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The use of diazepam as a pharmacological method for evaluating anxiety in sheep

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ABSTRACT

The aim of this study was to examine the effects of diazepam, an anti-anxiety drug, on the fear responses of sheep and its usefulness as a pharmacological method to study welfare. Fourteen, eight month old Romney ewes were randomly assigned to two treatments and injected via jugular venepuncture with diazepam (0.37mg/kg) or saline (controls) 20 minutes before exposure to a barking dog. Heart rate (bpm) and escape attempts were recorded throughout a 10 minute period before injection (baseline), 20 minutes post injection and 15 minutes following the dog. Average heart rate was lower in diazepam-treated sheep during the first 5 minutes following injection (P<0.01) and was significantly higher (P<0.01) for both diazepam and saline-treated sheep during the 5 minutes following exposure to the dog compared to baseline (+23.1 and +21.5 bpm for diazepam and saline respectively). Heart rate then decreased back to baseline levels during the last 10 minutes. Diazepam-treated sheep tended to have a higher number of escape attempts during the 5 minutes following exposure to the dog (P=0.08) compared to controls, suggesting that diazepam may have paradoxically increased anxiety in some sheep. The lack of differences between treatments suggests that diazepam did not modify the fear response of sheep to a dog. There appear to be interpretive difficulties associated with dose rates and the appropriate fear model in the use of diazepam to study anxiety in sheep.

Keywords: sheep; fear; diazepam; heart rate; behaviour.

INTRODUCTION

Fear and anxiety are emotional states that occur as coping mechanisms in situations that are perceived as being threatening or dangerous. Fear is considered to have a direct cause whereas anxiety is an overall, non-specific level of arousal (Boissy, 1995). The presence of these emotions are important in the assessment of animal welfare. Sheep are exposed to a range of potentially fearful experiences during routine farm management such as transport, exposure to unfamiliar conspecifics and mustering by humans and dogs. During lambing, human disturbance is one factor that may cause increased anxiety in ewes and can result in delayed parturition, desertion of lambs or premature movement from the lambing site, all of which can compromise ewe-lamb bonding and ultimately impact on lamb survival (Fisher & Mellor, 2002).

It is common for studies of animal welfare to use behavioural and physiological techniques to measure fear and anxiety. Pharmacological approaches are less commonly used, but manipulation of an animal’s emotions using drugs may be a useful way of studying fear-related responses to aversive experiences. Benzodiazepines, such as diazepam, are anti-anxiety drugs that facilitate the inhibitory activity of γ-aminobutyric acid (GABA) receptors, which balance the excitatory effects of glutamate and regulate general neural excitability (Roy-Byrne, 2005). Diazepam, administered intravenously, has a rapid distribution rate to and from the central nervous system resulting in a quick onset of action (approximately 1-5 minutes) (Horn & Nesbit, 2004; Mandelli et al., 1978). Benzodiazepines are frequently used to study anxiety and fear in laboratory animals, however, very few studies have used these drugs to study anxiety in farm animals.

Recently, diazepam has been used to study anxiety in cattle and pigs. Sandem et al. (2006) used diazepam as a tool to validate the degree to which the eyes are opened (i.e. the percentage of white in the eye area) as an indicator of ‘frustration’ in dairy cows. They induced ‘frustration’ by preventing cows from accessing visible food and found that the cows treated with diazepam showed a reduced percentage of white in the eye compared to untreated cows. A standard test for fear and anxiety in rodents is an elevated plus-maze in which anxious animals chose to avoid arms of the maze that have no sides and the increased risk of falling. Andersen et al. (2000) used this test and found that diazepam-treated pigs spent more time on open arms of an elevated plus-maze than pigs administered saline, which was consistent with results from rodent studies (Cole & Rodgers, 1995). Ferreira et al. (1992) found that treatment of parturient ewes with diazepam facilitated maternal behaviour and enhanced acceptance of alien lambs. They suggested that
Beausoleil causes increases in fear-related behaviour in the presence of a dog. There is evidence to show effects of diazepam on coping responses of sheep to the odour of the alien lambs. Diazepam may attenuate the ewe’s negative emotional response to the odour of the alien lambs.

