Oxytocin and prolactin suppress cortisol responses to acute stress in both lactating and non-lactating sheep

By CHRISTIAN J. COOK

Animal Stress and Welfare Programme, Meat Industry Research Institute of New Zealand (Inc.), PO Box 617, Hamilton, New Zealand

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Summary. Cortisol response to stress appears to differ between lactating and non-lactating animals. Lactating (14 d post partum) and non-lactating sheep were fitted with probes so that drugs and hormones could be infused directly into the posterior pituitary and paraventricular nucleus of the hypothalamus. The animals were also fitted with instruments to allow monitoring of heart rate, body temperature and blood cortisol levels. Their reactions to a source of acute stress (a barking dog) were then followed, with or without drug and hormone manipulation. Results in both lactating and non-lactating animals indicated shortcomings in the use of cortisol as a stress indicator. Infusing prolactin and oxytocin into either the posterior pituitary or the paraventricular nucleus of the hypothalamus suppressed cortisol responsiveness to stress in both lactating and non-lactating animals (the latter to a greater extent). In the absence of drugs, lactating animals had a slightly higher basal level of cortisol and a lower cortisol response to stress than their non-lactating counterparts. Despite suppression of cortisol responses, with or without drugs, other indicators of stress still changed with the presence of a barking dog, suggesting the complexity of control involved in stress responses.

Release of adrenal corticosteroids has been suggested as one of the main neurohumoral responses to acute stress (Sapolsky, 1992). In adult sheep, with certain situations of stress, cortisol release is often observed in parallel with other indicators of acute stress (Cook & Jacobson, 1995). However, cortisol changes alone are often poor predictors of stress, particularly in lactating animals (Higuchi *et al*. 1989), and considerable doubt surrounds the role of cortisol in stress responses. The regulation of cortisol secretion and its action on cells are both extremely complex and interact with many other neurohumoral axes (Plotsky *et al*. 1989). The release of oxytocin and prolactin both differ in the lactating and non-lactating states, and both may contribute to cortisol regulation (Walker *et al*. 1992). Definitive proof of this has not yet been provided.

The aims of the presented study were twofold: firstly to determine whether there were differences in stress response to an acute stressor (a barking dog) between non-lactating and lactating sheep and, secondly, to determine whether two hormones important in lactation (Shiu & Friesen, 1980), oxytocin and prolactin, had any influence on stress responsiveness.

MATERIALS AND METHODS

A flock of 34 sheep (Romney cross ewes, 18–24 months old, live weights 30–40 kg, non-lactating and non-pregnant) grazed together with water and feed available *ad lib*. Each animal underwent stereotaxic surgery as described previously (Cook *et al*. 1995), and modified microdialysis probes (base model: CMA 12; CMA/Microdialysis, S-104 05 Stockholm, Sweden) were implanted into both the posterior pituitary and the paraventricular nucleus of the hypothalamus (PVH). Surgery was performed aseptically using an anaesthetic combination of zolazepam and tiletamine hydrochloride (Zoletil 100; Techvet, Auckland, New Zealand) and xylazine hydrochloride (Rompun; Bayer, Petone, New Zealand). Zoletil was administered at 1 mg/kg live weight and Rompun at 0·3 mg/kg liveweight, both intramuscularly. Local areas were anaesthetized using 2 ml subcutaneous lignocaine hydrochloride (Xylocaine, 20 mg/ml; Astra Pharmaceuticals, Ryde, NSW 2113, Australia). Animals recovered within 6–8 h of surgical procedures and were treated prophylactically with 2 ml antibiotics intramuscularly (Tripen LA, Ethical Agents, Auckland, New Zealand). Post-surgical analgesia was administered every 4 h for 24 h in the form of 1500 mg oral salicylic acid (Solprin; Reckitt and Colman, Auckland, New Zealand) made up in a milk formula.

A further flock of 12 lactating sheep (same breed, age and weights as non-lactating animals), each with a singleton lamb, were similarly treated 7 d post partum. During post-surgical recovery each lactating sheep was individually housed with its lamb.

After 7 d recovery, individual animals were restrained in a polypropylene cradle. In the case of lactating animals the lambs were restrained alongside the ewe allowing body contact but not suckling. The sheep were fitted with probes as previously described by Cook *et al*. (1992) to allow measurement of skin and rectal temperatures, to obtain electrocardiograms (ECG), and to take blood samples from the jugular vein (via an implanted catheter). The microdialysis probes (adapted to contain a membrane with a molecular mass cut-off of 40 kDa, and o.d. 100 μm) were connected to a small syringe pump (MS 16A; Graseby Medical, Watford, UK) to allow infusion, at a rate of 1 μl/min, of sterile saline containing one of: ascorbic acid (Sigma Chemicals, St Louis, MO 63178, USA), oxytocin (Sigma), prolactin (luteotrophic hormone, 20–50 i.u./mg, Sigma) or a combination of oxytocin and prolactin. Ascorbic acid, prolactin and oxytocin were administered in amounts of 100 μg, 500 μg, 1 mg or 10 mg in 20 μl infusate. Prolactin–oxytocin combinations were administered at 50, 250 or 500 μg of each in 20 μl infusate. In a single experiment drugs were infused into either the posterior pituitary or the PVH. Drug concentrations were measured using radioimmunoassay as described by Kendrick *et al*. (1986, 1988) in both the dialysis probe inlet and outlet, making it possible to calculate how much of the infusate concentration was delivered.

