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Stress and travel sickness in pigs: effects of road transport on plasma concentrations of cortisol, beta-endorphin and lysine vasopressin

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Abstract

Two experiments were made to investigate the effects of road transport on stress hormone responses in pigs. In experiment 1, seven 40-kg pigs, prepared with jugular catheters, were loaded onto a livestock lorry and transported over a 2-day period on routes characterized, by means of an accelerometer, as rough or smooth. Two 100-min journeys, one rough and one smooth, separated by a 100-min rest period, were conducted each day. The experimenters travelled with the animals and blood samples were taken for hormone analysis from each pig at 20-min intervals. On the 3rd day, samples were collected from the pigs when housed in their home pen (control). Plasma concentrations of cortisol increased after loading, remained higher for longer on rough compared with smooth journeys and were higher during both journeys on day 1 compared with day 2. Concentrations of beta-endorphin increased after loading on day 1 but neither beta-endorphin nor lysine vasopressin showed clear changes in secretion pattern during rough or smooth journeys. On day 3 (control), mean concentrations of all three hormones were significantly lower than on days 1 and 2, indicating that the responses observed were not due to a diurnal rhythm. In experiment 2, six 35-kg catheterized pigs were loaded on a lorry (09.30 h) that remained stationary while blood samples were taken at 30-min intervals during the next 8 h (control). Two days later, this procedure was repeated with the vehicle in motion for 8 h. Plasma concentrations of lysine vasopressin during driving increased between 2 and 4·5 h which coincided with behavioural observations indicating that the pigs were travel sick.

Keywords: *endorphins, hydrocortisone, pigs, stress, transport, vasopressin.*

Introduction

Concern for the welfare of farm animals during transport is reflected by a growing interest in the behavioural and physiological effects of road journeys in pigs. Such research has tended to concentrate on the effects of the physical (Lambooy and Engel, 1991; Randall, 1993) or social (Guise and Penny, 1989; Warriss *et al.*, 1991; Bradshaw *et al.*, 1996a; Geverink *et al.*, 1996) environment, simple behavioural time budgets (e.g. Lambooy, 1988; Bradshaw *et al.*, 1996b) and concentrations of 'stress' hormones in the blood (e.g. Dalin *et al.*, 1988; Nyberg *et al.*, 1988; Dalin *et al.*, 1993; Geers *et al.*, 1994; Bradshaw *et al.*, 1996a). However, there have been few studies which have either taken blood samples from catheterized animals during transport or have examined the effects of a particular type of journey on patterns of hormone secretion.

A recent study by Bradshaw *et al.* (1996a) compared the behaviour and salivary cortisol concentrations of mixed and unmixed pigs during transport. Unfamiliar mixed pigs fought during short journeys and had higher levels of salivary cortisol compared with unmixed pigs. In addition, in catheterized pigs sampled during an 8-h journey, plasma cortisol concentrations were highest during loading and declined after 5 h to near control levels (recorded

when pigs were loaded on the vehicle which remained stationary). Bradshaw *et al.* (1996b) also compared the behaviour and salivary cortisol response of pigs and sheep during short (80 min) journeys which were defined as 'rough' and 'smooth', a study which was the first to investigate the effects of particular types of journey on behaviour and cortisol. Pigs were shown to be sensitive to different journey types as concentrations of cortisol were higher on 'rough' journeys. During the course of this study it was also found that pigs became travel sick when given food before transport. Normal commercial practice favours withholding food from pigs for a period before transportation and it has yet to be established whether pigs also become travel sick when not given food.

Fursling *et al.* (1984) have shown that exposure to vibration and noise leads to raised concentrations of plasma lysine vasopressin (LVP) in pigs. In addition, it is known that nausea is associated with enhanced vasopressin secretion in man (Rowe *et al.*, 1987). It has also been shown that vasopressin release is stimulated in man (Miaskiewicz *et al.*, 1989), monkeys (Verbalis *et al.*, 1987), sheep (Ebenezer *et al.*, 1989) and pigs (Parrott *et al.*, 1991) following i.v. injection of cholecystokinin, a gut/brain peptide that induces emesis (Verbalis *et al.*, 1987; Levine *et al.*, 1984; Parrott *et al.*, 1991). Thus, because vasopressin is a physiological correlate of nausea in animals that vomit, increased plasma LVP concentrations in pigs during transport may signal motion sickness.

Two studies are described in this paper. The first investigated the effects of short (80 min) road journeys (characterized as 'rough' or 'smooth' by means of an accelerometer) on plasma concentrations of cortisol, beta-endorphin and LVP. The second investigated whether pigs deprived of food before transport exhibit signs of travel sickness and an associated increase in plasma LVP concentrations. Information on plasma cortisol and beta-endorphin concentrations from the second study have been described in a previous report (Bradshaw *et al.*, 1996a).

