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Determination of highest no effect dose (HNED) for local anaesthetic responses to procaine, cocaine, bupivacaine and benzocaine

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Summary

The highest no effect doses (HNEDs) for the local anaesthetic (LA) effects of procaine, cocaine, bupivacaine and benzocaine were determined using the heat lamp/hoof withdrawal model of Kamerling *et al.* (1985b) and the abaxial sesamoid block model of local anaesthesia. The heat lamp rapidly (4 or 5 s) increased the temperature of the superficial skin layers of the pastern to about 90°C, at which point the animal sharply withdrew its hoof. Effective LA blockade precluded this response and superficial skin temperatures exceeded 120°C. Thermal stimulus experiments were routinely terminated after 10 s of exposure to prevent undue tissue damage.

Following abaxial sesamoid block with bupivacaine, the HNED for that drug was about 0.25 mg/site. Increasing the dose to 2 mg/site apparently produced complete and prolonged LA blockade.

Analogous work showed that the HNED for procaine was about 2.5 mg/site. Similarly, the dose response curve for procaine was parallel with that of bupivacaine but was shifted 10-fold to the right. The duration of the LA response following procaine injection was less than for bupivacaine with the statistically significant response following 40 mg/site injection lasting less than 45 min.

Cocaine was less potent than procaine, showing a shallower dose response curve. The HNED for cocaine was less than 5 mg/site, although at this dose the duration of action was extremely short (<7.5 min). Benzocaine had no significant LA action when a dose of 800 mg was applied topically as a 5% preparation.

These results show that the HNEDs for bupivacaine and procaine are remarkably low, that cocaine is somewhat less potent as a LA than might be expected, and that 5% topical benzocaine has no significant pharmacology. The small doses of bupivacaine and procaine producing effective local anaesthesia suggests that developing plasma thresholds for these agents is likely to be very challenging.

Introduction

Within the last decade, the sensitivity of equine drug testing has increased dramatically. Furthermore, the ability of chemists to detect traces of therapeutic medications in post race samples has also improved greatly. For example, procaine is one of the most commonly detected drugs in post race urine samples (Tobin and Blake 1977), with 73 identifications during the last 2.5 years (R. Gowen, personal communication). Many of these identifications appear to be inadvertent, resulting from administration of procaine as procaine penicillin or other legal therapeutic agents. Because procaine penicillin is a legitimate therapeutic agent used widely by veterinarians and since procaine is notoriously persistent in urine, inadvertent procaine identifications are a major problem for equine veterinarians, horsemen and regulatory officials. Less likely sources of procaine in equine urine are illegal administrations of this drug as nerve or joint 'blocks', the uses of procaine that are of regulatory concern (Tobin and Blake 1976; Tobin *et al.* 1977a,b; Tobin 1981).

Other LAs that may also yield significant residual concentrations include benzocaine, a topical LA found in leg braces and liniments (7 positives in last 2.5 years; R. Gowen, personal communication); bupivacaine, a high potency LA used in veterinary practice (1 positive during past 2.5 years; R. Gowen, personal communication) and cocaine, which has both LA (Lumb and Jones 1984) and stimulatory actions (McKeever *et al.* 1993). Although the number of positives for cocaine during the past 2.5 years is unavailable, recent identifications involving prominent horsemen has focused attention on the ease with which cocaine contamination of forensic samples can occur. All of these agents have been detected in saliva, serum, or urine of racing horses and the significance of trace or residual concentrations has been debated.

Currently, many racing jurisdictions use arbitrary thresholds based on the experience, skills, or analytical capabilities of their analysts instead of scientifically established thresholds. One approach to interpreting the significance of trace or residual

TABLE 1: National Institute of Drug Abuse (NIDA) thresholds

Drug	Screening threshold	Confirmation threshold
Amphetamines	1000 ng/ml	500 ng/ml
Cocaine metabolites	300 ng/ml	150 ng/ml
Phencyclidine	25 ng/ml	25 ng/ml
Opiates	300 ng/ml	300 ng/ml
THC (marijuana)	50 ng/ml	15 ng/ml

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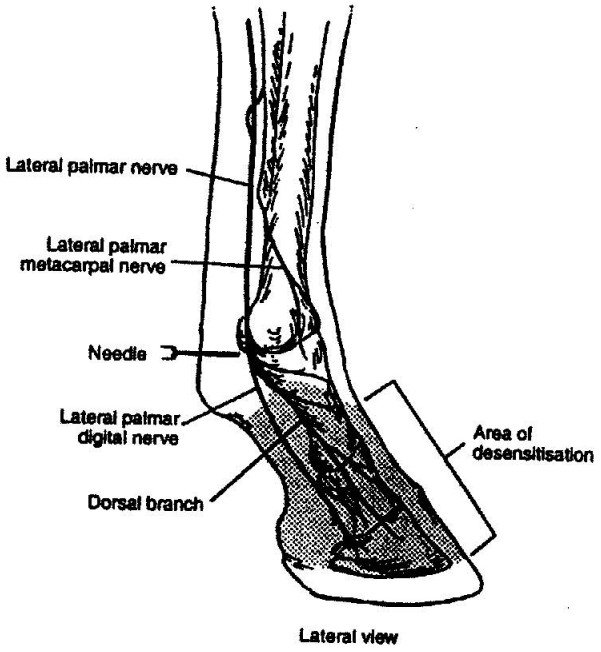


