HEART RATE AND STRESS HORMONE RESPONSES OF SHEEP TO ROAD TRANSPORT FOLLOWING TWO DIFFERENT LOADING PROCEDURES

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Abstract *Animal Welfare 1998, 7:* 257-267

This study was designed to investigate the physiological responses induced in sheep (n = 18) by two different loading techniques followed by a short road journey. All animals were prepared with venous catheters, to minimize the disturbing effects of blood sampling, and nine sheep were fitted with heart rate monitors. The animals were loaded onto a transport vehicle in groups of three, alternately using a conventional tailgate ramp or a crate raised with a hydraulic lift. When all of the sheep were loaded, they were taken on a journey lasting 195min. Blood samples were collected in the home pen, directly after loading, and at 15min intervals during the journey. Measurements were made of plasma concentrations of cortisol, prolactin and catecholamines (adrenaline and noradrenaline). The results indicated that heart rate increased during loading, regardless of the method used. No changes in concentrations of cortisol or the catecholamines were detected, although a small increase in prolactin was noted when animals were loaded using the ramp. During transport, all sheep exhibited increases in plasma cortisol concentrations which were greatest during the first 2h of the journey. The results suggest that, under the conditions employed in this experiment, the effects of the two loading procedures were similar and that transport appeared to be more stressful than loading.

Keywords: *animal welfare, handling, heart rate, road transport, sheep, stress hormones*

Introduction

The effects of long-distance road transport on the welfare of sheep represent a cause for concern. Accordingly, several groups of researchers have used a variety of behavioural, physiological and endocrine indices to examine how sheep respond to journeys of different lengths. However, before sheep can be transported they needed to be loaded onto the vehicle. This procedure, which involves the gathering of the flock and the driving of animals up a ramp into the transporter may, in itself, be stressful. In two reports which examined the effects of loading onto a vehicle that subsequently remained stationary, one found that loading induced stress hormone responses (Broom *et al* 1996) whereas the other did not (Cockram *et al* 1996). However, when loading was followed by transport, both investigations reported an increase in plasma cortisol concentrations. Moreover, in a more recent experiment (Parrott *et al* 1998) involving a two-stage journey separated by a rest stop, the cortisol response to the second loading was considerably smaller than that induced on the first occasion. Whether sheep perceive loading as being aversive may therefore depend upon a variety of factors.

Stress hormones, measured in blood samples obtained from catheterized sheep at regular intervals during transport, are present at their highest concentrations during the first few hours of travel (Broom *et al* 1996; Cockram *et al* 1996; Parrott *et al* 1998). Thus, while sheep eventually appear to adapt to motion stimuli during extended periods of transport, the stressful effects of the early part of the journey may summate with the response to loading. In consequence, protocols which reduce the distress of loading can be expected to improve welfare in animals undergoing long-distance road transport.

Although sheep are normally loaded via a ramp, climbing a steep and often slippery gradient may be an aversive experience, especially for lowland breeds. If the flock is driven quickly, some animals may risk injury and many may become distressed. An alternative procedure would be to load sheep onto the transporter using an hydraulic lift. However, this potentially attractive solution to the loading problem represents a highly novel situation for sheep and may have its own disadvantages. Hence, the aim of the present study was to compare the physiological responses of loading using a ramp or a lift, in two groups of sheep which were subsequently transported for a 3¼h (195min) period. To examine how the sheep reacted to these procedures, the animals were prepared with heart rate monitors and indwelling venous catheters. Blood samples thus obtained were used to determine plasma concentrations of hormones (cortisol, prolactin and the catecholamines, adrenaline and noradrenaline) known to be stress-responsive in this species (Houpt *et al* 1988; Parrott *et al* 1988, 1994; Parrott and Thornton 1989).

Methods

All procedures were conducted under Home Office Project Licence No 80/00579 and Personal Licence 70/02655. Eighteen mixed-breed lambs (6 wethers, 12 ewes) weighing between 23 and 36kg were obtained from a commercial supplier and held in a paddock for 2 weeks. They were then housed in groups of three (one wether, two ewes) in straw-lined pens and provided with hay, and water *ad libitum*. On the day before the experiment, each animal was prepared with a temporary catheter inserted into the jugular vein under local anaesthesia, as previously described (Parrott *et al* 1998). On the morning of the day of testing, nine lambs were prepared with subcutaneous electrodes connected to non-interacting heart rate monitors (Polar Vantage NV HRM Sport Tester, Polar Electro Oy, Kempele, Finland).

