/kg the increase in latency time peaked at 15 seconds, 10 minutes after injection and returned to control after 1 hour. After the medial and lateral palmar metacarpal nerves were blocked with 5 cc of 2% mepivicaine (Carbocaine®), the latency increased to 15 seconds and lasted for 3 hours. Injection of normal saline did not change latency from control values. Horses were administered 3 g/10 lbs of phenylbutazone (I.V.) and tested for their responses at 0, 12, 24 and 36 hours after phenylbutazone. Phenylbutazone pretreated horses were indistinguishable from saline pretreated horses. The data show that the cutaneous response time of these horses to a painful stimulus was extended by pretreatment with xylazine or carbocaine, but were not affected by phenylbutazone or normal saline.

INTRODUCTION

Nociception in conscious animals has been studied using a variety of methods. The noxious thermal method (Hardy *et al.*, 1940) was chosen in our study of pain perception in the horse. When a noxious thermal stimulus is applied to the forelimb of the horse, the hoof withdrawal reflex is elicited upon reaching the pain threshold. This thermal stimulus can be administered repeatedly and a defined end point can be reproducibly obtained. This method causes little or no tissue damage since the ratio between the stimulus eliciting a threshold response and that causing tissue destruction is about 2 to 1 (Hardy *et al.*, 1940). Since previous studies have shown dissociation between drug induced changes in body or skin temperature and pain threshold (Beecher, 1957), the experiments described below measure pain perception rather than heat perception. This technique is sufficiently sensitive to measure the antinociceptive effects of a variety of analgesic drugs (Pippi *et al.*, 1979 a, b).

Phenylbutazone (PBZ) is a potent nonsteroidal anti-inflammatory drug (NSAID) which suppresses the inflammatory response and reduces the hypersensitivity of inflamed tissues by inhibiting the formation of prostaglandins (Tobin, 1981). Our experiments were designed to determine whether PBZ alters pain perception in normal non-lame horses in a way similar to the narcotics or the centrally or peripherally acting analgesics, xylazine and mepivacaine, respectively.

MATERIALS AND METHODS

Mature Thoroughbred and Standardbred mares and geldings, weighing 400 - 500 kg, were used. They were brought into a barn 24 hours before an experiment for acclimation to their stalls. Prior to the application of the noxious heat stimulus, they were confined to equine stockades. Radian thermal stimuli were delivered by a heat lamp which consists of a projector bulb and condenser lens. A beam of light was focused into the metacarpophalangeal joint when the bulb was illuminated (Figure 1). Upon perception of the noxious heat stimulus the horse withdrew the forelimb. The time from illumination of the bulb to withdrawal of the limb was designated as the hood withdrawal reflex latency. This latency was measured by an electronic timer which was synchronized to the heat lamp and operated manually. Lamp intensity was controlled rheostatically and adjusted to produce around a 5 second reflex latency.

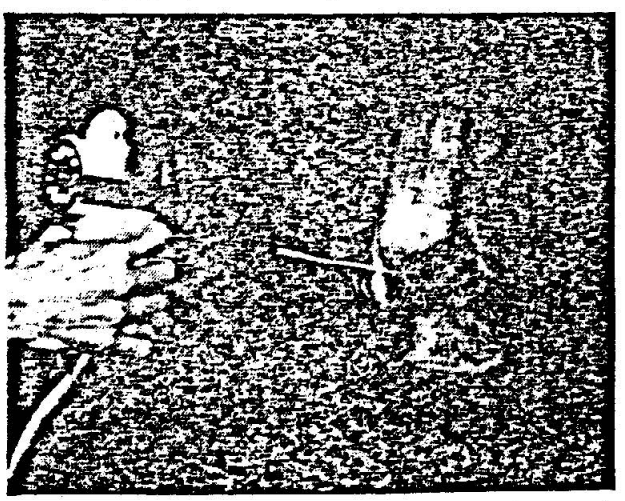

Fig. 1: Application of noxious thermal stimulus to the metacarpophalangeal region. Left side: Experimenter is holding heat lamp. Right side: Focused light beam on right forelimb.