Fig 1: Abaxial sesamoid block model. The needle indicates the site of injection and the shaded area illustrates the approximate area of effective LA block.

concentrations of a therapeutic medication is to determine it's highest no effect dose (HNED), defined as 'the highest dose of a drug at which there is no possibility of the horse having been pharmacologically influenced by the drug during a race'. Once the HNED is established, the highest plasma/urinary concentration or highest no effect threshold (NET) can be determined, that is 'the highest plasma or urinary concentration of a drug or specified metabolite following HNED administration'.

The NETs should be sufficiently low to preclude any performance-altering effect of subthreshold concentrations of a medication. Subthreshold post race samples could, therefore, be chemically positive but, by definition, would be forensically negative.

A broadly similar approach is standard in human forensic science, where positives for the 'NIDA' 5 (Table 1) are not identified if certain 'cutoffs' (thresholds) are not exceeded. Application of the 'thresholds' concept in veterinary science is simply an extension of an already well established practice in analysis of human drug abuse.

To establish such NETs, HNEDs to insure no pharmacological effect from the medication in question must first be verified. In this report, we describe the establishment of HNEDs for 4 LAs in horses: procaine, cocaine, bupivacaine and benzocaine.

Materials and methods

Horses

A total of 6 mature Thoroughbred mares weighing 413–602 kg were used for several dose response experiments; although, not all mares were used for each drug evaluated. All horses were acclimatised to their stalls 24 h prior to experimentation. Because of the critical role of superficial skin temperature in these experiments, no LA quantification experiments were performed when ambient temperature was less than 10°C. At least 4 and,

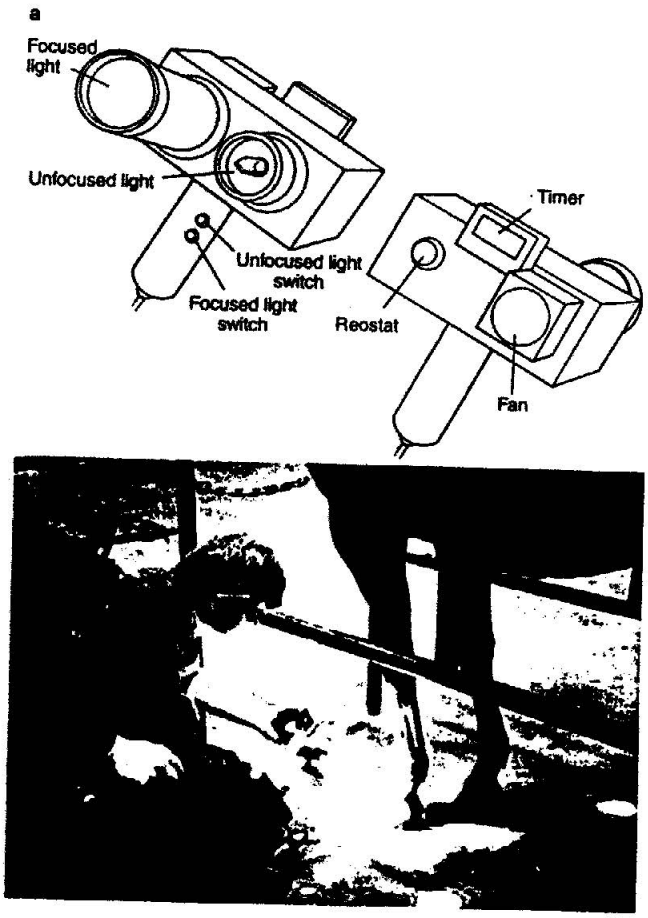


Fig 2: a) Hand held KHT radiant heat lamp. Focused light caused unanaesthetised skin to react in about 4 s. Unfocused light reduced conditioning response to visual stimuli. b) Light focused on pastern area.

more commonly, 7 days elapsed between individual LA dose response curve experiments.

Site preparation

Before each experiment in which a LA (procaine, cocaine, bupivacaine) was injected, the hair on the dorsal and lateral sides of the fore leg pasterns was clipped and the pastern was blackened with stamp pad ink (Dennison Manufacturing Co, Framingham, Massachusetts, USA) to insure equal and consistent heat absorption for all horses. Before each topical anaesthetic (benzocaine) experiment, the hair on the dorsal and lateral aspects of the metacarpus and pastern was clipped and the leg was blackened with stamp pad ink. Contralateral legs were also clipped, blackened and tested to assess any systemic effect of the LAs.

Drug administration

All injectable drugs were administered subcutaneously in a standard volume of 2 ml with matching doses of 2 ml of saline administered to the contralateral leg. The site of injection was into the area of the lateral palmar nerve where it passes lateral (abaxial) to the lateral sesamoid bone, as indicated in Figure 1. This block is referred to in clinical practice as an abaxial sesamoid block. Following injections, the horses were confined to the stall. Therefore, the conclusions of the study are directly applicable for unexercised horses.