The experimental protocol was as follows. A blood sample was taken in the home pen from each group of sheep before the heart rate monitors were attached. Then, starting at 1230h and continuing until 1255h, groups of three sheep were blood-sampled again and loaded alternately by ramp and lift. In the first case, the sheep were loaded via the metal tailgate ramp of the vehicle, a 9t cattle lorry (Figure 1a) whereas, in the second case, the animals were transferred into a wooden crate which was then raised via an hydraulic lift to the floor height of the vehicle (Figure 1b). The ramp was 2.03m wide with an 18° gradient; it had a 0.2m step at the foot and at the top, giving a floor height of 1.03m and there were 8 wooden cleats (0.05m wide x 0.025m high) separated by 0.25m gaps. In both situations, the groups of sheep were placed in separate pens (0.8x1.2 m) near the front of the vehicle and a further blood sample was taken as soon as possible. Because of the delays associated with the alternating treatments, the loading process

(a)

(b)

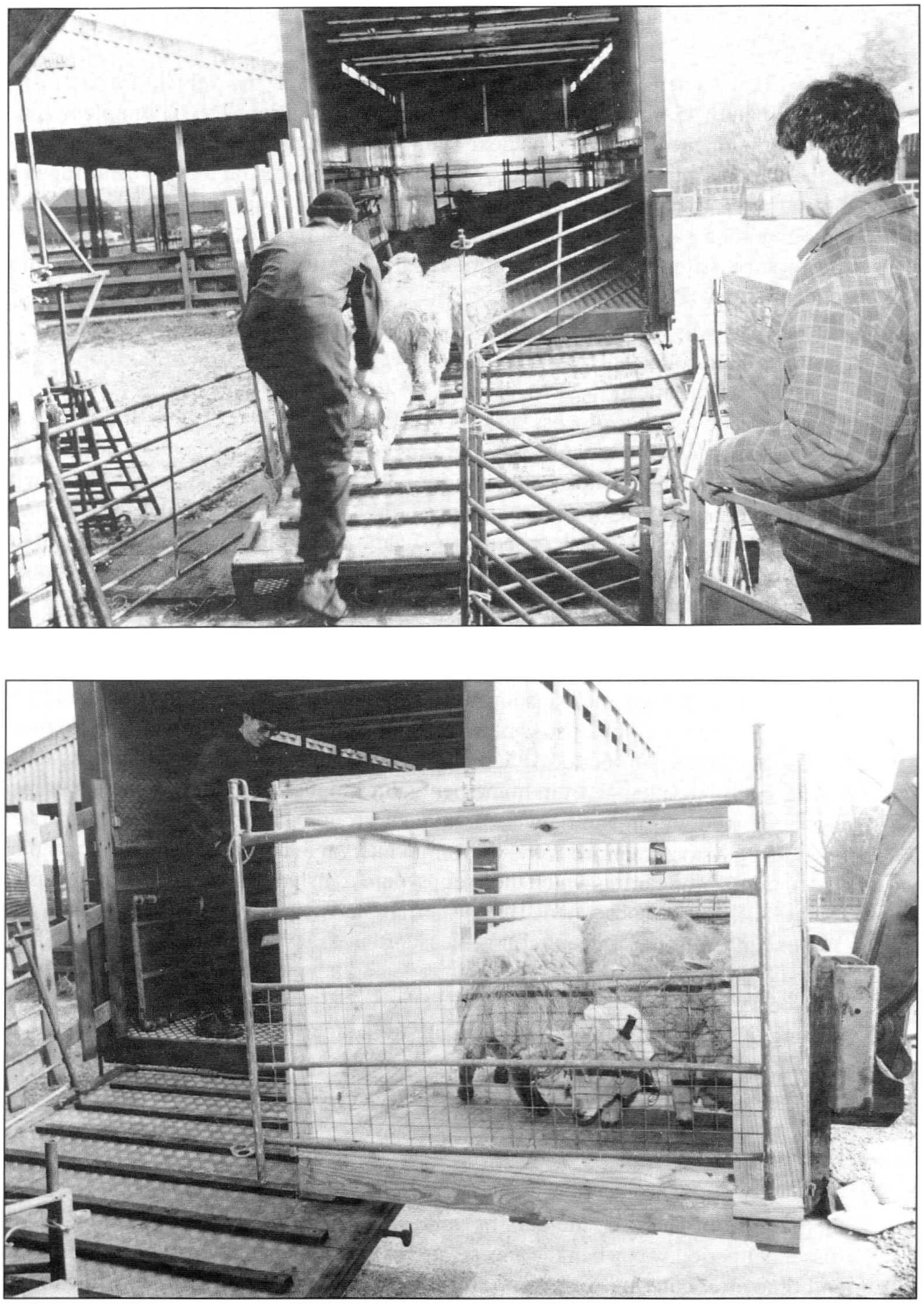

Figure 1 **(a) Sheep being loaded via the ramp. (b) Sheep being loaded using the lift.**

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was slow, although for individual groups transfer times varied between about 1min (ramp) and 5min (lift).