Baseline heart rate (derived from the electrocardiogram by using the interval between each QRS complex, which represents the electrical events of ventricular emptying), rectal and skin temperatures were recorded continuously during 30 min restraint alone. Three blood samples (2 ml each) were taken at 10 min intervals during this time.

Following this 30 min period, drugs (20 μl) were infused via the microdialysis probes into either the posterior pituitary or PVH. A further blood sample was taken immediately after infusion and another 10 min later. At 20 min after infusion animals were exposed to a moving, barking dog for 5 min. Continuous ECG and

temperature (skin and rectal) values were collected and blood samples taken every 10 min for 40 min after exposure to the dog. In each animal this protocol was repeated 3, 6, 9, 12 and 15 d later (a total of six experiments per animal). Each animal received, via the posterior pituitary probe, one ascorbic acid dose in one of the experiments, one dose of either oxytocin or prolactin in the second, and in the third a combination of prolactin and oxytocin. This was also repeated using the probe within the PVH. The order of drug presentation was randomized as were dosages and the use of either posterior pituitary or PVH probe. Blood samples were centrifuged and separated and plasma cortisol assessed in duplicate, with random duplicates being assessed in another laboratory, using displacement radioimmunoassay techniques based on labelled iodinated cortisol (Fell *et al.* 1985).

Four of the non-lactating animals and two of the lactating animals underwent a stress protocol (restraint and dog) identical to the above six times over 12 d without any infusion into the microdialysis probes.

In ten additional animals (non-lactating) similar instrumentation was connected to a telemetric backpack (author's design) that relayed continuously, using radio frequency bandwidths, information on rectal and skin temperature and ECG to a remote receiver connected to a personal computer allowing analysis using a Labview 3.0 package (Mathtec National Instruments, Austin, TX 787350-5039 USA). Blood collection and pump infusion were remotely controlled via a set timer (author's design). Once fitted with instruments, these animals were released back into the field and the above protocol repeated with the dog being introduced into the field for 5 min. The dog was restrained but allowed to bark and the sheep were free to move within the constraints of the paddock. At completion of the trial, animals were rounded up and the instruments removed. The same drug protocols were repeated, except that only two doses of ascorbic acid, prolactin or oxytocin were used, these being 2 or 10 mg in 20 μl infusate. In the case of the prolactin–oxytocin combination, only one dose containing 1 mg each of prolactin and oxytocin in 20 μl was used. Drug delivery was via microdialysis probes. The protocol was repeated four times (each 2 d apart) for each animal (one dose each of ascorbic acid, oxytocin, prolactin, and oxytocin–prolactin in a randomized order) using the posterior pituitary probe, and four times using the PVH probe. Probe usage was also randomized.

At the completion of the experiment the animals were killed using sodium pentobarbitone (barbital, Sigma), brains were removed, and probe sites verified histologically. Immediately before this, in three non-lactating and three lactating animals oxytocin was introduced into the posterior pituitary probes for infusion as described above and collections were made from the PVH probes. Collections from PVH probes were made for 40 min prior to the start of infusion (to track baseline oxytocin) and for 40 min after infusion to attempt to track the diffusive spread of infused oxytocin. Individual samples in each period were collected over 10 min. Samples were analysed by radioimmunoassay using the method described by Kendrick *et al.* (1986, 1988). In a similar trial with the two non-lactating and two lactating animals methylene blue dye was used. Immediately after this 40 min, the animals were killed. The animals that received methylene blue treatments were not perfused but their brains were immediately removed from the cranium, frozen in liquid nitrogen and later sectioned on a microtome to determine dye spread.

Results were analysed using a number of statistical methods. All horizontal values were subjected to two way repeat measures analysis of variance (ANOVA), and across individual data points χ^2 tests, nonlinear regressions and Student's t tests

were used. All results were also analysed for interactions between different treatments and sequencing of treatments.

RESULTS

Cortisol response to stress

Lactating animals had slightly higher ($P < 0{\cdot}05$) basal levels of cortisol during restraint, with and without infusions, than did non-lactating animals. However, cortisol levels 10 and 20 min after introduction of a dog were significantly ($P < 0{\cdot}05$) greater in non-lactating than in lactating animals with infusions of ascorbic acid into either the posterior pituitary (Fig. 1) or the PVH (Table 1). These values were not significantly different from those found in response to a dog and with no infusions (Fig. 1). Whether or not ascorbic acid was infused, cortisol values following exposure to a dog were significantly greater in both lactating ($P < 0{\cdot}01$) and non-lactating ($P < 0{\cdot}01$) animals than basal levels during restraint alone.