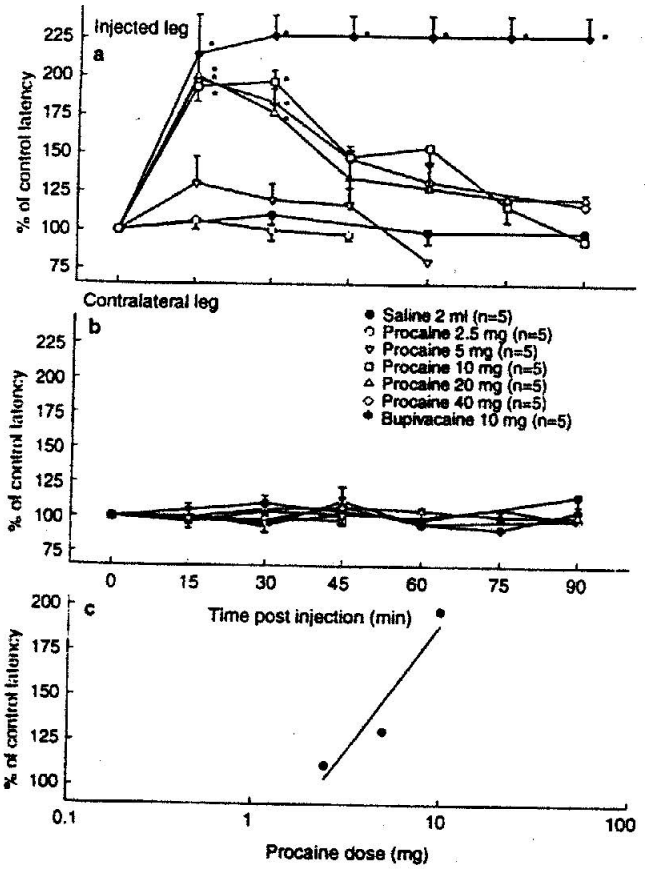


*Fig 4: a) Mean ± s.e. % increase in HWRL following injection of procaine doses. b) Per cent change in contralateral leg following saline injection. c) Procaine dose response curve. *significantly different from control values.*

distance. Data from each image was recorded on videotape during thermographic analysis.

Superficial skin temperatures of control and anaesthetised limbs were recorded at 1.75 s intervals. Temperature measurements began 3.5 s before heat stimulation using a focused light source (described below). Temperature measurements continued throughout heat stimulation and until skin temperature returned to baseline. A digital thermal image processing system was used to record the temperature of the superficial skin at the site of thermal challenge.

Determination of local anaesthetic effect

Dose and time response relationships for procaine, cocaine, benzocaine and bupivacaine were determined with a heat projection lamp (Fig 2a) adapted from that described by Kamerling *et al.* (1983; 1985a, b). Briefly, focused radiant light/heat was used as a noxious stimulus and was directed onto the pastern (Fig 2b) to elicit the classic flexion-withdrawal reflex. Hoof withdrawal reflex latency (HWRL) is defined as the time between lamp illumination and withdrawal of the hoof. These times could be adjusted by varying the intensity of the heat output of the lamp with a rheostat. In general, the intensity of the light beam was adjusted so that HWRL period was about 3–4 s, with the actual HWRL recorded on an electronic timer built into the lamp. In the anaesthetised leg, the duration of light exposure was limited to 10 s to prevent undue damage to the skin. A secondary unfocused light beam (sham light) was used to confound the horse, reducing the possibility that the flexion-withdrawal reflex was to visual rather than thermal perception of the focused light beam.

Dose and time response relationships

The HWRL was measured at -30 and -15 min and immediately before injection or topical application of the LAs. These 3 HWRL times were used to establish a control value for HWRL in each horse. The HWRL was also measured at 7.5, 15, 30, 45, 60, 75, 90, 120, 150 and 180 min after the limb areas had been 'blocked' by the different doses of LA. The HWRL is expressed as a percent of control values. Using this model, full dose response curves for the LA actions of procaine, cocaine, and bupivacaine were developed.

Statistical analysis

Data are presented as mean ± s.e. Paired *t* tests were used to compare HWRL saline and treatment values for procaine, benzocaine, cocaine, and bupivacaine at each measuring time. Significance was set at $P<0.05$.

Dose response curves were determined from the regression of the peak HWRL for each dose and the injected doses. The slopes of the regressions for the dose-response curves were compared for parallelism. The dose of drug required to produce 50% of maximal response was used to compare potencies of the LAs.