At 1315h, when all the animals had been penned in the vehicle for at least 10min (and approximately 45min after the start of loading), an additional blood sample was taken and the journey commenced. During the next 195min, experimenters travelling with the animals took further blood samples at 15min intervals which were stored on ice and centrifuged at the end of the journey. The resultant plasma was divided into aliquots and stored at –30°C pending radioimmunoassay to determine cortisol and prolactin concentrations (Parrott & Goode 1992) and high pressure liquid chromatography (HPLC) to measure adrenaline and noradrenaline concentrations (Parrott *et al* 1994).

Heart rate was recorded from four sheep loaded via the ramp and five raised in the lift. The digitized data collected by each monitor were downloaded onto a computer and the data expressed as the mean ± SEM beats min^{-1} (bpm), both in response to loading, and during transport. The loading process was recorded using a video camera and a time mark on the videotape was used to identify when groups of sheep were moved out of the home pen. This information enabled the heart rate data for individual sheep to be synchronized with respect to loading for the purpose of data analysis. During the data transfer, files from one animal loaded using the ramp were lost; in consequence, heart rate results for this treatment are based on data from only three sheep. Results were, however, obtained from all five animals raised in the lift and the endocrine data are based on results from nine animals per treatment.

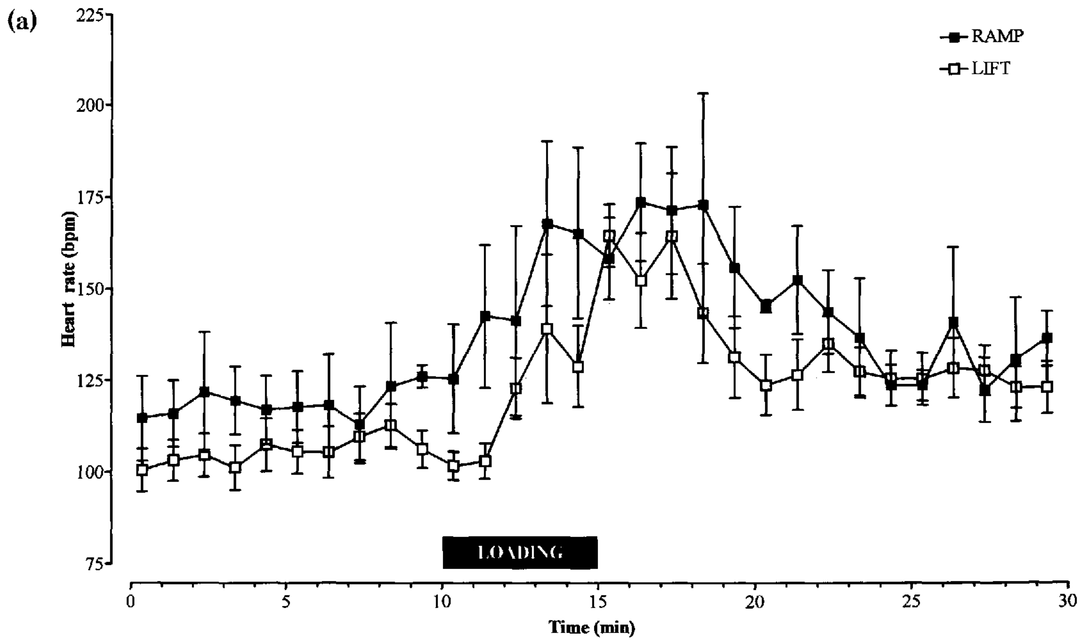
Statistical analysis

The effects of loading and transport were examined using paired and unpaired Students *t*-tests, where appropriate. Comparisons between ramp and lift treatments were made when the animals were in the home pen (period 1) and also during two experimental periods, as specified below. Additionally, the change from home pen values (net change) during the experimental periods was compared within and between treatment groups. In the case of the heart rate data (mean bpm), the periods examined were: the 10min interval in the home pen prior to loading (baseline); the 10 min period during which the groups were transferred from their home pens to the vehicle (period 2); and the subsequent 10min (period 3). The analysis of the hormonal data, however, used the mean of the concentrations measured in the two samples taken in the home pen, ie at 0945h (before fitting the heart rate monitors) and between 1230 and 1250h (before loading), to provide a baseline value for each group. These results were then compared with the response to loading, a single sample, and the response to the journey – based on the mean results from 13 (cortisol, prolactin) or 6 (catecholamines) samples taken from each sheep during the 3h of the transport period between 1330 and 1630h.