With oxytocin infusions into the posterior pituitary, cortisol levels rose significantly ($P < 0{\cdot}01$) following exposure to a dog in non-lactating and to a lesser extent in lactating animals. However, at higher concentrations of oxytocin this increase in cortisol was significantly ($P < 0{\cdot}05$) less than with either ascorbic acid, or no, infusion (Fig. 1). Oxytocin infusions into the PVH were associated with cortisol responses to a dog that were not significantly different from those with infusions of ascorbic acid (Table 1). At the highest concentrations of oxytocin infusion in lactating animals, cortisol response to stress actually fell significantly ($P < 0{\cdot}01$) below basal levels (although basal levels themselves were not changed by this infusion).

Cortisol responses to the presence of a dog during prolactin infusions was significantly ($P < 0{\cdot}05$) less than with either ascorbic acid, or no, infusion. Infusions were equally effective in both posterior pituitary and PVH (Table 1). Lactating animals had a lower ($P < 0{\cdot}05$) cortisol response to a dog than did non-lactating animals with prolactin infusion. Again, in lactating animals at the highest concentrations of prolactin infusion, cortisol responses to the dog fell below basal (with infusion) levels.

The combination of both prolactin and oxytocin in the infusate appeared most efficacious at suppressing cortisol increases in response to a dog, the response being significantly less than with ascorbic acid ($P < 0{\cdot}01$), oxytocin ($P < 0{\cdot}01$) alone or prolactin ($P < 0{\cdot}05$) alone (Fig. 1). The effect of prolactin–oxytocin combined infusions on cortisol response was slightly, but not significantly, less with infusion into the posterior pituitary than in the PVH (Table 1). In both lactating and non-lactating animals the highest concentrations of prolactin–oxytocin resulted in cortisol responses to a dog that were below the basal levels.

Effects of drug concentration in the infusate

Effects of infusate concentration are summarized in Table 1. Increasing concentrations of ascorbic acid had no significant effects upon cortisol response following presentation of a dog and no difference in cortisol levels was seen between any concentration of ascorbic acid and no infusion alone. At low concentrations of oxytocin, cortisol responses to a dog were slightly, but not significantly, higher than with ascorbic acid. However, at higher concentrations of oxytocin, cortisol responses to a dog were significantly less than with the equivalent dosages of ascorbic acid. Cortisol responses to a dog were significantly less with prolactin infusion than with equivalent concentrations of either ascorbic acid or, at lower concentrations,

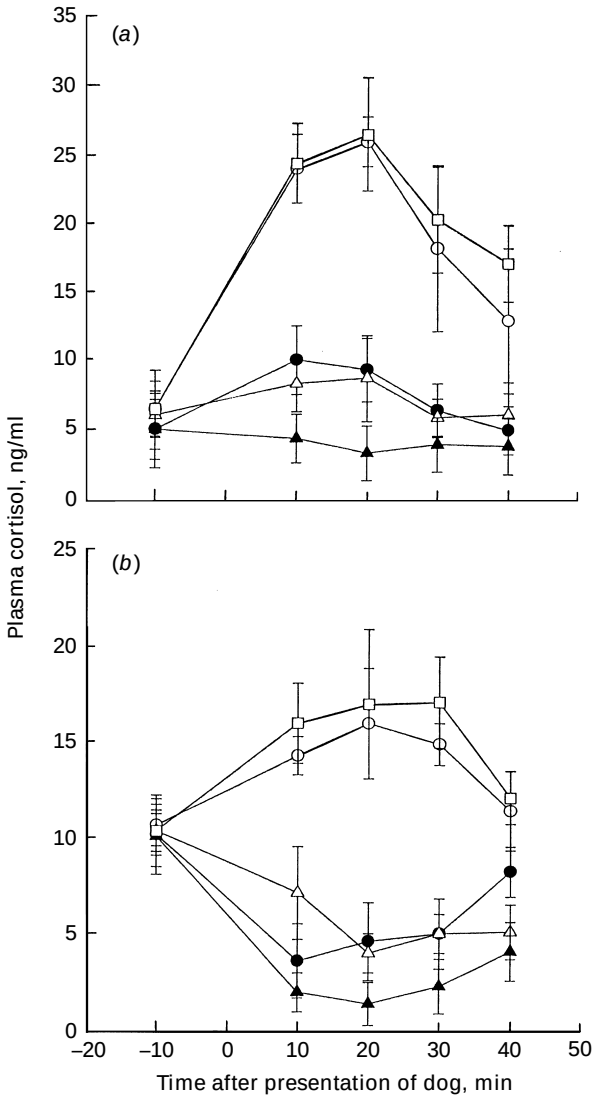

Fig. 1. Time course of plasma cortisol response following exposure to a dog, with posterior pituitary infusion of ○, ascorbic acid; ●, oxytocin; △, prolactin; ▲, prolactin plus oxytocin or □, no infusion in (*a*) non-lactating and (*b*) lactating sheep. Drugs were infused into restrained animals at 1 mg in 20 μl over 20 min. Values are means for $n = 12$ with SD indicated by vertical bars. The values at −10 min (10 min before presenting the dog) were not significantly different from basal levels prior to drug infusion.