Material and methods

Experiment 1

Seven 40-kg male Large White pigs were surgically prepared under general anaesthesia, using sterile precautions, with jugular vein catheters. Surgery was carried out 2 weeks before the experiment and the pigs were kept in individual cages during this period where they became accustomed to close contact with people. Catheter patency was maintained by daily flushing with sterile heparinized saline and catheters were protected by elasticated bandages.

The vehicle used was a 17-t four-wheeler cattle lorry with ventilation louvres (open), metal floor and sides (straw was provided) with internal penning (bars) such that pigs were individually confined so as to prevent damage to catheters. The pigs were transported over 2 days on 'rough' or 'smooth' journeys. On day 1, the schedule consisted of loading (10.00 h) followed immediately by a rough journey (10.10 to 11.50 h), a stationary period (11.50 to 13.30 h), a smooth journey (13.30 to 15.10 h) and a stationary period (15.10 to 16.50 h), after which pigs were unloaded to individual home pens provided with straw bedding, food and water. On day 2, the schedule consisted of loading followed by a stationary period (10.10 to 11.50 h), a smooth journey (11.50 to 13.30 h), a stationary period (13.30 to 15.10 h) and a rough journey (15.10 to 16.50 h), after which pigs were once again unloaded to individual home pens. Food was withdrawn at 18.30 h on the day before transport. Due to the different journey times for each day, the interval of food withdrawal differed for each journey (on day 1, 15 h 30 min before the rough journey and 19 h before the smooth journey; on day 2, 17 h 20 min before the smooth journey and 20 h 40 min before the rough journey) and the physiological status of the pigs may therefore have differed. The lorry travelled at an average speed of 64 km/h on rough journeys and 80 km/h on smooth journeys. The rough condition involved journeys on minor roads while the smooth condition consisted of journeys on a motorway. Pigs remained on the vehicle during rest periods and no food or water was provided. On day 3, pigs remained in their home pen with no food or water available.

During days 1 and 2 the experimenters travelled with the pigs in the body of the vehicle taking blood samples every 20 min from each pig. On day 3, hourly blood samples were taken from each pig between 09.20 and 18.20 h. In all cases, blood was centrifuged and the resultant plasma divided into aliquots which were frozen in dry ice and subsequently stored at -30°C pending hormone analysis. Plasma cortisol was assayed as described in Parrott and Goode (1992) and beta-endorphin was assayed as described by Fordham *et al.* (1989) using porcine beta-endorphin (Bachem, USA) as iodinated tracer and standards; the antiserum was supplied by Dr G. Lincoln (Edinburgh). The radioimmunoassay for LVP was conducted as described in Thornton *et al.* (1987).

The journeys were characterized by means of a triaxial accelerometer (EDR — 1S), a temperature probe (Grant type CTU thermistor), sound meters (Cirrus CRL 222A integrating meter) and a relative humidity probe (Vaisala HMP35A). Sensors recording air temperature (°C), sound (dBA) and

relative humidity reported to Grant Squirrel data loggers. A reading was taken every minute and every 10 min these were averaged and logged. The accelerometer was set to log every event greater than 0·707 g which had a duration exceeding 0·012 s, in one or other of the planes *x* (fore and aft), *y* (side to side) or *z* (vertical); there was a period of 0·48 s after a reading before another reading could be logged.

Two statistical comparisons were made using paired *t* tests and expressed as two-tailed probability values. First, for each type of journey (rough or smooth), total concentrations of plasma cortisol, beta-endorphin and LVP were compared between days 1 and 2. Secondly, hourly samples on day 3 (control) were compared with corresponding hourly samples during days 1 and 2 (transport).

Experiment 2

A summary of the methodology is presented below as full details have already been described (Bradshaw *et al.*, 1996a). Six 35-kg male Large White pigs were prepared under general anaesthetic with jugular vein catheters. A commercial livestock lorry (four-wheeler rigid), different from that used in experiment 1, was hired and internal penning was constructed such that pigs could be individually confined. On day 1, pigs were loaded onto the lorry at 09.30 h which remained stationary. (food was withdrawn at 17.00 h the previous evening); blood samples were taken every 30 min starting at 09.30 h and finishing at 17.30 h. Two days later, pigs were loaded at 09.30 h and driven continually on 'A' roads and motorway between 09.30 and 17.30 h, travelling a distance of 455 km in 8 h. Blood samples were taken at 30-min intervals and centrifuged in transit with the resultant plasma frozen on dry ice. The assay for LVP and characterization of the journey was the same as in experiment 1.

