A xylazine infusion regimen to provide analgesia in sheep

C. Grant[1], G. E. Summersides[2] & T. R. Kuchel[2]

[1]Department of Anaesthesia and Intensive Care, University of Adelaide, Adelaide, South Australia 5005 and [2]Division of Veterinary Services, The Institute of Medical and Veterinary Science, Frome Rd, Adelaide, South Australia 5000

Summary

The efficacy of continuous low-dose xylazine infusion following an initial loading dose in providing analgesia in sheep was examined using an algesimetry method based on a leg lifting response to an electrical stimulus. Sheep received a 5 mg intramuscular injection of xylazine followed by continuous infusion of intravenous xylazine (2 mg/h) for 90 min. This treatment resulted in significant increases in the level of current required to elicit a leg lifting response (287% of baseline) and steady state analgesia was maintained from 10 min after the start of the infusion until the end of the experimental period. This protocol appears to be a simple and effective regimen for providing steady state analgesia in sheep.

Keywords Sheep; analgesia; xylazine; nociception; algesimetry

Providing pain relief for experimental animals is an important issue confronting researchers, particularly in the light of growing public awareness and interest in the animal welfare implications of animal-based research. The ideal analgesic administration regimen would involve the use of a readily available agent whose pharmacological properties have been well examined, and whose relative cost encouraged its use. Its application should be simple and provide effective analgesia with no detrimental side effects, such as sedation or respiratory depression, which may compromise the well-being of the animal.

In many commonly used experimental animals such as cats and dogs these criteria are close to being realized, and analgesics and associated protocols for their use have been extensively validated to provide pain relief following surgical treatment and other painful procedures. The use of analgesics in sheep, however, is a smaller and relatively new area of study, and drugs have often been selected for use on the basis of their effectiveness in other species. Unfortunately inter-species variability in drug effects (Baggot 1992) means that standard treatments cannot necessarily be assumed to be effective in different species. Previously we have shown that many commonly used analgesics such as opioids are in fact ineffective in sheep (Grant *et al*. 1996). Grant *et al*. (1996) also showed that low-dose intramuscular injection of the alpha-2 adrenoceptor agonist xylazine significantly increased analgesia in sheep without negative side effects such as sedation.

However, this method of bolus xylazine administration only provides a period of analgesia of approximately 60 min (Nolan *et al*. 1987). Continuous intravenous infusion of drugs is a method of providing long-term steady state drug concentrations.

Correspondence to: Cliff Grant
E-mail: cgrant@medicine.adelaide.edu.au

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Unfortunately the time required to achieve effective drug concentrations during the wash-in period means that under-dosing can occur initially. The use of an initial bolus or loading dose, given prior to commencing the infusion is a simple method of achieving higher drug concentrations more rapidly than by infusion alone (Baggot 1995).

The aim of this investigation was to determine whether the administration of a set dose of intramuscular xylazine followed by continuous low-dose intravenous infusion could provide predictable, effective analgesia in sheep.

Methods

Experimental

The experimental protocol was approved by the Animal Ethics Commitees of both the University of Adelaide and The Institute of Medical and Veterinary Science. Six adult castrated male Merino sheep weighing between 45 and 60 kg were used. Prior to the study the animals were housed indoors in metabolic crates with free access to food and water to acclimatize to experimental conditions. Twenty-four hours prior to the experimental procedures a 16 G intravascular catheter (Angiocath, Becton-Dickinson Infusion Therapy Systems Inc, Utah, USA) was inserted into the right jugular vein under local anaesthesia and kept patent with a heparin lock. On the day of the experiment the sheep were placed in a sling within their crate, which prevented them from lying down but otherwise caused no discomfort. The experimental room was kept quiet and free from distractions during the course of each study and companion sheep were always present to avoid isolation stress in the experimental animal.