Results

Changes in heart rate in response to the two loading procedures are illustrated in Figure 2a. For the purpose of analysis, the data have been divided into three, 10min time blocks. The first (baseline) period was when the sheep were in the home pen, the second period includes loading and time after entering the pen on the vehicle, and the third period is when the animals were waiting in the stationary vehicle before the start of the journey.

Heart rate in period 1 appeared to be higher in the animals that were due to be loaded using the ramp (Figure 2a), however, this difference was not statistically significant. Both types of



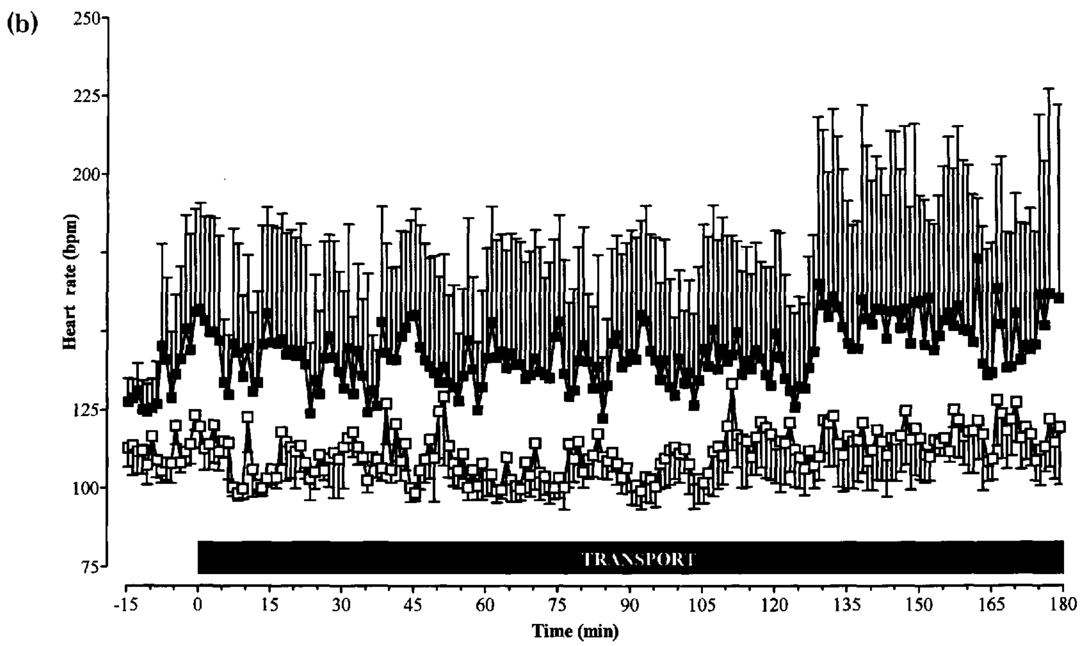


Figure 2 (a) Heart rate (bpm, mean ± SEM) in sheep loaded using the ramp (n=3) or the lift (n=5). (b) Heart rate (bpm, mean ± SEM) in the two groups of sheep just before, and during transport (indicated by the black bar).

loading increased heart rate, with maximum values recorded following transfer to the pens on the vehicle. A decrease in heart rate was then observed in both groups of sheep during period 3, although activity remained above home pen levels. The analysis showed that mean heart rates did not differ between groups at any of the time periods. The net change in rate that occurred in period 2 (period 2 minus period 1) was significant ($P < 0.02$) for animals loaded using the lift, but not for those loaded via the ramp; this lack of significance was probably due to the small number of data sets available (n = 3) for sheep in the ramp group. However, both groups responded in a similar way because the net change in heart rate did not differ between treatments. Although heart rates in period 3 were apparently greater than those observed in period 1, the net increase was not statistically significant either within, or between, groups.