Behaviour was observed at each sampling point and an assessment made of the number of animals showing symptoms of travel sickness. While incidences of retching and vomiting clearly indicate the pigs became travel sick, other behaviours associated with travel sickness have also been described: chewing, foaming at the mouth and sniffing the air while standing (Bradshaw *et al.*, 1996b). Thus a period of travel sickness was identified, defined as one or more animals exhibiting all or any one of these described symptoms of travel sickness at sequential sampling points. A comparison was then made using a paired *t* test to establish whether total concentrations of LVP during the period of travel sickness were significantly higher than corresponding control values (when the vehicle was stationary). Results were expressed as two-tailed probability values.

Results

Experiment 1

Cortisol. Concentrations of plasma cortisol increased markedly after loading and during both types of journey on days 1 and 2 (see Figure 1a and b). However, the effects of loading cannot be separated from those of the initial rough journey. In addition, habituation to the transport stimulus during the course of the day indicates that any comparison of the effects of types of journey (rough or smooth) on patterns of cortisol release must be made with caution. Concentrations of cortisol during both types of journey were significantly higher on day 1 compared with day 2, indicating that some habituation had occurred ($P < 0·05$). Concentrations, measured at the same time points, were significantly higher on days 1 and 2 compared with day 3 (see Figure 1c; $P < 0·05$).

Beta-endorphin. Concentrations of plasma beta-endorphin increased in response to loading on day 1 (see Figure 2a). Concentrations during the rough journeys were significantly higher on day 1 compared with day 2 although this difference may have been due to the effects of loading on day 1 which immediately preceded the initial rough journey (see Figure 2a and b; $P < 0·05$). Concentrations during smooth journeys showed no significant difference between days. Concentrations were significantly higher on day 1 compared with day 3 (see Figure 2c; $P < 0·05$) and approached significance on day 2 compared with day 3 ($P < 0·06$).

LVP. Concentrations of LVP (Figure 3a and b) were variable and showed no clear response to either rough or smooth journeys and no significant difference between days 1 and 2 for either type of journey ($P > 0·05$ — see Figure 3a and b). However, concentrations were significantly higher on days 1 and 2 compared with day 3 (see Figure 3c; $P < 0·01$).

Physical measurements. There were no substantial changes in weather conditions between days and the mean physical characteristics recorded were as follows: temperature, 12·9°C; relative humidity, 0·843; sound levels, 83·8 dB. The mean number of acceleration events for rough journeys was 66 and for smooth journeys was 38.

Experiment 2

Plasma concentrations of LVP and the number of pigs showing symptoms of travel sickness are shown in Figure 4. Two hours after the start of the journey three of the six pigs began to exhibit behaviour possibly associated with travel sickness which lasted for 2·5 h, one of these pigs retched during this period (after 4 h). After 4·5 h of the journey all pigs continually lay in sternal recumbency, immobile an