Analgesia was quantified using the method of a leg lifting response to electrical stimulus. Full details of this method have been published elsewhere (Ludbrook *et al*. 1995) but a brief description of its use is as follows. Two 26 G needles were placed subcutaneously 3 cm above the fetlock on the anterior plane of the metatarsal bone, to act as electrodes between which a current could pass. A modified peripheral nerve stimulator (Digistim 3, Neuro Technologies, Houston, TX, USA) was used to deliver a 50 Hz pulsed DC current to the needles, automatically increasing stepwise between 0 and 50 mA. The endpoint was taken as a deliberate lifting of the leg, at which point the current was stopped and the peak current recorded. Measurements were taken every 60 s, the ramp time for each reading being approximately 5 s. This algesimetry method provides a highly sensitive and repeatable index of analgesia, the stimulus is brief and causes no damage to the testing area. Examination of baseline stability has shown that the threshold current required to produce a leg lifting response does not change over time.

After a period of at least 5 min of stable readings, which was then considered to be the baseline, 5 mg of xylazine (Xylazil-20, Troy laboratories Pty Ltd, Smithfield NSW, Australia) made up in 2.5 ml of 0.9% saline was injected intramuscularly into the contralateral leg to which the electrodes were attached. A continuous infusion (Atom Adult/Neonatal syringe pump 1235, Atom Medical Corp., Japan) of xylazine (2 mg/h) through the jugular catheter was also commenced at this time and analgesia readings continued to be taken every 60 s for the next 90 min.

During the course of each study the sheep were observed for indications of sedation, including increases in salivation and degree of ptosis, and changes in alertness and response to external stimuli such as noise. At times of maximal drug effect when threshold currents were at their highest values, sheep were given an innocuous non-painful stimulus (touching the leg) to examine any depression of the central reflexes.

Data analysis

To reduce the clutter of the data representation over the 90-min period, endpoint current readings were averaged over 5 min to give an average value for each 5-min period (Fig 1, solid circle) all values are represented as mean and standard error of the mean (SEM) (Fig 1, dashed line). Dunnett's test was used to identify which time points were

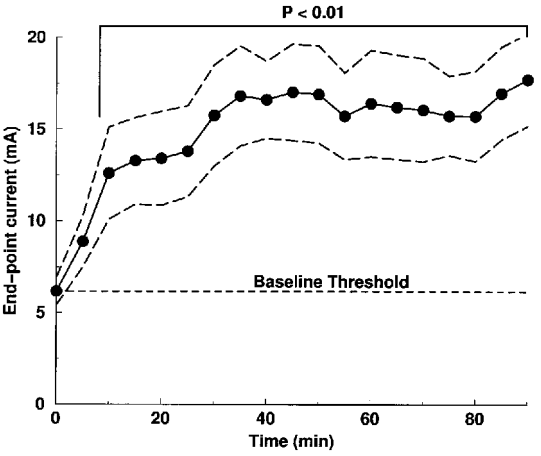

Fig 1 Algesimetry changes (mean ± SEM) in response to 5 mg i.m. xylazine followed by 2 mg/h continuous i.v. administration of xylazine. Time 0 represents the time of injection and start of infusion

significantly different from the baseline, a P value of less than 0.05 was considered significant.

To identify the origin and duration of steady state analgesia, a multiple comparison ANOVA was performed using the Newman-Keuls method, assuming a P value of 0.05 as significant (Statistica for Windows 5.1, Statsoft Inc., Tulsa, USA). The period when time series data were significantly different from the baseline but not significantly different from any of the ensuing points was assumed to be a period of steady state analgesia.

Results

This regimen of xylazine bolus followed by continuous infusion caused a significant increase in the threshold current required to produce leg lifting. From an average baseline value of 6.17 ± 0.76 mA, endpoint current gradually increased to a maximum of 17.72 ± 2.53 mA or 287% of baseline at 90 min (Fig 1). All peak current values were significantly higher than baseline from 10 min onwards ($P < 0.01$). Multiple comparison ANOVA revealed that there were no statistical differences between any points from 10 min onwards, and so this can be assumed to be a period of steady state analgesia.

Although there was a slight increase in the degree of ptosis and salivation in some sheep during the course of these studies, no signs of heavy sedation were noted. Even during the periods of highest anti-nociception no animal failed to respond to mild visual, auditory or other sensory cues. All sheep produced a leg lifting response when gently touched on the foreleg.

One animal was excluded from the dataset and the experiment terminated prematurely, due to the animal's refusal to elicit a leg lift in response to increases in current.

Discussion

From the data presented in this study the use of continuous infusion i.v. xylazine appears to be an effective and simple method for providing and maintaining analgesia in sheep.