oxytocin. Increasing concentrations of prolactin–oxytocin, within the range used, did not significantly increase the suppressive effect upon cortisol responses to a dog above that seen with the initial dosage. All concentrations did, however, significantly suppress the cortisol responses to a greater extent than equivalent concentrations of ascorbic acid or oxytocin, and at lower concentrations than prolactin. The effects of differing concentrations of ascorbic acid were similar with infusion into both the posterior pituitary and the PVH. Oxytocin at high concentrations had a significantly greater suppressive effect on cortisol responses to a dog when infused into the posterior pituitary whereas prolactin was slightly more effective in the PVH.

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Table 1. *Cortisol response measured as plasma cortisol concentrations 20 min after exposure to a dog in lactating and non-lactating sheep with and without drug infusions in the posterior pituitary or the paraventricular nucleus of the hypothalamus (PVH)*

(Values are ng/ml, means ± SD for $n = 12$)

	Lactating		Non-lactating	
Infusion	Posterior pituitary	PVH	Posterior pituitary	PVH
None	17·0 ± 3·9		26·5 ± 4·1	
Ascorbic acid				
100 μg	14·1 ± 2·8	15·2 ± 2·9	27·9 ± 4·5[*]	24·4 ± 1·5[*]
500 μg	19·0 ± 1·0	17·4 ± 1·9	24·1 ± 3·6[*]	28·3 ± 5·0[*]
1 mg	16·0 ± 2·9	18·7 ± 3·0	26·0 ± 1·8[*]	25·1 ± 3·9[*]
10 mg	15·1 ± 3·4	15·8 ± 2·9	22·3 ± 3·4[*]	23·0 ± 4·1[*]
Oxytocin				
100 μg	15·9 ± 4·1	17·9 ± 3·1	37·3 ± 8·5[*]	25·9 ± 3·4[*]
500 μg	13·3 ± 1·4	18·4 ± 3·0(*)	24·1 ± 4·1[*]	29·0 ± 5·1[*]
1 mg	4·6 ± 2·0*	16·2 ± 2·9(*)	9·3 ± 2·0*[*]	22·4 ± 4·0(*)[*]
10 mg	2·9 ± 2·0*	14·5 ± 3·1(*)	9·1 ± 1·0*[*]	20·1 ± 2·8(*)[*]
Prolactin				
100 μg	10·0 ± 1·2*	5·0 ± 1·2	13·3 ± 2·9*	12·1 ± 1·1*[*]
500 μg	6·4 ± 2·5*	5·3 ± 1·1*	12·9 ± 4·1*[*]	10·8 ± 2·3*[*]
1 mg	4·0 ± 1·0*	2·9 ± 1·0*	8·7 ± 3·1*	6·5 ± 2·9*
10 mg	2·0 ± 1·3*	1·1 ± 1·0*	3·6 ± 2·0*	3·1 ± 1·0*
Prolactin plus oxytocin				
50 + 50 μg	2·1 ± 1·0*	1·5 ± 1·1*	4·0 ± 2·3*	3·1 ± 1·5*
250 + 250 μg	1·1 ± 1·0*	1·0 ± 1·1*	3·1 ± 1·1*	2·2 ± 1·0*
500 + 500 μg	1·4 ± 1·1*	1·2 ± 1·0*	3·4 ± 1·0*	2·0 ± 1·1*
Basal cortisol, no infusion†	10·3 ± 1·1		6·4 ± 2·0[*]	
Basal cortisol, all infusions‡	10·6 ± 1·0	10·1 ± 1·6	6·1 ± 2·5[*]	6·0 ± 1·7[*]

† Cortisol level 10 min prior to introduction of a dog, without infusions.

‡ Cortisol level 10 min prior to introduction of dog; averaged for all infusions at all concentrations.

* Value was significantly different from value with no infusion or ascorbic acid infusion: $P < 0{\cdot}05$.

(*) Value was significantly different from corresponding value in the same animal for the other infusion site: $P < 0{\cdot}05$.

[*] Value was significantly different from corresponding value for the same infusion site in lactating animals: $P < 0{\cdot}05$.

Combinations of prolactin and oxytocin also appeared slightly more effective at suppressing cortisol responses to a dog when infused into the PVH, but this was not significant. Comparisons between drug concentrations in probe infusate and probe effluent indicated that at all concentrations at least 90 % of the drug appeared to be diffusing into the brain.