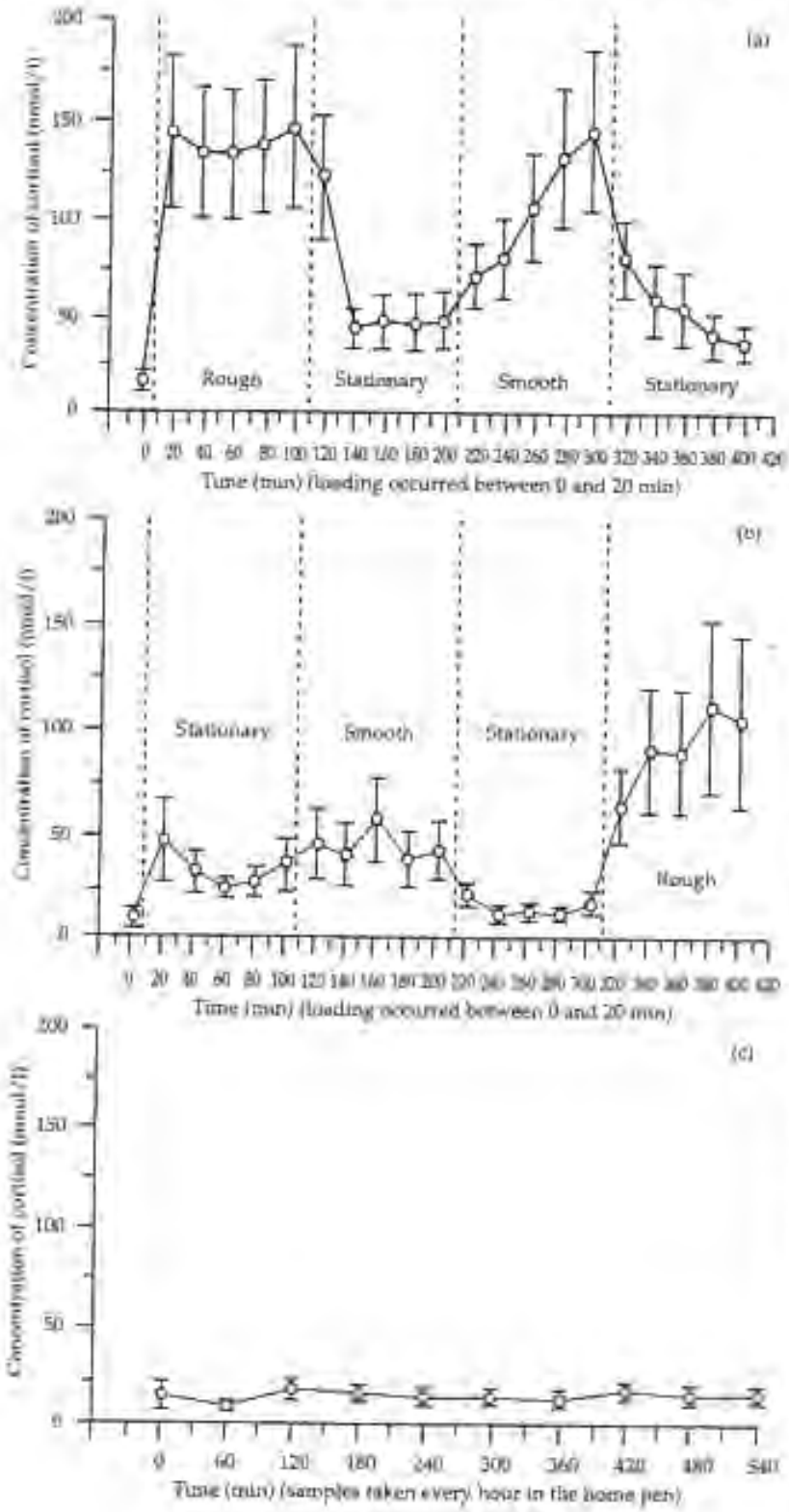

Figure 1 Concentration of plasma cortisol (nmol/l; mean with S.E.) in pigs (no. = 7) sampled every 20 min from 10.00 h (before loading) to 16.40 h (before unloading) showing response to rough and smooth journeys and stationary periods. (a) day 1, loading (10.00 h) followed immediately by a rough journey (10.10 to 11.50 h), a stationary period (11.50 to 13.30 h), a smooth journey (13.30 to 15.10 h) and a stationary period (15.10 to 16.50 h) after which pigs were unloaded to individual home pens with straw bedding, food and water. (b) day 2, loading followed immediately by a stationary period (10.10 to 11.50 h), a smooth journey (11.50 to 13.30 h), a stationary period (13.30 to 15.10 h) and a rough journey (15.10 to 16.50 h) after which pigs were once again unloaded to individual home pens. (c) day 3, pigs remained in their home pen. Food and water was withdrawn the previous evening on all 3 days. Concentrations of cortisol during both types of journey were significantly higher on day 1 compared with day 2 ($P < 0.05$). Concentrations, measured at the same time points, were significantly higher on days 1 and 2 compared with day 3 ($P < 0.05$).