The above procedure was employed to test 3 prototypic analgesic agents in 3 separate experiments. In the first experiment, xylazine (Rompun®) at a dose of 0.5 g/1000 lb. and an equal volume of saline were administered intravenously to the same 5 horses on separate occasions. Reflex latencies were obtained at 5 minute intervals for 20 minutes

prior to treatent and for 60 minutes following treatment. Data were analyzed using an analysis of variance comparing areas under the time action curves for both treatments.

In the second experiment a low volar nerve block was performed to anesthetize the cutaneous receptive field of the metacarpophalangeal joint which was subject to thermal stimulation. The medial and lateral palmar nerves were blocked at the level of the metacarpal bones two and four between the deep digital flexor tendon and the suspensory ligament by depositing 5 cc of mepivacaine (2%) subcutaneously. The medial and lateral palmar metacarpal nerves were blocked by subcutaneous deposition of a similar amount of mepivacaine at the distal ends of metacarpal bones two and four. The cutaneous desensitization of the lateral metacarpophalangeal region was provided by the lateral palmar nerve block (Derksen, 1980). Reflex latencies were obtained 15 minutes prior to and after treatment and subsequently at 30 minute intervals after treatment for 150 to 240 minutes. Separate groups of horses received either mepivacaine or an equal volume of saline. Post-treatment means at each time point were compared using a t-test.

In the third experiment, intravenous phenylbutazone (3 g/1000 lb.) and an equal volume of saline were administered to 4 horses on separate occasions. Each observation period consisted of 6 reflex latencies determined at 30 minute intervals for a total of 150 minutes. This 150 minute observation period was executed at 12, 24 and 36 hours after phenylbutazone and 12 hours after saline. Data were analyzed using an analysis of variance of area under time action curves for each treatment and time post-dose.

RESULTS

The time course of the effects of xylazine on the hoof withdrawal reflex is shown in Figure II. Prolongation of the reflex latency was observed within 5 minutes and became maximal over the next 15 to 20 minutes. Statistically significant analgesia persisted for 40 minutes at which time latencies returned toward control level. When averaged over the 40 minute analgesic period, the mean post-xylazine latency for 5 horses was 10.9 ±2.0 sec. These means were significantly different ($p<.025$).

Xylazine produced a roughly 2-fold increase in cutaneous pain threshold. In addition, xylazine produced sedation for nearly 2 hours. Sedative effects included head drop, ptosis and prolonged weight shift to one leg and position.

The time course of the effects of mepivacaine induced nerve block is shown in Figure III. Mepivacaine produced a rapid prolongation of the reflex latency within 15 minutes after injection. This effect persisted for at least 2½ hours (Table 2). Recovery of normal pain perception varied widely among horses as reflected by the increasing magnitude of the standard errors of the means obtained in the later measurements. Examination of the mean latencies obtained post-treatment show that mepivacaine produced around a 3-fold increase in reflex latency for the first 90 minutes post-treatment and roughly a 2-fold increase in latency during the remaining 60 minutes. The data suggest that normal pain perception is restored by 4 hours after local anesthetic administration.

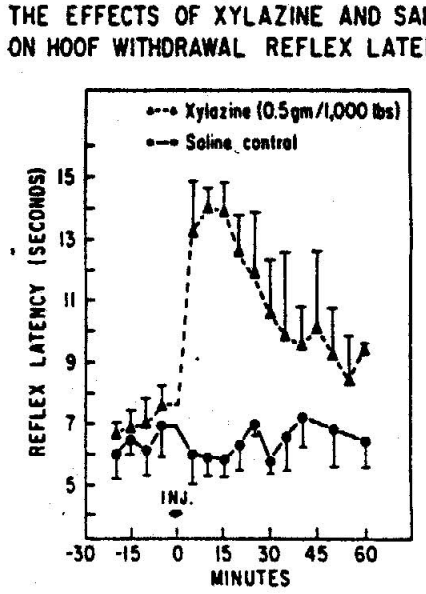

Fig. II: The effects of xylazine and saline on hoof withdrawal reflex latency. Each point represents the mean latency (±sem) of 5 horses.