Changes in other stress indicators

Heart rate increased significantly ($P < 0{\cdot}01$) in the presence of a dog, but returned to baseline values within 5 min of removing the dog (Fig. 2). None of the drug infusions had any obvious significant effects upon this, and no differences were found between any of the infusions and no infusion. Neither rectal nor skin temperatures significantly changed in the presence of a dog, and infusions of ascorbic acid, oxytocin and prolactin had no effect on this (Fig. 2). However, infusions of combined prolactin–oxytocin were associated with a slight decrease in rectal temperature ($P < 0{\cdot}05$) in the absence of a dog, and a slight ($P < 0{\cdot}05$) increase when

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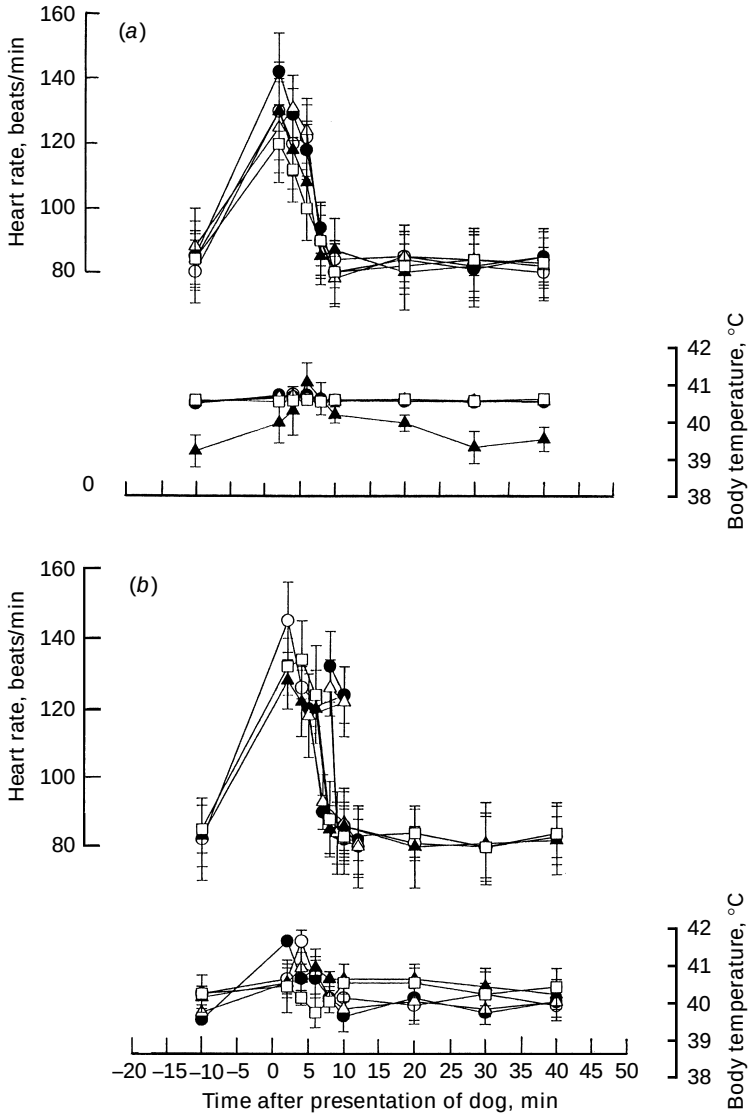

Fig. 2. Changes in heart rate and rectal temperature following exposure to a dog, with posterior pituitary infusion of ○, ascorbic acid; ●, oxytocin; △, prolactin; ▲, prolactin plus oxytocin or □, no infusion in (*a*) non-lactating and (*b*) lactating sheep. Drugs were infused into restrained animals at 1 mg in 20 μl over 20 min. Values are means for $n = 12$ with SD indicated by vertical bars.

a dog was present. Heart rates and body (skin and rectal) temperatures were not significantly different between lactating and non-lactating animals with any of the drug infusions.

Field measurements

Heart rates and body temperatures (skin and rectal), both basal and in response to a dog, were not significantly different in non-lactating animals in the field from those in restraint experiments. Drug infusions produced similar changes in these values (Fig. 3).

Basal cortisol levels were slightly, but not significantly, less than under restraint,