The aim of this study was to examine the effects of diazepam on coping responses of sheep in the presence of a dog. There is evidence to show that exposure to a dog is particularly stressful and causes increases in fear-related behaviour (Beausoleil et al., 2005; Hansen et al., 2001) and heart rate (Baldock & Sibly, 1990; MacArthur et al., 1979). A barking dog was therefore chosen as a fear stressor for sheep in this study. Our hypothesis was that diazepam administration would reduce fear responses, measured by heart rate and escape behaviour, of sheep exposed to a dog compared to saline-treated controls.

**MATERIALS AND METHODS**

Fourteen, eight month old Romney ewes (average weight 34.6 kg) were used in this study. Ten additional ewes of the same breed and age were used as companions. All the sheep were grazed on pasture under routine farm management. One month prior to treatment, the sheep were moved into an indoor barn each day (for approximately 3 hours) to allow acclimatisation to the indoor pens and handling and to reduce fear of human contact. The sheep were also acclimatised to restraint in a head bail and to wearing a heart rate monitor strap. The day prior to treatment, sheep were clipped around the girth and neck area to provide maximum contact for heart rate monitors and to facilitate jugular vein injection.

On treatment days, the sheep were randomly assigned to two treatments (7 sheep per treatment) and given a jugular intravenous injection of either diazepam (Pamlin, Parnell laboratories; 0.37mg/kg) from commercially available vials (5mg/mL) or an equivalent volume of saline (control treatment). Injections were administered while each sheep was manually restrained against the side of the pen by one other person.

One sheep was treated at a time and either 2 or 3 sheep were treated per day. During treatment, each sheep was held individually in the monitoring pen (1.0 x 1.4 m) and restrained in a head bail, in order to limit movement, facilitate a stronger fear response, due to the inability to escape from the dog, and maintain a consistent position and distance from the dog. Two unrestrained companion sheep were held in identical neighbouring pens either side of the monitoring pen. To prevent the companion sheep habituating to the dog, two different companion sheep were used each treatment day.

Polar S810i heart rate monitors (Polar Electro Oy, Finland) were fitted immediately after the sheep were moved into the monitoring pen prior to treatment. To optimise conductivity, ultrasound transmission gel was applied to the skin at each electrode contact point. The electrodes and transmitter were built into an elastic strap, designed for human use, which was strapped firmly around the sheep’s girth area, immediately behind the forelimbs, and a heart rate monitor attached. Once heart rate monitors were fitted, the sheep were given 5 minutes to settle before being restrained in the head bail. Continuous recordings of heart rate (bpm) and behaviour (via video recording) were then collected over a 45 minute sampling period. The sampling period included a 10 minute baseline period, followed by injection of diazepam or saline, a 20 minute period before exposure to a barking dog (0 minutes) and a final 15 minute recovery period. The dog, a 6 year old female huntaway farm dog, was led into the sampling area on a short leash and positioned approx 1 m in front of the sheep (average time, 15 s), commanded to bark 2 to 3 times, and then led away. Sheep were familiar with the handler but had no prior experience with the dog.

Behaviour was analysed from videos and the number of occurrences of attempts to escape (i.e. the number of times all four feet lifted off the ground) per min was recorded during the 5 minutes before and 5 minutes after exposure to the dog.

**Statistical analyses**

Heart rate and behaviour were expressed as the difference from baseline (i.e. the difference was calculated between the mean baseline pre-injection (the first 10 minutes) and various periods following exposure to the dog). A Student’s t test was used to compare differences between treatments and differences at various periods following exposure to the dog from baseline.