The effects of transport on heart rate are indicated in Figure 2b. During the 15min before the start of the journey, heart rates in the animals loaded via the ramp appeared to be higher than when the sheep were in the home pen (cf Figure 2a). Heart rates showed little change during transport in the animals loaded in the lift but seemed to rise in the group loaded via the ramp. However, this 'increase' was mainly due to the response of one animal, as indicated by the large SEMs. Furthermore, because there were only three sets of data for sheep in this group, these findings should be interpreted with caution.

The effects of the treatments on plasma hormone concentrations were assessed by considering data from samples taken at two time points in the home pen (basal values), directly after loading, and throughout the transport period. Changes in plasma cortisol in the two groups of sheep during these times are indicated in Figure 3.

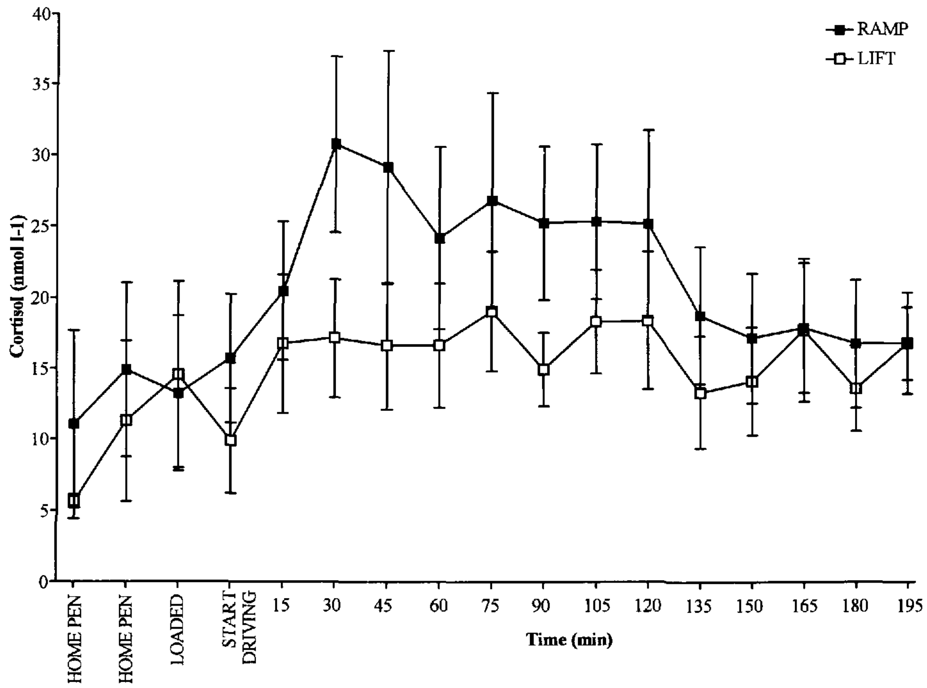


Figure 3 **Plasma concentrations (mean ± SEM) of cortisol (nmol l^{-1}) in sheep loaded via the ramp or the lift (n = 9 per group).**

The results suggest that plasma cortisol concentrations were greater in each of these periods in animals loaded using the ramp, however, none of these differences achieved statistical significance. There was also no significant effect of loading in either treatment group whereas, during transport, plasma cortisol concentrations were greater than basal values in sheep loaded using both the ramp ($P < 0.04$) and the lift ($P < 0.006$). In addition, the net change (transport minus basal values) did not differ between treatments, indicating that both groups of sheep responded to transport in a similar way.

The effects of the treatments on prolactin release are shown in (Figure 4). Prolactin concentrations did not differ between treatments, either in the home pen, or during transport. By contrast, loading produced contrasting effects on prolactin release in the two treatment groups (raising concentrations in the ramp group but decreasing them in the life group) with the result that prolactin concentrations at this time were significantly different ($P < 0.02$). Similarly, the net increase (loading minus basal values) after loading was significant ($P < 0.04$) in animals loaded via the ramp although no significant net change was detected among those loaded in the lift. The net change also differed between treatments ($P < 0.03$), confirming that the loading procedures had different effects on prolactin release.