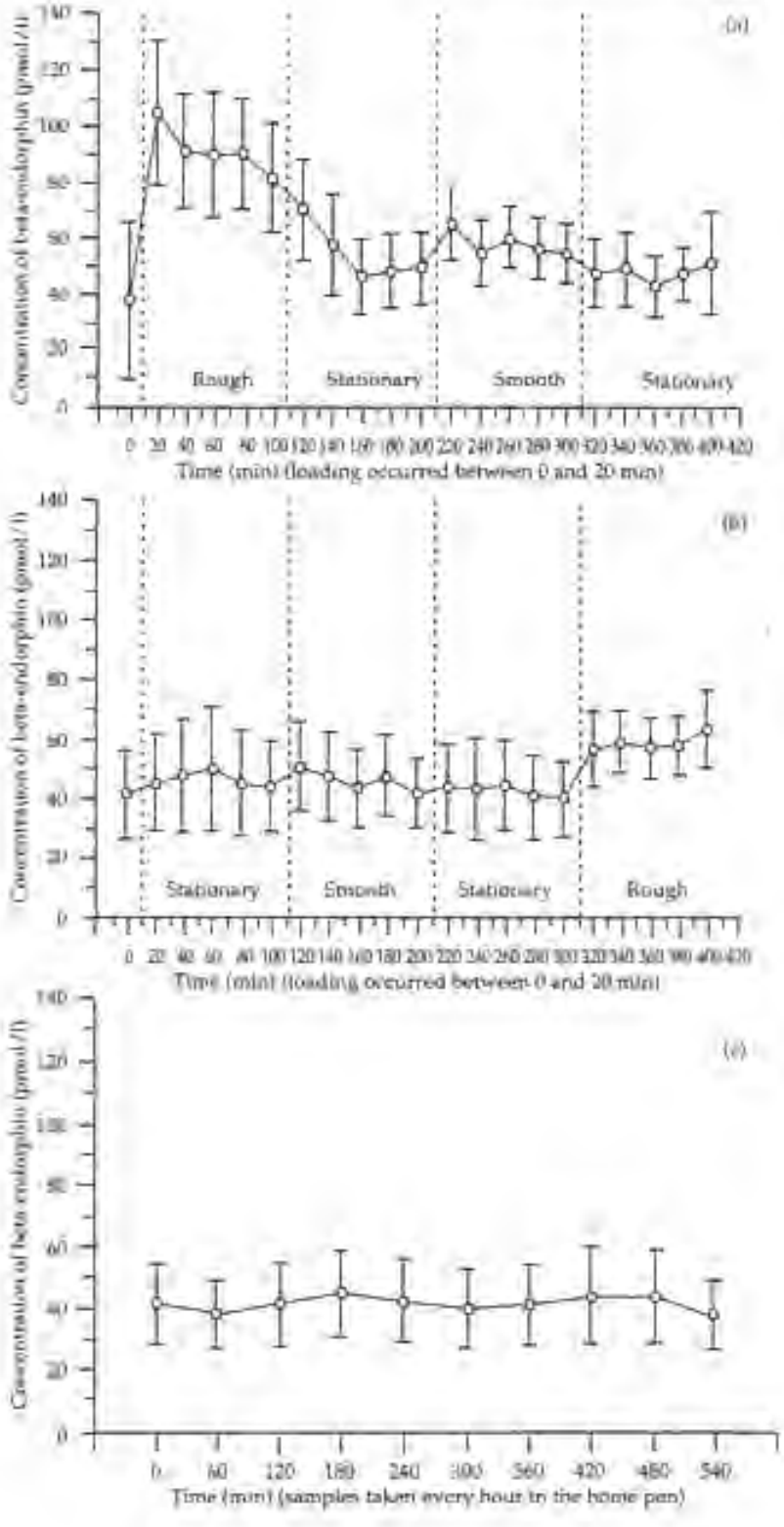

Figure 2 Concentration of plasma beta endorphin (pmol/l, mean with s.e.) in pigs (no. = 7) sampled every 20 min from 10.00 h (before loading) to 16.40 h (before unloading) showing response to rough and smooth journeys and stationary periods: (a) day 1, loading (10.00 h) followed immediately by a rough journey (10.10 to 11.50 h), a stationary period (11.50 to 13.30 h), a smooth journey (13.30 to 15.10 h) and a stationary period (15.10 to 16.50 h) after which pigs were unloaded to individual home pens with straw bedding, food and water; (b) day 2, loading followed immediately by a stationary period (10.10 to 11.50 h), a smooth journey (11.50 to 13.30 h), a stationary period (13.30 to 15.10 h) and a rough journey (15.10 to 16.50 h) after which pigs were once again unloaded to individual home pens; (c) day 3, pigs remained in their home pen: food and water was withdrawn the previous evening on all 3 days. Concentrations of beta-endorphins during the rough journeys were significantly higher on day 1 compared with day 2 ($P < 0.05$), while concentrations during smooth journeys showed no significant difference between days. Concentrations were significantly higher on day 1 compared with day 3 ($P < 0.05$) and approached significance on day 2 compared with day 3 ($P < 0.06$).