Results

Skin temperature measurements

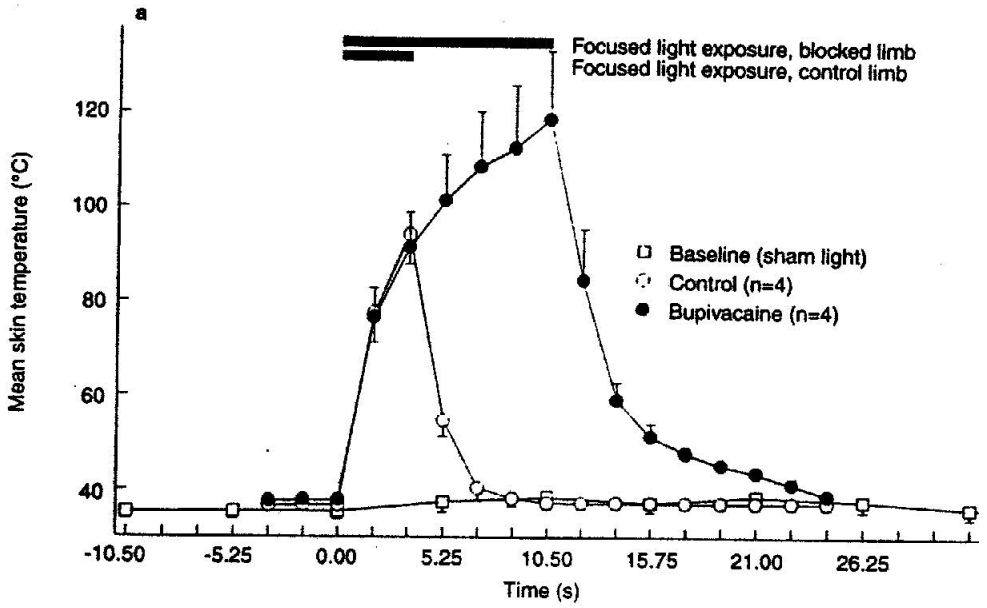
Figure 3a shows the effects on superficial skin temperature following exposure to the focused (control and bupivacaine) and unfocused (baseline) lights. Little change was seen in the surface skin temperature following exposure to the unfocused light. Similarly, little change was seen in the superficial skin temperature prior to activation of the focused light (Time= -3.50 to 0 s) on a specific portion of the pastern. Once the focused light was directed onto the skin, the temperature of the skin surface rose rapidly. The superficial skin temperature of unanaesthetised limbs (open circles, Fig 3a) increased to about 95°C, at which point the horse signalled it's perception of the noxious stimulus by withdrawing the hoof rapidly and replacing it sharply on the ground. At that point, the light was discontinued and the skin temperature rapidly returned to baseline.

The solid circles in Figure 3a show the increase in superficial skin temperature after blockade of the lateral palmar digital nerve with 10 mg of bupivacaine administered subcutaneously over the lateral sesamoid bone as described in methods. In the anaesthetised limb, the noxious stimulus was not perceived, and the superficial skin temperature continued to rise beyond peak control temperatures. After 10 s exposure, the focused light was discontinued and the temperature of the superficial skin rapidly returned to control values. Mean superficial skin temperatures of anaesthetised limbs reached about 120°C after 10 s exposure.

Figure 3b is a thermographic representation of the experimental site during exposure to the focused lamp, identifying the location and dimensions of the area of superficial skin temperature increase.

Dose and time response curves to procaine

The LA effect of procaine HCl is illustrated in Figure 4a. After administration of doses of 10, 20 and 40 mg procaine HCl, there was a significant difference between control and procaine values up to 30 min after injection. Additionally, there was a significant difference between negative (saline) and positive (bupivacaine, 10 mg) control



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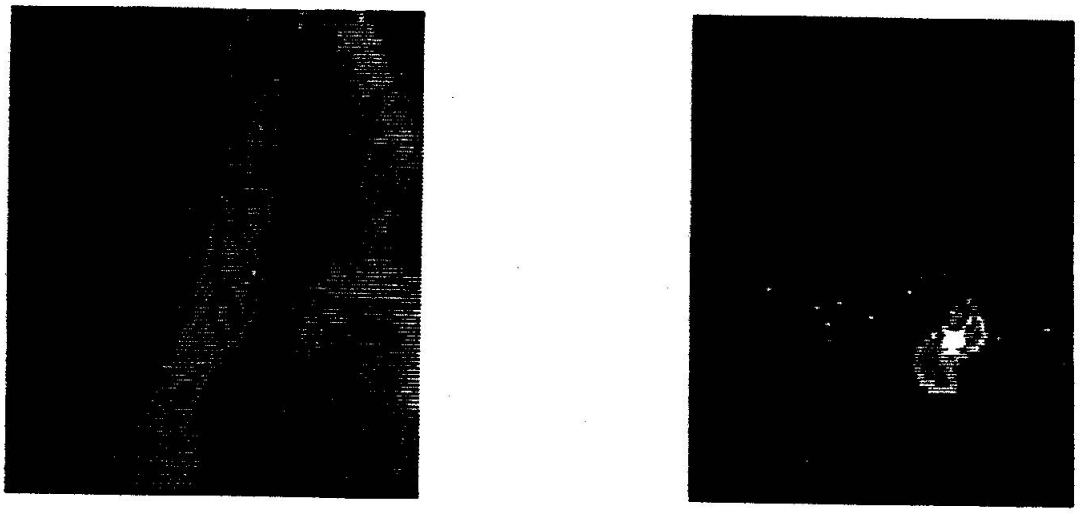

Fig 3: a) Mean ± s.e. change in skin temperature during heat lamp experiment after saline (control) and bupivacaine injections (10 mg). b) Thermograph of pastern control (left), heat lamp exposure (right).