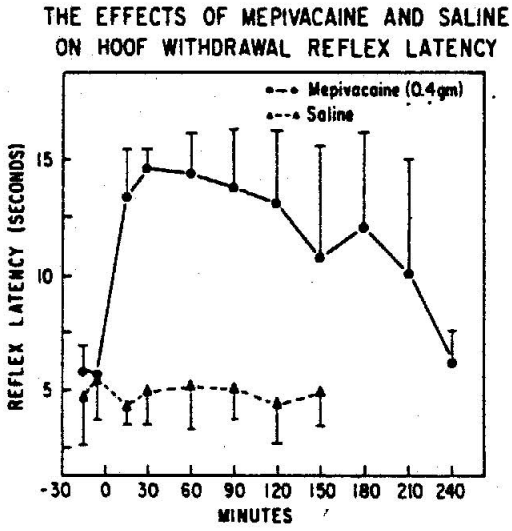

Fig. III: The effects of mepivacaine and saline on hoof withdrawal reflex latency. Each point represents the mean latency (±sem) of 3 to 7 horses.

The time course of the effects of phenylbutazone and saline 12 hours post-treatment is shown in Figure IV. An analysis of variance of area under the time action curve obtained for the 6 observations following saline and PBZ treatment indicated that these treatments did not significantly differ from one another. The mean of these 6 observations was 4.5 ±0.8 sec for saline and 5.0 ±0.8 sec for PBZ (Table 3). The time courses of the effects of PBZ, 12, 24 and 36 hours post-treatment are shown in Figure IV. These curves are nearly superimposable. An analysis of variance comparing areas under the time action curves for the 6 observations are three post-treatment periods indicated no signifiant variance among treatments. Mean latencies calculated for these post-treatment periods were of approximately equal value.

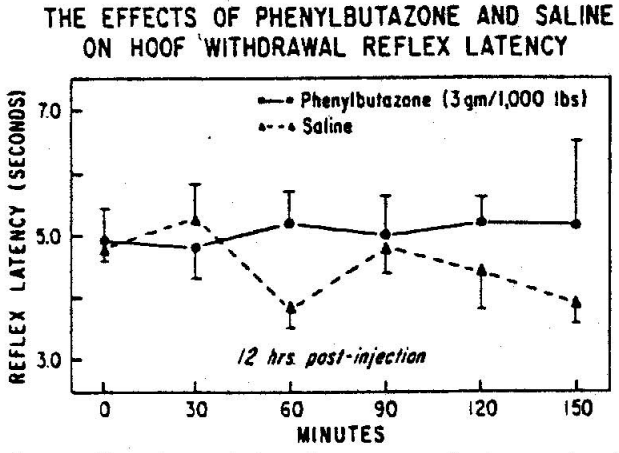

Fig. IV. The effects of phenylbutazone and saline on hoof withdrawal reflex latency. Each point represents the mean latency (±sem) of 4 horses.

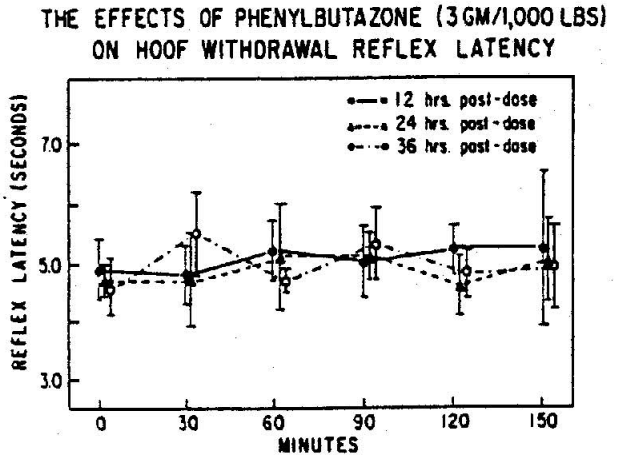

Fig. V. The effects of phenylbutazone (3 g/1000 lbs.) on hoof withdrawal reflex latency 12, 24 and 36 hours post-dose. Each point represents the mean latency (±sem) of 4 horses.