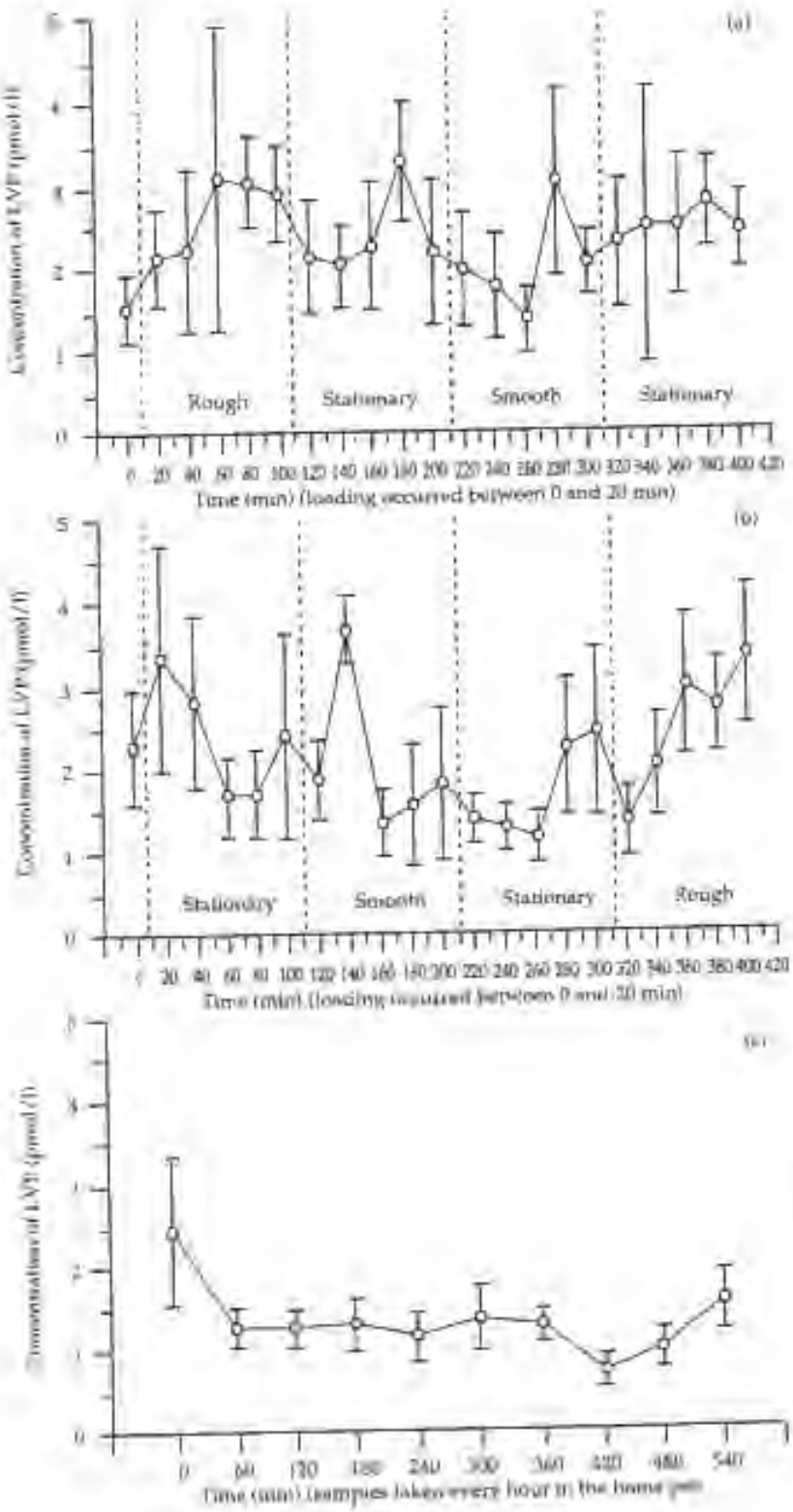

Figure 3 Concentration of plasma lysine vasopressin (LVP) (pmol/l, mean with s.e.) in pigs ($n_u = 7$) sampled every 20 min from 10.00 h (before loading) to 16.40 h (before unloading) showing response to rough and smooth journeys and stationary periods (*a*) day 1, loading (10.00 h) followed immediately by a rough journey (10.10 to 11.50 h), a stationary period (11.50 to 13.30 h), a smooth journey (13.30 to 15.10 h) and a stationary period (15.10 to 16.50 h) after which pigs were unloaded to individual home pens with straw bedding, food and water; (b) day 2, loading followed immediately by a stationary period (10.10 to 11.50 h), a smooth journey (11.50 to 13.30 h), a stationary period (13.30 to 15.10 h) and a rough journey (15.10 to 16.50 h) after which pigs were once again unloaded to individual home pens; (c) day 3, pigs remained in their home pen. Food and water was withdrawn the previous evening on all 3 days. Concentrations of LVP showed no significant difference between days 1 and 2 for either type of journey ($P > 0.05$), but were significantly higher on days 1 and 2 compared with day 3 ($P < 0.01$).