Randomly selected doses of 2% procaine HCl (2.5, 5, 10, 20 and 40 mg; Abbott Laboratories, North Chicago, Illinois, USA), 0.5% bupivacaine HCl (0.25, 0.5, 1, 2, and 10 mg; Abbott Laboratories) and doses (1.5, 5, 15 and 45 mg) of crystalline cocaine HCL (Sigma Chemical Company, St. Louis, Missouri, USA) were injected at the test site. Topical benzocaine (800 mg; EPF-5, Summit Hill Laboratories, Navesink, New Jersey, USA) was applied to the dorsal and lateral side of the metacarpus between the carpus and fetlock. Care was taken to insure no benzocaine contacted the pastern area. Saline (2 ml) and bupivacaine HCL (10 mg/2 ml) were injected as negative and positive controls, respectively.

During our initial experimental work, it was apparent that LAs were more effective in a larger rather than smaller volume of injection. For example, in commercial preparations of bupivacaine, 0.5 mg of the drug is available in a volume of only 0.1 ml. By diluting this dose with saline to a 2 ml bolus, the placement of the LA is not as critical and the chance of a significant LA effect is increased. For this reason, the volume of injection was standardised at 2 ml and all drugs and control injections were administered at this volume. To control for possible effects of pressure or volume, a similar volume of normal saline was injected into the contralateral leg, which was tested in parallel with the LA-treated leg.

Skin temperature measurements

An Agema Thermovision 870 infrared scanner (Agema Infrared Systems, Secaucus, New Jersey, USA) was used for infrared imaging of all horses. The superficial skin temperature was measured in an environmentally controlled laboratory (room temperature=22°C) and all images were recorded from a standard

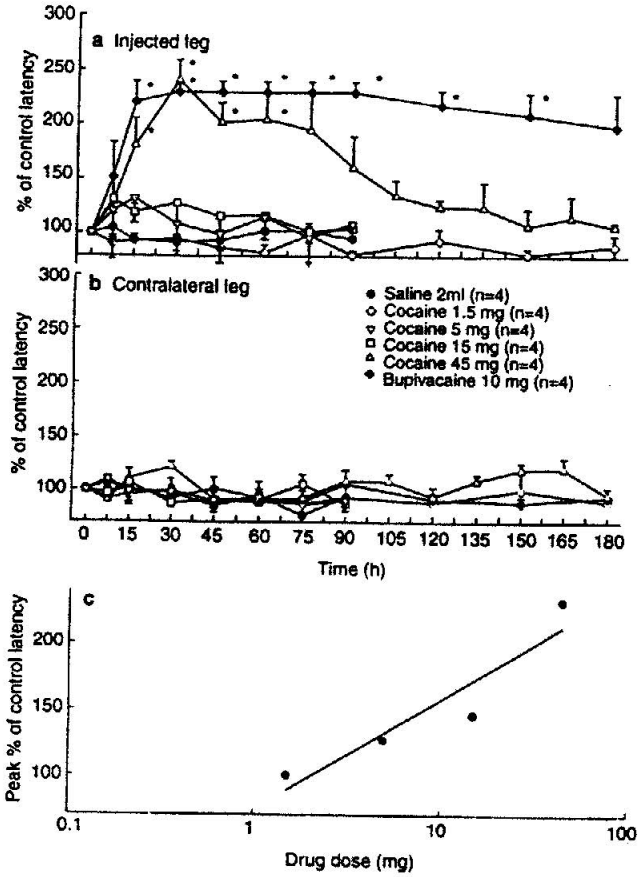

*Fig 5: a) Mean ± s.e. % increase in HWRL following injection of cocaine doses. b) Mean ± s.e. % change in contralateral leg following saline injection. c) Cocaine dose response curve. *significantly different from control values.*

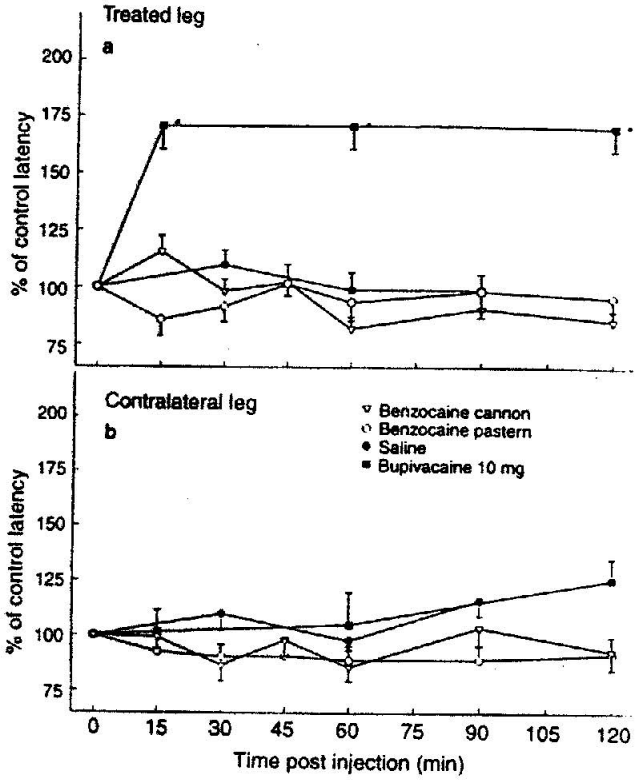

*Fig 6: a) Mean ± s.e. % increase in HWRL in cannon and pastern following benzocaine (800 mg/5% gel) application to cannon bone. b) Mean ± s.e. % change in contralateral leg. *significantly different from control values.*

values at every time point after anaesthetic injection. Although there was a slight increase in HWRL 15 min following injection of 5 mg of the drug, there was no statistically significant LA effect following injections of 2.5 and 5 mg procaine HCl.