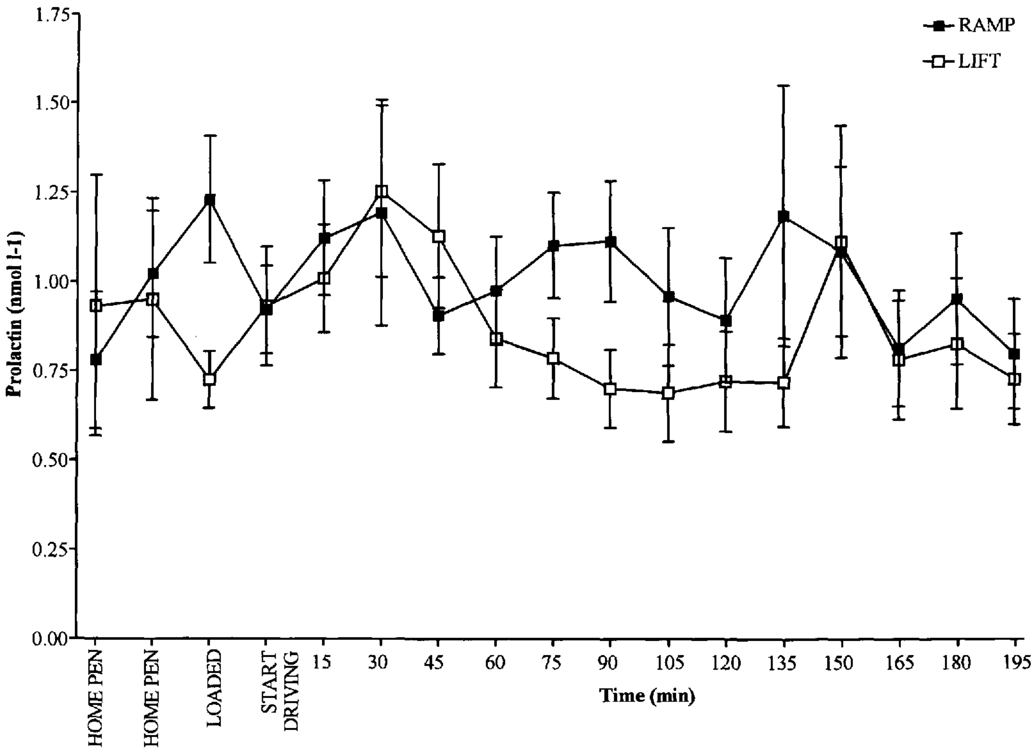


Figure 4 **Plasma concentrations (mean ± SEM) of prolactin (nmol l^{-1}) in sheep under the same experimental conditions as described for Figure 3.**

Noradrenaline concentrations (Figure 5) differed significantly between treatment groups after loading ($P < 0.01$) but not in the home pen, or during transport. Moreover, although the net change (loading minus basal values) also differed between treatments ($P < 0.05$) this result is probably of little consequence as no significant net change was detected at this time within either treatment group. There was also no evidence to suggest that noradrenaline concentrations altered in response to transport in either treatment condition. Similarly, analysis of the results for adrenaline (Figure 6) failed to detect any significant differences, either due to treatment, or in relation to time of sampling.