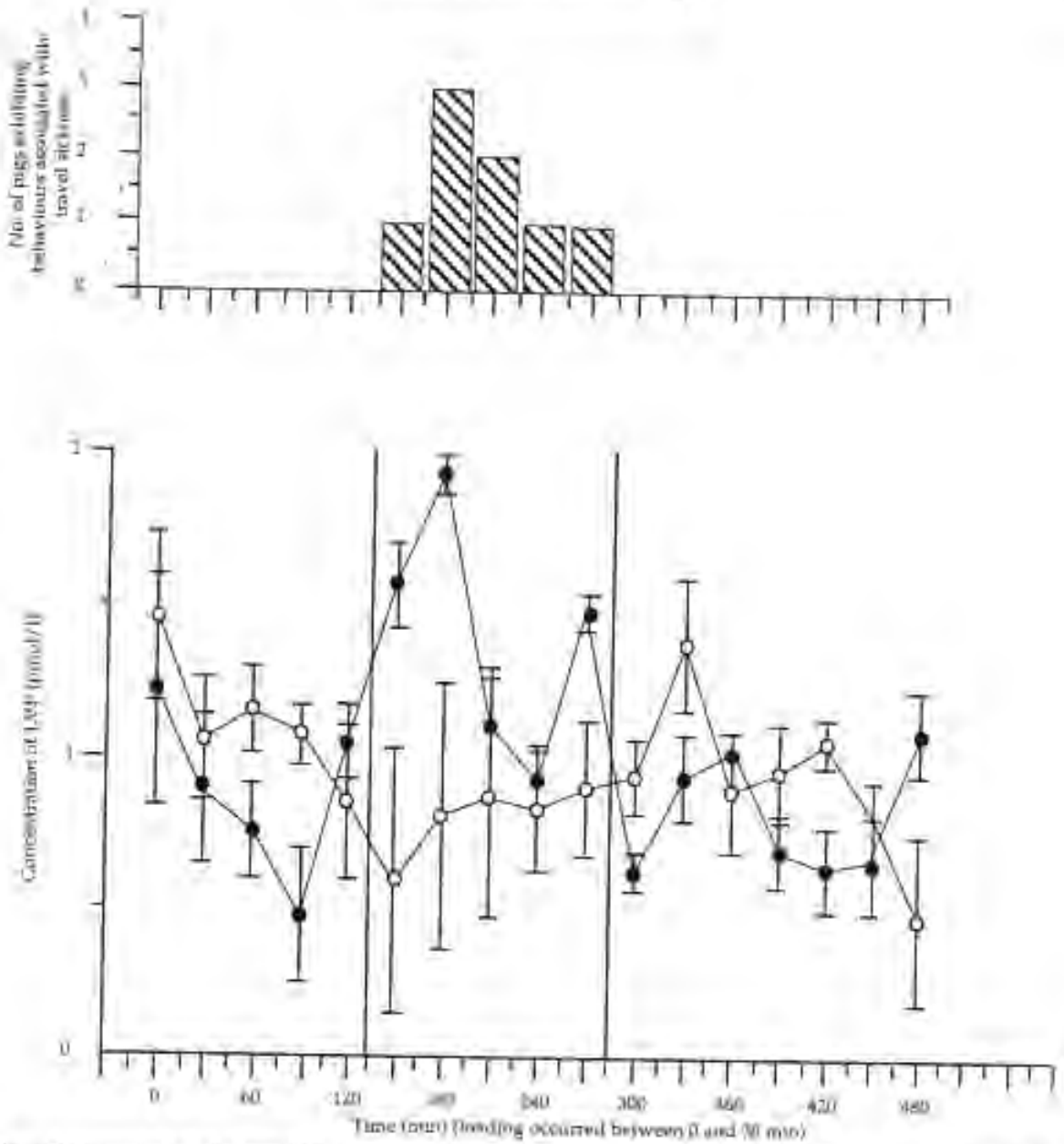

Figure 4 Concentration of plasma LVP (pmol/l; mean with s.e.) in pigs (no. = 6) sampled every 30 min from 08.30 to 17.00 h on experimental (moving) day (●) and control (stationary) day (○). Area between the two vertical lines indicates the period of elevated concentrations of plasma LVP (which approached significance: $P = 0.085$) coinciding with behaviours associated with travel sickness (2 to 4.5 h). Described behaviours associated with travel sickness were retching, chewing, foaming at the mouth and sniffing the air while standing

the floor of vehicle, showing no further obvious signs of travel sickness. The total period during which the described behaviours associated with travel sickness were displayed (2 to 4.5 h) appeared to be associated with elevated concentrations of plasma LVP (which approached significance: $P = 0.085$). There was no significant difference during days not designated as the period of 'travel sickness' and no overall difference between days. Concentration of LVP in the one pig which retched was particularly elevated

immediately before retching at 3·5 h (1·46 pmol/l). Physical characteristics (temperature, humidity and sound) have been reported by Bradshaw *et al.* (1996a).

Discussion

The results of experiment 1 are indicative of a generalized increase in hormone secretion on the days when the animals were transported. However, the changes in the concentrations of all three hormones (cortisol, beta-endorphin and LVP) may be seen as a response to four interacting factors: loading, type of journey, period of food withdrawal and habituation over the course of the experiment.