As shown in Fig 4b, administration of 2 ml of normal saline to the contralateral limb produced no significant changes in HWRL. The dose response curve for the peak LA response to procaine is presented in Figure 4c.

Dose and time response curves to cocaine

The LA effect of cocaine HCl is illustrated in Figure 5a. After administration of 45 mg cocaine HCl, there were significant differences between saline controls and cocaine values at 15, 30, 45 and 60 min after injection. There were no significant local anaesthetic effects following injections of 1.5 and 15 mg cocaine HCl; however, there was a significant LA effect at 15 min after injection of 5 mg cocaine HCl. There were significant differences between positive (bupivacaine, 10 mg) and negative (saline) control values at every time point after anaesthetic injection.

There was no apparent anaesthetic effect in the contralateral leg at any time point after administration of cocaine at any dosage rate (Fig 5b), suggesting no central nervous system effect of the administered cocaine. Also shown in Figure 5b, administration of 2 ml of normal saline to the contralateral limb produced no significant changes in HWRL. The dose response curve for the peak LA response to cocaine is presented in Figure 5c.

Dose and time response curves to benzocaine

The LA effect of topically administered benzocaine (800 mg in a 5% gel) is illustrated in Figure 6. There was no significant difference between control and benzocaine-treated cannon or pastern values at any point during the test (Fig 6a). There was a significant difference between negative (saline) and positive (bupivacaine, 10 mg) control values at every time point after the anaesthetic was injected. Furthermore, there was no anaesthetic effect in the contralateral leg (Fig 6b).

Dose and time response curves to bupivacaine

The LA effect of bupivacaine HCl is illustrated in Figure 7a. After administration of 10 mg bupivacaine HCl, there were significant differences between saline controls and bupivacaine values at every time point after anaesthetic injection. For doses of 2, 1 and 0.5 mg bupivacaine HCl, there were significant differences between saline controls and bupivacaine values from 15–90, 30–75 and at 30 min post injection, respectively. For doses of 0.25 mg, there was no significant difference between saline controls and bupivacaine values at any time post injection.

There was no apparent anaesthetic effect in the contralateral leg at any time point after administration of bupivacaine at any dosage rate (Fig 7b), suggesting no central nervous system effect of the administered bupivacaine.

Also shown in Figure 7b, administration 2 ml normal saline to the contralateral limb produced no significant changes in HWRL. The dose response curve for the peak LA response to bupivacaine is presented in Figure 7c.

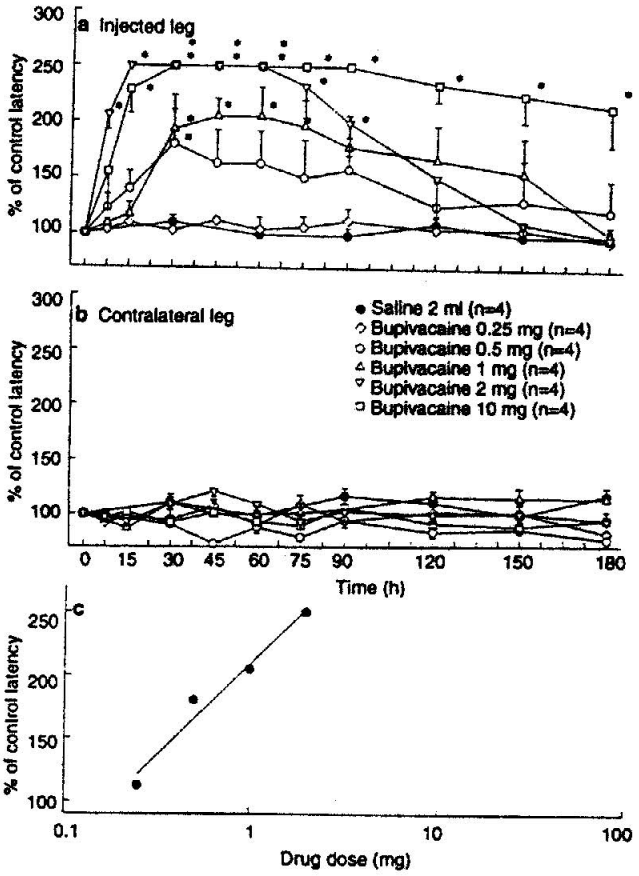

*Fig 7: a) Mean ± s.e. % increase in HWRL after injection of bupivacaine doses. b) Mean ± s.e. % change in contralateral leg after saline injection. c) Bupivacaine dose response curve. *significantly different from control values.*