DISCUSSION

In this preliminary study of pain perception in the horse, three different prototypic drugs were assayed for their analgesic effects. Xylazine is a non-opiate, centrally acting analgesic with potent sedative and muscle relaxant properties. Its depressant effects have been attributed to an action at alpha-II adrenergic receptors (Hsu, 1981). Our results indicated that therapeutic doses of zylazine produce a short-term period of analgesia and clearly alter normal pain perception. This analgesic effect is best described as an elevation in normal cutaneous pain threshold, as reflected by the observed increase in reflex latency. The xylazine treated group were clearly able to tolerate the painful stimulus for a longer period of time than the saline treated group.

Mepivacaine is a potent local anesthetic which reversibly prevents the generation and transmission of nerve impulses. Local anesthetics are analgesic in that they eliminate pain sensations by acting directly on neurons which transmit pain information. In our studies, mepivacaine clearly anesthetized the cutaneous receptive field upon which the thermal stimuli were applied. Pain threshold was raised markedly following drug administration. Analgesia was more profound and prolonged following local anesthetic than that observed after xylazine. The variability in recovery of normal pain perception among horses probably reflects intersubject differences in local anesthetic deposition as well as differences in the systemic absorption or metabolism of the drug.

Phenylbutazone is a widely used NSAID in equine medicine. However, it has been unclear whether drugs of this class can alter normal pain perception in non-inflammed tissue. The aspirin-like mild analgesics can raise cutaneous thermal pain threshold according to some studies. However, failure to distinguish the analgesic effects of these agents from placebo has been reported by others (see review by Beecher, 1957). In our studies doses of PBZ were employed which generally reduce signs of inflammatory pain. The peak anti-inflammatory action of PBZ is generally perceived as 10-12 hours (Tobin, 1979, Houdeshell & Hennessey, 1977). Since no differences in reflex latencies were seen between saline and PBZ treatments during the 12 hour post-dose observation period, we conclude that normal cutaneous pain threshold was not altered. The anti-inflammatory effects and plasma levels of PBZ are minimal, 24-36 hours following a single dose (Houdeshell & Hennessey, 1977; Tobin, 1979). These data agree with our observation showing no changes in pain threshold 24 to 36 hours post-dose.

In conclusion, centrally and locally acting analgesics can alter normal cutaneous perception of noxious thermal stimuli in the horse. The hoof withdrawal reflex latency was a sufficiently sensitive and reproducible measure of pain threshold to assay analgesic drugs. Projection of radiant heat according to the method described above is an effective means of delivering noxious thermal stimuli, which agrees with other pain studies (Pippi *et al.*, 1979 a, b). It appears that in the absence of local inflammation PBZ does not alter normal perception of painful stimuli in the horse.

REFERENCES

1. Beecher H.K. The measurement of pain: Prototype for the quantitative study of subjective responses. Pharmacol Rev., 9: 59-209. 1957
2. Derksen F.J. Diagnostic anesthesia of the equine front limb. Equine Prac, 2: 42-47, 1980.
3. Hardy J.D., Wolff H.G. and Goodell H. Studies on pain. A new method for measuring pain threshold. Observaions on spatial summations of pain. J Clin Invest, 19: 649-657, 1940.
4. Houdeshell J.W. and Hennessey P.W. A new nonsteroidal anti-inflammatory analgesic for horses. J Equine Med Surg, 1: 57-63. 1977.
5. Hsu W.H. Xylazine-induced depression and its antagonism by alpha-adrenergic blocking agents. J Pharmacol Exp Ther. 218: 188-192. 1981.
6. Pippi N.L. and Lumb W.V. Objective tests of analgesic drugs in ponies. Am J Vet Res. 40: 1082-1086. 1979a.
7. Pippi N.L., Lumb W.V., Fialho S.A.G., Scott R.J. A model for evaluating pain in ponies. J Equine Med Surg. 3: 430-435. 1979b.
8. Richie J.M. and Nicholas M.G. Local anesthetics. chapter 15 in *Pharmacological Basis of Therapeutics*. Gilman A.G., Goodman L.S., Gilman A. (ed.). Macmillan, New York, 1980. pp 300-320.
9. Tobin T. Pharmacology Review: The non-steroidal anti-inflammatory drugs. I. Phenylbutazone. J Eq Med Surg, 3: 253-258. 1979.
10. Tobin T. and Heard R. *Drugs and the Performance Horse*. Charles C. Thomas, Publishers, Springfield, Illinois. 1981.