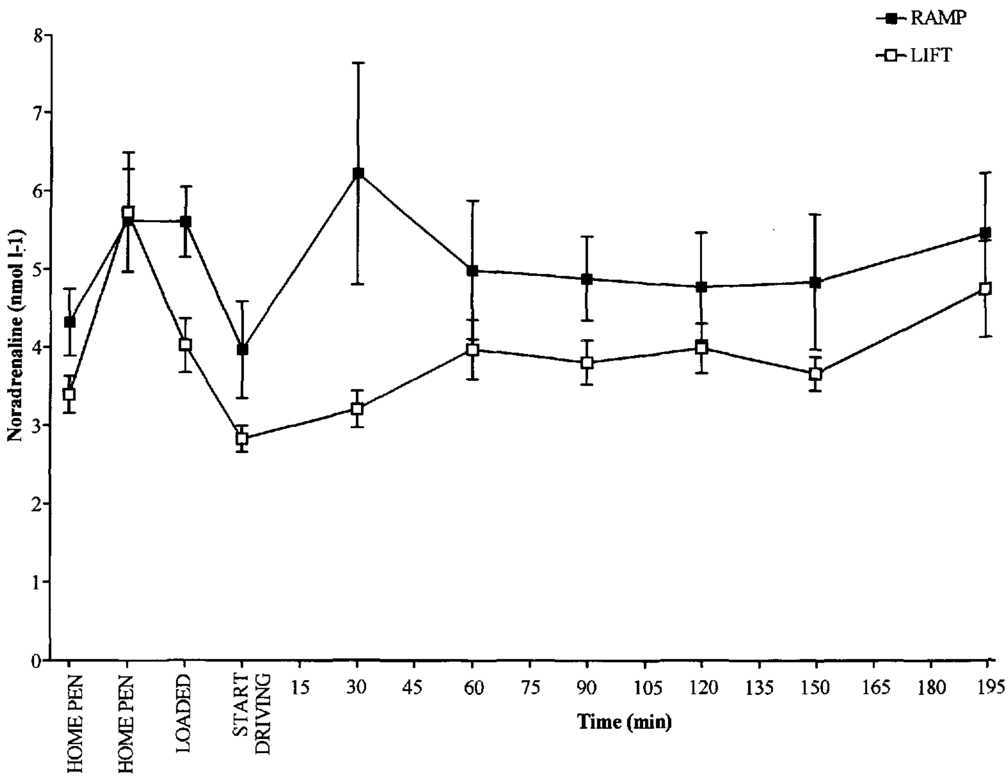


Figure 5 **Plasma concentrations (mean ± SEM) of noradrenaline (nmol l^{-1}) in sheep under the same experimental conditions as described for Figure 3.**

Discussion

In this study, it was anticipated that loading using the ramp would produce changes in heart rate and release of cortisol and prolactin similar to those previously described (Broom *et al* 1996) and that a different response pattern might occur when animals were raised in the lift. However, the results show that although both loading procedures increased heart rate, hormonal responses to loading were either small (prolactin) or absent (cortisol and the catecholamines).

Loading via a ramp can stimulate cortisol release (Broom *et al* 1996), although this is not always the case (Cockram *et al* 1996). In the present experiment, because groups of sheep were loaded alternately by ramp and lift, the whole process of transferring the sheep to the vehicle was carried out in an unhurried manner, therefore they may not have found loading particularly aversive. Furthermore, vision is known to be an important factor influencing the behaviour of sheep (Hulet *et al* 1975) and, therefore, additional visual information obtained by the animals in these experimental circumstances may have reduced the distress, or novelty, associated with the loading process. For example, many of the sheep were able to observe the loading procedure before being loaded themselves; also, all except the first group to be loaded would have found familiar individuals already penned in the vehicle after loading. Such factors may explain the failure of loading to stimulate cortisol or catecholamine release, although there did appear to be a small increase in prolactin in animals loaded using the ramp.