The level of analgesia, as indicated by the increase in threshold current, rose steadily during the study period until it reached a steady state at 10 min. Although this could be considered to be an adequate analgesic profile, particularly in response to intramuscular administration, in some situations an optimal analgesic profile may require steady state analgesia to be reached sooner than this. One method of achieving a faster wash-in would be to increase either the loading dose or the infusion rate. The use of low bolus and infusion doses, however, reduces the likelihood of the negative side effects associated with increased doses of alpha-2 agonists such as sedation, respiratory depression and changes in cardiovascular function (Klide *et al*. 1975, Waterman *et al*. 1987, Celly *et al*. 1997). The bolus is in fact administered to provide steady state analgesia levels faster than would be achieved by continuous infusion alone. Increasing the bolus dose whilst reducing the wash-in period also leads to an unnecessary overshooting of peak xylazine concentrations, providing levels of analgesia unnecessarily higher than those achieved later during steady state and increasing the risk of unwanted side effects. An argument could

also be made for administering the loading dose prior to commencement of surgery to obtain any possible benefits associated with pre-emptive analgesia. Opinion is divided on the value of pre-emptive analgesia, but there is evidence that the presence of existing chronic pain may reduce the analgesic efficacy of xylazine (Ley *et al*. 1991).

Normally in veterinary medicine, dosing is expressed in terms of dose per kg, this is done to provide an easy 'rule of thumb' for calculating dosing requirements across a broad range of body weights. We have found that in adult Merino sheep of the same genetic background the variation in body weight is small and dependent mostly upon fleece length and rumen contents. Normalizing doses for weight under such circumstances can contribute to a variability of effect rather than reduce it. The determinants of kinetic processes actually scale better to lean body mass rather than to total body weight, but measurement of lean body mass is difficult. With this knowledge, doses were administered based upon the average weight of the Merino sheep we use in experimental practice. Using a set dose in this manner provided a simple method of administration and resulted in a consistent analgesic profile amongst the sheep (Fig 1). This approach is also the most clinically practical in animal house situations when animals of similar age and genetic background are used and the weight range is known to be small. However, for animal groups with a large variation in body weight it is advisable to adjust doses according to body weight and to administer drugs in terms of dose per kg. Expressing the dosing from the present study in such terms, and assuming a 50 kg sheep, this equates to a loading dose of 100 μg/kg and an infusion rate of 40 μg/kg/h.

The point at which the sheep elicit a leg lifting response and terminate the application of current is entirely at the discretion of the sheep. Unlike some algesimetry methods which use a set level of stimulus and escape time latency as a point of reference, the ramped stimulus ensures that the animal dictates its own 'comfort zone', and at no point during the study could the sheep be considered to be suffering. This self-determination is exemplified by the refusal of one sheep in the experimental group to lift its leg in response to the painful stimulus. This behaviour is not entirely unexpected, as the leg lifting response used in this study is not a reflex but a cognitive voluntary behaviour and as such individual animals occasionally engage in displays of defiance. Testing of the device on the authors shows that the endpoint current at which the sheep respond, in the control phase, is similar to that of the authors, and could be equated to a mildly uncomfortable sensation. The 15 mA threshold reached by all the sheep in response to the regimen described is too painful for the unmedicated authors to bear. This increase in anti-nociception in sheep was determined to be a 'real' effect and not due to a depression of the central reflexes. Even when the current threshold required to produce a leg lift was at its highest, a novel, non-painful stimulus such as touching the leg would elicit a leg lifting response. This indicates that the transmission of sensory signals appears to remain unaffected and that increases in peak current values are not a result of inhibited locomotor activity. Thus this dosing regimen, whilst effectively altering the sheep's awareness of pain, does not interfere with its ability to respond to the testing stimulus.

Conclusion

The use of bolus doses of xylazine as an effective analgesic agent in sheep has been reported before (Flecknell 1987) but due to the variability in effect (Ley *et al*. 1990) and duration of action (approximately 60 min, Nolan *et al*. 1987) it has not been considered ideal for long-term pain control. The results of this study indicate that the concept of a loading dose of xylazine followed by continuous infusion can provide effective, predictable steady state analgesia in the sheep. This may provide researchers and animal carers with a simple technique for the control of longer-term pain in sheep.

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