FOOTNOTES

Published as Kentucky Agricultural Experiment Station Article 83-4-141, with the approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Publication #91 from the Kentucky Equine Drug Testing and Research Programs and the Department of Veterinary Science.

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DISCUSSION

SOMA: That's a neat study. I was wondering if you had considered more of an inflammatory model, such as the injection of lactic acid or carogene which is a more difficult thing to do but more mimics the natural condition that you're dealing with?

DeQUICK: That's an interesting suggestion. Our presentation consisted of preliminary studies, and we hope to look into those types of experiments in future studies.

SOMA: Do you have any comments on that kind of model?

DeQUICK: No not at this time.

KAMERLING: I'd be happy to take that comment for you. This is a model of acute pain perception and one of its weaknessess is that it does not reflect what occurs in a chronic condition, such as during chronic inflammation. It would be nice to have a good model of reproducible chronic lameness in which we could induce lameness, measure it in an objective way, show a difference between the untreated and treated conditions, and get dose related changes in the degree of lameness either as changes in gate, swelling or in the thermographic measurement of a joint for instance. This would give us more of a clue as to what's going on in a chronic state.

SOMA: The model that you have is a good model for incisional type pain which reproduce surgically or possibly post-surgically, it's not an efficient model for the pain which evolves 2 or 3 or 5 hours post-surgically following the inflammatory reaction, so you'd need some kind of an inflammatory and then assess stride length or pressure on the limbs of things of that sort, which is a little bit more difficult or you'd have to go to a navicular disease or get natural occurring pain and then use that. Now we at one time had a shoe which we tried with this kind of pain and we had moderate success by using a shoe on the front feet of an animal, but we were looking at horses with natural occurring pain, low grade which was amenable to the anti-inflammatory drugs.

KAMERLING There have been several models that have attempted to address this question, and I agree it needs to be addressed. Pratt and co-workers from M.I.T. have developed a force plate in which they measure lameness. They tie up a horse's forelimb and measure how much weight bearing occurs on the lame limb by measuring deviations about the mean in terms of weight shifting. If a horse is in fact lame through some joint inflammation it will waver and show a greater deviation about the mean in terms of its weight bearing ability. There is a Swedish group, I believe, who have done locomotor analyses using high speed cinematogrphy in which they measure gate parameters. However, they have never used this method to study lameness. Lame horses as one group could be compared to another group of non-lame horses and measured for changes in stride length, stride time, and suspension time. This type of model could then be used to assess gate parameters and chronic inflammatory lameness as well. That may have.answered your question.

SOMA: I don't want to hog the discussion, but can I ask another question? One of the problems with the force plate is you have to train your horse to the force plate, because if you hit the force plate at a diferent speed, and there is certainly a window of speed which you can increase the total amount of force you put down, if you go faster or slower, and your horse has to be well acquainted with that plate, because they'll tend to deviate away from it or with the force plate if they really hurt in the front they'll go back on their hind legs and they'll redistribute the weight distribution so you have a problem with the force plate too.

KAMERLING: Right.

BEAUMIER: In looking at your phenylbutazone study, did you try shorter times to see if there was any influence at say 3 and 6 hours?

DeQUICK: No we didn't, we felt that the 12 hour sampling time was significant since the peak therapeutic effect of phenylbutazone is approximately 12 hours.

BEAUMIER: Maybe one of the phenylbutazone effects, but it may have other effects too. I'm just thinking that would complete the thing.

KAMERLING: We based that time point on peak analgesic actions that have been reported in literature, but I think, we will redo this, we'll probably do it at earlier intervals. I think it's a good idea.

WEBER: Did you anticipate an analgesic effect with phenylbutazone, with this type of pain stimulant?

DeQUICK: No not originally.

KAMELING: It was based on literature regarding analgesic effectiveness using the non-steroidals aspirin-like drugs on models of this type, it's controversial, some people show it, some don't. What we need to do is, if we could have demonstrated hyperanalgesia and then tested these drugs we might have seen a more pronounced effect.