**RESULTS**

Baseline heart rate was similar for both groups (96.7 ± 2.7 and 102.9 ± 2.5 bpm for diazepam and saline respectively). For both treatments, average heart rate was significantly higher during the 5 minutes following exposure to the dog compared to baseline (P<0.01) and then decreased back to baseline levels during the last 10 minutes (Figure 1). Average heart rate increased during the first 5 minutes post injection but was lower for diazepam-treated sheep during this period (P<0.01) (Figure 1). Figure 2 shows the average number of occurrences of attempts to escape per min for the 5 min before and 5 min after exposure to the dog. All sheep stood calmly prior to the dog entering. Sheep administered with diazepam tended to have a
Figure 1: Average heart rate (bpm) ± S.E.M. for both diazepam (○) and saline (▲) treated sheep over the 45 minute sampling period. 0 minutes indicates the time that the dog entered and the treatment was administered via jugular injection at -20 minutes.

Figure 2: Average number of occurrences of attempts to escape per minute ± S.E.M for diazepam (□) and saline (□) treated sheep during the 5 minutes before and 5 minutes after exposure to the dog. 0 minutes indicates the time that the dog entered.
higher number of attempts to escape during the 5 minutes following exposure to the dog ($P=0.08$) compared to saline-treated sheep. There were no significant differences between treatments in heart rate or behaviour of sheep in response to the dog.

**DISCUSSION**

Exposure to a barking dog produced a stress response that was consistent with others studies that have measured heart rate responses of sheep to a dog and human handler (Baldock & Sibly, 1990; MacArthur et al., 1979). The lack of any differences in heart rate and behavioural responses to the dog between diazepam and saline-treated sheep suggests that diazepam did not modify the fear response of sheep to a dog. These results are inconsistent with studies in other farm animal species such as dairy cattle (Sandem et al., 2006) and pigs (Andersen et al., 2000). The results do not support our hypothesis that diazepam would reduce the fear response of sheep to a fearful experience and suggest that caution should be used when assuming that human drugs will have similar effect on other animals.

The lower heart rate of diazepam-treated sheep immediately after administration of the jugular injection compared to saline-treated sheep suggests that diazepam may have reduced anxiety and the fear response to handling required for the injection. There is little data on the effects of diazepam on heart rate, however, a reduction in heart rate following administration in humans has been reported (D’Amelio et al., 1973). The effects of the diazepam should have lasted long past the administration period as other studies have shown longer lasting effects of diazepam in sheep (Ferreira et al., 1992).

The tendency for diazepam-treated sheep to show more escape behaviours in response to the dog than saline-treated sheep suggests that diazepam may have actually increased anxiety in some animals. It was noted that some of the diazepam-treated sheep also appeared to be more anxious (e.g. more excited) after being released from the pens at the end of sampling. This stimulatory effect may not be totally unexpected as benzodiazepines can produce paradoxical stimulant effects that cause increased aggressive and hyperactive behaviour (Speth et al., 1980). It would have been useful to observe other fear-related behaviours, however, restraint in the head bail prevented other anti-predator behaviours, such as foot stamping and avoidance behaviours, which have been observed in other studies investigating behavioural responses to a dog (Baldock & Sibly, 1990; Beausoleil et al., 2005).

Our inability to distinguish any differences between treatments may be due to the dose rate or individual variability of responses to the dog. The dose rate used in this study was slightly lower than Ferreira et al. (1992) used to investigate the effects of diazepam on acceptance of ewes to alien lambs (20 mg/4 mL) and lower than doses used to induce sedation in sheep (0.5-1.5 mg/kg) (Bolte & Stupariu, 1978, cited in Ferreira et al., 1992). Ferreira et al. (1992) showed that the diazepam dose used in their study did not affect the level of activity of sheep in an open field test. However, it is evident that the optimal dose rates for the use of diazepam as a pharmacological method for measuring anxiety in sheep are unknown. Another possible explanation for the lack of effect of diazepam in this study may be the type of fear stressor that was used. For example, Sandem et al. (2006) prevented cows from accessing visible feed to induce ‘frustration’, which is a different type of stressor to the acute type of fear or fright response used in this study. It is possible that pre-treatment of diazepam to sheep exposed to a more chronic stressor that is more representative of anxiety, such as weaning or social isolation, may cause a different response.

In conclusion, the results from this study suggest that there are interpretive difficulties in the use of diazepam to study fear in sheep. The paradoxical and variable effects indicate that the responses to diazepam administration in sheep may differ depending on the dose or model of anxiety used.

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