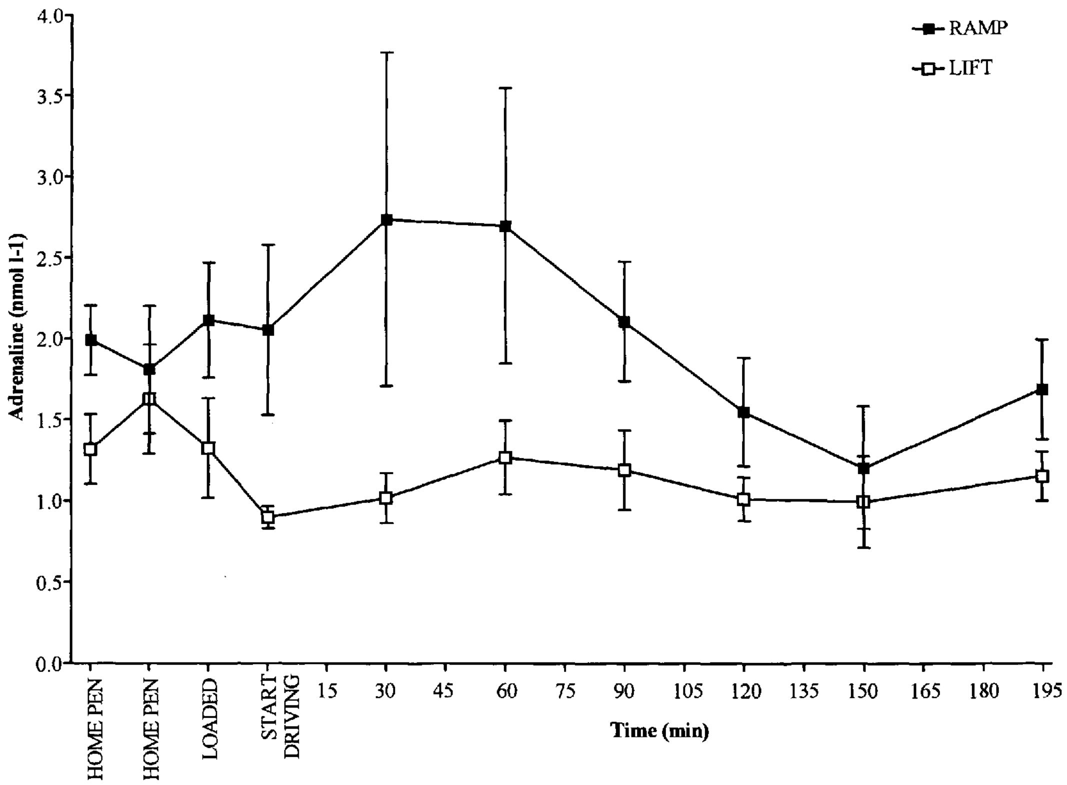


Figure 6 **Plasma concentrations (mean ± SEM) of adrenaline (nmol l^{-1}) in sheep under the same experimental conditions as described for Figure 3.**

Both loading techniques produced similar increases in heart rate. Unfortunately, records were lost from one sheep in the ramp group due to a technical failure. This group also started out with four animals monitored. The reason for this was that nine heart rate monitors were available and it was considered more important to collect data in the lift situation, as this had not been previously studied. Thus, while the sheep raised by the lift showed a net increase in heart rate after loading, the similar change in those climbing the ramp failed to achieve statistical significance. It would seem, therefore, that a minimum of five animals is required for reliable data to be obtained. Nevertheless, the analysis did show that both groups responded to loading in a similar fashion.

Possible explanations for the increase in heart rate include physical exertion associated with walking up the ramp and an emotional response to the novelty of the lift procedure. However, the fact that the changes induced were similar for all groups, suggests that other factors might have been more important as a stimulus to cardiac activity. For example, the process of driving the sheep from the home pen to either the ramp or the lift may have been largely responsible for the observed effects. It should also be borne in mind that loading is likely to be carried out with greater rapidity under commercial conditions; and that the steeper ramps linking decks on transporters may cause greater distress than the 18° incline used in this study. Furthermore, an additional issue not considered in the present investigation is the possibility that descent of a ramp may be more aversive for sheep than ascent.

In spite of the rather small effects of loading in this study, the results indicated that the animals were affected by transport. This is not apparent from the heart rate data, because those loaded using the lift showed no change in activity and the records from animals loaded via the ramp are probably unrepresentative. However, transport did affect the pattern of cortisol release. Concentrations of the hormone increased in both groups during the journey; when expressed as a percentage of home pen values, the increase was apparently, but not significantly, greater in the lift (93% increase, $P < 0.006$) than in the ramp (61% increase, $P < 0.04$) situation. Cortisol concentrations were raised for about 2h in both groups, supporting previous observations of a cortisol response to transport that decreases with time (Broom *et al* 1996). By contrast, changes in the concentrations of the other hormones were not significant, although different trends were apparent in the two treatment groups. For example, prolactin, noradrenaline and adrenaline concentrations tended to increase (+11%, +6%, +6%, respectively) in animals loaded via the ramp and to decrease (-8%, -15%, -24%, respectively) in sheep raised in the lift. This lack of effect of transport on catecholamine release contrasts with previous observations made using a transport simulator (Parrott *et al* 1994). A possible explanation for this difference is that the simulator produced unpredictable motion in a vertical and horizontal direction, whereas travel on main roads provides a motion stimulus that is relatively constant and unidirectional.

In conclusion, the available evidence suggests that, depending upon the particular conditions, loading can be distressing to sheep (Broom *et al* 1996). Moreover, the present study confirms observations (Cockram *et al* 1996) that transport can also induce stress hormone responses in situations where loading does not. In addition, these findings imply that the welfare benefits of employing an hydraulic lift to load sheep may be negligible when compared with the normal ramp technique. However, further studies using conditions closer to those prevailing commercially are needed to confirm this suggestion.

Animal welfare implications

Certain aspects of road transport induce poor welfare in sheep. In most studies, vehicular motion, as judged by a variety of physiological indices, seems to be stressful. However, on good roads and using a vehicle of an appropriate quality, animals appear to adapt within a few hours and the response evident during the remainder of a prolonged journey tends to be small. Nevertheless, loading can add to the distress of transport depending upon the particular circumstances under which it takes place. At present, it is not clear whether the effects of loading are due mainly to physical (eg handling) or psychological (eg novelty) factors. The use of a ramp appears to be satisfactory under experimental conditions and clearly, if the loading process is careful and unhurried, stress can be minimized.

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