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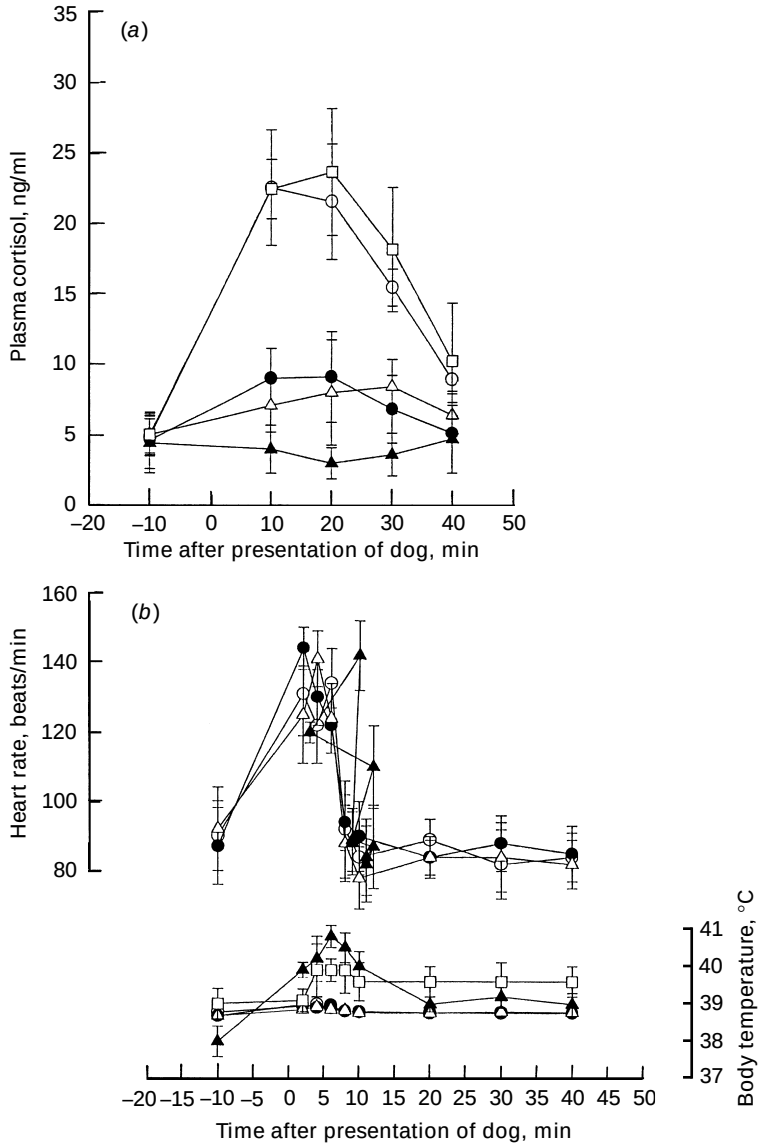

Fig. 3. Time course of (*a*) plasma cortisol response and (*b*) heart rate and rectal temperature following exposure to a dog, with posterior pituitary infusion of ○, ascorbic acid; ●, oxytocin; △, prolactin; ▲, prolactin plus oxytocin or □, no infusion in non-lactating sheep. Drugs were infused into unrestrained animals in the field at 2 mg in 20 μl over 20 min. Values are means for $n = 12$ with SD indicated by vertical bars. The values at -10 min (10 min before presenting the dog) were not significantly different from basal levels prior to drug infusion.

and cortisol response to a dog and its modification by drug infusions were similar to those under restraint (Fig. 3, Table 2).

Effect of repeated measurements

There was no significant effect of order, number or site of infusion upon repeated measures. Repetition of dog presentation was also without effect upon measured values. However, with repetition of restraint, basal but not dog-related cortisol values fell slightly. This fall was independent of other trial variables.

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Table 2. *Cortisol response measured as plasma cortisol concentrations after exposure to a dog in unrestrained non-lactating sheep in the field with and without drug infusions in the posterior pituitary or the paraventricular nucleus of the hypothalamus (PVH)*

(Values are ng/ml, means ± SD for $n = 10$)

Infusion	Posterior pituitary	PVH
None	23·6 ± 4·5	
Ascorbic acid		
2 mg	21·5 ± 4·1	24·1 ± 2·8
10 mg	23·9 ± 3·0	22·6 ± 1·9
Oxytocin		
2 mg	9·1 ± 3·2*	24·0 ± 6·4(*)
10 mg	8·4 ± 1·6*	22·1 ± 3·8(*)
Prolactin		
2 mg	8·0 ± 3·7*	8·4 ± 1·9*
10 mg	3·2 ± 1·5*	3·7 ± 2·8*
Prolactin plus oxytocin		
1 + 1 mg	3·0 ± 1·1*	3·2 ± 1·4*
Basal cortisol, no infusion†	5·0 ± 1·4	
Basal cortisol, all infusions‡	4·8 ± 2·1	6·1 ± 2·9

† Cortisol level 10 min prior to introduction of a dog, without infusions.
‡ Cortisol level 10 min prior to introduction of dog; averaged for all infusions at all concentrations.
* Value was significantly different from values with no infusion or ascorbic acid infusion: $P < 0·05$.
(*) Value was significantly different from corresponding value in the same animal for the other infusion site: $P < 0·05$.