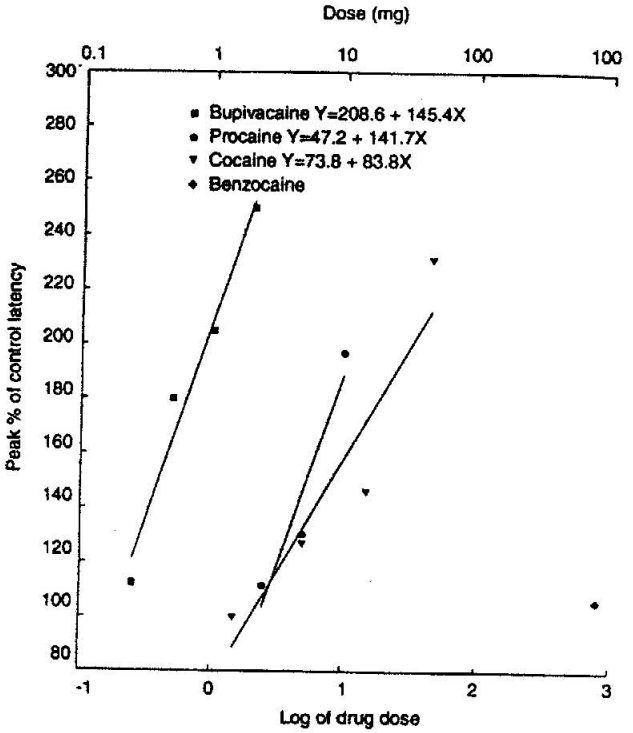

Fig 8: Dose response curves and regression equations for bupivacaine, procaine, cocaine, and benzocaine. Drug dose (mg) is on top x-axis and log of drug dose is on bottom x-axis.

Composite dose response curves

Figure 8 is a composite of the dose response curves for each LA. The vertical axis is arithmetic and represents a percentage of the control HWRL. The lower horizontal axis is a logarithmic scale of the drug dose. The top horizontal axis is an arithmetic scale of the logarithm of the drug dose. Because the dose-response curve for bupivacaine is farthest to the left, that drug is effective at a much lower dose than the other LAs. There was no significant difference between the slopes of the dose response curves for procaine and bupivacaine suggesting that the drugs act on the same receptor and have similar efficacies. The doses for 50% of the maximal effect (150% of control latency) for bupivacaine, procaine and cocaine were 0.77, 8.26 and 24.9 mg, respectively, suggesting bupivacaine is over 11 times more potent than procaine and 32 times more potent than cocaine.

The slope of the dose response curve for cocaine was significantly shallower than the slopes for procaine and bupivacaine, illustrating the wide dose range for cocaine. Note the lack of LA effect for benzocaine, even at the high dose of 800 mg/site.

Discussion

We have used a clinical model of local anaesthesia to evaluate the minimum doses of LAs required to produce a significant LA effect in the horse. The clinical model selected was a unilateral abaxial sesamoid block, in which the dose of the LA to be tested was administered in a 2 ml volume.

Bupivacaine is a highly effective LA and doses of only 2 mg/site produce complete local anaesthesia (Fig 7a). Furthermore, the dose must be reduced about 10-fold to eliminate a significant anaesthetic effect. From these data, it appears that the HNED for bupivacaine is about 0.25 mg/site, a surprisingly small quantity of drug. The significant local anaesthesia seen at doses of 0.5 mg/site illustrates the efficacy of modern LA agents. The high efficacy of bupivacaine is also evident from the steep dose-response curve illustrated in Figure 8.

Inadvertent detection of procaine in post race urine samples is a major problem for equine veterinarians, horsemen and regulatory officials arising from the improved ability of chemists to detect small traces of therapeutic medications in post race samples. The LA effect of procaine is considerably less potent than that of bupivacaine. The data show that no discernible local anaesthesia was produced with a dose of 2.5 mg/site (Fig 4a), the apparent NED, which again is a very small quantity of drug. A procaine dose of 5 mg/site produced a discernable, although statistically nonsignificant, effect, and a dose of 10 mg/site produced virtually full, though transient, local anaesthesia (Fig 4a). The dose response curve for procaine was parallel in slope but 11-fold less potent than observed for bupivacaine (Fig 8).

Cocaine is a major drug of abuse in man and is widely available among certain groups. Furthermore, cocaine is well absorbed across mucous membranes (Jaffe 1993), and little skill is required to apply the drug to mouth, nose, or genitalia of a horse. Such applications may be intentional or inadvertent. A recent study (McKeever *et al.* 1993) showed that the pharmacological effects of cocaine are short lived, suggesting limited usefulness for cocaine as a stimulant medication in racing horses.

Cocaine and its metabolites are commonly detected in post race urine samples Anon (1994). Furthermore, cocaine has

reportedly been applied to tongue ties prior to a race, suggesting intent to alter racing performance (R. Jensen, personal communication). While it is almost always unclear how cocaine gets into post race urine samples, one possible abuse of cocaine involves its misuse as an LA.