On the morning of day 1, cortisol release was stimulated by loading and the subsequent 100-min rough journey. The loading procedure was dissimilar to normal commercial practice as each pig was lifted from its cage, wheeled 100 m in a trolley to the vehicle and placed in an individual pen. Hence, this procedure would have been more stressful than commercial practice using a ramp or a lift, which is also known to be disturbing (Brown *et al.*, 1993).

A previous study (Bradshaw *et al.*, 1996a) found that cortisol concentrations in pigs that were loaded, but not transported, declined to pre-loading levels after 2·5 h (150 min). Thus, on day 1, while it is not possible to separate the effects of loading and the first (rough) journey on cortisol secretion, it might have been expected that any effects of loading would have largely disappeared by the time the smooth journey was conducted (after 200 min). Nevertheless, there was still a clear increase in hormone concentrations in response to the smooth journey, indicating that the pigs remained sensitive to the transport stimulus.

The results from day 2 indicate that adaptation had occurred because there was only a modest increase in cortisol after loading and during the smooth journey. While the effects of the loading procedure should have disappeared after 2·5 h (as explained above), it might have been anticipated that any increase in cortisol concentrations in response to the final (rough) journey would have been masked by the effects of habituation. It is, therefore, surprising that a distinct, although variable, increase in plasma cortisol concentrations occurred in this period. Thus, despite the effects of habituation, pigs appeared to remain sensitive to transport at the end of day 2. These results generally support previous findings (Bradshaw *et al.*, 1996b) in which pigs, repeatedly travelling for 80 min were found to remain responsive to transportation. Analysis of acceleration events revealed that the rough journeys were, on average, slightly smoother and smooth journeys slightly rougher in the present study.

The situation with beta-endorphin supports a less complicated interpretation. Concentrations increased in response to loading on day 1 and habituation appeared to occur much more rapidly than in the case of cortisol. As a consequence, responses to different types of journey could not be distinguished. This finding is in keeping with previous data (Bradshaw *et al.*, 1996a) showing that plasma concentrations of beta-endorphin increase in response to loading but show no clear response to transportation. Because beta-endorphin release is stimulated by an acute stressor (loading) but then either habituates rapidly, or is less sensitive to a chronic, but less severe, stressor (transport), this hormone may not provide a good welfare indicator under transport conditions. Therefore, its applications may be limited to the detection of relatively short-term acute stressors. Similar results have been reported in sheep (Fordham *et al.*, 1989).

The release of LVP in experiment 1 was not differentially affected by the type of journey, although the fact that hormone concentrations were higher on days 1 and 2 is suggestive of a generalized effect of transport. Similarly in experiment 2, there was no increase in LVP concentrations in response to loading but behavioural symptoms of travel sickness, apparent after 2 h coincided with a rise in hormone concentrations. This finding is in keeping with previous results from pigs subjected to noise and vibration (Forsling *et al.*, 1984). Moreover, as vasopressin release occurs in pigs in response to nausea (Parrott *et al.*, 1991), and in man under conditions of simulated motion sickness (Koch *et al.*, 1990), measurement of LVP concentrations may provide a useful welfare indicator in pigs undergoing transport. In the present study the period of travel sickness was defined as one or more pigs exhibiting behaviours previously described by Bradshaw *et al.* (1996b): retching, chewing, foaming at the mouth and sniffing the air while standing. Further research is required to establish whether these behaviours are exclusively associated with travel sickness since chewing, foaming at the mouth and sniffing the air while standing may also be associated with the pigs' response to a novel environment. Thus while these findings should be addressed with caution and do not reveal the relative importance of travel sickness in normal commercial practice, they do provide a basis against which future studies may be compared.

Randall *et al.* (1996) have measured vibration acceleration on the vehicle used in experiment 1 as part of a comparative study of the effects of four

different vehicles on pig welfare. These measurements indicate that the conditions employed in experiment 1 would result in a relatively uncomfortable ride. Thus, the 'ride' in experiment 1 may have been more unpleasant than that experienced during normal commercial practice. Further research is required to establish the precise vibrational characteristics of the lorry used in experiment 2 which may have caused travel sickness.

In conclusion, this study shows that pigs find loading and transport stressful, habituate to repeated transportation, but remain sensitive to a change in the physical characteristics—of the journey. In addition, under certain conditions, pigs become travel sick and these symptoms seem to be associated with raised plasma concentrations of LVP. Hence, measurements of plasma cortisol and vasopressin may provide some indication of stress and travel sickness, respectively, which is necessary for the assessment of welfare of pigs in transit. By contrast, beta-endorphin does not appear to provide a helpful welfare index in such situations.

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