Table 3. *Oxytocin concentration ranges in samples from a microdialysis probe in the paraventricular nucleus of the hypothalamus of lactating and non-lactating sheep with and without infusions of oxytocin into the posterior pituitary*

(Values are pg/ml, means ± SD for $n = 3$)

	Non-lactating		Lactating	
Infusion	Low	High	Low	High
None†	10·1 ± 7·3	40·2 ± 11·9	30·3 ± 12·5*	109·1 ± 14·6*
Infusion + 10 min‡	11·4 ± 9·8	44·6 ± 17·5	35·9 ± 11·2*	120·6 ± 17·3*
Infusion + 20 min‡	9·1 ± 7·6	43·4 ± 21·5	42·9 ± 14·1*	103·7 ± 26·5*
Infusion + 30 min‡	10·3 ± 8·1	44·9 ± 10·4	36·5 ± 9·3*	108·2 ± 29·1*

† Values for peaks (high) and troughs (low) averaged over a 40 min period (each sample being collected over 10 min) from the paraventricular nucleus of the hypothalamus prior to any infusion into the posterior pituitary.
‡ Values for peaks and troughs from the paraventricular nucleus of the hypothalamus during 10 min periods (0–10, 10–20 and 20–30 min) after the start of oxytocin infusion into the posterior pituitary. Infusions were carried out at 1 μl/min and sampling at 2 μl/min.
* Values were significantly different from the corresponding value for lactating animals: $P < 0·05$.

Histology

Histological examinations revealed that all probe sites were within 1 mm of their target for both the posterior pituitary and the PVH. Attempts at measuring changes in PVH levels of oxytocin, as measured by probe pickup, following infusion into the posterior pituitary were not conclusive as signal (infused oxytocin) seemed to be lost within the noise (background oxytocin). Oxytocin measurements from the PVH both prior to and after infusion into the posterior pituitary revealed peaks and troughs of considerable magnitude during the 40 min measurement period, particularly in lactating animals (Table 3). As each sampling period was of 10 min duration, these peaks and troughs represented averaging over that sampling period; they appeared

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independent of infusion, making determination of infusion spread inconclusive. In lactating animals some of the peaks appeared related to attempted suckling and vocalization from the lamb. Detection sensitivity of oxytocin was 5–10 pg/ml. The microdialysis probes were calibrated *in vitro* for relative recoveries (percentage of the external concentration recovered in the microdialysis sample) of oxytocin and this was found to be between 5 and 15%. In the animals where methylene blue was infused, the spread over 20 min infusion appeared to be of the order of 3–5 mm centrally.

DISCUSSION

Non-lactating animals under restraint had slightly higher baseline cortisol levels, and slightly greater cortisol responses to stress, than animals in the field. Drug infusions, however, had similar effects on non-lactating animals both under restraint and in the field. Restraint is mildly stressful to sheep and may have augmented further acute stress response, such as to the dog. Restraint-related baselines did fall with repetition, suggesting that the animals were becoming habituated. Values for cortisol both under restraint and in the field were in accordance with those in other sheep studies (Cook & Jacobson, 1995; Hopster *et al.* 1995).

Lactating animals had basal levels of cortisol higher than non-lactating animals, an observation noted in other studies (Meaney *et al.* 1989). Cortisol responses to stress, however, were less in lactating animals and this has also been reported in a number of other studies using different animals and stressors (Stern *et al.* 1973; Hopster *et al.* 1995; Rushen *et al.* 1995; Walker *et al.* 1995).

At high concentrations of hormone infusions in lactating animals cortisol response to stress actually fell slightly below basal levels. A fall in cortisol below basal levels has not, to my knowledge, been reported before and needs to be viewed with caution. The small sample of lactating animals ($n = 12$) has accompanying possibilities of bias and these results need further verification. If confirmed, they suggest that both basal and stress-related cortisol secretion are being affected by the hormone administered.

The method of administering hormones directly into the PVH or posterior pituitary allows relatively high concentrations to be delivered in a pulsatile manner, rather than the lower concentrations often injected into the brain ventricular system. Limited microdialysis sampling of endogenous oxytocin from the PVH suggests, tentatively, that the administered exogenous concentrations were within the range experienced by lactating animals on a pulsatile basis. There is a paucity of data concerning *in vivo* concentrations of oxytocin in the sheep posterior pituitary and PVH, although the measurements were of a similar order to those reported in the medial preoptic area and bed nucleus of the stria terminalis in lactating sheep (Kendrick *et al.* 1992). In a further series of experiments (C. Cook, unpublished observations) infusion of oxytocin or prolactin at these concentrations into the bloodstream had no effect upon basal or stress-related cortisol levels suggesting, perhaps, the importance of the pulsatile high concentration directly in (or from) PVH or posterior pituitary.

Endogenous levels and pulsatility of oxytocin were significantly greater in lactating animals and some of this appeared related to suckling attempts and vocalizations from the accompanying lamb. It would be extremely relevant to measure cortisol levels while these endogenous pulsatile changes are occurring.