As shown in Figure 5a, cocaine was relatively less effective than bupivacaine or procaine as a LA, with a very shallow dose response curve (Fig 8). To produce a 'full' LA response comparable to that seen with procaine or bupivacaine, a 45 mg/site dose of cocaine was required. This is a significant total dose of cocaine, and it raises the possibility that central effects of this drug might be significant. However, review of the HWRL for the contralateral leg (Fig 5b) showed no crossover effect, suggesting that this dose of cocaine produced no systemic effects detectable in the contralateral leg. Decreasing the injected dose of cocaine to 15 mg/site substantially reduced the LA response, while decreasing the dose to 5 mg reduced the local response even further. However, the 5 mg/site dose produced a transient and statistically significant increase in the heat lamp reaction time 15 min after dosing. Because of this, the NED for cocaine is about 1.5 mg/site, although the effect produced with 5–15 mg/site doses was small and transient.

It was interesting that significant local anaesthesia was produced by the 5 mg dose of cocaine but not by the 15 mg dose. This is partially explained by the fact that, with the smaller doses of local anaesthetic, the skill with which the injection was located progressively became more important as a determinant of the degree of anaesthesia. Although the same investigator (JDH) made all LA injections, the 5 mg injections were probably placed more accurately around the nerve than were the 15 mg injections. Additionally, the HWRL standard error for the 5 mg injections was relatively small.

Review of the LA data obtained after administration of cocaine suggests that abuse of cocaine as an LA should be easily controlled. The dose for effective local anaesthesia is apparently not less than about 45 mg/site. Such a high dose of cocaine is easily detectable after subcutaneous administration of this drug (Tobin *et al.* 1988). Use of cocaine as a LA to influence performance is therefore unlikely to occur in the face of sensitive immunoassay-based drug testing.

Benzocaine is a widely-used topical LA and is a common additive in leg braces and liniments used in racing horses. The drug is rapidly absorbed and reaches peak concentration in the urine between 1–3 h post dosing (Annan *et al.* 1983). Because benzocaine is classified as a LA, its detection in postrace samples has resulted in 7 identifications in North American racing during the past 2.5 years (R. Gowen, personal communication). As shown in Figure 6, topical application of benzocaine to the cannon bone had no effect on the heat lamp response times, neither in the area of local application (metacarpus) nor in areas distal to the local application (pasterns). The total dose of benzocaine applied at each test site was 800 mg and the absence of any LA effect suggests that the LA efficacy of benzocaine at the concentrations used in these tests is negligible.

For all LAs tested, the absence of clear indications of local anaesthesia in the contralateral legs suggested there was no central nervous effect. This was not surprising for procaine, bupivacaine and benzocaine. However, following 45 mg cocaine injection, there was a nonsignificant increase in HWRL for the contralateral leg at 30 min post injection. In 2 horses, there were increases in HWRL in the contralateral legs, but the other 2 horses demonstrated no increased effect. Therefore, there may be individual differences between horses regarding systemic sensitivity to relatively low concentrations of cocaine, as suggested by Shults *et al.* (1982).

From a regulatory perspective, the parent drug and/or its metabolites are detectable long after the LA effect has subsided. Although the LA effect of bupivacaine disappears by 10 h after injection (Lumb and Jones 1984), the major metabolite of bupivacaine (*para*-hydroxylated bupivacaine) persists in equine urine for 24 h (Anon 1991). Although the duration of local anaesthesia following benzocaine application was zero, benzocaine is detectable by thin layer chromatography, mass spectroscopy and high pressure liquid chromatography for longer than 48 h after application of the drug (Short *et al.* 1988). Procaine is an extreme example of detection long after the pharmacological effects have ebbed. The duration of LA effect was 30 min following procaine injection; however, procaine is detectable in urine for more than 30 days following procaine penicillin injection (S. Stanley, personal communication).

The clinical relevance of this study is debatable. The HNEDs are of more interest to regulatory agents who want to establish 'no effect thresholds' of plasma and urine concentrations for forensic purposes than to equine clinicians. The experiments were designed to determine the pharmacological and forensic significance of low concentrations of local anaesthetics/metabolites detected in post race urine samples. To evaluate this, threshold concentrations associated with the pharmacological effects of LA agents must be determined. Therefore, the radiant heat lamp model was used to measure the minimum dose for measurable pharmacological effects of these agents.

The estimated minimum dose for pharmacological effects is likely to be a conservative estimate. The model is based on a unilateral block, whereas clinical situations requiring local anaesthesia of a digit often require a bilateral block. Furthermore, 'deep' pain may be more difficult to block than cutaneous pain, which require a larger dose of LA agent. In both scenarios, the threshold dose developed in this study is probably a conservative estimate.

Finally, the fact that the horses remain unexercised is unlikely significantly to affect the rate of diffusion of the local anaesthetic away from the injection site. The Thoroughbred is exercised intensely for only 3 min or less, whereas dissipation of the local anaesthetic from the injection site requires at least 30 min for procaine and longer for other local anaesthetics. Conversely, the exercise status of the horse very probably affects the concentration of these agents/metabolites found in post race urine samples. This subject will be addressed in a subsequent investigation.

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