Reducing corticosteroid responses to stress during lactation have been reported widely (Algers *et al.* 1990; Rushen *et al.* 1995; Walker *et al.* 1995) and suckling stimuli

appear necessary to maintain this state (Walker *et al.* 1995). At low doses *in vitro*, oxytocin potentiates corticotrophin-releasing factor (CRF) release (Antoni *et al.* 1983) and in non-lactating animals low levels may act as adrenocortical axis secretagogues (Sapolsky, 1992). Levels of oxytocin are low in non-lactating as compared with lactating animals, and this was supported by the limited microdialysis studies. In lactating animals with higher levels and pulsatility, at least in part related to suckling stimuli, there may be a dose- (or pulsatility-) dependent suppression of corticosteroid release. Oxytocin may have dose-dependent effects that differ throughout the adrenocortical axis. Concurrent measurements of CRF, ACTH and cortisol, or the use of dexamethasone pulses, could prove useful in targeting sites and modes of action.

At low doses oxytocin had a greater cortisol suppressive effect in lactating as compared with non-lactating animals. If oxytocin levels are higher in lactating animals there may be a synergic effect between endogenous and exogenous oxytocin expressed at the lower end of the dose–response curve. Oxytocin appeared effective only when administered in the posterior pituitary and yet oxytocin neurones are present in the PVH, an area where oxytocin affects CRF in the non-lactational state (Sapolsky, 1992). Dosage, gender and lactational state all appear to influence the effects of oxytocin (Björkstrand & Uvnäs-Moberg, 1996).

Infusions of prolactin also had suppressive effects on the cortisol response to stress, both within the posterior pituitary and the PVH. This may be more suggestive of a hypothalamo-pituitary influence on CRF and/or ACTH or their secretagogues compared with oxytocin. Prolactin was more effective at lower doses in lactating as compared with non-lactating animals, and appeared slightly more suppressive on cortisol than oxytocin. Prolactin levels do exhibit greater pulsatility and magnitude in lactating as compared with non-lactating animals, but this varies with post partum period of lactation, suckling and lactation-related fluid balance (Ghosh & Sladek, 1995).

The site(s) of action of prolactin and oxytocin cannot be clearly defined from the present study. The volume of hormone infused and the 20 min latency between infusion and stress would have allowed considerable circulation both centrally and systemically. Other central changes appear to occur with lactation and these include serotonin release within the hypothalamus (C. Cook, unpublished results) and changes in γ-amino-n-butyric acid within the hypothalamus with both lactation and suckling (D. Grattan, pers. comm.). Given the roles of these neurotransmitters in hypothalamic control, oxytocin and/or prolactin could contribute to these changes.

The greater than additive effect of combining oxytocin and prolactin on suppressing stress-related cortisol response suggests that these two hormones, prominent in lactation, may be involved in lactation-related changes in cortisol stress-related responses. Oxytocin and prolactin vary in magnitude and pulsatility with numerous lactation factors including fluid balance, suckling stimuli and length of post partum period. These same factors affect the response of these two hormones to both catecholamines and corticosteroids (Higuchi *et al.* 1983, 1985, 1989; Ghosh & Sladek, 1995). Pulsatility of hormones can have acute and long-term effects on the cell receptors (Sapolsky, 1992) that control neurohumoral responses.

High pulsatile levels of cortisol can disrupt the lactation process and this may offer insight into lactation-related suppression of cortisol change. Slightly higher levels of cortisol, albeit stable, may be the cost of this process. Diurnal variation in maternal cortisol during lactation and its relationship to milk delivery would prove valuable.

Large fluctuations in maternal cortisol may also be delivered to the infant via milk, provided let-down occurs while cortisol levels are still high, and it has been speculated that fluctuating levels of cortisol can damage the developing nervous system (De Kloet *et al*. 1988). Neonatal infants do appear to have a suppression of their own cortisol response (Walker *et al*. 1986), despite a relatively functional pituitary corticograph axis. It would be useful to know if maternal factors contribute to this infant suppression, and how important it is in infants to prevent large cortisol fluctuations.

If the above speculations are valid it would be advantageous to have a lactation–suckling pattern of hormone pulsatility that could suppress cortisol stress-related responses.

Heart rate and body temperature responses to stress were not affected by drug infusion, except with combined prolactin–oxytocin infusion where the rise in body temperature in the presence of a dog was slightly greater than with other drug treatments. It seems likely that body temperature and heart rate responses to stress were not dependent on cortisol release. In lactating animals other stress responses, independent of cortisol, have been observed (Walker *et al*. 1995) so there is no generalized suppression of stress response in addition to cortisol. A combined oxytocin–prolactin infusate may have effects additional to suppressing cortisol on other aspects of physiology including body temperature. Other studies have suggested that oxytocin from the PVH influences tachycardic response to stress (Morris *et al*. 1995). Tachycardia was observed in response to the dog; however, infusions of oxytocin did not appear to change this. Possibly endogenous levels of oxytocin are enough to saturate this heart rate contribution fully.

This study further indicates the problems of using cortisol alone as a stress indicator. In dairy cows, where animals have undergone extensive genetic selection to achieve extended periods of milk production, these may be further exacerbated.

Roles of prolactin and oxytocin during lactation require further supportive study, but represent exciting areas of welfare and lactation physiology.

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